A NEUROHISTOLOGICAL STUDY OF THE SENSORI-MOTOR CORTEX

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CHAPTER 1

GENERAL INTRODUCTION

The cerebral cortex appears as a convoluted mantle of grey matter overlying most of the remaining structures of the primate brain. It was originally thought to act as a single unit with no localisation of function within it (see Walshe, 1943) but, starting with the experimental demonstration by Fritz and Hitzig (1870) of an area of cortex in the dog from which movements could be produced by electrical stimulation at a low threshold, much work over the last hundred years has been devoted to demonstrating both localisation of function within the cortex physiologically and structural subdivisions within it anatomically. In particular, differences in the shape, size and distribution of cell bodies within the cortex as seen with the Nissl method have been used as the basis for dividing the cortex into a large number of architectonic areas and of these systems of anatomical subdivisions that of Brodmann (1903, 1909) is the most widely recognised and used. The relationship between his histological areas and areas of differing functional significance was recognised early in the case of the motor cortex although there was debate as to whether the boundaries of the histological area 4 and the physiologically defined motor cortex corresponded exactly (see Walshe, 1942). The correspondence of an architectonic and functional area was shown more exactly in the sensory areas of the cortex using the method of evoked potentials (Adrian, 1941; Marshall, Woolsey and Bard, 1941) and has been confirmed in more

detail in recent years by the microelectrode studies on most of the sensory areas (e.g., Powell and Mountcastle, 1959b; Hubel and Wiesel, 1962, 1965; and Phillips, Powell and Wiesendanger, 1971). Powell and Mountcastle showed functional differences between the individual architectonic subdivisions of the somatic sensory cortex of the monkey, most cells in area 3 being activated by skin stimulation whereas most of those in area 2 were activated by stimulation of deep structures, and the proportion of cells of each type in the interposed area 1 was intermediate. Hubel and Wiesel similarly showed an increasing proportion of cells with more complex response properties in areas 18 and 19 of the visual cortex of the cat in comparison with the primary visual area, area 17. Much emphasis has therefore been given to the importance of the structural differences between different areas of the neocortex and to the correlation of these with differences in function.

The function of the motor cortex appears to be distinctly different from that of sensory cortical areas and there are marked differences between the sensory and motor cortical areas when seen under the light microscope. Previous electron microscopic studies of the neocortex have been mainly of sensory or parietal cortical areas (e.g., Colonnier, 1968; Szentágothai, 1969; Jones and Powell, 1970a-e; Lund and Lund, 1970; Peters and Kaiserman-Abramof, 1970; Peters, 1971; Garey, 1971; Garey and Powell, 1971; Cragg, 1976) with the exception of a description of the Betz cells and of the thalamo-cortical afferents to the motor

cortex, both in the cat (Kaiserman-Abramof and Peters, 1972; Strick and Sterling, 1974). The emphasis in this present study has therefore been placed on the motor cortex because it represents a distinctly different cortical area and because of the previous concentration on sensory cortical areas; the somatic sensory cortex has been studied only to give a direct comparison of sensory and motor cortical areas by the same observer in the same species. The physiology of the motor cortex has been investigated thoroughly in the primate and this region is frequently involved in pathological processes in man. For these reasons and because of the relatively large size of the motor cortex in the primate this study has been performed throughout on Rhesus monkeys.

An understanding of the functioning of a cortical area can only be obtained by a correlation of findings from anatomical and physiological methods. From this point of view the motor cortex provides a very good site for electron microscopic study because of the extensive attention it has received from physiologists. In particular it has a clearly identifiable output pathway, the pyramidal tract, which can be isolated and stimulated (although this is by no means its only significant output) and this has allowed study of the output cells of the motor cortex by antidromic stimulation. In this respect the motor cortex has been investigated in more detail than sensory areas and the effects of the connections made by the intracortical axon collaterals of

pyramidal tract cells have been determined (Phillips, 1956a,b, 1959; Stefanis and Jasper, 1964a, b; Brooks and Asanuma, 1965; Stefanis, 1969), giving information about the intrinsic organisation of the motor cortex which is not at present obtainable by anatomical methods. The functional relationships of the efferent connections of the motor cortex to the spinal cord have also been thoroughly studied (see Phillips, 1969) and this, apart from its own intrinsic interest, has provided valuable information about the physiological properties of the axon terminals of pyramidal tract cells. The effects of stimulation of the thalamic and commissural afferents to the motor cortex have also been investigated (Branch and Martin, 1958; Asanuma and Okada, 1962; Amassian and Weiner, 1966) and this has given information about some of the afferent fibre systems whose anatomical properties have been studied here. Since these afferent connections represent major inputs to the motor cortex their study is the logical next step in the analysis of cerebral motor function (Phillips, 1969).

A number of different approaches have been taken in this study although there is inevitably some overlap between them and the results may be broadly divided into those which are descriptive, those which are mainly concerned with the analysis of the synaptic connections of the cortex, those in which the motor and somatic sensory cortices are compared and finally some theoretical aspects of cortical organisation are considered.

The descriptive results are mainly related to the cytological features of neurons, and because of the similarity found here between the motor cortex and previous electron microscopic descriptions of sensory neocortical areas, only brief descriptions have been given where the structure of the motor cortex corresponds to these previous findings. More emphasis has been placed upon new observations, in particular on the two kinds of inter-neuronal connection found which had not previously been described in the neocortex, dendro-dendritic synapses and gap junctions. The opportunity has also been taken to study axon initial segments in some detail and to obtain quantitative data relating to their dimensions and to the synapses upon them, and also to study the relationships of their cisternal organs and those of the sub-surface cisternae found in cell somata and dendrites.

The electron microscope has made it possible to visualise the individual synaptic connections between neurons and so has opened the way to detailed investigations of the organisation of connections in the central nervous system, and the main aim of this study has been the analysis of the organisation of connections to and within the sensori-motor cortex. However it is necessary to study the complex three-dimensional structures of which the neocortex is composed by using very thin and therefore effectively two-dimensional sections, and to the observer the neocortex presents a bewildering picture of isolated parts of axons, dendrites and spines. These processes are frequently

connected to each other by synapses but give little clue as to which of the neurons scattered among the neuropil give rise to them. In the present study the unravelling of this puzzle has been approached in a number of ways. According to the neuron doctrine all the isolated axonal, dendritic and spinous profiles which make up the neuropil arise from neurons and an essential step in understanding the cortex is to define how many different types of this basic unit there are and in what proportions they exist in the neocortex. There is general agreement, on the basis of light microscopy using the Golgi technique, that cortical neurons may be divided into pyramidal and non-pyramidal types and that pyramidal cells have a triangular cell soma with apical and basal dendrites, large numbers of spines on the dendrites and an axon which is directed initially towards the white matter. There have been a number of attempts to classify non-pyramidal cells using the Golgi technique (e.g., Cajal 1909-11; Sholl, 1955, 1956; Szentágothai, 1973; Jones, 1975a) and certain definite subtypes such as the basket cell seem to be reasonably well established (Marin-Padilla, 1969, 1970). With the electron microscope there is agreement as to the features of pyramidal cell somata in the neocortex (Colonnier, 1968; Jones and Powell, 1970a; Peters and Kaiserman-Abramof, 1970) and also on the features of stellate or non-pyramidal cells although only one type appears to have been recognised (Colonnier, 1968; Jones and Powell, 1970a; Peters, 1971). A further classification

of neuronal cell somata has been attempted using features seen with the electron microscope and where possible correlations have been made with light microscopic findings, and a quantitative study has been made.

After definition of the features of the cell soma of neurons of a particular type the next step has been to try to recognise the dendrites arising from this cell type when they are seen in isolation in the neuropil. In certain sites such as the olfactory bulb and the cerebellar and hippocampal cortices this has been helped by the fact that it was known from findings using the Golgi technique that only dendrites of certain cell types occurred in certain regions. The less discrete nature of the organisation of the neocortex means that the features of the dendrites of each cell type have mainly to be worked out from the long lengths of dendrite which are on occasion found cut in continuity with the cell soma, although it has been possible in one case also to match the laminar distribution of a particular kind of cell soma to that of dendrites of a specific type. It has also been possible in certain instances to correlate the appearance of dendrites and particularly the presence of spines as seen with the Golgi technique with the appearance of dendrites as seen under the electron microscope.

The much greater lengths of axons compared to dendrites and the less distinctive appearances of different axon terminals under the electron microscope make the identification of the origin of the axon terminals seen in the normal cortex a very

difficult problem. Fortunately axon terminals undergo a characteristic series of changes visible under the electron microscope following section of their parent axons or removal of their cells of origin and the use of experimentally placed lesions is now a well established technique in electron microscopy as well as in light microscopy (Guillery, 1970). It is, however, an essential prerequisite for the study of the details of the connections of an area with the electron microscope that their broad outline has first been established with light microscopic techniques. Afferent connections to the motor cortex have been described from the ventrolateral nucleus of the thalamus, from the opposite motor cortex and from the ipsilateral somatic sensory cortex and area 6 (Walker, 1938; Jones and Powell, 1969a; Pandya and Kuypers, 1966; Pandya and Vignolo, 1971). The light microscope can, however, only indicate the presence of these connections and give some idea of their level of termination within the cortex; it cannot identify the exact sites of termination themselves. These afferent connections to the motor cortex have therefore been studied with the electron microscope after experimentally placed lesions and, for comparison, the thalamo-cortical and commissural connections to the somatic sensory cortex which have previously been studied with the electron microscope in the cat (Jones and Powell, 1970d, e) have also been studied. The aim of the experimental part of this study has been to identify the exact sites of termination and distribution of the degenerating axon terminals themselves and to identify

the types of synapse that they make and the types of profile with which they make synapses. By combining the findings from these experiments with those made on the normal structure of the cortex it has been possible in some instances to determine the types of neuron contacted by the different afferent connections rather than just the types of profile receiving synapses from degenerating axon terminals. It has thus been possible to work out some aspects of the organisation of the cortex.

However, it has been shown by undercutting the cortex (Szentágothai, 1964; Colonnier, 1966; Jones and Powell, 1970e) that afferent connections to the neocortex account for only a small proportion of the axon terminals seen in the neuropil. The remaining axon terminals must presumably therefore arise from the neurons within the cortex. Gray (1959) showed initially that axon terminals in the normal neocortex fixed with osmium tetroxide could be divided into Type I and Type II on the basis of the appearance of their synaptic membrane thickenings and this was extended to aldehyde-fixed material by Colonnier (1968) who called the two types asymmetrical and symmetrical synapses respectively and showed that the asymmetrical synaptic thickenings were associated with round synaptic vesicles whereas the symmetrical membrane thickenings were associated with flattened vesicles. (The flattening of synaptic vesicles has been shown to depend on the osmolarity of the fixative used but it is a constant artefact (Valdivia, 1971)). Round synaptic vesicles have been

correlated with excitatory function and flattened vesicles with inhibitory function of the synapse in the cerebellum (Uchinozo, 1965) and other sites (Gray, 1969) and this appears to hold for neocortical cells where this is testable (Chapter 10). It has also been shown that asymmetrical synapses occur predominantly on dendritic spines in the neocortex, although present on the dendritic shafts and cell somata of stellate cells, whereas only symmetrical synapses occur on the cell somata and initial segments of pyramidal cells (Colonnier, 1968; Peters, Proskauer and Kaiserman-Abramof, 1968; Jones and Powell, 1969c, 1970a; Peters and Kaiserman-Abramof, 1970). There is therefore a considerable amount of valuable descriptive information available relating to the axon terminals in the normal neocortex together with a tentative correlation of structure and function, and it is possible to identify the terminals of extrinsic afferents by experimental methods. By exclusion and from the results of undercutting the cortex it can be inferred that the remaining axon terminals are those of intrinsic connections. Small lesions placed within the neocortex can provide valuable information regarding the spatial distribution of these intrinsic connections (Fisken, Garey and Powell, 1975) but mechanical lesions cannot specifically involve one cell type within the cortex and so are not of direct assistance in identifying the individual cell types giving rise to particular intrinsic axon terminals and this problem of identifying the different types of intrinsic axon terminal and their corresponding cells of origin remains one of the major problems in the histological study of

the neocortex.

From an architectonic point of view the agranular motor cortex and the granular cortex of area 3b of the somatic sensory cortex represent two of the most divergent types of cortex described by light microscopy and the sensori-motor cortex is therefore an excellent site in which to make a comparison between different cortical architectonic areas. With the Nissl method the motor cortex is described as lacking distinct granular layers (II and IV) whereas the pyramidal layers III and V are well developed and most of the cells in the motor cortex appear to be pyramidal in type. In contrast, the cortex of area 3b, a primary sensory cortex, is described as being rich in granule cells, which have generally been equated with stellate cells, and layers II and IV are very prominent while the pyramidal layers III and V are poorly developed. The Nissl method only shows certain limited aspects of the structure of a cortical area, however, whereas the electron microscope can show much greater detail and also structures not otherwise seen. The electron microscope has therefore been used here to compare the general appearances of the motor and somatic sensory cortices and a number of particular aspects, namely the neuronal populations, the plexus of myelinated axons in layer I and the thalamic and commissural afferents have been compared in some detail. In some respects, particularly in regard to the neuronal populations,

the results of these comparisons have been surprising and have differed in a number of important respects from conclusions drawn on the basis of light microscopy.

There is now a wealth of physiological information relating to the motor cortex and in the general discussion the anatomical and physiological findings will be discussed together to see to what extent they can be correlated. On the basis of this evidence a simple circuit diagram of the cortex has been made. Although obviously an oversimplification, it will be shown that this neuronal network usually gives a limited response to an afferent input but under certain conditions it produces a sustained maximal discharge. The similarities between this and an epileptic fit will be discussed, as will the parallels between factors causing uncontrolled discharge in the model and conditions known to trigger fits in man. This similarity must however remain speculative at present although the model has provided a useful stimulus to further work.

Form of presentation

Following the chapter on the material and methods used, the normal structure of the motor and somatic sensory cortices is described in general. Particular aspects of the normal structure, namely the cell types, the axon initial segments, dendro-dendritic synapses and gap junctions are then described in detail in succeeding chapters. General aspects of the experimental results are considered, followed by a chapter giving

the details of the thalamo-cortical and commissural afferents to areas 4 and 3b and the association afferents to area 4 from the ipsilateral SI and area 6. Each chapter contains a discussion of its results and then the final general discussion attempts to bring these results together and correlate them with physiological findings and a theoretical model based on this evidence is described and its properties explored.

CHAPTER 2

MATERIAL AND METHODS

All observations were made on material from the motor cortex (area 4 of Brodmann) or area 3b of the somatic sensory cortex of twenty-one young adult Rhesus monkeys (Macaca mulatta). Most of the results to be described were obtained using the electron microscope and standard preparative techniques in conjunction with experimental lesions. Because these preparative techniques are now standard practice in E.M. laboatories they will not be described in detail, but greater detail will be given where these existing techniques have been developed or more specialised techniques have been used. In several instances such matters of technique have been of considerable importance in obtaining the results described.

Operations

Operations were performed under 'Nembutal' (sodium pentobarbital) anaesthesia using full aseptic precautions. Four basic operative procedures were used.

1. Large electrolytic lesions placed stereotaxically in the thalamus (three experiments).
2. Extensive removals of the cortex of both the motor and somatic sensory areas for the study of commissural connections (four experiments).
3. Partial or near complete removals of the somatic sensory cortex (SI) to study association connections to the motor cortex (ten experiments).

4. Partial removals of area 6 to study association connections to the motor cortex (four experiments).

For the lesions in the cortex a temporal skin flap was turned and the underlying connective tissues were incised and retracted, including the origin of the temporalis muscle. A burr hole was made and enlarged with bone forceps, a wide bone removal usually being made to minimise the effects of any cerebral oedema. The dura was opened carefully and reflected. The pia was removed with fine forceps over the appropriate area and the underlying cortex was removed by suction. For the study of the commissural connections most of the sensori-motor cortex was removed on one side and for the investigation of the association connections from SI to the motor cortex the surface of the post-central gyrus was removed but the posterior wall of the central sulcus was usually left in situ to protect the motor cortex although it was completely undercut by removal of the underlying white matter of the post-central gyrus. Lesions in area 6 were placed in front of the anterior end of the pre-central dimple and medial to the upper end of the arcuate sulcus, and the extent of these lesions was checked histologically to ensure that they did not involve area 4. After bleeding had been controlled, the dura was replaced and the connective tissues sutured, Penicillin powder was dusted into the wound and the skin incision closed.

Large electrolytic lesions were placed stereotaxically to involve the ventrolateral and ventral posterior nuclei of the thalamus using a horizontal approach passing through the occipital

pole. Needle electrodes insulated except at the tip were used and between nine and twelve parallel tracks were made to form a grid. The position of these lesions was confirmed by examination of the cut brain post-mortem.

Perfusion

Following survival periods of two to six days, the animals were again anaesthetised and then cooled with ice and alcohol to a rectal temperature of between 25°C and 30°C. The chest and pericardium were then opened and a mixture of 0.5 ml of Heparin and 0.5 ml of 1% sodium nitrite was injected into the left ventricle. The right and left sides of the heart were then opened and the animals were perfused through the aorta with White's saline followed by modified Karnovsky's fixative, both at room temperature (see Appendix I for details of composition). After one to two hours the brain was carefully removed and stored in fixative under refrigeration.

Light Microscopy

Normal Rhesus monkey material prepared by the Nissl method and various modifications of the Golgi technique was available for study in conjunction with the electron microscopic material. In addition, glutaraldehyde-fixed material of the lesions used for electron microscopy was prepared by the Nissl method using thionin and the fibre and terminal degeneration following various lesions was studied using the Nauta technique as modified by Wiitanen (1969) on frozen sections from blocks

adjacent to those studied with the electron microscope. For a light microscopic comparison of the plexuses of myelinated axons in layers I of the motor and somatic sensory cortices Weil's myelin stain was used slightly modified for frozen sections cut from glutaraldehyde-fixed material (see Appendix I for details).

Preparation of Material for Electron Microscopy

One millimetre thick slices were cut from the brains and care was taken to make them perpendicular both to the central sulcus and the pial surface; they were taken at various medio-lateral positions along the sulcus. Blocks about 1 mm wide and running through the whole depth of the cortex were then taken from the motor cortex and area 3b of the somatic sensory cortex by reference to Powell and Mountcastle (1959a), pp. 110 and 113, Figs. 1 to 5. These blocks were rinsed in 10% sucrose in phosphate buffer and then post-fixed in 2% osmic acid in phosphate buffer for one hour. After again rinsing in sucrose phosphate buffer the blocks were dehydrated through a graded series of alcohols, being also block-stained with uranyl acetate at the 70% alcohol stage, and then transferred to epoxy-propane for one hour. This was followed by a mixture of Epon-Araldite and epoxy-propane in equal parts in which the blocks were left overnight. The blocks were embedded in Epon-Araldite and polymerised at 37°C for 24 hours and 60°C for a further 48 hours.

One μm 'thick' sections were cut from the block face perpendicular to the pial surface and stained with a mixture of methylene blue and Azure II (Richardson, Jarrett and Finke, 1960). Using the 'thick' section as a guide a mesa, usually of approximately 1 x 0.5 mm, was trimmed and ultrathin sections of known depth and orientation were cut, mounted on Formvar-coated copper grids having a single 1 x 2 mm hole, and were stained with alkaline lead citrate (Reynolds, 1963) and uranyl acetate (5% solution in 50% ethanol). This procedure meant that the orientation and position of any ultrathin section in relation to the cortical laminae was always known and this was of great importance in this study. The cortex was routinely studied systematically through its depth and this required three mesas per block to cover the depth of the motor cortex, and two in the thinner somatic sensory cortex. These large sections were usually cut as short series of ten to fifteen serial sections so that any interesting structures could be followed through at least a few serial sections. Extensive use was also made of long series of up to a hundred small serial sections approximately 100 μ square taken from selected regions of a block. In some instances blocks were trimmed so that thick sections could be cut from the side as well as the front of a block and these were used to adjust the angle at which the block was cut so that sections could be taken accurately parallel to the 'grain' of the cortex as judged by the apical dendrites. This allowed long lengths of apical

dendrites and in particular axon initial segments to be studied in single or a small number of serial sections.

Sections were also cut parallel to the pial surface of the brain and to do this a thick section was first cut from the long face of a block and the block was then turned and cut parallel to the pia. The depth of any section then cut could be determined by taking further thick sections from the block perpendicular to the surface and the top edge of these thick sections, when compared to the original thick section, corresponded to the depth at which the sections cut parallel to the pia had been taken. It was thus possible to cut sections parallel to the pia at an accurately known depth in the cortex.

Phosphotungstic Acid staining for Electron Microscopy

In addition to the routine method described above, three other techniques were used to prepare blocks for electron microscopy in order to examine specific staining of certain structures by these methods (Gray, 1959; Bloom and Aghajanian, 1968; Gray and Willis, 1970).

(1) Block staining with Ethanolic Phosphotungstic Acid (E-PTA)

The blocks were dehydrated in alcohol and soaked in 1% Analar phosphotungstic acid in absolute alcohol for 3 hours after which they were embedded as described above. Sections were examined unstained.

(2) Osmium - E-PTA

Blocks were first postfixed in osmium tetroxide and

then dehydrated in alcohol, treated with E-PTA and embedded as above. Sections were examined unstained.

(3) Control method

Blocks were dehydrated in alcohol and embedded in Epon-Araldite. Sections were examined unstained.

E-PTA only stained approximately the outer 50 μ of the blocks used and so sections for all these three methods were taken from this region at the edge of the blocks.

Mapping and Quantitative Techniques for the Electron Microscope

The distributions of neurons and degenerating axon terminals in relation to the laminae of the cortex (Chapters 4 and 9) were plotted using the mapping technique described by Alkense, Blackstad, Walberg and White (1966) in which the microscope stage coordinates are recorded for the corners of the section and each cell soma or degenerating axon terminal and then these are plotted out on graph paper. The maps of individual sections were then collated by comparison of the thick sections of the individual mesas used with the thick section of the entire block used. The positions of the cortical laminae were determined from these thick sections and transferred to the maps so that a composite map showing the distribution of neurons or degenerating axon terminals through the depth of the cortex could be constructed (e.g., Figs. 4-30 and 9-28).

For a quantitative comparison of the numbers of myelinated fibres in layer I using the electron microscope, blocks were taken from the walls of the central sulcus so as to contain the superficial layers and apposed pial surfaces of both the motor cortex and area 3b of the somatic sensory cortex in the same block. Ultrathin sections were cut from these so that layer I of both cortical areas was present in the same section, with the two pial surfaces being apposed. The number of myelinated axons appearing on the microscope screen at a magnification of 6000 x was noted at a point 25 μ below the surface of the brain and 25 μ intervals below this on a line passing perpendicularly down through layer I, traverses being made alternately into the motor cortex and area 3b. The position and orientation of traverses were decided by plotting a map of the section being studied, including the positions of the surface and the deep boundary of layer I in each area and the required coordinates for each point were read off and set on the microscope stage. Ten traverses of ten points each were made in each area in five blocks from different hemispheres. In addition to the counts, photographs were taken at a proportion of points, with final prints at a magnification of 20,000 x and the external diameter of each myelinated fibre was measured to determine the size distribution of the axons in each plexus.

CHAPTER 3

ULTRASTRUCTURAL FEATURES OF THE SENSORI-MOTOR CORTEX

Introduction

This chapter is the first of a series describing an electron microscopic study of the normal structure of the motor cortex and area 3b of the somatic sensory cortex of the monkey and experimental studies of their connections. There have previously been a number of papers describing the normal structure of sensory areas of the neocortex, the visual cortex of the rat, cat and monkey (Colonnier, 1968; Lund and Lund, 1970; Garey, 1971), the parietal cortex of the rat (Peters and Kaiserman-Abramof, 1970; Peters, 1971) and Jones and Powell (1970a-c) have given a detailed description of the somatic sensory cortex of the cat. The emphasis in this study has therefore been on the fine structure of the motor cortex (area 4 of Brodmann), and area 3b of the somatic sensory cortex has been studied mainly for comparison with the motor cortex and to see if it differs markedly from the somatic sensory cortex of the cat.

The ultrastructure of the motor cortex closely resembles that of the other cortical areas described and therefore only a brief description of its general structure will be given and then several specific points will be described in more detail, emphasis being placed on new features and on comparison between motor and somatic sensory cortex. Detailed accounts of the features of the neuronal types, dendro-dendritic synapses, gap junctions and axon initial segments are given in subsequent chapters (Chapters 4-7) as are the results of the experimental studies (Chapters 8 and 9)

Results

Under the electron microscope the motor cortex and area 3b of the somatic sensory cortex of the monkey appear remarkably similar to each other and to the somatic sensory cortex of the cat (Jones and Powell, 1970a-c). The neuropil consists predominantly of dendritic spines and axon terminals; among these run dendrites and myelinated and unmyelinated axons and the somata of both neurons and glial cells are found at intervals. The triangular shape of pyramidal cell somata together with pyramidal apical dendrites give the cortex a definite grain so that the orientation of a section may be determined with little difficulty and the different laminae may be recognised by their differing features.

Only the occasional small neuron is found in layer I which in the motor cortex is about one and a third times the thickness of layer I in area 3b. It consists mostly of dendritic spines and axon terminals along with small dendrites and unmyelinated axons as in the somatic sensory cortex and most of the synapses are of the asymmetrical type although symmetrical synapses are also present. Layer I of both the motor and somatic sensory cortices also contains a plexus of small myelinated axons running parallel to the pia mater (Fig. 3-1). In light microscopic sections stained for myelin by Weil's method the fibre plexus in layer I of the motor cortex appears much more prominent than that in layer I of the somatic sensory cortex, this being particularly obvious in the walls of the central sulcus where the two layers I are

immediately adjacent (Fig. 3-2) but is also apparent when the exposed surface of the pre-central gyrus is compared with that of the post-central gyrus. As a control, the fibre plexuses in layer I in the anterior and posterior walls of the interparietal sulcus were carefully compared on the same sections and were found to be of approximately equal prominence and these observations were confirmed in a number of brains and over a wide range of staining density. These layer I fibre plexuses were compared quantitatively using the electron microscope. The density of the fibre plexus was measured by counting the number of myelinated axons appearing on the screen of the microscope at constant magnification, counts being made at equal intervals along a line running through layer I perpendicular to the pial surface of both area 4 and area 3b. Ten sample areas were counted at 25 μ intervals in each of a total of fifty columns in both area 4 and area 3b using five sections from four different brains. There was considerable variation in the density of fibres between individual counts (Fig. 3-3) but the pooled results confirm previous qualitative electronmicroscopic reports of the presence of a definite increased density of myelinated fibres in the upper third of layer I (Fig. 3-4) and they show that the density in the motor cortex is approximately twice that in area 3b. If the total numbers of fibres in layer I are compared, rather than their densities, layer I being thicker in the motor cortex, this difference is slightly more marked.

At a proportion of the points counted photographs were taken at

a standard magnification and the diameters of all myelinated axons on them were measured. The histograms of the distribution of fibre diameters in areas 4 and 3b are very similar (Fig. 3-5) as are the mean diameters, 0.71 μ for the motor cortex and 0.69 μ for area 3b. The difference between motor and somatic sensory cortex was therefore in the numbers of myelinated fibres present in layer I rather than in their sizes. There were no marked variations in axon diameters with depth from the surface in layer I.

Layer II in the motor cortex contains a considerable density of small neurons which may occur singly or in groups of two or three. Many are typical small pyramids, some of which may be seen to give rise to apical dendrites although their somata are less pyramidal in shape than those of larger pyramids. Other somata are frankly round or oval in outline with sparse clear cytoplasm, few dendrites and receive few synapses but these may be of the asymmetrical type. These have been identified as small stellate cells (Sloper, 1973a; Chapter 4). The apical dendrites of deeper pyramids run vertically through layer II and in sections taken parallel to the pia mater they may be seen to be uniformly distributed (Fig. 3-26). The neuropil between the apical dendrites consists predominantly of spines and axon terminals and only a few myelinated axons are seen, some of which run vertically through the layer and the occasional one may be seen to arise from a vertical axon initial segment above; in a few cases these have been traced in continuity with pyramidal cell somata (Sloper and Powell, 1973; Chapter 5).

Layer III in the motor cortex is a little thicker than in the somatic sensory cortex and may be identified by its medium and large pyramidal cells. Their somata are more obviously pyramidal in shape than those of layer II and give rise to apical dendrites passing vertically upwards and axon initial segments passing vertically down. These somata are frequently accompanied by a satellite glial cell. Many apical dendrites pass through layer III from below and because of their similarity to the dendrites of mitral cells in the olfactory bulb, which are myelinated in the monkey (Pinching, 1971), a careful search was made to see whether pyramidal apical dendrites were ever myelinated. None was found to be truly myelinated but in a few cases in experimental material an empty myelin sheath had become flattened and applied to an apical dendrite to give the appearance of a myelinated segment of dendrite in a single section (Figs. 3-6 and 3-7). However at the ends of the myelin the sheath was folded back on itself instead of having true end feet and serial sections confirmed that this was not true myelination. Layer III also contained varicose dendrites. These tended either to have a markedly varicose shape with empty-looking cytoplasm and to receive a moderate number of synapses of both kinds (Fig. 3-9) or else to receive a very high density of synapses and to contain a lot of organelles but to have a much less markedly varicose shape (Figs. 3-8 and 3-10). The latter type of dendrite was more frequent in the deep part of layer III. The neuropil of layer III

is dominated by dendritic spines which receive synapses from axon terminals. Most of these are asymmetrical and are from axon terminals of the small dark type but a proportion of the synapses are symmetrical and are made by rather paler axon terminals. Dendritic spines were found to receive one or two asymmetrical synapses or an asymmetrical and a symmetrical synapse (Fig. 3-11) but no dendritic spine was proved to receive only a symmetrical synapse. A few examples were found of spines which received synapses from three axon terminals or had unusual configurations (Figs. 3-12 and 3-13). It was not uncommon, both here and in other layers, for a single axon terminal of either the asymmetrical or symmetrical type to make a synapse both on to a spine and on to the shaft of the dendrite giving rise to that spine by way of a separate membrane specialisation (Figs. 3-14 and 3-15), but serial sections were often required to demonstrate this. Spines were also found occasionally on neuronal somata in several laminae (Figs. 3-16, 3-17 and 3-20). On pyramidal somata these have received symmetrical synapses but no clearly asymmetrical synapses and a terminal making a synapse on to a somatic spine may also make a synapse directly on to the parent cell soma (Fig. 3-20). In several laminae a rare but distinct type of axon terminal was found which had a remarkably varicose but uniform shape and occasionally more than one varicosity could be traced in continuity (Figs. 3-18 and 3-19). These terminals contained round vesicles, with a high proportion of large dense-cored vesicles, and made

asymmetrical synapses.

As conventionally described, layer IV in the motor cortex consists of a narrow strip containing rather more small cells, situated between the Betz cells of the upper part of layer V and the large pyramids in the lower part of layer III. Under the electron microscope layer IV in the motor cortex has no obvious boundaries. There is an increase in the number of myelinated axons in the region of its upper border and some sections show a dense plexus of unmyelinated axons in this region as in the somatic sensory cortex. Myelinated axons may be seen to give off branches at nodes of Ranvier (Fig. 3-21) particularly in the deeper cortical layers and in somatic sensory cortex pale axons making several 'en passage' symmetrical synapses are not infrequently seen (Fig. 3-22); these are rare in motor cortex. Large stellate cells appear prominent in layer IV as are the varicose dendrites of the type studded with synapses but both cells and dendrites extend well up into layer III and down through layer V. Small stellate cells also occur in layer IV but quantitatively in both motor and somatic sensory cortex of the monkey the majority of neurons in layer IV are small pyramids, although their presence is not conspicuous. In area 3b the large stellate cells appear to be more restricted to layer IV than in the motor cortex. Layer IV in both motor and somatic sensory cortex contains large pale axon terminals with round vesicles and making asymmetrical synapses which are often multiple in a single

section. An occasional finding has been a structure consisting of bundles of parallel densely staining fibrils, usually in dendrites (Figs. 3-23 to 3-25). Their significance is unclear but one example appeared to be in continuity with a mitochondrion. Apical dendrites pass up through layer IV from deep pyramids and in sections cut parallel to the pia in motor cortex these are seen to occur in bundles of about six to twelve (Figs. 3-27 and 3-28). These bundles extend up into the lower part of layer III and appear to be more prominent in some parts of the motor cortex than others.

The motor cortex of the monkey is between one and a half and two times the thickness of area 3b of the somatic sensory cortex and most of this difference is accounted for by the greater thickness of layers V and VI in the motor cortex. Layer V is characterised by the Betz cells which occur mainly in its upper part and may occur in groups of two or three. Very large examples of the large type of stellate cell are also found in layer V and may be as much as 30 μ in mean diameter. They are less common than Betz cells but are often found close to them. Most of the neurons in layer V are pyramidal but in the deeper part and in layer VI many neurons are fusiform in shape and often have large dendrites emerging from their upper and lower poles. Large stellate cells are prominent in the upper part of layer V and small stellate cells are found in layers V and VI. Dendrites of the varicose type studded with synapses are also frequent in layer V, although both these and large stellate somata appear to

be more prominent in some parts of layers IV and V than in others. In both layers dendrites of this type may make gap junctions with other dendrites, usually of the same type, or with large stellate somata (Sloper, 1972; Chapter 7) and gap junctions are occasionally also found in the lower part of layer III. In the deep layers of the motor cortex the occasional dendrite is also found which makes a dendro-dendritic synapse (Sloper, 1971; Chapter 6). These synapses have symmetrical membrane thickenings and small groups of synaptic vesicles. The presynaptic dendrites are varicose in shape and receive a number of both asymmetrical and symmetrical synapses. Myelinated axons are frequent in layer V; many run vertically through it and it is not infrequent for several of these vertical axons to occur together (Fig. 3-29). Towards the bottom of the cortex myelinated axons become even more frequent until the neuropil of layer VI merges into the white matter.

During this study of the motor cortex several features of cytological interest have emerged. Subsurface cisternae are a well recognised feature of neurons and their structure in the motor cortex corresponds to previous descriptions (Rosenbluth, 1962b; Siegesmund, 1968; Jones and Powell, 1970a). They are found both in neuronal somata and in dendrites. As in the somatic sensory cortex they show a considerable variation in structure from a simple dense plate which often opens into a sac of endoplasmic reticulum(Figs. 3-30 to 3-33) to a complex

structure of alternating dense plates and membranous sacs (Fig. 3-35). In the motor cortex those subsurface cisternae having a single dense plate may often be found with it closely applied to the plasma membrane of a cell soma or dendrite opposite a symmetrical axon terminal (Figs. 3-30 to 3-33). These terminals frequently make a symmetrical synapse with the cell or dendrite in the same section but the synaptic membrane complex occupies only a part of the area over which the symmetrical terminal is apposed to the cell or dendrite and the dense plate of the subsurface cistern is found opposite a different part of the symmetrical axon terminal and is thus separate from the synaptic complex. However, opposite the subsurface cistern the membranes of both dendrite or soma and terminal are also usually thickened, this being distinct and separate from the synaptic membrane thickening, and in most examples there is granular electron-dense material in the extracellular cleft opposite the cistern and between the dense plate of the cistern and the adjacent plasma membrane. These membrane specialisations opposite the cisternae therefore appear identical to a synaptic membrane complex of the symmetrical type but the cistern is attached below the 'postsynaptic' component, synaptic vesicles are not aggregated opposite the structure and the membrane thickenings are not always as prominent as those of a synapse. Such membrane specialisations have not been seen between a subsurface cistern and any other adjacent profile. Subsurface cisternae related to symmetrical axon

terminals occur in both pyramidal and stellate cells; pyramidal somata receive only symmetrical synapses but large stellate somata receive synapses of both types and so all identifiable synapses on to a series of large stellate somata selected at random were recorded and the presence or absence of a subsurface cistern opposite the terminal was noted to see whether the relationship was specific to symmetrical type axon terminals. Of 290 synapses recorded 120 were symmetrical and of these 18 (15%) had associated subsurface cisternae whereas none of the 170 terminals giving rise to asymmetrical synapses was related to a subsurface cistern. The dense plates of these cisternae may extend beyond the specialised region opposite a symmetrical axon terminal in some examples and in single sections they may be found opposite other components of the neuropil; extensive study of serial sections would be required to determine whether all cisternae of this type are related to symmetrical axon terminals in adjacent sections. The dense plates of these subsurface cisternae may often be seen to be connected to the endoplasmic reticulum of the cell soma and may also be closely related to a mitochondriom (Figs. 3-30 and 3-31).

It is not unusual for the plasma membranes of neuronal profiles in the sensori-motor cortex to show small coated pits and these have been described in various sites as part of the process of vesicle formation or discharge (Gray, 1961; Andres, 1964; Westrum, 1965; Kanaseki and Kadota, 1969; Gray and Willis, 1970; Gray and Pease, 1971). Although often no contents are visible, in some examples the cytoplasm of an adjacent profile is

invaginated into one of these coated pits; Figs. 3-36 and 3-37 show this happening between an axon terminal and a dendrite and it appears that part of the cytoplasm of the axon terminal is being taken up by the dendrite. Such a coated pit has also been seen in continuity with the plasma membrane of a myelinated axon within its myelin sheath (Fig. 3-38). This process appears to be the same as that involved in the phagocytosis of degenerating axons and axon terminals by neurons and glia (Chapter 8).

The motor cortex was also studied in material block-stained with ethanolic Phosphotungstic Acid (E-PTA) (Method 1). As well as staining synaptic thickenings and the spine apparatus as described in other sites (Figs. 3-39 and 3-40) this method stained specifically the dense plates of at least some subsurface cisternae (Fig. 3-34), cilia arising from both neurons and glia (Fig. 3-45), and nucleoli (Fig. 3-43). The undercoating of axon initial segments and nodes of Ranvier, cisternal organs and degenerating axon terminals were also specifically stained by the E-PTA and are described elsewhere (Sloper and Powell, 1973; Chapter 5). Comparison of nucleoli stained with E-PTA and in normal material showed that E-PTA stained the pars granulosa less heavily in relation to the other parts of the nucleolus than did the routine method (Figs. 3-43 and 3-44). The central filaments of cilia were densely stained by E-PTA and in addition a short length of membrane undercoating was stained which extended under the plasma membrane of the cilium for approximately the first

0.25 μ and was slightly flared away from the central tubules at the base of the cilium (Fig. 3-45). A similar undercoating may also be seen in cilia stained by the normal method (Fig. 3-42). Preosmication of the blocks (Method 2) partially inhibited the staining of the dense plates of the spine apparatus by E-PTA (Fig. 3-41). As a control, unstained sections from unosmicated unstained blocks were examined (Method 3); none of the structures described above was naturally electron-dense and therefore the density was due to staining by the E-PTA.

Discussion

The motor cortex and area 3b of the somatic sensory cortex of the monkey are basically very similar to each other when seen under the electron microscope and are very similar to other areas of the neocortex described in various species (Colonnier, 1968; Jones and Powell, 1970 a-c; Peters and Kaiserman-Abramof, 1970; Garey, 1971; Peters, 1971). The motor cortex has the same laminar pattern as these sensory cortical areas, although the deep layers are thicker, and the same features may be used to recognise the different laminae with the electron microscope. This degree of similarity is perhaps surprising considering the differences between the areas apparent in light microscopic sections stained by the Nissl method and in particular layer IV in the motor cortex is inconspicuous in such sections whereas in sensory cortical areas it appears well developed.

With the electron microscope the prominent presence of stellate cells studded with synapses in layer IV of sensory cortical areas has been emphasised but these are also prominent in layer IV of the motor cortex although they spread more into layers III and V; the overall impression given is that the components that make up the distinct layer IV of the sensory cortical areas have become more spread out and intermingled with the adjacent parts of layers III and V in the motor cortex but they are still present. The types of neuron present in the other laminae also appear qualitatively very similar in motor and somatic sensory cortices, although pyramids in particular were generally larger in the motor cortex. This qualitative impression of similarity was confirmed by a quantitative comparison of the neuronal populations in motor and somatic sensory cortices which is reported separately (Sloper, 1973a; Chapter 4).

Synaptic relationships in the motor cortex resemble very closely those in sensory cortical areas. Dendritic spines are a prominent feature of the neuropil and receive the majority of synapses and the combinations of asymmetrical and symmetrical synapses they receive are the same as in sensory areas. In particular symmetrical axon terminals were never found to give the only synapse on to a dendritic spine although they did so on to spines on axon initial segments (Sloper and Powell, 1973; Chapter 5), and possibly also on to those on pyramidal somata. As in sensory areas the majority of axon terminals in the motor cortex

are small and fairly dark with spherical vesicles and asymmetrical membrane thickenings and a minority are slightly larger and paler and have flattened vesicles and symmetrical membrane specialisations. Jones and Powell (1970a) described large pale axon terminals with round vesicles and asymmetrical thickenings in layers IV and I of the somatic sensory cortex, and thought that these represented the terminals of the thalamo-cortical afferents. Similar terminals are prominent in layer IV of both the motor and somatic sensory cortex of the monkey and correspond in distribution to the thalamo-cortical projection in this species. The features of early degenerating thalamo-cortical terminals in experimental studies of these areas also correspond to those of these terminals (Sloper, 1973b; Chapters 8 and 9). Occasional axon terminals containing large dense-cored vesicles were identified by Lund and Lund (1970) in the visual cortex of the rat. Similar terminals are occasionally found in the motor cortex and their appearance resembles that of terminals in the neocortex which take up 5-Hydroxytryptamine (Descarries, Beaudet and Watkins, 1975) and so these terminals may be part of the diffuse aminergic projection to the neocortex.

Of the rarer types of interneuronal connection, gap junctions were found in both motor and somatic sensory cortex and dendro-dendritic synapses found here in the motor cortex also occur in the somatic sensory cortex (Shanks, personal communication) and so it would appear that the synaptic organisation of motor and somatic sensory cortical areas is very similar. Although these unusual structures are not frequent, their occurrence in the cortex

indicates also that the synaptic organisation of the neocortex is not as simple as it at first appeared.

The differences that were apparent between the motor and somatic sensory cortices were mainly the same as those seen with the light microscope. The motor cortex is considerably thicker than area 3b and this difference is mainly due to the greater thickness of layers V and VI. Pyramidal cells are generally larger in the motor cortex and so have a more obviously pyramidal shape and the characteristic Betz cells are present in layer V. There was a clear difference in the fibre plexus of layer I of the two areas seen by both light and electron microscopy and the greater number of fibres in the motor cortex is considerably more than can be accounted for simply by the greater thickness of layer I there. The origin of this fibre plexus is unknown but following lesions involving layer I its fibres degenerate for a distance of five to ten millimetres from the lesion and this degeneration crosses architectonic boundaries, unlike that of other fibre systems (Jones and Powell, 1969a; Fisken, Garey and Powell, 1975). Cells from deeper layers of the cortex, particularly layers V and VI, are known to project to layer I (Szentágothai, 1964) and this plexus may represent their axons. However, although the deep layers are more developed in the motor cortex, the number of cells through the depth of the cortex is no greater in motor than in somatic sensory cortex so this may not account for the

greater number of axons in the motor cortex plexus. It might also be expected that the cells in the motor cortex would be larger and give rise to axons of greater diameter but the size distributions of axons in the two plexuses are very similar. There are however more synapses per cell in the motor cortex (Cragg, 1967) and it may be that the difference in these fibre plexuses is a reflection of the more extensive axonal arborisations that this implies. In addition there is evidence that this plexus of fibres in layer I arises in part from non-specific nuclei in the thalamus (Jones, 1975b) and it is possible that the difference in the density of the fibre plexuses reflects differing degrees of development of these thalamic nuclei in relation to different parts of the neocortex. The fact that this fibre plexus crosses architectonic boundaries would tend to support this suggestion but no firm conclusion can be reached at present.

Bundles of apical dendrites have been described in layer IV of both visual and somatic sensory cortices (Fifkova, 1970; Peters and Walsh, 1972) and the appearances described are very similar to those in the motor cortex. The distribution of apical dendrite bundles in the depth of the motor cortex corresponds to that of the thalamo-cortical afferents and degenerating thalamo-cortical axon terminals are found associated with the apical dendrites more frequently than in the neuropil between the bundles (Sloper, 1973b; Chapter 9). However this relationship to the

thalamo-cortical afferents is not necessarily the reason for the existence of the bundles but may be secondary to it. Apical dendrites in layer II are evenly spread but pyramids in layers II and III generally occur in groups and the occurrence of deeper apical dendrites in bundles may be a consequence of their having to pass upwards between these more superficial cell groups. However, whether the bundles of apical dendrites or the cell groups are simply a natural consequence of the three dimensional packing of the cortex or whether they have a particular functional significance remains to be determined.

Spines are a dominant feature of the synaptic organisation of the neocortex and their possible significance has been discussed by several authors (e.g., Jones and Powell, 1969d; Diamond, Gray and Yasargil, 1970). In this study it was not infrequent, particularly in serial sections, to find that a single axon terminal of either the asymmetrical or symmetrical type made a synapse on to a spine and also on to the shaft of the dendrite giving rise to that spine, usually by way of a separate membrane specialisation. A similar situation also occurs not infrequently in the thalamus (Harding and Powell, 1977). It would be difficult to determine whether this arrangement has any particular functional significance of itself but it must be taken into account in considering the possible functions of spines. In particular it is difficult to see how a spine in this situation could serve to isolate its input from the dendritic shaft when the same axon terminal also makes a

synapse on to the shaft directly, although possibly it could do so for another terminal with a synapse on to the same spine.

Subsurface cisternae are a well recognised feature of neurons and have a variety of structures of differing complexities (Rosenbluth, 1962b; Siegesmund, 1968; Jones and Powell, 1970a). The observations made here relate to the type of cistern having a single dense plate which is often seen to open out into a sac of endoplasmic reticulum. Observations of two different kinds have led to the conclusion that cisternae of this type are related to symmetrical axon terminals. First, where the dense plates of these cisternae are closely apposed to the plasma membrane of the cell there is a specific membrane complex consisting of thickening of the membranes of both the soma and the symmetrical axon terminal and there is dense granular material both between the membranes and between the cistern and the cell membrane. Although dense material has been described between the cistern and the membrane of the soma (Siegesmund, 1968) opposite other types of profile, the full membrane complex has only been observed in association with symmetrical axon terminals. The second line of evidence is the statistical association of cisternae and symmetrical terminals. The dense plates of these cisternae may extend beyond the region opposite the symmetrical terminal itself and so in single sections they may appear to be only opposite other profiles. It is also possible that some cisternae having this type of structure are not related to symmetrical terminals although they may either have been or would have become so. To overcome these difficulties a

comparison was made of the relationship to cisternae of asymmetrical and symmetrical axon terminals making synapses on to large stellate cell somata, a site where both types of terminal are present in approximately equal numbers. This showed clearly that the cisternae were specifically related to symmetrical rather than asymmetrical terminals. These subsurface cisternae therefore have both a statistical and a specific structural relationship to symmetrical axon terminals.

The exact relationship of the cisternae to symmetrical axon terminals is of importance in that the cistern does not occur beneath the synaptic complex but opposite a non-synaptic part of the terminal. Although such a cistern may be related to a terminal without a synapse being present in the section the possibility that in such cases the terminal makes synapses on to the cell or dendrite in an adjacent section has not been excluded. The position of the cistern at a site away from the synaptic membrane complex itself suggests that its function may not be directly related to synaptic transmission. Rosenbluth (1962b) has suggested that subsurface cisternae in general may be concerned with the transport of substances to and from the plasma membrane and the association of mitochondria with cisternae suggests that they perform an active metabolic role. In relation to these symmetrical axon terminals it is possible that they are concerned in the transport of some trophic factor between the pre- and post-synaptic structures and the linking of a synaptic activity to the metabolism and growth of the neuron. However, when fully developed

the membrane complex associated with these cisternae differs from a symmetrical synapse only in the presence of the cistern and the absence of a local aggregation of synaptic vesicles. In some examples the membrane specialisations are less well developed and in a few they are absent and these factors, together with the relationship of the cisternae to the endoplasmic reticulum and mitochondria, suggest that these cisternae may be involved in the synthesis of new synaptic membrane complexes. The presence of a fully developed synaptic zone in addition in some examples is in no way against this suggestion since a characteristic feature of these symmetrical axon terminals in the neocortex is the frequent occurrence of more than one membrane complex between a single axon terminal and cell soma. In support of this suggestion subsurface cisternae are present early in development, both in Xenopus retinal ganglion cells (Grillo and Rosenbluth, 1972) and in the neocortex of one day old rats (Siegesmund, 1968); the monkeys used in this study were not fully mature and it would be of interest to compare them with very young and fully adult animals. It is therefore possible that, following apposition of a symmetrical type preterminal bouton to a cell soma, a subsurface cistern is induced in the soma at that site and this synthesises or delivers the components which make up the synaptic membrane complex. It may also be of interest in this respect that the dense material of both cistern and synaptic complexes stains specifically with E-PTA. It has been shown that subsurface

cisternae are induced by the apposition of an isolated postsynaptic membrane thickening both in the olfactory bulb (Pinching and Powell, 1972) and the neocortex (Sloper, 1973c) but it is also possible that the cisternae are already present but inactive before the presence of symmetrical-type boutons and this could explain the cisternae found opposite other types of profile, particularly in young animals. Price and Powell (1970c) noted the occurrence of similar cisternae in relation to reciprocal synapses in the olfactory bulb; although they described them as being related to the presynaptic component of the asymmetrical synapse, these cisternae may also be considered to be postsynaptic to the symmetrical part of the reciprocal synapse which would make them analogous to those described here. Open sacs have also been described in spinal motoneurons beneath boutons containing flattened vesicles but these boutons appear to show no membrane specialisations (Conradi, 1969a; McLaughlin, 1972). Subsurface cisternae have, however, also been described in dorsal root ganglion and acoustic ganglion cell somata (Rosenbluth, 1962a,b), both of which receive no axo-somatic synapses and the more complex types of cistern in the cortex are not related to synapses. However there is no reason to expect all subsurface cisternae to perform the same function, particularly in view of differences in their structure, and it may well be that their function in relation to these synapses is a specific example of a more general function.

The relationship of the cisternal organ to symmetrical axon terminals making synapses on to axon initial segments appears to be the same as that of the subsurface cistern to them in the cell soma (Sloper, 1973c). The cisternal organ comes into close apposition with the plasma membrane of the initial segment opposite the non-synaptic parts of symmetrical terminals and the membranes in the region of apposition may show the same specialisations. Both cisternal organs and subsurface cisternae are also structurally similar, consisting of membranous sacs and dense plates which in both cases stain specifically with E-PTA (Sloper and Powell, 1973). The spine apparatus also consists of dense plates which stain specifically with E-PTA and which alternate with membranous sacs and these are connected to the reticulum of the cell (Peters and Kaiserman-Abramof, 1970) and the relationship of the spine apparatus to Type I or asymmetric synapses both on to spines and the shafts of dendrites is well known (Gray, 1959; Jones and Powell, 1970a). There is thus a remarkable parallelism between these three organelles in both structure and relationships and it therefore seems reasonable to suppose that they may perform similar functions in relation to the different types of synapse and different sites.

CHAPTER 4

A QUALITATIVE AND QUANTITATIVE STUDY OF THE NEURONS OF THE MOTOR AND SOMATIC SENSORY CORTICES

Introduction

The neuron is the basic unit of nervous tissue and so a knowledge of the different types of cell present in an area of brain and of their distribution within that area is essential for a full understanding of its functioning. Three main histological methods have been used to study cortical neurons, the Golgi technique, the Nissl method and electron microscopy. Impregnation of a neuron by the Golgi technique can demonstrate the ramifications of its dendrites and axon and the shape of its soma and the neurons of the neocortex have been classified by a number of investigators on the basis of differences in these features (e.g. Cajal, 1909-11; Lorente de Nó, 1949; Sholl, 1955, 1956; Marin-Padilla, 1969, 1970; Valverde, 1971; Szentágothai, 1973; Jones, 1975a). The division into pyramidal and stellate cell types is generally accepted and various sub-types of stellate cells have been described by the different authors. The Golgi technique, however, only demonstrates a small percentage of the cells present and so is of restricted use in determining their relative distributions and frequencies. In contrast the Nissl method stains all the cells present but only stains the cell soma. It can thus show the distribution of cell somata and their sizes and shapes, and so forms the basis of architectonics, but is of little help in classifying cell types. Electron microscopy gives a detailed picture of the cell soma and the proximal parts of the dendrites and axon. However the more distal parts of these structures are not seen in

continuity with the soma and so have to be identified by indirect methods. The neuronal somata of the neocortex have been studied qualitatively with the electron microscope by a number of authors, these studies being mostly in sensory areas (Colonnier, 1968; Szentágothai, 1969; Jones and Powell, 1970a-c; Lund and Lund, 1970; Peters and Kaiserman-Abramof, 1970; Garey, 1971; Peters, 1971; Cragg, 1976). Pyramidal cells have been extensively studied and so the only ones to be considered in detail here are Betz cells. In sensory areas criteria for distinguishing stellate or non-pyramidal cells from pyramidal cells have been determined and descriptions of "typical" stellate cells given by various of these authors, who are in general agreement, but different types of stellate cells have not been described using the electron microscope.

This electron microscope study may be divided into several parts. The motor cortex was examined extensively and a large number of cells was studied qualitatively. This showed typical pyramidal and stellate cells to be present but there was also a considerable proportion of neurons which could not be classified as pyramidal or "typical" stellate cells. Because of the inevitably selective nature of this approach, a quantitative study using a mapping technique was then performed to study all the cells in a strip running through the full depth of the motor cortex to try to confirm that these unclassifiable cells were a separate population and to study the relative

proportions and distributions of the different cell types in the motor cortex.

Architectonically the motor cortex has been classified as agranular cortex using the Nissl method and has been thought to consist predominantly of pyramidal cells. Functional differences have been shown between different architectonic areas (e.g. Powell and Mountcastle, 1959b; Hubel and Wiesel, 1965) and so it was thought to be of interest also to study an area of neocortex of very different architectonic character to compare the cells with those of the motor cortex using the electron microscope. Area 3b of the somatic sensory cortex was chosen because it is granular cortex, a primary sensory projection area and was thought to consist predominantly of stellate cells and to be very different in character from the motor cortex. Area 3b was studied qualitatively and then a quantitative study was done in the same way as in the motor cortex, and this allowed a detailed comparison of the cells of the two areas to be made.

The Golgi technique was also used to study comparatively complete neurons from both cortical areas and an attempt has been made in the discussion to correlate findings made with the Golgi technique with those from electron microscopy. Other indirect evidence has also been used to try to overcome the technical limitations of electron microscopy and to build as complete a picture as possible of the neurons of the neocortex.

A summary of some of the results in this chapter has appeared previously (Sloper, 1973a).

Material and Methods

The motor cortex (area 4 of Brodmann) was studied qualitatively in the brains of 21 young adult Rhesus monkeys used for experimental neuroanatomical studies described elsewhere (Chapter 9). Five of these brains were also used for the qualitative study of area 3b of the somatic sensory cortex. A quantitative study comparing the neurons of the motor cortex and area 3b was done in one block taken from each area at the same medio-lateral position along the central sulcus of one hemisphere to minimise any possible artefactual differences between the appearances of the two areas. 'Thick' sections for light microscopy were cut from these blocks for determination of the cortical layers and sections were cut which were accurately perpendicular to the cortical surface and parallel to the 'grain' of the cortex by ensuring that these 'thick' sections contained long lengths of pyramidal apical dendrites. A strip of equal width running through the full depth of the cortex was taken from both areas, requiring three sections in motor cortex and two in area 3b, and the position of every cell fulfilling the criteria defined below was recorded on a map using the technique of Alkense et al. (1966) and each of these cells was photographed. The criteria used were:-

1. That every cell must receive at least one synapse on the section studied.
2. That the nuclear membrane must not show nuclear pores face on (i.e. as circles) except at indentations. This excluded cells that were not sectioned approximately through the centre of the nucleus.
3. That the cell must be completely within the strip of cortex being studied.

Photographic prints were made at a standard magnification and the parameters tabulated below were determined from these. The cells were then classified according to the criteria described for the different types in the first part of the results. The correlation matrix was done on an ICL 1906A computer.

Results

Qualitative study of motor cortex

Pyramidal and stellate cells which resemble closely those described in sensory cortical areas may be recognised in the motor cortex of the monkey. The cells in the motor cortex are in general larger and the largest of the pyramidal cells are the Betz cells. However, in this study, a proportion of cells was found which had features differing from these previous descriptions. These have been termed small stellate cells and those stellate cells which correspond broadly to previous descriptions have been called large stellate cells. Although size is by no means the most important distinguishing feature,

these terms have been used because they are comparatively non-committal. These different groups of cells are described separately below.

Pyramidal cells

The somata, dendrites and axon initial segment of small pyramidal cells of the monkey motor cortex resemble closely those of small pyramidal cells described in sensory areas of the neocortex of the cat (Colonnier, 1968; Jones and Powell, 1969c, 1970a; Garey, 1971) and rat (Peters and Kaiserman-Abramof, 1970; Peters, Proskauer and Kaiserman-Abramof, 1968). There is, however, a greater proportion of larger pyramidal cells in the motor cortex of which the largest are the Betz cells. The soma of a pyramid is typically triangular in shape and often gives rise to one or more dendrites in a section (Fig. 4-1). The nucleus is pale, of even density and may have a single or occasionally a more complex indentation and the cytoplasm contains a moderate number of organelles and cisternae of endoplasmic reticulum, these features becoming more prominent in the larger cells. The soma and proximal dendrites receive a small number of exclusively symmetrical synapses. The initial segments of these cells are directed towards the white matter, may have spines which receive synapses and in a number of examples the initial segment has been shown to give rise to a myelinated axon of similar diameter to itself. These results relating to axon initial segments are described in detail in Chapter 5.

Betz cells

Betz cells are the characteristic feature of the motor cortex and have been described in the cat (Kaiserman-Abramof and Peters, 1972). In the monkey Betz cells occur singly or in clusters in layer V of the motor cortex (Fig. 4-2). They are the largest of the pyramidal cells, up to 50 μ in transverse diameter and, although they are basically the same as other pyramidal cells, certain features become more prominent with the larger size of the cells. Their ultrastructure is very similar to that of Meynert cells in the visual cortex (Chan-Palay, Palay and Billings-Gagliardi, 1974). The size of the Betz cell nucleus is small in relation to that of its cytoplasm and the nuclear membrane often shows a region of complex indentation (Fig. 4-4). The nucleolus is large and may exceed 5μ in diameter and it has a well developed internal structure. The cell soma is often more markedly pyramidal in shape than that of a small pyramid because the diameter of the apical dendrite is larger in proportion to the diameter of the cell soma. The cytoplasm of Betz cells contains considerable amounts of endoplasmic reticulum arranged in definite clumps with clear areas of cytoplasm between them (Fig. 4-5) and there is usually a cluster of endoplasmic reticulum in relation to an area of nuclear indentations (Fig. 4-4). The background cytoplasm of a Betz cell is light and when seen in close proximity to that of a large stellate cell it is clearly paler and the endoplasmic reticulum

and ribosomes stand out more strongly against it. The cytoplasm of a Betz cell contains a considerable number of mitochondria and often also small black membrane-bound bodies (Fig. 4-5). The soma generally receives up to six synapses and these are all of the symmetrical type (Fig. 4-6) and may be related to subsurface cisternae as in other pyramidal cells (Sloper, 1973c). The cell body and apical dendrite may give rise to spines which receive synapses and the same axon terminal may make a synapse on to the dendritic shaft or cell soma as well as on to a somatic spine. Many of the features described above may be seen with the light microsccpe using Araldite-embedded 'thick' sections and Fig. 4-3 shows a Betz cell in such a section. This same cell was subsequently studied with the electron microscope in serial ultrathin sections (Fig. 4-2).

The dendrites of Betz cells are generally parallel sided, may contain characteristic clumps of endoplasmic reticulum, and they have pale background cytoplasm like the cell soma. Although they generally receive few synapses, one Betz cell was found which gave off two small side branches from its apical dendrite and these received a considerable density of synapses.

The axon initial segments of Betz cells are of large diameter, in some examples exceeding 5 μ, and because of their large size the membrane undercoating and bundles of neurotubules are less prominent than in smaller cells (Chapter 5). One example was found to give rise to a spine of the type having a

narrow pedicle and expanded head, and which received symmetrical synapses from two axon terminals. This spine was situated immediately distal to the origin of the initial segment and one of the two terminals also made a synapse on to the initial segment and the other on to the axon hillock as well (Figs. 5-28 and 5-29). In two examples it was possible to trace the initial segment of a Betz cell from the soma to the point where it gave rise to a myelinated fibre at 45 μ and 47 μ from the soma respectively. It may be significant that this length is no longer than in small pyramidal cells (Sloper and Powell, 1973; Chapter 5). Only symmetrical synapses have been found on the initial segments of these Betz cells, as has been found with other pyramids.

Large stellate cells

The neurons described here as large stellate cells correspond broadly to the stellate or non-pyramidal neurons described in sensory and parietal areas of the neocortex by previous authors (Colonnier, 1968; Szentágothai, 1969; Jones and Powell, 1970a; Lund and Lund, 1970; Peters, 1971; Garey, 1971). The most obvious feature of cells of this type is the high density of synapses which they receive (Fig. 4-7) and approximately 60% of these synapses are of the asymmetric type. It is not unusual for the somata of the larger cells of this type in the motor cortex to receive twenty or more synapses in a single section and few receive less than five synapses. Serial sections have shown that this high density of synapses occurs consistently over the surface of these cells. The mean diameter of the soma is

usually 13 μ or greater and may be as large as 30 μ. Its outline is generally rounded and it is unusual for more than one dendrite to be seen arising from the soma in any one section through it. The nucleus is an even, moderately dark tone and is often indented; the nucleolus is prominent and the different parts are well differentiated. These cells have abundant cytoplasm which contains numerous prominent stacks of parallel cisternae of rough endoplasmic reticulum and abundant free ribosomes occurring both singly and in clusters. The cytoplasm also contains a high density of mitochondria, considerably more than in any of the other types of cell, and also frequent small electron-dense bodies.

The lengths of dendrite seen arising from the somata of large stellate cells are studded with asymmetric and symmetrical synapses (Figs. 4-7, 4-9 and 4-10). They do not have a markedly varicose shape and tend to run in a straight line for a considerable distance and their origin from the cell soma does not show a marked constriction. They contain prominent microtubules and a high concentration of organelles, particularly ribosomes and mitochondria, and may occasionally be seen to give rise to spines having a definite pedicle and head (Fig. 4-9). Dendrites seen cut in isolation in the neuropil often share the above features and have therefore been identified as being those of large stellate cells. The gap junctions which occur sparsely in the cortex are found predominantly on the dendrites and somata

of this type of cell and would appear to be a particular feature of it (Sloper, 1972; Chapter 7).

The initial segments of these large stellate cells generally resemble those of small pyramidal cells but may often be curved. They may arise either directly from the cell soma or from a dendrite at a point close to its origin from the soma (Fig. 4-8). They are often directed towards the cortical surface or obliquely. It has been possible using serial sections to trace two of these initial segments to the point where they gave rise to myelinated fibres of similar calibre to the initial segment and it would appear that the distance from the soma to the myelin sheath may be shorter in these cells than in pyramidal cells (Chapter 5).

Large stellate cells in the motor cortex occur mostly in a horizontal band from the lower part of layer III and including layers IV and V although the occasional example is found in the more superficial layers. The dendrites of the type identified as belonging to this cell type similarly occur in a somewhat wider band of cortex. Several cell somata of this type may be found close together and it appears that they may be clustered in some way and not homogeneously distributed in a horizontal plane. They also often occur close to pyramidal cells of similar diameter, and the very large ones often seem to occur close to Betz cells. The appearance of cells of this type is remarkably consistent and it therefore seems likely that large

stellate cells form a distinct population of cells within the cortex. Following lesions of the ventrolateral nucleus of the thalamus the cell somata found to be receiving degenerating axosomatic terminals are uniformly of this cell type and a proportion of the terminals of all the afferent pathways to the cortex studied with the electron microscope are found to make synapses on to the shafts of dendrites of large stellate cells (Sloper, 1973b; Chapter 9).

Small stellate cells

A considerable number of cells was found in the motor cortex which could not be classified as either of the types described previously. As they are of stellate rather than pyramidal type, they have been termed small stellate cells. They have small rounded or fusiform somata, most having a mean diameter of 9-12 μ. The somata receive few synapses, rarely more than three in a single section and usually only one or two, and study using serial sections has shown that a cell of this type may often receive no synapses at all on a number of sections in a series through it and it is then almost indistinguishable from a glial cell. The nucleus of these cells is often dark with rather granular chromatin that often occurs in dense clumps (Figs, 4-11, 4-15, 4-18 and 4-19) and the nuclear membrane may have complex indentations. The fusiform cells often have a rather angular nucleus and there is frequently a longitudinal nuclear indentation. The cytoplasm of small stellate cells is

sparse and in the rounded cells it has a very light background and contains very few organelles, although the Golgi apparatus is usually prominent. Often no dendrite is seen to arise from the soma in a given section and it is unusual to find more than two doing so. The origin of a dendrite from the cell soma is generally markedly constricted and may closely resemble that of an initial segment as there are often a few neurotubules aggregated in it (Fig. 4-14), but a definite initial segment may be found in addition. These features described above differentiate small stellate cells from the large type and in practice, using single sections, it is more difficult to distinguish them from small pyramidal cells. However a proportion of the synapses they receive are asymmetric (Figs. 4-12 and 4-17) and this has never been seen on the somata of definite pyramidal cells; the initial segment may be directed towards the surface of the cortex (Fig. 4-19), also not seen with undoubted pyramids with the possible exception of inverted pyramids in layer VI; these cells have been shown not to have apical dendrites by studying serial sections taken through the soma. Quantitative evidence for distinguishing small stellate cells as a separate population from the other cell types is given below.

Most of the rounded small stellate cells occur in layer II and occasional ones occur in layer I whereas the fusiform type is found more in the deeper layers of the cortex. The occasional

dendrites seen arising from these cells tend to have a more varicose shape than those of large stellate cells but receive a lower density of synapses; a proportion of these synapses are of the asymmetric type. It is unusual to cut any length of dendrite in continuity because they usually turn out of the plane of the section after a short distance, and in serial sections they may be seen to follow a tortuous course. The fusiform cells may however have a large polar dendrite which is not varicose in shape (Fig. 4-18). Because of the rarity with which lengths of dendrite are cut in continuity with small stellate somata it is difficult to define their characteristics but the dendrites having a very varicose shape and light background cytoplasm and receiving a moderate number of synapses of both asymmetric and symmetrical types which are seen in isolation in the neuropil probably arise from small stellate cells because they occur prominently in layer II where the majority of round small stellate cells are found.

The initial segments of small stellate cells are thin, usually about 0.5 μ in diameter, and are often directed towards the surface of the cortex (Figs. 4-19 and 4-20). They have the typical membrane undercoating and one or more bundles of neurotubules. It has not proved possible to follow these initial segments for any distance using serial sections, mainly because of their small size and variable orientation.

Small stellate cells do not appear to form one homogeneous

population. The round and fusiform cells appear to be two distinct groups with differing features and laminar distributions and it seems likely that further study will confirm the presence of several sub-types of small stellate cells.

Martinotti cells

In the deep part of layer V and in layer VI of the motor cortex a few neurons have been found which have axon initial segments directed towards the superficial layers of the cortex (Figs. 4-21 and 4-22). In general these cells are fairly large and often have a distinctly fusiform shape. They appear generally pale and contain a moderate number of organelles in their cytoplasm. The cell somata receive few synapses but more are found on large polar dendrites, some of which are of the asymmetric type. These cells are uncommon and do not fall into any of the groups described above. In view of their position in the deep layers of the cortex and their superficially directed axons it is possible that these neurons correspond to the Martinotti cells of light microscopy (Cajal, 1909-11).

Qualitative study of area 3b of somatic sensory cortex

Pyramidal, large stellate and small stellate cell types could all be identified in area 3b of the somatic sensory cortex and in general their features are the same as in the motor cortex. The main differences from the motor cortex appear to be due to differences in the mean sizes of the cell types (see quantitative results). There were fewer large pyramidal cells than in the

motor cortex as would be expected but, unlike the somatic sensory cortex of the cat (Jones and Powell, 1970a), monkey somatic sensory cortex contains a proportion of large pyramids which have definite clusters of endoplasmic reticulum in their cytoplasm. The large stellate cells in area 3b were also in general smaller than in the motor cortex and no very large examples were found. Small stellate cells (Fig. 4-14) were slightly smaller but there was considerably more overlap between the size ranges of the large and small stellate cells in the somatic sensory cortex than in the motor cortex. As in the motor cortex, the cell somata in area 3b which receive synapses from degenerating axo-somatic thalamo-cortical terminals are of the large stellate type (Chapter 9). The laminar distributions of the different cell types were basically the same in both areas but large stellate cells appeared to be more confined to layer IV in area 3b, probably because of the greater thickness of this layer in the somatic sensory cortex than in the motor cortex. Overall, however, the cell populations of the two areas appeared remarkably similar and this was confirmed by the quantitative study.

Quantitative study of motor cortex and area 3b of somatic sensory cortex

The results of a qualitative study such as that described above are inevitably biased by the observer, since the cells studied in detail are those noticed and thought to be 'interesting'.

To overcome this problem and to obtain quantitative information, every cell fulfilling certain basic criteria was studied in a randomly selected vertical strip of cortex from both the motor and somatic sensory areas. The criteria used were that the cell must receive at least one synapse, to ensure that it was a neuron; that the nuclear membrane must not show nuclear pores face on (i.e. as circles) except at indentations, to ensure that the section passed approximately through the centre of the cell and therefore that measurements such as cell diameter were accurate, and that the cell must be completely within the strip of cortex being studied. A strip of equal width and running through the full depth of the cortex was taken in the motor cortex and area 3b of the somatic sensory cortex in blocks from the same medio-lateral position along the central sulcus of the same hemisphere to minimise possible artefactual differences. Each eligible cell was photographed at a standard magnification and the synapses on to it studied at higher magnification and the position of each cell was recorded on a map. The strip of motor cortex contained 107 cells fulfilling the above criteria and the data relating to these is shown in Table 4-1; that of area 3b contained 110 cells and the data relating to these is shown in Table 4-2. All data refer to the single section through the cell which was studied and all subsequent results and tables except the maps are derived from the basic information in these two tables. The mean diameter of a cell was measured as the mean of the greatest

TABLE 4-1 Data relating to all neurons from the motor cortex

Cell No.	Type	Mean Diam. μ	Area Total	Area Nuc.	Cyt.	Cyt. Nuc.	Apic. Dend.	No. of Synapses Dend.	No. Asym.	Nucleus Ind.	Nucleus Drk	Cytopl. E.R.	Cytopl. Mer.	Layer
1	SS	11	88	53	35	.66	O	1	3	O	O	O	O	
2	SS	10	77	42	33	.79	O	O	2	O	1	O	O	
3	SS	10	90	57	33	.58	O	O	1	O	O	O	+	
4	P	11	104	63	41	.65	+	1	1	O	O	O	+	
5	P	10	108	74	34	.46	O	O	2	O	O	O	+	
6	SS	11	114	65	39	.60	O	1	1	O	M	+	O	
7	P	16	194	106	88	.79	+	2	1	O	O	O	+	
8	P	15	155	103	52	.49	+	1	2	O	O	O	+	
9	P	13	130	72	58	.81	+	2	1	O	O	O	O	
10	SS	12	115	63	52	.82	O	2	1	+	M	O	O	II
11	SS	13	122	69	53	.77	O	1	2	O	1	+	O	
12	P	15	151	77	74	.96	+	3	2	O	O	O	+	
13	P	16	215	105	110	1.02	O	1	1	O	M	O	+	
14	P	16	191	111	80	.73	O	1	4	O	O	O	+	
15	SS	11	72	46	26	.57	O	O	5	+	O	+	O	
16	SS	9	52	29	23	.79	O	1	3	O	O	+	O	
17	P	13	130	64	66	1.02	O	1	1	O	O	O	+	
18	P	16	206	91	115	1.26	+	1	2	O	O	O	+	
19	P	15	191	101	90	.82	+	1	2	O	O	O	+	
20	P	13	122	52	70	1.34	O	O	2	O	O	O	O	
21	P	16	210	111	99	.90	O	1	5	O	O	O	O	
22	P	16	200	100	100	1.00	+	1	2	O	O	+	+	
23	P	17	208	102	106	1.04	+	1	1	O	O	O	+	
24	SS	10	78	29	49	1.70	O	O	3	O	1	O	+	III
25	SS	11	86	33	53	1.60	O	O	3	O	M	+	O	
26	P	16	184	92	92	1.00	+	1	1	O	O	O	O	
27	P	18	272	127	145	1.14	+	1	1	O	O	+	O	
28	P	19	252	112	140	1.25	+	2	3	O	O	O	O	

TABLE 4-1 (continuation 1) Data relating to all neurons from the motor cortex

Cell No.	Type	Mean Diam. μ	Area Total	Area Nuc.	Area Cyt.	Cyt. Nuc.	Apic. Dend.	No. of Synapses Dend.	No. Asym.	Nucleus Ind.	Nucleus Drk	Cytopl. E.R.	Cytopl. Mer.	Layer
29	P	18	244	112	132	1.18	+	1	3	O	O	O	+	
30	P	16	219	107	112	1.02	+	2	3	O	O	O	+	
31	LS	14	141	65	76	1.17	O	O	7	+	M	O	+	+	
32	LS	18	243	98	145	1.48	O	O	5	+	O	O	+	+	
33	P	17	268	114	154	1.35	+	2	2	O	O	O	O	O	
34	P	11	98	65	33	.51	O	O	1	O	O	O	+	III
35	SS	9	58	29	29	1.00	O	1	5	O	1	+	O	+	
36	SS	11	84	52	32	.61	O	2	1	O	1	+	O	+	
37	P	20	308	130	178	1.37	+	1	4	O	O	O	+	O	
38	P	14	131	75	56	.75	O	O	1	O	O	O	O	+	
39	P	18	273	100	173	1.73	+	3	1	O	O	O	O	O	
40	LS	13	122	47	75	1.60	O	1	4	+	O	+	+	+	
41	SS	8	49	26	23	.88	O	O	1	O	O	+	O	+	
42	P	17	229	104	125	1.20	+	1	1	O	O	O	O	+	
43	P	12	123	84	39	.47	O	O	1	O	O	O	O	+	
44	P	12	98	49	49	1.00	O	1	2	O	O	O	O	+	
45	P	17	242	115	127	1.10	+	2	2	O	O	O	+	O	
46	SS	10	60	43	17	.40	O	O	1	O	O	O	O	+	
47	P	16	197	93	104	1.12	+	1	3	O	O	O	+	O	
48	P	15	164	84	80	.95	O	O	2	O	O	O	+	+	
49	P	15	186	66	120	1.82	+	2	1	O	M	O	+	+	
50	SS	10	93	51	42	.82	O	1	1	O	1	O	O	+	IV
51	LS	17	244	67	177	2.65	O	1	15	+	O	O	+	+	
52	SS	9	61	36	25	.70	O	O	1	O	1	O	O	+	
53	SS	13	113	50	63	1.26	O	O	2	O	O	O	O	+	
54	P	13	142	47	95	2.03	O	1	2	O	O	O	+	O	
55	P	18	248	115	133	1.15	O	1	4	O	1	O	+	O	
56	P	13	147	81	66	.82	O	1	2	O	O	O	O	O	

TABLE 4-1 (Continuation 2) Data relating to all neurons from the motor cortex

Cell No.	Type	Mean Diam. μ	Area Total	Area Nuc.	Cyt.	Cyt. Nuc.	Apic. Dend.	No. of Synapses Dend.	No.	Asym.	Nucleus Ind.	Nucleus Drk.	Cytopl. E.R.	Cytopl. Mer.	Layer
57	P	19	275	118	157	1.33	+	2	1	O	O	O	+	+	
58	P	14	155	70	85	1.22	+	2	1	O	O	O	O	O	IV
59	P	13	128	66	62	.94	O	O	3	O	M	O	+	O	
60	SS	9	54	24	30	1.25	O	O	2	O	O	+	O	+	
61	P	15	179	95	84	.88	O	1	1	O	O	O	+	+	
62	P	16	189	98	91	.93	+	1	1	O	1	O	O	O	
63	P	12	115	69	46	.67	+	1	1	O	O	O	O	+	
64	P	15	192	103	89	.86	+	1	2	O	O	O	O	+	
65	P	16	212	74	138	1.86	O	1	1	O	O	O	+	O	
66	P	15	200	93	107	1.16	O	2	2	O	O	O	O	+	
67	P	19	305	86	219	2.55	O	O	3	O	1	O	+	O	
68	LS	27	502	110	392	3.55	O	1	14	+	O	O	+	O	V
69	LS	13	128	55	73	1.32	O	1	5	+	O	O	+	+	
70	P	25	418	138	280	2.02	O	2	4	O	M	O	+	O	
71	P	17	303	116	187	1.62	O	1	2	O	M	O	O	O	
72	P	15	162	83	79	.95	O	1	1	O	M	O	+	+	
73	P	14	166	106	60	.57	O	1	1	O	O	O	O	+	
74	LS	15	167	74	93	1.26	O	1	10	+	O	O	+	O	
75	SS	11	91	45	46	1.02	O	O	1	O	M	+	O	+	
76	P	12	123	62	61	.99	+	1	2	O	M	O	O	O	
77	LS	16	184	58	126	2.17	O	1	11	+	O	O	+	O	
78	P	14	143	73	70	.96	O	O	2	O	M	O	+	+	
79	P	15	153	90	63	.70	+	1	1	O	M	O	+	+	
80	P	18	212	103	109	1.03	+	1	3	O	O	O	O	+	
81	SS	11	93	57	36	.63	O	O	2	O	1	O	O	O	
82	P	14	151	71	80	1.12	+	1	1	O	O	O	+	O	
83	P	15	157	80	77	.96	O	O	4	O	O	O	+	+	
84	P	15	152	92	60	.65	+	1	2	O	M	O	+	O	

TABLE 4-1 (Continuation 3) Data relating to all neurons from the motor cortex

Cell No.	Type	Mean Diam. μ	Area Total	Area Nuc.	Area Cyt.	Cyt. Nuc.	Apic. Dend.	No. of Synapses Dend.	Synapses No.	Synapses Asym.	Nucleus Ind.	Nucleus Drk.	Cytopl. E.R.	Cytopl. Mer.	Layer
85	P	12	124	65	59	.91	O	1	1	O	O	O	O	O	
86	P	15	171	100	71	.71	+	1	3	O	1	O	+	O	
87	P	14	168	96	72	.75	O	3	1	O	O	O	+	+	
88	P	13	138	64	74	1.16	O	1	1	O	O	O	+	O	
89	P	15	176	92	84	.91	+	2	2	O	M	O	+	+	
90	P	12	109	59	50	.85	O	1	1	O	M	O	O	O	
91	P	13	151	71	80	1.12	O	O	1	O	O	O	+	O	
92	P	13	125	76	49	.64	O	O	1	O	O	O	+	O	
93	P	15	121	56	65	1.16	+	1	1	O	O	O	O	O	
94	P	15	152	77	75	.98	O	O	3	O	1	O	+	O	VI
95	SS	10	62	23	39	1.70	O	O	5	+	M	+	+	O	
96	P	14	129	70	59	.84	+	1	1	O	1	O	+	O	
97	P	14	159	91	68	.75	+	2	1	O	O	O	O	O	
98	P	14	155	85	70	.82	O	1	2	O	1	O	+	O	
99	SS	10	78	43	35	.81	O	O	2	O	O	+	O	O	
100	P	17	224	96	128	1.33	+	1	1	O	M	O	+	+	
101	P	14	146	78	68	.87	+	1	2	O	M	O	O	O	
102	P	16	197	124	73	.59	O	O	1	O	M	O	O	O	
103	P	11	80	45	35	.78	O	O	2	O	O	O	O	O	
104	P	14	152	74	78	1.06	O	O	1	O	M	O	+	O	
105	P	17	217	119	98	.82	+	2	1	O	M	O	O	O	
106	P	10	73	32	41	1.28	+	1	1	O	O	O	O	O	
107	P	14	164	87	77	.89	O	1	2	O	M	O	O	O	

TABLE 4-2 Data relating to all neurons from area 3b of the somatic sensory cortex

Cell No.	Class	Mean Diam. μ	Apic. Dend.	No. of Synapses Dend.	No. of Synapses No.	No. of Synapses Asym.	Nucleus Ind.	Nucleus Drk	Cytopl. E.R.	Cytopl. Mer.	Laminae
1	SS	12	O	1	3	+	1	+	+	O	
2	P	9	O	O	2	O	O	O	O	+	
3	SS	10	O	1	3	+	1	+	O	O	
4	SS	9	O	1	2	O	1	+	O	O	
5	SS	7	O	1	1	O	M	+	O	O	
6	SS	10	O	O	1	O	O	O	O	O	
7	P	10	+	1	1	O	O	O	O	O	
8	SS	12	O	O	2	O	M	O	O	O	
9	P	10	+	1	2	O	O	O	O	+	
10	P	12	+	1	1	O	O	O	O	+	
11	P	13	+	2	2	O	O	O	O	O	
12	SS	8	O	O	2	O	O	O	O	+	
13	SS	9	O	O	1	O	O	O	O	O	
14	P	13	+	2	1	O	O	O	O	+	II
15	P	11	O	O	2	O	O	O	O	+	
16	P	13	O	O	2	O	O	O	O	+	
17	P	11	O	O	1	O	O	O	O	+	
18	P	13	O	1	3	O	O	O	O	+	
19	SS	8	O	1	2	O	O	O	O	+	
20	P	17	+	2	4	O	O	O	+	+	
21	P	14	+	1	2	O	O	O	+	O	
22	P	10	O	1	2	O	O	O	O	O	
23	P	10	O	O	1	O	O	O	O	+	
24	SS	8	O	O	1	O	O	O	O	+	
25	P	11	O	1	1	O	O	O	O	+	
26	P	10	O	1	2	O	O	O	O	O	
27	P	12	O	1	2	O	O	O	O	+	
28	P	10	+	1	1	O	O	O	O	O	
29	P	12	O	1	3	O	O	O	O	+	

TABLE 4-2 (Continuation 1) Data relating to all neurons from area 3b of the somatic sensory cortex

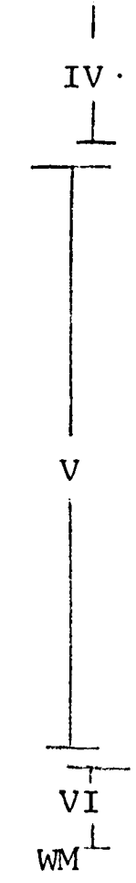
Cell No.	Class	Mean Diam. μ	Apic. Dend.	No. of Synapses Dend.	No.	Asym.	Nucleus Ind.	Drk	Cytopl. E.R.	Mer.	Laminae
30	SS	12	O	O	1	O	O	O	O	O	
31	P	13	O	1	2	O	O	O	O	+	II
32	SS	12	O	1	3	O	1	O	O	O	
33	P	16	+	2	2	O	O	O	O	O	
34	SS	11	O	O	4	O	1	O	O	O	
35	P	13	O	1	2	O	O	O	O	+	
36	P	10	O	O	1	O	O	O	O	+	
37	P	10	+	1	1	O	O	O	O	+	
38	P	11	O	1	1	O	O	O	O	O	
39	SS	9	O	O	1	O	O	O	O	+	
40	SS	12	O	O	1	O	M	O	O	+	
41	P	13	O	O	1	O	O	O	O	+	
42	P	10	O	1	1	O	O	O	O	+	
43	P	14	+	1	1	O	O	O	O	O	
44	SS	10	O	2	2	O	M	+	O	O	
45	P	12	O	O	1	O	O	O	+	+	III
46	P	12	O	O	3	O	O	O	O	O	
47	P	16	+	2	1	O	O	O	O	O	
48	P	15	+	2	1	O	O	O	+	O	
49	SS	8	O	O	2	+	M	+	O	+	
50	P	14	O	1	1	O	O	O	O	O	
51	P	15	+	1	3	O	O	O	+	O	
52	P	11	O	1	1	O	O	O	O	+	
53	P	14	+	2	1	O	O	O	+	+	
54	P	10	O	1	1	O	O	O	O	O	
55	P	11	O	1	2	O	O	O	O	+	
56	P	14	O	1	3	O	O	O	O	O	
57	SS	11	O	1	1	+	1	+	O	O	
58	P	12	+	2	2	O	O	O	O	O	IV

TABLE 4-2 (Continuation 2) Data relating to all neurons from area 3b of the somatic sensory cortex

Cell No.	Class	Mean Diam. μ	Apic. Dend.	No. of Synapses Dend.	No.	Asym.	Nucleus Ind.	Drk	Cytopl. E.R.	Mer.	Laminae
59	SS	9	O	O	2	O	O	O	O	O	
60	P	10	O	O	2	O	O	O	O	O	
61	P	16	+	2	2	O	O	O	O	O	
62	P	11	+	1	2	O	O	O	O	+	
63	P	11	O	1	3	O	O	O	O	O	
64	P	17	O	2	2	O	O	O	+	O	
65	P	12	+	2	1	O	O	O	O	O	
66	P	13	+	2	1	O	O	O	O	O	
67	P	11	O	O	1	O	O	O	O	+	
68	P	12	+	1	2	O	O	O	O	O	
69	LS	11	O	1	6	+	1	+	+	+	
70	P	12	+	1	2	O	O	O	O	O	
71	P	9	+	1	1	O	O	O	O	+	IV
72	SS	9	O	O	1	O	M	+	O	+	
73	P	14	O	1	2	O	O	O	+	O	
74	P	13	+	1	3	O	O	O	+	O	
75	LS	9	O	2	6	+	1	+	O	+	
76	P	10	O	1	1	O	O	O	O	O	
77	LS	12	O	O	10	+	1	+	+	+	
78	P	10	O	O	1	O	O	O	O	+	
79	SS	7	O	O	1	O	1	+	O	+	
80	P	11	O	1	3	O	O	O	O	+	
81	P	9	O	O	1	O	O	O	O	+	
82	P	9	O	O	2	O	O	O	O	O	
83	P	16	O	O	1	O	1	O	O	O	
84	P	10	O	1	1	O	O	O	O	+	
85	P	10	O	O	1	O	O	O	O	O	
86	P	10	O	O	1	O	O	O	O	O	
87	LS	12	O	O	4	O	O	O	+	+	
88	SS	8	O	O	3	O	1	+	O	+	

TABLE 4-2 (Continuation 3) Data relating to all neurons from area 3b of the somatic sensory cortex

Cell No.	Class	Mean Diam. μ	Apic. Dend.	No. of Synapses Dend.	No.	Asym.	Nucleus Ind.	Drk	Cytopl. E.R.	Mer.
89	LS	10	O	1	5	+	1	O	O	O
90	SS	9	O	1	1	O	1	+	O	+
91	P	10	O	1	1	O	O	O	O	+
92	P	20	+	2	3	O	1	O	+	O
93	SS	10	O	1	3	+	1	+	O	+
94	P	11	O	1	1	O	1	O	O	O
95	SS	11	O	O	1	O	1	O	O	+
96	P	12	O	1	3	O	1	O	O	+
97	SS	9	O	O	3	+	M	+	O	+
98	P	15	+	3	1	O	M	O	O	O
99	P	12	O	O	1	O	O	O	O	O
100	P	13	O	1	1	O	O	O	O	O
101	P	11	O	O	2	O	O	O	O	O
102	P	16	O	1	2	O	1	O	O	O
103	P	15	O	1	1	O	1	O	O	O
104	P	14	+	2	1	O	O	O	O	O
105	P	13	O	1	2	O	O	O	O	O
106	P	12	O	O	3	O	O	O	O	O
107	P	12	O	O	3	O	O	O	+	O
108	P	13	O	O	3	O	M	O	+	O
109	P	12	O	O	2	O	M	O	O	O
110	P	15	O	O	1	O	O	O	O	O



and least diameters for a round or oval cell and for a cell with an apical dendrite it was taken as the mean of the transverse diameter and the height of the cell to an arbitrary but consistent division between soma and apical dendrite, taken to be at the level where the outline of the cell changed from convex to concave. The presence or absence of an apical dendrite was recorded as was the number of dendrites arising from the soma, including an apical dendrite if present. The number of synapses on the soma was counted, excluding any on lengths of dendrite arising from the cell, the boundary being determined as for apical dendrites. The presence of asymmetric synapses was recorded only if one or more synapses clearly identifiable as being asymmetric was present; as a proportion of synapses are inevitably sectioned in such a way that they cannot be definitely typed, a negative does not imply that the cell necessarily received only symmetrical synapses. Nuclear indentations were recorded as absent, single or multiple and a nucleus was classified as 'dark' if it contained dense chromatin as in Fig. 4-14. The presence of one or more stacks of at least four cisternae of endoplasmic reticulum was recorded as a positive and finally the general impression of whether the cell cytoplasm merged with the background neuropil or not was noted. Some of these criteria are inevitably subjective, although a number have been used by previous authors, but they were applied consistently by the same observers to all the cells in the samples and so should be useful at least for making comparisons between the two cortical areas studied here.

The results were analysed in two stages. Before any classification of the cells was done a number of analyses were performed on the whole sample of cells from each area (Figs. 4-23 to 4-29). This produced several pieces of evidence which were not in any way dependent on a previous classification of cells and gave independent support from an unselected sample of cells to the classification made above using the qualitative results. The cells in the study were then classified using the criteria described in the first section of the chapter to provide quantitative information about each class of cell and to make a detailed comparison of the characteristics, proportions and distributions of the cells in the two areas of cortex being studied.

Pre-classification analysis

Histograms comparing the mean diameters of all the cells studied in the motor cortex and area 3b of the somatic sensory cortex are shown in Fig. 4-23. It can be seen that in the motor cortex the cells are in general rather larger and that they also have a wider range of mean diameters. In the motor cortex in particular there is a suggestion that the size distribution is bimodal and subsequent classification in fact showed that most of the cells in the peak below 12 μ in diameter were small stellate cells and most of those above 12 μ were pyramids and large stellate cells. This was not as clearly shown in area 3b, possibly being blurred because of the smaller overall spread of size. The mean

of the diameters of all cells in the motor cortex was 14.16 μ and in area 3b was 11.55 μ. The frequencies with which cells of different sizes were found to have apical dendrites in motor and somatic sensory cortices are shown in Figs. 4-24 and 4-25 respectively. It can be seen that about half (57%) of the cells in the motor cortex of more than 13 μ in diameter have apical dendrites whereas only a few (13%) of those with a mean diameter of less then 13 μ have one. This suggests strongly that there is a population of cells present in the motor cortex of less than 13 μ in mean diameter which does not have apical dendrites, and on subsequent classification most of these cells smaller than 13 μ and without an apical dendrite were found to be small stellate cells. Fig. 4-25 shows that there is similarly a marked reduction in the proportion of cells having apical dendrites below a mean diameter of about 11 μ in area 3b.

Histograms of the numbers of synapses found on each cell profile in the two cortical areas are shown in Fig. 4-26. The most striking feature of these in both areas is the remarkably small proportion of cells receiving the large numbers of synapses associated with 'typical' stellate cells. This suggests that this type of cell is rather less common than had been previously supposed. It should be noted that neurons receiving no synapses in the section studied were excluded by the criteria used but a statistical estimate of their numbers is given under the individual cell types below.

To investigate the relationship between the diameter of a cell and the number of synapses it receives, these two parameters were plotted against each other for all the motor and somatic sensory cells (Figs. 4-27 and 4-28). The number in each square represents the number of cells having that combination of size and number of synapses. It can be seen in both areas that a small proportion of the cells receives a large number of synapses but in general there is not a marked increase in the number of synapses per cell with size and this confirms that the large numbers of synapses which are found on a proportion of cells is a reflection of a genuinely high synaptic density and not just a consequence of the large size of some cells.

A group of cells is usually recognised as a distinct class if the cells in it tend to have a certain set of features occurring together. A correlation matrix was performed by computer on all the cells of the motor cortex sample (Fig. 4-29) to see which features, if any, of these cells tended to occur together and to see if any particular groupings of features emerged from the unclassified cells to give independent evidence as to what the groups of cells present were. A '+' in the matrix indicates that the features shown in the two intersecting columns were found together in the sample more frequently than would be predicted by chance, this being statistically significant at at least the 5% level. Similarly a '-' indicates that the two features were found together less frequently than chance would predict at the

same probability level, that is that the features tended to be mutually exclusive.

The presence of an apical dendrite can be seen from the matrix to correlate with increasing mean cell diameter and increasing total, nuclear and cytoplasmic area, but not with a high ratio of cytoplasmic to nuclear area; an apical dendrite is therefore found more commonly on the larger cells of the sample. An apical dendrite also correlates with cells having more dendrites (although in itself it must mean that the cell has at least one) and the presence of an apical dendrite is inversely correlated with the number of synapses received by the cell and with the presence of asymmetrical synapses. This grouping of features is in fact that of pyramidal cells and so this confirms the presently accepted classification.

The matrix shows that the number of synapses received by a cell increases with mean cell diameter and total area. In contrast to apical dendrites, however, the number of synapses correlates with increase in the area of cytoplasm and in the ratio of cytoplasmic to nuclear area, but not with the area of the nucleus, so that it is cells with abundant cytoplasm rather than just large cells which tend to receive large numbers of synapses. The presence of large numbers of synapses can also be seen to be associated with asymmetrical synapses and clumps of endoplasmic reticulum. This grouping of features corresponds to that described on the basis of qualitative evidence to the

features of large stellate cells.

Neither the presence of an apical dendrite nor the number of synapses received by the cell correlates with the presence of a dark nucleus so this does not appear to be a significant feature of either of the first two groupings. The presence of a dark nucleus can however be seen to correlate with a number of other features. It is inversely correlated with the depth of the cell from the pial surface and so occurs more frequently in cells from the more superficial layers of the cortex. It is also inversely correlated with mean cell diameter and total, nuclear and cytoplasmic area and so occurs mainly in small cells. A dark nucleus also correlates inversely with the number of dendrites and the presence of clumps of endoplasmic reticulum and so cells with dark nuclei tend not to have dendrites arising from the soma and to have sparse endoplasmic reticulum. A dark nucleus also tends to be indented. This cluster of features shown to occur together in the cells of this unselected sample corresponds to the features attributed to small stellate cells on qualitative evidence and none of these features shows a similar correlation with an apical dendrite or the number of synapses present. These results therefore confirm independently that these small stellate cells form a separate distinct population in the cortex.

It is of interest that nuclear indentations, as well as being found more frequently in dark nuclei which occur towards the pial surface, also increase overall with depth from the pia.

This apparent contradiction occurs because, although a greater proportion of dark nuclei are indented, in fact in absolute terms most indentations occur in pyramidal nuclei, these forming the greatest proportion of the cells, and for some reason these indentations increase with depth (see below). The matrix also confirms quantitatively that an increase in cell diameter goes with an increase in the ratio of cytoplasmic to nuclear area and also a greater frequency of clumps of endoplasmic reticulum. This confirms observations made qualitatively on pyramids above. It may also be noted that there was a very close correlation between the mean diameter of a cell and its total area as measured here and they were effectively interchangeable. Therefore, because mean diameter is the simpler measurement to make, this was the only measure of cell size used in the somatic sensory cortex.

In summary therefore, the correlation matrix shows three significant clusters of features which occur together in the cells of this unselected sample and these three sets of features correspond to the descriptions of pyramidal, large stellate and small stellate cell types which were made using qualitative evidence.

Results classified by cell type

The proportions of the different cell types and their laminar distributions

After the data in the previous section had been obtained,

the cells from both areas were classified into pyramidal, large stellate and small stellate types using the criteria described above under the qualitative results. When the classification of the micrographs was done the numbers of cells of each type were not added up until classification was complete. The proportions of each type of cell in the two cortical areas are shown in Table 4-3 where it can be seen that the numbers of each cell type present in the motor cortex and area 3b of the somatic sensory cortex are remarkably similar; pyramidal cells account for more than two-thirds of the cells in each area and small stellate cells form the majority of the remainder. The distributions of the cells through the depth of the cortex in the two areas are shown in the maps of Fig. 4-30, and the histograms of Figs. 4-31 and 4-32. It can be seen that the smaller thickness of the somatic sensory cortex is compensated for by the denser packing of its cells so that the total numbers of cells in the two equal-width strips are remarkably similar. The cell shown as being in the white matter of the somatic sensory map was an anomaly since the area surrounding it, shown as white matter on the map, consisted of densely packed myelinated axons and was clearly not cortex; it is included with layer VI for analysis. From the map and histograms of the motor cortex it can be seen that there are relative concentrations of cells in layer II and layer IV and that there are definite sparse regions at the boundary of layers III and IV and in the middle of layer V. Although care must be

TABLE 4-3

The proportions of the different cell types in the motor and somatic sensory cortices.

	Motor Cortex	Somatic Sensory Cortex (Area 3b)
Total number of cells	107	110
Pyramidal	77 (72%)	79 (72%)
Large Stellate	8 (7%)	5 (5%)
Small Stellate	22 (21%)	26 (23%)

taken in interpreting the results from a narrow strip of cortex, it seems likely that these gaps are genuine and that they correspond to the outer and inner bands of Baillarger. The somatic sensory cortex shows similar concentrations of cells in layers II and IV and similar gaps at the junction of layers III and IV and in the upper part of layer V. Histograms of the depth distributions of the individual cell types in the two areas are shown in Figs. 4-33 and 4-34. It can be seen in both areas that pyramidal cells occur in all layers except layer I and are more densely packed in layers II, IV and VI. In motor cortex the large stellate cells are in a band extending from the middle of layer III through layer IV to the middle of layer V whereas in area 3b of the somatic sensory cortex they are restricted to layer IV. The small stellate cells are most frequent in layer II in both areas but also occur in all the other cortical layers of the maps except layer I. The proportions of each cell type in the different layers of the cortex are shown in Tables 4-4 and 4-5. Although the numbers in some groups are small, it is clear that up to half the cells in layer II of both cortical areas are small stellate cells and that this proportion falls off at greater depths from the pial surface. The proportion of pyramids increases progressively in the deeper layers in the motor cortex while in the somatic sensory cortex there is a relatively greater localisation of stellate cells to layer IV with a correspondingly smaller proportion of stellate cells in layer III. A study was also made of a section taken

TABLE 4-4

The numbers and proportions of the different cell types in the different layers of the motor cortex.

Layer	Pyramid	Large Stellate	Small Stellate	Total
I	0	0	0	0
II	12 (60%)	0 (-)	8 (40%	20
III	14 (64%)	3 (14%)	5 (22%)	22
IV	12 (66%)	1 (-)	5 (28%)	18
V	18 (75%)	4 (17%)	2 (8%)	24
VI	21 (91%)	0 (-)	2 (9%)	23
	77	8	22	107

TABLE 4-5

The numbers and proportions of the different cell types in the different layers of the somatic sensory cortex (area 3b)

Layer	Pyramid	Large Stellate	Small Stellate	Total
I	0	0	0	0
II	22 (63%)	0	13 (37%)	35
III	16 (80%)	0	4 (20%)	20
IV	25 (69%)	5 (14%)	6 (17%)	36
V	13 (81%)	0	3 (19%)	16
VI	2	0	0	2
WM	1	0	0	1
Totals	79	5	26	110

parallel to the pial surface from layer IV of the motor cortex to obtain a larger sample of cells and so a more reliable estimate of the proportions of the different cell types in this layer. The results of this are shown in Table 4-6 where it can be seen that the proportion of large stellate cells is in agreement with that in the adjacent laminae III and V of the first motor cortex map and is close to the proportion of large stellate cells in layer IV of the somatic sensory cortex.

Cell sizes

The relative sizes of the populations of pyramidal cells in motor and somatic sensory cortices are shown in Fig. 4-35 and Table 4-7. In the motor cortex it can be seen that most of the pyramids form one distribution and there is only one cell which is markedly larger than the rest, as is also the case in the somatic sensory cortex. The pyramidal population in the motor cortex has a greater mean diameter than that in the somatic sensory cortex (Table 4-7), the mean of the pyramidal cell diameters in motor cortex being 15.2 μ and in the somatic sensory cortex 12.3 μ, giving a ratio of diameters of 1.21 to 1. Cell somata are however three-dimensional objects. If one assumes that they approximate to a sphere then their volume is proportional to the cube of their radius and on this basis the mean volume of motor cortex pyramids is 1.77 times that of those in area 3b of the somatic sensory cortex.

TABLE 4-6

Numbers and proportions of the different cell types in a map of the motor cortex parallel to pia in layer IV

Pyramids	17 (52%)		
Large Stellate	5 (15%)	)	
		)	48%
Small Stellate	11 (33%)	)	
	33		

TABLE 4-7

Means of the diameters of the different cell types in the motor and somatic sensory cortices

	Motor Cortex μ	Somatic Sensory Cortex (Area 3b) μ
Pyramids	15.2	12.3
Large Stellate	16.6	10.8
Small Stellate	10.4	9.6

The size distributions of large and small stellate cells in the two cortical areas are shown in Fig. 4-36. These histograms confirm the size ranges described in the qualitative results and it can be seen that in the motor cortex there is little overlap between the size distributions of the two stellate cell types. The mean diameter of large stellate cells in the motor cortex was 16.6 μ and of small stellate cells 10.4 μ and so size is a useful distinguishing feature in this area (Table 4-7). In the somatic sensory cortex the small stellate cells were slightly smaller than in the motor cortex, with a mean diameter of 9.6 μ. The large stellate cells were however considerably smaller than in the motor cortex, with a mean diameter of 10.8 μ, and so there is considerable overlap between the size ranges of the two stellate cell types in the somatic sensory cortex. The two types are however still distinguished by the other features described above, in particular by the difference in the numbers of synapses that the cells of the two groups receive.

The diameters of cells differ with the depth of the cell from the pia; this is shown in Fig. 4-37 for the motor cortex and in Fig. 4-38 for the somatic sensory cortex, and the mean diameters of cells in the different laminae of the two areas are shown in Tables 4-8 and 4-9. The smallest cells occurred in layer II and in layer IV in both areas, with a few immediately above the conventional narrow layer IV in the motor cortex. The concentrations of cells at these levels seen in

TABLE 4-8

Mean diameters of neurons in different layers of the motor cortex

Layer	Mean cell diameter	
	Overall μ	Pyramids μ
I	-	-
II	12.8	14.1
III	14.9	16.7
IV	13.6	14.8
V	15.6	15.6
VI	13.6	13.9

TABLE 4-9

Mean diameters of neurons in different layers of the somatic sensory cortex (area 3b)

Layer	Mean cell diameter	
	Overall μ	Pyramids μ
I	-	-
II	11.2	12.0
III	11.9	12.4
IV	11.1	11.6
V	12.9	13.6
VI	12.5	12.5

the maps and depth distribution histograms (Figs. 4-30 to 4-34) can be seen here to consist predominantly of small and medium sized cells, this being more marked in the somatic sensory cortex. These two bands of small and medium sized cells probably correspond to the granule cell bands; analysis of the cell types present in them showed that they were predominantly small stellate cells and small pyramidal cells in similar proportions, with the small pyramidal cells being generally slightly larger than the small stellate cells. In layer IV of the somatic sensory cortex large stellate cells were also present in the band but were generally slightly larger in size.

The largest cells in both areas occurred in layers III and V and the mean diameter of all cells and of pyramids was greatest in these two laminae in both cortical areas (Tables 4-8 and 4-9). It seems, however, at least in this strip of motor cortex, that the mean diameter of pyramidal cells in layer V does not differ significantly from that of those in layer III or is possibly smaller, although in somatic sensory cortex the mean diameter of layer V pyramids is greater than that of those in layer III. It may be that the observed differences in cell sizes between these two layers in sections stained by the Nissl method is due to a small proportion of very large cells being present in layer V, especially in the motor cortex, rather than to the general population of cells in layer V being larger.

Numbers of synapses

The distributions of the numbers of synapses occurring on pyramidal cells in motor and somatic sensory cortices are shown in Fig. 4-39. It can be seen that most pyramids in both areas receive only one or two axo-somatic synapses in a single section. There are more pyramids receiving four or five synapses in the motor cortex, this occurring on larger cells, and the mean number of synapses received by a pyramid in a single section of motor cortex is 1.79 (Table 4-10) which is slightly greater than the somatic sensory cortex with 1.68.

Being a small positive integer, it would be expected that the distribution of the numbers of synapses on pyramidal cells found by this sampling method should follow a Poisson distribution if the density of synapses on pyramidal cell somata is constant, and analysis of these distributions from both cortical areas shows that they in fact do so (χ^2 motor cortex 0.694, 3df; somatic sensory cortex 0.297, 2df). The observed and predicted distributions for each area are compared in Fig. 4-40. The Poisson distribution is however truncated because those neurons which happened not to receive a synapse in the section studied were not included in the sample because they could not be reliably distinguished from glial cells. This number can be estimated by mathematically completing the Poisson distribution and in the motor cortex was 21 cells and in the somatic sensory cortex was 25 cells. This means that about 21% of the pyramids in the motor cortex and 24% in the somatic

TABLE 4-10

Mean numbers of synapses on single profiles of neurons of each type

	Motor Cortex	Somatic Sensory Cortex (area 3b)
Pyramidal	1.8	1.7
Large Stellate	8.9	6.2
Small Stellate	2.2	1.8

TABLE 4-11

The proportions of the different cell types in the motor and somatic sensory cortices corrected to allow for the predicted numbers of cells receiving no synapses in the section studied

	Motor Cortex	Somatic Sensory Cortex (area 3b)
Pyramidal	98 (74%)	104 (73%)
Large Stellate	8 (6%)	5 (4%)
Small Stellate	26 (20%)	33 (23%)

sensory cortex received no synapse in the section studied and were therefore excluded from the sample. The estimates of the numbers of pyramids in the two areas are therefore correspondingly low but the difference is very similar for the two areas (Table 4-11, cf. Table 4-3).

If the stellate cell somata formed a single population with a single synaptic density like the pyramidal cells, the distribution of the numbers of synapses per cell profile should likewise follow a Poisson distribution. The distributions for all the stellate cells in motor cortex and all those in the somatic sensory cortex are shown in Fig. 4-41 and are compared to Poisson distributions having the same mean in Fig. 4-42. In the motor cortex it can be seen that the observed population differs from the predicted population in having an excess of cells with very few synapses or a large number of synapses and a relative deficit of cells around the mean value of four of the predicted Poisson distribution. This deviation from the predicted Poisson distribution is statistically significant ($P < 0.02$). In the somatic sensory cortex there are similar but less marked differences between the observed and predicted populations and these do not reach statistical significance although the fit between the two curves is poor ($\chi^2 = 2.77$ 3df), particularly when compared to those for the pyramidal cells in both areas. This therefore strongly suggests that the stellate cells do not form a single homogeneous population. Fig. 4-43 shows the

distributions in motor cortex and Fig. 4-44 that in the somatic sensory cortex after splitting into large and small stellate cell types. The distributions of both cell types in both areas can now be fitted by Poisson curves (Figs. 4-45 and 4-46). The fit for small stellate cells in both areas is satisfactory (χ^2 motor cortex 0.39, 2 df; somatic sensory cortex 0.62, 2 df) while for large stellate cells the number of cells is too small to test statistically although the fit appears reasonable visually. The mean number of synapses on a small stellate cell in the motor cortex is 2.2 (Table 4-10) while on a large stellate profile it is 8.9 and in the somatic sensory cortex the mean for a small stellate profile is 1.8 and for a large stellate profile is 6.2; this is one of the most important distinctions between the two stellate cell types. As in the case of the pyramidal cells, the numbers of each cell type in both areas which receive no synapses can be estimated by completing the Poisson distribution. For small stellate cells it is 4 in the motor cortex and 7 in the somatic sensory cortex, while it is very unlikely that any large stellate cells in either area will fail to receive a synapse in any particular section. This means that overall the proportion of large stellate cells will have been overestimated in both areas in relation to both the other cell types because of the criteria which have to be adopted but since this will be similar in both areas a fair comparison of the proportions of the different cell types can be made. The proportions corrected for the predicted

numbers of cells of each type without synapses in the section studied are given in Table 4-11, where it can be seen by comparison with Table 4-3 that this in fact makes very little difference to the overall figures.

The relationship between the numbers of synapses received by a cell and mean cell diameter is shown for the three cell types in the two cortical areas in Figs. 4-47 to 4-50. The number at each point indicates the number of cells of that type having that combination of number of synapses and cell diameter. By comparison with the overall graphs of these two parameters (Figs. 4-27 and 4-28) it can be seen that the three different cell types occur predominantly in different regions of the graph. Most small stellate cells occur in the region corresponding to a small mean diameter and a small number of synapses in both areas; the pyramidal population overlaps with this at the lower end of its size range but is mostly larger and there is only a slight tendency for the number of synapses to increase with the size of the pyramidal cells. In contrast to these two groups the large stellate population extends across a region of moderate to large numbers of synapses but the size range of the cells is very similar to that of the pyramids. Use of these two parameters will therefore separate to a large extent the three cell types in both cortical areas. In particular when the two stellate cell populations are plotted on the same graph they may be separated by a straight line as shown in the samples from both the motor

and the somatic sensory cortices (Figs. 4-51 and 4-52). Thus the cells of the two stellate populations which overlap with regard either to mean diameter (Fig. 4-36) or number of synapses (Figs. 4-43 and 4-44) do not overlap with regard to the other of these parameters and no cells in the two groups in fact overlap with respect to both diameter and number of synapses received.

Number of dendrites per cell

The frequencies with which cell somata of the different types were found to give rise to one or more dendrites in the section studied are shown in Fig. 4-53, with mean values in Table 4-12. It can be seen that the distributions in the two cortical areas are very similar but that there are clear differences between the cell types. Whereas most pyramidal cells give rise to at least one dendrite in a section, as do the majority of large stellate cells, the majority of small stellate cells do not. Even when pyramids with apical dendrites are excluded the remaining pyramids are still more likely to give rise to a dendrite than is a small stellate cell. It should be emphasized that these results refer to sections cut accurately perpendicular to the pial surface and do not necessarily apply to sections not so cut.

Nuclear indentations

The frequencies with which nuclei in the different types of cell were found to have a single or multiple nuclear indentation are shown in Table 4-13. The majority of small stellate nuclei can be seen to be indented in both cortical areas. There appears

TABLE 4-12

Mean numbers of dendrites arising from a cell profile for each of the cell types

	Motor cortex	Somatic sensory cortex (area 3b)
Pyramids - total	1.01	0.89
- with apical dendrite	1.33	1.56
- without apical dendrite	0.65	0.54
Large Stellate	0.75	0.60
Small Stellate	0.45	0.42

TABLE 4-13

The frequencies of nuclear indentations in neurons of each type

Motor Cortex	Total	Single indentation	Multiple indentation	Proportion with indentation
Pyramidal	77	7	18	32%
Large Stellate	8	0	1	(12.5%)
Small Stellate	22	8	5	59%
Somatic Sensory Cortex (area 3b)				
Pyramidal	79	6	3	11%
Large Stellate	5	4	0	(80%)
Small Stellate	26	11	7	69%

to be a difference between the two areas with respect to large stellate cells but since the numbers involved are small this is probably not significant. The majority of indentations found in the motor cortex were in pyramids because of the large proportion of the cells which are of this type but the fraction of pyramids with an indentation is only just over half the fraction of small stellate nuclei which are indented while in the somatic sensory cortex a small stellate nucleus was six times as likely to be indented as a pyramidal nucleus. Comparison of the pyramids from motor and somatic sensory cortices (Table 4-13) shows that almost three times as many pyramidal nuclei in motor cortex have an indentation and that the majority of these are multiple, in contrast to those in the somatic sensory cortex.

The frequency of nuclear indentations in pyramids from both cortical areas was further analysed with respect to the cortical layer in which the cell occurred and the results of this are shown in Table 4-14, with the mean diameter of the cells in each group shown in brackets. This table shows a striking difference between the supra- and infragranular layers in both cortical areas; whereas only one pyramid in layers II and III from either area has a nuclear indentation, half or more of those in layers V and VI have one. This explains the relationship between nuclear indentations and depth found in the correlation matrix (Fig. 4-29) and may also explain the lower incidence of indentations in pyramidal nuclei in the somatic sensory cortex

TABLE 4-14

The frequency of indentations in pyramidal nuclei in different cortical laminae

Motor cortex

Layer	Without indentation	With indentation Single	Multiple	% with indentation
I	-	-	-	-
II	11 (13.9μ)	0	1	8%
III	14 (16.7μ)	0	0	0%
IV	9 (14.6μ)	1	2	25%
V	9 (14.9μ)	2	7 (16.2μ)	50%
VI	9 (12.8μ)	4 (14.5μ)	8 (14.9μ)	57%

The mean sizes of the cells in each group are shown in brackets

Somatic sensory cortex (area 3b)

I	-	-	-	-
II	22 (12.0μ)	0	0	0%
III	16 (12.4μ)	0	0	0%
IV	24 (11.5μ)	1	0	4%
V	7 (12.4μ)	5 (14.8μ)	1	46%
VI + WM	1	0	2	(67%)

since, although almost the same proportions of pyramids in layers V and VI are indented in the two cortical areas, these two layers contain many more cells in the motor cortex. This very low incidence of nuclear indentations in pyramids in layers II and III contrasts with the small stellate cells in these layers, seven out of the twelve small stellate cells in layers II and III of the motor cortex and ten out of seventeen in these layers of the somatic sensory cortex having indented nuclei.

This analysis of the frequency of nuclear indentations in the different laminae was repeated for only those cells which had an apical dendrite and comparable results were obtained (Table 4-15) to those for the whole pyramidal population. This rules out the possibility that the observed difference in frequency of nuclear indentations in different laminae was due to mis-identification of cells.

It can be seen from Table 4-14 that the mean diameter of the cells has some relationship to the incidence of nuclear indentations since within any one layer of the cortex the mean size of cells with nuclear indentations is greater than that of those without and overall those cells with nuclear indentations in either cortical area are larger than those without (Table 4-16). However there are clearly other factors which differ between the different laminae because the mean size of pyramids in layer III is greater than that of those in layer V although the incidence of nuclear indentations is much greater in the

TABLE 4-15

Proportions of cells with apical dendrites having nuclear indentations in each lamina

Motor cortex

Layer	Total	Single	Multiple	% with indentation
I	-	-	-	
II	7	0	0	0
III	11	0	0	0
IV	5	0	1	(20%)
V	8	1	3	50%
VI	9	2	4	67%
Somatic sensory cortex (area 3b)				
I	-	-	-	-
II	9	0	0	0
III	6	0	0	0
IV	9	0	0	0
V	3	1	1	(67%)
VI + WM	0	0	0	0

TABLE 4-16

Mean sizes of pyramidal cells with and without nuclear indentations

	With indentations (single or multiple)	Without indentations
Motor Cortex	15.5μ	14.8μ
Somatic sensory cortex (area 3b)	14.4μ	12.0μ

latter layer. Also cells with indentations in layer VI of motor cortex have the same mean diameter as those without indentations in layer V and the group of cells with the largest mean diameter is that of cells without indentations occurring in layer III of the motor cortex. Overall those cells without indentations in the motor cortex are larger than those with indentations in the somatic sensory cortex. The results in the motor cortex were also analysed in relation to nuclear area and cytoplasmic area of the cells but the results did not differ significantly from those above and it would appear that there is some definite difference with respect to nuclear indentations between pyramids in the supra- and infragranular layers.

Endoplasmic reticulum

All the large stellate cells in the motor cortex and three out of five of those in the somatic sensory cortex contained one or more stacks of endoplasmic reticulum, but only one small stellate cell in each area did so (Table 4-17). More than twice as many pyramids contained a stack of endoplasmic reticulum in the motor cortex than in the somatic sensory cortex (Table 4-17). Analysis of this by laminae in the motor cortex (Table 4-18) showed a situation very similar to that with nuclear indentations in that only a few cells in layers II and III contained a stack of endoplasmic reticulum whereas about half of those in layers V and VI did so. As with nuclear indentations, size would appear not to be the only factor in this as the mean size of pyramids in layer III of motor cortex exceeds that of those in

TABLE 4-17

The frequencies of stacks of endoplasmic reticulum in neurons of each type.

Motor cortex

	Total	With stack of endoplasmic reticulum	
Pyramidal	77	32	42%
Large stellate	8	8	100%
Small stellate	22	1	5%

Somatic sensory Cortex (area 3b)

Pyramidal	79	12	15%
Large stellate	5	3	60%
Small stellate	26	1	4%

TABLE 4-18

Incidence of clumps of endoplasmic reticulum in pyramidal cells in different laminae

Motor cortex

Layer	Without stack of Endoplasmic Reticulum (mean diam.of cells in each group in brackets)	With stack of Endoplasmic Reticulum	% with
I	-	-	
II	12 (14.1μ)	0	0%
III	11 (16.3μ)	3 (18.0μ)	21%
IV	4 (12.8μ)	8 (15.7μ)	67%
V	8 (14.9μ)	10 (16.3μ)	56%
VI	10 (13.5μ)	11 (14.3μ)	52%

Somatic sensory cortex (area 3b)

I	-	-	-
II	20 (11.6μ)	2	9%
III	12 (11.8μ)	4 (14.0μ)	25%
IV	22 (11.2μ)	3 (14.6μ)	12%
V	11 (13.1μ)	2	15%
VI + WM	2	1	(33%)

layer V in this sample and the mean diameter of those with a stack of endoplasmic reticulum in layer V of the motor cortex is the same as those without one in layer III. However, unlike the situation with nuclear indentations, the somatic sensory cortex does not show a similar increase in the incidence of stacks of endoplasmic reticulum in the pyramids of the deep cortical layers. The mean size of pyramids without a stack of endoplasmic reticulum in motor cortex is however very close to that of pyramids with a stack of endoplasmic reticulum in the somatic sensory cortex (Table 4-19).

Asymmetric synapses

The frequencies with which clearly identifiable asymmetric synapses were found on the different cell types in the two cortical areas are shown in Table 4-20. It can be seen that no pyramids were found to receive any asymmetric synapses but that they were present on almost all the large stellate cells in both areas. They were found on a proportion of small stellate cells but the small number of synapses on each cell reduced the chance of finding a clearly identifiable asymmetric one.

Dark nuclei

The nuclei of thirteen small stellate cells in each cortical area were judged to be dark (Table 4-20), this meaning that their chromatin appeared dense and occurred in clumps (Fig. 4-14). This total was about half the small stellate cell population in each area. No systematic differences were found in the incidence of dark nuclei in the different cortical laminae. No

TABLE 4-19

Mean sizes of pyramidal cells with and without stacks of endoplasmic reticulum (ER)

	With E.R.	Without E.R.
Motor cortex	15.6 μ	14.5 μ
Somatic sensory cortex (area 3b)	14.7 μ	11.9 μ

TABLE 4-20

Frequencies of asymmetrical synapses, dark nuclei and merging cytoplasm for neurons of each type

Motor cortex

	Total	Asymmetric synapses	Dark nuclei	Merging cytoplasm
Pyramidal	77	0	0	35
Large stellate	8	8	1	5
Small stellate	22	3	13	11

Somatic sensory cortex (area 3b)

Pyramidal	79	0	0	32
Large stellate	5	4	3	4
Small stellate	26	6	13	13

pyramidal nuclei were dark but one large stellate nucleus in motor cortex and three in somatic sensory cortex were judged to be dark.

Merging of cytoplasm with the background neuropil

The cytoplasm was judged to merge with the neuropil in about half of the cells of each type in each area (Table 4-20). However when analysed with respect to the different cortical layers some differences emerge (Table 4-21). In layer II of both areas small stellate cells tend to stand out from the background, whereas pyramidal cells do not, but in the deeper layers of both cortical areas the situation reverses and the pyramids tend to stand out from the background. With the possible exception of layer V of the motor cortex, the large stellate cells merged with the background neuropil in both cortical areas.

Discussion

Classification of cells

The basic division of neocortical neurons into pyramidal and non-pyramidal cells was established originally by workers using the Golgi technique to impregnate whole neurons for light microscopy (e.g. Cájal, 1909-11; Lorente de Nó, 1949) and the non-pyramidal cells have been further subdivided to differing degrees by different authors using this technique (e.g. Sholl, 1955, 1956; Marin-Padilla, 1969, 1970; Szentágothai, 1973; Jones, 1975a). This same division of neocortical neurons into pyramidal and non-pyramidal types has now been established using electron microscopy and descriptions of cells of the two groups have been given by a number of authors (Colonnier, 1968;

TABLE 4-21

Incidence of cytoplasm which merged with the background neuropil in the different cell types and different laminae

Motor cortex

	Pyramids		Large stellate		Small stellate	
	Merging	Not merging	Merging	Not merging	Merging	Not merging
I	-	-	-	-	-	-
II	10	2	0	0	1	7
III	7	7	3	0	4	1
IV	5	7	1	0	5	0
V	10	8	1	3	1	1
VI	3	18	0	0	0	2

Somatic sensory

I	-	-	-	-	-	-
II	15	7	0	0	3	10
III	8	8	0	0	3	1
IV	8	17	4	1	4	2
V	1	12	0	0	3	0
VI + WM	0	3	0	0	0	0

Szentágothai, 1969; Jones and Powell, 1970a; Lund and Lund, 1970; Peters and Kaiserman-Abramof, 1970; Garey, 1971; Peters, 1971). However in concentrating on those features which distinguish pyramidal and non-pyramidal cells at the electron microscope level, these descriptions of non-pyramidal cells appear to be mainly of those cells which are most strikingly different from pyramidal cells and in particular those receiving numerous axo-somatic synapses and clearly identifiable asymmetric synapses. This present study has confirmed these previous observations made in sensory cortical areas and has extended them to the motor cortex and the primate. However a considerable proportion of the cells found in this study clearly did not correspond to previous descriptions of stellate cells, but also had features which made it clear that they were not pyramidal cells; these cells have been termed small stellate cells. They may be distinguished from pyramidal cells because they can be shown in serial sections to lack an apical dendrite, because they receive asymmetric synapses, because the dendrites they give rise to are usually beaded and because they may have an axon initial segment directed towards the cortical surface. The presence of a population of small cells lacking apical dendrites is also shown by the graphs of the frequency of occurrence of an apical dendrite against size in the overall cell population (Figs. 4-24 and 4-25). A number of other features have also been found to distinguish the population of small stellate cells from that of pyramidal

cells, including their small size (Table 4-7 and Figs. 4-35 and 4-36), their incidence of dark nuclei(Table 4-20), the low frequency of dendrites found arising from their somata in a single section (Table 4-12, Fig. 4-53), their rounded shape and, in the superficial laminae, their high incidence of nuclear indentations (Tables 4-13 and 4-14).

This group of small stellate cells also clearly differs from previous descriptions of typical stellate cells (Colonnier, 1968; Szentagothai, 1969; Jones and Powell, 1970a; Lund and Lund, 1970; Peters, 1971; Garey, 1971). In particular small stellate cells do not receive the large number of synapses described as contacting typical stellate cell somata and they lack the abundant cytoplasm and endoplasmic reticulum and the high density of cytoplasmic organelles described as typical of these cells. Also the majority of small stellate cells occur in layer II of the cortex whereas previous descriptions have concentrated largely on those stellate cells which occur in layer IV of sensory cortical areas. The motor cortex also contains stellate cells which correspond closely to previous descriptions and confirm them and these cells have been termed here large stellate cells. In the motor cortex, where the distinction was first made, there is a clear size difference between the two stellate cell types (Table 4-7 and Fig. 4-36) but it should be emphasised that this is by no means the most important difference and in the somatic sensory cortex these other differences become

much more important in differentiating the two types of cell because the size difference is much less marked (Table 4-7 and Fig. 4-36).

Having established that the sensori-motor cortex contains a proportion of non-pyramidal cells which do not correspond to previous descriptions of stellate or non-pyramidal cells, it is necessary to determine whether these cells represent an extension of the stellate cell population described previously or whether they form a separate population. The impression gained from studying the cortex qualitatively is that the stellate cells form two distinct groups with different sets of features rather than forming a continuum, but there is a risk of selecting 'typical' cells of each type for study in this way and so gaining an artificial impression of a split. This danger is avoided by studying a random sample of cells and, although there was inevitably a little overlap, most stellate cells in the samples were clearly of one or other type, particularly in the motor cortex. The correlation matrix showed this objectively in that a cell with a large number of synapses, a feature of large stellate cells, also tended to have the other features described for this type and a cell with a dark nucleus, a feature mostly of small stellate cells, tended to have the other features of small stellate cells. Conversely, there was no significant association of features of one stellate type with the features of the other type. If the stellate cells formed a single population, the matrix should show

neither of the effects since the chance of finding a particular feature should then be the same in any stellate cell and all the features of the cell type should correlate together. This is therefore evidence that the features of a random sample of cells analysed by an objective method fall into two distinct groups and therefore that large and small stellate cells form two distinct populations.

A second approach to the problem of whether the stellate cells form one or more than one population is to examine parameters of a random sample of the stellate population, such as those taken, to see whether those parameters are distributed as would be expected for a single population. The random samples of the pyramidal populations serve as useful controls for this. In a single population of cells it would be expected that the density of axo-somatic synapses on different cells would be very similar. A single section through the centre of one of these neurons should sample these axo-somatic synapses randomly and because the numbers of synapses found in a single section is a small positive integer the numbers of synapses seen per cell profile (Figs. 4-39 to 4-46) should approximate to Poisson distributions. This is so for pyramidal cells in both areas (Fig . 4-40) but, when all the stellate cells are taken together, the distribution differs significantly from a Poisson distribution in the motor cortex (Fig. 4-42); the deviation is due to the combination of the proportion of the cells having large numbers

of synapses with the majority of cells which have only a small number of synapses. To illustrate this point, the mean of the number of synapses on all motor cortex stellate cells is four. With this mean it is statistically unlikely that the four stellate cells having ten or more synapses are part of the same population as the fifteen cells having only one or two synapses when there is only one cell which actually has the mean number of four synapses. By comparison, the mean number of synapses on a motor cortex pyramid is 1.79 and most of the population are clustered about this value. These findings therefore indicate that, unlike the pyramids, the stellate cells do not form a single population. When split into large and small types, however, the distributions for both large and small stellate cells in both areas do not differ significantly from the Poisson distributions (Fig. 4-45). This therefore confirms that there is more than one population of stellate cells.

When the number of synapses per cell is plotted against mean cell diameter for all stellate cells (Figs. 4-51 and 4-52) it can similarly be seen in both areas that there is a cluster of cells in the region of small size and low number of synapses and then a number of cells spread across the region of moderate to large size and large number of synapses. This contrasts with the more uniform distribution of the pyramidal population and it seems unlikely that this sort of cluster and spread would be produced by a single population of cells. It would however be explained

by there being two distinct stellate cell populations.

It is evident therefore in normal material that two types of stellate cell may be recognised on the basis of clusters of features which tend to go together and examination of the distributions of various parameters in a randomly selected population of stellate cells also indicates that this population consists of more than one component. In experimental material, following thalamic lesions, only large-type stellate cells, as recognised on the above basis, have been found to receive degenerating axo-somatic terminals (Sloper, 1973b; Chapter 9), and the data in Fig. 4-54 relate to all those cells in the motor cortex which were found to receive synapses from degenerating axo-somatic thalamo-cortical terminals in that study. The whole depth of the cortex was scanned systematically and equally in the experimental material, as here, and so these cells represent a random sample of those cells in the motor cortex which receive synapses from axo-somatic thalamo-cortical terminals. Comparison of these data with that of Figs. 4-36 and 4-43 therefore gives a fair reflection of how the population of cells receiving synapses from degenerating thalamo-cortical terminals in experimental material compares with the large stellate population as defined here by features seen in normal material and it can be seen that the populations as defined in the two different ways correspond. Therefore under the right conditions it is possible to demonstrate a clear qualitative difference in afferent connections between

cells of the large and small stellate populations and they are thus two distinct cell types.

Using the appropriate method it is therefore possible to demonstrate clear qualitative differences between cells of the three different types in the cortex and the main features of each cell type are summarised in Table 4-22. However when classifying a cell in a single section of normal electron-microscopic material the information available about a particular cell is restricted by technical factors, for example an apical dendrite being missed by the plane of the section, and so a cell has to be classified by considering only those features which it does show in the particular section studied. Most cells may in fact be confidently classified in this way and there was real doubt in only about 10% of the cells in the two samples taken and in most of these cases the difficulty was in deciding whether a particular example was a small pyramidal or a small stellate cell. The same criteria for classification were used by the same observers in the two different areas of cortex studied and so the results should give a reasonably accurate comparison of the proportion of each cell type present in each cortical area.

Comparison of different cortical areas

It has generally been thought on the basis of light microscopy, using mainly the Nissl technique, that the motor cortex consisted predominantly of pyramidal cells whereas most of the cells in the somatic sensory cortex were stellate cells. The finding in this study that the motor cortex and area 3b of the

TABLE 4-22 Summary of the features of the different types of cell somata

	Apical Dendrites	Nucleus	Cytoplasm	Synapses	Size	Shape	Connections	Layers
Pyramids	Yes	Light, often indented in layers IV, V and VI	Amount varies with cell size. Larger cells contain stacks of endoplasmic reticulum	Only symmetrical. Few.	Motor 10-50μ Somatic 9-20+μ	Pyramidal		II-VI
Large Stellate	No	Moderately dark. Some dark in somatic sensory cortex. May be indented	Abundant high concentration of mitochondria. Frequent stacks of endoplasmic reticulum, especially in the motor cortex.	Many, both asymmetric and symmetrical	Motor 13-30μ Somatic 9-12μ	Rounded.	Receive thalamocorticals and other afferents. Gap junctions	Motor Lower III IV and upper V Somatic IV
Small Stellate	No	Often dark. Often indented.	Sparse. Pale background and few organelles especially in round type.	Few. Both asymmetric and symmetrical	Motor 9-12 μ Somatic 7-12 μ	Round or fusiform		II and some in all other laminae

somatic sensory cortex contain very similar proportions of pyramidal and stellate cells as judged by electron microscopic criteria was therefore unexpected. In view of the importance of this the cell classification has been scrutinised very closely. It can be seen from the data taken before classification however that both cortical areas contain only a small proportion of cells with the large number of synapses typical of previous descriptions of stellate cells, or of large stellate cells as described here, and both areas contain similar proportions of cells with dark nuclei, a feature predominantly of small stellate cells. Following classification the parameters of each of the three cell types in the two areas are also very similar, for example the numbers of dendrites per cell and the proportions of each type with asymmetrical synapses or dark nuclei, and most of the differences appear to be secondary to size. There can therefore be little doubt that the two samples of cells from the different cortical areas are very similar and it is most unlikely that the proportion of stellate cells in the sample from the somatic sensory cortex could have been grossly underestimated.

Because these samples of cells represent only one strip from each cortical area there must be some caution about regarding them as typical of each area. However the results from them are in general accord with the impression gained from comparing the two cortical areas qualitatively and it seems likely therefore that these samples are representative. The same three cell types

have now been recognised in area 17 of the visual cortex (Tömböl, 1974) and a quantitative survey was performed taking only those cells having nucleoli in the section studied. Although the other criteria used were the same, this difference is likely to give a slight bias towards smaller cells and so the data in this study were analysed with respect only to those cells having nucleoli to make the results comparable. Thirty-six per cent of the cells in the motor cortex and 41% of those in the somatic sensory cortex had nucleoli in the section which confirms the slight bias towards smaller cells. The proportions of the three cell types using this additional criterion are compared with the results for the visual cortex in Table 4-23 where it can be seen that they are very similar in all three areas of cortex. Since these different cortical areas represent what were thought to be the extreme architectonic types, it seems a strong possibility that the proportions of the cell types may be very similar in intermediate cortical areas such as the parietal lobe and it is possible that this is so throughout the cerebral neocortex.

The motor and somatic sensory cortices do however appear different when stained by the Nissl technique and there are undoubtedly many more cell somata in the motor cortex which appear pyramidal in shape. It seems likely that this is due to two factors: first there is a greater proportion of larger pyramids in the motor cortex (Fig. 4-35) and it has been noted that the diameter of the apical dendrite is larger in proportion

TABLE 4-23

The proportions of the different cell types in Motor, Somatic sensory and visual cortices for cells with nucleoli only

	Motor cortex	Somatic sensory (area 3b)	Visual cortex* (area 17)
Pyramidal	64%	60%	59%
Large stellate	10%	9%	7.4%
Small stellate	26%	31%	33.6%

* from Tömböl (1974)

to that of the cell soma in larger cells. This therefore means that larger pyramids have a more obviously pyramidally-shaped soma and this would be apparent in material stained by the Nissl method. The second factor is that the Nissl technique stains clumps of endoplasmic reticulum as Nissl substance and it is often these which outline the shape of the cell soma and apical dendrite. It has been found here that endoplasmic reticulum is more frequent in larger pyramids and since it often extends into the beginning of the apical dendrite it will make it more conspicious. It therefore seems that the differences between the motor and somatic sensory cortices when stained by the Nissl technique are due to differences in the sizes of cells rather than to differences in cell type.

The similarity of the cell populations in the different cortical areas is of considerable significance to both the understanding of the embryological development of the neocortex and of its functioning. The whole neocortex develops initially as one uniform sheet of cells. Since these different areas of the adult neocortex contain the same numbers of cells through the depth, now confirmed in other neocortical areas (Rockel, Hiorns and Powell, 1974), and the same proportions of the different cell types, now confirmed in the visual cortex (Tömböl, 1974), these results indicate that there is no subsequent divergent differentiation of the cell populations in the areas studied but that the basic uniformity of the neocortex is preserved. It would

appear that there may be a general principle that a cylinder of constant cross-section taken through the full depth of the cortex of any area will contain the same number of cells with the same proportions of each type and it may be that this is a reflection of a constant functional subunit within the cortex and that the basic mechanism of neuronal processing is the same throughout the neocortex.

The close similarity in the populations of neurons in the motor cortex and area 3b of the somatic sensory cortex means that the differencs which are apparent between these two areas must be due to other factors. The average volume of cell somata in the motor cortex is almost twice that of those in area 3b and it would be expected that the volumes of the dendritic ramifications of these cells will similarly be larger. This will to some extent account for the greater volume of neuropil in the motor cortex and so the greater spacing of cells and the greater thickness of this cortical area. There must presumably be a correspondingly greater number of axon terminals in the motor cortex since they do not obviously form a smaller proportion of the neuropil, and it has been shown that there are many more synapses per cell in the motor cortex of the monkey compared to its visual cortex (Cragg, 1967). Although there may be more extrinsic afferents to the motor cortex, there does not seem to be any striking difference in the connections studied (Sloper, 1973b; Chapter 9) and all the extrinsic afferents together form only a

small proportion of the terminals in the neocortex (Szentágothai, 1964; Gruner, Hirsch and Sotelo, 1974). It therefore seems that there must be more extensive ramifications of the axons and axon collaterals of cells within the motor cortex and that the main difference between motor cortex and the somatic sensory cortex is that the cells of the motor cortex are more extensively interconnected with each other by these.

The other factor affecting the appearance of the cortex in sections stained by the Nissl method is the relative development of the different cortical laminae and the relationships of the fibre bands to these. In as far as can be judged from this study, the fundamental laminar pattern is qualitatively the same in both cortical areas but the deeper cortical layers are considerably thicker and contain a greater proportion of the cells in the motor cortex, as has been described previously (Bonin and Bailey, 1947). That this is a real difference is confirmed by the higher relative position of the band of thalamo-cortical degeneration in the motor cortex (Chapter 9) and possibly by the difference in nuclear indentations in the two areas. Nuclear indentations occur in about half those pyramids in layers V and VI of both motor and somatic sensory cortices and the proportions falls off markedly in layer IV and above in both areas but, because of the much smaller thickness of layers V and VI in area 3b of the somatic sensory cortex, there are less than half the total number of pyramids with nuclear indentations in this area compared to the

motor cortex. It is suggested below that this difference may be due to the pyramids of layers V and VI having more extensive axonal arborizations and this would be consistent with the evidence, both anatomical (Fisken, Garey and Powell, 1975; Jacobsen and Trojanowski, 1974, 1975) and physiological (Naito, Nakamura, Kurosaki and Tamura, 1969) that most cortical efferents arise from these deep layers. The differences in lamination of the two cortical areas would then be due to the motor cortex giving rise to a larger efferent projection than area 3b of the somatic sensory cortex and this would not be unexpected in view of its large contribution to the pyramidal tract and its motor function.

Another difference in the laminar arrangement of cells in the two cortical areas, observed here but not apparent in Nissl-stained material, is that the band of large stellate cells is wider in the motor cortex than in area 3b of the somatic sensory cortex, extending about halfway into layers III and V whereas that in the somatic sensory cortex is restricted to layer IV, although occasional cells are found outside the bands in both areas. The dense band of thalamo-cortical degeneration is no wider in the motor cortex than in the somatic sensory cortex (Chapter 9) and this suggests that the large stellate cells are not primarily related to this main band of degeneration. The pyramids are however spread over a greater depth in the motor cortex and it may well be that the distribution of the large

stellate cells is related to this.

In summary therefore these results suggest that the differences between the motor and somatic sensory cortices are due to a more extensive development of connections within the motor cortex rather than to differences in the populations of neurons. The structural similarities in turn suggest that the basic neuronal mechanisms involved in such apparently dissimilar functions as the processing of sensory information and the control of movement may be very similar, and it seems that the motor cortex differs from the somatic sensory cortex in that its cells are more extensively interconnected and a greater proportion of them give rise to axons which leave the cortex.

Nuclear indentations and endoplasmic reticulum

The significance of nuclear indentations is unknown although it seems likely that they serve to increase the surface area of the nuclear membrane and this may be related to transport of substances between the nucleus and cytoplasm. There are obvious differences between different types of neuron, for example the bipolar neuron of the retina has a regular nuclear outline whereas that of the amacrine cell has many complex infoldings (Dowling and Boycott, 1966), but it is very difficult to assess the significance of such differences because of the number of variable factors involved. However in this study marked differences in the frequency of occurrence of nuclear indentations were observed between cells of one type found in different laminae.

Nuclear indentations were observed with much greater frequency in the pyramids of layers V and VI than in those of layers II and III in both cortical areas studied here and, although indentations are more frequent in larger cells, the differences between the laminae could not be explained by size differences since they occurred between cells of similar sizes. There was also a comparable difference in the frequency of clumps of endoplasmic reticulum in pyramids of the different laminae which could not be explained by size differences and so it is possible that this is in some way related. Although the pyramids of the supra- and infragranular layers appear remarkably similar, there is evidence from anatomical findings (Fisken, Garey and Powell, 1975) that the supragranular pyramids have a less extensive axonal arborisation than those in the deep layers and evidence from both anatomical and physiological work that the cortical efferents arise mainly in the deep layers of the cortex (Nakamura et al. 1969; Jacobsen and Trojanowski, 1974, 1975; Fisken, Garey and Powell, 1975). With a smaller volume of axoplasm in relation to that of the cell soma, the soma would presumably have to synthesise less protein to maintain it and this may be reflected in the paucity of endoplasmic reticulum and nuclear indentations in these cells since the former is known to be involved in protein synthesis, and protein synthesis involves transport of substances between the nucleus and cytoplasm (see Harris, 1974). Such a difference in rate of protein synthesis between supra- and

infragranular pyramids would presumably be reflected in the uptake of radioactively labelled amino acids and might well be demonstrable by autoradiography.

Correlation of electron microscopic results with other evidence

Different histological techniques reveal different aspects of the structure of the neocortex and the information obtained from these is in many ways complementary. In electron microscopic material the cell soma is usually seen in isolation but it may only account for 5% or less of the total volume of the cell and receive only a small and possibly selected proportion of the afferent synapses to that cell. Some estimate of the extent of the dendritic and axonal arborisations of cortical neurons may be arrived at from published results. The soma of a Betz cell has been estimated to receive on average a total of 870 synapses (Kaiserman-Abramof and Peters, 1972) whereas neurons in the motor cortex have been estimated to receive on average a total number of 60,000 synapses (Cragg, 1967). It is clear therefore that the great majority of the synapses received by a cortical cell are on its dendrites and the study of the soma alone can only give a very restricted idea of the afferent connections of a neuron. It is also possible from these figures to obtain an indirect estimate of the axon arborisation of a cortical cell. Most of the axon terminals in the neocortex have been shown to be of intrinsic origin because they persist after undercutting of

the cortex (Szentágothai, 1964; Colonnier, 1966; Jones and Powell, 1970e) and only a very small proportion of the terminals in the neocortex are found to degenerate after lesions affecting all the extrinsic afferents to the cortex (Gruner, Hirsch and Sotelo, 1974). Each synapse received by a cell in the cortex must arise from the terminal of another cell and so, if the small proportion of extrinsic afferents is neglected, all the synapses received by the whole population of cortical neurons arise from that same population of cells and so the average number of efferent synapses made by the axon of a single cortical neuron will be approximately equal to the average number received by a single neuron in the cortex. Although a single axon terminal may give rise to more than one synapse, it is unlikely that on average they give rise to more than four or five synapses and so on the basis of these published figures it would appear that an average neocortical neuron gives rise to more than 10,000 axon terminals. The technical problems of following a given cell soma to its axon terminals are therefore immense, particularly as the most distant terminals may, at the magnifications used, be as far as several miles from the cell soma and so in this discussion an attempt has been made to do this using evidence mainly from indirect methods, to try to give a composite picture of each cell type and to correlate this with physiological findings.

Pyramidal cells

The appearance of the cell soma and apical dendrites

of this cell type have been described on a number of occasions (e.g. Colonnier, 1968; Peters and Kaiserman-Abramof, 1970; Jones and Powell, 1970a; Garey, 1971) as have the features of the more proximal dendrites and the dendritic spines. The initial segment of the axon has also been described (Peters, Proskauer and Kaiserman-Abramof, 1968; Jones and Powell, 1969c; Sloper and Powell, 1973; Chapter 5) and in the latter study 12 out of 14 examples were shown to give rise to a myelinated axon at between 30 and 55 μ from the cell soma. In one example the myelinated axon was shown to give off two collaterals at the first node of Ranvier and in one example myelination did not occur until 120 μ from the cell soma and the unmyelinated axon between this and the initial segment gave off two collaterals. This example also gave the only direct evidence as to the nature of the terminals of pyramidal cells since the unmyelinated axon of this pyramid made an en passage synapse having round vesicles and an asymmetric membrane thickening on to a dendrite. The nature of pyramidal terminals is confirmed by indirect evidence obtained from experimental studies since the degenerating terminals in other sites following ablation of cortical areas will be the terminals of cortical efferents, most if not all of which arise from pyramids (Cajal, 1909-11; Nakamura et al. 1969; Jacobson and Trojanowski, 1974, 1975). These efferents from the motor cortex have been studied in the caudate nucleus (Kemp and Powell, 1971c), the ventrolateral nucleus of the thalamus (Harding and

Powell, 1977), and the contralateral motor cortex (Sloper and Powell, 1973b; Chapter 9), and in all these sites the degenerating terminals were found to have asymmetric membrane thickenings and these are known to occur in relation to terminals containing round vesicles in these sites. Terminals were both of the terminal bouton and en passage types. Similar results have also been obtained in the pontine nuclei (Hollander, Brodal and Walberg, 1969) and projections from both somatic sensory cortical areas (SI and SII) and the visual cortex to a number of sites have also been shown to end in terminals which have asymmetric membrane specialisations (Jones and Powell, 1969e, 1970d, e; Lund and Lund, 1970). Even if the pyramidal cell is not the only source of cortical efferents to these sites it must still give rise to terminals with asymmetric membrane thickenings and round vesicles because no other type of degenerating terminal has been found in these sites following cortical ablation.

The effects of stimulating cortical efferents have been studied in a number of sites including the contralateral motor cortex (Asanuma and Okada, 1962) and the spinal cord (Phillips, 1969) and the only monosynaptic effects observed in these sites were excitatory. Similarly the shortest latency and therefore probably monosynaptic effects from pyramidal collaterals stimulated by antidromic excitation of the pyramidal tract are excitatory (Phillips, 1959; Stefanis and Jasper, 1964b). It may therefore be deduced that pyramidal terminals are excitatory and the

correlation of this with their having asymmetric membrane specialisations and round vesicles is in accord with the observations of Uchizono (1965).

Large stellate cells

The somata and dendrites of these cells as seen with the electron microscope have been described in detail above. The cell soma is of medium to large size and usually round and the dendrites arise without an initial constriction, their outline is not markedly varicose and they tend to run in straight lines for some considerable distance. They also give rise to occasional spines, an uncommon feature for a non-pyramidal cell type, and they make gap junctions with dendrites or somata, usually of the same cell type (Sloper, 1972; Chapter 7). In the motor cortex large stellate cells are found mainly in a horizontal band extending from the middle of layer III, through layer IV to the middle of layer V. The initial segment of these cells is similar in structure to that of a pyramid but may be orientated in any direction and is often curved. In the two examples where it has been possible to trace the initial segment far enough, it has been shown to give rise to a myelinated axon of similar diameter to itself at 23 μ and 24 μ from the cell soma (Chapter 5), rather closer than with pyramidal cells.

Of the types of stellate cell described using the Golgi technique, the basket cell or Type I of Jones (1975a) has a number

of features which correspond to those of the large stellate cell. It has a rounded cell soma with a similar size range, and its dendrites arise without an initial constriction and have only a slightly varicose shape and may arise as a tuft; they also tend to run in straight lines and have occasional spines (Marin-Padilla, 1969, 1970). Basket cells are found from the middle of layer III down to the middle of layer V and this laminar distribution corresponds to that of large stellate cells. There is therefore a close correspondence between basket and large stellate cells in both morphology and distribution. If the large stellate cell is the basket cell this implies that it gives rise to terminals which form synapses on to pyramidal cell somata and which are therefore symmetrical in type. Of interest in this regard are incidental observations relating to 'dark' cells and dendrites (Chapter 8). These occur rarely in normal material and are almost exclusively of the large stellate type, easily identified because of the large number of asymmetric synapses they receive and the high concentration of organelles which is still visible in the dark cytoplasm. In normal material a 'dark' symmetrical axon terminal has on occasion been found in the same region as a 'dark' large stellate dendrite or soma. More significantly, in one otherwise rejected brain in which the blood supply to the motor cortex was probably impaired at operation, the motor cortex contained a considerable number of 'dark' symmetrical axon terminals making synapses on pyramidal somata, dendrites and spines as well as the

expected asymmetrical degeneration from a lesion of SI. Although the general quality of the material was good, this motor cortex also contained a considerable number of 'dark' dendrites and a few 'dark' somata, and both dendrites and somata could mostly be identified as being of the large stellate type. Symmetrical axon terminals were therefore undergoing darkening together with large stellate dendrites and somata and this suggests that these terminals arise from large stellate cells. Anatomical evidence from two different sources therefore suggests that large stellate cells give rise to symmetrical axon terminals making synapses on to pyramidal somata and dendrites.

Large stellate cells in the motor and somatic sensory cortices are mostly 13 μ or more in diameter and some have a diameter as large as 30 μ. They form between five and ten per cent of the cells in the cortex and occur mainly in layers IV and V. It seems likely from this that they form a proportion of the units recorded in microelectrode studies. 'Pyramidal tract interneurones', defined physiologically as neurons activated synaptically but not directly antidromically by pyramidal tract stimulation, have been described in the motor cortex of the cat (Stefanis, 1969) and rat (Stone, 1973) and a number of points suggest strongly that these are the physiological equivalent of large stellate cells; it has previously been suggested that they correspond to the stellate cells receiving synapses from thalamo-cortical afferents (Stone, 1973). 'Pyramidal tract interneurons'

are situated fairly deeply in the cortex, at a depth corresponding to the larger pyramidal tract cells, and may be found in close proximity to them, and both these observations are also true for large stellate cells. One of the distinctive properties of 'pyramidal tract interneurons' is that they are powerfully activated by specific thalamic afferents, giving a train of spikes lasting for up to one second; large stellate cells receive a considerable density of thalamo-cortical terminals, including many on the soma and proximal dendrites, up to three degenerating terminals having been found on a single soma and attached length of dendrite in a single section following a thalamic lesion, and large stellate cells are the only type of cell other than pyramids which have been shown to receive thalamo-cortical afferents by electron microscopy (Chapter 9). It is also interesting to note the high mitochondrial content of large stellate cells which suggests that they are very active metabolically and this might be expected from their powerful response to an afferent input. Finally 'pyramidal tract interneurons' are activated synaptically by antidromic stimulation of the pyramidal tract and a pyramidal axon within the cortex has been shown to make a synapse on to the dendrite of a large stellate cell (Chapter 5); many of the terminals making synapses on to large stellate cells are intrinsic in origin (Szentágothai, 1964) and in view of the widespread arborisation of pyramidal axon collaterals within the cortex (Cajal, 1909-11; O'Leary, 1941)

and the similarity of many of the terminals making synapses on to large stellate cells with those of cortical efferents, it seems likely that they receive a considerable projection from pyramidal collaterals. It is interesting to consider the possibility that the gap junctions which connect dendrites and somata of cells of this type may be a factor in prolonging the effects of afferent stimulation on individual large stellate cells. Also of interest in this respect is the difficulty experienced by Stone (1973) in blocking the pyramidally evoked synaptic activity of 'pyramidal tract interneurons' by direct iontophoretic application of glutamate antagonists. Although L-glutamic acid diethyl ester hydrochloride (GDEE) administered systemically,usually by intraperitoneal injection,effectively blocked the spikes induced synaptically in these cells by pyramidal tract stimulation, direct iontophoretic application of GDEE to them did not do so. This was in spite of the fact that these same cells were very powerfully activated by the iontophoretic application of glutamate. The explanation suggested for this anomaly was that the synapses involved were more than 100 μ from the cell soma and so beyond the range of the iontophoretically applied GDEE, although not beyond that of the glutamate. However if pyramidal tract interneurons are large stellate cells then they are connected to other large stellate cells by gap junctions and the direct electrical transmission by these gap junctions of activity from other large stellate cells also activated by the

pyramidal tract stimulation could explain the failure of the local pharmacological blockade. Intraperitoneal injection of the antagonist would presumably affect all the 'pyramidal tract interneurons' and so the blockade would then be effective.

'Pyramidal tract interneurons ' are thought to mediate the recurrent inhibition of pyramidal tract cells (Stefanis, 1969) following antidromic stimulation of the pyramidal tract and this would imply that large stellate cells are inhibitory. Further evidence as to the nature of large stellate cells may be obtained from other experimental results. Commissural terminals from the contralateral motor cortex have been shown to make synapses on to spines, most of which are probably pyramidal in origin, and on to the dendrites and somata of large stellate cells (Sloper and Powell, 1973b; Chapter 9). Stimulation of the contralateral motor cortex produces monosynaptic excitatory potentials and disynaptic inhibitory potentials in pyramidal tract cells as well as polysynaptic effects of both types; there is a predominance of excitatory effects in a small mirror focus opposite the stimulated area and this is surrounded by a ring of predominantly inhibitory effects (Asanuma and Okada, 1962). The monosynaptic effects must be mediated directly by the commissural afferents, or possibly by pyramidal collaterals stimulated antidromically, but, since all the monosynaptic effects are excitatory, the afferents themselves must be excitatory. The disynaptic effects must be mediated by one of the cell types

contacted directly by the afferents; since pyramidal terminals are excitatory the inhibitory effects on pyramidal tract cells are probably mediated by the large stellate cells as they are the only other known recipients of synapses from commissural terminals. There is a point to point connection of commissural axons (Jones and Powell, 1969b) and the monosynaptic excitatory effects in the central mirror image area must be mediated directly by the commissural afferents to both pyramidal and large stellate cells. Axons within the cortex giving rise to symmetrical terminals are on average longer than those giving rise to asymmetric terminals (Fisken, Garey and Powell, 1975) and basket cell axons in the Golgi technique may extend laterally for 4-5 mm (Marin-Padilla, 1969, 1970), so it is likely that the inhibitory surround is produced by the activation of the large stellate cells. Similar monosynaptic excitatory and disynaptic inhibitory effects are observed in pyramidal tract cells following thalamic stimulation (Branch and Martin, 1958; Amassian and Weiner, 1966) and the afferents similarly end on spines, probably of pyramidal cells, and large stellate somata and dendrites (Sloper, 1973b; Chapter 9).

In view of the similarities of structure between the neocortex and hippocampus, it is interesting to note that very similar conclusions have been reached regarding the basket cell in the hippocampus where it has been possible to make detailed correlations between the anatomical and physiological findings (Andersen, 1966; Andersen, Gross, Lømo and Sveen, 1969). The

neocortical basket cell as described here may be very similar in function to the cerebellar basket cell and it is interesting to note the remarkable similarities in structure between the two cell types. Both have abundant cytoplasm full of mitochondria and endoplasmic reticulum and appear very similar, both cell types in the mammal are interconnected by dendrodendritic and dendrosomatic gap junctions, a very unusual feature (Sotelo and Llinás, 1972) and both have terminals with flattened vesicles and symmetrical membrane specialisations. It seems likely by analogy that gap junctions will be found between the dendrites and somata of the hippocampal basket cells. Both cerebellar and neocortical basket cells are also interesting in that they appear to have inhibitory axon terminals but also have gap junctions which would be expected to mediate both excitatory and inhibitory afferent and efferent effects. The possible significance of the large stellate cell in the functioning of the neocortex, including the possible role of the gap junctions, is discussed in detail in a subsequent chapter (Chapter 10).

Small stellate cells

Unlike the large stellate cells, the small stellate cells do not give the appearance of forming a homogeneous population and in particular there is electron microscopic evidence of differences between those with round and fusiform somata. Impregnation with the Golgi technique shows clear differences in the dendritic ramifications of round and fusiform

small stellate cells and it would appear that most of the sub-divisions of non-pyramidal cells recognised by light microscopy (e.g. Cajal, 1909-11; Sholl, 1956; Jones, 1975a) fall within this electron microscopic group. Although it has not been possible to make detailed correlations, it seems likely that the round small stellate cells seen particularly in layer II correspond to the neurogliaform cells of Cajal (1909-11). A general description of the features of the somata and dendrites of small stellate cells has been given above and the Golgi technique confirms the more varicose shape of their dendrites and the tendency for them to arise with an initial constriction. The pre-synaptic dendrites giving rise to the dendro-dendritic synapses seen in the motor cortex have the cytological features of dendrites of small stellate cells and are found in the deep layers of the cortex (Sloper, 1971; Chapter 6). Although it has not been possible to obtain any direct evidence as to their origin, it seems most likely that they arise from an infrequent type of fusiform small stellate cell and the laminar distribution and a detailed comparison of the shape of dendrites in Golgi and electron microscopic material suggests that this could be the 'cellule a double bouquet protoplasmique' (Cajal, 1909-11; Colonnier, 1966; Jones, 1975a). Further elucidation of these problems however requires techniques by which the same cell may be studied by both light and electron microscopy.

CHAPTER 5

A STUDY OF THE AXON INITIAL SEGMENT AND PROXIMAL AXON OF NEURONS IN THE MOTOR AND SOMATIC SENSORY CORTICES

Introduction

The axon initial segment is thought to be the point at which all the influences acting upon a typical neuron are finally integrated and its output determined and so the structure and function of this region of the cell is of particular interest. The fine structure of the axon initial segment has been studied with the electron microscope in a number of sites (Conradi, 1966, 1969b; Palay, Sotelo, Peters and Orkand, 1968; Peters et al. 1968; Jones and Powell, 1969c; Westrum, 1970; Kemp and Powell, 1971a, b) and it has been shown that the initial segment may be recognised by its membrane undercoating and bundles of neurotubules. Initial segments have been found to receive synapses and to contain cisternal organs, consisting of an alternation of parallel membranous sacs and electron-dense plates, and spines have been described in the pyriform cortex (Westrum, 1970) and caudate nucleus (Kemp and Powell, 1971a, b). In this study a large number of initial segments belonging to the different types of neocortical neuron have been studied mainly in the motor cortex, and certain differences between those of the different cell types noted. The distribution of synapses along the length of the initial segment has been determined and a relationship demonstrated between synapses and the cisternal organs. Spines have been found on the initial segments of cells in the neocortex and in a number of examples the complete length of the initial segment has been studied which has enabled certain quantitative observations to

be made. Extensive use of serial sections has made it possible to obtain information about the axon beyond the initial segment and to trace the origin of the most proximal axon collaterals. The ultrastructure of the initial segment has also been studied in material stained in the block with Ethanolic Phosphotungstic Acid (E-PTA) and certain specific staining properties of the initial segment noted.

Results

Quantitative aspects

The results in this quantitative section are based on the initial segments of 14 pyramidal cells, including Betz cells, and 2 large stellate cells which were cut in continuity from the cell soma to the end of the initial segment in single or serial sections, examples of which are shown in Fig. 5-1 to 5-16 and Fig. 5-27. The initial segments of both the large stellate cells and 12 of the pyramids gave rise to myelinated axons immediately and the other 2 pyramids to unmyelinated axons by loss of the membrane undercoating and dispersion of the bundles of neurotubules; both of these unmyelinated axons were shown to become myelinated considerably further from the cell soma. For each of these cells the mean diameter of the cell soma was measured by the method described previously (Chapter 4); the length of the initial segment was measured from its origin at the soma to the point where it became myelinated or, in the two examples having

a length of unmyelinated axon, to the end of the membrane undercoating or last afferent synapse if this was further from the soma The diameter of the myelinated or unmyelinated axon was also measured, as was that of the initial segment at about 10 to 15 μ from the soma, beyond any initial tapering. The distance of each afferent synapse from the cell soma was measured and the presence of cisternal organs noted.

The relationship of the mean diameter of the cell soma to the diameter of its myelinated or unmyelinated axon for these cells is shown in Fig. 5-17. This shows that there is a clear relationship between these two parameters with the cells of larger mean diameter giving rise to larger diameter axons, at least for that part of the axon closest to the cell soma. However the same is not true when the length of the initial segment is plotted against the mean cell diameter (Fig. 5-18); all the initial segments of the 14 pyramidal cells are between 30 and 55 μ in length and, although no initial segments of larger cells were found at the lowest end of the length range, there is no tendency comparable to that for the axon diameter for the larger cells to have longer initial segments. The length of the initial segment would therefore appear to be largely independent of the diameter of the cell soma. Fig. 5-19 shows that there is similarly no clear relationship between the length of the initial segment and the diameter of the axon to which it gives rise and over a five-fold range of axon diameter the length of the initial segment of

some examples was almost identical.

In so far as can be judged from two examples, this independence of the length of the initial segment from the diameters of the cell soma and axon is also true for large stellate cells (Figs. 5-18 and 5-19). However the initial segments of both large stellate cells are considerably shorter (23 and 24 μ) than any of those of the pyramidal cells and are only just over half of the mean of the lengths of all the pyramidal initial segments (43 μ, Table 5-1), although they do not differ greatly in the diameters of soma or axon. There would therefore appear to be a difference in the length of initial segment between these two cell types which cannot be explained by difference in cell size. (See below for the relationship of mean cell diameter and initial segment diameter for the different cell types, Fig. 5-25).

The distribution of the distances from the cell soma of afferent synapses on to the 14 pyramidal initial segments is shown in Fig. 5-20. This shows the synapses to be evenly distributed for the first 35 μ from the cell soma and then to fall off progressively in density. This falling off, however, corresponds to the myelination of the shorter initial segments which starts at about 35 μ. If the length of each initial segment is divided into ten equal parts and the numbers of synapses in each tenth plotted, in order to compensate for this inequality of length, it can be seen (Fig. 5-21) that synapses occur with

TABLE 5-1 Complete Pyramidal Initial Segments

Cell No.	Layer	Mean cell diameter μ	Axon diam. μ	I.S. diam. μ	I.S. length μ	No. of synapses found	No. of cisternal organs	Synaptic density	Estimated total no. of synapses
Supragranular									
1	II/III	17	0.8	0.8	31	14	1	0.65	50
2	II/III	17	1.0	1.1	39	12	3	0.44	59
3	II/III	17	0.6*	0.9	52	9	2	0.25	36
4	II	23	1.2	1.7	54	19	0	0.50	145
5	II/III	17	1.1	1.2	42	14	2	0.48	76
6	III	14	0.9	1.1	40	15	1	0.54	74
11	III	15	0.6	0.8	31	12	1	0.55	43
14	II/III	17	0.6*	0.8	40	12	2	0.43	43
Infragranular									
7	V	20	1.0	1.0	36	5	1	0.20	22
8	V	32	2.5	3.3	50	7	5	0.20	104
9	V	37	2.5	2.7	47	6	0	0.18	73
10	IV	14	1.1	1.2	42	2	0	0.07	11
12	V	15	1.0	1.0	49	7	1	0.20	31
13	V	35	3.0	3.2	45	2	1	0.06	29

Mean Initial Segment Length
- Overall = 43 μ
- Supragranular = 41 μ
- Infragranular = 45 μ

Mean synaptic densities
Supragranular = 0.48 synapses/μ^2
Infragranular = 0.15 " "

* Gave rise to unmyelinated axons.

equal frequency along the entire length of the initial segment right to the end. Insufficient synapses were found on the two large stellate initial segments to give a comparable distribution but synapses occur right up to the point of myelination in this cell type as well.

Although the three largest of these fourteen pyramids occurred in layer V, there are no systematic differences between the pyramids of the supra and infragranular layers in the length of the initial segment (Figs. 5-18 and 5-19, Table 5-1) or the distribution of synapses along it (Fig. 5-21). There is however a clear difference in the number of synapses found on the initial segments of these two groups of pyramids (Tables 5-1 and 5-2); on average the total length of an initial segment of a supragranular pyramid receives 13.4 synapses in a single section, one every 3.1 μ, whereas that of an infragranular pyramid receives only 4.8, one every 9.3 μ, and this difference in the number of synapses found occurs irrespective of the diameter of the initial segments (Fig. 5-22). Using the formulae derived in Appendix 2, the density of synapses on the surface of each initial segment and the estimated total number of synapses received by each initial segment may be calculated (Table 5-1). On average the infragranular pyramids receive a smaller total number of synapses, with a mean of 45 compared to 65 for the supragranular pyramids. This difference is less than that found in single sections but when the total number of synapses is plotted against the diameter

TABLE 5-2 Comparison of frequencies of synapses and cisternal organs of initial segments of the different cell types

Cell type		No. of Cells	Total Initial Segment Length μ	Total Initial Segment Area μ^2	No. of Synapses	Average Synaptic Spacing μ	No. of Cisternal Organs	Average Cisternal Organ Spacing By Length μ	Average Cisternal Organ Spacing By Area μ^2
Pyramids	Overall	14	598	929	136	4.5	20	30	46
	Supragranular	8	329	358	107	3.1	12	27	30
	Infragranular	6	269	571	29	9.3	8	34	78
Large stellate		14	129	117	13	9.9	0	-	-
Small stellate		10	54	24	3	18.0	0	-	-

of the initial segment (Fig. 5-23) or cell soma (Fig. 5-24) this is seen to be due to the three large pyramids in the infragranular group. The measurement of the density of synapses on the surface membrane of the initial segment compensates for these size differences and overall the density of synapses on the initial segments of supragranular pyramids is more than three times that of synapses on those of infragranular pyramids.

Whereas synapses occur only on the surface of an initial segment and so the number found is proportional to its length (see Appendix 2) the cisternal organs may be found anywhere in the cytoplasm of the initial segment and the chance of finding one will be proportional to the area of initial segment cytoplasm found on a given section. The frequency of cisternal organs was therefore measured both in relation to length of initial segment and to the area on the section of the initial segment profiles, this being calculated as the product of length and diameter and so the total areas of supra and infragranular initial segments were obtained for these fourteen pyramids. The numbers of cisternal organ profiles seen in these initial segments are shown in Tables 5-1 and 5-2; in the initial segments of supragranular pyramids there were cisternal organs in 358 sq.μ, one every 30 sq.μ of initial segment profile, and in the initial segments of infragranular pyramids there were 8 in 571 sq.μ, an average of one every 78 sq.μ (Table 5-2). Cisternal organs were thus 2.6 times as frequent per unit area in the initial segments

of supragranular pyramids.

Qualitative features of initial segments and a comparison of the different cell types

The membrane undercoating and bundles of neurotubules in the axon initial segments of cells of the motor and somatic sensory cortices of the monkey have the same appearance as those described in sensory cortical areas of other species (Peters et al. 1968; Jones and Powell, 1969c; Westrum, 1970) and these features allow an initial segment to be identified when seen in isolation in the neuropil. Initial segments of pyramidal cells arise from the base of the perikaryon or from a basal dendrite and one cell was found where the initial segment arose from a dendrite 30 μ from the soma. Pyramidal initial segments are often very straight, although the first part may taper slightly, and they are always directed towards the white matter. They are accurately aligned with the 'grain' of the cortex so that continuous long lengths of initial segment are mostly found in sections which are cut accurately perpendicular to the cortical surface and contain very long lengths of apical dendrite. There is often, however, a kink in the initial segment, usually towards the lower end, but the part of the axon below the kink is generally in line with that above so that in a single section an otherwise continuous initial segment has a short break and serial sections are required to establish the continuity. A similar kink is often present in initial segments of pyramidal

cells which have been impregnated by the Golgi technique. The initial segments of Betz cells are similarly orientated to those of smaller pyramids but in the larger pyramids the first part of the initial segment often arches away from the soma before becoming straight.

The initial segments of large stellate cells may arise from the cell soma or from a dendrite; those of small stellate cells have only been found arising directly from the cell soma but it is possible that this is because of the relative infrequency with which dendrites of this cell type are cut in continuity with the cell soma (Chapter 4). The relationship of the mean diameter of the cell soma to the diameter of the initial segment is shown for large and small stellate cells in Fig. 5-25, where they are compared with the 14 complete pyramidal initial segments. Both dimensions are in general slightly smaller than for pyramidal cells, particularly in the case of small stellate cells, but the relationship between them is similar for all three cell types. The initial segments of both stellate cell types have a more pinched-off origin than those of pyramids. They do not have a single preferred orientation and consequently long lengths of them are cut in continuity with the cell soma less frequently than with pyramidal cells. The initial segments of large stellate cells in particular are also often curved, which reduces the chance of long lengths being found in a single section. Because of these factors, the isolated vertical lengths of initial segment often seen in the cortical neuropil must

arise mainly from pyramidal cells.

The membrane undercoating of the initial segment appears to be the same in the different types of neuron in the neocortex. In examples where an initial segment gives rise to a myelinated axon (e.g. Fig. 5-3) the undercoating stops at this point; when the initial segment gives rise to an unmyelinated axon the undercoating extends for a similar distance from the soma and then stops in a somewhat ragged fashion. In one example, described in full below, the undercoating stopped some distance proximal to the last afferent synapse on to the initial segment. In these examples where the initial segment became an unmyelinated axon the bundles of neurotubules became dispersed approximately at, or just proximal to, the end of the undercoating. Although the typical undercoating and bundles of neurotubules are present in the initial segments of Betz cells, they are much less obvious than in those of smaller pyramids because of the much greater overall size of the initial segment (Figs. 5-26 to 5-31). Complex vesicles are often seen in the cytoplasm of initial segments and often appear to be budding from the surface membrane. They may sometimes be seen to be taking in cytoplasm from adjacent structures, this presumably giving rise to the double-walled complex vesicle sometimes seen (Chapters 3 and 8).

The initial segments of pyramidal and both large and small stellate cells receive symmetrical synapses from axon terminals and these terminals may in addition make a synapse on to other components of the neuropil such as dendrites (Fig. 8-42). The synapses on

to pyramidal initial segments are evenly distributed along the whole length of the initial segment (Fig. 5-21) and those on to large stellate initial segments may occur right up to the beginning of the myelin sheath. Qualitatively large stellate initial segments appear to receive fewer synapses than pyramidal initial segments and those of small stellate cells fewer than either. This was studied quantitatively by measuring lengths of initial segment cut in continuity with the somata of a number of large and small stellate cells and by counting the number of synapses on to them seen in a single section (Table 5-2). This will give a sample in which that part of the initial segment nearest to the soma is most heavily represented. This was compared to the results for the complete pyramidal initial segments described above in which the synapses were shown to be evenly distributed along the length of the initial segment. This comparison showed that the synapses on all the pyramidal initial segments taken together occurred with an average spacing of 4.5 μ, those on large stellate initial segments at an average spacing of 9.9 μ and those on small stellate initial segments at an average spacing of 18 μ (Table 5-2). However when pyramidal cells are divided into supra and infragranular groups it can be seen that most of the difference in density is in fact between supragranular pyramids and large stellate cells. For at least their proximal part the initial segments of large stellate cells receive less than a third of the linear density of synapses of supragranular pyramidal cells and, since there was no concentration

of synapses in the distal part of the complete initial segments of the two large stellate cells, it is likely that this difference in synaptic density is present for the whole length of the initial segment. Although the synaptic density on the initial segments of infragranular pyramids and large stellate cells is similar, however, the two full length initial segments of large stellate cells are only just over half as long as those of the infragranular pyramids and so they will receive only about half the total number of synapses that initial segments of the same diameter arising from infragranular pyramids receive.

Cisternal organs have been noted in the initial segments of cells in a number of sites and their structure of alternating membranous sacs and dense plates described. They are commonly found in the initial segments of pyramidal cells, with an average spacing of one every 30 μ (46 sq.μ) in the 14 complete initial segments described (Table 5-2). None has been found, however, in the initial segments of large or small stellate cells although a total of 129 μ (117 sq.μ) of large stellate initial segment and 54 μ (24 sq.μ) of small stellate initial segment were closely examined in the study of synaptic density. These stellate cell initial segments do however sometimes contain single sacs which may serve a similar function; similar single sacs are also found in pyramidal cell initial segments.

The above measurements refer to cisternal organs as they are seen in single sections and in single sections they may

be very large and extend for a considerable distance along the initial segment (Fig. 5-32). Serial sections confirm that they may extend for a considerable length and show that their position in the initial segment may vary between the periphery and centre. They often become closely apposed to the plasma membrane and, although any component of the neocortical neuropil may be found adjacent to an initial segment, this close apposition is found almost exclusively opposite the non-synaptic region of a symmetrical axon terminal and this terminal may usually be shown to make a synapse with the initial segment elsewhere (Figs. 5-32 to 5-37). This specificity of apposition was confirmed quantitatively; of the large number of cisternal organs studied, 62 were found to come into close apposition with the plasma membrane of the initial segment in a single section. This apposition occurred opposite the non-synaptic part of a symmetrical axon terminal in 56 examples (90%), with 5 being opposite glia and one opposite an unmyelinated axon. Six of the 56 examples opposite symmetrical terminals also extended opposite glia and in serial sections examples found opposite glia were shown to be related to symmetrical terminals. The neuropil apposed to the initial segment was analysed in a single section of two of the complete supragranular and one of the infragranular pyramidal initial segments; the non-synaptic part of symmetrical axon terminals was found to occupy 31%, 28% and 23% of the outlines of these three initial segments with glia occupying 40%, 26% and 25%. Ninety per cent of the cisternal organs were therefore found

closely apposed to the plasma membrane opposite a profile that occupied only about 30% of the outline of the initial segment and it seems likely that those found opposite other structures in single sections are related to terminals in serial sections. The close apposition does not extend beneath the synaptic membrane complex itself, although the cisternal organ may, this being seen particularly well in transverse section (Figs. 5-33 to 5-36), but a single cisternal organ has been found to come into close apposition with the non-synaptic parts of as many as three symmetrical axon terminals. The cisternal organ may also be closely associated with a mitochondrion (Fig. 5-32). In a number of examples the membranes of both the initial segment and symmetrical terminal appear denser over the area of apposition of the cisternal organ and there is granular material in the extracellular cleft between them, but in the majority of examples the membranes are unspecialised. There is no aggregation of vesicles in the terminal opposite the area of apposition. This relationship of cisternal organs to symmetrical axon terminals may explain their scarcity in or absence from initial segments of large stellate cells as these receive fewer synapses than do those of pyramidal cells.

In a few examples an exposed postsynaptic membrane specialisation has been found apposed to an axon initial segment (Figs. 5-38 and 5-39) and in these examples a subsurface cistern has been found in the initial segment immediately apposed to the

exposed thickening.

Some initial segments in the neocortex are found to have spines (Figs. 5-40 to 5-46). When these initial segments have been cut in continuity with their cell somata they have been pyramidal cells in all cases and one example was found on the first part of the initial segment of a Betz cell (Figs. 5-28 and 5-29). The form of the spine varies considerably; most of those found are small sessile pegs but there is a complete range of forms from these pegs through to large pedunculated spines. The relative infrequency with which the spines with thinner stalks are found may be due to technical factors. Spines have been found to receive synapses from one or two axon terminals and, where identifiable, these have been of the symmetrical type. It is not unusual for a terminal to make a synapse both on to a spine and directly on to the shaft of the parent initial segment and examples occur where a terminal makes synapses on to two adjacent spines on the same initial segment (Fig. 5-46). Two or more spines are frequently found close together on an initial segment. The undercoating of the initial segment usually continues into the spine and sometimes appears to be giving rise to a complex vesicle within the spine. A cisternal organ may extend into a sessile spine (Figs. 5-42 and 5-44) and, particularly in the longer spines, it may closely resemble the spine apparatus of dendritic spines (Gray, 1959).

In addition to the specific features described above, initial segments contain mitochondria and ribosomes both free and

in clusters. These components are more prominent in the initial segments of large stellate cells than pyramids and this would seem to be a reflection of the similar difference in the cell somata.

The axon beyond the initial segment

Twelve of the 14 pyramids and both of the large stellate cells in which the whole length of the initial segment was obtained gave rise to typical myelinated axons of similar diameter to the parent initial segment. The first part of the myelinated axon contains a greater concentration of mitochondria and ribosomes than do most myelinated axons and may therefore be recognised when cut in isolation. In one pyramidal cell (Figs. 5-1 to 5-13) the myelinated axon was followed towards the white matter and the myelin sheath was found to finish at 120 μ from the soma, the length of the myelinated internode being approximately 80 μ and of the initial segment 42 μ; the mean diameter of the parent cell was 17 μ and the axon diameter was 1.3 μ. The axon gave off a collateral immediately beyond the end of the myelin sheath, continued unmyelinated and gave off a second collateral 5 μ more distally and then the main axon became myelinated beyond this point. The first collateral left the main axon at approximately a right angle and was unmyelinated for the 1.5 μ for which it could be followed and it had a diameter of 0.4 μ at that point. The branching point was slightly expended and the membrane had an undercoating as described in nodes of Ranvier in the central nervous system (Peters, 1966). This

undercoat however diminished in the unmyelinated length of axon between the two collaterals and in the collaterals themselves. A complex vesicle appeared to be budding from this undercoat at the origin of the first collateral, and there was a cluster of ribosomes in the axoplasm at the point of junction. The second collateral left the opposite side of the parent axon and was directed obliquely downwards. It became myelinated immediately, the myelin being contiguous, if not continuous, with that of the parent axon, and it had an external diameter of 0.55 μ. At this point of divergence the axoplasm contained a structure of alternating sacs and dense plates (Fig. 5-9) which is similar to a spine apparatus but there was no spine or synapse present on this part of the axon.

In two of the pyramidal cells, both of 17 μ mean diameter and from layer II of the motor cortex, the initial segment gave rise to an unmyelinated axon which became myelinated considerably further from the cell soma. In one of these two examples the cell gave rise to a typical descending initial segment which curved forward through a number of serial sections and then became parallel to the plane of the sections (Figs. 5-47 to 5-59). Five spines were found on this region of the initial segment and also a number of synapses directly on its shaft. The membrane undercoating ended rather patchily at about 43 μ from the cell soma and the bundles of neurotubules became dispersed at about this level (Figs. 5-51 to 5-53). Two afferent symmetrical synapses were found on the initial segment distal to this point, the last at 52 μ from the

cell soma. Immediately proximal to this last afferent synapse the initial segment/unmyelinated axon made an efferent synapse on to a dendrite (Figs. 5-51, 5-53, 5-55 and 5-56). This was within 0.5 μ of the last afferent synapse and proximal to it and about 3.5 μ from the next most proximal afferent symmetrical synapse and so there was a serial axo-axonic synaptic arrangement within a small region. The membrane specialisation of the efferent axo-dendritic synapse had the typical structure of a synaptic complex, including having presynaptic projections, and it was of the asymmetric type. The synaptic vesicles were round and were fairly clustered in the region of the membrane complex but were not as restricted as in a typical dendro-dendritic synapse in the motor cortex (Chapter 6). The dendrite postsynaptic to this efferent synapse was followed through 41 serial sections and in this length of approximately 2 to 3 μ it received 8 synapses, a number of which were asymmetric (Figs. 5-53 and 5-55 to 5-57). Its shape was slightly varicose and it contained prominent microtubules, a considerable number of ribosomes and a mitochondrion and so was typical of a fairly small dendrite of a stellate cell of the large type (Chapter 4). Beyond these afferent and efferent synapses the 'initial segment' continued as an unmyelinated axon with a diameter of approximately 0.6 μ. It contained prominent evenly spaced microtubules, a few ribosomes, a number of mitochondria, a few small elongated membrane-bound dense bodies, scattered vesicles and a multivesicular body. At 65 μ from the soma an exposed membrane

thickening on a spine was apposed to the axon and had induced a small sac in the axon (cf. Pinching and Powell, 1972). The axon had a bulbous expansion to 0.7 μ diameter at 75 μ from the soma and the serial sections showed this to be the origin of a collateral of 0.5 μ diameter. The junctional region contained ribosomes and scattered vesicles but generally appeared empty and the comparatively regular arrangement of the tubules of the axon became disorganised. The continuation of the main axon beyond this collateral contained fewer organelles than did the more proximal parts but still contained occasional ribosomes and dense-cored bodies and the neurotubules were still quite prominent. The axon diameter became as small as 0.3 μ at some points in this part of its course, this being confirmed as the full diameter by the serial sections. At 95 μ from the soma the axon expanded and gave rise to a second collateral. The axonal membrane had an undercoating in this region which was not present above and below and the tubules became disorientated as at the first collateral and could be seen to pass into both the main axon and the collateral, although individual tubules were not seen to branch. The main axon continued beyond the origin of the collateral for a further 8 μ and then acquired a typical myelin sheath and became a myelinated axon of 0.5 μ diameter at 103 μ below the parent soma (Fig. 5-59). This cell and axon are shown diagrammatically in Fig. 5-58.

The second of these two pyramidal cells also gave rise to a typical descending initial segment which received symmetrical synapses and had a spine. Its undercoating finished at approximately 40 μ from the soma, distal to the last afferent synapse; no efferent synapse was found. The axon continued unmyelinated for 50 μ and then became myelinated at 90 μ from the cell soma. The cytological features of the unmyelinated axon were very similar to the previous example and an exposed thickening on a dendrite had become apposed to this axon and had induced a sac in it as in the other example (Fig. 8-31). The axon gave off a collateral immediately proximal to the myelin sheath and there was also a bulbous expansion of the axon at 62 μ from the soma which was similar to the origin of a collateral but insufficient serial sections were available to establish this. This example thus confirmed closely the features described in the first example.

The staining of axon initial segments by Ethanolic Phosphotungstic acid

Examination of unstained sections from blocks stained with Ethanolic Phosphotungstic Acid (E-PTA) confirmed previous observations that this method stains specifically the membrane specialisations of the synaptic complex (Bloom and Aghajanian, 1968) and the dense plates of the spine apparatus (Adinolfi, 1971) (Fig. 5-70). In this material certain profiles were found which were outlined in black and stood out clearly from the rest of the neuropil. These were identified as axon initial segments by the

typical way they emerged from cell somata, particularly from the bases of pyramidal cells (Fig. 5-60), by their shape and predominantly vertical orientation, by the faintly stained bundles of neurotubules they contained and by the synapses they received (Fig. 5-61); one example was found which both received synapses and entered a myelin sheath (Fig. 5-64), a combination of features in the neocortex which only occurs on axon initial segments. The identification of these profiles as axon initial segments was also confirmed by examination of similar sections counterstained with uranyl acetate and lead citrate.

Detailed examination of the initial segments in material stained by PTA alone showed that their black outline was due to specific staining of a membrane undercoating by the PTA (Figs. 5-61 and 5-63). This undercoating is found immediately beneath the plasma membrane (electron lucent in PTA-stained material) and corresponds in position and fine structure to that seen in normally prepared material. At high magnifications the undercoat can be seen to have a dentate appearance (Figs. 5-63 and 5-65) and when seen face on it has a discontinuous granular structure (Fig. 5-64). There is also a less densely stained layer of granular material immediately outside the plasma membrane of the initial segment (Fig. 5-63). This has been described in normal sections (Peters et al. 1968) and is particularly well shown in this material where the membrane itself is unstained. The membrane undercoating in this material starts a short distance from the cell soma and continues

to the beginning of the myelin sheath; it continues into at least some spines (Fig. 5-68) but appears to stop immediately adjacent to the postsynaptic specialisations of synapses. As in normal material, complex vesicles can be seen both in axon initial segments and apparently budding from their surfaces (Fig. 5-69).

In sections cut both perpendicular and parallel to the pial surface from material block-stained by E-PTA the initial segments were frequently found to contain a single or several parallel plates of electron-dense material which resemble the appearance of the spine apparatus in this material (Figs. 5-61, 5-65, 5-67 and 5-68). Comparison with normal material (Figs. 5-32 to 5-37) shows that these correspond in size and position to the dense plates of the cisternal organ. On occasion they appear to be related to synaptic complexes but, because the membranous components do not stain, the exact relationship cannot be determined in this material.

The myelin sheaths of axons are not stained by E-PTA but show up as white against the general granularity of the background. This enables nodes of Ranvier to be identified and these show a similar undercoating to that of the initial segment as has previously been described in conventionally stained axons (Peters, 1966) and they also have a granular layer outside the plasma membrane in this material like that of the initial segment. Patches of what appear to be a membrane undercoating similar to that at the nodes of Ranvier may also be seen below the plasma membrane of axons within their myelin sheath but the significance of this is unclear.

The effect of pre-osmification on this staining by E-PTA was studied in blocks prepared by Method 2. This gave much better tissue preservation than PTA alone and in particular it showed up the plasma membranes. However, although the synaptic complexes were still specifically stained by the E-PTA to at least some extent, the specific staining of the membrane undercoating and the dense plates of the cisternal organ was inhibited by this treatment (Fig. 5-66) in regions of the blocks which were comparable to those well stained by E-PTA alone. It would therefore seem that pre-treatment with osmium tetroxide inhibits the binding of PTA to these structures, possibly by competing with it for the same binding sites.

As a control, to ensure that the electron density of the membrane undercoating and dense plates of the cisternal organ in E-PTA-stained material was due to the E-PTA treatment, unstained sections from unosmicated, unstained blocks (Method 3) were examined. Neither of these two structures could be found and so this confirmed that the specific staining was due to the PTA and that the structures were not naturally electron-dense.

Discussion

Axon initial segments have now been studied in a number of sites throughout the central nervous system (Conradi, 1966, 1969b; Palay et al. 1968; Peters et al. 1968; Jones and Powell, 1969b; Westrum, 1970; Kemp and Powell, 1971a, b) and their basic features, in particular the membrane undercoating and the bundles of neuro-tubules, are now well established. In the neocortex Peters et al.

(1968) have studied the initial segments of pyramidal cells in layers II and III of rat parietal cortex, and the initial segments of neurons in the somatic sensory cortex of the cat have been described by Jones and Powell (1969c). This present study in the motor cortex and area 3b of the somatic sensory cortex of the primate has confirmed these descriptions of axon initial segments in the neocortex and a number of new features have been described. Technical factors have been of considerable importance in this, in particular the accurate orientation of sections perpendicular to the pial surface of the cortex and the extensive use of serial sections; quantitative analysis has also provided new information and these results are dealt with first because they form a basis for the interpretation of a number of the qualitative findings.

Isolated lengths of axon initial segments have previously been reported to give rise to myelinated axons in several sites (Westrum, 1966, 1970; Peters et al. 1968; Palay et al. 1968) and in a few cases initial segments have been traced in continuity from the cell soma to the beginning of the myelin sheath (Palay et al. 1968; Conradi, 1969b), but little quantitative data is available on the dimensions of axon initial segments or about the synapses on them. The fourteen pyramidal cells with complete initial segments described here provide such data and in particular they form an interesting group because the diameters of the cell somata vary over a considerable range in these cells of the same type; this allows the relationships between the various dimensions

of the soma and initial segment to be studied without complications due to differences between cell types.

It has generally been accepted that larger cells give rise to initial segments and axons of larger diameter and this is borne out by measurements made on these pyramids and also by those on stellate cells (Figs. 5-17 and 5-25). However the length of the initial segment of pyramidal cells is not proportional to the diameter of the soma, as might be expected, but varies between 30 μ and 55 μ apparently at random and independently of the diameters of both the cell soma and axon (Figs. 5-18 and 5-19). It is interesting to compare these measurements with those made on spinal motoneurons by Conradi (1969b); the distance from cell soma to the myelin sheath (his AH + IS in Table 1) is plotted against initial segment diameter in Fig. 5-71 and compared to the measurements made here of initial segment length and diameter. (The initial segment diameter of the pyramids is used instead of the axon diameter used previously so that the results are directly comparable (Table 5-1); this initial segment diameter was measured at about 10 μ to 15 μ from the soma and distal to any marked tapering of the initial segment and is generally slightly larger than the axon diameter of the same cell). It can be seen from Fig. 5-71 that, although the initial segments of the spinal motor neurons have diameters considerably greater than those of the largest pyramids and there is a five-fold range of initial segment diameter overall, the lengths of all the initial segments fall within this same range. It is also interesting

that, in the two pyramids which had lengths of unmyelinated axon between the initial segment and the myelinated axon, the length over which the undercoat and bundles of neurotubules were present was similar to that in the cells which gave rise to a myelinated axon directly. This suggests that this length, and so by present criteria the length of the initial segment, is determined by factors other than the proximity of the myelin sheath to the soma. What these factors may be is unclear but it is likely that the dimensions of the initial segment will have a considerable effect on its electrical properties. If, as has been thought, the initial segment is the site of initiation of the action potential then the relative constancy of its length, in contrast to the variation of the diameters of the soma and axon, may have important implications for the understanding of the electrical properties of the neuron and of spike initiation. In this respect it is again interesting that it is the length over which the undercoating and bundles of neurotubules extend which is constant rather than the distance from the soma to the beginning of the myelin sheath.

The synapses on the initial segments also only extend for this same distance from the cell soma even if myelination does not occur immediately and, because of the key position which the initial segment occupies, these synapses are likely to exert a powerful influence on the cell and so their properties are of considerable interest. The synapses are evenly distributed along the whole length of the initial segment of both supra and infragranular

pyramids and this would seem to indicate that they must have some influence even when immediately adjacent to the myelin sheath, a factor which must be taken into account in relation to the mechanism and site of generation of the action potential. To determine this distribution only requires a sampling technique which is uniform along the length of the initial segment, a condition satisfied by the single longitudinal section used. The mathematics of this sampling technique is considered in Appendix 2 where it is shown that, by making slight approximations, this technique of counting the number of synapses per unit length in a single longitudinal section in fact gives a result which is proportional to the density of synapses on the surface of the initial segment and which is independent of its diameter provided that this exceeds a certain minimum value (0.22 μ with a synaptic diameter of 0.35 μ, considerably smaller than any initial segment found in this study). From this data the total number of synapses received by each initial segment was then calculated using the formulae derived in Appendix 2. Until much more is known about the electrical properties of the initial segment it is not possible to determine whether it is the density of synapses on its surface or the total number of synapses received, regardless of initial segment size, which determines their effectiveness although it seems likely that the former is the more important. Comparison of the results from supra and infragranular pyramids shows a clear difference in the density of synapses on their initial segments and in the total numbers of

synapses on initial segments of cells of comparable size. This difference appears to be restricted to the initial segment since the somata of pyramids of comparable sizes from the supra and infragranular layers receive similar numbers of synapses in a section (Chapter 4). Although the exact significance of this difference is not clear, it may be an indication of some difference in function between the supra and infragranular layers.

The possibility of an association of cisternal organs with synapses has been discussed by a number of previous authors without any definite conclusions being reached. Although Westrum (1970) and Kemp and Powell (1971b) thought that there was some association, Peters et al. (1968) did not. The fact that cisternal organ profiles occurred with a higher density in the initial segments of supragranular pyramids, which receive more synapses, is indirect evidence of an association but close study shows that the association is not in fact with the synapse itself but with the symmetrical axon terminal at a site away from the membrane complex. This close apposition of cisternal organs to the non-synaptic region of symmetrical axon terminals occurs much too frequently to be a chance finding and has in fact been illustrated previously (Westrum, 1970, Figs. 3 and 9; Peters et al. 1968, Fig. 9) and in the rare examples where the apposition occurs opposite another profile in a single section it seems likely that this is due to extension of a cistern from a region of apposition to a symmetrical terminal. The specificity of the relationship of cisternal organs to symmetrical

terminals is also shown by the fact that a proportion of examples shows a degree of membrane specialisation in this region of apposition which has not been seen elsewhere. The relationship of the cisternal organ to symmetrical synapses is very similar to that of somatic subsurface cisternae to them and is also analogous to the relationship of the spine apparatus to asymmetric synapses. The structure of all three organelles is similar, the dense material of all three stains with phosphotungstic acid and all three are related to the reticulum of the cell (Chapter 3; Peters and Kaiserman-Abramof, 1970) and so it seems likely that they may all serve a similar function. The relationship of the cisternal organ to the non-synaptic region of the terminal suggests that this function is related to some interaction between the terminal and cell and that it is not directly related to synaptic function; this is discussed in detail elsewhere (Chapter 3).

Although spines have not been previously described on axon initial segments in the neocortex, they have been found on the initial segments of neurons in the prepyriform cortex (Westrum, 1970) and the caudate nucleus (Kemp and Powell, 1971a, b). Jones and Powell (1969c) did however describe "small side branches" which did not receive synapses and which they identified as the possible origin of axon collaterals on neocortical initial segments. The spines described here closely resemble those described in the pyriform cortex and caudate nucleus in their ultrastructure, synaptology and in the variety of their forms, illustrated

diagrammatically in Fig. 5-72, but although the sessile type of spine has been found most commonly this may be due to the reduced chance of sectioning a spine with a thin pedicle in continuity with its parent initial segment. Although a large number of spines has been studied here, often with serial sections, there has been no suggestion that any of these protruberances was anything more than a spine. Their structure is very different from that of the collaterals found arising from the axon more distally and, although the spines may contain one or more vesicles, there has been no suggestion of an efferent synapse from one. It is therefore very likely that the 'small side branches' of Jones and Powell represent the pedicles of axonic spines and not the origin of the collaterals. It is interesting to note that, where it was possible to identify the cell of origin, the axonic spines found here were all on the initial segments of pyramidal cells, the dendrites of which give rise to many spines. These pyramidal initial segments also receive an unusually large number of synapses compared to initial segments of other types of neuron (Palay et al. 1968) and both these factors may be related to the presence of spines on their initial segment. However, although no spines have been found on the initial segments of non-pyramidal cells, the relative infrequency with which these initial segments are seen makes it impossible to be sure that they do not occur.

There has been considerable speculation as to the function of dendritic spines (e.g. Diamond, Gray and Yasargil, 1970)

and some features of axonic spines noted here may be of relevance. Although dendritic spines always receive at least one synapse from an asymmetric axon terminal (see Chapter 3) the axon initial segment receives only symmetrical synapses and this is equally true for spines on the initial segment, even when these are of the type with a narrow pedicle and expanded head. Such a spine may receive two symmetrical synapses and no asymmetric ones, this having been demonstrated in serial sections. Serial sections have also demonstrated that it is relatively frequent for a single terminal to make a synapse both on to an axonic spine and the shaft of an axon initial segment, an arrangement seen with dendritic spines (Harding and Powell, 1977; Chapter 3). Although there may be differences in the functioning of spines in these different locations, these factors should be taken into account in the consideration of their physiology.

The membrane undercoating of the initial segment is structurally similar to that found at nodes of Ranvier (Peters, 1966), at the base of neuronal cilia and around complex vesicles and in all these sites it is specifically stained by E-PTA (Sloper and Powell, 1973; Chapter 3). It has been suggested that the function of the undercoating found around complex vesicles is to cause the bending movements of the membrane necessary for their formation (Kanaseki and Kadota, 1969) and it is certainly present at sites where vesicles are being formed (Chapter 8). The initial segment undercoat frequently appears to be giving rise to complex

vesicles and there can be little doubt that they are being formed, rather than discharged, in examples where a small portion of the cytoplasm of an adjacent structure is being taken in. The initial segment undercoat forms a complete sheet, however, whereas usually only isolated patches are seen giving rise to complex vesicles elsewhere. Although this may be no more than a reflection of the large numbers of vesicles which appear to arise from this region, it is interesting to consider the possibility that, as well as being involved in the membrane movements of vesicle formation, the undercoat may be concerned in larger scale movements of the membrane. Apparent constrictions of the initial segment are not uncommonly seen in fixed material and it is possible that these are a sign of peristaltic movements of the initial segment membrane and that these drive the axoplasmic flow which is known to occur; the undercoating at nodes of Ranvier could serve to boost this flow at points more distant from the cell soma. Solution of this problem however requires direct visualisation of the axon initial segment in vivo.

Technical factors make it very difficult to follow the axon of a cell beyond the initial segment and to do this requires fortunate orientation of the section in relation to the axon and the extensive use of serial sections. Consequently this was only achieved in a few examples. The finding of an efferent synapse from the distal end of an initial segment was surprising and there appears to be no previous report of this (except cf. Pinching and Brooke, 1973). This efferent synapse was proximal to the most

distal afferent synapse on to the initial segment, although distal to the end of the membrane undercoating and bundles of neurotubules, and so it is a matter of semantics as to whether it should be considered as arising from the end of the initial segment or the beginning of the unmyelinated axon, but in either case a serial axo-axonic synaptic arrangement was present. In contrast to the serial dendro-dendritic synapses found in the neocortex (Sloper, 1971; Chapter 6) this efferent synapse was in the superficial layers of the cortex and had round vesicles and an asymmetric membrane specialisation, whereas dendro-dendritic synapses are found in the deep cortical layers and have flattened vesicles and a symmetrical membrane thickening; the presynaptic processes making dendro-dendritic synapses have also been followed for considerable distances in serial sections but have never shown any features suggestive of an initial segment. There is however a previous report which describes a few examples of serial axo-axonic synapses in the motor cortex of the rat (Artyukhina, 1966). These were found in the superficial cortical laminae and the efferent synapses were of type I of Gray (1959), with the afferent synapses being Type II (equivalent to asymmetrical and symmetrical synapses respectively in aldehyde-fixed material). Only short lengths were found of the process that was both pre- and postsynaptic and different methods of fixation and preparation were used from those employed here, but the appearance of this process is suggestive of an initial segment. It seems likely therefore that these

descriptions were in fact of a similar arrangement to that described here but seen in isolation. Care must be exercised in interpreting the significance of this single axo-axonic synaptic arrangement described here since it could be atypical in some way. If this previous report is of the same arrangement, however, this makes it less likely to be so and since this region of the axon is so rarely cut in continuity with the cell soma there is no reason to suppose that it is a particularly unusual finding. Even if the position of the efferent synapse is unusual, its structure is entirely typical of a synapse and it is unlikely that it differs in type from the other efferent synapses made by the same cell. It therefore provides the only direct evidence as to the nature of the axon terminals of pyramidal cells and this agrees with evidence obtained indirectly (see Chapter 4) and also provides direct evidence that pyramidal cells make synapses upon the dendrites of large stellate cells within the same cortical area.

These examples where long lengths of axon were followed from the cell soma also showed a number of other interesting features. The two examples where the axon did not become myelinated immediately distal to the initial segment both originated from cells in layer II and were of small diameter and it would appear that the absence of a myelin sheath on the first portion of the axon may be related to the superficial position of these cells in the cortex; most of the other examples of complete initial segments were somewhat deeper in the cortex but all except one had axons of larger diameter. The

presence of collaterals arising from the axons of pyramidal cells is of course well known from Golgi studies (e.g. Cajal, 1909-11) but this technique does not show their relationship to the myelin sheath or initial segment. Although collaterals do not appear to arise from the initial segment itself, they were found proximal to the beginning of the myelin sheath in both examples where myelination did not occur immediately and two were found arising from the first node of Ranvier of the myelinated axon followed. These findings confirm those obtained with the Golgi technique and emphasise the extensive nature of the axonal arborisation of pyramidal cells within the cortex.

Although it is the initial segments of pyramidal cells which have been studied most extensively both here and by previous authors, the initial segments of other cortical neurons do not differ fundamentally from them. Apart from the differences in synaptic density and cisternal organs which are discussed above, the other differences would appear to be secondary to differences in size or general features between the cell types. The undercoating and bundles of neurotubules in Betz cell initial segments only appear less prominent because of the large diameter of the initial segment and the greater density of cytoplasmic organelles in large stellate initial segments compared to those of pyramidal cells is similar to the differences in the cytoplasmic contents of these two cell types. This therefore confirms that the axon initial segment has a basically similar structure in neurons of different types but this basic pattern

is modified to some extent by the specific features and connections of the parent neuron.

CHAPTER 6

DENDRO-DENDRITIC AND RECIPROCAL SYNAPSES IN THE MOTOR CORTEX

Introduction

Dendro-dendritic synapses in the mammalian central nervous system were first described in the olfactory bulb of the rat (Rall, Shepherd, Reese and Brightman, 1966) and in the primate retina (Dowling and Boycott, 1966). In the olfactory bulb they form a prominent feature of the synaptic organisation and characteristic reciprocal synapses with an asymmetric and a symmetrical component are frequently found. In the retina the dendro-dendritic synapses arise from the processes of amacrine cells, known from light microscopy to have no axons. Although it was initially thought that dendro-dendritic synapses were confined to these neurologically primitive sites, they have now also been described in the superior colliculus (Lund, 1969), various thalamic nuclei (e.g. Ralston and Herman, 1969; Famiglietti, 1970; Wong, 1970; Harding, 1971; Ralston, 1971; Lieberman and Webster, 1972), the hypothalamus (Güldner and Wolff, 1974) and in the motor cortex (Sloper, 1971); this chapter describes in detail and extends the findings relating to dendro-dendritic synapses in the motor cortex of the primate.

Results

Extensive study of the motor cortex with the electron microscope has shown that it contains an infrequent type of process which makes synapses on to other neuronal profiles, but which otherwise has the typical morphology of a dendrite. The synapses made by these processes have therefore been identified as dendro-dendritic synapses and the processes which make them

will be described as presynaptic dendrites (PSDs). The results in this chapter relate to 27 examples of single dendro-dendritic synapses and two reciprocal dendro-dendritic synaptic arrangements.

Dendro-dendritic synapses in the motor cortex have the typical structure of a synapse, consisting of pre- and postsynaptic membrane specialisations together with synaptic vesicles in the presynaptic process. The presynaptic processes themselves however receive a considerable density of synapses of both the asymmetrical and symmetrical types and in this study no synapse has been identified as a dendro-dendritic synapse unless the presynaptic process was itself shown to receive a synapse in the same or a serial section. In longitudinal sections of the presynaptic dendrite and in the number of examples where a considerable length of PSD has been followed in serial sections these afferent synapses greatly outnumber the dendro-dendritic synapses (Figs. 6-1 to 6-5); in an example in which the presynaptic dendrite was reconstructed from serial sections it received fifteen synapses over the length in which it made two dendro-dendritic synapses and no other PSD has been observed to make more than one dendro-dendritic synapse although considerable lengths have been studied. Afferent synapses usually occur close to the dendro-dendritic synapse but do not seem to be particularly concentrated in that region. In some examples both asymmetrical and symmetrical axon terminals have been observed to make synapses on to both the PSD and the process on to which the PSD itself makes a synapse, thus forming

a triplet arrangement although this does not appear to be a common feature.

As well as receiving numerous synapses the PSDs have the typical ultrastructural features of dendrites (e.g. Fig. 6-6). They often have a diameter in excess of 2 μ, this being maintained over long lengths in some examples, and they frequently have an irregular outline. They contain numerous ribosomes both singly and in clusters and also small sacs of smooth endoplasmic reticulum and they contain abundant mitochondrial profiles (e.g. Figs. 6-4 and 6-8), more than in other types of dendrite in the neocortex. Multivesicular bodies have also been observed in them as have complex vesicles, these latter having been seen apparently budding from the membrane of the PSD close to the dendro-dendritic synapse (Fig. 6-40). The background cytoplasm of the PSDs is clear, particularly in their varicose expansions, and microtubules are often seen funnelling into the constrictions (Fig. 6-9). When present in the wider parts of PSDs microtubules often appear rather disorganised. The varicose shape of some of these dendrites has been confirmed by serial sections; the constrictions are as small as 0.2 μ and are very much narrower than the varicose expansions (Fig. 6-9) which appear rather bulbous in shape. Study of long lengths of these PSDs in serial sections has shown that they consist of long straight wide segments together with very varicose portions which tend to follow a tortuous course and these different configurations may occur together in a single dendrite. Adjacent

to a dendro-dendritic synapse long lengths of PSD occur which do not make such synapses although studied fully in serial sections, and these lengths may include several varicosities. No PSD has ever been seen to become myelinated or has shown any feature suggestive of an initial segment and no example has contained widespread vesicles or vesicles at any distance from a synapse. In contrast, axon terminals in the motor cortex usually have vesicles scattered throughout their cytoplasm as well as in the synaptic region (Fig. 6-11).

Many of these dendro-dendritic synapses were found during systematic study of the full depth of the motor cortex and all were found in the deep layers of the cortex, being distributed from the bottom of layer III through layers IV and V to layer VI. Although usually found alone, in several instances two or three dendro-dendritic synapses were found in close proximity to each other in single or serial sections but arising from apparently independent dendrites. PSDs were found in all orientations relative to the cortical surface although on occasion they appeared to be aligned with the vertical grain of the cortex. The relative orientation of the pre and postsynaptic dendrites also varied, the two dendrites being parallel to each other in some examples but converging at any angle up to a right angle in others.

The ultrastructural features of the membrane specialisations of these dendro-dendritic synapses are typical of those of a synapse of the symmetrical type and presynaptic projections were present

in some examples (Figs. 6-12 to 6-22). There is the usual cleft between the membranes of the pre and postsynaptic dendrites and this contains electron-dense extracellular material. With occasional exceptions the vesicles form a compact group close to the synaptic complex, rarely extending laterally further than the width of the membrane specialisations or far into the dendritic cytoplasm, so that the dendro-dendritic synapse only occupies a small part of the PSD and this was shown by serial sections to be consistent throughout the full extent of the synapse (Figs. 6-14 to 6-22). There were often remarkably few vesicles at a synapse, only 6 to 8 in a single section although occasional examples had rather more, and these small clusters of vesicles have an electron-dense fuzz between them in addition to the presynaptic projections seen in some samples. The majority of the vesicles at these dendro-dendritic synapses appear round, with a small proportion being clearly flattened (e.g. Figs. 6-18 and 6-19), and they appear somewhat larger than the flattened vesicles in symmetrical axon terminals in the same material. Tilt analysis of the flattened vesicles at dendro-dendritic synapses showed them to be discoid in shape but the majority of vesicles at these synapses were found to be spherical or nearly so by this technique (Figs. 6-23 to 6-32). A number of synapses had one or two dense-cored vesicles of the 80 - 120 nm variety in the vesicle cluster (Figs. 6-10 and 6-23) but serial sections showed this not to be a feature of every dendro-dendritic synapse. In some examples a small flattened membranous sac was

also present near the vesicle cluster.

Dendro-dendritic synapses often occurred apparently as a simple synapse between a dendrite and another dendrite or a spine, but in a number of examples they occurred in more complex arrangements (Fig. 6-33). In some examples a single axon terminal made synapses on to both the pre and postsynaptic processes forming a triplet arrangement; this was seen with an asymmetric terminal alone and with an asymmetric and a symmetrical terminal together both making synapses on to the same two dendrites (Fig. 6-33, D and E). In the five examples where dendro-dendritic synapses occurred on to spines, the spines were all found to receive in addition a synapse from an asymmetric axon terminal and some received one from a symmetrical axon terminal as well (Fig. 6-6), but no spines were found which only received a dendro-dendritic synapse and a synapse from a symmetrical axon terminal. In several examples a dendro-dendritic synapse was found to be associated with a gap junction occurring between the same two dendrites, it being immediately adjacent or a small distance away (Figs. 6-1 to 6-3, and 6-23) and in some examples a desmosome was present as well (Figs. 6-1 to 6-3). Three examples were found of very complex synaptic arrangements involving dendro-dendritic synapses. For one of them a reconstruction was made which is shown in Fig. 6-34 and diagrammatically in Fig. 6-33E. A single presynaptic dendrite D1 made two dendro-dendritic synapses on to spines (Sp1 and Sp3), the latter of which is shown in Figs. 6-14

to 6-22. The presynaptic dendrite itself received 15 axo-dendritic synapses in the length studied in serial sections, these being both of the asymmetric and symmetrical type, and one terminal of each type made synapses on to both pre and postsynaptic dendrites (Figs. 6-33E and 6-34), T6 making asymmetric synapses and T5 making symmetrical synapses. The other two examples of complex synaptic arrangements involved both reciprocal dendro-dendritic synapses and gap junctions and one also contained a serial dendro-dendro-dendritic synaptic arrangement (Figs. 6-33G and H and 6-35 to 6-43). The dendro-dendritic synapses of these reciprocal synaptic arrangements had the same ultrastructural features as the dendro-dendritic synapses found singly; both membrane specialisations were of the symmetrical type and the vesicles at both the components of the reciprocal dendro-dendritic synapses were the same as those at single dendro-dendritic synapses.

Most of the dendro-dendritic synapses found made synapses on to other dendrites while a few made synapses on to spines. The arrangements of other terminals making synapses on to these spines are described above. The form of these spines was typical of spines in the cortex (Jones and Powell, 1969d; Peters and Kaiserman-Abramof, 1970) and several contained a spine apparatus; one spine postsynaptic to a dendrite was very large and contained mitochondria in addition to a spine apparatus. In three examples the spine postsynaptic to a dendrite was followed to its parent dendrite (Figs. 6-6 and 6-7) which in all three cases was of medium to fairly

large diameter. These dendrites received only sparse synapses and those that could be clearly identified were all of the symmetrical type. One of these dendrites was found to give rise to a second spine close to the one receiving the dendro-dendritic synapse and in this example the dendritic shaft at the origin of these spines was somewhat expanded and contained many mitochondrial profiles. In one other example the dendro-dendritic synapse was presynaptic to the shaft of a medium-sized dendrite near the bases of two typical spines receiving asymmetrical axo-spinous synapses; this dendritic shaft received no other synapses. The majority of the dendritic shafts receiving dendro-dendritic synapses were, however, those of varicose dendrites. These were often fairly large, received a moderate number of synapses, contained comparatively few organelles and had a moderately varicose shape; a proportion of these varicose dendrites received rather more synapses and contained a high density of organelles and appeared to be of the type identified as belonging to large stellate cells (Chapter 4). Dendro-dendritic synapses contacted both the wide and narrow parts of these varicose dendrites and most of the postsynaptic dendrites were of the va ricose type in those examples where a simple dendro-dendritic synapse was associated with a gap junction. In a few examples the postsynaptic dendrite was very thin and the presynaptic dendrite and dendro-dendritic synapse appeared to wrap round it (Figs. 6-9 and 6-10) and in serial sections these very thin dendrites became somewhat wider away from the postsynaptic region.

In two examples the postsynaptic dendrite was shown to be a PSD because it made a dendro-dendritic synapse itself on to a further profile and in the reciprocal synapses the postsynaptic process was of course also a PSD. In the remaining few examples the characteristics of the postsynaptic dendrite could not be determined, usually because insufficient serial sections were available and the orientation was unfavourable.

Discussion

The classification of nerve cell processes into axons and dendrites was originally made by light microscopy, particularly by use of the Golgi technique. In material prepared by this method the axon was recognised as a single, slender, usually long process which had a smooth contour and usually a fairly even diameter and originated from a conical axon hillock, whereas dendrites were characterised as shorter, multiple processes of larger diameter, which had a wider base and an irregular outline and often had spines. The dendrites were thought to be the site where the cell received activity and this was transmitted to other cells by the axon. With the electron microscope dendrites have been identified from the above features and have been characterised as the processes which receive afferent synapses whereas the axon makes efferent synapses on to the processes of other neurons. Dendrites have also been shown often to have an irregular plasma membrane and to contain organelles, in particular ribosomes, both singly and in clusters, and sacs of smooth endoplasmic reticulum.

These inclusions are rarely seen in axons which, in addition to making synapses, are often myelinated and have an origin from the cell soma which shows the specific structural features of the axon initial segment (Peters et al. 1968; Chapter 5).

The processes described here from the motor cortex, however, both receive and make synapses and so they cannot be immediately identified as axons or dendrites on the basis of their synaptic relations. Their non-synaptic characteristics are, however, those of dendrites, in particular their large size and dendritic outline, the frequency and number of single ribosomes and clusters of ribosomes in them and the sacs of smooth endoplasmic reticulum in some examples (Peters, Palay and Webster, 1970). The arrangement of their afferent synapses is also typical of a dendrite, especially in that many afferent synapses are found on to them and may occur on long lengths of these processes which are not locally presynaptic. These long non-presynaptic lengths are typical of varicose dendrites in the motor cortex and must frequently be identified as such in the absence of an efferent synapse in the section or length seen. The efferent synapse itself is also similar to the known dendro-dendritic synapses in large dendrites in the olfactory bulb in having only a few synaptic vesicles restricted to the region of the dendrite close to the synaptic membrane complex while the rest of the cytoplasm is free of them (e.g. Pinching and Powell, 1971) and the vesicles at dendro-dendritic synapses in the cortex are considerably more restricted

than has ever been seen in an axon there. These processes in the motor cortex which both make and receive synapses have therefore the characteristics of dendrites on present criteria and have never shown clearly axonal features; they have therefore been identified as presynaptic dendrites (PSDs).

The identification of these processes as PSDs is confirmed by the contrast between them and the occasional serial axo-axonic synapses reported in the motor cortex (Artyukhina, 1966; Chapter 5). These have been found only in the superficial cortical laminae and have round vesicles and an asymmetric or Type I membrane thickening, with the synapses on to the presynaptic process itself being symmetrical or Type II; in one example the process in question was also traced in continuity with an axon initial segment in one direction and a myelinated axon in the other. These features contrast with the deep location, flattened vesicles, symmetrical membrane specialisation, asymmetric and symmetrical afferent synapses and lack of axonal features of these PSDs and dendro-dendritic synapses.

In the mammalian central nervous system dendro-dendritic synapses were first described in the olfactory bulb of the rat (Rall, Shepherd, Reese and Brightman, 1966) and in the primate retina (Dowling and Boycott, 1966). Although they were originally thought to be confined to these more primitive parts of the central nervous system they have now also been described in the superior colliculus of the rat (Lund, 1969), in a number of thalamic nuclei

in various species (e.g. Ralston and Herman, 1969; Famiglietti, 1970; Wong, 1970; Harding, 1971; Ralston, 1971; Lieberman and Webster, 1972), in the hypothalamus (Güldner and Wolff, 1974) as well as in the motor cortex of the primate (Sloper, 1971). Dendro-dendritic synapses are therefore now known to have a wide distribution both in terms of level of the neuraxis and species and so must be considered to be a general feature of the organisation of nervous tissues although there are considerable differences in the frequency of their occurrence in different sites. There are as yet no published reports of them in other neocortical areas but it is possible that this is accounted for by their apparent rarity in relation to other types of synapse in the neocortex and possibly also by the less marked development of the deep laminae which contain them in areas of cortex other than that of the precentral gyrus. In view of the qualitative similarities of different cortical areas it seemed unlikely a priori that dendro-dendritic aynapses were restricted to the motor area of the cortex, and an example has recently been found in the somatic sensory cortex of the monkey (Shanks, personal communication).

Comparison of cortical dendro-dendritic synapses with those in other sites shows a number of interesting points. The similarity of vesicle morphology at dendro-dendritic synapses in various sites including the motor cortex has been noted by Lieberman and Webster (1972) and this has been confirmed here by the demonstration by tilt analysis that the flat vesicles at dendro-dendritic

synapses in the motor cortex are discoid. There must however be some slight reservation about this similarity since many vesicles at these cortical synapses are not flattened although it is well known that the flattening of vesicles is dependent on conditions of fixation used (e.g. Valdivia, 1970) and this may be the explanation for the small proportion of vesicles which are flattened at these synapses. It is also of interest that the membrane specialisations of all these dendro-dendritic synapses in different sites are symmetrical, with the exception of one of the types in the olfactory bulb. (Shepherd, 1972). The reciprocal synapses in the motor cortex are similar to those in the thalamus in that both components have symmetrical membrane specialisations and flattened vesicles and this contrasts with the characteristic reciprocal synapses in the olfactory bulb with their asymmetric and symmetrical components. The structure of PSDs in the neocortex and the arrangement of dendro-dendritic synapses within them, however, differs quite markedly from those in the thalamus; although PSDs in both sites may be markedly varicose in shape, those in the cortex are generally much more obviously dendritic in nature and the synaptic vesicles at the cortical dendro-dendritic synapses are much more localised to the region of the synaptic membrane specialisation and in this way they resemble closely the dendro-dendritic synapses of the olfactory bulb. Another similarity to the olfactory bulb is the occurrence of gap junctions between dendrites involved in dendro-dendritic and reciprocal

synapses (Pinching and Powell, 1971). Because of technical factors it is difficult to judge the relative frequency of dendro-dendritic and reciprocal synapses in different sites. It is clear however that both dendro-dendritic and reciprocal synapses in the motor cortex are very much less frequent than in the olfactory bulb and would appear also to be less frequent than in thalamic nuclei. In this context the importance of the extensive use of serial sections in finding and studying dendro-dendritic synapses in the cortex cannot be overemphasised.

Another point of interest in relation to the vesicles at these dendro-dendritic synapses was the finding of complex vesicles apparently budding from the surface membrane of cortical PSDs close to dendro-dendritic synapses. Similar appearances have been observed in the receptor cells of the retina, and Gray and Pease (1971) have postulated that this is the mechanism of formation of synaptic vesicles in this site. A similar phenomenon may be occurring here and it is possible that transmitter is taken up again from the synaptic cleft by these vesicles and it is also interesting to speculate that the dense-cored vesicles found at some of these synapses may represent storage sites for a transmitter substance.

It has not proved possible to trace any PSDs in the motor cortex to their parent cell soma and so the cell type giving rise to them cannot be directly identified. PSDs are however clearly dendrites of the varicose type and so probably originate from a type

of stellate cell. Together with their relative infrequency and distribution in the deep layers it seems likely that they originate from a relatively infrequent cell type which occurs predominantly in the deep cortical laminae. Some correlation may also possibly be drawn between the shape of the long lengths of PSDs seen in serial sections with the electron microscope and the appearance of varicose dendrites in Golgi-stained material. PSDs have an unusual combination of straight parallel-sided lengths and very varicose and somewhat tortuous parts which occur together in the same dendrite and this may correspond to the appearance of the dendrites of a variety of the 'cellule à double bouquet protoplasmique' seen in Golgi preparations (Cajal, 1909-11; Colonnier, 1966; Jones, 1975a) This cell type is also an infrequent type of stellate cell found predominantly in the deep cortical laminae and so may be the origin of the PSDs in the neocortex. If so, this cell type has an axon and its axon terminals should be present in the cortical neuropil and would be expected to contain vesicles having the same morphology as those at the dendro-dendritic synapses. However if this type of 'cellule à double bouquet protoplasmique' is the source of cortical PSDs then these dendrites might be expected to have a predominantly vertical orientation. It is therefore not possible as yet definitely to identify the cell of origin of cortical PSDs or to determine whether it has an axon or normal dendrites in addition to its presynaptic dendrites or is a cell of the amacrine type.

Although the functional significance of these dendro-dendritic synapses cannot be determined without physiological study, the anatomical findings may give some relevant indications. Five examples were found of dendrites which made synapses on to dendritic spines, all of which were studied in serial sections. In all examples the postsynaptic spine also received a synapse from an asymmetric axon terminal and in some cases a symmetrical axon terminal also made a synapse on to the same spine. In no example, however, did only a dendrite and a symmetrical axon terminal make synapses on to a spine. The PSDs therefore appear to follow the same rule in relation to spines as do symmetrical axon terminals in that they appear never to make a synapse on to a dendritic spine unless an asymmetric axon terminal does so as well. In the few triplet and complex synaptic arrangements seen it has also been either an asymmetric terminal alone or both an asymmetric and a symmetrical terminal which have made synapses on to both pre- and postsynaptic dendrites, never a symmetrical terminal alone, and this may be in some way comparable to the situation in relation to spines. In the reciprocal synapses between PSDs both synaptic components have the same morphology and so it seems likely that they are also functionally the same, both being either excitatory or inhibitory. If both components are excitatory, this arrangement would appear to have the potential for self re-excitation, with activity passing indefinitely from one synapse to the other and back again in an uncontrolled manner. It is possible of course

that they could interact in a more complex way but it would seem more likely that both components are inhibitory. This would be in accord with the similarity of PSDs to symmetrical axon terminals in their relationship to spines since symmetrical axon terminals with flattened vesicles are themselves thought to be inhibitory at least in certain sites (Uchizono, 1965; Gray, 1969). The morphology of the dendro-dendritic synapses themselves, having flattened vesicles and symmetrical membrane specialisations, is also of the type correlated with inhibitory function elsewhere (Uchizono, 1965; Gray, 1969; Price and Powell, 1970b) and so it will be of interest to see if these speculations can be confirmed by physiological study; this will however present very great technical problems.

A proportion of dendro-dendritic synapses in the neocortex are associated with gap junctions although the majority of both appear to occur alone and it is not clear whether this association has any particular significance. Gap junctions have not been found in association with synapses made by axon terminals in the neocortex (Chapter 7) but this may be because they only occur between dendrites for other reasons rather than because of a fundamental difference in the synapses made by axons and dendrites in the motor cortex.

There are now a considerable number of instances in which the conventional anatomical and physiological conecpts of neurons have proved inadequate. In addition to the descriptions

of dendro-dendritic synapses mentioned there have been reports of myelinated dendrites in the monkey olfactory bulb (Pinching, 1971) and of dendrites with regenerative spike mechanisms in the alligator cerebellum (Llinás and Nicholson, 1971) as well as the example of the dorsal root ganglion cell. It is therefore suggested that the interrelationship of the different types of nerve cell process which have been observed may be better understood if neurons are considered to have arisen by progressive steps from a single 'primitive' type of cell having only one type of process. This process is envisaged as a simple extension of the neuronal cytoplasm which both receives and makes synapses, since the cell must have both afferents and efferents, and is thus equivalent to a presynaptic dendrite and has no regenerative spike mechanism or myelin. Conduction of activity within this type of cell would therefore be by passive electronic spread of current and efferent synapses near an activated afferent synapse would be more powerfully influenced by it than distant efferents on other dendrites. This factor would limit the size of the cell. From this 'primitive' cell type development could occur in two ways: some of its processes could lose their efferent synapses and this would give rise to cells such as the granule cell of the olfactory bulb with both 'normal' and presynaptic dendrites (Price and Powell, 1970a). Alternatively one of the processes could take on the characteristics of an axon making a cell type like the mitral cell of the olfactory bulb with presynaptic dendrites and an axon (Price and Powell, 1970c). The prime

characteristic required for this appears to be the development of a membrane capable of generating and transmitting a nerve impulse and the spike mechanism seen in the main dendrite of the Purkinje cell of the crocodile (Llinás and Nicholson, 1971) may be regarded as an early instance of this development, with the dorsal root ganglion cell being a further specialisation. The development of a spike mechanism is an essential requirement before the axonic process of the cell can become substantially longer than a dendrite because of the limits imposed by the attenuation of passive electrical spread by distance. Myelination of this elongated process may then occur but it is of interest that this also is not exclusively a characteristic of axons (Pinching, 1971). The development of a specialised process for the conduction of efferent activity from the cell may then make the dendritic efferents redundant and subsequent loss of these would give rise to a neuron of the conventional type having 'normal' dendrites and an axon, examples being the spinal motoneuron and the pyramidal cell. It is therefore possible to see how the variety of neuronal types with their differing combinations of processes could have arisen from a common stem neuron.

This view has implications for consideration of the significance of dendro-dendritic synapses. If these were present before axons and axonic synapses developed then, rather than seeking reasons for the presence of dendro-dendritic synapses in certain sites, it is more logical to ask why they have been largely replaced

by axonic synapses, particularly in the phylogenetically newer parts of the mammalian nervous system. The key factor in this would appear to be the length of connection formed by most neurons in the mammal, particularly those giving rise to the long tracts but most other connections are longer than the extent of the dendrites of the cells concerned. However this view that dendro-dendritic synapses have remained where there are very short connections does not preclude the possibility that there may also be more specific positive reasons for the continued existence of dendro-dendritic synapses in particular sites in the nervous system.

CHAPTER 7

GAP JUNCTIONS BETWEEN DENDRITES AND SOMATA OF NEURONS IN THE SENSORI-MOTOR CORTEX

Introduction

Although synaptic transmission was originally thought to occur by direct electrical conduction, the existence of chemical transmitter substances and their role in normal synaptic transmission is now generally recognised (e.g. Katz, 1966). By analogy with the neuromuscular junction and other sites, the vesicles and membrane specialisations seen in the nervous system are considered to be the sites at which chemical synaptic transmission takes place. There are, however, a number of sites in the nervous systems of lower vertebrates where physiological evidence indicates that direct electrical transmission of activity occurs between certain neuronal processes (Washizu, 1960; Martin and Pilar, 1963; Furshpan, 1964; Grinnell, 1966; Bennett, Nakajima and Pappas, 1967a, b; Bennett, Pappas, Aljure and Nakajima, 1967; Bennett, Pappas, Gimenez and Nakajima, 1967) and regions of close membrane apposition, defined as gap junctions by Brightman and Reese (1969), have been consistently found between these same neuronal structures (Robertson, Bodenheimer and Stage, 1963; Charlton and Gray, 1966; De Lorenzo, 1966; Bennett, Nakajima and Pappas, 1967a, b; Bennett, Pappas, Aljure and Nakajima, 1967; Bennett, Pappas, Gimenez and Nakajima, 1967; Sotelo and Taxi, 1970; Waxman and Pappas, 1971). This has therefore led to the gap junction being identified as a low resistance pathway between cells which allows direct electrical transmission of activity to take place (see Bennett, 1972). Gap junctions have only been identified more recently in the nervous

systems of mammals but they have now been described in the retina (Dowling and Boycott, 1966), the olfactory bulb (Pinching and Powell, 1971), the lateral vestibular nucleus (Sotelo and Palay, 1970), the sensorimotor cortex (Sloper, 1972), the cerebellar cortex (Sotelo and Llinás, 1972) and the inferior olive (Sotelo, Llinás and Baker, 1974), and in the lateral vestibular nucleus and inferior olive there is physiological evidence of electronic conduction between the cells connected by the gap junctions (Korn, Sotelo and Crepel, 1973; Wylie, 1973; Llinás, Baker and Sotelo, 1974). This chapter describes in full the findings in the sensorimotor cortex of the primate.

Results

Gap junctions have been observed in the motor cortex (area 4 of Brodmann) and in area 3b of the somatic sensory cortex of the Rhesus monkey (Macaca mulatta) in this study. Since, with the exception of the association with dendro-dendritic synapses, the findings in both areas were the same, they will be described together. Most of the 25 gap junctions found occurred between two dendrites (e.g., Figs. 7-1 to 7-5) but they have also been found between a dendrite and a dendritic spine and between a dendrite and a cell soma. None has been found in which an axon terminal was directly involved.

The ultrastructure of these gap junctions corresponds to that described by Brightman and Reese (1969); the plasma membranes of the two processes making the gap junction are closely

apposed, there being a 'gap' of about 2 nm between their adjacent outer leaflets and these outer leaflets appear somewhat attenuated (Fig. 7-4) whereas the leaflets of each membrane which are nearest the neuronal cytoplasm appear somewhat denser than those of non-junctional membrane. This gives an overall seven-layered structure of four dark and three light bands which has an overall width of about 15 nm and there is electron-dense material in the adjacent cytoplasm on both sides of the junction. The central 'gap' shows a periodicity in transverse section (Fig. 7-4) and, in an example in which a single gap junction twisted through a right angle in relation to the plane of the section, it was possible to demonstrate the pattern of hexagonal sub-units previously described in 'en face' sections (Figs. 7-6 and 7-7) (Robertson, 1963; Revel and Karnovsky, 1967). Serial sections showed that the area of the gap junction varied considerably from one example to another (e.g. Figs. 7-10 to 7-12, cf. Fig. 7-13), with maximum diameters in serial sections ranging from 0.1 μ to 0.6 μ, and the gap junction did not always occupy the full area over which two dendrites were immediately adjacent.

The majority of the gap junctions described here were studied in serial sections. Many appeared to occur without any associated membrane specialisation but in a number of examples a desmosome was found adjacent to the gap junction (Figs. 7-8, 7-9 and 7-17 to 7-19). These desmosomes linked the same two neuronal structures as the gap junction and had the typical structure of a

desmosome with the usual cleft containing dense extracellular material between the profiles, in contrast to the structure of the gap junction. In five examples from the motor cortex a dendro-dendritic synapse and a gap junction were found adjacent to each other and between the same two dendrites and in four of these cases there was also an associated desmosome. One example showed a reciprocal dendro-dendritic synapse, a desmosome and a gap junction all together at the interface between two dendrites (Fig. 6-40 to 6-43). The structure of these dendro-dendritic synapses showed the typical arrangement of vesicles and membrane thickenings with an extracellular cleft containing a band of dense material as described previously in the motor cortex (Sloper, 1971; Chapter 6) but no vesicles were ever found opposite the gap junction itself in any example. Although there did not appear to be any consistent synaptic arrangement related to gap junctions, in a few examples a single axon terminal was found to make synapses on to both dendrites connected by a gap junction (Figs. 7-1 to 7-3). These different arrangements described above are shown diagrammatically in Fig. 7-15.

Nineteen of the twenty-five gap junctions found in the sensori-motor cortex were between two dendrites, with four being found between a dendrite and cell soma (Figs. 7-16 to 7-20) and two between a dendrite and a dendritic spine (Figs. 7-10 to 7-12 and 7-22 to 7-24). A large proportion of the dendrites making gap junctions received a high density of synapses of both the asymmetric

and symmetrical types and many of them contained a relatively high concentration of ribosomes and mitochondria and had prominent microtubules. When cut in longitudinal section these dendrites usually had a somewhat varicose shape, they ran in a straight line for some distance and were studded with synapses (Figs. 7-8 and 7-22). These dendrites were therefore typical of those identified as arising from stellate cells of the large type (Chapter 4). Most of these dendrites making gap junctions were of small to medium size but a number were large and appeared to be main stem dendrites (Figs. 7-13 and 7-14, and 7-22 to 7-24). Of the remaining dendrites making gap junctions most made an adjacent dendro-dendritic synapse and were typical of the presynaptic dendrites found in the motor cortex (Chapter 6). They received fewer synapses than those of the large stellate type and often had a markedly varicose shape. A small number of the dendrites involved in gap junctions could not be positively identified as belonging to one of these two types but they had no features to suggest that they were of any type other than these. . In most examples both dendrites were identifiable and of these gap junctions occurred most frequently between two dendrites of the large stellate type, with a few between a large stellate dendrite and a presynaptic dendrite and one between two presynaptic dendrites.

Four examples were found of gap junctions between a dendrite and cell soma (Figs. 7-16 to 7-21) and all the cell somata could be clearly identified as large stellate cells because of the

large number of axosomatic asymmetric and symmetrical synapses they received and because of their abundant cytoplasm containing a high concentration of organelles (Chapter 4). In one of these examples the gap junction could be seen to be situated within about 5 μ of the origin of the axon initial segment, which was directed towards the cortical surface (Fig. 7-21). Two of the dendrites making a gap junction with a cell soma were of the large stellate type (Figs. 7-19 and 7-21), this being confirmed in serial sections, and the other two were probably also of this type.

Two gap junctions were found between a dendrite and a dendritic spine. One of these was sessile and contained a small sac (Figs. 7-10 to 7-12); it arose from a dendrite of the large stellate type which received a number of normal synapses but no normal synapses were present on the spine in addition to the gap junction, this being shown by a complete series of sections through the spine. The other spine receiving a gap junction contained a spine apparatus and had flocculent background cytoplasm but could not be traced to its parent dendrite (Figs. 7-22 to 7-24). This spine received a synapse from an asymmetric axon terminal which also made a synapse on to the dendrite which formed the other component of this gap junction. This example of a gap junction was found in an experimental brain in which a thalamic lesion had been placed five days previously and the dendrite involved in this gap junction, which was clearly of the large stellate type, was found to receive synapses from two degenerating thalamo-cortical axon terminals (Fig. 7-23).

Most gap junctions in both cortical areas were found in layers IV and V, with the occasional one occurring in the deep part of layer III or in layer VI. Although the full depth of the cortex was studied systematically, none was found in the more superficial layers. Gap junctions are an infrequent feature of the neocortical neuropil taken overall but were noticeably more common in regions where there was a concentration of large stellate dendrites and they were found to occur at a considerable proportion of the sites where two dendrites of the large stellate type came into apposition. All the cell somata making gap junctions were found in layers IV and V of the cortex, somato-dendritic gap junctions having been found in both the motor and somatic sensory cortices.

Discussion

Areas of close membrane apposition are a not uncommon feature of nervous tissue seen with the electron microscope and they have been differentiated into gap junctions, tight junctions and labile membrane apposition by Brightman and Reese (1969) who discussed their identification and the criteria by which they may be distinguished, as did Sotelo and Palay (1970). The fine structure of gap junctions as seen in 'en face' sections has been described by Robertson (1963) and by Revel and Karnovsky (1967). The areas of close apposition described here are gap junctions because of their seven-layered structure with an overall width of 15 nm and with a central 'gap' containing hexagonal sub-units when

viewed 'en face' and because of the associated electron-dense material in the adjacent cytoplasm. Their restricted extent and specific occurrence in relation to certain neuronal profiles and in certain cortical laminae also distinguishes them from labile membrane appositions. This contrasts with the five-layered structure of tight junctions and the lack of specific structure and non-specific distribution of labile membrane apposition.

Gap junctions have been described in a number of sites in the nervous systems of lower vertebrates including various nuclei in the central nervous systems of a number of fish (Robertson, Bodenheimer and Stage, 1963; Bennett, Nakajima and Pappas, 1967a, b; Bennett, Pappas, Aljure and Nakajima, 1967; Bennett, Pappas, Gimenez and Nakajima, 1967; Waxman and Pappas, 1971), the ciliary ganglion of the chick (De Lorenzo, 1966) and the spinal cord, oculomotor nuclei and cerebellum of various amphibia (Charlton and Gray, 1966; Sotelo and Taxi, 1970; Waxman and Pappas, 1971; Sotelo and Llinás, 1972) and they have been described between axon terminals and dendrites or somata, between two dendrites or between a dendrite and a cell soma. In some cases the gap junctions occurred together with a 'chemical' synapse and in other examples they were found alone. Many of these sites have been studied physiologically and evidence of direct transmission of activity between the profiles linked by the gap junctions has been obtained. In fish, electrical transmission has been demonstrated at the Mauthner cell club endings in the goldfish (Furshpan, 1964)

and in brainstem nuclei or the spinal cord of a series of different types of fish which were also studied histologically (Bennett, Nakajima and Pappas, 1967a, b; Bennett, Pappas, Aljure and Nakajima, 1967; Bennett, Pappas, Gimenez and Nakajima, 1967) and evidence indicating direct electrical transmission has also been obtained in the chick ciliary ganglion (Martin and Pilar, 1963) and in the spinal cord of the frog (Washizu, 1960; Grinnell, 1966). All these are sites where gap junctions have been demonstrated histologically and on the basis of this evidence the gap junction has been identified as a low resistance pathway between cells, acting as an electrotonic synapse (e.g. Bennett, Nakajima and Pappas, 1967; Peters, Palay and Webster, 1970; Bennett, 1972).

Gap junctions had initially been thought to be restricted to the nervous systems of lower species, but they have now been described in a number of sites in the nervous systems of mammals. They were first described in fairly peripheral parts of sensory pathways, the primate retina (Dowling and Boycott, 1966) and the lateral vestibular nucleus and olfactory bulb of the rat (Sotelo and Palay, 1970; Pinching and Powell, 1971) but they have now been described in both the cerebellar cortex of the cat (Sotelo and Llinás, 1972) and the motor and somatic sensory cortices of the monkey (Sloper, 1972) and the inferior olive of the cat (Sotelo et al. 1974). Gap junctions would therefore appear to be a general, if infrequent, feature of the organisation of mammalian nervous systems. Physiological evidence indicating electrical

coupling of neurons in the mammal has been obtained in the lateral vestibular nucleus of the rat (Korn et al. 1973; Wylie, 1973) and the inferior olive (Llinás et al. 1974) but the other sites where definite gap junctions have been described in the mammal have not been closely studied in this respect. However, in view of the correlation between gap junctions and electrical transmission in all the sites described above it seems very likely that these gap junctions in the primate neocortex represent low resistance pathways which permit direct electrical transmission to occur between the dendrites and somata of the neurons involved.

In considering the possible functional significance of the gap junctions in the neocortex the most similar situations studied physiologically would appear to be those studied by Bennett and others in the electromotor nuclei of Mormyrid fish (Bennett, Pappas, Aljure and Nakajima, 1967) and of Gymnotid fish (Bennett, Pappas, Gimenez and Nakajima, 1967). In both cases dendro-dendritic and dendrosomatic gap junctions were present between cells of a single type and there was clear evidence of electrical coupling between these cells, so that if one cell was depolarised or hyperpolarised this was transmitted to adjacent coupled cells in a graded manner. In the first case the coupling was powerful enough for activation of one cell to be able to excite all the other cells in the group, and very extensive gap junctions were present between them histologically. In the gymnotid fish, however, the coupling was weaker so that a single impulse in one cell would not

excite all the others but activity that occurred tended to be equalled out among all the coupled cells. This would appear to be a closer parallel to the situation in the motor and somatic sensory cortices as the gap junctions there are not extensive. They do however occur on large dendrites and cell somata, on occasion close to the initial segment, and so it is likely that they will have a clear effect on the activity of these neurons. As in the electromotor nuclei, current will be expected to flow through the gap junctions between a more depolarised cell and a less depolarised cell and this will tend to even out activity between the coupled cells so preventing any one cell being much more or less active than its coupled neighbours.

Most of the coupled cells in the neocortex are large stellate cells while a few are the unidentified cells which give rise to presynaptic dendrites. It is not possible to rule out the possibility that a few cells of another type may also be coupled but since most of the dendrites giving rise to gap junctions were clearly identifiable these could only form a small proportion. Large stellate cells receive synapses from a proportion of the afferent axon terminals to the sensori-motor cortex (Chapter 9) and it was directly demonstrated in one case here that thalamic afferents end on large stellate dendrites having gap junctions. The coupled cells are therefore receiving a proportion of the afferent input to the cortex. Evidence has been advanced elsewhere (Chapter 4) on the basis of observations made with correlation of

the Golgi technique and the electron microscope that large stellate cells are the cortical basket cell of light microscopy (Cajal, 1909-11; Marin-Padilla, 1969, 1970; Jones, 1975a) and it is interesting to note that it is also the basket and stellate cells in the cerebellar cortex which are linked by gap junctions (Sotelo and Llinas, 1972). It would therefore appear that electrical coupling may be a feature of basket cells and it would seem likely that gap junctions will be present between the dendrites and somata of basket cells in the hippocampus. The possible significance of the coupling of large stellate cells in relation to the physiology of the neocortex is discussed elsewhere (Chapters 4 and 10).

CHAPTER 8

OBSERVATIONS ON THE PROCESS OF DEGENERATION IN THE SENSORI-MOTOR CORTEX

Introduction

This chapter reports a number of observations made on the features of degenerating axons and axon terminals as seen in the sensori-motor cortex of the primate with the electron microscope. These observations were made during the course of a systematic experimental study of afferent connections to the sensori-motor cortex reported elsewhere (Chapter 9). Terminal degeneration following lesions of the parent cell soma or axon has been widely used as an experimental tool in the study of the nervous system with the electron microscope (see Guillery, 1970) but the process of degeneration and the fate of its products is also of interest in itself and has been emphasised by several authors (e.g., Gray and Hamlyn, 1962; Colonnier, 1964; Guillery, 1965; Jones and Powell, 1970d; Pinching and Powell, 1972). In particular it must form a part of any postulated process of reorganisation of connections following injury to the central nervous system and so may provide information relevant to the problem of healing in the brain. The changes occurring in the axon terminal following its separation from the cell soma may also give some information about the dependence of the terminal on the soma, including the role of axoplasmic flow, and histochemical changes may be of particular relevance here. Finally, study of the process of degeneration has provided information relevant to the formation of vesicles, a process of general importance in many tissues.

Results

Following the placement of lesions in the thalamus, the contralateral motor cortex and the ipsilateral somatic sensory (SI) and premotor cortex (area 6) of the Rhesus monkey, the axons and axon terminals of the projections from these sites to the motor cortex undergo a characteristic series of degenerative changes. The basic features of this process of degeneration in the primate motor cortex appear to be the same as those in the somatic sensory cortex of the cat (Jones and Powell, 1970d) and so will not be described in detail; the earliest change seen is the swelling of synaptic vesicles in the axon terminals and this is followed by darkening of the background cytoplasm of the terminals and subsequently by the progressive disruption of the internal structure of the axon terminals and their engulfment by glia. Following both cortical and thalamic lesions a few terminals have also been found which show the neurofilamentous hypertrophy type of degenerative reaction (Gray and Hamlyn, 1962).

The basic process of degeneration is the same in the terminals of both the thalamo-cortical and cortico-cortical connections studied here and in many instances degenerating terminals of the two types of connection are indistinguishable. However certain differences are often apparent and a comparison of early, medium and late degeneration in thalamo-cortical and commissural cortico-cortical axon terminals is shown in Figs. 8-1 to 8-6. A proportion of degenerating thalamo-cortical terminals contains

prominent glycogen granules at most stages of degeneration whereas glycogen is very rarely seen within degenerating cortico-cortical terminals, although abundant in surrounding glia in both types of degeneration. Some degenerating thalamo-cortical terminals also contain prominent neurofilaments until late in the degenerative process (Fig. 8-1) although rarely showing true neurofilamentous hypertrophy, whereas neurofilaments are rare in degenerating cortico-cortical terminals (Figs. 8-7 and 8-8). Degenerating thalamo-cortical terminals often appear larger than cortico-cortical terminals at a similar stage of degeneration and long lengths of degenerating thalamo-cortical axons making 'en passage' synapses may be found.

There is some evidence that the rate at which the process of degeneration proceeds is related to the length of axon left between the site of section and the axon terminal (Vaccareza, Reader, Pasqualini and Pecci-Saavedra, 1970) and so it is of interest to compare the rate of degeneration of the different cortico-cortical connections in this study. Survival periods of 4, 5 and 6 days were used for the commissural connections, 1¼, 2, 3, 4, 4¾, 5 and 6 days for connections from the ipsilateral somatic sensory cortex and 4 and 5 days for ipsilateral area 6 lesions. A little early degeneration was present in the motor cortex as early as 2 days after a lesion of the ipsilateral somatic sensory cortex and more was present at 3 days, with some terminals at later stages. The peak of the degenerative process appeared to be at

four to five days following this lesion, with the majority of terminals at a medium or late stage of degeneration at four days, and at five and six days most terminals were at the late stages of degeneration although all stages could still be found. Although degeneration following area 6 lesions was not as dense, the stages of degeneration at four and five days appear to correspond to those following somatic sensory lesions. After lesions of the contralateral motor cortex the same progression of degenerative changes was apparent in the terminals of commissural axons as in association axon terminals. However at four days' survival most of the degenerating commissural terminals in the motor cortex were at an early stage, at five days considerably more degeneration was present with medium stages predominating, whereas at six days most of the terminals were at a late stage of degeneration. It therefore appears that following removal of the contralateral motor cortex the onset and timecourse of degeneration is delayed by about a day compared to that following removal of the ipsilateral somatic sensory or pre-motor (area 6) cortex, although once started the process seems to progress at the same rate.

The engulfment and removal or degenerating terminals by glial profiles is well recognised at later stages of the degenerative process. Detailed study of some of the degenerating terminals seen in this study has shown the presence of small portions of late degenerating terminals which extend into invaginations of the membrane of the surrounding profile. These invaginations vary

in the extent to which the portion of the degenerating terminal appears to be pinched off by the surrounding profile (Figs. 8-9 to 8-14) and the side of the membrane of the profile away from the degeneration consistently has an undercoating like that of a complex vesicle. These invaginations would appear to represent a process of micropinocytosis of the degenerating terminals by adjacent profiles; all stages may be seen from an almost flat membrane with a short length of undercoating adjacent to a degenerating profile (Figs. 8-9 and 8-11) through all degrees of pinching off of the degeneration to coated vesicles containing dense material in the cytoplasm of the adjacent profiles (Fig. 8-14). These profiles are most commonly glial but unequivocally neuronal profiles, including neuronal somata (Figs. 8-15 to 8-20), dendrites and dendritic spines have been found to show this phenomenon. It therefore appears that a process of micropinocytosis by glia and neurons is responsible, at least in part, for the removal of degenerating terminals in the neocortex.

Exposed postsynaptic membrane thickenings have been described following terminal degeneration in a number of sites (see Pinching and Powell, 1972). They have been found following these lesions in the neocortex although they also occur in material which otherwise shows only early stages of degeneration, which suggests that they may not always have been the result of the experimental lesions. However their origin as a result of the loss of the presynaptic component of the synapse by degeneration

is supported by the finding of examples in which only a thin sliver of cytoplasm is left adherent to the postsynaptic profile (Fig. 8-21) and by the occasional example in which glial cytoplasm appears to be invading the synaptic cleft (Fig. 8-22). Exposed postsynaptic thickenings have occasionally been found on spines which also receive synapses from degenerating axon terminals (Fig. 8-23) but this does not indicate that the spine originally received synapses from two degenerating terminals because a single degenerating terminal may occasionally make two separate synapses on to one spine (Fig. 8-24). The structure of exposed postsynaptic membrane thickenings is typical of asymmetric postsynaptic membrane specialisations(Fig. 8-26), with the occasional one being of the symmetrical type (Fig. 8-28), and the cleft material is still adherent to the postsynaptic plasma membrane. Exposed thickenings may be found opposite any component of the neuropil (Figs. 8-25 to 8-32), both neuronal and glial, including axon initial segments and unmyelinated axons (Fig. 8-31). When opposite neuronal profiles there is often a subsurface cistern related to them in the profile with its dense plate apposed to the region of the postsynaptic specialisation, but this has not been seen when the exposed thickenings are opposite a glial profile. As with other subsurface cisternae, those apposed to these exposed membrane thickenings were shown in some examples to be continuous with the rough endoplasmic reticulum in the cell soma (Figs. 8-29 and 8-30) and may be closely associated with mitochondria (Fig. 8-32) (Chapter 3).

Degenerating myelinated axons were frequently seen in this experimental material. The axoplasm undergoes a series of degenerative changes corresponding to those seen in the axon terminals and leading to shrinkage and varicosity of the axon within its myelin sheath. Not uncommonly the dark degenerating axon may be seen surrounded within the myelin sheath by pale cytoplasm with the features of glia and even a glial nucleus has been found within a continuous sheath of myelin. One example was found showing a glial cell with its cytoplasm passing into the end of a myelin sheath, presumably at what was a node of Ranvier, but another example showed glial cytoplasm which appears to break through a myelin sheath and surround the degenerating axon within it (Fig. 8-33). These glial cells presumably remove the degenerating material from within the myelin sheaths and empty myelin sheaths were found in this material. They were usually collapsed and on occasion they became applied to various structures including the apical dendrites of pyramids, a situation which gave the appearance in single sections of lengths of myelinated dendrite (Chapter 3) (cf. Pinching, 1971). The myelin was however doubled back on itself at the ends of the "myelinated" segment of dendrite, instead of having typical end feet, and serial sections confirmed that the double thickness myelin did not form a true sheath round the dendrite. No true myelinated dendrites were seen in this material.

Blocks from two brains in which experimental lesions had been placed were block-stained with ethanolic Phosphotungstic Acid

(E-PTA) with no further staining (Method I). In one of these brains a stereotaxic lesion had been placed in the thalamus and in the other the contralateral motor cortex had been removed. In both of these brains the distribution and density of degeneration had been studied with the electron microscope in conventionally prepared blocks taken adjacent to those stained with PTA. Degenerating axon terminals and degenerating myelinated and unmyelinated axons were found to be specifically stained by the E-PTA and showed up dark against the pale background (Figs. 8-34 to 8-37) and the distribution and density of degeneration as shown by the E-PTA corresponded in both brains to that seen in adjacent conventionally prepared blocks. The dark staining by the E-PTA appeared to be mainly cytoplasmic and some terminals appeared considerably darker than others. From the degree of shrinkage and deformation of these terminals it would appear that these more darkly stained terminals correspond to the later stages of degeneration as in conventionally prepared material. Mitochondria generally appeared pale against the dark cytoplasm and their membranes were unstained (Fig. 8-37). The postsynaptic membrane specialisations could be seen attached to degenerating axon terminals with the plasma membranes of both pre- and post-synaptic components showing up as white unstained lines having the stained cleft material between them. A spine apparatus was occasionally seen immediately beneath the post-synaptic membrane specialisation and so presumably in the postsynaptic profile (Fig. 8-35). The outline of vesicles

is occasionally apparent in the later degenerating terminals but in general they appear to be obscured by the very dense cytoplasmic staining; in the paler terminals they are presumably not well seen because of the lack of membrane staining by E-PTA and the comparatively poor definition of this type of material. Degenerating myelinated axons were stained by the E-PTA in the same way as the degenerating terminals and showed the same shrinkage and irregularity as in conventionally prepared material. The membranes of the myelin sheath were not stained by the E-PTA and so showed up as pale against the generally granular background.

Sections from completely unstained blocks were examined as a control to ensure that the degeneration was not naturally electron-dense. Blocks which had been post-fixed with osmium tetroxide before block staining with E-PTA were also examined. This technique stained the myelin sheaths of the axons but did not appear to affect the staining of the degenerating axons or terminals by the E-PTA.

Most of the degenerating axon terminals in this study have been found to make synapses on to dendrites and these are usually seen in isolation in the neuropil. In order to obtain more information about the cell types from which these spines arise serial sections have been used extensively to trace them to their parent dendrites. Examples of this are shown in Figs. 8-39 to 8-41 and the detailed results obtained by this method are given in Chapter 9. Serial sections have also

demonstrated that a single degenerating axon terminal may make a synapse on to both sides of a dendritic spine, either as two separate synaptic contacts (Fig. 8-24) or as one synapse which wraps round the spine (Figs. 8-40 and 8-41), and so it would be difficult to prove that a spine received synapses from two separate degenerating axon terminals without a complete series of sections through the spine. A single degenerating axon terminal may be found to make synapses on to two or three different postsynaptic profiles in a single section (Fig. 8-42). Study of degenerating terminals in serial sections has shown that, in addition to these, a proportion of degenerating terminals which make only one synapse in a single section in fact make multiple contacts when studied in serial sections and so the proportion of terminals found to make multiple contacts in a single section is an underestimate of the true incidence of multiple contacts.

Occasional dark dendrites have been found in the material studied here. These dendrites have been predominantly of the large stellate type (Figs. 8-44 and 8-45), so identified because they receive a large number of synapses including asymmetric ones. Rare dark cell somata have been found, including one Betz cell and one example of a cell soma at an obviously late stage of degeneration (Fig. 8-46). With the exception of one brain, however, dark dendrites and somata have been a rare finding. In this one brain in which the somatic sensory cortex had been removed the motor cortex contained a considerable number of dark dendrites,

predominantly of the large stellate type and a large dark stellate soma was also present (Fig. 8-43). This material contained degenerating terminals making asymmetric synapses as would be expected in the motor cortex following a lesion of the somatic sensory cortex. However, in addition, a considerable number of degenerating axon terminals were present which made symmetrical synapses, the nature of the membrane specialisations being confirmed in serial sections. These degenerating symmetrical terminals occurred particularly in layer V and made synapses on to pyramidal cell somata (Figs. 8-47 and 8-48) and large dendrites of the pyramidal type (Fig. 8-50) as well as a few which made synapses on to spines (Fig. 8-49). This degeneration occurred over most of the medio-lateral extent of the motor cortex in both the wall of the central sulcus and on the exposed surface of the precentral gyrus. The motor cortex was examined very carefully both macroscopically and histologically for direct damage but none was found. The most likely explanation for these observations would seem to be that at operation the blood supply to the motor cortex was compromised and this seems to have produced a selective degeneration of some of the large stellate cells and of symmetrical axon terminals.

Discussion

Degeneration of axons and axon terminals has now been studied with the electron microscope in many parts of the central nervous system including the neocortex (see Guillery, 1970) and

its general features are now well known. The findings of this study are fully in agreement with those of previous authors working on the neocortex (e.g. Colonnier, 1964; Lund and Lund, 1970; Jones and Powell, 1970d; Garey and Powell, 1971), but have extended them in a number of ways.

The association and commissural connections to the sensori-motor cortex all arise from other areas of neocortex. Commissural connections have been shown to arise from pyramidal cells (Jacobson and Trojanowski, 1974) and it is likely that association terminations do so as well whereas the thalamo-cortical afferents arise from relay cells within the thalamus. Being terminals of different cell types it is therefore not unexpected that there should be differences in their appearance when they degenerate. The absence of glycogen from within the obviously degenerating cortico-cortical terminals is however of interest since the appearance of glycogen has frequently been considered as an integral part of the degenerative process. It is likely that the neurofilaments and larger size of some of the degenerating thalamo-cortical terminals is a reflection of the appearance of these terminals in normal material and tends to confirm the tentative identification of the large pale type of terminal seen mainly in layer IV as the terminal of thalamo-cortical afferents (Jones and Powell, 1970a; Chapter 3).

It is well known that the terminals of different fibre systems degenerate at different rates following axonal section and

that in some systems the changes in different terminals appear to occur at approximately the same time whereas in other sites including the neocortex terminals showing all stages of degeneration are present simultaneously and the overall progression is prolonged (Jones and Powell, 1970d). Increase in the length of the distal axonal stump has been shown to delay the process of degeneration in the lateral geniculate nucleus of the cat (Vaccarezza et al. 1970), this being possibly related to the phenomenon of axoplasmic transport, and differences in axon diameter and in the degree of terminal branching of the degenerating axons have also been suggested as factors affecting the rate and synchronisation or otherwise of the degenerative change. The degenerative changes in the terminals of both commissural and association axons studied here were asynchronous as in previous studies in the cortex (Lund and Lund, 1970; Jones and Powell, 1970d, e) but both the onset of degeneration and its peak were delayed by about 24 hours in the motor commissural connection as compared with the association connections to the motor cortex from the somatic sensory cortex (SI) and area 6. All these connections arise from the neocortex and so probably from the same cell type. There is no information regarding differences in fibre diameter or degree of terminal branching in commissural and association fibres in the monkey although in the cat the somatic commissural axons appear to be of comparable size to the association fibres between SII and SI (Jones and Powell, 1970e). However the commissural connection

of the trunk area of the motor cortex in the monkey is approximately 5 cm long (measured on the cut, fixed brain) whereas the two association connections are 1 to 2 cm long. This gives a delay of approximately 6 to 8 hours for each centimetre difference of axon length compared to the 12 hours per centimetre delay found in the retino-geniculate projection of the cat (Vaccarezza et al. 1970)and it seems likely that this length difference is the main factor delaying the degeneration of the commissural terminals.

Although the reasons for the specific staining of various structures by ethanolic phosphotungstic acid (E-PTA) are not clear, a change occurs in axons and axon terminals during the process of degeneration which makes them take up the E-PTA, since normal axons and terminals are not similarly stained. The staining appears to be cytoplasmic and so it seems likely that some change occurs in the biochemical properties of the cytoplasm of the axons and terminals during degeneration. A similar change in the staining of axons and terminals is of course seen in material prepared conventionally but normal profiles are also stained and so the degeneration does not stand out as prominently as with E-PTA staining. This specific staining may possibly provide a method for the study of the distribution of degeneration with the electron microscope.

The general involvement of glia in the removal of degeneration has been widely described although, with the exception of Walberg (1963), the role of micropinocytosis in this does not

appear to have been previously recognised. The process of micropinocytosis with the appearance of a spike-like fringe on the membrane and its subsequent pinching off has been described in relation to normal profiles in the central nervous system (Andres, 1964); the appearances described here in relation to degenerating material are very similar and the process would appear to be identical. All stages of the process have been seen, from a simple fringe of spikes on the membrane through all degrees of pinching off, to dense-cored coared vesicles in profiles adjacent to the degeneration. The involvement of dendrites in the removal of degeneration has been previous described (Walberg, 1963) and this has been clearly confirmed here and in addition neuronal somata and dendritic spines have been found to phagocytose degenerating terminals by micropinocytosis. What the ultimate fate of this phagocytosed material may be is however not clear.

The presence of glial cytoplasm surrounding degenerating axons within their myelin sheaths has been previously described (Jones and Powell, 1970d). It would seem from this present study that glia invade degenerating myelinated axons at both nodes of Ranvier and by breaking through the myelin at other points and that they remove the degenerating axoplasm to leave empty myelin sheaths. The eventual fate of these empty sheaths is unclear but those seen to have become applied to pyramidal apical dendrites appear at first sight remarkably like the myelinated dendritic segments seen in the olfactory bulb of the monkey (Pinching, 1971).

However they clearly are not so as they lack the typical end feet of a true myelin sheath and are instead doubled back on themselves and they also cannot completely surround the dendrite as the true myelin sheath does.

Exposed postsynaptic membrane specialisations have been found not uncommonly in this material. In the olfactory bulb they have been shown to be left after the complete removal of the degenerating axon terminal (Pinching and Powell, 1972) and it seems likely that they are similarly left in the cortex, although the possibility of their existence in normal material, perhaps as a developmental remnant, cannot be ruled out. In this study exposed postsynaptic thickenings have been seen applied to all components of the cortical neuropil, apparently at random, with the exception of axon terminals; this is probably due to the great difficulty, if not impossibility, of differentiating them from a normal synapse at this site. In a proportion of all the neuronal components to which they have become apposed, including axon initial segments and unmyelinated axons as well as somata and dendrites, the exposed membrane thickenings often occur opposite subsurface cisternae as has been reported in the olfactory bulb (Pinching and Powell, 1972). This has not been seen with exposed membrane thickenings opposite glial profiles. The subsurface cisternae seen are similar to those related to symmetrical axon terminals in the same material (Chapter 3) but their function is not clear. It may be significant however in the context of the

discussion about the possible reinnervation of these exposed postsynaptic sites, that when an exposed asymmetric membrane thickening has been seen apposed to an unmyelinated axon making an asymmetric synapse elsewhere (Chapter 5) the exposed thickening had induced a subsurface cistern opposite itself in exactly the same way as those opposite cell somata or dendrites. If it had however induced a new synapse it would presumably have been indistinguishable from a normal synapse and so not recognisable.

CHAPTER 9

AN EXPERIMENTAL ELECTRON MICROSCOPIC STUDY OF AFFERENT CONNECTIONS TO THE MOTOR AND SOMATIC SENSORY CORTICES

Introduction

The electron microscopic study of terminal degeneration following the placement of lesions in the nervous system is now well established as a technique (Guillery, 1970) and has been used by a number of authors to study afferent connections to different sensory areas of the neocortex in various species (e.g. Colonnier and Rossignol, 1969; Jones and Powell, 1970d,e; Lund and Lund, 1970; Garey and Powell, 1971; Fisken, Garey and Powell, 1975) and the thalamo-cortical projection to the motor cortex has been studied in the cat (Strick and Sterling, 1974). Because these previous studies have been mainly of sensory cortical areas this study has been primarily of the motor cortex. A systematic study has been made of the afferent connections to the motor cortex from the thalamus, contralateral motor cortex, the ipsilateral somatic sensory cortex (SI) and the ipsilateral premotor cortex (area 6), these connections being known from light microscopic studies (Walker, 1938; Jones and Powell, 1969 a,b; Pandya and Kuypers, 1969; Pandya and Vignolo, 1971). The motor and somatic sensory cortices have been thought to be very different in character and so the thalamo-cortical and commissural afferents to area 3b of the somatic sensory cortex have been studied to give a direct comparison of these afferent connections and also because they have not previously been studied in the monkey.

Knowledge of the normal ultrastructure of the sensori-motor cortex was essential for the interpretation of these

experimental findings and it has been studied in conjunction with this experimental work. It is reported in Chapters 3 to 8 and the normal and experimental findings have been discussed together (Chapter 10) to see what light they can throw on the organisation and functioning of the neocortex.

Results

Following the placement of lesions in the thalamus, the contralateral sensori-motor cortex and the ipsilateral primary somatic sensory cortex (SI) and premotor cortex (area 6) typical degenerating axon terminals were found in the sensori-motor cortex of the monkey. These terminals showed swelling of synaptic vesicles, cytoplasmic darkening and progressive disruption of their internal structure with engulfment by glia as described previously in various sites (e.g. Colonnier, 1964; Jones and Powell, 1970d; Pinching and Powell, 1972); the features of the process of degeneration in the axon terminals studied here are described in detail elsewhere (Chapter 8). No structure was identified as a degenerating axon terminal in this study unless it showed clear evidence of degenerative change as described above and also had an unequivocal postsynaptic specialisation. Throughout the examination of this material the whole depth of the cortex was studied systematically and all degenerating terminals recorded, so that an unbiased sample of terminals was obtained; this allows valid quantitative comparisons to be made between the different connections.

Thalamo-cortical afferents

Following large thalamic lesions, dense degeneration was found in both motor cortex and area 3b of the somatic sensory cortex. After a survival period of four days much of the degeneration seen was at an early to medium stage (Figs. 9-1 to 9-3) and at 5 days medium and late stages of degeneration predominated (Figs. 9-4 and 9-5). Extensive sampling and mapping were therefore done at the later survival period.

Degenerating thalamo-cortical terminals were found to have asymmetric membrane specialisations in all cases where the synaptic type was clearly identifiable. Of a total of 553 degenerating terminals studied in the motor cortex 486 (88%) made synapses on to a single identifiable postsynaptic profile, 40 (7.2%) on to two postsynaptic profiles and 6 (1.1%) on to three postsynaptic profiles in a single section. In the remaining small proportion of cases the postsynaptic profile could not be clearly identified. Of those terminals making synapses on to a single identifiable postsynaptic profile, 89.5% made synapses on to dendritic spines (Figs. 9-1 to 9-5), 9% on to dendritic shafts and 1.5% directly on to cell somata (Table 9-1), the whole depth of the cortex having been sampled systematically (see below). Those degenerating terminals making synapses on to more than one profile in a single section contacted two or three spines (Figs. 9-6 and 9-7), a spine and one or two dendritic shafts (Fig. 9-12) and in one case two spines and a cell soma, there being also a few

TABLE 9-1

Proportions of the different types of postsynaptic profile receiving synapses from degenerating axon terminals.

Connection	Proportions of degenerating axon terminals making synapses on to each type of post-synaptic site (as a percentage of the total number of single identifiable postsynaptic profiles)		
	Spine %	Dendrite %	Soma %
Thalamo-cortical			
Motor	89.5	9	1.5
Area 3b	89	11	-
Commissural			
Motor	96	3	< 1
Area 3b	97	3	-
Association			
SI -> Motor	82	18	-
Area 6 -> Motor	76	24	-

examples where one profile could not be identified. Examples were also found where a single spine received synapses from both a degenerating thalamo-cortical terminal and a normal asymmetric or symmetrical axon terminal (Figs. 9-8 to 9-10).

In area 3b of the somatic sensory cortex, of a total of 223 degenerating terminals studied 183 (84%) made synapses on to a single identifiable postsynaptic profile and 26 (12%) on to two profiles. Of those making synapses on to a single identifiable postsynaptic profile 89% contacted dendritic spines (Figs. 9-13 and 9-14) and 11% dendritic shafts (Table 9-1); one example made a synapse on to a cell soma and a spine. Of those making double contacts examples were found where a single degenerating terminal made synapses on to two spines (Fig. 9-16) or a spine and a dendritic shaft in a single section and examples were also found where a spine received synapses from both a degenerating terminal and a normal asymmetric or symmetrical terminal (Fig. 9-16).

In fortunate single sections and by extensive use of serial sections 32 spines receiving synapses from degenerating thalamo-cortical terminals were traced to their parent dendrites in the motor cortex and 7 were traced in area 3b of the somatic sensory cortex. Most of these parent dendrites were of small or medium size, between 0.2 and 1.0 μ in diameter, and rarely received other synapses (Figs. 9-11 and 9-15). They were not varicose in shape and contained few organelles although they contained a number of microtubules. A few of the dendrites were

larger, around 2 μ in diameter, and contained a few more organelles (Fig. 9-8) and these dendrites characteristically contained a considerable number of relatively evenly spaced microtubules and had a regular outline. Some of these larger dendrites were orientated obliquely in relation to the cortical surface but in two examples found in sections cut parallel to the cortical surface in layer IV of the motor cortex the parent dendrites of such spines appeared to be the apical dendrites of deep pyramidal cells cut in transverse section.

The majority of the 44 dendritic shafts found receiving synapses from degenerating thalamo-cortical terminals in the motor cortex (Figs. 9-17 to 9-20) and the 20 from the somatic sensory cortex (Figs. 9-21 to 9-23) received a high demsity of other synapses, with a proportion of these being asymmetric and in a few examples two degenerating terminals were found to make synapses on to the same dendrite. Most of these dendrites contained abundant mitochondria and ribosomes and, when cut in longitudinal section, they were frequently varicose in outline and ran in a straight line for considerable lengths. All these features identify these dendritic shafts as arising from large stellate cells and on one occasion a dendrite of this type receiving synapses from two degenerating thalamo-cortical axon terminals was traced in continuity with a large stellate cell soma (Fig. 9-17). A similar dendrite was also found which received synapses from two degenerating thalamo-cortical terminals and made a gap junction,

the latter being a feature predominantly of large stellate cells in the sensori-motor cortex (Chapter 7). Most of the dendritic shafts receiving synapses from degenerating thalamo-cortical terminals were between 0.5 μ and 2 μ in diameter but a few were larger. In the motor cortex two degenerating thalamo-cortical terminals were found making synapses on to the shafts of fairly large dendrites each of which also had a spine receiving a synapse from a normal axon terminal; only one other synapse was present on the shaft of either dendrite and so these dendrites were probably pyramidal in origin. In area 3b one degenerating terminal was found to make a synapse on to the shaft of a very large (diameter 6 μ) vertically orientated dendrite at the base of a side branch (Figs. 9-22 and 9-23); this dendrite received only two other synapses in spite of its large size and had the typical appearance of a pyramidal apical dendrite.

Seven cell somata were found to receive synapses from degenerating thalamo-cortical axon terminals in the motor cortex and one was found in the somatic sensory cortex. All received numerous synapses both asymmetric and symmetrical and all had abundant cytoplasm containing a high concentration of mitochondria and ribosomes and clusters of rough endoplasmic reticulum (Figs. 9-24 to 9-26). All these cell somata were therefore typical of the large type of stellate cell seen in both the motor and somatic sensory cortices (Chapter 4). The histograms of the size distribution of these cells and of the numbers of synapses on to

them are shown in Fig. 9-27.

Degenerating thalamo-cortical axon terminals were found in all layers of the cortex in both the motor cortex and area 3b of the somatic sensory cortex but there were striking differences in the density of degenerating terminals in the different laminae. In the motor cortex a few degenerating terminals were present in layers I and II and in the upper half of layer III; there was a very dense band of degenerating terminals occupying the lower half of layer III and the upper two-thirds of layer IV; some degenerating terminals were present in the lower third of layer IV and the upper two-thirds of layer V, and there was a moderately dense band of degenerating terminals in the lowest part of layer V and the top of layer VI with a few below this level. This distribution of degenerating axon terminals was mapped in three sections taken accurately one below the other from one block of motor cortex so that an accurate representation of the distribution through the whole cortical depth was obtained (Fig. 9-28).

The relationship of the degeneration to the laminae of the motor cortex was also studied with the light microscope using the Wiitanen modification of the Nauta technique, a technique which shows cell somata and so the cortical laminae. Study of this material confirmed that the dense band of degeneration was in the lower half of layer III and the upper two-thirds of layer IV and also confirmed the presence of a second much less dense band of degeneration in the boundary region between layers V and VI.

The degeneration in both bands consisted mostly of fine granules thought to represent degenerating axon terminals and preterminal fragments.

With the electron microscope area 3b of the somatic sensory cortex also showed a very dense band of degenerating thalamo-cortical axon terminals in the lower half of layer III and the upper two-thirds of layer IV. Above this level there were a few scattered degenerating terminals and below it there were rather more than above, but there did not appear to be a second concentration of degenerating terminals at the boundary of layers V and VI. This distribution of degenerating terminals was mapped as in the motor cortex, two sections being required to cover the full cortical depth (Fig. 9-29).

Light microscopy of Wiitanen-stained sections from area 3b of the somatic sensory cortex showed a dense band of finely granular degeneration in the lower half of layer III and the upper two-thirds of layer IV and also confirmed the lack of a second band of degeneration in the deep laminae.

In the electron microscopic maps of degeneration described above the nature of the postsynaptic profile or profiles was recorded for each degenerating thalamo-cortical terminal. The distribution of the different postsynaptic profiles receiving synapses from degenerating thalamo-cortical terminals is shown in the maps of the motor cortex (Fig. 9-30) and area 3b (Fig. 9-31) and their depth distribution is analysed in the adjacent histo-

grams. Those degenerating terminals classed as ending on spines are those ending on one or more spines but not those ending on a spine plus a dendrite or cell soma; those classed as ending on dendritic shafts or cell somata similarly exclude those ending on more than one type of profile. It can be seen from these histograms that the degenerating terminals in the dense band in layers III and IV make synapses mostly on to dendritic spines and the distribution of degenerating terminals contacting spines corresponds approximately to the overall distribution of degenerating terminals. However those degenerating terminals making synapses on to dendritic shafts do not follow this overall pattern but are fairly evenly spread through the deeper layers of the cortex, occurring mainly below the dense band of degeneration in layers III and IV, this being better shown in the motor cortex where the deep laminae are thicker. The two cell somata receiving synapses from degenerating thalamo-cortical terminals belong to the same type of neuron as the majority of the dendritic shafts and appear to occur within the same distribution. The difference between the distribution of degenerating thalamo-cortical axon terminals making synapses on to spines and those making synapses on to dendrites and somata was tested statistically by comparing the mean depths of the two distributions. This showed a significant difference between the two distributions in the motor cortex (Student's t-test $t = 2.697$ $P < .01$); in the somatic sensory cortex although the means depths were different this was not

statistically significant. This difference in the distribution of degenerating terminals ending on spines and dendrites or somata means that the proportions ending on the different post-synaptic sites vary greatly between different laminae. If the histograms (Figs. 9-30 and 9-31) are divided immediately below the dense bands of degeneration (below bin 12) and the proportions making synapses on to each site above and below this level are calculated, it is found that in the motor cortex 5% of the degenerating terminals contact dendrites or somata within and above the dense band whereas 29% do so below it and in the somatic sensory cortex 9% contact dendrites within and above the dense band and 18% below it.

When studied in sections taken parallel to the pial surface of the motor cortex the apical dendrites of deep pyramidal cells may be seen to occur in groups as they pass through layer IV and the lower part of layer III, this being more marked in some parts of the motor cortex than others (Chapter 3). Sections of this experimental material from the motor cortex were therefore taken parallel to the cortical surface at this level, corresponding also to the dense band of degeneration in layers III and IV, in order to study the relationship between the apical dendrites and degenerating thalamo-cortical axon terminals. Qualitative study showed that many of the degenerating terminals occurred close to or within the bundles of apical dendrites and all but a few of them made synapses on to dendritic spines. Although some

degenerating terminals were found in the intervening neuropil, there appeared to be some specific association between the degenerating thalamo-cortical terminals and the bundles of apical dendrites and the degenerating terminals often occurred in very close proximity to apical dendrites (Fig. 9-32). Three maps were therefore done of sections taken parallel to the pia at about the level of the boundary of layers III and IV from two brains and the positions of all the degenerating terminals and apical dendrites were plotted. Fig. 9-33 shows one example and this confirms the impression that the degenerating terminals frequently occur in relation to the apical dendrites and measurement of all the maps showed that about three-quarters of the degenerating thalamo-cortical terminals occurred within 10-15 μ of an apical dendrite. The maps were analysed statistically by superimposing a grid of squares randomly on to each map and noting the numbers of apical dendrites and degenerating terminals in each square. If the position of degenerating thalamo-cortical terminals is independent of that of apical dendrites then the proportion of squares containing degenerating terminals should be the same whatever the number of spical dendrites in the square. The actual results are shown in Table 9-2 for the map illustrated, Tables 9-3 and 9-4 for the other two maps, and the overall results are shown in Table 9-5. From these tables it can be seen that the proportion of the squares containing degeneration in fact increases with the number of apical dendrites in a square and that overall a square containing one or more apical dendrites is

about twice as likely to contain a degenerating terminal as a square with no apical dendrites. These differences in the frequency of degeneration between those squares containing no apical dendrites and those containing one or more apical dendrites were tested statistically using Student's t-test. For maps 1 and 2 the differences in frequency of degeneration between squares with no apical dendrites and those with one or more are significant (Map 1, $t = 2.88$ $P < .01$; Map 2, $t = 2.655$ $P = .01$) and for these same maps the differences between squares containing no apical dendrites and those containing two or more apical dendrites are also significant (Map 1, $t = 2.73$ $P < .01$; Map 2, $t = 2.65$ $P < .01$). The differences for map 3 do not reach statistical significance. This map was from a deeper level in the cortex than the other two maps and may be below the optimal level for the association. When all three maps are pooled the differences are highly significant for both the comparison between squares with no apical dendrites and one or more apical dendrites ($t = 3.65$, $P < .001$) and between squares with no apical dendrites and those containing two or more apical dendrites ($t = 4.54$ $P < .0001$). There is thus some factor causing apical dendrites and degenerating thalamo-cortical axon terminals to occur together in layer IV of the motor cortex more often than would be expected on a random basis.

TABLE 9-2

Map 1

Number of apical dendrites per square	Number of squares with degeneration	Number of squares without degeneration	Total	% of squares with degeneration
0	6	57	63	9.5
1	16	42	58	28
2	13	31	44	34
3	3	14	17	18
4	4	7	11	36
5	2	3	5	(40)
6	0	2	2	-
⩾ 1	38	99	137	28
⩾ 2	22	57	79	28

TABLE 9-3

Map 2

Number of apical dendrites per square	Number of squares with degeneration	Number of squares without degeneration	Total	% of squares with degeneration
0	13	174	187	7.1
1	23	148	171	13.5
2	12	61	73	16.4
3	4	17	21	19.0
4	1	4	5	(20.0)
5	0	1	1	-
⩾ 1	40	231	271	14.0
⩾ 2	17	83	100	17.0

TABLE 9-4

Map 3

Number of apical dendrites per square	Number of squares with degeneration	Number of squares without degeneration	Total	% of squares with degeneration
0	18	68	86	21
1	9	56	65	14
2	13	33	46	28
3	8	16	24	33
4	6	5	11	55
5	0	1	1	-
6	0	1	1	-
7	0	1	1	-
⩾ 1	36	113	149	24
⩾ 2	27	57	84	32

TABLE 9-5

Pooled Results

Number of apical dendrites per square	Number of squares with degeneration	Number of squares without degeneration	Total	% of squares with degeneration
0	37	299	336	11
1	48	246	294	16
2	38	125	163	23
3	15	47	62	24
4	11	16	27	41
5	2	5	7	(29)
6	0	3	3	-
7	0	1	1	-
⩾ 1	114	443	557	21
⩾ 2	66	197	263	25

Commissural connections

The commissural connections of both the somatic sensory and motor cortices have been shown by light microscopy to be restricted to those areas of cortex corresponding to the more midline parts of the body (Jones and Powell, 1969b; Pandya and Vignolo, 1971) and so material for the electron microscopic study of the commissural connections was taken from the trunk area of the motor cortex and area 3b of the somatic sensory cortex, this being at about the level of the lower end of the intraparietal sulcus. Following removal of the contralateral sensori-motor cortex typical degenerating axons and axon terminals were found in both the motor cortex and area 3b of the somatic sensory cortex (Figs. 9-34 to 9-47). At four days' survival the degeneration was fairly sparse with early forms predominating, at five days' survival more degeneration was present with a greater proportion of medium and late forms and at six days' survival the degeneration was fairly dense and predominantly late in character. Extensive sampling and mapping were therefore done at the six day survival period.

In all cases where the synaptic membrane complex of degenerating commissural terminals could be clearly classified it was of the asymmetric type in both motor and somatic sensory cortices. Of a total of 468 degenerating commissural terminals studied in the motor cortex 394 (84%) made synapses on to a single identifiable postsynaptic profile. Of these 96% contacted a

dendritic spine (Figs. 9-34, 9-36 and 9-37) and 3% a dendritic shaft while one terminal was found to make a synapse on to a cell soma directly (Table 9-1). Twenty-eight (6%) of the 468 motor commissural terminals made synapses on to two postsynaptic profiles in a single section; 23 of these contacted two spines (Fig. 9-35), two contacted a spine and the shaft of a varicose dendrite and three a spine and an unidentifiable process. In about 10% of all examples the postsynaptic process could not be identified. Examples were also found of spines receiving synapses from both a degenerating terminal and a normal asymmetric or symmetrical axon terminal (Fig. 9-38).

In area 3b of the somatic sensory cortex 183 degenerating commissural terminals were studied of which 158 (86%) made synapses on to a single identifiable postsynaptic profile. Of these 97% contacted dendritic spines (Figs. 9-39 and 9-41) and 3% dendritic shafts (Table 9-1). Fifteen (8.2%) of the total of somatic sensory commissural terminals made synapses on to two spines (Fig. 9-40) and one contacted three spines in a single section (Fig. 9-42) and examples were also found of both a normal and a degenerating terminal making synapses on to the same spine.

Ten spines receiving degenerating commissural terminals were traced to their parent dendrites in the motor cortex (Fig. 9-36) and six in the somatic sensory cortex (Fig. 9-42). All the parent dendrites in both areas were of small or medium size, between 0.3 and 1.2 μ in diameter, and were in general smaller than the parent

dendrites of spines receiving synapses from degenerating thalamo-cortical terminals. The shafts of these parent dendrites rarely received other synapses and they contained few organelles and were generally of approximately constant width when cut longitudinally. They had therefore the features of dendrites of pyramidal cells and one dendrite was found in the somatic sensory cortex which had one spine receiving a synapse from a degenerating commissural terminal and a second spine receiving one from a normal asymmetric terminal.

Of the dendritic shafts receiving synapses from degenerating commissural terminals in both areas of cortex about half received a number of other asymmetric synapses and contained numerous ribosomes and mitochondria and so could be identified as the dendrites of large stellate cells (Figs. 9-43 to 9-45). These were in general of similar diameter to the comparable dendritic shafts receiving synapses from degenerating thalamo-cortical terminals except that no very large examples were found. Of the remainder of the dendritic shafts receiving synapses from degenerating commissural terminals the majority received asymmetric synapses or had a varicose outline and so were also stellate in origin; some were probably of the large stellate type but the markedly varicose shape and lack of organelles of some of the others suggest that they belong to small stellate cells (Chapter 4). One degenerating commissural terminal from the motor cortex made a synapse on to the shaft of a small dendrite which had a spine but received no other synapse.

The cell soma found to receive a synapse from a degenerating commissural terminal in the motor cortex was fairly large. It received a number of other synapses, including a clearly asymmetric one, and had abundant cytoplasm full of mitochondria, ribosomes and endoplasmic reticulum. It was therefore the soma of a large stellate cell (Figs. 9-46 and 9-47).

Degenerating commissural axon terminals were found in all layers of the cortex in both the motor cortex and area 3b of the somatic sensory cortex. The distribution of these degenerating terminals was mapped as for the thalamo-cortical projection. Fig. 9-48 shows a map of an individual section of the superficial third of the motor cortex to give the actual density of degenerating terminals found and it also appears to show some tendency for the degenerating terminals to occur in clusters. The composite map of area 4 consists of three individual maps superimposed at each level and so of nine maps in all and that of the somatic sensory cortex consists of three superimposed maps of each of the superficial and deep halves of the cortex, a total of six maps. When the maps have been superimposed it can be seen in the motor cortex that the degeneration is present in all layers but is comparatively more dense in layer I, the upper part of layer III, the upper part of layer V and the lowest part of layer V with layer VI, there being relatively clear bands in layer IV and the middle of layer V (Fig. 9-48). In area 3b of the somatic sensory cortex there is also degeneration in all the cortical layers but there is a

dense concentration of degeneration in layers II and III and this is much more pronounced to one side of the mapped area (Fig. 9-49). A further section taken of the same area of the superficial half of the cortex but further into the block showed that this patch of degeneration was still present but was markedly less dense. It would therefore appear that this was an isolated patch of dense degeneration. However at its most dense it did not involve more than a small percentage of the terminals in the area and no degenerating symmetrical axon terminals were found. The material was well preserved and there was no evidence of direct damage to the cortex in this area, this region not having been exposed at operation. The degeneration was qualitatively the same as other commissural degeneration and it would therefore appear that there was a genuine difference in the density of degenerating commissural axon terminals in different parts of layers II and III in this material of the somatic sensory cortex. Such differences in the density of degeneration have been confirmed by light microscopy of the somatic sensory cortex following contralateral cortical lesions (Shanks, Rockel and Powell, 1975).

The small proportion of degenerating commissural terminals making synapses on to dendritic shafts occurred at all levels in the cortex and there were no differences apparent in the proportions of the different types of postsynaptic process in the different laminae (Figs. 9-50 and 9-51). The cell soma receiving a degenerating commissural axon terminal was in the upper part of layer V of the motor cortex.

Association connection from SI to the motor cortex

Following the removal of the ipsilateral primary somatic sensory cortex (SI, areas 3b, 1 and 2 of Brodmann) typical degenerating axon terminals were found in the motor cortex. A few early signs of degeneration were present at 1¼ days' survival and after 2 days a few unequivocal degenerating terminals showing marked cytoplasmic darkening and internal disruption were present. Rather more degeneration was present after 3 days and the peak density appeared to be at 4 and 5 days' survival, with a predominance of medium and late forms of degeneration. At 6 days most of the degeneration was of the late variety. Mapping was therefore done on 5-day survival material.

In all cases where the synaptic type was clearly identifiable degenerating axon terminals from SI had asymmetric membrane specialisation. Of 142 degenerating terminals studied 119 (84%) made synapses on to a single identifiable postsynaptic profile. Of these 82% contacted dendritic spines (Figs. 9-52 to 9-54 and 9-56) and 18% dendritic shafts. Seven (4.9%) of all the terminals made synapses on to two profiles and one on to three spines in a single section (Fig. 9-55); of those contacting two profiles, 4 made synapses on to two spines and 3 on to a spine and a dendritic shaft, one of these dendrites being of the large stellate type and the other two having clearly varicose shapes.

Three spines receiving synapses from degenerating terminals from SI were traced to their parent dendrites. These

dendrites received few other synapses, contained few organelles and did not have a varicose outline. Of the 22 dendritic shafts found to receive degenerating terminals from SI almost all could be identified as being of the varicose type because they received other asymmetric synapses or had a markedly varicose shape (Figs. 9-57 to 9-59). The majority received a number of synapses and contained prominent mitochondria and ribosomes and so were of the large stellate type and some of these were large and ran in straight lines for some considerable length (Fig. 9-58). The markedly varicose shape and lack of organelles of a few suggested that they were dendrites of small stellate cells.

Degenerating terminals from SI were found scattered through all the cortical laminae. They showed no marked differences in density between the different layers but in general more were present in the superficial half of the cortex. The individual map of the middle third of the cortex (Fig. 9-63) shows the density of degeneration found and the composite map made up from two superimposed sections at each level confirms the greater density of degeneration in the superficial half of the cortex (Fig. 9-63). The dendritic shafts receiving degenerating terminals were distributed through the depth of the cortex and there were no marked differences in the proportions of each type of postsynaptic profile contacted at different depths (Fig. 9-64).

Association connection from area 6 to the motor cortex

Because of the necessary restriction of area 6 lesions

to prevent direct damage to the motor cortex the degeneration found was sparse and so a detailed study could not be made. The material was well preserved and no evidence to suggest any direct damage to the motor cortex was found.

Degenerating terminals of medium and late stages were present in the motor cortex 4 and 5 days after area 6 lesions and, when classifiable, their postsynaptic thickenings were of the asymmetric type. Of 43 terminals found 38 (88%) made synapses on to a single identifiable postsynaptic process, 76% on to spines (Figs. 9-60 and 9-61) and 24% on to dendritic shafts (Fig. 9-62). Two degenerating terminals made synapses on to two spines in a single section and one spine was found receiving a synapse from a degenerating terminal and a symmetrical synapse from a normal axon terminal.

Two spines receiving synapses from degenerating association terminals from area 6 were traced to their parent dendrites. Both of these dendrites were small, received few other synapses and did not have a varicose shape or contain many organelles. Of the 9 dendritic shafts receiving synapses from area 6 nearly all were varicose in type from their shape or because they received asymmetric synapses. The majority of them were of the large stellate type because they received a number of synapses and contained a high concentration of mitochondria and ribosomes and several of them were of quite large diameter.

Degenerating terminals from area 6 were found scattered

through all layers of the cortex, with no obvious differences in density between different laminae. They were however very sparse and because of this mapping was not carried out.

Discussion

There have previously been a number of experimental electron microscopic studies of projections to sensory areas of the neocortex from various sites in different species (e.g., Colonnier, 1964; Colonnier and Rossignol, 1969; Jones and Powell, 1970d,e; Lund and Lund, 1970; Garey and Powell, 1971; Fisken, Garey and Powell, 1975) and the thalamo-cortical projection to the motor cortex has been studied in the cat (Strick and Sterling, 1974). The results of these studies indicate a basic similarity between the different sensory cortical areas. The motor cortex has however been considered on the basis of light microscopy to be a different type of neocortex from the primary sensory areas (see Chapter 4) and so it is of interest to compare its connections with those of the sensory areas. The electron microscopic study of the normal structure of the motor cortex, done in conjunction with this experimental work, has indicated that the motor cortex is in fact remarkably similar to area 3b of the somatic sensory cortex in many respects. It is therefore not surprising that this experimental study should have shown that the afferent projections to the motor cortex are likewise very similar to the corresponding projections to sensory areas of the neocortex. The main differences between the projections to the motor and

sensory cortices would appear to be in their relationships to the different cortical laminae and these are discussed in detail below. They would appear to be reflections of differences in the degree of development of the various laminae in different areas and are present between different sensory areas of cortex as well as between motor and sensory areas.

In considering these findings in relation to those of other experimental studies of the neocortex a number of technical points should be mentioned. The features used to recognise degenerating axon terminals in this study were those generally accepted (Guillery, 1970), including in all cases a definite synaptic membrane specialisation. The full depth of the cortex was always studied systematically and every degenerating terminal found was recorded, usually photographically, and so the samples of terminals obtained are representative of the whole of the projection studied; this is particularly important in relation to the thalamo-cortical projections where a sample biased towards a particular cortical layer would significantly alter the proportions of terminals found to make synapses on to the different types of postsynaptic structure because of the variation of these proportions at different depths from the cortical surface. Recognition of the various types of postsynaptic structure is of course dependent on a knowledge of the normal ultrastructure. The criteria used to distinguish spines and dendrites were those generally accepted (Jones and Powell, 1969d; Peters, Palay and Webster, 1970) and

used by previous authors and confirmed by study of the normal ultrastructure of the motor cortex (Chapter 4). In general dendrites were easily recognisable because of the number of positive features they show and it is very unlikely that any significant number was misidentified. The positive identification of isolated spines is more difficult because in the absence of a spine apparatus they are featureless profiles. In most cases small featureless profiles with flocculent cytoplasm were classified as spines and the correctness of this was confirmed by serial sections in a considerable number of examples. Between about 5 and 10% of profiles receiving synapses from degenerating terminals of each type were considered unclassifiable. It is likely that almost all of these postsynaptic profiles were in fact spines; recalculation of the proportions of degenerating terminals contacting each type of postsynaptic site making the assumption that all those unclassified were spines increases the proportion ending on spines by less than 2% where this proportion is less than 90% and by less than 1% where it exceeds 90%. The influence of this unidentifiable group is therefore negligible. A significant proportion of degenerating terminals end on more than one profile in a single section. In calculating the proportions of degenerating terminals making synapses on to each type of postsynaptic profile these have been excluded so that the proportions calculated are of those terminals ending on a single identifiable postsynaptic profile. These proportions have therefore been calculated on a consistent basis for all the

projections studied here and this, together with uniform sampling, allows useful comparisons to be made between the different connections.

Thalamo-cortical connections

The findings of this study in relation to the thalamo-cortical projections are similar in most respects to those of electron microscopic studies of the thalamic projections to the somatic sensory cortex of the cat (Jones and Powell, 1970e) and the visual cortical areas of the cat and monkey (e.g. Colonnier and Rossignol, 1969; Garey and Powell, 1971) as well as of that to the motor cortex of the cat (Strickland and Sterling, 1974). The type of terminal undergoing degeneration appears very similar in all the areas studied although there may be slight differences in the rate of degeneration between areas or species. The proportions of degenerating terminals ending on spines, dendrites and cell somata in the monkey are very similar in the motor cortex and area 3b of the somatic sensory cortex. Although differences in sampling methods make comparison less useful, they would also appear to be similar in area 17 of the monkey visual cortex where Garey and Powell (1971) found 84% of degenerating thalamo-cortical terminals making synapses on to spines with 14% on to dendrites and 2% on to cell somata. Results in areas 17, 18 and 19 of the visual cortex and in the somatic sensory cortex of the cat are also within the same range with spines receiving more than 75% of all degenerating thalamo-cortical terminals in these areas (Jones and Powell, 1970e;

Garey and Powell, 1971). It will be of interest to see whether the differences found between areas 17, 18 and 19 in the cat are confirmed by samples taken through the whole depth of the cortex rather than just from layer IV in view of the differences between laminae and, if so, to see whether comparable differences exist between areas 3, 1 and 2 of the somatic sensory cortex.

Because most of the spines in the neocortex are seen in Golgi preparations to arise from pyramidal cells, Jones and Powell (1970e) considered that the spines receiving synapses from degenerating thalamo-cortical axon terminals were those of pyramidal cells. The failure of impregnation of spines on pyramidal cell dendrites following eye removal was also interpreted as showing this (Globus and Scheibel, 1967b) although this was almost certainly a transneuronal effect. However the existence of stellate cells with spiny dendrites has been emphasised by Garey (1971), Garey and Powell (1971) and Lund (1973) and they have suggested that, although these stellate spines form only a small proportion of the total number of spines in the cortical neuropil, it is possible that thalamo-cortical terminals end specifically on them. However, spiny stellate cells are much less frequent in the somatic sensory cortex (Jones, 1975a). To attempt to resolve this question a considerable number of examples have been collected in which the parent dendrites of spines received synapses from degenerating thalamo-cortical terminals were identified. With rare exceptions

these dendrites received few other synapses, contained few organelles and were not varicose in outline. They were therefore clearly not the dendrites of large or small stellate cells although the former do have occasional spines (Chapter 4). Although the possibility that these parent dendrites are those of an as yet unrecognised cell type cannot be completely ruled out, the characteristics of these parent dendrites are those of the small and medium sized dendrites of pyramidal cells (Jones and Powell, 1970a; Chapter 4) and the few large parent dendrites found were almost certainly main apical or basal dendritic branches of pyramidal cells. There would seem to be little doubt that most of the spines receiving synapses from thalamo-cortical axon terminals arise from pyramidal cells and, since most thalamo-cortical terminals contact spines, it would seem that most thalamo-cortical afferents make synapses directly on to pyramidal cells. This conclusion is in agreement with the finding in physiological studies of the thalamic projection to the motor cortex that thalamic stimulation produces monosynaptic EPSPs in pyramidal tract cells (Amassian and Weiner, 1966).

The remainder of the thalamo-cortical terminals made synapses on to dendrites and cell somata. Most of these dendrites received a high density of other synapses, with a considerable proportion of them being asymmetric, and they contained a high concentration of mitochondria and other organelles. They were often varicose in shape and tended to run in straight lines for

some considerable distance and a proportion of them were of large diameter. These dendrites were therefore clearly of the large stellate type and this was confirmed by an example in which a dendrite of this type which received synapses from two degenerating thalamo-cortical terminals was traced to a large stellate cell soma. The finding of a gap junction on a dendrite of this type which also received synapses from two degenerating thalamo-cortical terminals was also further confirmation of the large stellate nature of these dendrites (Chapter 7). The features of dendritic shafts found to receive synapses from degenerating thalamo-cortical axon terminals in the somatic sensory cortex of the cat (Jones and Powell, 1970e) and in the visual cortices of the cat and monkey (Garey and Powell, 1971) also appear to correspond to those of large stellate cells. Three examples were found in this study in which degenerating thalamo-cortical terminals made synapses on to dendritic shafts which were clearly not of the large stellate type. Two of these had spines and all three were probably of pyramidal origin.

All the cell somata receiving synapses from degenerating thalamo-cortical axon terminals were undoubtedly those of large stellate cells because of the numbers of synapses they received, including asymmetric ones, because of their abundant cytoplasm full of mitochondria and ribosomes and because of their content of stacks of endoplasmic reticulum, and if the histograms of the mean cell diameters and numbers of synapses received by this group of cells are compared to the corresponding histograms for large

stellate cells studied in normal material (Chapter 4) it can be seen that the populations correspond. Those cell somata found to receive synapses from degenerating thalamo-cortical terminals in the visual cortex also appear to be of this large stellate type (Garey and Powell, 1971). The thalamo-cortical projection therefore appears to consist of two main components; pyramidal cells would appear to receive synapses from the majority of thalamo-cortical axon terminals by way of their spines and large stellate cells are contacted by a small proportion of the terminals by way of synapses on their dendrites and somata.

There is general agreement that the great majority of degenerating thalamo-cortical terminals are found in a band in the region of layer IV in the adult animal (e.g., Jones and Powell, 1970e; Garey and Powell, 1971; Strick and Sterling, 1974). However this study indicates that in the monkey this band of degeneration extends well up into layer III in both the motor cortex and area 3b of the somatic sensory cortex and the electron microscopic findings were confirmed by light microscopy using a technique by which the laminae can be clearly identified. Using similar techniques Hubel and Wiesel (1969, 1972) and Garey and Powell (1971) have described a separate band of degeneration in layer IIIb of area 17 of the monkey visual cortex following thalamic lesions and its separation from the main band of degeneration in layer IV has been ascribed to the presence of the Stria of Gennari in layer IIIc. In area 3b of the somatic sensory

cortex of the monkey where the outer band of Baillarger is not prominent there is only one band of dense degeneration which extends well up into layer III but in the motor cortex of the monkey there is a separate band of degeneration in layer V. This is below the well developed inner band of Baillarger and this degeneration would appear to have been separated from the main band in a similar way to that in the visual cortex but by the deep fibre plexus. It would therefore appear that the basic laminar pattern of the thalamo-cortical projection in the monkey is that seen in area 3b of the somatic sensory cortex of a single dense band extending from the middle of layer III to about two-thirds of the way through layer IV with some scattered degeneration below this level, and that this has been modified in the other two cortical areas by the prominence of the inner or outer bands of Baillarger. In the cat the laminar termination of thalamo-cortical afferents has been studied in areas 17, 18 and 19 of the visual cortex (Colonnier and Rossignol, 1969; Garey and Powell, 1971) and in the somatic sensory cortex (Jones and Powell, 1970e). In both visual and somatic sensory cortices the degeneration appears to extend less into layer III than in the monkey and no separate band is apparent in area 17. This absence of a separate band may be a reflection of the less marked development of the Stria of Gennari in the cat. There also appear to be more degenerating thalamo-cortical terminals in layer I in the cat, forming a separate band,

whereas in the monkey this is much less apparent.

The difference in the laminar distribution of those thalamo-cortical terminals contacting spines, compared to those contacting dendrites and somata does not appear to have been previously reported. The dendrites receiving synapses from degenerating thalamo-cortical terminals are mostly of the type arising from large stellate cells and their laminar distribution corresponds to that of large stellate cell somata in normal material (Chapter 4) although it is probably a little wider. Those cell somata receiving synapses from degenerating thalamo-cortical terminals are found within the band of dendrites receiving them and within the normal distribution of large stellate somata. It therefore appears that those large stellate cells receiving synapses from degenerating thalamo-cortical terminals on their dendrites and somata are distributed evenly throughout the total population of large stellate cells. With their different laminar distribution, it seems unlikely that the spines receiving synapses from degenerating thalamo-cortical terminals arise from the same cell type. The reasons for believing that these spines are those of pyramidal cells are discussed above and it is not surprising that the two components of the thalamo-cortical projection should have different laminar distributions if they contact two cell types which themselves have different distributions.

The interpretation of the association between degenerating thalamo-cortical axon terminals and apical dendrites requires some discussion. Such a statistical effect could arise by a negative influence if some component of the sections excluded both degenerating terminals and apical dendrites and this would lead to both of them being concentrated in the remainder of the section and so appearing to be associated. Although of course blood vessels do exclude both types of structure the area occupied by them was found to be negligible and in fact most of the area containing neither apical dendrites nor degenerating terminals was normal neuropil. It appears therefore that there is some positive reason for the degenerating terminals to occur near the apical dendrites and it is relevant to note that the bundles of apical dendrites appear to be most prominent at the level of the dense band of thalamo-cortical degeneration (Chapter 3). The most obvious suggestion is that a proportion of the spines receiving synapses from the degenerating terminals in fact arise from the apical dendrites and the failure of impregnation of a proportion of the spines on the apical dendrites of pyramidal cells in the visual cortex following enucleation or destruction of the lateral geniculate nucleus has been interpreted as showing this (Globus and Schiebel, 1967b; Valverde, 1968). However if all the spines receiving synapses from degenerating thalamo-cortical terminals were on apical dendrites all the degenerating terminals would be closely associated with them. In fact the strength of

the association overall is that which would be found if about one-third of the degenerating terminals were specifically and closely related to apical dendrites and the remainder were unrelated and distributed evenly throughout the section both inside and outside the apical dendrite bundles. However this is of course a hypothetical figure and the evidence from those examples where a spine receiving a synapse from a degenerating thalamo-cortical terminal has been traced to its parent dendrite indicates that most of the parent dendrites are of small to medium diameter and of pyramidal type, although in a few instances both in this study and that of Garey and Powell (1971) they do appear to be apical dendrites. It therefore seems that for the most part the association between the degenerating terminals and the apical dendrites is a less direct one and the most likely interpretation is that a small proportion of thalamo-cortical terminals end on spines on the main shafts of apical dendrites and that a considerable further proportion end on side branches of apical dendrites. This small proportion of the thalamo-cortical terminals making synapses on to spines on apical dendritic shafts may however contact a large proportion of the relatively few spines at this site and so produce the considerable reduction in spine density seen in Golgi material following enucleation or lateral geniculate nucleus lesions whereas the greater proportion which make synapses on to the side branches of apical dendrites may only contact a small proportion

of the high density of spines present and produce a small percentage reduction. Which of these groups of terminals has the most powerful influence on the activity of the cell is however a separate issue.

In summary therefore the thalamo-cortical projection to the sensori-motor cortex of the monkey appears to contact two distinct cell types. About 90% of the terminals make synapses on to spines, probably of pyramidal cells and these occur mostly in a dense band in the lower half of layer III and the upper two-thirds of layer IV. The remaining 10% or so of the terminals make synapses on to dendritic shafts and cell somata and are distributed through much of the deep half of the cortex; most of these dendrites and all of the somata can be identified as those of large stellate cells.

Commissural connections

The findings here that all but a few commissural terminals in both the motor and somatic sensory cortices of the monkey make synapses on to spines is in line with findings in the visual cortex of the rat (Lund and Lund, 1970), cat and monkey (Fisken, Garey and Powell, 1975) and the somatic sensory cortex of the cat (Jones and Powell, 1970e). A few commissural terminals have been found to make synapses on to dendritic shafts in these studies as here and Lund and Lund (1970) reported cell somata receiving synapses from commissural terminals but in view of their rarity in the motor cortex (1 out of 468) no significance should be attached to their

not having been found in the somatic sensory cortex here or in other studies.

As with the thalamo-cortical projection, when spines receiving synapses from degenerating commissural terminals were traced to their parent dendrites these rarely received synapses, contained few organelles and were not varicose in shape. Most were therefore clearly not large or small stellate dendrites but had the characteristics of pyramidal dendrites (Jones and Powell, 1970 a-c; Chapter 4). All those found were of small or medium size which suggests that commissural terminals, unlike thalamo-cortical terminals, may not make synapses on to spines on the main dendrites of pyramidal cells. As with the thalamo-cortical projection there is also a reduction in the number of pyramidal spines impregnated in Golgi sections following callosal section but on side branches of apical dendrites (Globus and Scheibel, 1967a). Although the dendrites seen here could be side branches of apical dendrites on the basis of size there must be some reservation about this because of the proportion of commissural terminals found in the deep layers of the cortex, below the level of most of the pyramidal cell bodies.

Of the dendritic shafts receiving synapses from commissural terminals about half could be positively identified as those of large stellate cells using the criteria discussed above. Of the remaining dendritic shafts receiving commissural terminals some were probably those of large stellate cells but lacked sufficient

features to be clearly identifiable; this may have been in part due to their small size. However some of these dendrites had a very markedly varicose shape and contained few organelles and these were probably the dendrites of small stellate cells.

The one cell soma receiving a synapse from a degenerating commissural terminal was a large stellate cell and so this cell type receives contacts from commissural terminals on both its dendrites and somata. The commissural projection thus appears to make synapses on to the spines of pyramidal cells and the dendrites and somata of large stellate cells as does the thalamo-cortical projection but it also appears to contact the dendrites of small stellate cells.

Light microscopic studies of Wiitanen-stained sections of the motor commissural afferents indicate that the laminar pattern shown by the electron microscopic maps is fairly typical of that part of the motor cortex receiving commissural afferents. It is interesting that the commissural afferents have a less dense band in the middle of layer V as do the thalamo-cortical afferents to the motor cortex and this would appear to correspond to the inner band of Baillarger. However in layers III and IV where both projections are more dense the commissural and thalamo-cortical afferents appear to have a reciprocal relationship; the commissural afferents are dense in the upper half of layer III but the lower border of this density appears to correspond to the upper border of the very dense band of thalamo-cortical afferents.

Reasons have been given above for considering that the dense patch of degeneration in layers II and III of the somatic commissural map is genuine, namely that the material is well preserved with no sign of direct damage, that the degenerating terminals were only a small proportion of all the terminals present, that all the degenerating terminals were of the asymmetric type unlike those resulting from local lesions (Fisken, Garey and Powell, 1975) and that the degenerating terminals almost all made synapses on to spines like the rest of the commissural afferents but unlike the intrinsic (Fisken and Powell, 1975) thalamo-cortical or association connections. Lateral variations in the density of commissural degeneration have been described in the somatic sensory cortex using light microscopy (Shanks, Rockel and Powell, 1975). However such variations could also arise from the incomplete removal of the contralateral cortical area and this must be excluded before conclusions are drawn from such lateral variations. There appear however to be two interesting points in respect of the depth distribution of the commissural connections of area 3b. It seems clear that there is considerably less degeneration in the deep layers of the somatic sensory cortex than in the motor cortex and this would seem to correspond to the greater development of the deep layers in the motor cortex. It also seems significant that the lower border of the dense degeneration in layer III of area 3b again corresponds to the upper border of the dense band of the thalamo-cortical afferents and this confirms the apparent reciprocal

relationship between the commissural and thalamo-cortical afferents at this level.

Association connections

Both the somatic sensory cortex and area 6 are immediately adjacent to the motor cortex and so it was of great importance in placing lesions in them to ensure that no direct damage to the motor cortex occurred. In making lesions of the somatic sensory cortex the superficial laminae of the cortex of the posterior wall of the central sulcus were left *in situ* to protect the motor cortex, although the white matter of the postcentral gyrus deep to this cortex was removed. Since it is known that the association fibres to the motor cortex pass through the white matter (Jones and Powell, 1969a) this is equivalent to removing the whole somatic sensory cortex (SI). In addition it was usually possible to avoid opening the dura mater over the motor cortex at operation. Lesions of area 6 were confined to the cortex anterior to the posterior end of the precentral dimple at operation to avoid encroaching on the motor cortex. For both types of experiment the lesions and the motor cortex were examined macroscopically, in brain slices cut perpendicular to the central sulcus and in Nissl-stained sections. No evidence of direct damage to the motor cortex was found in any brain and in the case of area 6 lesions Nissl sections always showed that part of area 6 was present between the lesion and the anterior border of area 4. With area 6 lesions the necessary restriction of the size of the lesion resulted in sparse degenera-

tion but it was felt that this was preferable to the risk of direct damage to the motor cortex. As further confirmation of the fact that the motor cortex was undamaged, no symmetrical degenerating terminals were found except in one discarded brain. Symmetrical degenerating terminals are found in area 17 after lesions intrinsic to that area (Fisken, Garey and Powell, 1975) and it seems unlikely that direct damage could have occurred to the motor cortex without similarly causing symmetrical terminals to degenerate.

Although the degeneration following area 6 lesions was sparse it was very similar to that found after somatic sensory lesions and no differences were found between these two association connections. The majority of the terminals of both projections made synapses on to spines and when the parent dendrites of these spines were identifiable they were of the type arising from pyramidal cells as with the thalamo-cortical and commissural connections. A greater proportion of the terminals of both these association connections made synapses on to dendritic shafts than of the thalamo-cortical or commissural connections (Table 9-1). The proportions of each type of postsynaptic profile contacted correspond to the findings in relation to the association connections from SII to SI in the cat (Jones and Powell, 1970e) and to those between areas 18 and 17 of the visual cortex of the cat (Fisken, Garey and Powell, 1975). All the dendritic shafts receiving synapses from terminals from SI or area 6 were of the vaticose type and the majority had

the features of large stellate dendrites. As with the commissural terminals, a few of the remainder appeared to be the dendrites of small stellate cells and the others could have been of either type. The association connections therefore appear to make synapses mainly on to pyramidal spines, with some contacting large stellate dendrites and a few probably contacting dendrites of small stellate cells.

In spite of the large size of the lesions and the wide range of survival period used for study of the association connections from SI this degeneration was less dense than motor commissural degeneration; this is in accord with other studies (Jones and Powell, 1970e; Fisken, Garey and Powell, 1975). The lack of a distinct laminar pattern for these association connections is also in accord with these other studies although when compared with the histogram of the motor commissural connections, that of the association connections from SI has a suggestion of concentrations of terminals at some levels which correspond to those on the commissural histogram and it could well be that a larger study would produce sufficient degenerating terminals to demonstrate a laminar distribution similar to that of the commissural connections.

General discussion

All the afferent connections to the motor and somatic sensory cortices studied here share a number of common features. All have terminals with asymmetric membrane specialisations. In the motor cortex pyramidal tract cells defined by physiological

criteria have been shown by dye injection to be pyramidal cells (Naito et al. 1969) and the monosynaptic effects of both thalamo-cortical and commissural afferents on pyramidal tract cells have been shown to be excitatory (Amassian and Weiner, 1966; Asanuma and Okada, 1962). These correlations of terminals having asymmetric membrane specialisations and round vesicles in the cortex with excitatory effects confirm the correlation of structure and function made in the cerebellum (Uchizono, 1965) and elsewhere (Gray, 1969). The majority of terminals of each connection make synapses on to dendritic spines which probably arise from pyramidal cells. A proportion of the terminals of each afferent connection makes synapses on to the dendrites of large stellate cells and in some instances also on to the somata of these cells. In the case of the cortico-cortical projections (association and commissural) there also appears to be a small projection on to the dendrites of small stellate cells.

The results relating to each type of connection have been compared to those obtained by other authors in the previous sections and are qualitatively similar, but because sampling techniques have differed the usefulness of making quantitative comparisons with these other studies in limited. A uniform sampling technique has been used for the six connections studied here, allowing more detailed comparison of quantitative results and these are summarised in Table 9-1. For both the thalamo-cortical and commissural connections the proportions of terminals

making synapses on to spines, dendrites and cell somata in the motor and somatic sensory cortices are very similar and, considering the relatively small sample of association terminals from area 6, the two association connections to the motor cortex are comparable. There are however clear differences in these proportions between the thalamo-cortical, commissural and association connections and it would appear therefore from these results that the proportions of each type of postsynaptic process contacted is a feature of the type of connection rather than whether it goes to the motor cortex or area 3b of the somatic sensory cortex; this is presumably related in some way to the differing functions of these connections.

Where spines receiving synapses from degenerating commissural axon terminals were traced to their parent dendrites these were of small or medium size and so the commissural projection probably contacts spines mainly on the more peripheral dendritic branches of pyramidal cells. Most of the parent dendrites of the spines receiving synapses from degenerating thalamo-cortical terminals were of similar size to those receiving the commissural projection but a few of these parent dendrites were large and it would therefore appear that the thalamo-cortical projection extends to include spines on the larger dendritic branches of pyramids near the cell soma as well as occurring on the more peripheral sites. It is interesting that this difference is paralleled by the distribution of degenerating terminals on large stellate cells; whereas the commissural terminals are found

mostly on the smaller dendritic branches and rarely on the cell somata the distribution of the thalamo-cortical terminal extends on to the larger dendrites and the somata of large stellate cells although the majority are still found on the smaller dendritic branches. As has been shown with spinal motor neurons (see Shepherd, 1974) such a difference in the distribution of synapses upon the postsynaptic cell is likely to have a considerable bearing on their functional effect.

CHAPTER 10

GENERAL DISCUSSION

In the previous chapters a number of aspects of the structure and connections of the sensori-motor cortex have been described and discussed individually. In this chapter these findings will be briefly reviewed and discussed in the context of an overall view of the motor cortex and an attempt will be made to combine them with the physiological and anatomical findings of other studies to see to what extent the extrinsic and intrinsic connections of the sensori-motor cortex can now be described. On the basis of these anatomical and physiological data a simple model of the neocortex has been formulated and its characteristics examined to see what features of cortical function it can simulate.

Although there have been a number of electron microscope studies of sensory neocortical areas, the motor cortex of the primate has not been described in detail. Light microscopic appearances suggest that there are marked differences between the motor cortex and sensory cortical areas, particularly in regard to the neuronal populations present. Probably the most important finding from this study has therefore been the striking similarity of the ultrastructural features of the motor cortex to those of the somatic sensory cortex and this similarity is found both for the neuronal populations and also in the types of synaptic connection present between them. A number of features were also found in the motor cortex which had not been previously described in neocortex and these were for the most part subsequently also

found in the somatic sensory cortex, further confirming the similarity of these different cortical areas. Of these new features, the recognition of a new type of stellate cell with the electron microscope is not surprising in view of the variety of stellate cell types described using the Golgi technique. More than one type of interneuron might also be expected in the neocortex by analogy with the retina, olfactory bulb and thalamus which share a common pattern of having a relay cell and at least two types of interneuron (see Shepherd, 1974; Harding and Powell, 1977). It may be that this represents a basic unit of neuronal organisation in all three sites, and in the neocortex it seems likely that there are more than two types of interneuron. It is also of considerable interest that afferent connections to the neocortex have been found to end only on one of the two types of stellate cell in addition to contacting pyramidal cells and in this again the neocortex resembles the thalamus (e.g., Harding and Powell, 1977) and olfactory bulb (Pinching and Powell, 1971) where the afferent connections make synapses on to the relay cell and one of two interneurons; the possible significance of this dual termination of cortical afferents is discussed in detail below.

In contrast to the small stellate cell, the occurrence of dendro-dendritic synapses and gap junctions in the primate neocortex was unexpected, particularly as these had been considered to be features only of more primitive types of neuronal organisation.

Their occurrence at what has been considered to be the highest level of the primate nervous system suggests that they may be a much more general feature of neuronal interaction than had been previously supposed and also indicates that the synaptology of the neocortex is not as simple as it at first appeared. Both structures are found relatively infrequently in the neocortex, although technical factors may be to some extent responsible for this. The significance of these dendro-dendritic synapses in the neocortex may therefore be more in relation to a general understanding of neuronal mechanisms than to the understanding of the neocortex in particular. Gap junctions however, although not frequent, are often strategically placed on the large dendrites or somata of large stellate cells and it seems likely therefore that they have a considerable effect on the functional properties of these cells, and it may be that direct electrical transmission plays an important role in the physiology of the primate neocortex (see below). With present techniques it would appear to be difficult to demonstrate electrical transmission in the neocortex by direct means, although evidence has been presented regarding the possible physiological equivalent of the large stellate cell (Chapter 4).

The qualitative appearance of the axon initial segment has previously been described in detail both in the neocortex and other sites. This region of the neuron has been of considerable interest to both physiologists and anatomists because it is thought

to perform a key role in integrating the afferent activity of a cell and initiating its efferent impulses. Its electrical properties are therefore of considerable importance and its physical dimensions will play an important role in determining these. It was for this reason that particular attention was directed to the quantitative study of axon initial segments and the finding that the length of an axon initial segment is not related to the size of its parent cell soma or to whether it gives rise to a myelinated or unmyelinated axon has important implications for the understanding of the mechanism of impulse initiation and its propagation into both myelinated and unmyelinated axons. As well as impulse initiation, however, the axon initial segment also receives synapses which would seem to be in a key position to influence the efferent activity of the cell. That these synapses are evenly distributed along the whole length of the initial segment is therefore of interest as are the differences in density of these synapses between different cell types and between the same cell type in different layers of the cortex.

The nerve impulse is a short-lived phenomenon. In the processes of learning and memory this short term electrical activity of the brain must lead to more lasting changes in neurons or their connections and these are likely to be of a structural or biochemical nature, or both. Any link between the synapse and the protein synthetic apparatus of the neuron is therefore of interest. The association between asymmetric synapses and the

spine apparatus has been known for some time (Gray, 1959) and the spine apparatus is connected to the reticulum of dendrites (Peters and Kaiserman-Abramof, 1970) although this is not often seen. The finding here of an association between symmetrical synapses and subsurface cisternae or cisternal organs means that both recognised synaptic types in the neocortex are now known to be related to similar types of structure and thence to the reticulum of the cell and for subsurface cisternae in particular, the relationship to the endoplasmic reticulum of the cell soma is very close. These specialised arrangements of membranes therefore provide a close structural link between the synapse and the metabolic machinery of the cell and must therefore be very strong candidates for a role in any long-term modification of neuronal activity by synaptic inputs.

The experimental findings of this study in relation to the afferent connections to the motor cortex closely resemble previous findings in sensory neocortical areas both in the types of terminal undergoing degeneration and the postsynaptic sites contacted. The proximity of the motor and somatic sensory cortices and that of the thalamic nuclei and contralateral cortical areas giving rise to their afferents provided an excellent opportunity for making detailed comparisons between these systems and this further confirmed the similarity between motor and somatic sensory cortices. The main differences found appeared to be due to quantitative differences in the laminar arrangements of the two areas rather than to fundamental differences between them. A

number of new points emerged in this study, particularly regarding the thalamo-cortical projections. The relationship of thalamo-cortical terminals to bundles of apical dendrites is of interest both because it supports the relationship of the afferents to pyramidal cells but also because it may indicate the segregation of afferents into groups related to groups of dendrites and so possibly to groups of pyramidal cells. If so, this situation would not be unlike the glomeruli of the olfactory bulb (see Pinching and Powell, 1971) and it may also be of importance in relation to the columnar organisation of neocortex. The differing laminar distributions of thalamo-cortical terminals making synapses on to spines and dendritic shafts is also of interest and is evidence for the dual termination of cortical afferents discussed below. However, probably the most useful information has come from the correlation of experimental and normal findings which has allowed the identification of the postsynaptic structures receiving afferent connections. This is an essential step in the analysis of the neocortex and an attempt will be made in the next section to correlate the conclusions reached from these anatomical findings with the results of physiological experiments.

Correlation of anatomical and physiological evidence

Physiological studies of the motor cortex have concentrated largely on the organisation of its efferent connections and in particular on its relationship to spinal motorneurons through the pyramidal tract (see Phillips, 1969) whereas this study has been

concerned primarily with the intrinsic organisation of the motor and sensory cortices. There are however a number of physiological findings which may be correlated with the anatomical results and an attempt will be made here to consider systematically the anatomical and physiological evidence concerning the connections to and within the motor cortex and to build up a picture of intracortical circuitry. It is recognised that the evidence for some aspects of these connections is not conclusive and that some correlations must be speculative but it was considered useful to try to obtain an overall picture, in particular as a guide to future work.

The anatomical evidence that the majority of terminals of each of the afferent connections to the motor cortex make synapses on to the spines of pyramidal cells has been discussed in detail above (Chapter 9). Physiologically, pyramidal tract (PT) cells are considered to be pyramidal cells (although the converse is not necessarily true) and this has been confirmed histologically by experiments in which dye has been injected into PT cells identified by antidromic stimulation (Naito et al. 1969). Stimulation of both the ventrolateral nucleus of the thalamus (Amassian and Weiner, 1966) and the contralateral motor cortex (Asanuma and Okada, 1962) result in monosynaptic excitation of PT cells and so there is thus close agreement between the anatomical and physiological findings and it seems reasonable to conclude that the majority of afferent terminals to the motor cortex excite pyramidal cells monosynaptically.

Many pyramidal cells have axons which leave the cortex and these will transmit the afferent activity that they receive and integrate to subcortical sites, including in some cases spinal motorneurons where the effects of pyramidal axon terminals have been shown to be excitatory (Phillips, 1969). Such efferent activity may in due course also arrive back in the motor cortex in modified form by way of several subcortical sites (e.g., see Kemp and Powell, 1971d). Using the Golgi technique pyramidal axons are seen also to have extensive collateral ramifications within the cortex and the presence of such collaterals has been confirmed here by electron microscopy. With the one exception where a pyramidal axon was traced to where it made a synapse on to a large stellate cell dendrite, anatomical methods have not as yet demonstrated the sites of termination of these pyramidal axon collaterals within the cortex. There are, however, many axon terminals in the neocortex whose morphology closely resembles that of the terminals of cortical projections to other sites (see Chapter 4). Although a few of these terminals are extrinsic in origin it has been demonstrated by experiments in which the cortex was undercut that most of the terminals in the neocortex are intrinsic in origin (Szentágothai, 1964; Colonnier, 1966; Jones and Powell, 1970e; Gruner et al. 1974). There are, therefore, many intrinsic terminals in the cortex whose ultrastructural features are like those of known pyramidal axon terminals. In contrast to the indirect nature of the anatomical data, the

physiological properties of pyramidal axon collaterals have been studied directly using antidromic stimulation of the pyramidal tract (Phillips, 1956a,b, 1959; Stefanis and Jasper, 1964a,b; Brooks and Asanuma, 1965; Stefanis, 1969). These studies have shown that these collaterals make short-latency excitatory connections with other pyramidal tract cells. The activity received from its extrinsic afferents by a pyramid may therefore be passed on to a number of other pyramids by way of its excitatory axon collaterals.

The excitatory effect of the pyramidal collaterals is followed in most cases by a marked inhibitory effect which has been presumed to be mediated through an interneuron both because of its reversed effect and its greater latency. 'Pyramidal tract interneurons' defined as non-pyramidal tract cells excited by pyramidal tract stimulation and having certain distinctive properties have been described (Stefanis, 1969; Stone, 1973) and it was thought that it was these cells which mediated the inhibitory effects of pyramidal collaterals on other pyramidal cells. These same 'pyramidal tract interneurons' are also powerfully excited by specific thalamic stimulation (Stefanis, 1969; Stone, 1973). Physiological evidence therefore shows convergence of extrinsic afferents and pyramidal collaterals on to a non-pyramidal tract cell which is thought to be an inhibitory interneuron which makes synapses on to pyramidal tract cells. Evidence that these 'pyramidal tract interneurons' are equivalent to large stellate

cells has been discussed above and this includes a pyramidal cell axon which was shown directly to make a synapse on to a large stellate dendrite in the cortex, thus demonstrating a possible path for the activation of large stellate cells by antidromic stimulation of the pyramidal tract.

In summary there is thus good evidence that the afferent connections to the motor cortex make synapses on to pyramidal cells and on to inhibitory interneurons which in turn make synapses on to pyramidal cells. There is also good evidence that pyramidal cell axons are excitatory and that they make widespread synapses within the cortex on to other pyramidal cells and on to the same class of inhibitory interneurons as the afferents, as well as giving rise to axons leaving the cortex.

Model of the neocortex

On the basis of the connections discussed in the preceding section a simple model of cortical connections has been formulated and its properties explored. It should be emphasised that the purpose of this model is not primarily to explain the specific processing functions of individual neurons but rather to see how a population of neurons with connections of the type described will behave and to try to relate this to the normal and abnormal functioning of the neocortex. It is thus in many ways complementary to the theory of neocortex proposed by Marr (1970). The model is necessarily incomplete because neither the afferent nor efferent connections of small stellate cells are known; it

does, however, allow the interactions of pyramids and the inhibitory interneuron to be explored.

The essential features of the model are:-

1. Extrinsic afferents are excitatory and make synapses on to pyramidal cells and an inhibitory interneuron. This inhibitory interneuron is envisaged as being the large stellate cell and being equivalent to the pyramidal tract interneuron and basket cell although this is not essential to the model.
2. Pyramidal axons have excitatory axon terminals and make widespread connections with other pyramids and the inhibitory interneurons by way of local collaterals within the cortex.
3. The inhibitory interneurons make synapses on to pyramidal cells.
4. The cells add and subtract excitation and inhibition linearly.

A simplified diagram showing this arrangement for a small number of cells is shown in Fig. 10-1. For the derivation of the model each cell in the diagram represents all the cells with the appropriate connections. Thus pyramid A represents the set of pyramids receiving the afferent input x and B represents all those pyramids receiving synapses from cells of set A. The sets do not necessarily contain the same numbers of neurons. When the term activity is used it implies the total activity in the group of

cells referred to and so it is the sum of the activities of each of the individual cells.

Definition of terms

x = level of afferent activity.

θ = excitatory coefficient. This is the ratio of the activity in a set of neurons produced by the activity in the previous set to that activity in the previous set, assuming the excitatory connections to be the only ones present. It is thus a measure of the strength of the excitatory connection between two sets of neurons. Its value will depend on such factors as the number of these connections and their strength. For mathe matical simplicity it has been assumed to be the same for the synapses of afferent connections to pyramids as for pyramid to pyramid connections although this is not essential to the derivation of the model.

ϕ = inhibitory coefficient. This is the ratio of the reduction in the activity of a set of pyramids produced by the activity in the previous set of pyramids acting through the inhibitory interneurons, to the activity in the previous set. It is thus a measure of the strength of the inhibitory connection between two sets of pyramids and will depend on the numbers and strengths of both the synapses between pyramidal collaterals and inhibitory interneurons

and those between inhibitory interneurons and the next set of pyramids, as well as other factors.

Derivation of the model

Consider the set of pyramids A which receive the input x.

The excitation these pyramids receive will be the input x times the excitatory coefficient Θ.

i.e. Θx

Similarly the inhibition they receive through the inhibitory interneuron as a result of the input will be the input x times the inhibitory coefficient ϕ.

i.e. ϕx

And by subtracting the inhibition from the excitation the total activity of the pyramids of setA will be

$$\Theta x - \phi x$$

which simplifies to

$$x(\Theta - \phi) \qquad (1)$$

Now consider the set of pyramids B which receives connections from A. The excitation these pyramids receive from set A will be the activity of set A times the excitatory coefficient

i.e. $\Theta x(\Theta - \phi)$ from (1)

and similarly the inhibition that set B receives from A via the inhibitory interneurons will be the activity of A times the inhibitory coefficient

i.e. $\phi x(\Theta - \phi)$

and the overall activity of set B will be

$$\Theta x(\Theta - \phi) - \phi x(\Theta - \phi)$$

which equals

$$x(\Theta - \phi)^2 \qquad (2)$$

Similarly the activity in set C will be

$$x(\Theta - \phi)^3$$

In set D it will be

$$x(\Theta - \phi)^4$$

And in the nth set it will be

$$x(\Theta - \phi)^n \qquad (3)$$

We therefore have an expression which gives the activity produced in the nth set of neurons by an input x and its properties will now be considered. Changes in the value of x simply produce a proportionate change in the expression at each stage and a large input will produce a proportionately large response. Changes in the values of Θ and ϕ have a more complex effect. When the difference between Θ and ϕ is one, the value of $x(\Theta - \phi)$ will always be x whatever the value of n. Thus the same level of activity will be passed on from one set of cells to the next indefinitely. If the difference between Θ and ϕ is less than one the value of $x(\Theta - \phi)^n$ will decrease as n increases.

e.g. for $\Theta = 1$, $\phi = \frac{1}{2}$

the expression becomes $x(\frac{1}{2})^n$

and when n = 1 the activity is $\frac{1}{2}x$

when n = 2 the activity is $\frac{1}{4}x$ and so on.

Therefore when the inhibitory coefficient approaches the excitatory coefficient activity dies away as it passes from one set of pyramids to the next and this would appear to correspond to the normal state of affairs in the neocortex.

If, however, the difference between Θ and ϕ is greater than one the expression becomes larger as n increases.

e.g. for $\Theta = 3$, $\phi = 1$

the expression becomes $x(2)^n$

and when n = 1 the activity is 2x

when n = 2 the activity is 4x and so on.

Therefore as the margin between the excitatory and inhibitory coefficients increases activity grows as it passes from one set of neurons to the next and will in theory increase to infinity. The behaviour of the term $x(\Theta - \phi)^n$ for different values of $(\Theta - \phi)$ and of n is shown graphically in Fig. 10-2 to illustrate the above points.

Under normal conditions afferent activity does die away in the neocortex and so this would correspond to values of $(\Theta - \phi)$ of less than one. However in a epileptic seizure the cortex appears to behave in a similar way to the model for values of $(\Theta - \phi)$ of greater than one. The model would therefore predict that factors which impair inhibition on enhance excitation within the cortex would provoke seizures.

The expression as derived so far (Equation 3) allows the activity of a set of neurons to rise to infinity. In practice this cannot occur for two reasons: firstly there is a maximum rate at which any neuron can discharge, this being determined by its refractory period, and secondly a set must contain a finite number of neurons because it cannot exceed the total number of neurons in the cortex. These constraints may be included in the model by defining two further terms Emax and Imax.

Emax = Maximum excitatory output of a set or sets of neurons. It will be determined by the number of neurons available, their maximum rate of firing and the excitatory coefficient.

Imax = Maximum inhibitory output of a set or sets of neurons. It will similarly be determined by the number of neurons available, their maximum rate of firing and the inhibitory coefficient.

Thus at each stage of processing (i.e. for each term) the excitatory and inhibitory components of activity must be less than their respective maxima.

i.e. For the first stage

$\Theta x \leqslant$ Emax

$\phi x \leqslant$ Imax

For the second stage

$\Theta x(\Theta - \phi) \leqslant$ Emax

$\phi x(\Theta - \phi) \leqslant$ Imax

And for the nth stage

$$\Theta x(\Theta - \phi)^{n-1} \leqslant \text{Emax}$$

$$\phi x(\Theta - \phi)^{n-1} \leqslant \text{Imax}$$

Before exploring the effects of these constraints on the model it is necessary to get some idea of the likely relative values of Emax and Imax and of their relationship to normal levels of cortical activity and in doing this several points are of relevance.

1. Analysis of cell populations in the neocortex has produced consistent ratios of about ten pyramidal cells to each large stellate cell, and pyramids constitute about 70% of cells in the neocortex. There would therefore appear to be more excitatory than inhibitory cells available in the cortex.

2. In order to give a powerful enough inhibition to maintain the cortex in a stable state with this much smaller density of cells the effectiveness of individual inhibitory interneurons must be much greater than that of individual pyramids. This may occur in several ways:-

   (i) The inhibitory synapses may be so placed as to have a more powerful effect than the excitatory synapses on pyramids of the next set. There is physiological evidence that the inhibitory synapses on pyramids are on the cell soma and this would also be the case if

the inhibitory interneuron is the basket cell (see Shepherd, 1974). Symmetrical synapses on the axon initial segment may also arise from basket cells.

(ii) A given afferent input may produce relatively more activity in an individual inhibitory interneuron than in a pyramid. This would mean that the total inhibitory activity at any time would consist of the activity of a small number of highly active inhibitory interneurons balanced against the activity of a large number of less active pyramids. One of the characteristic features of 'pyramidal tract interneurons' is that they produce a high rate of firing to a thalamic stimulus and they also produce three or four spikes to antidromic stimulation of the pyramidal tract (Stefanis, 1969; Stone, 1973) so there is direct physiological evidence to support this idea.

The maximal firing rate of a neuron is determined mainly by its refractory period and is unlikely to differ greatly between pyramids and the inhibitory interneuron. As individual inhibitory interneurons are more powerfully driven by an input they will reach their maximum firing rate at a lower level of input than will pyramids. This, together with the small number of large stellate cells compared to pyramidal cells would make it likely that the maximum possible activity of inhibitory interneurons in an area of cortex is considerably smaller than the maximum

possible excitatory activity in that same area.

i.e. Emax > Imax.

The behaviour of the model with the addition of these constraints has been explored and leads to an interesting conclusion. With a difference between the excitatory and inhibitory coefficients of less than one ($(\Theta - \phi) < 1$), activity died away in the previous model and the cortex was stable. With the limitations of total excitatory and inhibitory activity, however, activity dies away at lower levels of input and the cortex is stable, but as the input level is increased the cortex passes into an unstable state and activity increases to the excitatory maximum where it is maintained. This is shown graphically for $\Theta = 2$, $\phi = 1.5$ and a ratio of Emax to Imax of 4 to 1 in Fig. 10-3. The exact conditions determining this instability may be defined as follows:-

Instability occurs when the input produces more activity than itself. For the first stage this is

$$\Theta x - \phi x > x$$

But instability occurs because the Imax has been reached so ϕx = Imax,

$$\text{therefore } \Theta x - \text{Imax} > x$$

$$\text{and so } \qquad \text{Imax} < x(\Theta - 1)$$

Thus the cortex will become unstable if x is increased far enough provided that Θ is greater than 1 and the greater is the value of Imax the more will x or Θ have to be increased before the level

of instability is reached.

The model therefore shows that stimulation of an area of cortex above a certain strength results in an uncontrolled discharge of activity at the maximum rate possible. It is also interesting to note however that maximal inhibitory activity is occurring at the same time but is masked by the excitatory activity.

The behaviour of the model under these conditions parallels closely observations made on isolated slabs of neocortex by Burns (1951). Single shocks of low intensity applied to the surface of the cortex produced a response which died away but when the strength of the shock was increased above a certain threshold level it produced a response which spread throughout the slab of cortex and resulted in a prolonged afterdischarge. This threshold was increased by anaesthetic agents and this would be reproduced in the model by a reduction in the excitatory coefficient. In the human, what appears to be a parallel situation occurs in electro-convulsive treatment; in this an electric shock is applied to the cortex through the scalp and this results in an epileptiform seizure.

The model has so far considered the activity of only one set of neurons at a time with an input being passed from one set to the next. In practice inputs occur continuously and many sets of neurons will be active simultaneously. The model can be modified to simulate the effect of a sequence of inputs by using

a quantal time assumption like that of McCulloch and Pitts (1943).

At time zero (t = 0) afferent activity is x.

At time 1 (t = 1) this produces activity $x(\Theta - \phi)$ in set A as before (1) and another afferent volley x arrives. Total activity at time 1 is the sum of these,

i.e. Total activity $t_1 = x + x(\Theta - \phi)$

At the next time interval (t = 2) the activity of set A is passed to set B as before (2) and the activity in set B will be $x(\Theta - \phi)^2$. Simultaneously the input x produces activity $x(\Theta - \phi)$ in set A and another afferent input x arrives. Total activity at t_2 is the sum of these,

i.e. Total activity at $t_2 = x + x(\Theta - \phi) + x(\Theta - \phi)^2$

Similarly at time interval n

Total activity at $t_n = x + x(\Theta - \phi) + x(\Theta - \phi)^2 \ldots + x(\Theta - \phi)^n$ (4)

We now have an expression for the total activity produced at any time by a continuing input of strength x. This expression has been explored in the same way as the single term $x(\Theta - \phi)^n$ above. If the balance of excitation and inhibition is changed as before, when the difference between Θ and ϕ is less than one the series has a finite sum and the activity reaches a stable maximum value. If however the difference between Θ and ϕ is more than one the value of each term of the expression is larger than that of the previous one and the series to infinity has an infinite sum. These two types of behaviour are shown

in Fig. 10-4. Comparing this with the effect of a single input (Fig. 10-2) it can be seen that for values of $(\theta - \phi)$ of less than one the effect of a single input dies away whereas the sustained input produces continuing activity. In both cases however the activity has no tendency to increase indefinitely and if the continuous input is removed after an interval of time activity dies away. When $(\theta - \phi)$ is greater than 1 the level of activity increases indefinitely in both cases and this occurs even if the continuing input is removed. The activity of the cortex thus becomes autonomous in both instances.

The series for continued input was also explored with maximum possible values of excitatory and inhibitory activity as before, these values applying to the total activity present in all the sets active at any time. In order to apply the maxima to the series for continued input the positive and negative terms of the series must be kept separate, the series (4) being rewritten as

$$\text{Total activity } t_n = x + \theta x - \phi x + \theta x(\theta - \phi) - \phi x(\theta - \phi) + \theta x(\theta - \phi)^2 - \phi x(\theta - \phi)^2 \ldots\ldots + \theta x(\theta - \phi)^n - \phi x(\theta - \phi)^n \quad (5)$$

From this it can be seen that both the excitatory and inhibitory (i.e. positive and negative) terms form series, that for the inhibitory terms being

$$-\left[\phi x + \phi x(\theta - \phi) + \phi x(\theta - \phi)^2 \ldots\ldots + \phi x(\theta - \phi)^n\right]$$

In calculating the response of the model the sum of this series

cannot exceed the maximum for the inhibitory activity Imax and so in the model if this value was reached further inhibitory terms were disregarded. The response of the model to different levels of input using values of $\Theta = 2$, $\phi = 1.5$ as before and the same maxima is shown in Fig. 10-5. At the lower levels of the sustained input ($x = 2$ and $x = 3$) activity increases to a maximum level and remains stable at this value. However as the input level is increased the resulting activity suddenly increases disproportionately and becomes autonomous, rising to a level limited only by the total possible excitatory Emax and remaining at this level indefinitely. The way in which the activity becomes independent of the input is shown for $x = 4$. If the input is stopped after t_3 the activity left in the cortex dies away as shown but if the input continues to t_5 and then stops the activity in the cortex become autonomous and rises to maximum. If the input is stopped at t_4 activity continues indefinitely but neither increases nor decreases and this represents the threshold duration for this level of input. An input level which will not produce uncontrolled activity if only maintained for a short period may therefore do so if it is sustained beyond a critical duration.

The mathematical condition determining whether an indefinitely maintained input will cause uncontrolled activity is the relationship of the sum of the series of inhibitory terms

$$\phi x + \phi x(\Theta - \phi) + \phi x(\Theta - \phi)^2 \ldots\ldots \phi x(\Theta - \phi)^n$$

to the maximum inhibitory level Imax.

When the sum to infinity of this series of inhibitory terms is less than Imax the total series is as above (5) and this corresponds to the original series without maxima (4). These series have finite sums when $\Theta - \phi$ is less than one (Fig. 10-4). When the sum of the inhibitory terms exceeds Imax then subsequent negative terms are omitted. At the term at which this occurs the value of the excitatory term is calculated in the usual way as is that of the negative one but only that part of the negative term which does not take the total of the inhibitory terms above Imax is subtracted and the resulting term is therefore larger than it would otherwise have been. If this term is taken as y, the next term will be obtained by multiplying it by Θ, there being no further inhibitory terms and each subsequent term is obtained similarly by multiplying the previous term by Θ. The series from the point at which Imax is exceeded therefore becomes

$$y + \Theta y + \Theta^2 y + \Theta^3 y \ldots + \Theta^n y \qquad (6)$$

The worked example for x = 4 is given below

$\Theta = 2$ Emax = 40 x = 4

$\phi = 1.5$ Imax = 10

From (5) Activity = $x + \Theta x - \phi x + \Theta x(\Theta - \phi) - \phi x(\Theta - \phi)$

$$4 + \underbrace{8 - 6}_{2} + \underbrace{4 - 3}_{1}$$

$$+ \Theta x(\Theta - \phi)^2 - \phi x(\Theta - \phi)^2 \ldots\ldots$$

$$2 - 1\tfrac{1}{2}$$

With this last term the sum of the inhibitory (negative) terms is $10\frac{1}{2}$. Since Imax is ten the negative component of this last term can only be 1 and subsequent inhibitory terms vanish. The value of this last term of the series therefore becomes 1 (2 - 1 instead of 2 - $1\frac{1}{2}$) and this is by definition y. Since $\Theta = 2$ and $y = 1$, subsequent terms in this series are 2, 4, 8 etc. and the series has an infinite sum. The total activity in the cortex at each time is obtained by summating the individual terms up to that time interval and this is shown graphically in Fig. 10-5 (curve for x = 4 with sustained input).

The graph (Fig. 10-5) shows that the cortex is stable for an input of x = 3 but unstable for an input of x = 4 (with $\Theta = 2$, $\phi = 1\frac{1}{2}$). Between these values there is a threshold for instability which may be determined as follows. When the sum of the negative terms is less than Imax the cortex is stable. If it exceeds Imax the negative terms disappear from the series and leave a last term y. However small the value of y, if Θ is more than one the series

$$y + \Theta + \Theta y^2 + ... + \Theta y^n$$

has an infinite sum. Therefore for the cortex to be stable the inhibitory terms must not disappear from the series, i.e. their sum must not exceed Imax. The threshold is the value of the input x at which the sum to infinity of the negative terms equals the maximum inhibitory activity

$$\text{i.e. } Imax = \phi x + \phi x(\Theta - \phi) + \phi x(\Theta - \phi)^2 + \phi x(\Theta - \phi)^3 .....$$

For the example quoted of $\Theta = 2$, $\phi = 1\frac{1}{2}$ this series becomes

$$Imax = 1\frac{1}{2}x + x + 3/8x + 3/16x \ldots\ldots$$

The sum to infinity of this series is 3x.

therefore $Imax = 3x$

$$3x = 10$$

$$x = 3.3\dot{3}$$

i.e. the threshold of the model under these conditions is at an input of $3.3\dot{3}$.

The model of the cortex as finally developed is therefore stable at lower (and presumably normal) levels of input but has a threshold level of input above which it produces sustained maximum activity. The factors determining whether the cortex remains stable or not are the power and numbers of the excitatory connections (Θ), the power and numbers of the inhibitory connections (ϕ), the maximum level of inhibitory activity possible (Imax) and both the strength and duration of the afferent input x.

Speculations regarding epilepsy and the role of the basket inhibitory interneuron in the neocortex

The behaviour of the model with its threshold above which it produces maximal uncontrolled activity has an obvious parallel in the production of epileptiform seizures by the neocortex. It seems likely that anticonvulsant drugs such as phenytoin act by reducing the effectiveness of excitatory connections within the neocortex either directly or by reducing

post-tetanic potentiation (Woodbury, 1969) and this is paralleled in the model by the effect of reducing Θ and so increasing the stability of the system. There is no doubt that afferent activity may trigger epileptic fits (Bickland and Klass, 1969), the best known example of this being photically induced seizures. Photic stimulation has to be maintained for a period of time to cause a fit, paralleling the model, and it seems likely that the intense flashing lights used produce a large input into the cortex although there is no proof that it is the quantity rather than the quality of the input which is important. However, in the model the most important factor determining whether the cortex is stable or not is the effectiveness of the inhibitory systems, represented by the terms ϕ and Imax. This effectiveness is determined primarily by the properties of the inhibitory interneurons. The evidence for this interneuron being equivalent to the large stellate cell and basket cell has been discussed above. Factors which affect the functioning of large stellate cells or their synapses will therefore be expected to have a marked effect on the stability of the neocortex. These fall into two main groups, pharmacological, presumably acting at the nerve terminal and synapse, and metabolic, affecting the whole cell. There is now considerable evidence for gamma-aminobutyric acid (GABA) being an inhibitory transmitter in the neocortex and it has been suggested as the transmitter of the basket cell (Curtis and Felix, 1971). If so, then the observations that the content of GABA is

reduced in the cortex of epileptics (Van Gelder, Sherwin and Rasmussen, 1972), that thiosemicarbazide which reduces GABA levels increases seizure susceptibility (Curtis, 1969) and that GABA itself is a potent anticonvulsant would be expected as would the effects of bicuculline. This is a specific GABA antagonist; it antagonises the inhibition of PT cells both by recurrent pyramidal tract stimulation and by direct cortical stimulation (Curtis and Felix, 1971). More significantly it is a potent convulsant agent. If GABA is the basket cell transmitter then these pharmacological facts are in accord with the present model.

One of the most striking ultrastructural features of large stellate cells is the very high concentration of organelles and particularly mitochondria in their cytoplasm (Figs. 4-7 and 4-10). This suggests that they are metabolically very active and have a high consumption of oxygen and glucose. They would also be expected to be more sensitive to the effects of hypoxia and hypoglycaemia than other neocortical cells. Thus both hypoxia and hypoglycaemia would be expected to depress these inhibitory interneurons before they affected other cells and so cause fits, which indeed they do. Episodes of hypoxia and hypoglycaemia may also cause a permanent liability to fits and it is important to distinguish two mechanisms here. First, hypoxia or hypoglycaemia may cause acute impairment of inhibitory interneuron function and so cause a fit as above, with full recovery

occurring when the hypoxia or hypoglycaemia is reversed. Secondly, however, the hypoxia or hypoglycaemia may be sufficiently severe or prolonged to cause actual death of inhibitory interneurons but be reversed before pyramids are killed. This would result in a deficit of inhibitory interneurons and a permanent epileptic tendency, reproduced in the model by a reduction in Imax and ϕ. Similarly, following cerebral infarction partial and selective necrosis occurring at the edge of the infarct could explain the occurrence of epilepsy following a stroke.

Finally it is possible to formulate a more complete theory of the role of the neocortical basket cells. I+ is suggested that they receive a sample of all the activity in the neocortex, both afferent and intrinsic, and apply a level of inhibition proportional to the mean level of cortical activity to all pyramids in the local area. In doing this they perform the same role in relation to cortical pyramidal cells as Marr (1969) has suggested cerebellar basket cells perform in relation to Purkinje cells. He has suggested that the cerebellar basket cell plays an important role in information processing in the cerebellum by sampling the level of activity in the parallel fibres and then applying a level of inhibition to the Purkinje cells corresponding to the average level of parallel fibre activity. This means that only those Purkinje cells excited by a high <u>proportion</u> of parallel fibres active at any time are activated enough to overcome the inhibition and so specificity

is maintained although the absolute level of parallel fibre activity may fluctuate widely. It is suggested here that the neocortical basket cells act in a similar way but in relation to the processing of the afferent input to pyramidal cells instead of the parallel fibre input to Purkinje cells. However the neocortex differs from the cerebellum in that pyramidal cells are excitatory, whereas Purkinje cells are inhibitory, and pyramidal cells have a more extensive recurrent collateral system leading to the possibility of autonomous activity as shown above. In averaging neocortical activity and applying inhibition to pyramidal cells basket cells are acting in the same way as the inhibitory interneuron in the model and will thus stabilize the cortex. To do this, effective averaging is required since a local deficit of inhibition could result in a group of pyramidal cells becoming excessively active and triggering a fit. The gap junctions between the large stellate cells are a low resistance pathway (see Chapter 7) and current will flow through them and tend to equalise the membrane potential of adjacent cells. This will also tend to equalise their activity and differences in activity between different large stellate cells will tend to be evened out and local inhibitory deficits prevented. Direct electrical transmission may therefore play an important role in maintaining the stability of the neocortex.

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APPENDIX 1

Buffered salt solution

Prepare two stock solutions:

A Dissolve in sequence in about 80 ml of distilled water:

NaCl	14.00 gm
KCl	0.75 gm
$MgSO_4$ (anhydrous)	0.55 gm
$Ca(NO_3)_2.4H_2O$	1.50 gm

Add distilled water to make 100 ml. Store in refrigerator.

B Dissolve in sequence in about 80 ml of distilled water:

Dextrose	17.00 gm
$NaHCO_3$	1.10 gm
$Na_2HPO_4.7H_2O$	0.22 gm
KH_2PO_4 (anhydrous)	0.052 gm
Phenol red	0.01 gm

Add distilled water to make 100 ml. When dissolved at room temperature, saturate solution B with CO_2. Filter if cloudy and store in refrigerator.

Mix 1 volume of A with 8 volumes of distilled water, and then add slowly, with stirring, 1 volume of B. If B is magenta it must be resaturated with CO_2 before use. The pH should be about 7.4. Brick-red colour is neutral.

Fixative (Modified from Karnovsky, 1965).

Add 16 gm paraformaldehyde to 200 ml distilled water. Heat to 60° C with stirring. Add 4 to 10 drops of 1N NaOH and leave until the solution is clear. Cool and add 16 ml 25% glutaraldehyde. Make up to 400 ml with 0.1M phosphate buffer.

Phosphate buffer

Stock solutions:

A	0.2M $NaH_2PO_4.H_2O$	(27.6 gm/litre)
B	0.2M Na_2HPO_4	(28.4 gm/litre)

To make buffer at pH 7.3:

Solution A	23 ml
Solution B	77 ml

Dilute to 200 ml with distilled water. Add 2 drops of 1% $CaCl_2$ per 10 ml of buffer. If this precipitates, start again.

Epon-Araldite

Epon 812	3.1 ml
Dodecenylsuccinic anhydride	6.9 ml
Araldite resin CY212	1.9 ml
Araldite accelerator DMP-30	0.25 ml
Dibutyl phthalate	0.375 ml

Weil's Myelin Stain

As used on frozen sections cut from material fixed by perfusion with modified Karnovsky's solution.

1. Wash in distilled water.
2. Stain for 15-30 minutes at 50° C in a mixture of

   50 ml 4% Aqueous Iron Alum and

   50 ml 1% Haematoxylin.
3. Wash in distilled water.
4. Differentiate in 4% Iron Alum until grey and white matter are distinguishable.
5. Wash in tap water.
6. Continue differentiation in Weigert's solution (2.5 g Pot. Ferricyanide and 2.0 g Sodium Biborate in 100 ml distilled water).
7. Wash in tap water.
8. Wash in tap water containing a few drops of ammonia or saturated lithium carbonate.
9. Dehydrate, clear and mount.

APPENDIX 2

The relationship of the number of synapses seen in a single longitudinal section of an initial segment to the synaptic density and total number of synapses upon it.

A single section along the length of an initial segment will demonstrate only those synapses on it which extend unto or through the plane of the section. The relationship of this number to the synaptic density and total number of synapses on the initial segment may be determined if the following approximations are made:

1. That sufficient of the dense material of a synaptic membrane complex is present in a section to allow the synapse to be identified as such when the edge of the synaptic disc extends through at least half the thickness of the section. This is equivalent to a synapse touching or crossing the plane midway between the two surfaces of the section.
2. The initial segment is treated as a cylinder.
3. The plane of section is parallel to the long axis of the initial segment.
4. Synapses are treated as discs of a constant diameter applied to the surface of the cylinder and distributed homogeneously round its circumference without overlap. (They are known to be evenly spread along the length of the initial segment; Chapter 5).

Fig. A-1 shows a cross-section of an initial segment with a longitudinal plane of section AA' perpendicular to the page.

Any synapse whose edge touches or crosses this plane will be seen in the section of which this is the plane midway between its two surfaces. Thus any synapses which lie at or between the extreme positions VX and XZ or vx and xz will be detected, that is any synapse whose centre lies between W and Y or w and y will be seen in the section AA'. This diagram is extended into three dimensions in Fig. A-2 and it can be seen that any synapse whose centre is in the shaded area on the surface of the cylinder bounded by WYY'W' will be seen in the section AA' (extending back through X') and there will be an identical area on the opposite side of the cylinder corresponding to the projection of wy.

Let T = Total number of synapses on an initial segment

D = Density of synapses on the surface of an initial segment

d = Diameter of the initial segment

l = Length of the initial segment

s = Diameter of the synaptic discs.

WX = XY = W'X' = X'Y' = $\frac{1}{2}$s (by definition)

The area of WYY'W' is therefore s x l and the total area in which the synapses are detected (i.e. both sides of the cylinder) is 2sl.

It is not necessary for the section to pass through the centre of the initial segment to obtain this result. Fig. A-3 shows an off-centre section BB'. This will detect all the synapses within one synaptic width of it, i.e. completely between V" and Z" and v" and z" and therefore with centres between W" and Y" and w" and y" and the area on the surface of the cylinder in which synapses are detected will be W"Y" x l + w"y" x l. Since W"Y"

= w"y" = s the area in which synapses are detected is 2sl as above. This will be true for any off-centre section provided that Z" and v" or z" and V" do not meet or overlap, when the total area in which detection occurs is reduced by the area of the overlap.

A section along the length of an initial segment will therefore detect all synapses on an area of 2sl on the surface of the initial segment and the number of synapses divided by this area will be the synaptic density (D) on this surface.

$$\text{i.e. } D = \frac{\text{Number of synapses detected}}{2sl}$$

The measurement of the synaptic density is therefore independent of the diameter of the initial segment and this is because the synapses detected by this method are contained in a constant width strip (2s) whatever the diameter of the initial segment. It is therefore only necessary to divide the number of synapses seen on an initial segment by its length in order to compare the synaptic density on different initial segments (provided that the synaptic diameter is constant).

The total number of synapses on an initial segment (T) may be calculated by multiplying the density (D) by the total area of the curved surface of the initial segment ($\widetilde{II}dl$),

$$\text{i.e. } T = \widetilde{II}dlD$$

and the proportion of the total number of synapses on an initial segment which is observed in a particular case is given by the area in which synapses were detected divided by the total area

$$\text{i.e. Proportion observed} = \frac{2sl}{\widetilde{II}dl} = \frac{2s}{\widetilde{II}d}$$

The proportion of the total number of synapses observed is therefore inversely proportional to the diameter of the initial segment and this relationship is shown graphically in Fig. A-4. However since more than the total number of synapses cannot be observed $\widetilde{II}d$ must always be greater than, or equal to, 2s. (When $\widetilde{II}d$ is less than 2s synapses may be counted on both sides of an initial segment and so the result invalidated). In practice however the synaptic diameter is about 0.35 μ and the inequality may be solved:-

$$\widehat{II}d \geqslant 2 \times 0.35\ \mu$$

$$d \geqslant \frac{0.7}{\widehat{II}}\ \mu$$

$$d \geqslant 0.22\ \mu$$

The expression therefore holds for initial segments of diameter 0.22 μ and above, which includes all those in this study.

The total number of synapses on an initial segment may also be calculated as the number seen multiplied by the reciprocal of the proportion observed at that diameter.

$$\text{i.e. } T = \frac{\widetilde{II}d}{2s} \text{ X number seen.}$$

ABSTRACT

The electron microscope has been used to study the normal structure of the motor cortex (area 4 of Brodmann) and area 3b of the somatic sensory cortex in the monkey. The termination of the thalamo-cortical and commissural afferent fibres to both cortical areas, and the association connections to the motor cortex from the somatic sensory cortex and area 6 have been studied in experimental material. Because previous electron microscope studies have been mostly of sensory or parietal cortical areas the emphasis throughout this study has been on the structure and connections of the motor cortex and the somatic sensory cortex has been studied primarily to give a comparison with a different cortical area.

The general ultrastructural appearance of the motor cortex and area 3b of the somatic sensory cortex is remarkably similar. The neuropil of all layers consists predominantly of axon terminals and dendritic spines together with dendritic shafts, myelinated and unmyelinated axons. Layer I of both cortical areas contains a plexus of myelinated axons which was shown by light and electron microscopy to be markedly more dense in the motor cortex than in the somatic sensory cortex, although the diameters of the myelinated axons in both areas were similar. In layers II and III there are numerous neurons between which run vertical lengths of apical dendrite and these layers contain relatively few myelinated axons compared to the deeper layers of the cortex. Layer IV, in

both the motor and somatic sensory cortices, contains conspicuous stellate cell somata which receive large numbers of synapses and in the motor cortex these extend both above and below the narrow layer IV which is conventionally described. There is a considerable increase in the number of myelinated axons at and below this level in the cortex and in sections of layer IV cut parallel to the pial surface the apical dendrites which run vertically through layer IV may be seen to occur in bundles of six to twelve dendrites. Layer V contains the prominent Betz cells and similar, but smaller, large pyramidal cells are present in layer V of the somatic sensory cortex. Both layers V and VI contain predominantly pyramidal and fusiform cells and these layers in the motor cortex are considerably thicker than in the somatic sensory cortex, this difference accounting for most of the difference in overall thickness between the two cortical areas.

Pyramidal and stellate cell types have previously been recognised in the neocortex with the electron microscope. A detailed study has been made here of the neuronal somata in the sensori-motor cortex. Pyramidal cells in the motor cortex are very similar to those described previously in sensory and parietal cortical areas. The largest pyramidal cells in area 4, the Betz cells of layer V, are up to 50 μ in transverse diameter. Although basically resembling smaller pyramidal cells, the nucleus of a Betz cell often has a complex indentation and is smaller in relation to the overall size of the cell soma than is that of a smaller pyramid

and the cytoplasm of Betz cells contains discrete clumps of endoplasmic reticulum. As with other pyramidal cells, the synapses on to Betz cell somata are all of the symmetrical type. Previous descriptions of stellate cells have been of cells receiving a high density of axosomatic synapses of both the asymmetric and symmetrical type. Cells like this are found both in the motor and somatic sensory cortices and have been termed here large stellate cells. In addition to their high density of axosomatic synapses, they have abundant cytoplasm full of organelles and usually containing stacks of endoplasmic reticulum. Their dendrites similarly receive a high density of asymmetric and symmetrical synapses and contain prominent organelles and have a moderately varicose shape. Large stellate cells occur predominantly in layer IV in area 3b but in the motor cortex they are also found commonly in the lower part of layer III and the upper part of layer V.

A third class of neuron has been described in both the motor and somatic sensory cortices and cells of this type have been termed small stellate cells. These receive a low density of axosomatic synapses, but some of these are of the asymmetric type, and they have sparse cytoplasm with few organelles. They have a small rounded or fusiform soma, they frequently have a dark nucleus, they have no apical dendrite and their axon initial segments are thin and may be directed towards the cortical surface. Most of the rounded small stellate cells occur in layer II whereas those with fusiform somata occur more in the deeper layers of the cortex.

A quantitative study was made of the cells in a strip of the same width running through the full depth of the cortex in both cortical areas. The absolute numbers of cells in the strips of the motor and somatic sensory cortices were very similar as were the proportions of each type of neuron, 72% being pyramidal in each area with 21% being small stellate and 7% being large stellate in the motor cortex and 23% small stellate and 5% large stellate in area 3b. The quantitative study also provided evidence that large and small stellate cells form two distinct populations rather than being a continuum.

The axon initial segments of cells of all three types have a membrane undercoating and bundles of neurotubules. Those of pyramidal cells are directed towards the white matter whereas those of large and small stellate cells often run obliquely or towards the cortical surface and may be curved. Pyramidal initial segments may have spines which receive symmetrical synapses as do the shafts of the initial segments. The full length of the initial segment was studied for fourteen pyramidal and two large stellate cells. All gave rise to myelinated axons although two pyramidal cells had lengths of unmyelinated axon between the initial segment and myelinated axon. One of these lengths of unmyelinated axon made an asymmetric synapse on to a dendrite just after losing its initial segment features. Quantitative analysis of these complete initial segments showed that, whereas the diameter of the initial segment and the axon it gave rise to were approximately proportional to the

size of the parent cell soma over a considerable range of cell diameters, the length of the initial segment appeared to be unrelated to either its diameter or the size of its parent soma but varied between 30 μ and 55 μ apparently at random. Synapses were evenly distributed along the full length of the complete pyramidal initial segments, but the density of synapses on the initial segments of supragranular pyramids was about three times that on those of infragranular pyramids and cisternal organs were similarly more frequent in the initial segments of supragranular pyramids.

Both cisternal organs in axon initial segments and subsurface cisternae in cell somata and dendrites have been found to be related to axon terminals making symmetrical synapses on to these same structures. The cisternal organ or the dense plate of the subsurface cistern is closely apposed to the overlying plasma membrane opposite the non-synaptic part of the symmetrical axon terminal and the membranes of both the terminal and initial segment, soma or dendrite may show some degree of specialisation at this site. The dense plates of both cisternal organs and subsurface cisternae are specifically stained by ethanolic phosphotungstic acid, thus resembling the spine apparatus, and it is suggested that the role of all three structures is similar. Ethanolic phosphotungstic acid also stains the membrane undercoating of axon initial segments and nodes of Ranvier, cilia, nucleoli and degenerating axons and axon terminals as well as the synaptic membrane complexes.

Dendro-dendritic synapses have been observed infrequently

in the deep layers of the motor cortex. The presynaptic dendrites are of a varicose type and themselves receive a considerable density of synapses both of the asymmetric and symmetrical type. The ultrastructure of the dendro-dendritic synapse itself shows the typical arrangement of pre- and posrsynaptic membrane densities, often with presynaptic dense projections, and the membrane specialisation is of the symmetrical type. There is the usual cleft containing electron-dense material between the pre- and post-synaptic profiles. The synaptic vesicles occur in a small cluster confined to a region close to the presynaptic membrane specialisation; some of the vesicles are flattened and were shown by tilt analysis to be of the discoid type. Two examples were found of reciprocal dendro-dendritic synapses, both components being of the symmetrical type, and a single axon terminal may make a synapse on to both dendrites involved in a dendro-dendritic synapse.

Gap junctions have been found infrequently between two dendrites or a dendrite and a cell soma in the deep layers of both the motor and somatic sensory cortices. At these junctions the outer leaflets of the plasma membranes of both profiles are intimately apposed with a gap of 2 nm between them which shows a structure of hexagonal subunits in tangential sections. These gap junctions occur mainly between the dendrites or dendrites and somata of large stellate cells but are also associated in some examples with a dendro-dendritic synapse and thus occur between large stellate dendrites and presynaptic dendrites; a desmosome may also occur in

association with a gap junction and dendro-dendritic synapse. Gap junctions have been identified as sites of electrical transmission between cells in a number of sites and it is therefore suggested that some neurons in the sensori-motor cortex are electrotonically coupled.

Study of the process of degeneration in the sensori-motor cortex has shown the degeneration to be mainly of the dark type with shrinkage and engulfment by glia. Micropinocytosis of degeneration has been observed and both glial profiles and dendrites, spines and neuronal somata have been observed to be taking up degeneration by this process. Similar micropinocytosis has also been observed in which a normal neuronal profile is apparently engulfing a small portion of another normal neuronal profile. Exposed postsynaptic membrane specialisations have been seen following lesions and are frequently associated with a sub-surface cistern in an apposed profile.

Following large, stereotaxically placed thalamic lesions the degeneration in the motor and somatic sensory cortices was studied with the electron microscope at survival periods of 4 and 5 days. Degenerating thalamo-cortical terminals had asymmetric membrane specialisations. In the motor cortex 89.5% made synapses on to dendritic spines, 9% on to dendritic shafts and 1.5% on to cell somata and in area 3b 89% made synapses on to spines, 11% on to dendritic shafts and one example contacted a cell soma and a spine. A considerable number of the spines receiving synapses

from degenerating thalamo-cortical terminals were traced to their parent dendrites and these were of the pyramidal type whereas the dendritic shafts and cell somata contacted by degenerating thalamo-cortical terminals were mostly of the large stellate type. Most of the thalamo-cortical degeneration in both cortical areas occurred in a dense band in the upper two-thirds of layer IV and the lower half of layer III but a number of degenerating terminals were found deep to this and in the motor cortex a second, less dense band of degeneration was present in the lower part of layer V and top of layer VI. Degenerating thalamo-cortical terminals making synapses on to dendritic shafts and cell somata were scattered through the deep half of the cortex and not concentrated in the dense band of degeneration and so formed a greater proportion of the degeneration in the deep layers, particularly in the motor cortex. Sections cut parallel to the pial surface in layer IV of the motor cortex showed a statistically significant association between the degenerating thalamo-cortical axon terminals and the bundles of apical dendrites present at this level.

Degeneration of commissural fibres was studied at 4, 5 and 6 days after removal of the contralateral sensori-motor cortex. Degenerating terminals had asymmetric membrane specialisations. In the motor cortex 96% made synapses on to dendritic spines, 3% contacted dendritic shafts and one example made an axosomatic synapse; in area 3b, 97% made synapses on to dendritic spines and 3% contacted dendritic shafts. A number of the spines receiving synapses from

degenerating commissural axon terminals were traced to their parent dendrites and these were of the pyramidal type. The cell soma and the majority of the dendritic shafts receiving synapses from commissural terminals were of the large stellate type although some of the dendritic shafts were probably those of small stellate cells. In the motor cortex degenerating commissural axon terminals were found in all cortical layers but were relatively more dense in layer I, the upper part of layer III, the upper part of layer V and the lowest part of layer V with layer VI; in area 3b most degenerating commissural terminals were found in the superficial half of the cortex.

Following lesions of the primary somatic sensory cortex (SI) or of area 6 of the premotor cortex degenerating terminals making asymmetric synapses were found in the motor cortex. Of the terminals of association fibres from SI, 82% made synapses on to dendritic spines and 18% on to dendritic shafts and of those of fibres from area 6, 76% made synapses on to dendritic spines and 24% on to dendritic shafts. For both these association fibre connections a proportion of the dendritic shafts contacted were clearly identifiable as those of large stellate cells. Terminals of both association connections occurred in all cortical layers with no obvious concentrations at any particular depth.

The normal and experimental results have been discussed together and in relation to the physiology of the motor cortex.

On the basis of these results a simple model of cortical circuitry has been proposed and its properties have been explored to see what aspects of normal and abnormal cortical function can be simulated.