AN INVESTIGATION OF CERTAIN CONNECTIONS OF THE VISUAL CORTEX

by

R. A. FISKEN

St John's College,

being a thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in the University of Oxford

1975

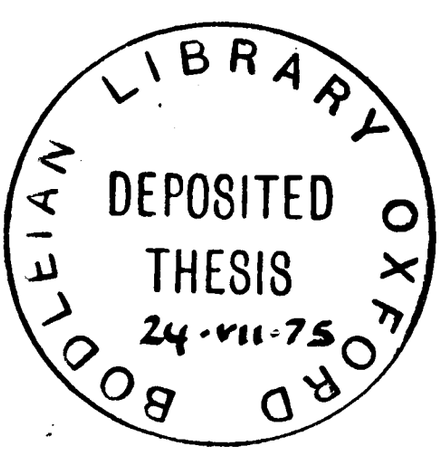


TABLE OF CONTENTS

ACKNOWLEDGEMENTS

Even a humble work such as this one depends for its successful completion on the collaboration and assistance of a great many people, and I should like to extend my warmest thanks to all those who have contributed to this project. During the period of my research I was privileged to work in the Department of Human Anatomy under the late Professor G. W. Harris, F.R.S., and subsequently under Dr A. G. M. Weddell.

There is no doubt that the greatest formative influence on this work was that of my supervisor, Dr T. P. S. Powell, to whom I owe my deepest debt of gratitude. His high standards of scientific excellence, dogged persistence and encyclopaedic knowledge of the nervous system have consistently been at hand to further this project and to rescue it from the abyss into which it not infrequently threatened to fall. I must also thank my collaborator, Dr L.J.Garey, who put in a good deal of the spade-work in the present research, and my numerous friends in the department for their helpful comments and advice.

Throughout this work I have derived great benefit from, and have been saved immense trouble by, the high standards of technical work which I have been able to rely on. I must pay an especial tribute to Mr R. N. L. Brooke for his great skill in the provision of consistently good light and electron microscopic material and for

his endless patience in the face of seemingly impossible demands. Mr M. Sanders, Mrs J. Corrigan and Mrs C. Warr have been his most able team. I must also thank the electron microscope technicians, Mr D. Taylor and Mr K. Reynolds, for their skilled assistance.

The presentation of the results of this work owes a great deal to several people: to Mr B. Archer and his staff for the unfailing provision of first-class photographs, to Miss C. Court for her skill and intelligence in working up our line drawings into valuable figures, and to Mrs M. Collen for her great skill and patience in the typing and re-typing of the manuscript.

I am grateful to the Medical Research Council for financial support during the course of this work.

Finally, I must say a word of thanks to my wife, Jenny, who has been a constant source of comfort and encouragement throughout this project, and especially at times when things were going wrong.

CHAPTER 1

General Introduction

The systematic study of the functions of the cerebral cortex could be said to have begun in 1870, when Fritsch and Hitzig challenged the prevailing view that the cortex as a whole has some "action propre", so that no localisation of different functional roles to different cortical regions is possible. They reported experiments in which electrical stimulation of certain cortical areas in the dog resulted in the movement of muscles of the opposite side of the body, and concluded that the areas stimulated exercised a primary function in the cortical control of movement. The idea that the cortex could be divided up into regions with different properties was further promoted by the work of Brodman, Campbell and the Vogts just after the turn of the century. (This work was anatomical, but it is part of the credo of any anatomist that the features he describes reflect functional properties, so that their work can be regarded as pointing in the same scientific direction.) These workers (Brodmann, 1903, 1909; Campbell, 1903, 1905; Vogt and Vogt, 1903), using the Nissl technique, reported that the cerebral cortex of a number of mammalian species is not homogenous but can be divided into a number of different areas on the basis of the size, shape and arrangement of the cells within them. Since that time, the question of what exactly is meant by "functional localisation" in the cerebral cortex has been debated

at length; however, a consensus of opinion seems to have emerged to the effect that certain areas of the cortex have a primary, though not an exclusive, part to play in the exercising of particular functions, and terms such as "visual cortex", "motor cortex", "somatic sensory cortex" have passed into common usage. It may, perhaps, be of interest to trace briefly the evolution of this point of view. Following the cytoarchitectonic work of the early 1900s, Vogt and Vogt attempted to correlate their findings with functional properties in physiological experiments. In the 1930s a new approach was adopted: workers set out to show that the various areas of the cortex differ in their precise connections with other parts of the nervous system. Thus, Poliak (1932), using the Marchi technique, and Le Gros Clark and Boggon (1935) and Walker (1938), using the method of retrograde cell degeneration, established that there are specific connections to the cortex from certain thalamic nuclei. A few years later, the newly developed method of evoked potential recording showed that well delimited cortical fields could be mapped out (Adrian, 1941; Marshall, Woolsey and Bard, 1941; Talbot and Marshall, 1941) and that, in a number of cases, these corresponded well with the cytoarchitectonic areas described by Brodmann. A direct correlation of anatomy and physiology was first made in the auditory system (Rose, 1949; Rose and Woolsey, 1949), where it was shown that the area of cortex responsive to cochlear nerve stimulation corresponded to the auditory region as defined on morphological grounds. Further work has served to strengthen this idea of functional primacy and

functional specialisation: thus, it has been shown that the different architectonic subdivisions of the first somatic sensory area receive differential projections from the thalamus (Le Gros Clark and Powell, 1953). It has also been shown that there are differences between the connections of areas 17, 18 and 19 with the lateral geniculate nucleus, both in respect of thalamocortical (Wilson and Cragg, 1967; Garey and Powell, 1968, 1971; Rossignol and Colonnier, 1971) and corticothalamic (Guillery, 1967; Garey, Jones and Powell, 1968) connections. Moreover, Hubel and Wiesel (1965) have shown by electro-physiological means that there are three topographically organised maps of the retina on to the cerebral cortex of the cat, and that these three areas correspond closely with the cytoarchitectonic areas 17, 18 and 19. Finally in this regard, one may cite the work of Jones and Powell (1970f) who showed that there is a stepwise progression of anatomical connections from each primary sensory area to successive areas in both frontal and parietotemporal cortex, and that at each stage the projection areas correspond closely to cytoarchitectonic areas as defined by Brodmann.

Once the idea had become more widespread that areas were functionally specialised, it became more feasible to investigate cortical function by studying the activity of small groups of cells or individual units. Three electrophysiological findings of particular interest to the anatomist have emerged from such studies: the first of these is the close correlation of physiological "maps"

and functional zones on the cortex with architectonic areas; the second is the organisation of the cortex into well-defined columns of cells which are related in certain of their response properties; thirdly, evidence has begun to emerge in recent years that the laminar arrangement of cortical cells, a feature which has long been regarded as distinctive of the cortex, has functional importance.

As has been mentioned, the pioneering observations in the correlation of cytoarchitecture with function were those of Adrian (1941) and Marshall et al. (1941). These workers showed that, when the sensory areas of the cortex were mapped with the evoked potential method, a close correlation could be found between the maps so obtained and cytoarchitectonic areas. Subsequently Rose (1949) and Rose and Woolsey (1949) reported that there was a good correlation between the auditory area defined morphologically and the area responsive to cochlear nerve stimulation, and also between the first auditory area defined physiologically and the anatomically determined projection of the anterior two thirds of the medial geniculate nucleus. That such functional specialisation exists at the level of individual architectonic areas was first shown by the work of Powell and Mountcastle (1959) on the somatic sensory cortex of the monkey. They showed that two thirds of the cells in area 3 were activated by cutaneous stimuli, whereas ninety per cent of cells in area 2 responded to stimulation of deep tissues. In area 1 the proportion is intermediate, thirty per cent of cells responding to skin stimulation. This gradient

across the first somatic sensory area is matched by a gradient of morphological change from the bottom of the central sulcus to the posterior margin of area 2. The functional specialisation of architectonic areas in the visual system was reported by Hubel and Wiesel (1962, 1965). They showed by electrophysiological means that the visual cortex of the cat contains three representations of the retina and that each representation corresponds with a cytoarchitectonic area, successively areas 17, 18 and 19 (Hubel and Wiesel, 1965). They also classified cortical cells, on the basis of their response properties, into "simple", "complex" and "lower-" and "higher-order hypercomplex" *, and were able to show that the proportions of these cells differed markedly between the three areas 17, 18 and 19, tending towards successively higher levels as one passed from area 17 outwards.

The second physiological finding of note, namely the arrangement of cortical cells as functional columns, was first reported by Mountcastle (1957) in his studies of the somatic sensory cortex of the cat. He observed that, when a microelectrode penetration is made perpendicular to the surface of the cortex, all cells encountered are of the same "modality" type, i.e., they respond to stimulation of skin or deep tissues or joints, but not more than one of these structures. Oblique penetrations, however, showed sudden, stepwise changes of modality as the electrode was advanced. Mountcastle concluded that "the elementary pattern of organisation in the cerebral cortex is a vertically oriented column or cylinder of cells capable of input-output functions of

* The meaning of these terms is discussed in more detail on page 9.

considerable complexity, independent of horizontal, intragriseal activity". This notion was further strengthened by work in the monkey (Powell and Mountcastle, 1959); it became clear that neurones encountered in vertical penetrations are related not only to the same modality but also to nearly identical peripheral receptive fields. Furthermore, for maximally effective stimuli of particular receptive fields, the latency of cells in all cortical layers falls within a very narrow range, of the order of 2-4 msec. This suggests that the immediate fate of afferent activity is to be transmitted up and down a vertical column.

The existence of functional columns in the cortex was further supported by the work of Hubel and Wiesel in the visual system. They showed that columns exist in which preferred stimulus orientation is the same for all cells (Hubel and Wiesel, 1962). They were also able to demonstrate a columnar organisation for eye preference; although cells differ in the degree to which they are dominated by one or other eye, the balance of activity consistently favours one eye within a given column. Interestingly enough, this columnar mosaic seems to be quite independent of the mosaic for stimulus orientation. The columns, of both types, seem to be variable in width but are of the order of 0.5 - 1.0 mm in the cat; in the monkey, eye preference columns are some 300-500 μm wide, while orientation columns are a good deal narrower, approximately 40 μm wide (Hubel and Wiesel, 1963, 1968). (They are not necessarily columnar in shape, in fact, but may be arranged as sheets or whirls (Hubel and Wiesel, 1969); however, their walls are always perpendicular to the surface of the cortex.) The cortex of area 17 of the monkey provided Hubel and

Wiesel with the idea of searching for the anatomical basis of the column: they observed (Hubel and Wiesel, 1968) that the cells of layer IV, which appeared to receive the incoming geniculocortical impulses, were driven exclusively by one eye; consequently, they made lesions in individual laminae of the lateral geniculate nucleus and were able to show (Hubel and Wiesel, 1969, 1972) that Nauta degeneration was produced in layers IV and IIIb of area 17 in regular patches, alternating with areas of unaffected cortex. Unfortunately, this cannot strictly be regarded as an anatomical demonstration of columns, but only of an eye-preference mosaic in layers IV and IIIb; however, it is highly suggestive of the existence of anatomical columns. With the exception of this experiment, there is no strictly anatomical evidence to support the existence of the column, so that it must remain, at present, a functional concept.

The question of which cells are connected together in what order has been pursued at an even more refined level in recent work which has begun to shed light on the functional importance of a cortical feature which has long been recognised: the well-defined laminar arrangement of cells. Hubel and Wiesel (1968) showed that there is a distinct difference in the properties of cells in different laninae of area 17 of the monkey, and in particular that the great majority of cells in layer IV are simple and are driven exclusively by one eye, whereas the cells in layers III and V are complex or hypercomplex and are binocularly activated. As it has been shown with both the light (Hubel and Wiesel, 1969,

1972; Garey and Powell, 1971) and electron (Garey and Powell, 1971) microscopes that the geniculocortical input to area 17 terminates predominantly in layer IV, it is reasonable to suppose that there are connections from the cells of layer IV to the complex and hypercomplex cells above, below and obliquely alongside them. Such laminar differences in response properties have also been reported in the somatic sensory cortex (Whitsel, Roppolo and Werner, 1972), where it appears that directionally selective cells for stimuli moving across the skin are present in layer III but not in layer IV.

As well as these functional findings, a number of anatomical reports exist of differences in connections between laminae of the cortex. For example, it was found by Globus and Scheibel (1967) in the rabbit and confirmed by Valverde (1968) in the mouse, that removal of an eye in young animals leads to loss of dendritic spines from the apical dendrites of pyramidal cells in layer V, but that such spine loss is essentially confined to the portions of the dendrites passing through layers III and IV; this suggests that the input from the lateral geniculate nuclei terminates predominantly in these layers. Also, it has recently been shown in the cat (Jones and Powell, 1970c) that the laminar patterns of termination of thalamocortical, association and commissural fibres to the somatic sensory cortex are different.

It has been pointed out, then, that an anatomical study of the visual cortex would probably be especially concerned with the anatomical basis of columnar organisation, the functional

significance of cytoarchitectonics and the nature of laminar specialisation. It has to be admitted, however, that such a study will inevitably be drawn towards the question of exactly what cortical circuitry exists and what it might be doing.

The accounts of visual cortical neurophysiology which have attracted the greatest attention in recent years are those of Hubel and Wiesel (1959, 1962, 1963, 1965, 1968). A full account of their findings would be inappropriate at this stage, but it may be of value to review their data briefly: these authors set out to determine whether the cells of the cat visual cortex could be classified according to their optimal stimulus ~~parameters~~ properties, and they found that the cells did indeed fall into groups, which differed in the size, shape, orientation and direction of movement of these optimal stimuli, and in their preferred arrangement of light and dark areas. They also differed in the size and shape of their receptive fields (defined as those regions within which a stimulus caused a change in the firing rate of the cell). The four types of cell which they described are referred to as "simple", "complex" and "lower-" and "higher-order hypercomplex", and their properties can be summarised as follows:

Simple cells have receptive fields consisting of two or more antagonistic regions; adjoining regions will cause the cell to fire to a light being turned on or off and are therefore known as "on" or "off" areas (though these cannot be simply equated with excitatory or inhibitory regions, respectively). The arrangement of antagonistic areas varies, but the receptive field usually

consists of an "on" or "off" area flanked or surrounded by areas of the opposite type, which may be of unequal potency on the two flanks. Characteristically, these receptive fields are elongated, so that the cells tend to respond best to slits of light, edges between areas of different brightness, or dark bars. The orientation of such a stimulus is specific for the cell, and moving stimuli evoke a brisker response than stationary ones; the preferred direction of movement is always at right angles to the long axis of the receptive field, and movement in one such direction may evoke a much brisker response than movement in the other direction. Simple cells make up the great majority of cells in area 17 of the cat, though in area 17 of the monkey they are, in relative terms not so numerous.

Complex cells are again specific for type of stimulus (slit, edge or dark bar) and for orientation; however, their receptive fields may be uniform or may consist of two regions, one excitatory and the other inhibitory, the size of which is unrelated to the size of the optimum stimulus. The position of the stimulus within the receptive field is quite unimportant, in contrast to the finding with simple cells; however, the width of the stimulus is critical, activity falling off rapidly if the stimulus becomes too wide. The movement sensitivity of these cells resembles that of simple cells, except that they usually have an absolute requirement for a moving stimulus. Complex cells form a small percentage of the population of area 17 of the cat and constitute the majority in area 18 and about half the cells in area 19. They are also found

in area 17 of the monkey.

Hypercomplex cells resemble complex cells but are, in addition, sensitive to the length of the stimulus, which, for optimal response, has to be limited in one or both directions. Some hypercomplex cells have two preferred stimulus orientations at right angles. Hypercomplex cells are rare in area 17 of the cat, but increase in frequency in area 18 and again in area 19. They are relatively common in area 17 of the monkey.

The major cells types in the visual cortex show differing degrees of eye dominance, tending towards a greater degree of binocular interaction the more complicated the cell's response properties are. In the monkey (Hubel and Wiesel, 1968), the simple cells of area 17 are driven exclusively by one eye; indeed, the great majority of cells in both the cat and the monkey show preference for one or other eye and, as has been stated, they are arranged in eye-preference columns.

The conclusion which Hubel and Wiesel came to was that, while the cells of the cortex were undoubtedly arranged in a highly sophisticated manner, the predominant pattern might be one in which lateral geniculate neurones converged on "simple" cortical cells, which, in turn, converged on "complex" cells, these on to "hypercomplex" cells, and so on, the stimulus parameters necessary for activating the cell becoming subtler and more selected at each stage (see Hubel and Wiesel, 1965). Further information about the properties of cortical cells has shown that they are indeed more complicated than Hubel and Wiesel's model might be taken to imply (see Barlow, Blakemore and Pettigrew, 1967; Pettigrew, Nikara and Bishop, 1968; Bishop, Coombs and Henry,

1971 a, b). Nevertheless, their model continues to play an important part in discussions on the arrangement of visual cortical cells, and it was partly in the hope of shedding some light on this model and on this area of enquiry in general that the present study was undertaken.

So far, then, we have been concerned with the scientific background to the present work; however, in order to place it in a more precise context, it may perhaps be useful to review briefly the present state of understanding of the anatomy of the visual cortex. Interestingly enough, a good deal of our present store of information concerning the gross anatomy of the visual area has come from electrophysiological studies. It has been known for many years that the retina is topographically represented on the visual cortex, but a detailed study of the nature of this representation has been undertaken only recently. Daniel and Whitteridge (1961) took note of the fact that the visual cortex of the primate is highly convoluted over much of its extent and reasoned that a full understanding of the geometry of the retinocortical projection could only be gained by finding a method of "unfolding" the cortex. Accordingly, they made a series of long penetrations through the occipital lobe of the monkey and the baboon and correlated the retinal positions of the units from which they recorded with their anatomical positions within the folded extent of the visual cortex. In this way they were eventually able to produce a convex rubber model representing the "unfolded" visual cortex. They were also

to show that the "magnification factor", i.e. the linear extent of cortex devoted to a certain visual angle, is the same for all points at the same radial distance from the macula and that it decreases progressively with increasing eccentricity. At the fovea, 1^{o} of visual angle is represented by 6 mm of cortex, so that the minimal angle of resolution (about 0.67') is represented by 67 μm, or about the distance occupied horizontally by five cortical cells. Daniel and Whitteridge also pointed out that, as one moves peripherally, the magnification factor in the monkey decreases in a similar way to the visual acuity in Man. Another mapping study which is of interest in this context is that of Cowey (1964) in the squirrel monkey. This author mapped the retina on the striate and peristriate areas and was able to show that the magnification factor at the fovea is the same in the squirrel monkey as in the macaque, so that the representation of the periphery is very much compressed in the squirrel monkey as compared with its larger relative. Cowey suggested that this might lead to a difference in peripheral acuity between the two species, a suggestion which was later confirmed (Cowey and Ellis, 1969). Cowey was also able to show (1964) that the input to peristriate cortex from area 17 is more important than any direct projection, since areas in the peristriate cortex cannot be excited by visual stimuli after removal of area 17 but can be excited by electrical stimulation of area 17. Whitteridge and his co-workers (Bilge, Bingle, Seneviratne and Whitteridge, 1967) have also published a retinotopic map of the visual cortex in the cat.

The microscopic anatomy of the visual cortex has been investigated by several methods: in addition to the Nissl technique, which has been the basis of cytoarchitectural studies, the Golgi and Nauta methods have been of great value, and in recent years the fine structure of the cortex has been studied with the electron microscope. The cellular architecture of the neocortex is commonly considered in terms of the six laminae of the classical Nissl section. That such laminae exist and that, broadly speaking, there is present a superficial molecular layer, deep to which are alternating laminae of pyramidal and non-pyramidal neurones are well known facts; however, the definition and analysis of these layers require a certain care, for the specification of a lamina in terms of cell types and the delimitation of laminae are often somewhat arbitrary matters. Fortunately, the laminae of the primary visual cortex, area 17, with which the present study is concerned, are relatively well defined and homogenous by comparison with those of other areas. (The question of nomenclature is discussed in Chapter 3). The types of cells which compose these layers can only be satisfactorily classified if at least one other experimental tool, the Golgi method, is employed, and the account given by its foremost user, Ramon y Cajal (1911), of the cell types of the primary visual cortex is of such quality that it remains unsurpassed as a description, though it has been extended in certain aspects in regard to classification. The pyramidal cell, with its pyramidal or goblet-shaped soma, long apical dendrite reaching superficially

over several layers and possibly up to layer I, characteristic basal dendrites and numerous dendritic spines is the predominant cell type of the layers adjoining the main thalamocortical receiving layer, that is to say layers III and V; because of its overwhelming occurrence in area 4 of the motor cortex, it is generally believed to be the main projection cell type of the cortex. Those cells which are frankly non-pyramidal in shape and in the arrangement of their dendrites are classed loosely as "stellate", although the pattern of their dendrites shows a wide variation. Until recently the classification of stellate cells into subgroups was at best a partial matter; Cajal himself, while he had described the dendritic and axonal arborisations of many, if not all, types of stellate cell, was unable to say much more than that certain patterns existed. However, in view of the fact that certain authors have recently attempted to interpret the appearance of stellate cells in terms of their function (Colonnier, 1966; Valverde, 1971), a recent systematic account of the Golgi appearance of stellate cells (Szentagothai, 1973) may usefully be cited.

In order for cortical connections to be traced to their precise terminations, it is necessary to have some knowledge of the detailed anatomy of cell processes and of the synapses which they make, and it is in this area that the electron microscope can make its contribution. Although it is inappropriate to go into the details of the ultrastructure of the cortex at this point (a fuller description will be given in Chapter 4), it is

worth mentioning the major contributions which electron microscopy has made to an understanding of cortical organisation. The neurone types of the cortex have been extensively studied and some success achieved in identifying certain morphological groups, notably the pyramidal and large stellate cells. The morphological appearances of different laminae are also well described. Moreover, the features of cell processes are sufficiently well known that spines can be distinguished from dendrites, certain stellate cell dendrites identified (by their varicose appearance and the presence of large numbers of asymmetrical synapses on the dendritic shaft) and axon initial segments distinguished (by the presence of bundled neurotubules and membrane undercoating). In addition, the structure and arrangement of the constituents of synapses are now well described, so that the presynaptic element can be distinguished (by the presence of vesicles) from the postsynaptic, and synapses can be classified into groups, in particular into Gray's type I (spherical vesicles and asymmetrical membrane thickenings) and type II (flattened or pleomorphic vesicles and symmetrical membrane thickenings). Since there is now a certain amount of evidence that these types may be excitatory and inhibitory respectively (Uchizono, 1965, 1968; Walberg, 1968; Colonnier, 1968), the importance of this type of ultrastructural study is clear.

Finally, having briefly set down the normal anatomy underlying the present work, one might mention certain experimental anatomical work which has contributed to our understanding of the

fine structure of the visual cortex. Amongst the earliest of such studies was that of Le Gros Clark and Sunderland (1939), who reported that, when slabs of area 17 of the visual cortex of the monkey were chronically isolated by undercutting, the stria of Gennari remained apparently unchanged, so that the stria probably does not consist of the terminal arborisations of geniculocortical fibres, but is rather made up of intrinsic axons. A more recent observation, already mentioned, is the one that geniculocortical fibres appear with both the light and electron microscopes to terminate in layers IV and IIIb of area 17 of the monkey (Hubel and Wiesel, 1969, 1972; Garey and Powell, 1971), i.e., on either side of the stria of Gennari. Thirdly, evidence has been presented (Spatz, Tigges and Tigges, 1970) to suggest that the supragranular layers of area 17 of the monkey project heavily on to layer V.

In the present study an attempt has been made to investigate the organisation of the intrinsic fibre connections of area 17 of the monkey. After the placement of small lesions within the cortex, the Nauta method of axonal degeneration and the electron microscope have been used to determine the laminar origin and termination of certain of these connections. It should be emphasised that the results will be described and interpreted in terms of the laminae of the cortex rather than of particular types of neurone, but from a correlation with Golgi studies, certain suggestions about the latter may be made.

Ancillary Studies

Although the intrinsic organisation of the cortex was the main concern, it was considered that, for a further understanding of sensory mechanisms as a whole, more detailed studies of the extrinsic connections of the sensory areas would be of value. Consequently an attempt has been made to provide some information about certain aspects of the association and commissural connections of the visual cortex of the cat and monkey.

The question of whether the primary visual cortex (striate cortex) is commissurally connected has received attention from a number of workers using a variety of techniques and spanning a period of some sixty years, but it is only in the last decade or so that the organisation of this commissural connection has been satisfactorily understood. One of the earliest attempts to approach this problem was that of Polyak (1927), using the Marchi technique in the cat. He concluded that the whole striate cortex was callosally connected, a view which was supported by Marchi studies in the monkey (Mettler, 1935). Curtis (1940), using the evoked potential method, concluded that commissural connections did exist in area 17 of the cat but not in the same area of the monkey, while Garol (1942), also using electrophysiological techniques, concluded that there were no commissural connections in the striate area of the cat. In 1962, Myers opened the modern period of study of this subject with his work in the monkey using the recently developed Nauta technique. He reported that, after removal of the occipital lobe of one side, a zone,

some 2-3 mm wide, of commissural fibre degeneration appeared around the edge of area 17. This zone was narrow at all points except at the area representing macular vision, where it was continuous anteriorly with degeneration in other zones. However, Myers did not believe that any of this degeneration lay within area 17 itself. The Nauta technique was also employed by Polley and Dirkes (1963), who reported that in the cat parts of both area 17 and area 18 receive commissural connections. This view was challenged by Ebner and Myers (1965) on the basis of very similar experiments; however, their placing of the degeneration in relation to cytoarchitectonic areas was in accordance with an older description of these areas which differed from that now generally held to be correct, viz. Otsuka and Hassler (1962). Hubel and Wiesel (1965) considered that the lateral part of area 17 of the cat probably did show some degeneration after commissural lesions, but the matter was finally put into perspective by the work of Choudhury, Whitteridge and Wilson (1965). They showed that, if one optic tract is cut in the cat, the deafferented side shows early responses to visual stimuli only in the region of the 17/18 boundary; as would be expected, the stimuli exciting such responses must lie near the vertical meridian. These responses could be abolished by cooling the corresponding portion of areas 17 and 18 on the opposite side or by cutting the corpus callosum. In the baboon they used the technique of strychnine neuronography to provide similar information: a visual stimulus would provoke a strychninisation discharge from one side to the other provided

that the point strychninised lay near the boundary of area 17; if it was more than a few millimetres behind that boundary, no response could be obtained. The idea therefore emerged that the region of the 17/18 boundary, which is known to be the site of representation of the vertical meridian, is callosally connected with the corresponding zone on the other side so that the two 17/18 boundaries make up a matched pair of functional units, but that the rest of area 17 is devoid of commissural connections. This concept has received support from experimental anatomical work (in the cat, Wilson, 1967; Garey and Powell, 1968; in the monkey, Zeki, 1970); it should be pointed out, however, that Wilson believed that, while the 17/18 boundary in the cat was the zone of maximum commissural degeneration, such degeneration, though sparse, extended through area 17 well down the medial margin of the hemisphere.

In view of these findings, we have used the light and electron microscopes to investigate the laminar pattern of degeneration found at the 17/18 boundary after lesions involving the corresponding area on the opposite side, but we have not examined material from the major part of area 17 or from the more peripheral parts of area 18. We have also refrained from studying the various patches of commissural degeneration further out in peristriate cortex, as this would require an extensive investigation.

With regard to association cortico-cortical connections, one can say that although the peristriate cortex has long been supposed to serve a visual function in a number of species (Campbell,

1905), it is only in recent years that it has been convincingly shown to be connected by association fibres to the striate cortex. A projection from area 17 to the more lateral areas of cortex was demonstrated anatomically by Polley and Dirkes (1963), and the existence of such a projection was supported by the findings of Hubel and Wiesel (1965). More recently Wilson (1967), Garey and Powell (1968), and Heath and Jones (1970, 1971) have provided evidence for projections back into area 17 from more lateral areas. In the present study we hoped to look at the projection from area 18 to area 17 of the cat in a little more detail, using light and electron microscopic methods.

Form of Presentation

After an account of the material and methods used, the results will be dealt with under three headings, namely light microscopy of the visual cortex (area 17) of the monkey after small, intrinsic lesions; electron microscopy of the same area; and light and electron microscopy of area 17 of the cat and monkey after lesions in certain other cortical areas. Under each heading a brief introduction of the topic will be given, outlining the way in which the results have been obtained and the ways in which they will be analysed and interpreted, followed by a statement of the results themselves. After all the results have been described, a discussion will be presented of the issues raised by the findings and of any possible conclusions. A summary, a list of references and an account of technical procedures are appended.

CHAPTER 2

Material and Methods

For the present studies young adult monkeys (Macaca mulatta) or adult cats were used. The operative procedures and neuroanatomical methods used in the different studies were largely similar, so that it is not necessary to give a full account for each. Accordingly, a detailed account will only be given for the experiments involved in the investigation of the intrinsic connections of area 17 of the monkey, and the procedures used in other experiments will be described in detail only where they differ from the first account.

(a) Intrinsic organisation of monkey area 17

Young adult monkeys were used for this study, seven for light microscopy and eight for electron microscopy.

Operative procedures

The animals were anaesthetised with intravenous sodium pentobarbitone in doses of 30-40 mg per Kg of body weight. Full aseptic precautions were taken. A curved incision was made in the scalp, the skin reflected and the periosteum retracted. A burr hole was made in the bone and enlarged so as to expose the lateral surface of the occipital lobe on one or both sides. The dura was opened and lesions, restricted as far as possible to the cortex, were placed with a needle or fine knife, or with a tungsten microelectrode. In both cases the instrument making

the lesion was kept under constant observation with a dissecting microscope, and care was taken to insert the instrument between vessels so as to minimise the degree of vascular damage. Preliminary experiments showed that the extent of axonal degeneration was limited to a few millimetres on either side of the lesion. Therefore, as it is known that area 17 proper neither sends nor receives commissural fibres, it was arranged that, in the later experiments, several small lesions would be placed on each side of the brain.

In the early experiments two types of lesion were made: either a slit, some 2-3 mm long, through the whole depth of the cortex, made with a needle or fine scalpel blade, or an electrolytic lesion at a particular depth within the cortex. In the case of microelectrode lesions, considerable care was taken to try to place lesions at known depths within the cortex; using the dissecting microscope, the electrode was lowered mechanically on to the surface of the cortex, contact being judged by the beginning of the appearance of "dimpling". In the later experiments, in which current was passed continuously, contact was also judged by the reading of the ammeter, which indicated that current had begun to flow. The reading of the micrometer was noted at the point of contact so that the lesions could be placed at known depths, although there was inevitably some slight error in these estimations because of the dimpling of the cortex initially and the subsequent recoil as the electrode penetrated the pia mater. Although with

the electrolytic method localised lesions of approximately 100-200 μm diameter were found in individual laminae, it was clear that the traverse of the electrode through the more superficial layers had caused a certain amount of degeneration, which made interpretation of the findings difficult. The procedure for making an electrolytic lesion was therefore changed so that the current was turned on before the introduction of the electrode into the cortex and was allowed to run continuously while the electrode was slowly driven to a particular depth and during its withdrawal to the surface.

It was possible with the naked eye to identify post mortem some of the lesions placed at operation by small spots of blood on the surface of the brain. The remaining lesions were identified in the following way: as the lesions were positioned with the aid of a stereotaxic apparatus, they could be set at known distances from known landmarks such as the lunate sulcus anteriorly and the medial margin of the hemisphere medially; in addition, accurate drawings were made at the time of operation, and the lesions at adjoining points were deliberately placed at distinctly different depths.

It should also be pointed out that, with the exception of two lesions, all the observations were made on that part of area 17 containing the representation of the central ten degrees of the visual field (Talbot and Marshall, 1941; Daniel and

Whitteridge, 1961); for technical reasons we did not attempt to place lesions within the cortex of that part of area 17 which is buried within the walls of the calcarine sulcus, since in order to do so we would have had to pass through the cortex and white matter lying superficial to it, without knowledge of the consequences of this incidental destruction. Also, as the electrode would have had to be inserted more deeply, there was more likelihood of error in the estimation of the depth at which the lesion was being placed.

At the end of the experiment the dura was replaced and the muscle sutured over the bone opening. The area was lightly dusted with penicillin powder and the skin incision closed. After a survival period of 1-6 days the animals were again anaesthetised. For light microscopy, the animals were perfused through the ascending aorta with 0.9% saline followed by 10% neutral formalin. The brains were removed and stored in fixative for several weeks. For electron microscopy, the animals were cooled to 25^{o} C (recorded rectally) by wetting the fur with 70% alcohol and covering the animal with crushed ice. The chest wall and pericardium were opened and a mixture of 0.5 ml 1% sodium nitrite and 0.5 ml heparin was injected into the left ventricle. The animal was perfused through the ascending aorta, the descending aorta being clamped immediately before the start of the perfusion so that only the head and forelimbs were fixed. The perfusion fluids were a buffered salt solution followed by a mixture containing 1%

glutaraldehyde and 4% formaldehyde in phosphate buffer at pH 7.0 - 7.3; this mixture is a modification of that used by Karnovsky (1965). (The composition of both the salt solution and the aldehyde mixture is given in the appendix). After several hours of further fixation, the brain was removed and stored in fixative at about 4° C.

Histological techniques

For the study of degeneration with the light microscope, the occipital lobe was cut off just in front of the superior temporal sulcus and put into a solution containing 30% sucrose, either in 10% formalin or in water, for about ten days. When the block had sunk to the bottom of the pot, i.e., the fixative within it had reached a high osmolarity, it was sectioned on a freezing microtome at 25 μm in the coronal or sagittal plane, all sections being collected. Spaced series of sections were stained, either singly or by means of a compartmentalised sieve of the type described by Wilson and Cragg (1967), with a reduced silver method; the methods of Nauta and Gygax (1954), Fink and Heimer (1967) and Wiitanen (1969) were used. The procedure employed was as follows: when the brain had been sectioned, a spaced series (usually 1 in 20 or 1 in 40) of sections was stained by one or other method and the sections examined for the presence of lesions. An alternate series, stained by the same or a different method, was taken, this process being repeated until all the lesions had been found and could be examined on closely adjoining sections stained by more than one method. In certain cases, serial sections through

a microelectrode lesion were stained and examined.

Preparation of material for electron microscopy

Slices of cortical tissue approximately 0.5 mm thick were taken at right angles to slit lesions, each slice then being divided into blocks approximately 2 mm by 2 mm, beginning at the edge of the lesion and extending for some 3-4 mm from it. Blocks were bevelled and separately numbered so that the position and orientation of each block relative to the lesion could be known. In the case of microelectrode lesions, the procedure was to cut out a 0.5 mm thick slice of tissue which contained the lesion and then trim this down to a single block; in a number of cases this consisted of the lesion and the cortex 1 mm to either side of it. Blocks were rinsed briefly in 10% sucrose in phosphate buffer, then transferred to 2% osmic acid in phosphate buffer for 1 hour. After rinsing in sucrose phosphate buffer, the blocks were dehydrated through graded alcohols (including block staining with uranyl acetate at the 70% alcohol stage) and transferred from absolute alcohol to epoxy-propane for 1 hour. This was replaced by a mixture of Epon-Araldite and epoxy-propane (equal parts, see appendix) and left overnight. The blocks were embedded in Epon-Araldite, placed in an oven at 37° C for 24 hours, and were then transferred to an oven at 60° C for 48 hours to polymerise the epoxy resins.

In order to identify the exact region required for thin sections, a "thick" section of 1-2 μm was taken from the whole block face, mounted in water and stained with a mixture of

methylene blue and Azure II (Richardson, Jarett and Finke, 1960). An appropriate area for thin sections was chosen and the block trimmed to this area. Thin sections of about 700 Å (silver) were cut, mounted on a film of Formvar on copper grids with a single hole 1 mm X 2 mm, and stained on the grid with alkaline lead citrate (Reynolds, 1963) and uranyl acetate (5% solution in 50% ethanol).

Electron microscope mapping techniques

It is perhaps appropriate at this point to describe the way in which detailed electron microscopic maps of perpendicular sections of the cortex were made. The aim of this part of the study was to collect information about the distribution of degenerating terminals in the different laminae at varying distances from a particular lesion, i.e., to produce a two-dimensional record, such as that shown in Fig. 4:40. The region to be surveyed was usually of the order of two millimetres (the approximate depth of the cortex) by three millimetres; however, the sections for survey could not be larger than about 1.5 mm X 0.75 mm, since larger sections were difficult to cut and to mount on the grid, and took so long to survey that there was grave danger of the section rupturing. Accordingly, the first need was to arrange a way of dividing up the face of the whole block into areas for thin sections so that the region to be surveyed could be covered without any appreciable gaps between areas. This arrangement having been decided upon, the block was trimmed to

several areas, in succession; at each stage the size of the area and its position relative to the edges of the whole block face were recorded by means of a drawing made with the aid of a dissecting microscope fitted with an eyepiece micrometer. Each section was surveyed in the electron microscope using a conventional "square search" procedure: the stage micrometer co-ordinates of the corners of the section were recorded and then the section was examined systematically, the position and features of any degenerating terminals found being recorded. Finally, a representation of each area, with its degenerating terminals, was plotted out on graph paper and the "maps" so formed fitted together to form a representation of the whole region occupied by the block.

(b) Commissural connections of the juxta-striate cortex of the monkey

Six monkeys were used in this study. Many of the procedures employed were the same as those described in the preceding section, so that only points of difference will be described here.

Animals were anaesthetised and subjected to lesions of most of the superomedial part of the peristriate cortex on the lateral surface of the brain (areas OB and OA of von Bonin and Bailey, 1947). Lesions were made by suction, and so far as possible the cortex of the whole of the prelunate gyrus and of both walls of the lunate sulcus was removed. After survival

periods of 3-5 days the animals were again anaesthetised, cooled and perfused with buffered saline followed by a mixture of 1% glutaraldehyde and 4% paraformaldehyde. Blocks of tissue, reaching through the depth of the cortex and 1 mm square in cross section, were taken for electron microscopy from the posterior wall of the lunate sulcus of the opposite side, the orientation of the blocks with respect to the area 17/area 18 boundary being known. In some cases a block of tissue several millimetres thick and oriented in a para-sagittal plane was taken from the contralateral side, immediately alongside the slices taken for electron microscopy, sectioned on a freezing microtome at 25 μm and stained by reduced silver methods; in this way the position of the focus of maximum degeneration in the posterior bank of the lunate sulcus could be determined and a more systematic search of the electron microscopic material made. Maps of the distribution of the degeneration with depth in the cortex could then be made with the electron microscope. The regions surveyed were 0.5 mm-wide strips through the depth of the cortex. The procedure for mapping individual sections was the same as that described earlier for the study of the intrinsic connections of area 17.

(c) Association and commissural connections of the visual cortex of the cat

Five cats were used in the investigation of commissural connections, and in three of these animals material was also studied from the ipsilateral area 17 for association connections.

Once again, the procedures used in this section will be described in detail only where they differ from those described above.

Cats were anaesthetised by intraperitoneal injection of sodium pentobarbitone and subjected to lesions of the visual areas on one side. In two animals, used for commissural connections, the whole of areas 17, 18 and 19 were removed by suction. In the remaining three animals of this group needle lesions were placed in area 18 on the lateral gyrus, care being taken not to encroach on adjoining areas.

After survival periods of 2-5 days, the animals were again anaesthetised, cooled and perfused with aldehyde mixture for electron microscopy. The brain was removed and 0.5 mm thick coronal slices were taken at the level of the lesion. Blocks were taken from area 17 on the ipsilateral side and from area 17, area 17/18 boundary and area 18 on the contralateral side. Once again, blocks some 2-3 mm thick were taken for light microscopy immediately next to the slices taken for electron microscopy; 25 μm frozen sections of these blocks were stained by reduced silver methods.

CHAPTER 3

A light microscopic study of the intrinsic connections of area 17 of the monkey

Introduction

A brief account of the objectives and design of this study has been given in Chapter 1. In order that the results may be assessed critically, however, some further account of precautions taken and of limitations on interpretation is necessary at this point. The study is based on the degeneration observed after 67 lesions, of different depths, in the cortex of area 17 on the exposed lateral surface of the hemisphere, and upon two in the same architectonic area on the banks of the calcarine sulcus on the medial surface. Fifty-one of these lesions have been found to be useful, the remaining 16 being discarded because of poor impregnation, excessive sideways spread of the lesion or undue extension into the underlying white matter. The experiments will be divided into groups, depending upon the depth of the lesion and the laminae involved; the depth of the lesions will be described in terms of those laminae, from the surface. Although the sections have been examined as carefully as possible to determine the maximum depth of the lesions, such an assessment is bound to contain an element of uncertainty, and where a lesion encroached on the superficial margin of a lamina, we have tended to assume that the lamina was involved.

Preliminary experiments showed that the extent of axonal degeneration was limited to a few millimetres on either side of the lesion, and it is known that area 17 proper neither sends nor receives commissural fibres. Accordingly, it was arranged that, in the later experiments, several small lesions would be placed on each side of the brain. It should be emphasised, however, that the lesions were always sufficiently separated to prevent confluence of the degeneration, which, indeed, was never found on the sections. The lesions were made either with a fine knife (slit lesions) or with a tungsten microelectrode; these microelectrode lesions were of two types: in the early experiments, focal lesions were made by passing a current through the tip of the electrode when it was at a particular depth within the cortex, whereas in the later experiments the current was allowed to run continuously during the traverse of the electrode so as to avoid variations in the amount of damage present along the electrode track. Lesions could be identified individually by comparing their appearance in the sections with the records made at operation of their exact positions and depths.

As has been mentioned in the "Material and Methods" section (Chapter 2), three different versions of the Nauta technique were used to stain adjoining sections of most of the lesions, and as the study progressed it was found that the use of more than one of these staining techniques was extremely valuable. Each of them had certain advantages and disadvantages in that, for example, the original Nauta-Gygax method showed

the degenerating axons very clearly but was less useful for the finer, terminal degeneration, whereas the Fink-Heimer technique had the opposite characteristics; the particular advantage of the Wiitanen method, in our hands, was that in addition to staining the degeneration, it permitted such remarkably clear delimitation of the laminae that there was no need for the staining of adjoining sections by the Nissl method, which was important because the lesions were frequently so small that it was necessary to stain all sections over their extent for fibre degeneration.

As many of the lesions were very small and localised, and as in most experiments the degeneration was limited to a few millimetres in extent, certain points may be mentioned regarding the validity of the observations. In each experiment, all of the sections of the occipital lobe were kept, in order, and repeated series of sections over the extent of the lesions were stained by two or three of the different techniques. Furthermore, the sections were examined carefully for the possibility of any small incidental lesion or focal necrosis due to involvement of small pial vessels. Finally, the results of preliminary experiments using the recently developed method of injection of labelled L-leucine (Cowan et al. 1972) are in general agreement with the observations made on material impregnated for axonal degeneration.

It should be emphasised that, with the exception of two lesions, all of the observations were made on that part of area 17 containing the representation of the central ten degrees

of the visual field (Talbot and Marshall, 1941; Daniel and Whitteridge, 1961).

To facilitate the description of the results of individual experiments, a brief account of the cytoarchitecture of area 17 will be given. A recent analysis by Hassler and Wagner (1965) divides the cortex of area 17 into six major laminae of which two, the main pyramidal cell layers III and V, are further subdivided. The important new feature of their classification as compared with that of von Bonin (1942) is that Hassler and Wagner consider that the sublayer containing the stria of Gennari, which in the monkey is quite separate from the layers receiving geniculocortical afferents (Hubel and Wiesel, 1969; Garey and Powell, 1971), should be thought of as the deepest subdivision of layer III, called by Hassler and Wagner layer IIIc, rather than as the superficial part of layer IV (layer iva of von Bonin). The cellular composition of the various laminae is as follows: layer I is a molecular layer containing a few stellate cell somata, while layer II is a layer of predominantly small pyramidal cells with some scattered small stellate cells. Layer III is wide and is divisible into a superficial lamina of small and medium-sized pyramidal cells and a deeper band of larger, darker pyramids. Deeper still the cell density decreases noticeably, the pyramidal cells continuing to be present but in reduced numbers, and a considerable number of small and medium-sized stellate cells appearing between them.

It is this heterogeneous, sparse-celled band which contains the overwhelming majority of the fibres of the stria of Gennari and which is designated layer IIIc. Layer IV proper (ivb of von Bonin) stands out remarkably at low magnification as a dark band of closely packed large and small stellate cells. The superficial, more loosely packed part of this layer also contains the deepest fibres of the stria of Gennari. Layer V is again much lighter and is a narrow strip of small pyramidal cells with a few very large, dark pyramidal cells, the cells of Meynert, on its deep aspect. Layer VI is composed of deeply staining, medium-sized pyramidal and fusiform cells and presents the appearance of a further dark band, with a more sparsely celled zone on its deep aspect. Occasional Meynert cells are also found in that part of layer IV adjacent to layer V. A fuller review of the structure of the visual cortex has been given recently by Garey (1971), while the Golgi architecture of this region has been described in detail by Szentágothai (1973).

Results

Group A

In the first group of thirteen experiments, all made with the needle or fine scalpel, the damage extends through most of the depth of the cortex, either without involvement of underlying white matter (4 cases) or with slight extension into it (9 cases).

As an example of the first type of lesion, that in

experiment 126R-1 (Fig.3:1;3:9),made by inserting a needle to the estimated depth of the cortex, will be described. It is situated in the lateral part of area 17, approximately 10 mm behind the lunate sulcus and about 4 mm medial to the inferior occipital sulcus as it turns backwards. In the sagittal sections of this hemisphere the lesion is seen as a narrow, well defined slit reaching to the deep part of layer VI; over no part of its extent does it involve the underlying white matter. Its mediolateral extent is approximately 2 mm, and it remains remarkably constant in both width and depth. (It is possible to define the anterior limit of area 17 in these sections both by the change in the laminae and by the sudden appearance of a few unusually large cells at the margin of layers III and IV; the lesion is approximately 5 mm behind this boundary.)

In the sections stained by the Fink-Heimer method, degeneration can be seen throughout the depth of the cortex, but for a surprisingly limited extent on each side. It can be said immediately that, except for approximately 200 μm on each side of the lesion, where there is very dense, fine degeneration throughout all layers, there are distinct differences both in the appearance and the extent of the degeneration in different laminae. In layer I there is a little fine granularity and some rows of granules indicative of fibre degeneration, but only distributed over a distance of 0.5 - 1.0 mm from the lesion. Layers II and III have a largely similar appearance to each other,

with an even, dense distribution of fine, granular generation. This is densest for 100-200 μm on either side of the lesion, then remaining more or less uniform throughout the depth of these layers for a distance of 1.5 mm or so, where it stops relatievly suddenly. The extent of spread is different on the two sides, being slightly greater towards the boundary of area 17. In the deep part of layer III much sparser degeneration of slightly coarser granules can be seen extending for a further 1-2 mm, being continuous on its deep aspect with much heavier degeneration in the stria, from which an occasional degenerating fibre can be seen passing into this layer. A notable feature of the degeneration in layers II and III is the marked paucity of obvious fibre degeneration, and in only a few cases can granules be seen to be arranged in rows as fragments of obliquely disposed fibres. The degeneration in layer III is directly continuous with degeneration in the stria of Gennari, but that in the latter is somewhat coarser, is intermingled with more fibre fragmentation and clearly extends for a greater distance. On the side towards area 18 the degeneration, although gradually diminishing, reaches for 3.5-4.0 mm, i.e. almost up to the 17/18 boundary, whereas on the opposite side it extends for about 2 mm. Except for approximately 200 μm immediately to the side of the lesion, where there is dense, fine granularity in layer IV, the deep aspect of the stria is clearly delimited by the relatively clear appearance of layer IV; beyond the immediate vicinity of the lesion there is only very sparse granular degeneration and an

occasional, vertically disposed, fragmenting fibre in this layer. This lack of degeneration in layer IV, in contrast to that on its deep and superficial aspects, is very striking and makes the layer stand out even at relatively low magnifications, as is seen, for example, in experiment 110L - 2 (Figs. 3:10 and 3:11). The appearances of layers V and VI are similar to each other, except that the quantity of degeneration in layer VI is somewhat less. The terminal, granular degeneration is coarser in these layers as compared with layers II and III, and the number of degenerating fibres is much greater; the latter are also quite coarse, are seen to run in all directions and are perhaps more numerous in layer V. In the latter layer there is also a greater proportion running horizontally, in about the middle of the layer, giving, under low magnification, a general appearance of degeneration affecting the inner band of Baillarger. Although there is a marked decrease in the number of degenerating fibres at about 1 mm away from the lesion, sparse degeneration can be traced for a distance approximately the same as that in the stria.

In sections over this lesion stained with the Nauta-Gygax method there is less impregnation of the fine, granular degeneration, but more of coarser fragmenting fibres. The degenerating fibres in layer I are seen more clearly, and because of the diminished granularity of layers II and III, the fibre degeneration in the stria of Gennari stands out more prominently as a horizontal band. In layer V the tendency of the

horizontally disposed fragments to be collected together in the position of the inner band of Baillarger can be seen more definitely. A further point brought out in these sections is that, in the two deepest layers, V and VI, the degenerating fibres close to the lesion are predominantly vertically arranged, giving the appearance of a "cuff" around the slit-like lesion, whereas further out the fibres are equally disposed in all directions. In the three other lesions of this group, the findings are essentially the same, and in particular the asymmetric spread of the degeneration on the two sides of the lesion is seen in each case. Only a few additional points need to be mentioned: in one of the experiments, 117L-C8, the horizontal fragments in layer V are clearly seen to be disposed in three or four rows in the position of the inner band of Baillarger; in another lesion, 117L-2, which only partially involved layer VI, the vertically arranged degenerating fibres in the deeper part of this layer, which are passing into the white matter, are concentrated in a narrow band in the direct line of the lesion. Another feature of this last experiment, in which the lesion is situated near the anterior edge of area 17, is that the terminal degeneration in layer IV extends appreciably further anterior to the lesion than it does posteriorly, and more than would be expected from the asymmetry found in the other lesions: posterior to the lesion the degeneration in this layer, as in the experiment described in detail, extends for only 200 μm or so, but anteriorly it is

distributed for almost a millimetre; this difference is no doubt due to the part of the lesion in layers V and VI having interrupted the thalamocortical fibres which must have entered at this point, as the lesion is at the anterior margin of the underlying white matter.

In the nine lesions in which the underlying white matter was involved to a small extent, certain slight differences were found in the pattern of degeneration (117-R2, Fig. 3:1). More degenerating fibres were found in layers V and VI, some of which were quite long and could be seen to take a very oblique course away from the lesion. The second notable feature was the difference in the amount of degeneration in layer IV on the two sides of the lesion: on one side it was similar to that seen after lesions confined to the cortex, in that there was granular degeneration in the immediate vicinity of the lesion, but beyond this it was relatively clear. On the other side, however, the degeneration extended further (Figs. 3:12, 3:13) and was accompanied by some fibre degeneration, which could be seen entering it from the deeper layers. It is almost certain that this difference is due to the interruption of some thalamocortical fibres in their subcortical course, as the side more extensively affected was always that which would be predicted from a knowledge of the course of the incoming thalamic fibres, that is, anterior or medial to the lesion. The suggestion that this involvement of thalamocortical fibres occurred in the white matter and not within the cortex is

supported by the findings in one experiment (120R) in which the lesion was made electrolytically and barely encroached on the white matter. The sections happened to be cut at an oblique angle to the line of the track; consequently, the lesion was seen as a small focus of necrosis at different depths on succeeding sections (Fig. 3:2). It was striking that, when the lesion was in the middle portions of the cortex, the major fibre degeneration, both above and below the lesion, was in a band the width of the lesion and the fibres were vertically disposed; on either side of the lesion some fibres passed out horizontally and these were more obvious when the lesion was at the level of the outer or inner band of Baillarger in layer IIIc or layer V. The finding of degenerating fibres superficial to the lesion over a width only approximately equal to that of the damage suggests strongly that these thalamocortical* fibres run vertically for most of their intracortical course, and this is supported by the finding of no obvious asymmetry in the terminal degeneration in layer IV. No significant qualitative difference was seen in the layers superficial to layer IV after these lesions involving the white matter, for the degeneration in these layers was so dense after lesions confined to the cortex that whatever slight increase there might have been in the amount of degeneration

---

* as there are no commissural or association cortico-cortical fibres to this part of area 17

when the white matter was also involved was not noticeable.

Group B

The first group of experiments have shown the maximum extent and the total pattern of fibre and terminal degeneration after lesions of the cortex of area 17. They have, however, given little information about the origin of the fibres in the individual laminae. In the experiments of this and the subsequent groups, the lesions affect differentially two or more laminae and provide some information about the laminar, if not the cellular, origin of certain of these fibres. In this second group of three experiments, the lesion is confined to layers I and II. (We have no lesion which is restricted to layer I). The three lesions are similar in that each is only 0.3 - 0.5 mm in diameter (e.g., experiment 126R-2, Fig.3:3). In the sections stained with the Fink-Heimer and Wiitanen methods, fine fibre and granular degeneration is seen in layer I and, while densest in the immediate vicinity of the lesion, it extends out for one or two millimetres on either side (Fig. 3:3; see also experiment 114R-2, Fig. 3:23). In layer II there is dense, very fine granular degeneration surrounding the lesion, but this granularity rapidly diminishes in amount away from the site of damage (Figs. 3:14 and 3:15). The fine granules are not arranged in any pattern, but are quite uniformly distributed. The horizontal extent is approximately one millimetre, while vertically, sparse degeneration extends into the superficial part of layer III. It does not extend deeper than IIIa, as there is a clear area between the deep

margin of the degeneration and the level of the stria in IIIc. With these stains there is no obvious degeneration of fibres, and no degeneration can be seen deep to the superficial part of layer III. No fibres can be seen passing vertically out of the cortex or running horizontally in the stria. In the Nauta-Gygax preparations a few degenerating fibres are seen running down into the upper part of layer III, but again the predominant feature is the uniform distribution of the granular terminal degeneration in layer II.

Group C

This group is made up of nine lesions which, in addition to damaging layers I and II, have involved layers IIIa and IIIb but not IIIc, in which the stria runs. It has not been possible in this material to distinguish with certainty between involvement of the subdivisions a and b of layer III.

The first experiment to be described in this group is experiment 139R-3. The lesion was produced electrolytically and forms a small area of necrosis, approximately 500 μm in diameter, in layers I, II and the superficial part of layer III. The Wiitanen sections are exceptionally well stained and show both the degeneration and the laminae very clearly (Fig. 3:4). There is dense, fine granular degeneration immediately around the lesion in each of the affected layers, but it becomes rapidly sparser; the fine terminal degeneration extends outwards for

about 1.5 mm on one side of the lesion, while on the other side it extends for about 2 mm in layer I, but for only 0.5 - 1 mm in layers II and III. From this narrow zone of dense degeneration around the lesion a few fine degenerating fibres can be seen radiating outwards. Although it is difficult to be certain about small differences in the density of degenertion in different experiments, it is probable that the amount of terminal degeneration in layer III due to small lesions reaching down only to this layer is less than after one extending through the deeper layers as in the first group. Deep to the lesion, in the deep half of layer III and the underlying stria, there is moderate granular degeneration, but the major feature is a narrow band of fragments of degenerating, vertically disposed fibres; these fragments are slightly larger than the fibres around the lesion. It should be pointed out, however, that the side-to-side extent of the degeneration in the deep half of layer III, including the stria, is no wider than at the level of the lesion; in addition, both in the stria and throughout the depth of layer III above it, there is only an occasional degenerating horizontal or obliquely disposed fibre. In layer IV the vertically disposed degenerating fibres can be clearly seen, but they again occupy a relatively narrow side-to-side extent; interspersed among them is a little fine granularity. In layer V there is a slight increase in the fine terminal degeneration, together with a little degeneration of the horizontal fibres in the inner band of Baillarger. A few

degenerating vertical fibres continue through layer VI to enter the white matter, and in this layer there is also a little granular degeneration. There is no doubt that the number of degenerating fibres in layer VI is considerably less than the number passing through the stria and layer IV.

Experiment 117L-5 (Fig. 3:4) will be described as an example of a lesion which extends to the deep margin of layer IIIb, but which does not encroach on the stria lying in IIIc; in addition, it provides a closer comparison with the experiments described in the first group, because it is in the form of a well-defined, narrow needle track no more than 50 μm in width. The pattern of degeneration in the different laminae is similar to that in the previous experiment, but because of the narrowness of the lesion, certain features are well displayed. In the stria and in layer IV, the restricted extent of the vertical degenerating fibres is quite definite, and in the latter of these there is a sharp diminution in the amount of degeneration on either side. In layer V the degeneration in the inner band of Baillarger is well impregnated (Fig. 3:19) and can be seen to extend more widely than the degeneration in layer IV, being almost as wide as that in layer III. The decrease in the number of fragmented fibres and in terminal degeneration in layer VI is again quite definite.

Two further experiments will be described together as they illustrate, in different ways, the same points (Fig. 3:5). In experiment 114R-3, the lesion was made by passing a current

of a few microamps during the traverse of the needle through the superficial layers. The sections were cut obliquely to the lesion; consequently portions of the latter at different depths appear on different sections as small foci a few hundred microns in diameter. The deepest extent of the lesion is in IIIb immediately above the stria (Fig. 3:17). The interesting point is that, on the individual sections, on which the lesion appears in layer II and the superficial part of layer III on the one hand, and in the deep part of layer IIIb on the other, the fine granular degeneration in layer III is more or less restricted to the vicinity of the lesion, and it is worth noting that the width of the degeneration in layer IIIc is no greater than that in IIIa and b. In the other experiment, 126R-3, three small foci, each about 50 μm wide, were made at different depths along the same oblique needle track and the three foci have been found on the same section (Fig. 3:16); they are all within the region of layers II and IIIa and b. Again there is a definite appearance of three circular areas of fine degeneration, one around each, with the superficial and deep margins continuous. The narrow bands of vertically disposed degenerating fibres from each of these are clearly separated and the number of fibres from each focus increases progressively with its depth.

Group D

In this group of ten experiments, in which the lesion has extended to involve the stria of Gennari in layer IIIc, an

important new feature of the pattern of degeneration appears, namely that the fibre degeneration in the stria is more severe and extensive than in the previous groups of more superficial lesions. There are two distinct types of lesion in this group: the first is similar to those which have already been described in that all of the affected layers have been damaged more or less equally by a needle stab or by the current being passed from a microelectrode during its entire traverse, whereas the second is a small focus of necrosis strictly confined to layer IIIc, with no appearance in the superficial layers suggestive of damage due to the passage of the fine electrode.

In the experiments with the first type of lesion (117L- 4, Fig.3:6), the resulting degeneration in the layers superficial and deep to the stria is similar, in both appearance and extent, to that in the previous group (group C) with small lesions. In the stria itself, however, the pattern is distinctly different: there is more fragmentation of fibres and a large proportion of these are clearly running horizontally; although the intensity of this degeneration is maximal for a few hundred microns on either side of the lesion, it extends out on both sides to beyond the limit of the fine granular degeneration occupying most of the depth of layers IIIa and b. In all of the experiments the degeneration is clearly asymmetrical in extent and is uaually present for 1-2 mm more on one side than the other; although the number of degenerating fibres diminishes away from the lesion, undoubted fragments can be seen over a total extent

(from end to end) of 5-6 mm. From this degeneration in the stria fibres can be seen passing both superficially into the deep part of layer IIIb, and deeply into the superficial, loosely cellular half of layer IV; in these layers a little coarser terminal degeneration is present over the extent of the degeneration in the stria.

In experiment 111L-4 (Figs. 3:6, 3:21 and 3:22) the damage is in the form of a small, flask-shaped focus of necrosis approximately 150 μm in width and is confined to the stria and the immediately adjoining part of layer IIIb; its deep margin is certainly within the limits of the stria. There is no sign of the electrode track passing through the more superficial parts of the cortex, even on adjoining serial sections, and this is in accord with the small amount of fine, granular degeneration in these layers. From the site of damage degenerating fibres can be seen running in the stria, and on each side, in addition to those running horizontally, there are some fibres diverging obliquely down to the superficial half of layer IV. There is again a little terminal degeneration in this part of layer IV and in the deep part of layer IIIb over the extent of the degeneration in the stria. Two points may be noted: first, even after this small, focal microelectrode lesion, the degeneration in the stria extends over a distance of some 6 mm from end to end, and is again clearly asymmetrical; secondly, the amount of degeneration, both of fibres and of terminals, in layer V appears to be appreciably

less after this lesion than after those, of this and previous groups, in which there was damage to the layers superficial to the stria; this difference is almost certainly not technical because the sections are very well impregnated.

In experiment 110R-6 (Figs. 3:6 and 3:18) a small microelectrode lesion is mainly within the stria of Gennari, but has encroached upon the superficial half of layer IV. Again there is no sign of an electrode track in the superficial layers, and only a little sparse degeneration is present superficial to the site of damage. The additional involvement of layer IV has not resulted in any significant difference in the pattern of degeneration as compared with a lesion restricted to the stria.

As far as can be judged, the degeneration resulting from a focal area of damage of comparable size, but confined within layer IV, is also similar in distribution (experiment 111R-9, Figs. 3:7 and 3:20). Immediately around the small focus of damage in this experiment there is granular degeneration throughout the depth of layer IV, and, superficially, degenerating fragmented fibres can clearly be seen to enter the stria. The oblique direction and divergence of these fibres on either side of the lesion as they pass into the stria of Gennari is quite obvious. Away from the neighbourhood of the lesion, terminal degeneration is seen only in the superficial part of layer IV and in the part of layer IIIb adjoining the stria. The only further points that need be emphasised about these two experiments is the surprising intensity of fibre degeneration in the stria

after such a small amount of damage (Fig. 3:24), and that the spread of the degeneration is of the same order as after larger lesions, partly, no doubt, because the sections were fortunately very well impregnated.

Group E

In the final group of ten experiments the lesions penetrate the cortex to the depth of layer V. Most of these lesions are in the form of narrow slits, some of which are at quite an oblique angle to the surface of the cortex. In the latter there are a number of degenerating, vertically disposed fibres in the layers superficial to the lesion; these probably represent the degeneration of afferent fibres and of axon collaterals of cells in the deeper layers. This increase in fibre degeneration is the only difference seen in layer III in these experiments as compared with the previous ones. The intensity and spread of the degeneration in the stria does not appear to be any different after involvement of layer V, but in layer IV there are somewhat coarser granules of terminal degeneration and of fragmented fibres; however, this degeneration does not extend for more than 100-200 μm on either side of the lesion. In layer V, however, the number of degenerating fibres in the inner band of Baillarger is greater than after lesions reaching down only as far as layer IV, but are still much fewer than those in the stria. The extent of spread of these fibres in layer V is essentially the same as that of fibres in the stria,

but those in layer V are numerous only for about 1 mm from the lesion, at which there is a sharp reduction in density and the fibre degeneration is very sparse thereafter. There is also an undoubted increase in the number of degenerating, vertically disposed fibres passing through layer VI to enter the white matter. In these experiments there is less granular, terminal degeneration and far fewer obliquely arranged fibres, in both layers V and VI, than in the first group (group A) in which the damage involved layer VI; consequently it has been possible to define more clearly the disposition and spread of the degenerating fibres in the inner band of Baillarger.

In one experiment (135-L, Fig.3:8) a narrow slit lesion was made within a couple of millimetres of the lateral part of the lunate sulcus; the slit was 2-3 mm long, ran parallel to the sulcus and was close to the junction of the representations of the horizontal and vertical meridians. On the sections the lesion was found to be at the junction of areas 17 and 18 and extended slightly into the underlying white matter. The pattern and distribution of the degeneration in area 17 is similar to that found after the comparable lesions described earlier in group A, and the intensity of the degeneration within the stria and the inner band of Baillarger is particularly severe in these well impregnated sections; consequently the relative absence of degeneration in layer IV beyond the immediate vicinity of the damage stands out prominently. On the opposite side of the lesion, in area 18, there is degeneration of approximately the

same intensity throughout all layers of the cortex, with more fragmenting fibres in the deeper layers but with no appreciable concentration in the positions corresponding to the stria and inner band of Baillarger. The degeneration on this side of the lesion is almost certainly due partly to the interruption of afferent fibres in the underlying white matter. This degeneration extends for a few millimetres into area 18. There is well stained degeneration in other portions of the peristriate cortex, but as this is not relevant to the present investigation, it will not be described. ~~in detail.~~

Group F

The lesions in all of the groups which have been described so far have been in that part of area 17 which is on the exposed lateral surface of the hemisphere, and they therefore lie within the representation of the more central parts of the retina (Talbot and Marshall, 1941; Daniel and Whitteridge, 1961). In two experiments small lesions were placed in the cortex of area 17 on the anterior bank of the inferior ramus of the calcarine sulcus, which should be in the representation of part of the visual field some 15-20 degrees out from the macula (Daniel and Whitteridge, 1961). The histological sections show that both lesions are within area 17, that one of them extends as far deeply as the stria and that the other passes through the entire thickness of the cortex to involve slightly the underlying white matter. The degeneration after each of these lesions is

comparable to that found after lesions of similar depth in the part of area 17 involved in the previous groups. In the lesion in which white matter was involved there is severe terminal degemeration in layer IV, and close to the lesion, where this merges with that in the stria, the granules are seen to be coarser than those in the stria; this degeneration of thalamo-cortical fibres extends further towards area 18 than does the degeneration in the stria, and as a result its bilaminar distribution, in the deep part of layer IIIb and in layer IV, on either side of the clear stria, is quite distinct.

Fig. 3:1 The lesion and the resulting fibre degeneration in experiment 126R - 1 (upper) and 117R - 2 (lower). In this and subsequent figures the lesion is shown in solid black, the fibre degeneration by short lines and the terminal degeneration by fine dots. The laminae of the visual cortex are indicated by the Roman numerals on the right of the figure. The horizontal extent of the degeneration has been indicated as accurately as possible, and the scale of the figure is given by reference to the length of the 1 mm shown below.

3:1

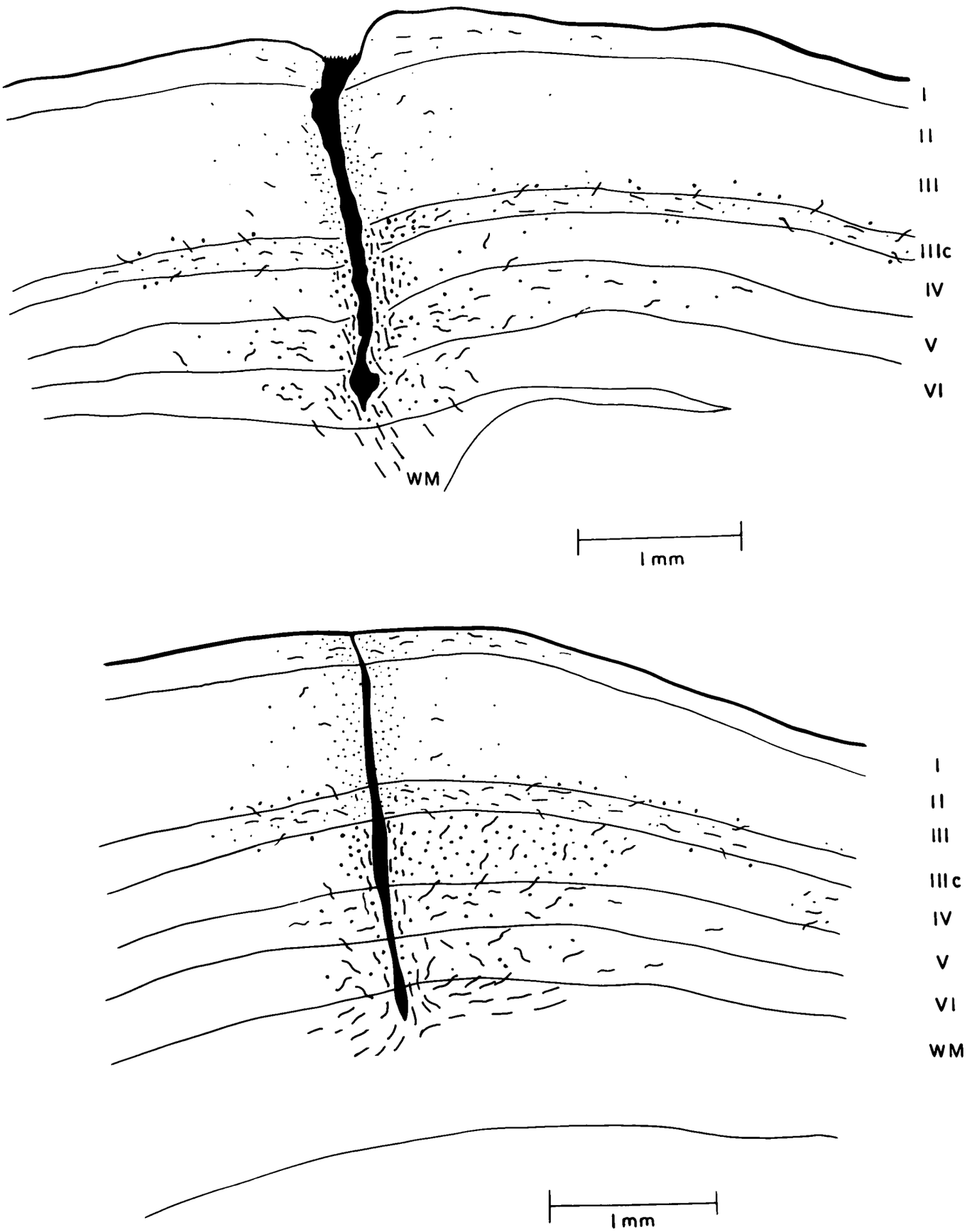


Fig. 3:2 The site of the damage and the resulting fibre degeneration in experiment 120R in which the lesion appeared at different depths on adjoining sections. It should be noted that the fibre degeneration superficial and deep to the lesion is of approximately the same width as the lesion itself.

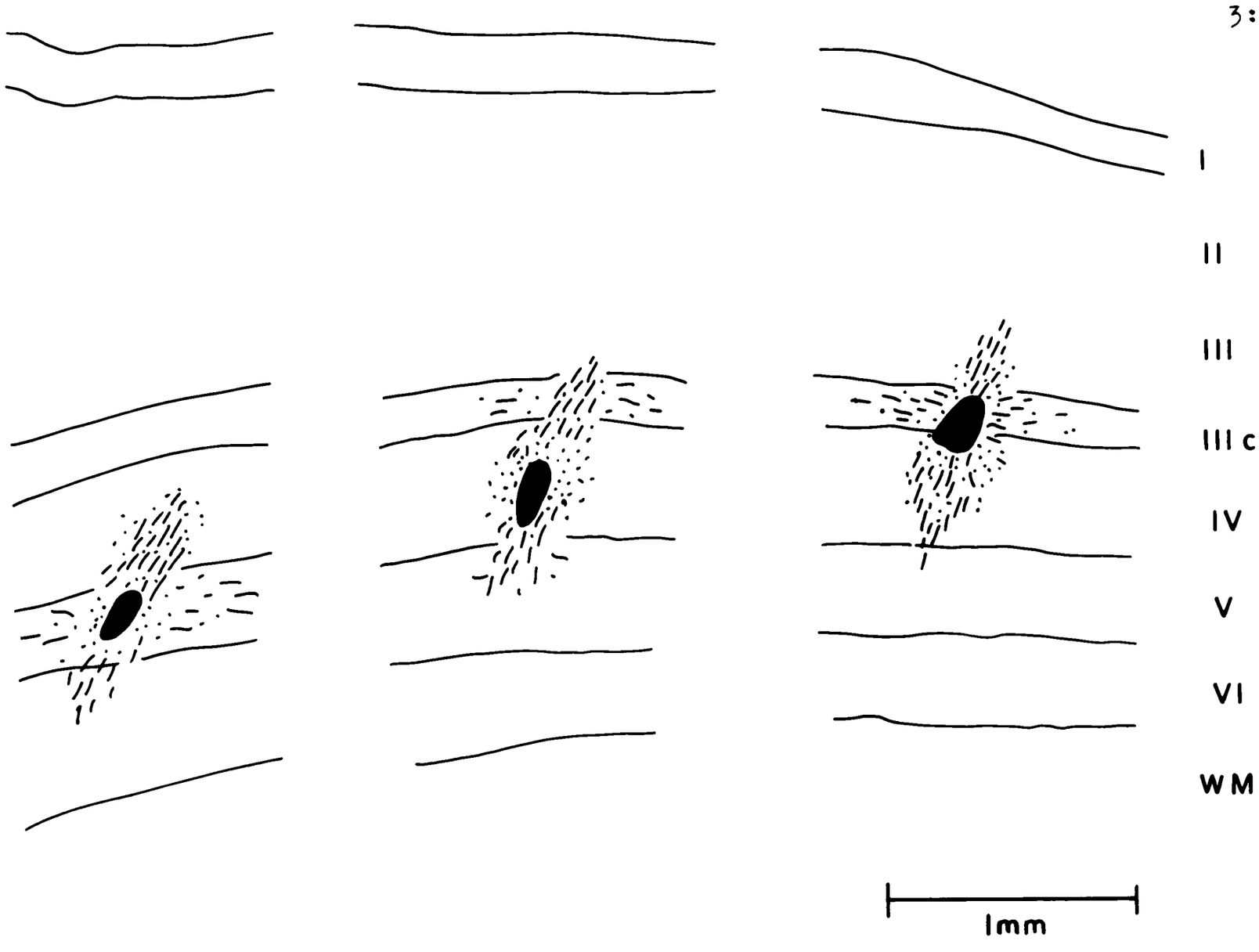

3:2
I
II
III
III c
IV
V
VI
WM
1mm

Fig. 3:3 The extent of the damage and the resulting degeneration in experiment 126R - 2, in which only laminae I and II were involved.

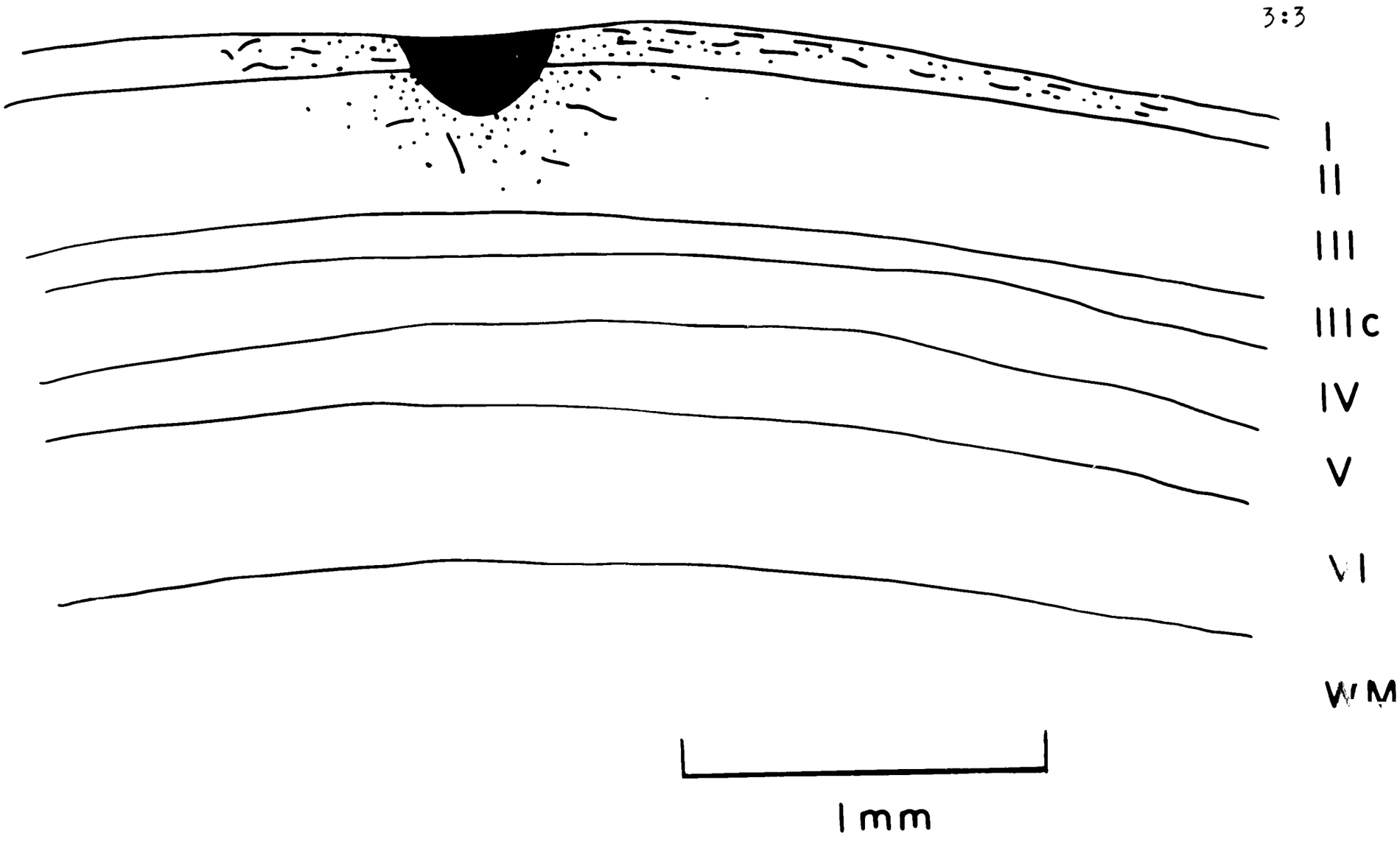
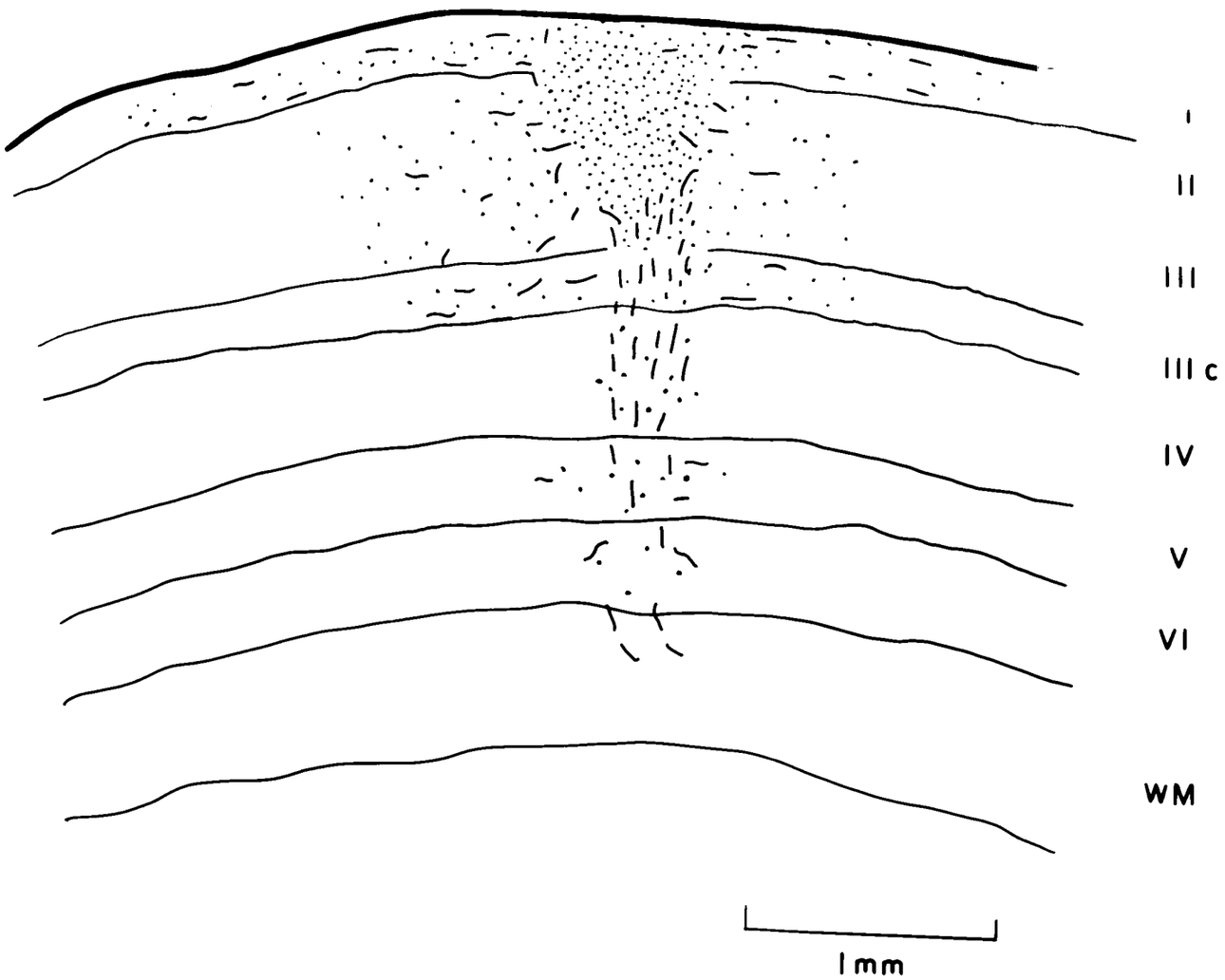

3:3
I
II
III
IIIc
IV
V
VI
WM
1mm

Fig. 3:4 The lesion and degeneration in experiment 139R - 3 (upper) and 117L - 5 (lower). In experiment 139R - 3 the extent of the lesion is indicated by the dense stipple.


I
II
III
III c
IV
V
VI
WM
1 mm

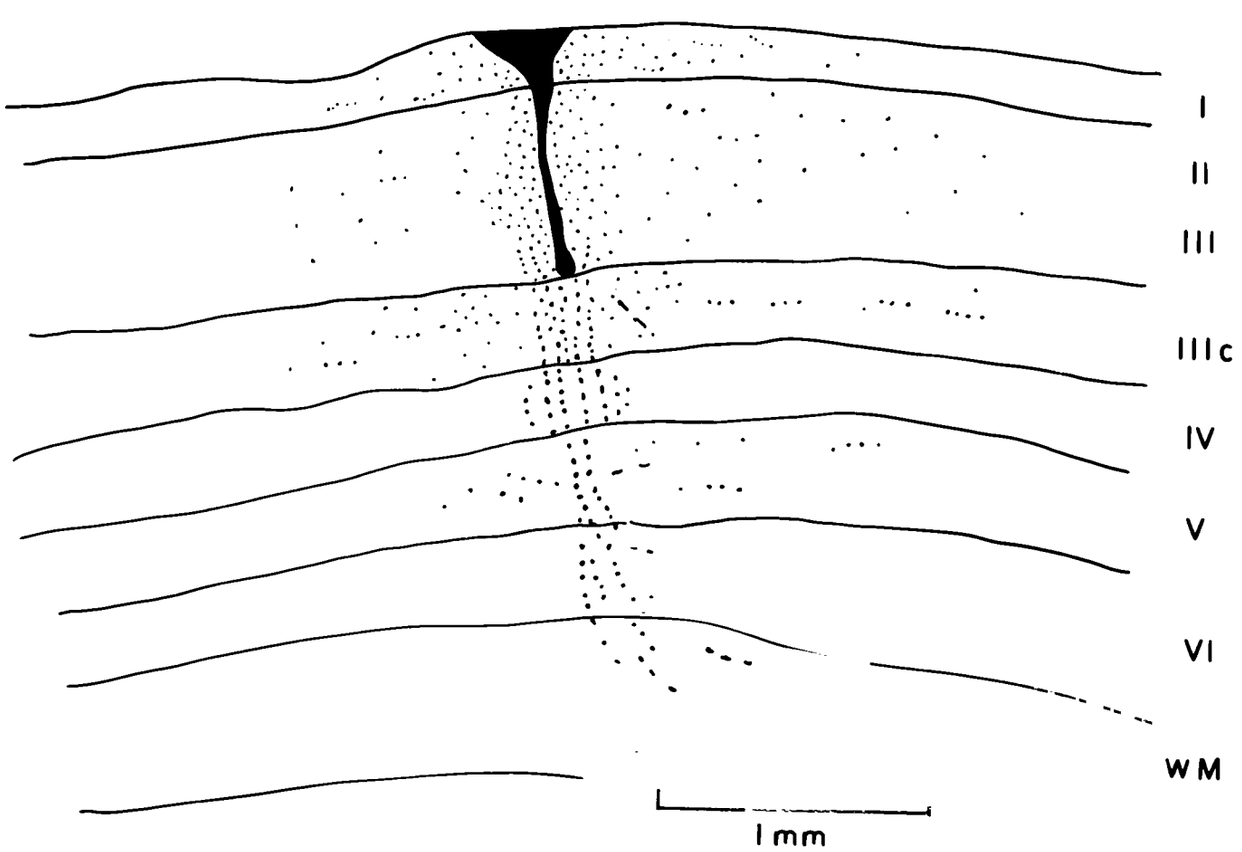

I
II
III
III c
IV
V
VI
WM
1 mm

Fig.3:5 The lesion and degeneration in experiment 126R - 3 (left) and 114R - 3 (right). In 126R - 3 three small focal lesions were made at different depths along the same penetration, and all were found on the same section.

3:5

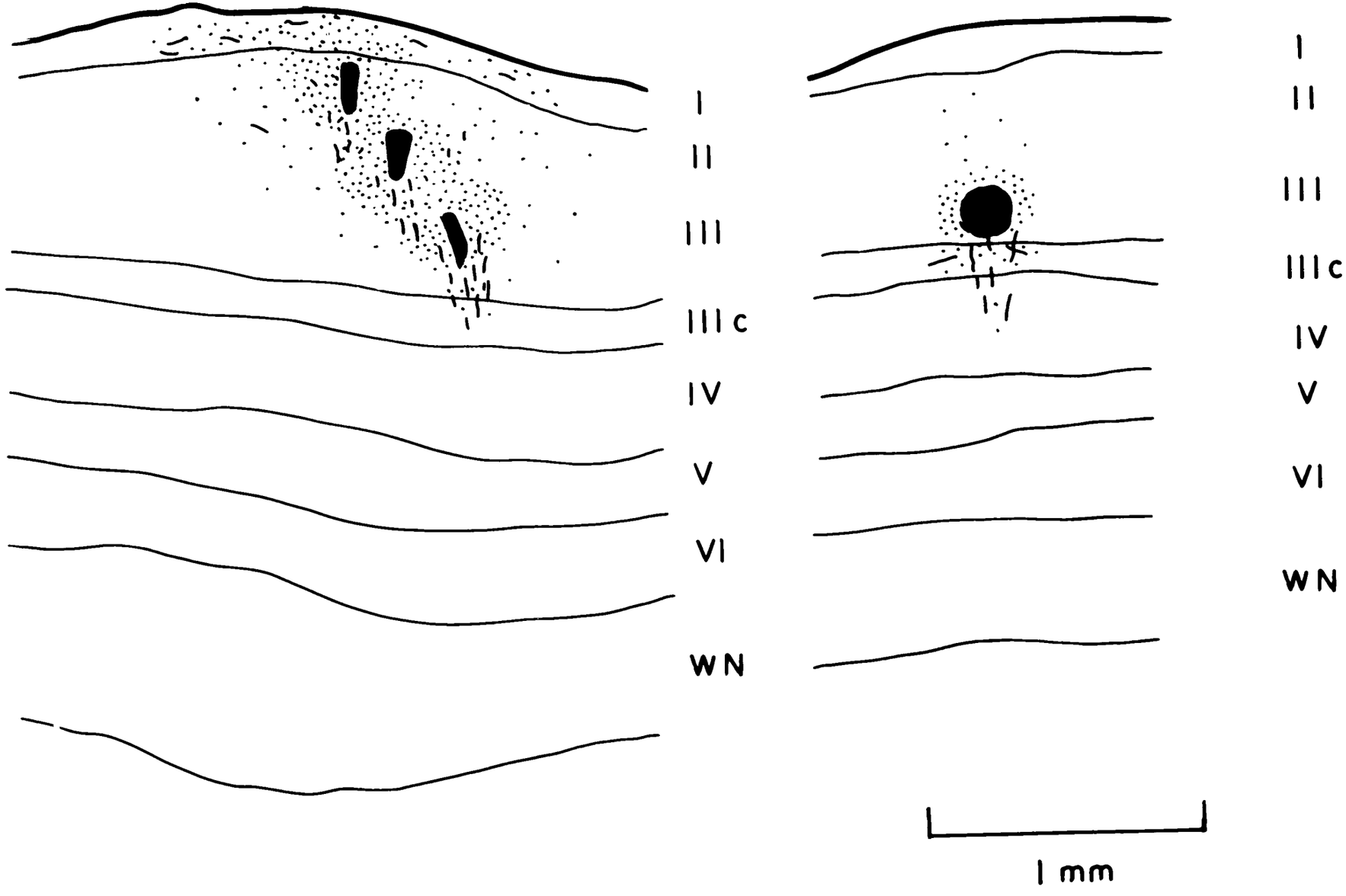


Fig.3:6 The extent of the damage and the resulting degeneration in experiment 117L - 4 (upper), experiment 111L - 4 (middle) and experiment 110R - 6 (below). In each of these experiments the damage extended down to involve the stria in lamina IIIc. In experiments 111L - 4 and 110R - 6 there was very little degeneration in the superficial layers due to the passage of the electrode.

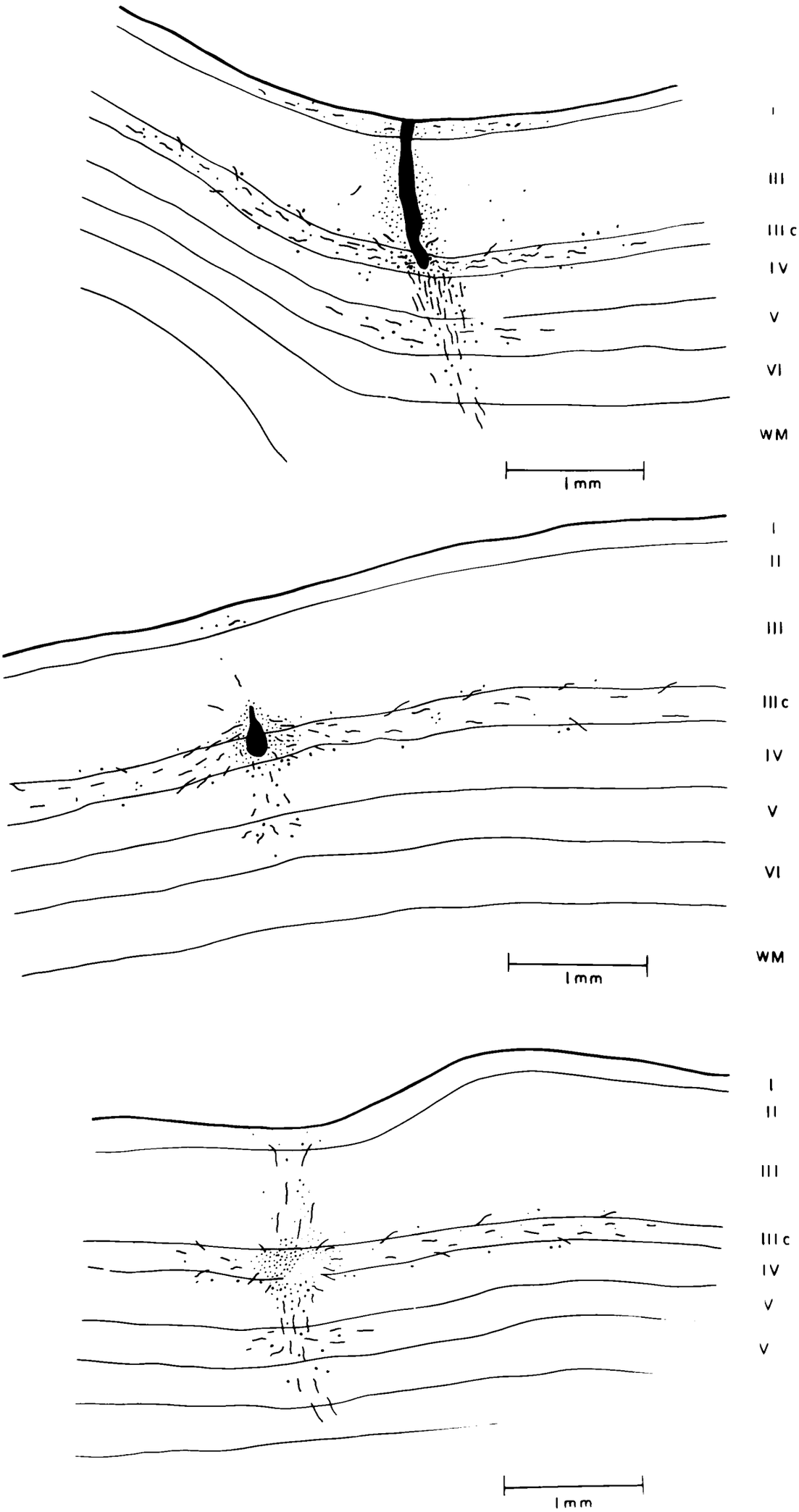

I
III
III c
IV
V
VI
WM
1 mm
I
II
III
III c
IV
V
VI
WM
1 mm
I
II
III
III c
IV
V
V
1 mm

Fig.3:7 The small focal area of damage within lamina IV and the ensuing degeneration, mainly in the stria, in experiment 111R - 9. In this experiment also there was very little degeneration in the more superficial layers due to the passage of the electrode.

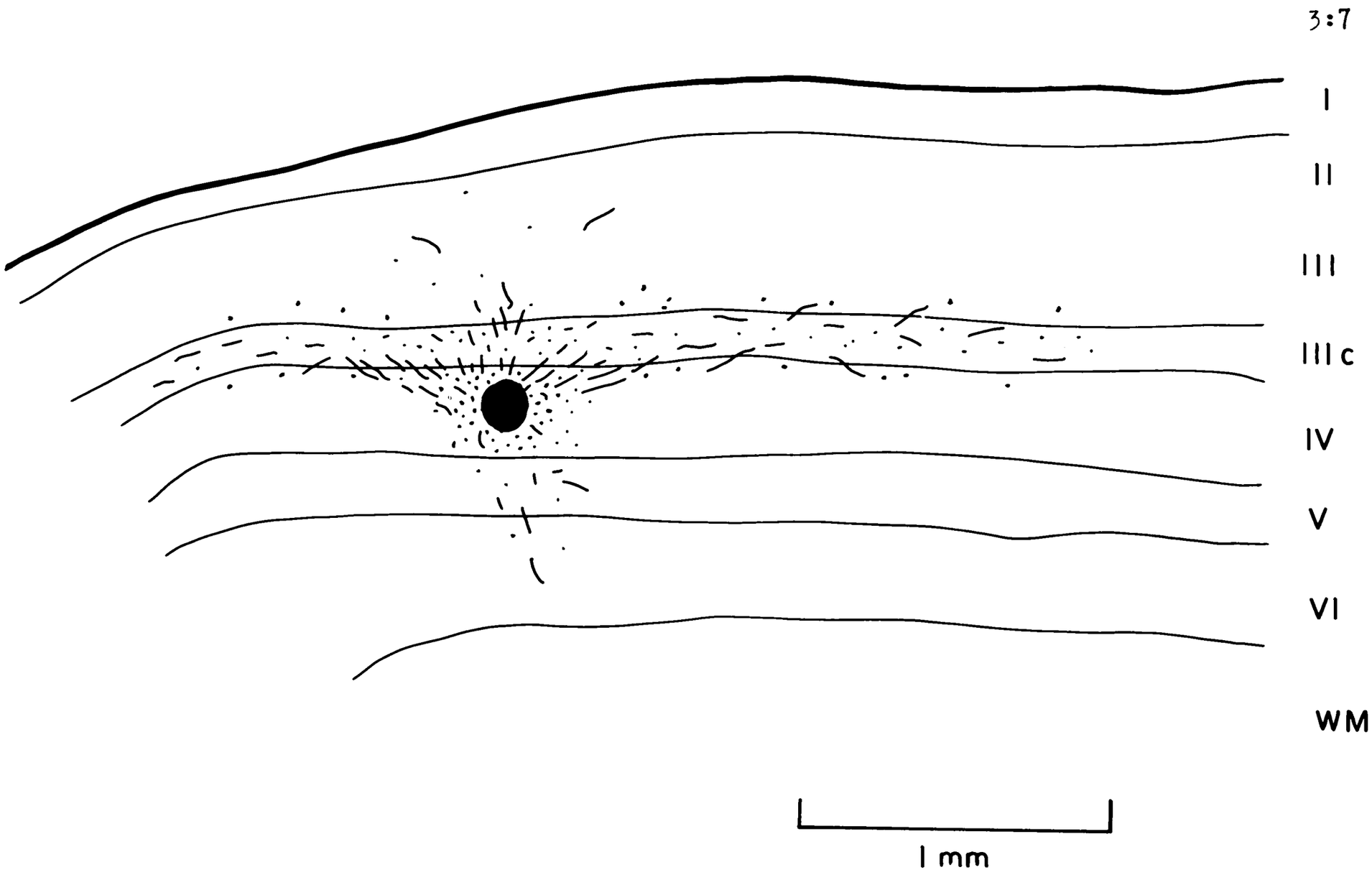

3:7
I
II
III
III c
IV
V
VI
WM
1 mm

Fig. 3:8 The narrow slit lesion in the cortex of area 17, close to the boundary of areas 17 and 18, and the resulting fibre and terminal degeneration in experiment 135 - L. Note the different pattern of degeneration in area 17 on the right (in which lamina IV is almost devoid of degeneration beyond the immediate vicinity of the damage) as compared with that in area 18 on the left, in which there is the same intensity of degeneration throughout all layers of the cortex (partly due to interruption of afferent fibres in the underlying white matter).

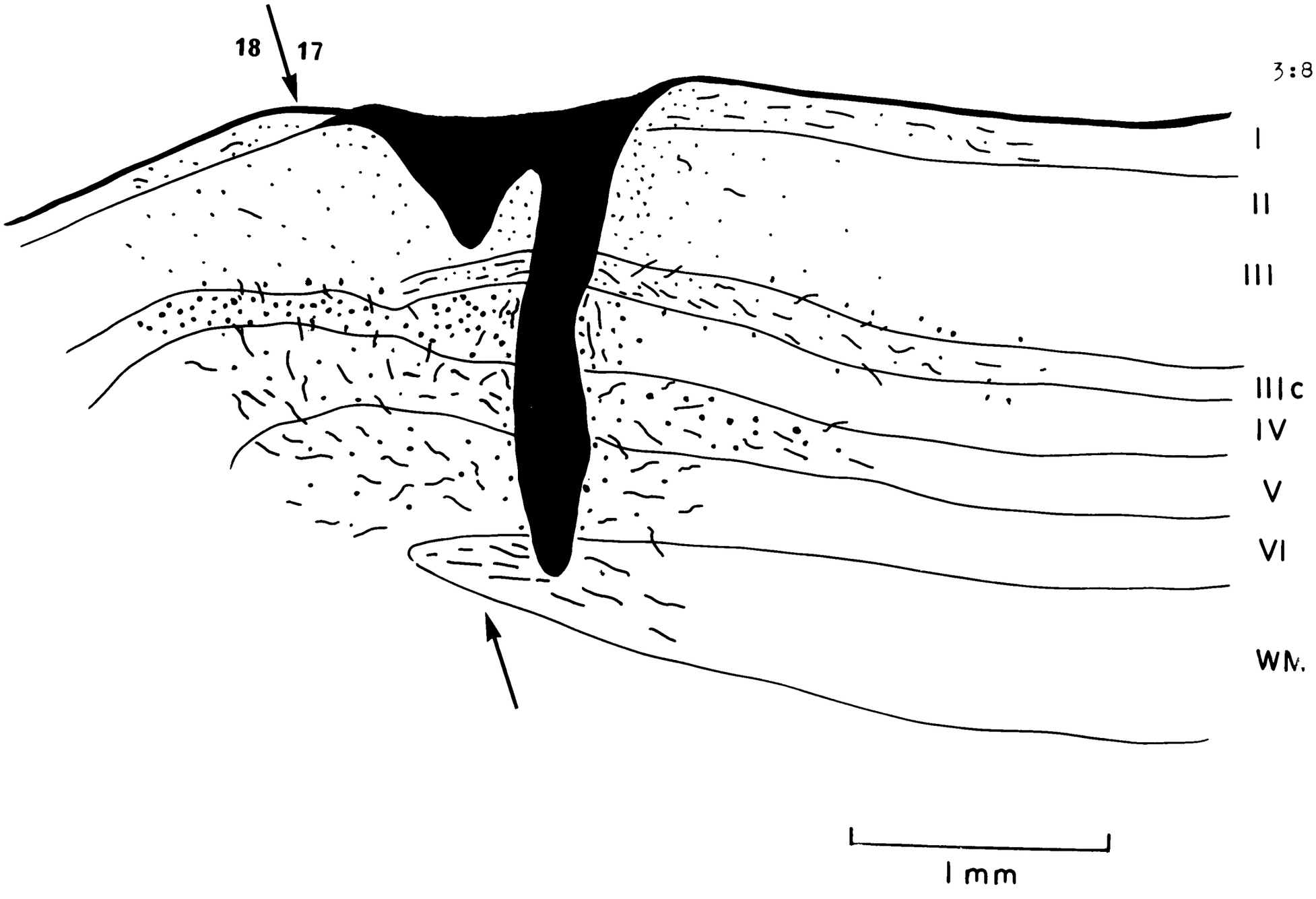

18
17
3:8
I
II
III
IIIc
IV
V
VI
WM.
1 mm

PLATE I

All photomicrographs shown in the plates have been taken from area 17 of the visual cortex within 2 mm of the lesion in the cortex. Sections have been stained by either the Nauta-Gygax (1954) method, the Fink-Heimer (1967) or the Wiitanen (1969) modification of the Nauta technique.

Fig. 3:9

The slit lesion in the cortex of area 17 of experiment 126R-1. The lesion is strictly confined to the cortex and does not invade the underlying white matter. Fink-Heimer stain. X 50.

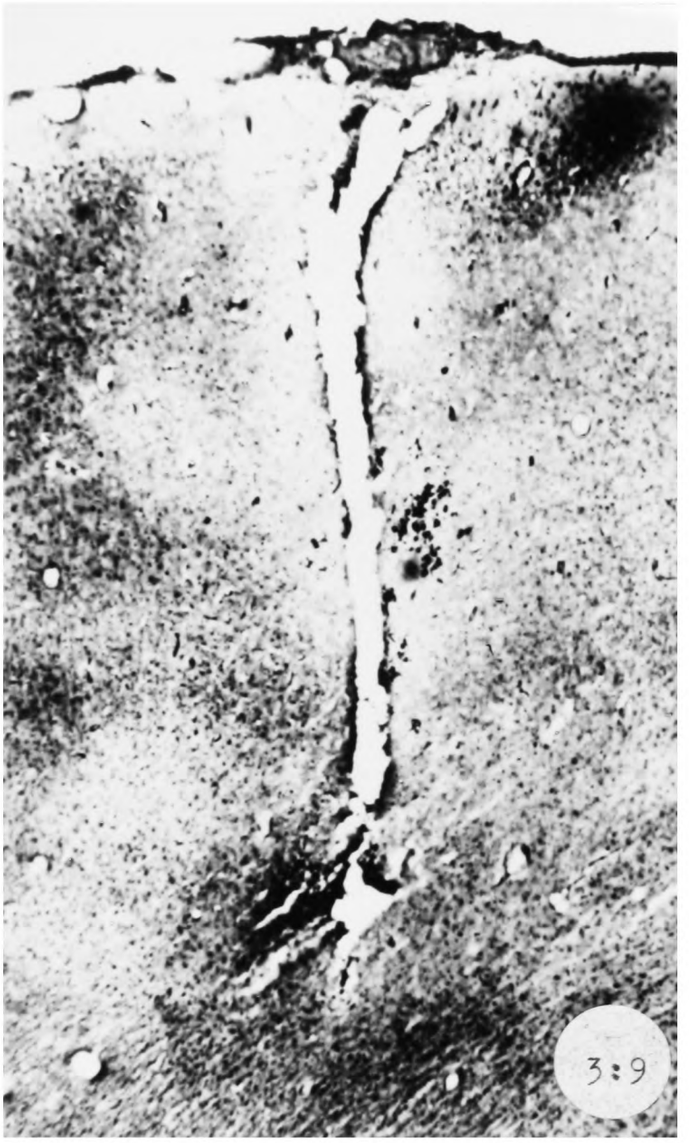
Fig. 3:10

The slit lesion in experiment 110L-2. The slit is wider than in the previous experiment, but does not extend deeper than layer V. Wiitanen stain. X 75.

Fig. 3:11

Part of the previous figure at higher magnification, to show the absence of fibre and terminal degeneration in layer IV except in the immediate vicinity of the lesion. The absence of degeneration in layer IV is in marked contrast to the severe degeneration present in layers IIIc and V. X 180.

Plate 1.



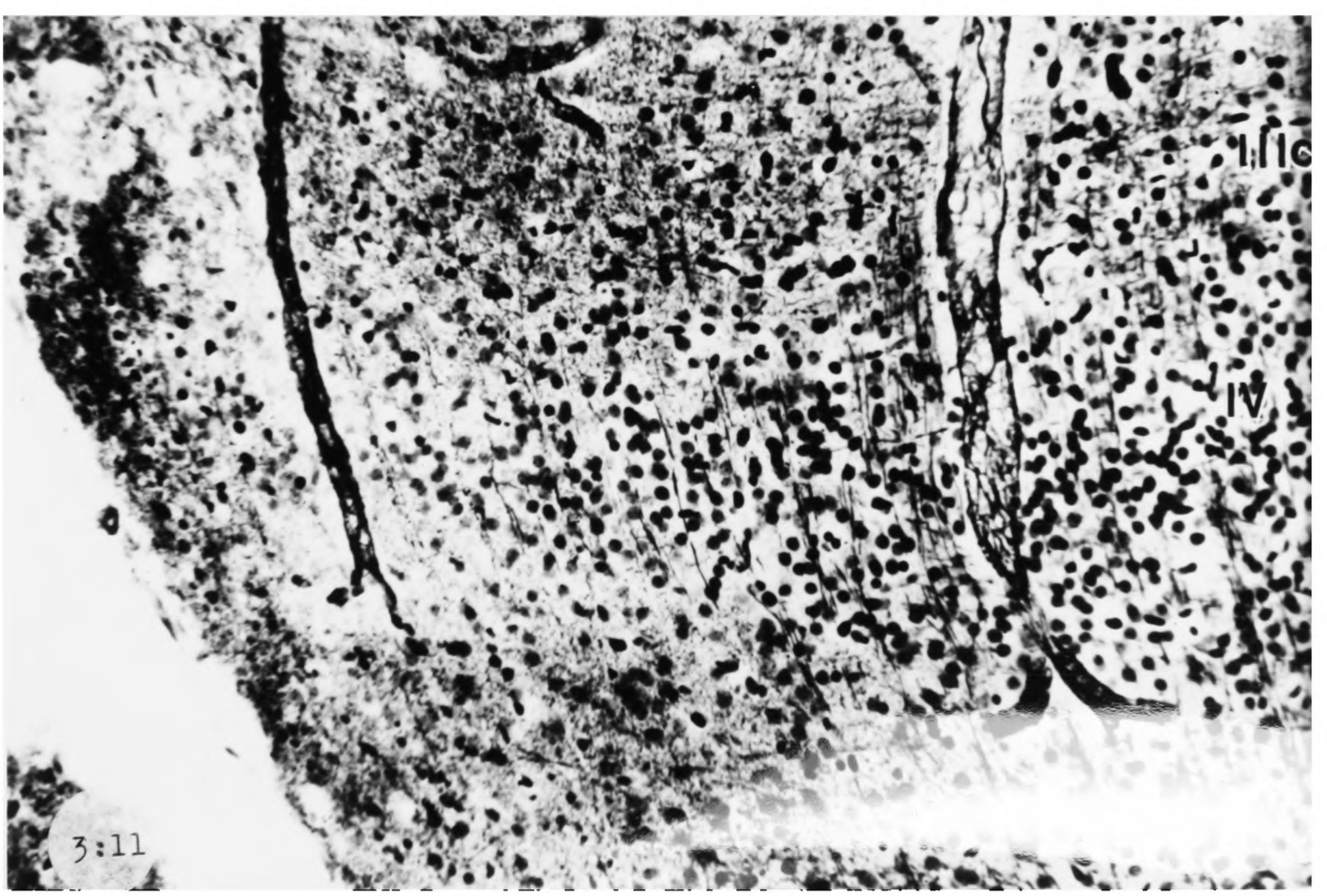


PLATE 2

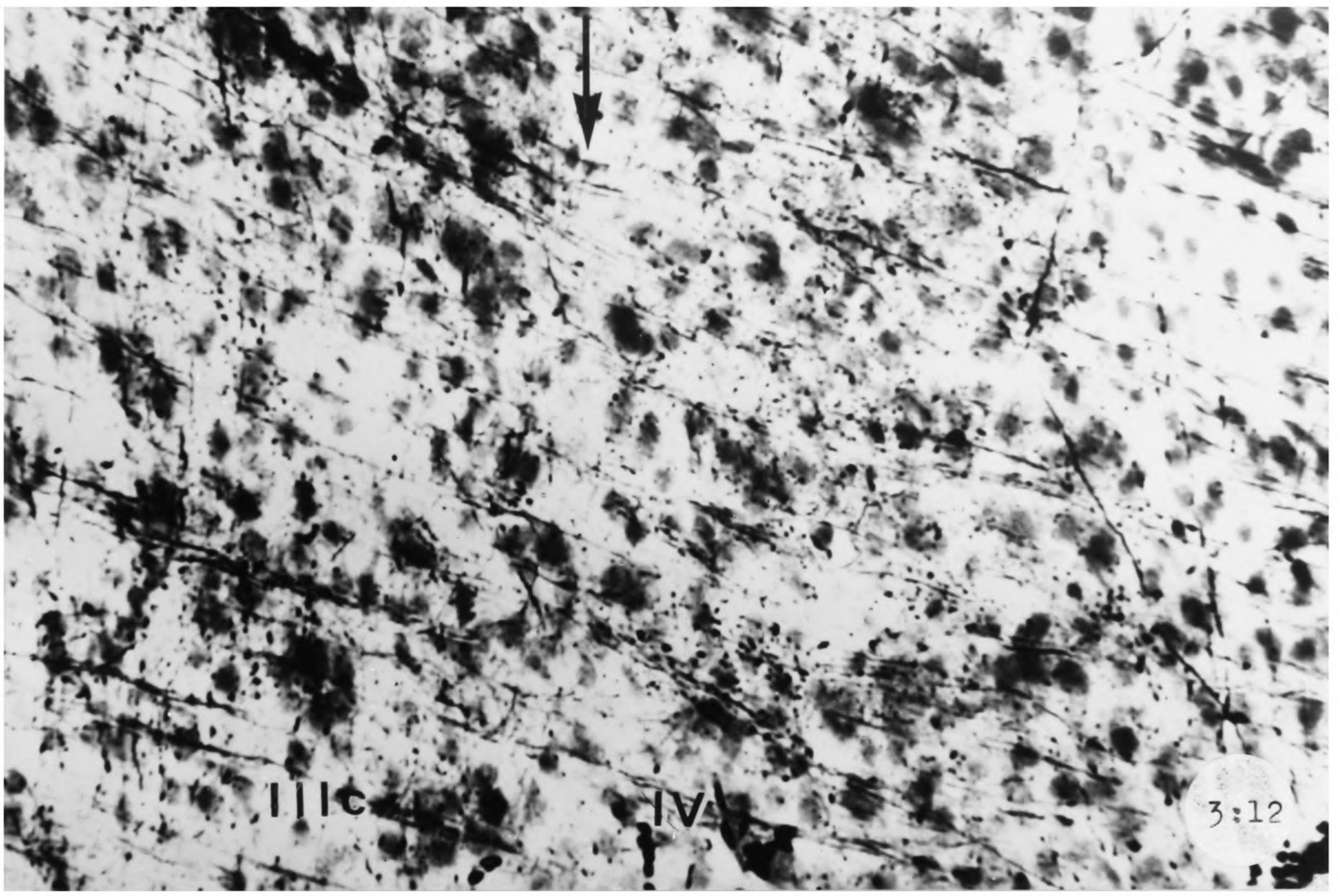
Fig. 3:12

The fibre and terminal degeneration in the stria (IIIc) and in layer IV in experiment 117R - 2. The arrow demarcates layer IIIc from layer IV, and it should be noted that the superficial aspect of the cortex is to the left of the figure and the deep margin to the right. X 400.

Fig.3:13

To show the degeneration of afferent thalamo-cortical fibres in layer IV beyond the point at which degeneration is present in the stria and layer IIIc in experiment 117R - 2. The arrow demarcates layer IIIc from layer IV and the disposition of the cortex is as seen in Fig. 3:12. It can be seen that the stria in layer IIIc is free of fragmentation. X 400.

Plate 2.



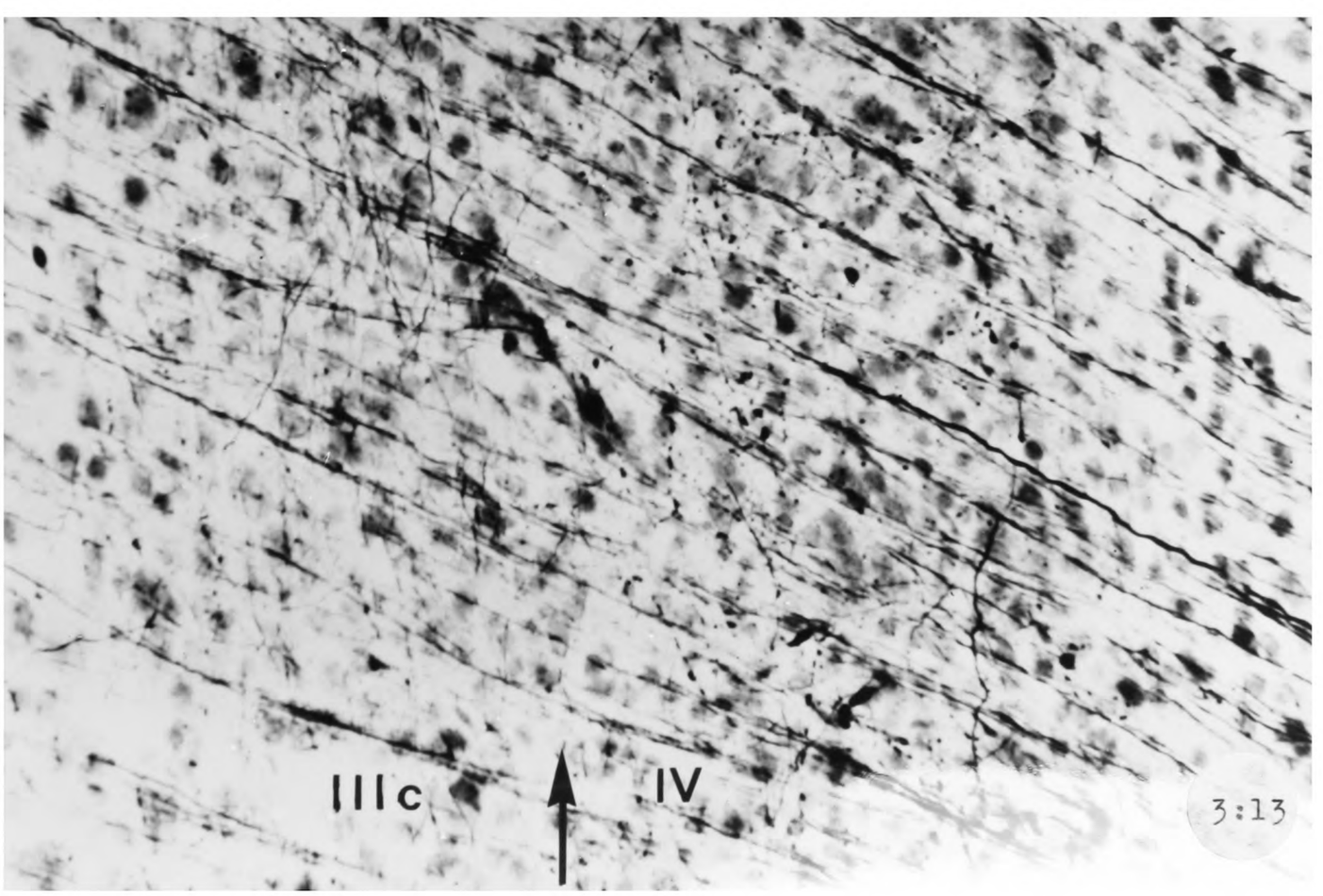


PLATE 3

Fig.3:14

The small lesion in layers I and II in experiment 114R - 2. X 50.

Fig.3:15

The same lesion as in the previous figure, at a higher magnification. X 150.

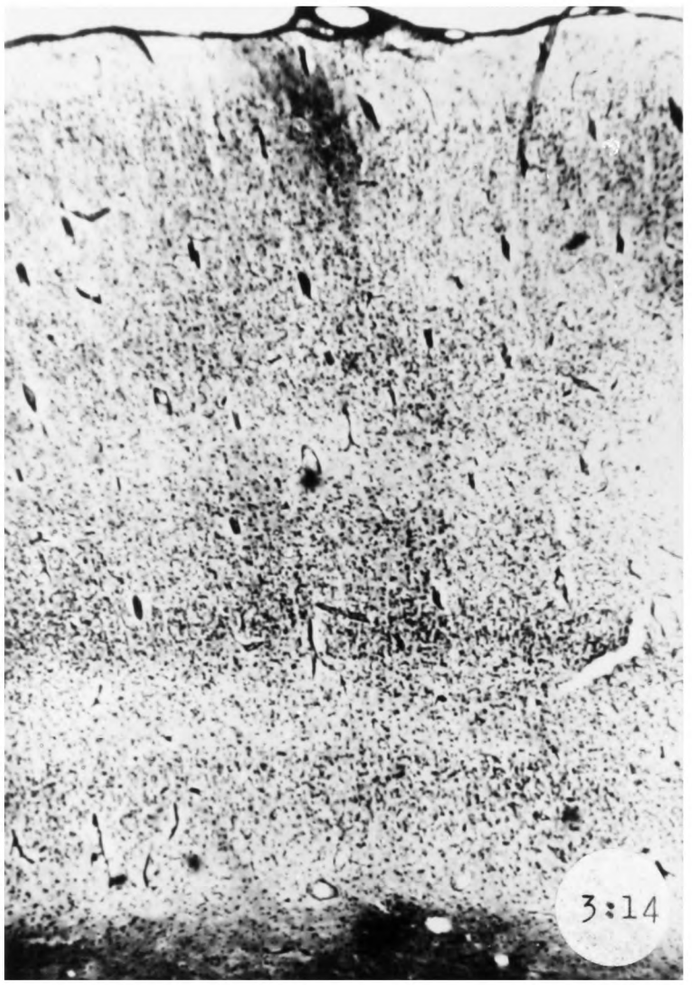
Fig.3:16

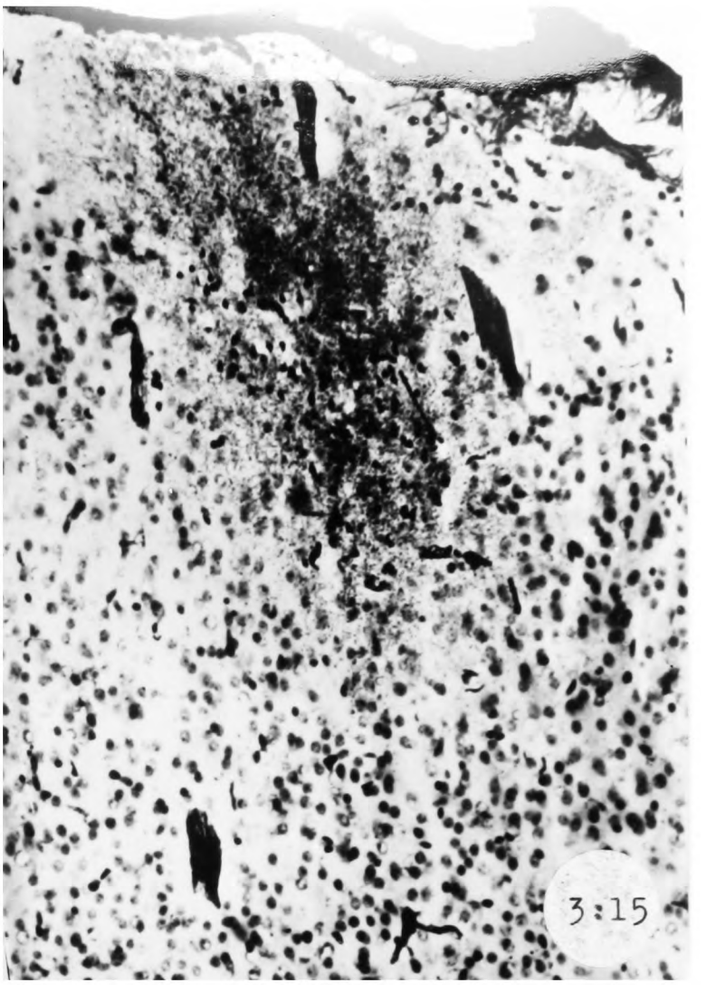
The three small lesions in layers II and III in experiment 126R - 2. X 50.

Fig.3:17

To show the small numbers of fibres running vertically downwards through layer IIIc from the deep aspect of the lesion in experiment 114R - 3. The lesion is indicated by the dense granularity at the top of the figure. X 450.

Plate 3.





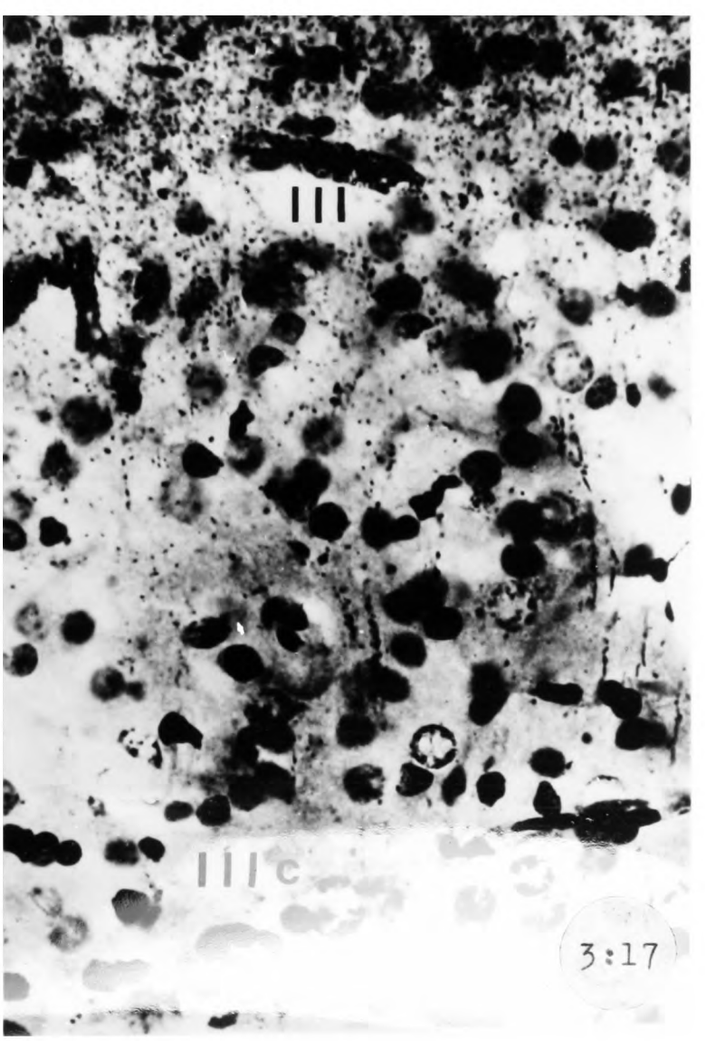


PLATE 4

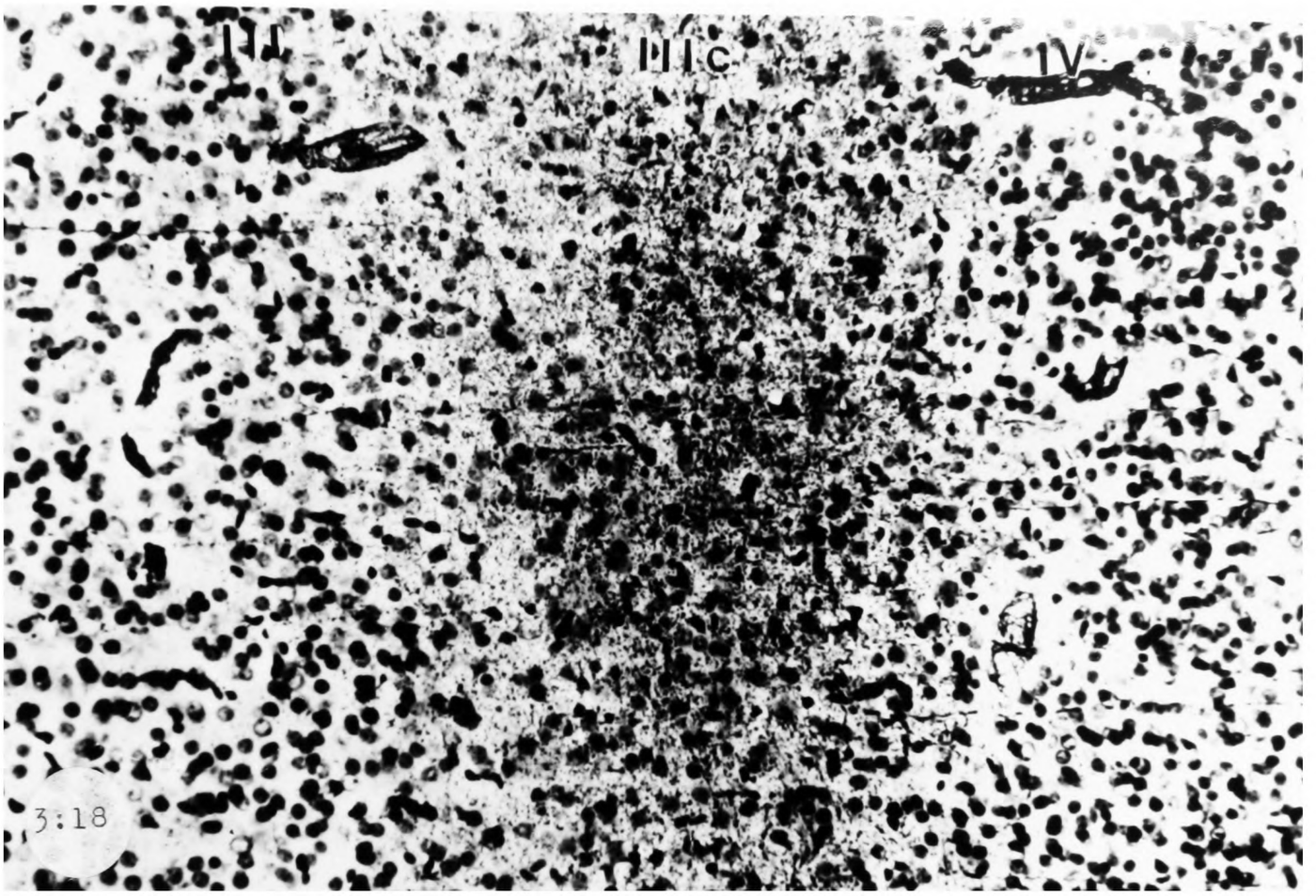
Fig. 3:18

The lesion in the striá (IIIc), as indicated by the very dense granular degeneration in experiment 110R - 6. There is very little degeneration in layer III superficial to it and in layer IV deep to it. The surface of the cortex is to the left and the deep aspect to the right. X 200.

Fig.3:19

The degenerating fibres passing horizontally in layer V, due to the lesion in experiment 117L- 5. Again it should be noted that the surface of the cortex is to the left and the deep aspect to the right. X 450.

Plate 4.



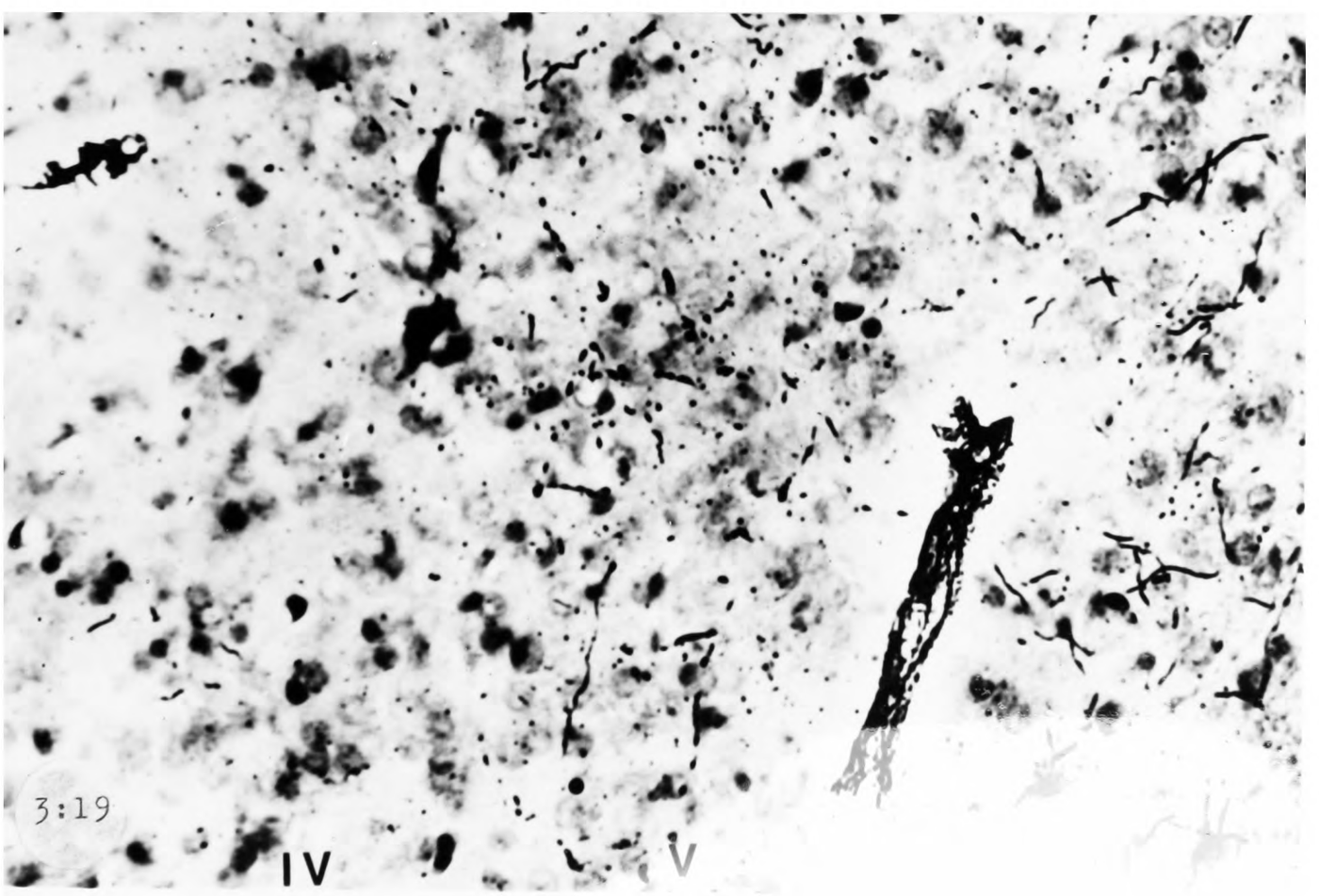


PLATE 5

Fig. 3:20

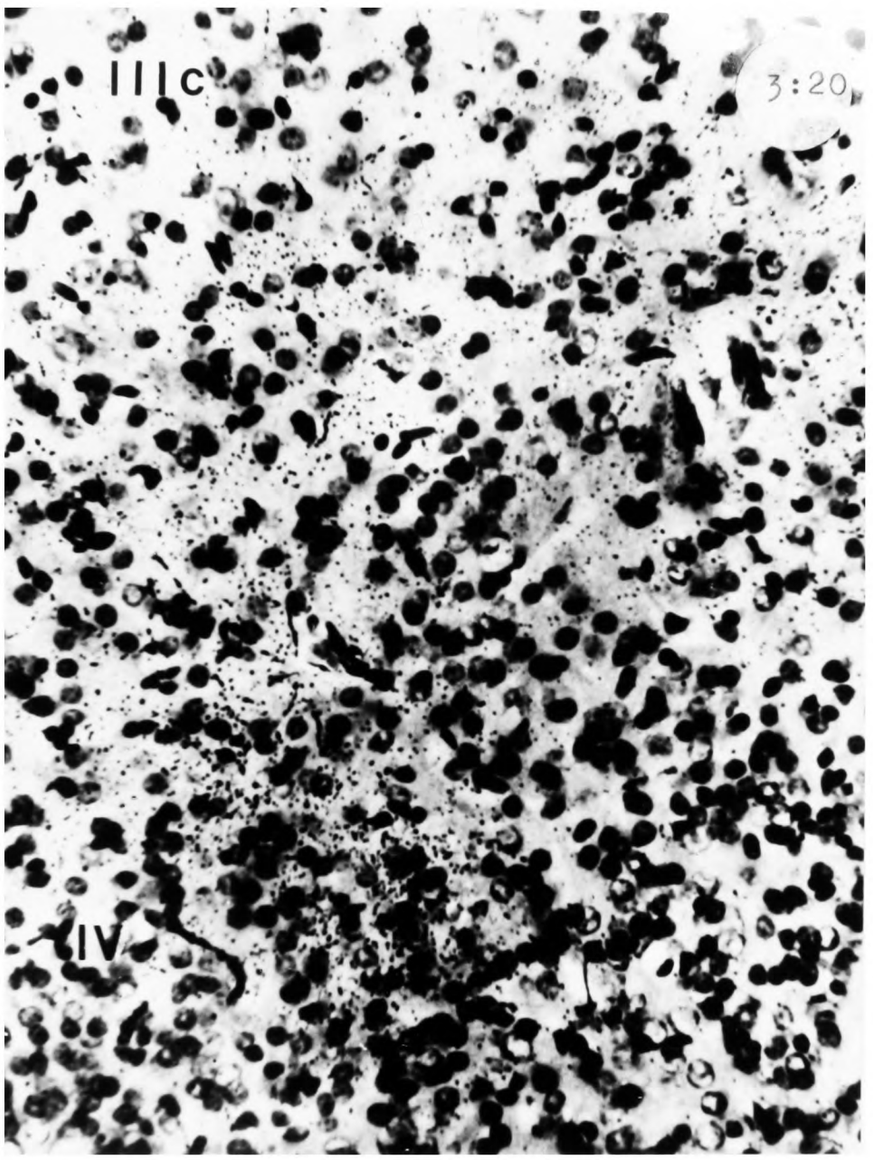
The lesion in layer IV in experiment 111R - 9, shown by the focus of granularity in this layer. The degenerating fibres due to this lesion can be seen extending superficially into the stria in layer IIIc. X 300.

Fig. 3:21

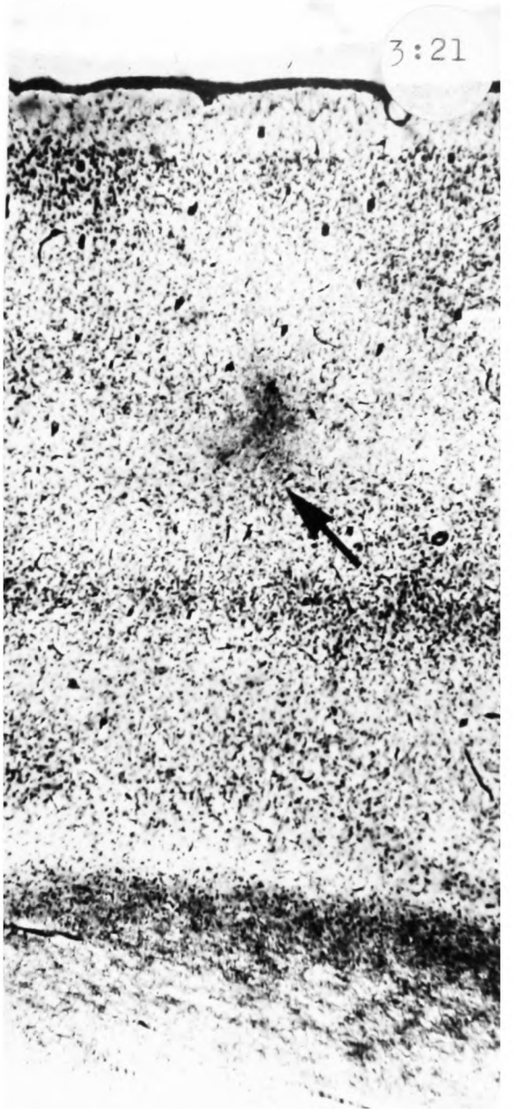
The small focal lesion in the deep part of layer IIIb and in IIIc in experiment II1L - 4. X 40.

Fig. 3:22

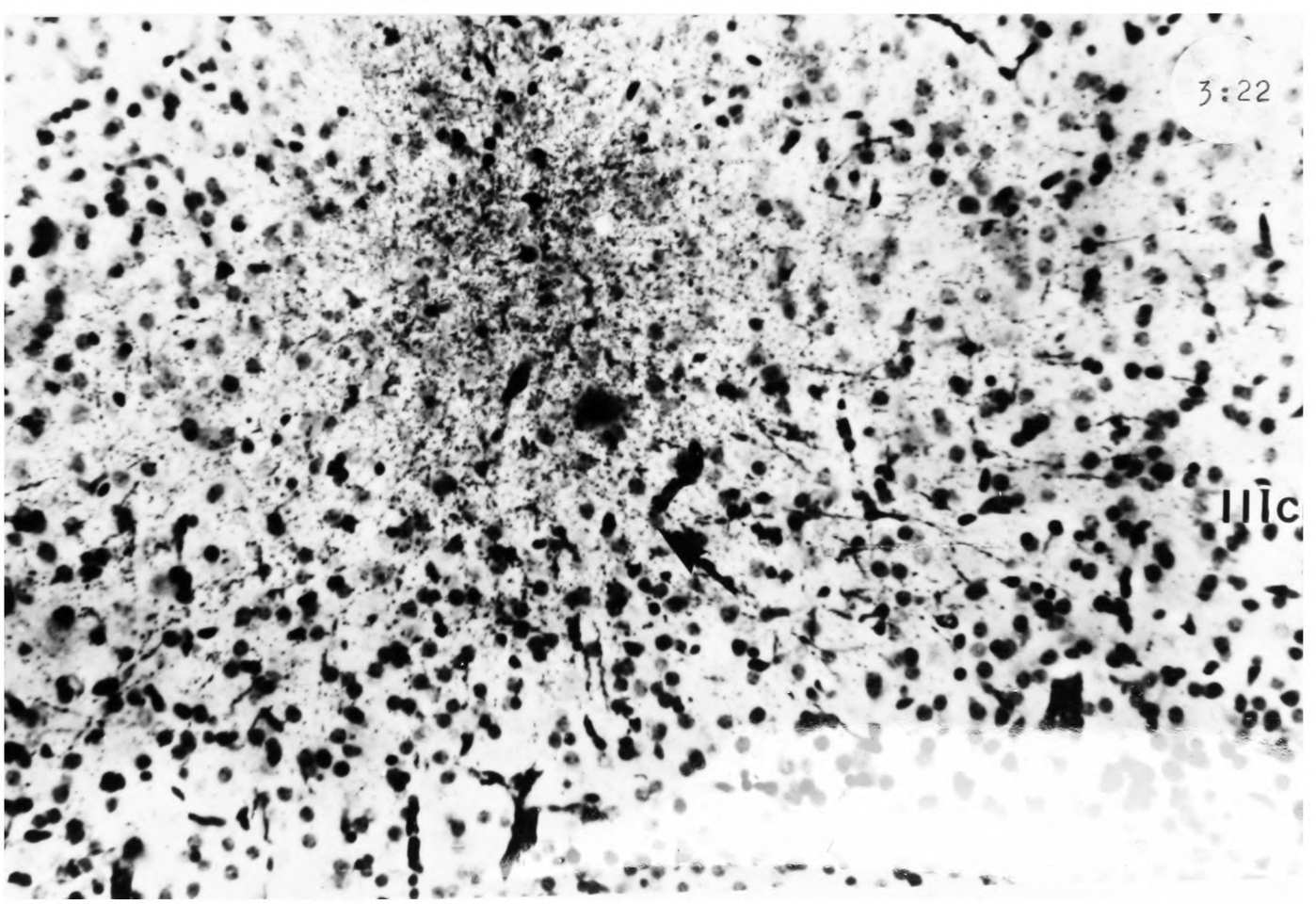
The deep aspect of the same lesion at higher magnification, and the degenerating fibres resulting from this can be seen extending horizontally into the stria (IIIc) on either side. X 240.



3:20



3:21



3:22

PLATE 6

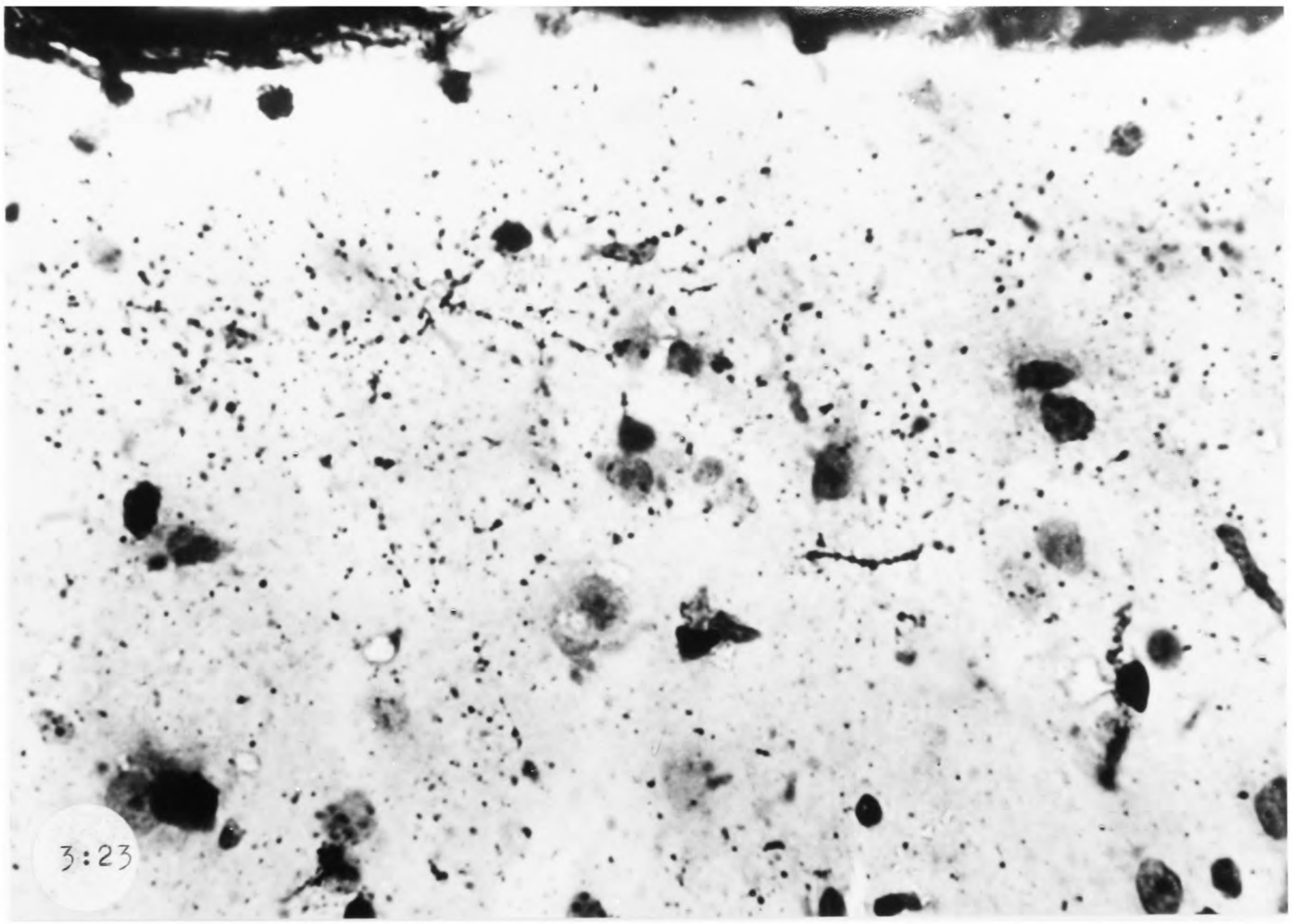
Fig. 3:23

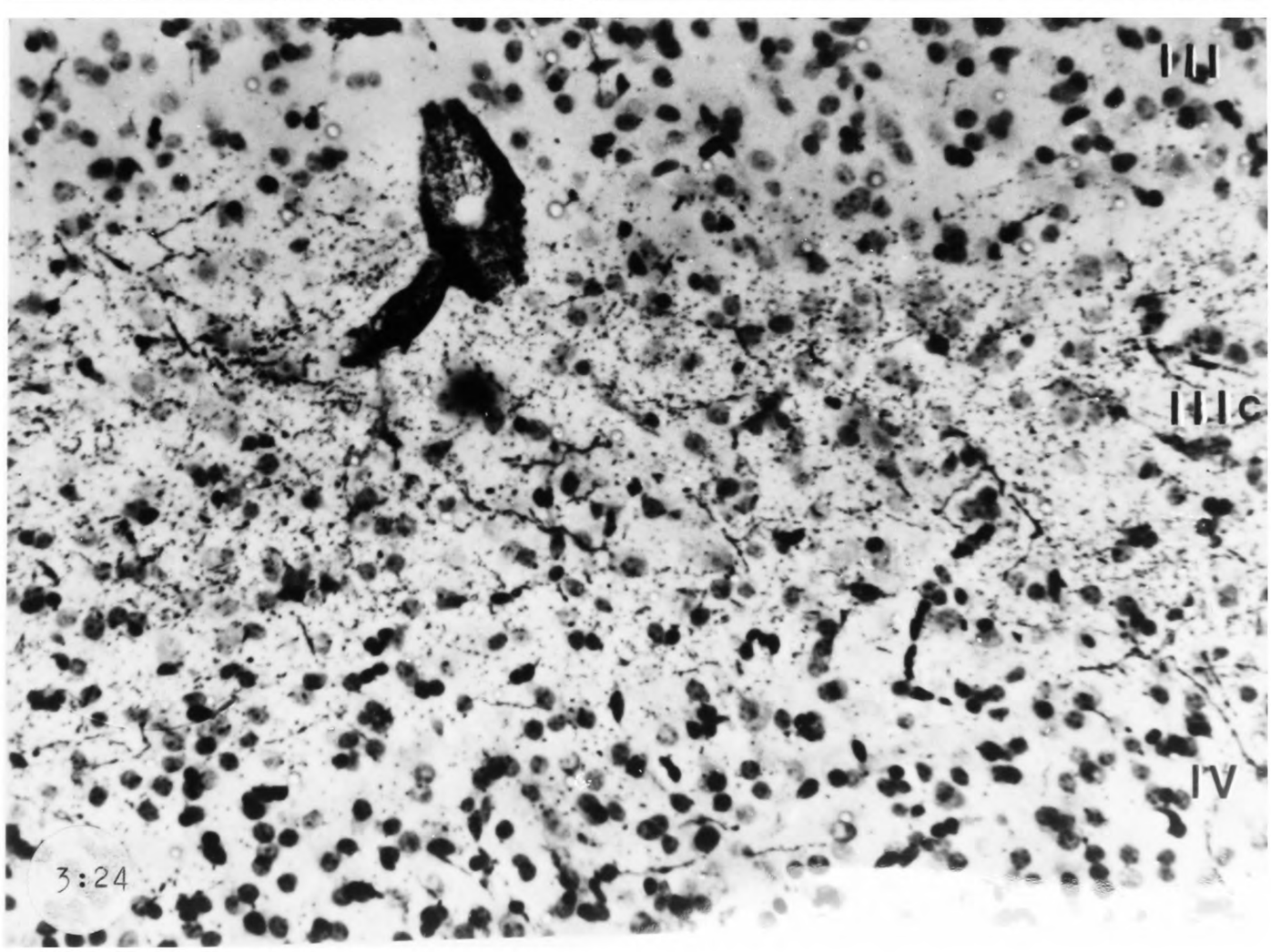
Terminal and fibre degeneration in layer I of the cortex 1.5 mm away from the small superficial lesion of layers I and II, in experiment 114R - 2. X 700.

Fig. 3:24

The dense degeneration in the stria in layer IIIc, 0.5 mm away from the focal lesion in layer IV in experiment 111R - 9. X 320.

Plate 6.





CHAPTER 4

An electron microscopic study of the intrinsic connections of area 17 of the monkey

Introduction

This study is complementary to that which has been described in Chapter 3, and is intended to bring out some of the features of cortical connections at an ultrastructural level. Accordingly, it may be useful at this point to describe briefly the main ultrastructural features of area 17 (a fuller account is given by Garey, 1971) which, indeed, do not differ greatly from those described for the cortex by earlier workers (see Jones and Powell, 1970 a, 1970 b, 1970 c for references; also Gray, 1959; Colonnier, 1968; Peters, Palay and Webster, 1970).

The two main neurone types, pyramidal and non-pyramidal, can readily be distinguished in an electron microscope section : the pyramidal cell, which typically possesses a large apical dendrite into which the cell soma tapers, shows a rather light cytoplasm with few organelles. The nucleus often has a single indentation. Rather few synapses are seen on the cell soma and proximal parts of the dendrites, and such as are present are all of the symmetrical (vide inf.) type (type II of Gray), with flattened or pleomorphic vesicles. The non-pyramidal neurone, as it has been described hitherto, lacks an apical dendrite and can also be recognised by

the comparative density of its cytoplasm, which results from the presence of appreciable numbers of mitochondria and considerable amounts of both rough and smooth endoplasmic reticulum. This density frequently makes the cell difficult to define at low magnification, as it merges very readily into the surrounding neuropil. The other characteristic feature of such cells is that the cell soma and proximal dendrites receive a large number of synapses, roughly half of which are of the asymmetrical type (type I of Gray), with spherical vesicles. The dendrites of such cells are frequently varicose or tortuous in outline and bear variable numbers of spines, in contrast to the dendrites of pyramidal cells, which are straight and uniform and bear a large number of spines, especially in their more peripheral parts (this property is inferred from Golgi rather than electron microscope studies; see Valverde, 1967). Recently a second type of non-pyramidal neurone has been described in the sensorimotor cortex of the monkey by Sloper (1973 a). Cells of this type, referred to as "small stellate" (the non-pyramidal neurones described above being referred to as "large stellate") are usually some 8-12 μm in diameter and are round. The nucleus is dark, with dense clumps of chromatin, and is frequently indented. The cytoplasm is sparse and pale, with a low density of organelles, although the Golgi apparatus is often prominent. The soma receives few synapses, usually less than three in a single section, but these may be of either type. The

dendrites of these cells are usually very varicose, often having an initial constriction; they receive a moderate number of both types of synapse. Small stellate cells are commonest in layer II, though they may occur in any cortical layer. While we are not able to provide full information about the cell types of the visual cortex as seen with the electron microscope, it is nevertheless our impression that small stellate cells do exist in area 17 and that they are commonest in layer II. Examples of such cells are shown in figs. 4:1, 4:2 and 4:3. Fig. 4:30 shows an axosomatic symmetrical terminal ending upon a cell which is probably small stellate in nature.

The neuropil of area 17 consists of dendrites, myelinated and non-myelinated axons, axon terminals and dendritic spines. Synapses are common and can be recognised by the presence of synaptic vesicles and of synaptic membrane thickenings at the contact zone between the axon terminal and the postsynaptic structure. It is now generally accepted that almost all synapses can be classified as being either asymmetrical (type I) in which the postsynaptic thickening is denser than the presynaptic and the synaptic vesicles appear round, or symmetrical (type II), in which the postsynaptic thickening is equal to or only very slightly thicker than the presynaptic and the vesicles appear discoid, cigar-shaped or pleomorphic. The synaptology of different parts of the neurone varies: a dendritic spine invariably receives one,

and may receive two, asymmetrical synapses. In addition, some 10-20% of spines receive a symmetrical terminal (Jones and Powell, 1970 a). Pyramidal cell dendrites probably receive a mixture of the two synaptic types; however, close to the cell soma, where their nature can be more confidently determined, they receive rather few synapses, virtually all of which are symmetrical. Stellate cell dendrites, by contrast, receive a large number of synapses, roughly half of which are asymmetrical. The differences in synaptology between the soma of the pyramidal cell and those of the two types of stellate cell have already been mentioned. Finally, recent descriptions (Palay et al. 1968; Peters et al. 1968; Jones and Powell, 1969 c) have served to clarify the appearance of axon initial segments and to render them readily distinguishable from dendrites. Inital segments are characterised by the abrupt narrowing of the cytoplasm of the parent cell as they arise, by the presence underneath the membrane of a greyish fuzz or undercoating, which differs from synaptic membrane specialisations in being lighter and less clearly delimited, and by the presence within the cytoplasm of neurotubules arranged as well-defined bundles. Initial segments receive a few synapses, but it is noteworthy that these are invariably of the symmetrical type.

Most of the results to be described below were obtained from experiments in which lesions were placed throughout the whole depth of the cortex either in the form of a slit, made with a knife

or a needle, or by allowing the current to run continuously during the downward and upward traverse of a microelectrode. As in the preceding light microscopic study, all the lesions were placed in that part of area 17 which is on the exposed, lateral surface of the hemisphere, containing the representation of the central part of the retina. Only material adjacent to lesions which involved virtually all of the depth of the cortex but which did not penetrate the white matter was used for electron microscopic study, after a careful examination of "thick" sections of several blocks. This restriction was necessary for two main reasons: first, despite accurate measurements of the site of placement of small microelectrode lesions in relation to known landmarks, it proved more difficult than anticipated to know where they were precisely situated in order to include them in the small blocks necessary for electron microscopic study; and secondly, the density of degeneration even within a few millimetres of larger lesions proved to be surprisingly small. However, some results of the study of material in relation to partial lesions will be presented.

Blocks of approximately 0.5 mm thickness were cut in such a way as either to include the whole lesion and approximately 1 mm or so on each side (microelectrode lesions), or the edge of a lesion and 2-3 mm of the cortex on one side. Depending upon the orientation of the lesion, blocks were cut either in the sagittal or coronal plane and, in agreement with the light microscopic observations, no obvious differences were found between sections

from blocks cut in these different dimensions.

In the "thick" sections, the edge of the lesion was seen to be surrounded by a narrow zone, approximately 200 μm wide, which was densely stained and showed disorganisation of structure with intense gliosis. Usually, this region was sharply demarcated from the rest of the section, which appeared essentially normal; the neurones and their dendritic processes stained normally and were not separated by oedematous swelling. The pia mater was intact; this indicated that the aim at operation to minimise interference with blood supply, by inserting the needle between blood vessels, had been achieved.

In the thin sections used for electron microscopy these observations were confirmed. Except for the region of 200 μm immediately adjoining the lesion the tissue was remarkably well preserved; the overall appearance of the neuropil was no different from that of experimental material from brains in which lesions had been placed in a distant site, such as the lateral geniculate nucleus or, except for the degenerating terminals and glial reaction, from normal tissue. Within the 200 μm width immediately adjoining the edge of the lesion, however, the structure is completely different: the majority of profiles are abnormal, either in being grossly enlarged, pale and with widely dispersed organelles or in being very dark and shrunken. All parts of the neurone: soma, dendrites and spines or axons and their terminals may show either

of these two distinctly different appearances. In addition, there is an intense glial reaction; most of the glial processes are very dark, with large amounts of glycogen. In view of this marked disorganisation of tissue, probably partly due to direct damage and partly to vascular involvement, no study of terminal degeneration has been made within this region, and all the results refer to the essentially normal tissue beyond it. In the latter, only a very occasional dark neuronal soma, dendrite or spine has been encountered, and the observation that most of these are found within the first millimetre strongly suggests that they represent parts of neurones directly damaged by the lesion.

All of the affected axon terminals showed the same type of degenerative change, in that, after the earliest sign of enlarged vesicles, they underwent a progressive darkening and shrinkage; no evidence of filamentous degeneration of any terminals at any survival period has been obtained. In order to determine whether there were any differences in pattern and density of degeneration with changes of survival period, comparable material was examined at 24-hour intervals from 1 to 6 days. After 24 hours there was early degeneration of relatively few terminals within 1 mm of the lesion, shown either by enlargement and irregularity of the synaptic vesicles, but without much change in the axoplasm, or by slight increase in density of the axoplasm, closer packing of the vesicles and slight shrinkage and irregularity of the outline of the terminal; glycogen granules may be present in

terminals at these stages. Over the next few days there was a progressive increase in the number of terminals showing later stages of degeneration in the form of increasing degrees of shrinkage, disruption of vesicles and mitochondria and increased electron density. The final stages of the degenerative process were seen either as engulfment of the dense terminal and preterminal fibre by reactive glial processes, or as the presence of the remnant of the terminal as a dense sliver adjoining the postsynaptic membrane; occasionally, an exposed postsynaptic thickening was seen, with no evidence of a presynaptic structure remaining. At all intervals studied after the first two days, terminals at all stages of degeneration may be found, and even at 6 days an occasional terminal may be seen, usually a few millimetres from the lesion, showing the earliest stages of degeneration. The period at which there seemed to be the maximum number of terminals showing unequivocal signs of degeneration was four days; consequently, material from experiments with this survival period was used for the quantitative studies. It is perhaps worth mentioning, in addition, that for the purposes of this investigation certain strict criteria were set down for the rejection and acceptance of possible degenerating terminals seen with the electron microscope. Firstly, only those dark profiles with synaptic membrane specialisations polarised away from them have been accepted, for although axon terminals can be distinguished from dark dendrites by other means (in the early stages of degeneration by the presence of synaptic vesicles and in

the later stages by the considerably greater electron density of the axon terminal), the appearance of the synaptic thickenings has been found to be the most reliable guide. Secondly, in regard to classification, it may be noted that during degeneration the shape and size of synaptic vesicles changes, so that their features cannot be used for differentiating between asymmetrical and symmetrical synapses. Once again, the characteristics of the membrane thickenings have been used; however, although in the majority of cases it has been possible on this basis to classify terminals with a reasonable degree of confidence, a certain proportion have been left unidentified.

Results

Qualitative Observations

Most of the degenerating terminals seen were undoubtedly of small size, but an occasional large terminal with asymmetrical membrane thickenings and several synaptic contacts (Fig. 4:6) was observed. The majority of degenerating boutons appear to be true terminals, but examples of terminals "en passage" have been seen (Fig. 4:4); however, these are not sufficiently numerous to permit conclusions to be drawn concerning their distribution within the cortex or in relation to particular types of cell. Axon terminals undergoing degeneration have been found to make synaptic

contact with all the main parts of neurones: the soma, proximal and distal dendrites, spines and initial segments. Occasionally, within the first millimetre or so of the lesion, the postsynaptic profile (small dendrite or spine) has been found to be dark and undergoing degeneration. Examples of such dark profiles are given in Figs. 4:7, 4:9 and 4:10. Just as there was difficulty in some cases in the identification of the type of axon terminal according to its membrane thickening, so in respect of some of the smaller postsynaptic profiles there has been uncertainty about their identification as either small peripheral dendrites or spines.

The density of degeneration was greatest within the first millimetre or so from the lesion, but even here, at the optimal survival period of four days, the number of terminals which were degenerating formed a very small proportion of the total, certainly not more than 5%. At a distance of 1 mm the density of degeneration showed a sudden and marked decrease, and beyond this level, up to the distance examined (3 mm), only an occasional degenerating bouton was found. Within the first millimetre the density of degeneration was sufficient to permit valid comparisons between the laminae, and the most striking feature of these comparisons is the virtual absence of degenerating terminals in lamina IV. In repeated surveys the only evidence of degeneration in this lamina was found in the first half-millimetre from the lesion; this finding agrees very well with our light microscopic observations (see p. 39). Laminae I and VI also showed relatively few affected

axon terminals, whereas laminae II, III and V contained most of the degeneration. Within the areas of maximum degeneration there was some evidence of clustering of degenerating terminals (see Fig. 4:19), due, in part at least, to involvement of "en passage" and terminal boutons of the same axon. Degeneration of myelinated fibres and non-myelinated pre-terminal axons was found in most layers of the cortex and was maximal close to the lesion. The myelinated fibres were mostly fine, varying in diameter from 0.5 to 1.0 μm, and in these sections, cut perpendicular to the surface, they are seen in transverse, longitudinal and oblique section.

Degeneration of Asymmetrical Terminals

In all the experimental material examined, either in general survey of sections or in the more systematic, quantitative studies, the vast majority of degenerating terminals ended with asymmetrical membrane thickenings. Furthermore, of these the majority were of small size. Although such terminals have been found to end upon cell somata, dendrites and spines, the greatest proportion of postsynaptic profiles consisted of dendritic spines. Of the latter, all varieties of size and form were found to be contacted by terminals undergoing degeneration, but it may be significant that the largest proportion of the spines were small and contained only an indistinct spine apparatus. (Examples of such small spines receiving degenerating asymmetrical terminals

are shown in Figs. 4:12 to 4:16; Fig. 4:8 shows such a small spine, this time containing vestiges of spine apparatus, being contacted by a degenerating terminal and being partly engulfed by glia). There were several examples of degenerating terminals making synaptic contact with two spines, which were either on opposite sides of the terminal (Figs. 4:11, 4:19) or immediately adjoined each other with their synaptic contacts on the same aspect of the terminal (Figs. 4:15, 4:19). (Further examples of multiple synaptic contacts are those of Figs.4:17, where a degenerating asymmetrical terminal ends upon two dendrites, and 4:18, where a degenerating terminal ends upon two structures, one of which is a dendrite while the other is probably a dendritic spine). A degenerating asymmetrical terminal may contact a spine upon which there is another, normal terminal of either the asymmetrical (Fig. 4:16) or symmetrical type, but there was no instance of a spine receiving two degenerating terminals. Degenerating preterminal axons with asymmetrical boutons "en passage" have been seen (Fig. 4:4), and the latter synapse upon spines. As has already been mentioned, dark dendritic spines have occasionally been seen (Figs. 4:7, 4:9, 4:10), and these also may receive a synapse from a degenerating axon terminal.

Degenerating asymmetrical axon terminals may also make axodendritic synapses, as has already been noted; the dendritic profiles may be of medium or small size, and those of medium size

have often been found to have two or more other, normal asymmetrical synapses close to the degenerating terminal, when the dendrite has been cut in either transverse or longitudinal section. These features of the dendrites strongly suggest that they originate from stellate cells. In one such example (Fig. 4:22), a stellate dendrite appears to receive two asymmetrical degenerating terminals. No degenerating terminals with asymmetrical membrane thickenings have been found to make synaptic contact with a large or medium-sized dendrite which could be definitely identified as belonging to a pyramidal cell, though that of Fig. 4:18 could possibly be such, as it has a straight, uniform appearance in longitudinal section. Degenerating terminals of this type have, however, been found to terminate on small dendritic profiles, but it has not been possible to identify their type, and it is in relation to these dendrites and to small spines that there has been the greatest difficulty of identification. A few examples have been found of degenerating terminals making asymmetrical synaptic contact upon stellate cell somata of the large type, upon which there are other, normal asymmetrical axon terminals (Figs. 4:20 and 4:21). No degenerating asymmetrical terminals were seen ending on small stellate cells or on pyramidal cells; however, the somata of the latter are believed not to receive any asymmetrical terminals of any kind.

Degeneration of Symmetrical Terminals

It is probably true to say that it is more difficult

to be confident that an axon terminal makes a symmetrical synapse than it makes an asymmetrical one, and for this reason degenerating terminals were only identified as having a symmetrical membrane thickening if the synaptic contact zone was cut perpendicularly and showed an absence of dense material subjacent to the postsymaptic membrane (cf. Gray, 1959; Colonnier, 1968). Although terminals which were identified as symmetrical formed only a small proportion of the total, they were found to end upon all the constituent parts of a neurone, including the soma and axon initial segment; the great majority, however, were found to end upon dendrites. Degenerating symmetrical terminals were found infrequently upon dendritic spines; in single sections they were either the only synapse upon the spine or were associated with another normal terminal making an asymmetrical synapse (Fig. 4:23); no examples were found of a degenerating symmetrical terminal making synaptic contact with two spines or with a spine upon which there was a degenerating asymmetrical terminal.

Degenerating symmetrical terminals were found to make axodendritic synapses with both pyramidal- and non-pyramidal-type dendrites, including, in contrast to the degenerating asymmetrical terminals, large proximal portions of pyramidal dendrites, identified by their straight outlines and few synapses (see Figs. 4:27 and 4:28; other examples of large dendrites receiving degenerating symmetrical terminals are shown in Figs. 4:32 and 4:36). Of the

medium-sized dendrites, some were also pyramidal, while others were regarded as possibly being of stellate origin in view of the presence of other asymmetrical synapses (up to three) in close proximity to the degenerating terminal; one of the latter dendrites, within 1 mm of the lesion, was dark (Fig. 4:26). An example of a small dendrite receiving a degenerating symmetrical terminal is shown in Fig. 4:24. Several examples were seen of one degenerating terminal making symmetrical synaptic contacts with two dendritic profiles of large or medium size, and in several such cases at least one dendrite appeared to be of pyramidal origin (Fig. 4:29). A further example of a degenerating terminal making symmetrical contacts with two dendrites is shown in Fig. 4:31; here, however, the cell type to which the dendrites belong cannot be identified with certainty. The site of the axodendritic synapse was often close to the origin of a spine, and sometimes immediately opposite (Fig. 4:25). In one instance a degenerating terminal with symmetrical membrane thickenings was found upon a basal dendrite close to the cell soma in layer V, and because of its large size and the relatively small number of synapses upon it, this has been tentatively identified as a Meynert cell (Fig. 4:33). This type of degenerating terminal also makes axosomatic synapses, and in the five examples found the cells concerned have been pyramidal in two cases (e.g. Fig. 4:34), of the large stellate type in two cases and apparently of the small stellate type in the remaining case (Fig. 4:30).

In one experiment, a degenerating terminal was found at 250 μm from the lesion, ending upon the axon initial segment of a pyramidal cell in layer II (Figs.4:37 to 4:39). This terminal and initial segment were studied in twelve serial sections and other normal symmetrical endings were found making synaptic contact, but the degenerating terminal did not synapse with any other profile.

Degenerating fine non-myelinated fibres have been seen making multiple synaptic contacts with symmetrical thickenings, and in the example shown in Fig. 4:29 one axospinous and one axodendritic synapse can be distinguished.

Quantitative Comparisons

In addition to the examination of numerous sections, taken from several blocks at different survival periods, for the qualitative study of the type of axon terminal and postsynaptic structure and for a survey of the density of degeneration, more detailed information was obtained in two ways: first, the whole depth of the cortex was examined, in either one or two perpendicular sections, at precisely known distances from the lesion as measured from the thick sections; secondly, three maps were made of the whole depth of the cortex, from the edge of the lesion to a distance of 3 mm away. As described in Chapter 2, the region to be surveyed was mapped as several areas, each about 1.5 mm by 0.75 mm, arranged so that there were no appreciable gaps between areas. The

block of tissue was trimmed to each area in succession and at each stage the size of the area and its position relative to the whole block face were recorded by means of a drawing made with the aid of a dissecting microscope. When each area had been studied with the electron microscope, it was mapped out on graph paper and the individual maps were fitted together to form a representation of the whole region occupied by the block. From the qualitative survey and from individual sections at known distances, the conclusions drawn from the light microscopic investigation were confirmed, in that the maximum degeneration was found within the first millimetre or so, that it then diminished in quantity rapidly and that there were certain differences in the density of degeneration between the laminae, notably that lamina IV was virtually devoid of degenerating terminals. The distance of 3 mm was chosen for the systematic mapping because the light microscopic study (Chapter 3) showed that little spread occurred beyond this distance and that it was essentially restricted to layer IIIc, and also because electron microscopic study of thin sections beyond this distance showed only an occasional degenerating terminal or fibre. The depth of a degenerating terminal was correlated with the cortical laminae on the basis of the electron microscopic features of the individual laminae, together with a careful comparison of the micrometer measurements made during the study of the thin sections with measurements of the "thick" sections of the same block.

While most of the laminae have features which allow them to be identified with reasonable confidence, and some have margins which are reasonably sharp, others merge insensibly into each other.

Figure 4:40 shows one of the three systematic maps made of the full depth of the cortex for a distance of three millimetres from the lesion; there are only slight differences between it and the other two maps made in a similar way. It is also representative of the findings made both in the qualitative surveys and in individual sections at known distances from the lesion. It can be seen clearly that the greatest density of degeneration in all layers is within the first 1 mm or so; it then suddenly diminishes in amount and there is a more gradual decrease for the remainder of the distance. The histogram of the number of degenerating terminals at half-millimetre intervals (Fig. 4:41) taken from the data of all three maps, also shows clearly the sharp fall-off at about 1 mm, and suggests further points about the distribution of degenerating symmetrical terminals: though few in number, these terminals show an appreciably more gradual diminution in density with distance than do asymmetrical terminals, so that they form an increasing proportion of the total at progressively further positions from the lesion; these combined data also indicate that axons with the two types of terminal extend, overall, for the same distance from the lesion. Table 1 gives the proportions of asymmetrical and symmetrical terminals at different distances from the lesion, as recorded in these mapping studies.

Within the first millimetre the distribution of the degeneration is more or less uniform throughout the depth of the cortex, with the notable exception that there are very few degenerating terminals in layers I and IV. Beyond this distance there is little discernible difference between the different laminae, except that, possibly, layers IIIa and b show a relatively greater diminution in density than the other layers. It can also be seen that there are examples of both types of axon terminal in all laminae, and this is confirmed by the combined data from all of the quantitative studies, which will be discussed in more detail below. Even with the qualification that the extent of the tissue studied was determined by information obtained in the concurrent light microscopy studies, it is striking how well the findings made with the light and electron microscopic techniques agree, and in particular the virtual absence of terminal degeneration in layer IV beyond the first few hundred microns may be noted.

Table 2 includes all of the degenerating terminals found in the three mapping studies and also in those sections taken at known distances from the lesion. This shows, first, that degenerating terminals with asymmetrical membrane thickenings form over 90% (91.4%) of the total, and secondly that the density of degenerating terminals is greatest in layers IIIa and b and V; furthermore, in these layers the proportions of asymmetrical and symmetrical terminals are approximately the same as the proportion overall, whereas in

layers I and II the proportion of symmetrical terminals is rather low (5.6% and 2.7% respectively), while in layer VI it is high (18%). However, because of the known difficulty of drawing precise boundaries between adjoining laminae, and also because of the relatively high proportion of terminals about which it was difficult to be certain of the type of synaptic membrane thickening, too much emphasis should not be placed upon slight differences in proportions in the different laminae; moreover, as it is generally agreed that in thin sections it is difficult to distinguish between layers V and VI, it would probably be more reasonable to pool the results for these two layers, when the symmetrical terminals would form some 12% of the total. Nevertheless, despite this qualification, the relative number of symmetrical terminals in layers I and II does appear to be low, and this is in accord with the proportions of these types of terminal in normal material.

An analysis of the postsynaptic profiles on which the degenerating terminals end (Table 3) shows that two thirds terminate on dendritic spines, approximately one third on dendrites and less than 1% on cell somata and initial segments. Of the identified terminals ending upon dendritic spines, 98% (Table 4) were asymmetrical, while of those ending upon dendrites, 75% were asymmetrical.

A further analysis of the degenerating terminals which have been classified as asymmetrical or symmetrical shows (Table 3) a striking difference between the two types, as 75% of the former

end upon spines and 24% on dendrites, whereas 78% of symmetrical terminals end upon dendrites and only 13% on spines. Although both types of terminals end on cell somata, the numbers observed are too small to permit any comparison.

Although most of the results which have been described have been obtained from the study of material on the side of a lesion through the whole depth of the cortex, three other varieties of material have been examined, but less systematically. In the first of these studies, material in the angle between two slit lesions placed at right angles to each other showed a higher density of degeneration than is found in material taken from a single lesion, but, although no quantitative studies were made, there did not appear to be any obvious differences in the proportions of the types of terminals affected, the density in the different laminae or the proportions of postsynaptic profiles. Secondly, in a few blocks which included the lesion, the latter was found to extend down only to about the level of layer IV, and therefore sections were taken from the cortex deep to the lesion. In these sections the density of degeneration was less than after a lesion throughout the whole depth of the cortex, but again degeneration of both types of axon terminals was found. Finally, in one experiment in which a lesion had been placed in the peristriate cortex of the prelunate gyrus for a study of the association cortical connections into area 17, an unusually large number of degenerating terminals with symmetrical membrane thickenings were

found. They were commonest in layer III, though occasional examples could be found in all other layers below layer II, and were found to synapse predominantly on large and medium-sized dendrites, with smaller numbers on dendritic spines and cell somata; all of the somata contacted in this way appeared to be pyramidal cells. In these sections there were also present "dark" cell somata, which were mainly in layer III and could be identified as large stellate cells on the basis of the number and type of synapses on the cell soma; dark dendrites were also seen. These features were present on sections taken from several blocks at distances up to 5 mm from the area 17/18 boundary. Although the interpretation and possible significance of these findings will be discussed more fully later (Chapter 6), it should be emphasised that such a large proportion of symmetrical degenerating terminals has been found only in this one brain and that such terminals were not present in other experiments with comparable lesions of the peristriate cortex or with slit-like lesions of area 17 close to the 17/18 boundary. A careful examination of the "thick" sections of some of the blocks from this brain and of adjoining regions of area 17 showed small foci of gliosis and dark cells in the superficial layers of the cortex; this strongly suggests that the high proportion of degenerating symmetrical terminals was in some way a consequence of incidental damage to the small blood vessels of the overlying pia mater.

PLATE 7

All of the micrographs are from area 17 of the visual cortex of the monkey and are of material taken within 2-3 mm of a lesion in the cortex.

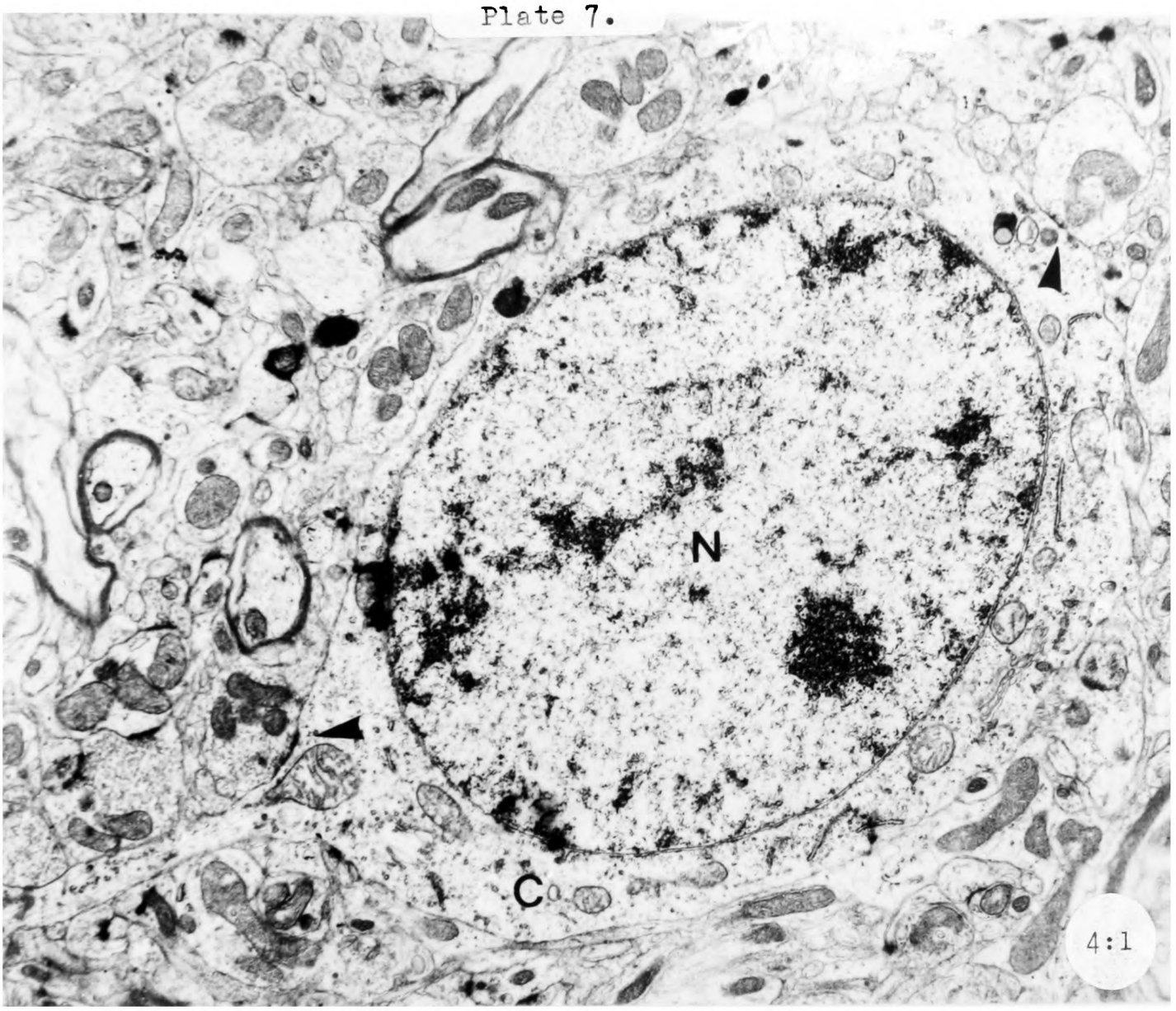
Fig. 4:1

A small stellate cell in layer III of area 17 of the visual cortex. The cell has a rather dark nucleus (N), with prominent chromatin clumps, while the cytoplasm (C) is sparse and pale. Synapses upon the cell (arrowheads) are few in number. The cell gives rise to a rather narrow dendrite. X 8,400.

Fig. 4:2

A small stellate cell in layer III of the visual cortex. The nucleus (N) shows chromatin clumps and the cytoplasm (C) is sparse with relatively few organelles. The cell gives rise to a dendrite (D) from the root of which the axon initial segment (A) can be seen arising. X 6,000

Plate 7.



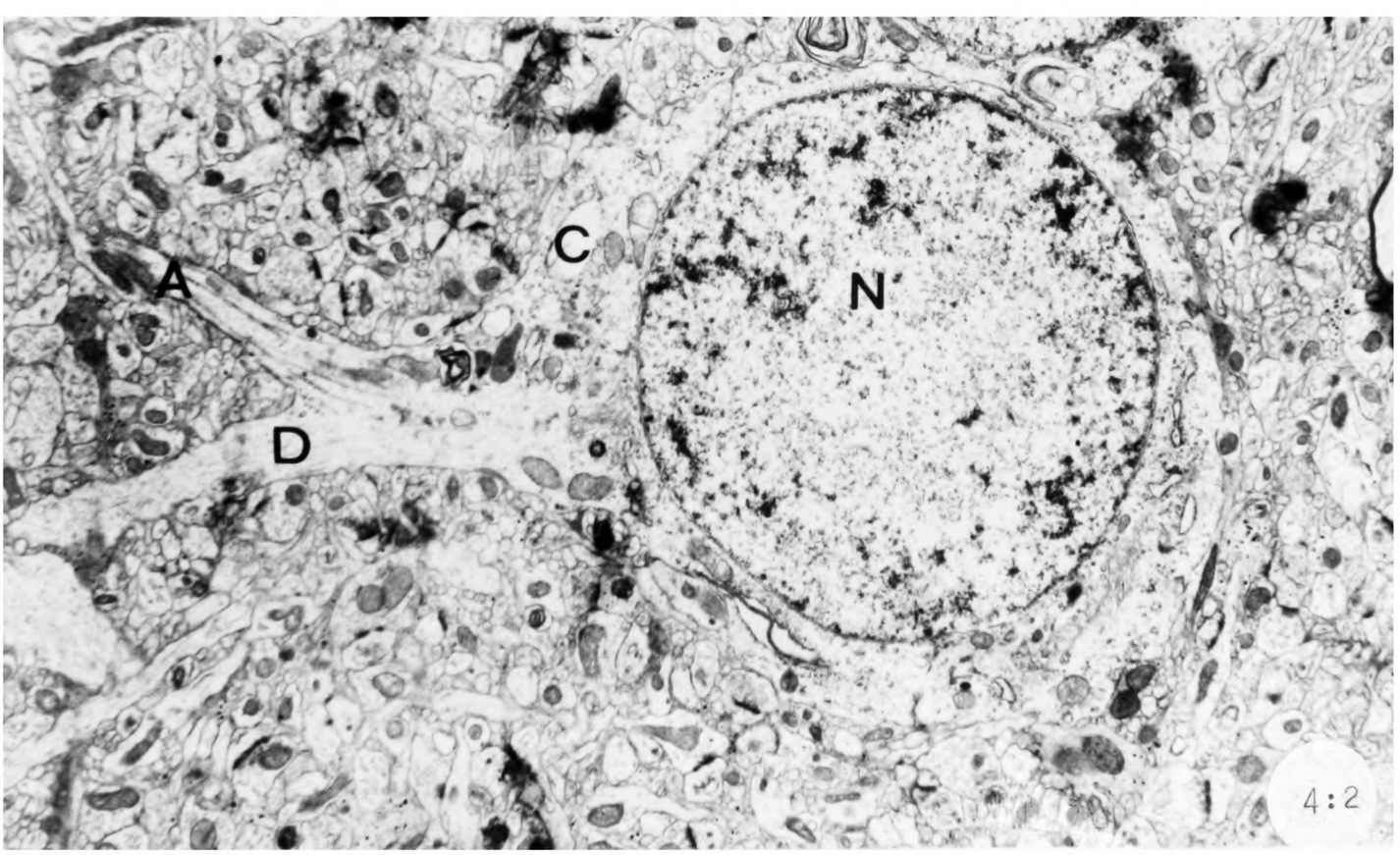


PLATE 8

Fig. 4:3

A small stellate cell in layer IV. This cell is one of a group of similar cells. N, nucleus; C, cytoplasm. X 10,000

Fig. 4:4

A degenerating intrinsic nerve axon (A) in the deep part of layer III, with a terminal *en passant* making an asymmetrical synapse (arrowhead). X 9,500

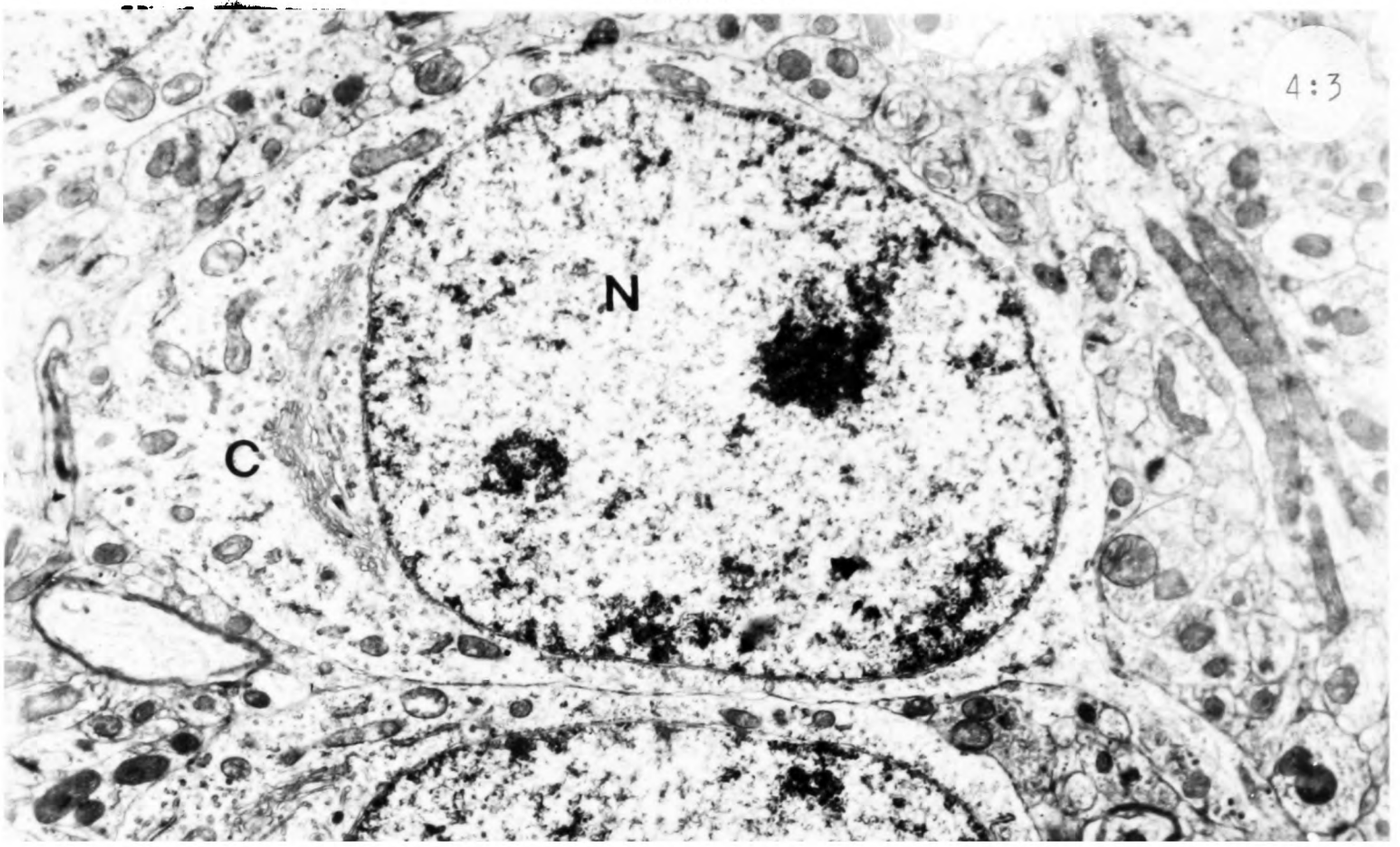
Fig. 4:5

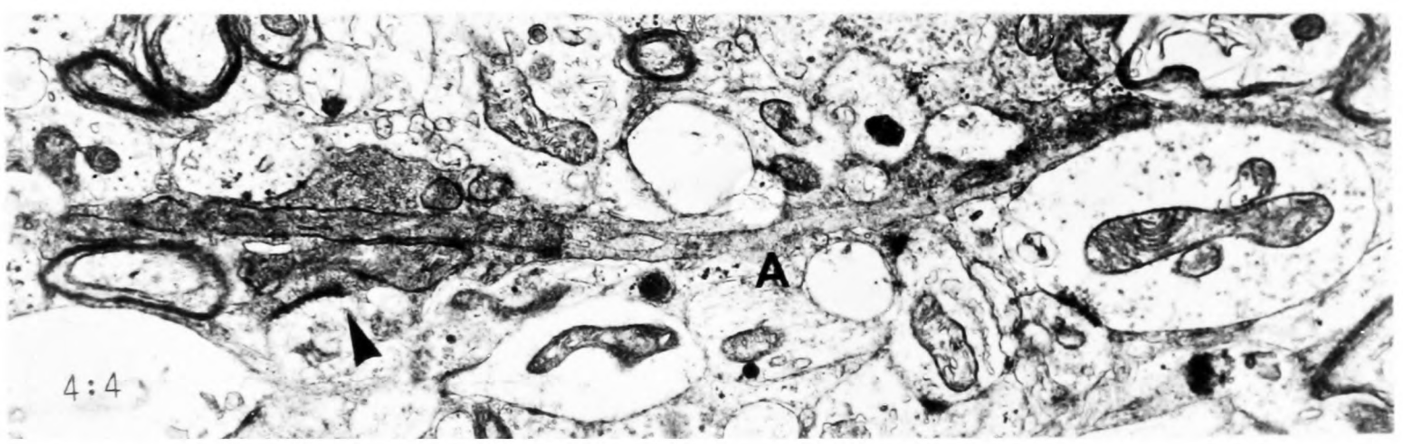
The terminal *en passant* of Fig. 4:4 and its asymmetrical synapse (arrowhead) at higher magnification. X 23,000

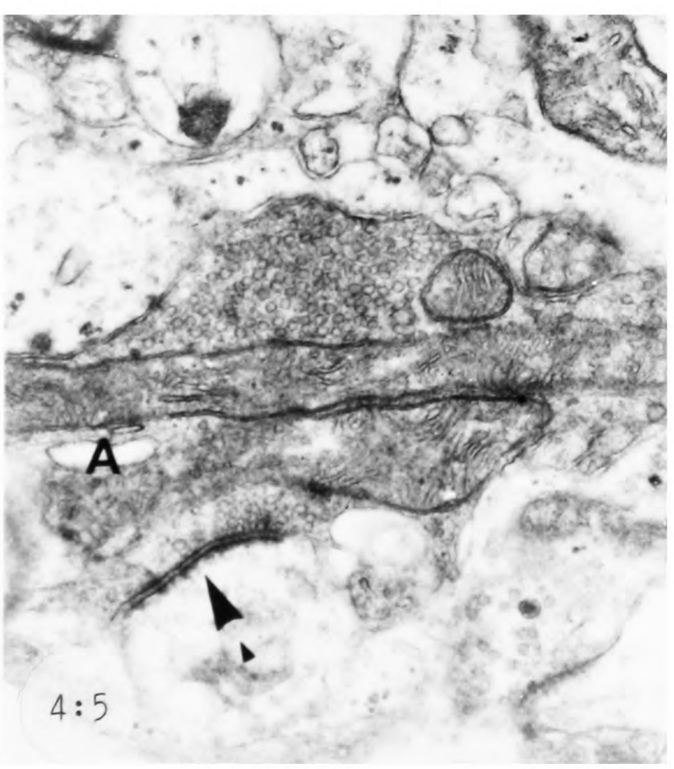
Fig. 4:6

A degenerating large terminal in layer VI. The terminal appears to have three synaptic thickenings, two of which (the larger indicated by an arrowhead) contact a dendrite (D). X 24,000

Plate 8.







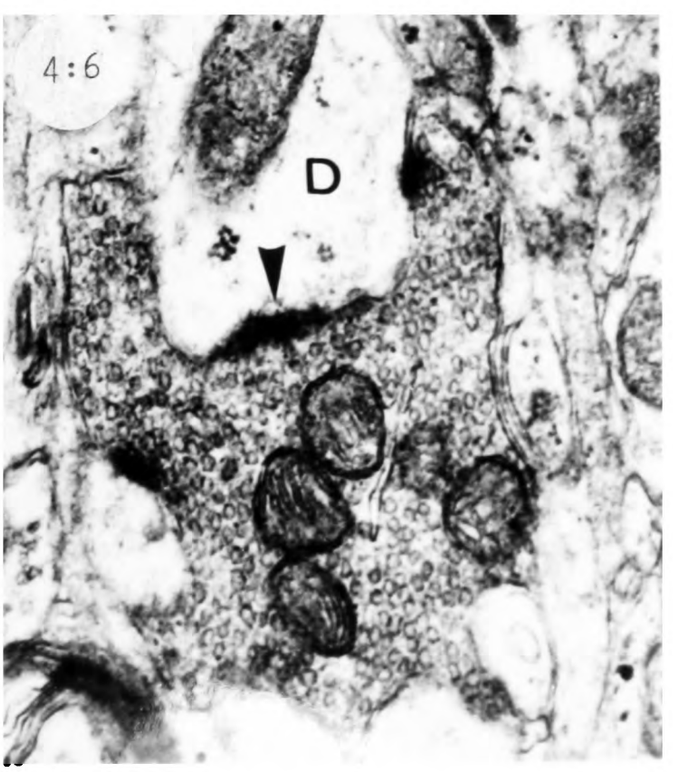


PLATE 9

Fig. 4:7

Two spines (S) in layer III close to a lesion. One spine is dark and has an asymmetrical synaptic contact (arrowhead) with a degenerating axon terminal. X 48,000

Fig. 4:8

A degenerating axon terminal in layer IV, making an asymmetrical synapse with a spine (S). Both processes are engulfed by the cytoplasm of a glial cell. (G) X 48,000

Fig. 4:9

A dark spine (S) close to a lesion; it has an asymmetrical contact with a degenerating nerve terminal (T) which is at a relatively early stage of degeneration. X 48,000

Fig. 4:10

A dark spine (S) in which the spine apparatus can still be seen, making synaptic contact (arrowhead) with a disrupted nerve terminal (T) in layer III. X 48,000

Fig. 4:11

A degenerating nerve terminal in layer III making asymmetrical contact with each of the two spines (S). X 48,000

Fig. 4:12

A degenerating terminal in layer V making an asymmetrical synapse (arrowhead) with a small spine. X 48,000

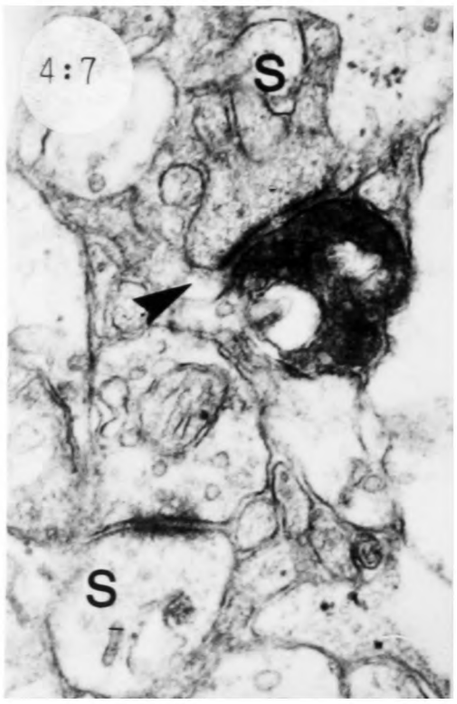
Fig. 4:13

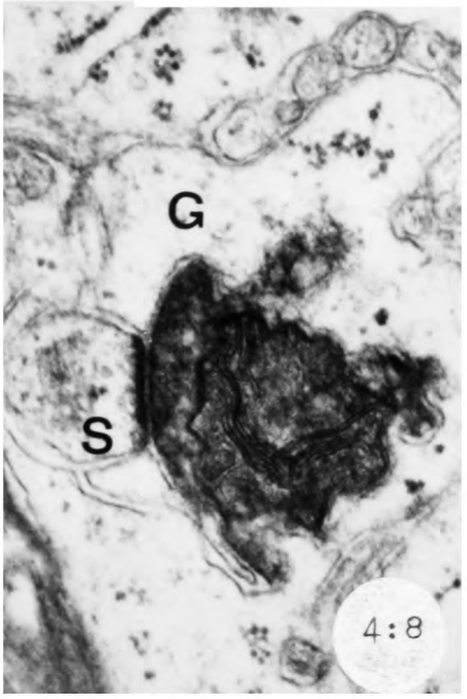
A degenerating terminal in layer III making an asymmetrical synapse with a small spine (S). X 48,000

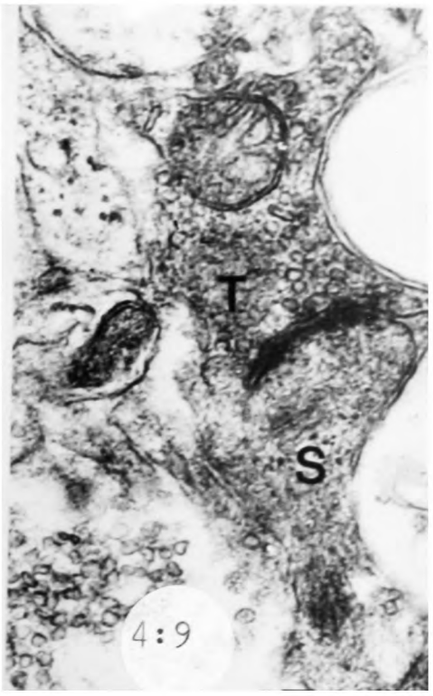
Fig. 4:14

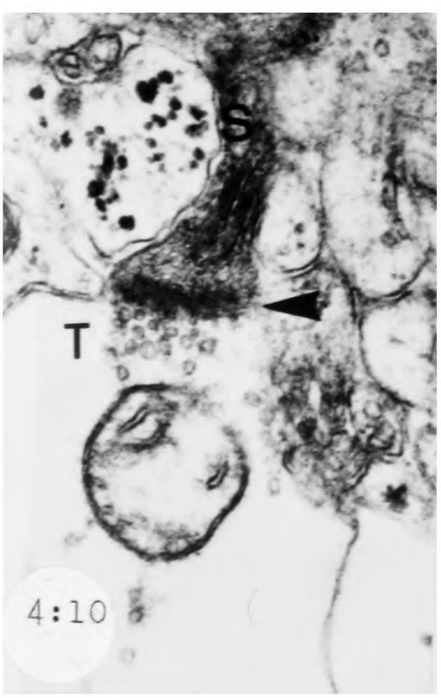
A small spine in layer III receiving an asymmetrical synapse (arrowhead) from a degenerating terminal (T), seen here at a late stage of degeneration and surrounded by glial processes (G). A dark dendrite (D) is seen nearby; this also receives an asymmetrical synapse (arrow). X 42,000

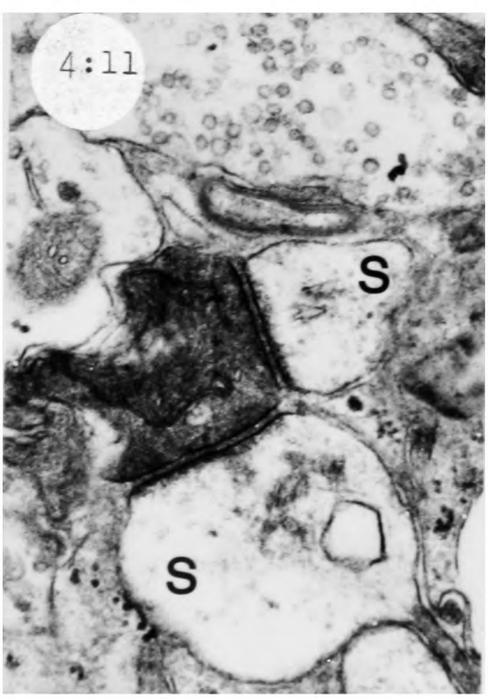
Plate 9.

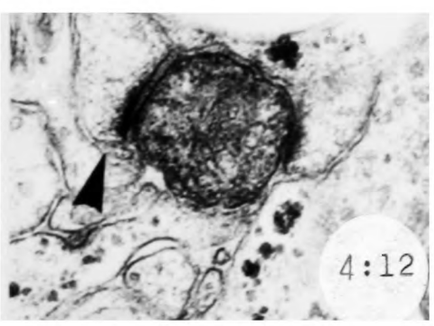


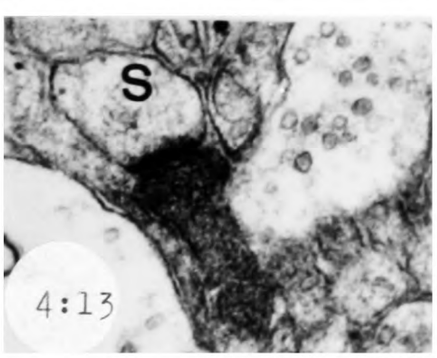












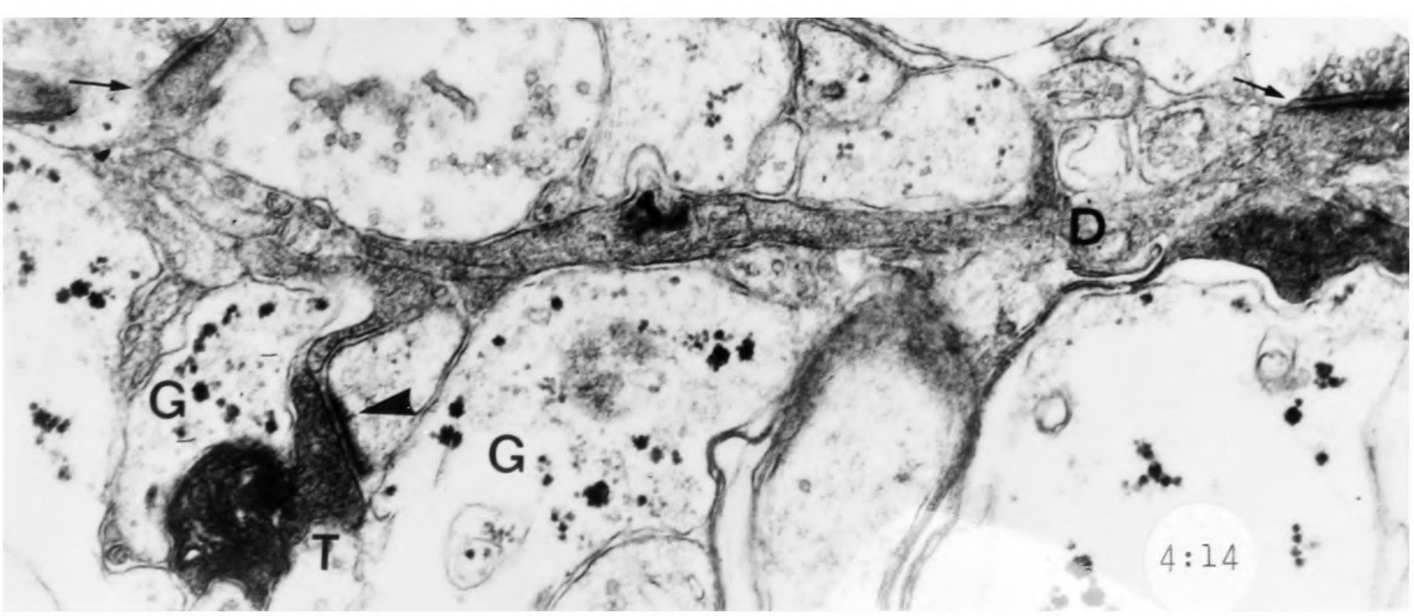


PLATE 10

Fig. 4:15

A degenerating terminal in layer III, with asymmetrical synapses on to two adjacent small spines. The terminal is partly engulfed by a glial process (G). X 48,000

Fig. 4:16

A degenerating terminal in layer III making asymmetrical contact (arrowhead) with a small spine (S) which also receives an asymmetrical contact (arrow) from a normal terminal (T). The degenerating terminal is again partly surrounded by glia (G). X 48,000

Fig. 4:17

An axon terminal at a relatively early stage of degeneration, making asymmetrical contact (arrowheads) with two dendrites (D), one of which receives a further asymmetrical synapse (arrow) from another axon terminal (T). X 55,000

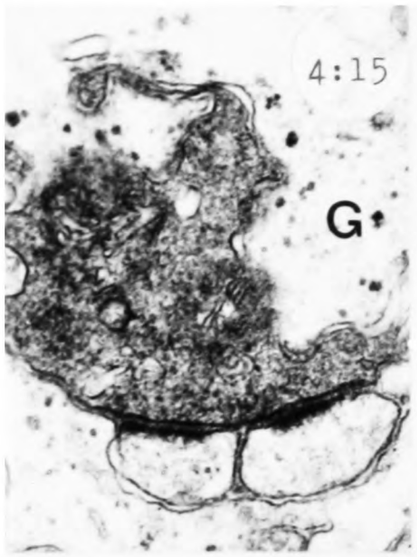
Fig. 4:18

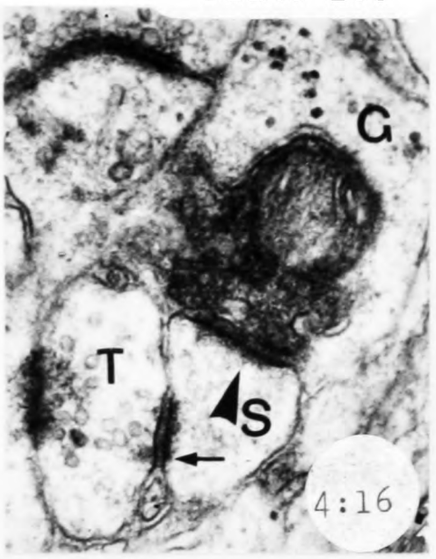
A degenerating axon terminal, at a rather early stage of degeneration, making asymmetrical contact with two structures in layer V, one of which (arrowhead) is a long straight dendrite (D), which also receives two normal synapses (arrows). The other post-synaptic profile is probably a spine. X 26,000

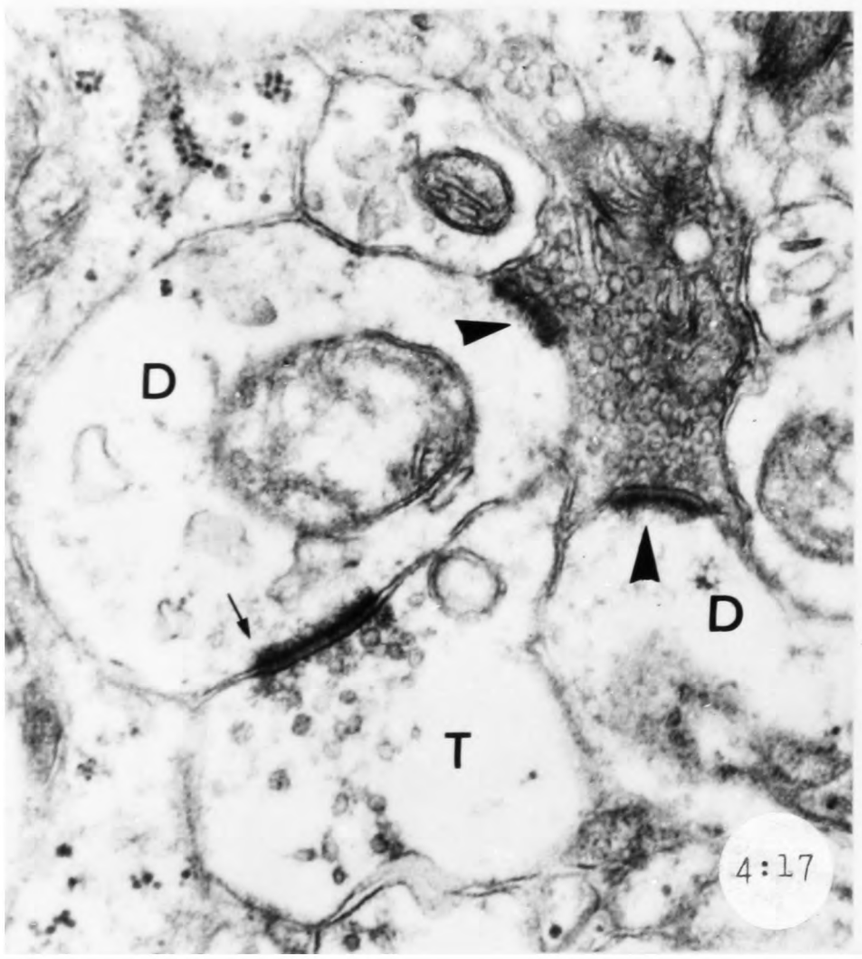
Fig. 4:19

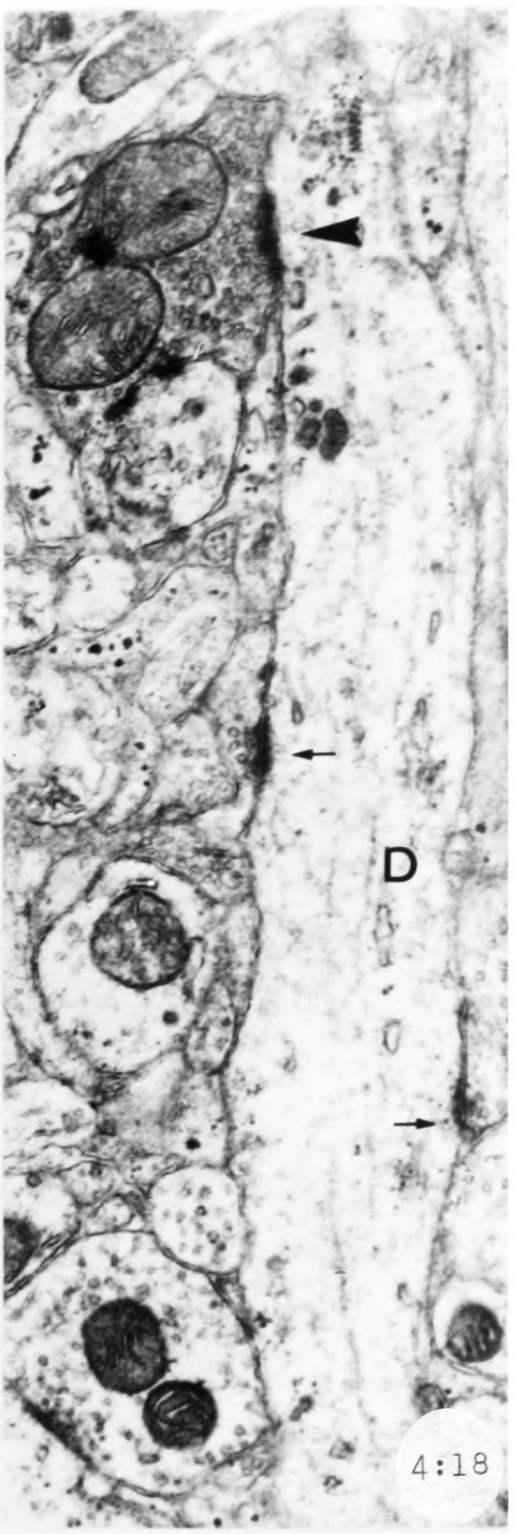
Two degenerating terminals (T) close to each other in layer III. Each terminal makes an asymmetrical synaptic contact with a dendritic spine. A degenerating pre-terminal fragment (arrow) is seen nearby. X 35,000

Plate 10.









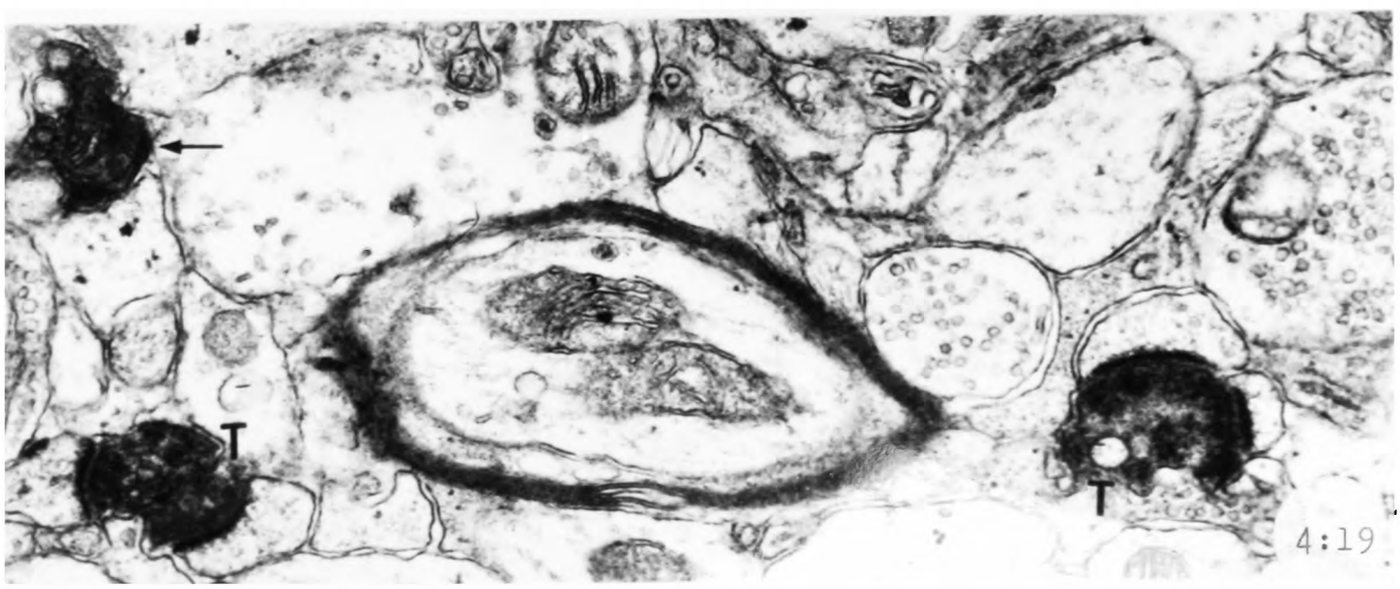


PLATE 11

Fig. 4:20

A large stellate cell at the junction of layers II and III of the cortex. The nucleus (N) is rather dark but uniform, while the cytoplasm (C) contains many organelles especially mitochondria. A degenerating axon terminal is seen making an asymmetrical contact (arrowhead) with the cell. A degenerating myelinated axon (paired arrows) is seen nearby. X 10,000

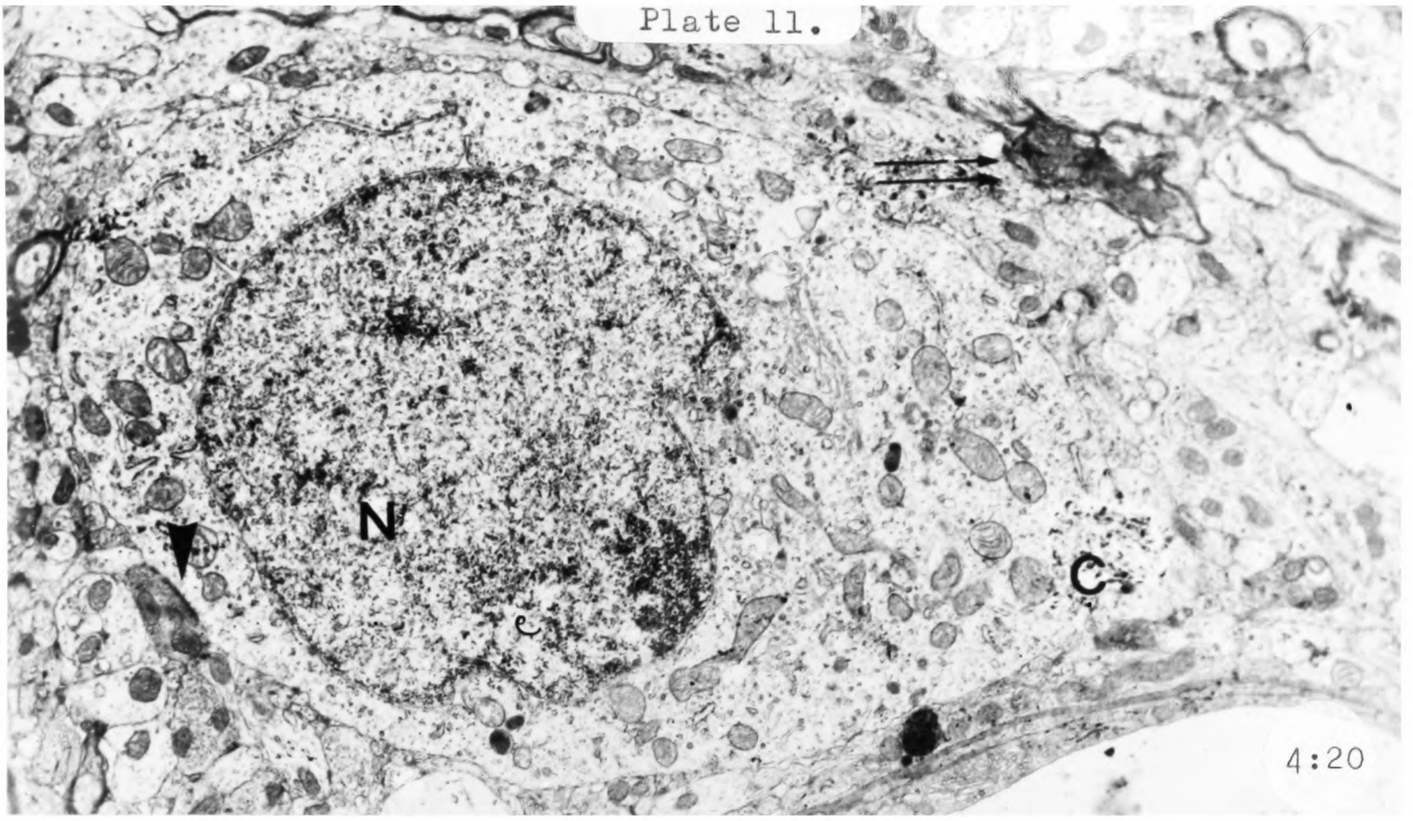
Fig. 4:21

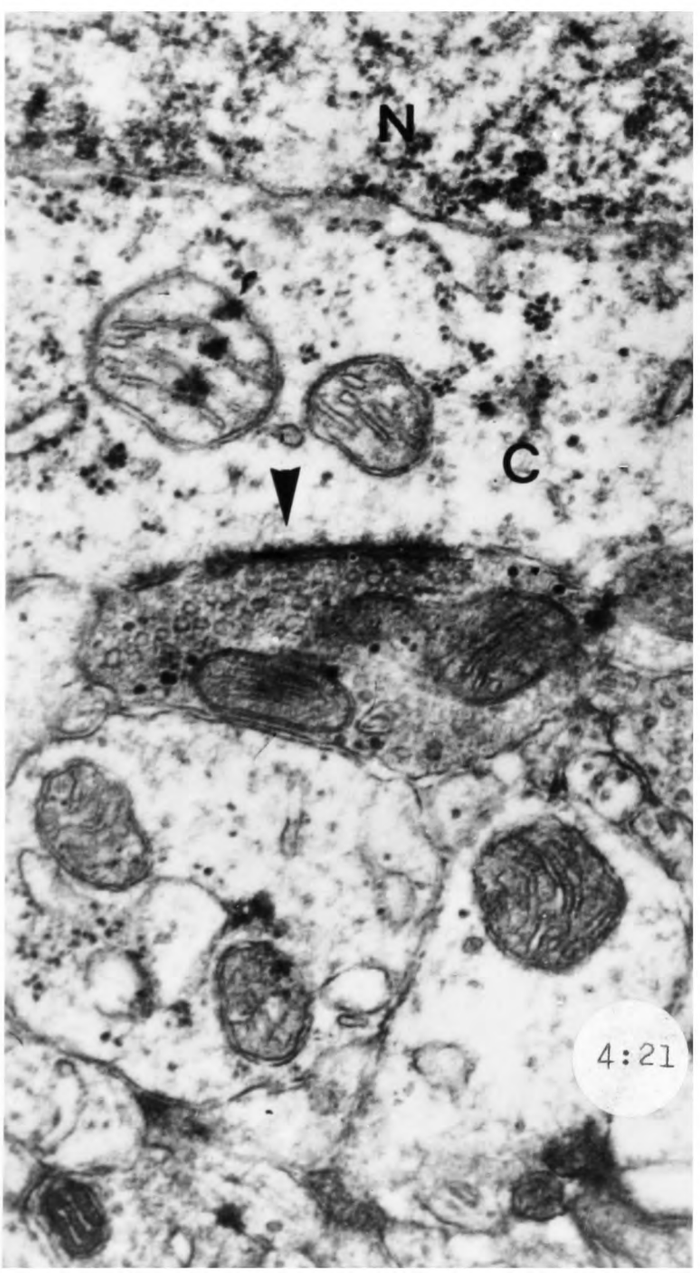
The degenerating axon terminal (arrowhead) of the previous figure, with an asymmetrical synapse, at higher magnification. X 46,000

Fig. 4:22

A dendrite (D) in layer III on to which two degenerating axon terminals make asymmetrical synapses (arrowheads). A further synaptic contact is present on the dendrite (arrow). X 44,000

Plate 11.





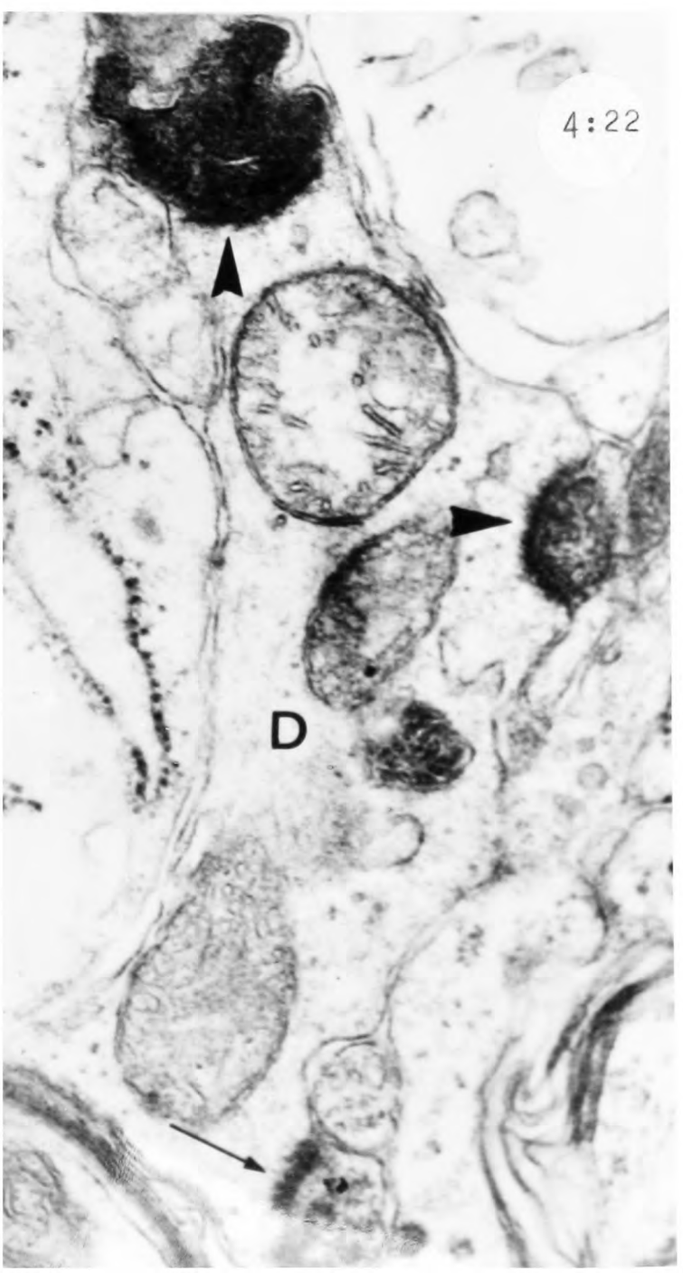


PLATE 12

Fig. 4:23

A degenerating terminal in layer III making a symmetrical synaptic contact (arrowhead) upon a small spine, which also receives a normal, asymmetrical terminal (arrow). X 42,000

Fig. 4:24

A degenerating terminal in layer IV making a symmetrical synapse (arrowhead) with a small dendrite (D). X 42,000

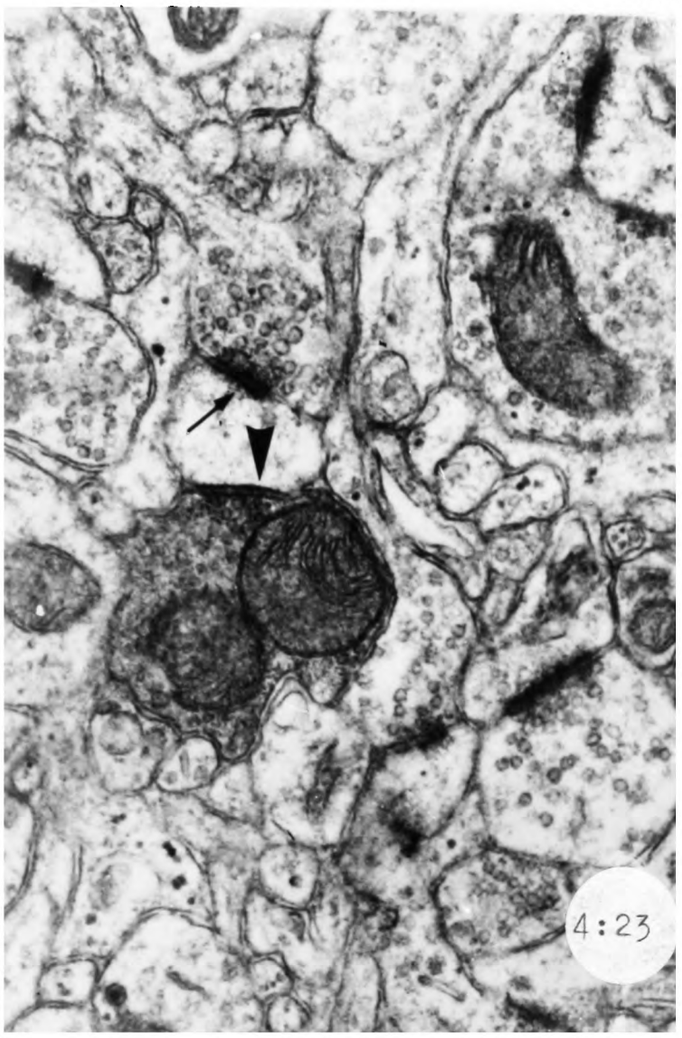
Fig. 4:25

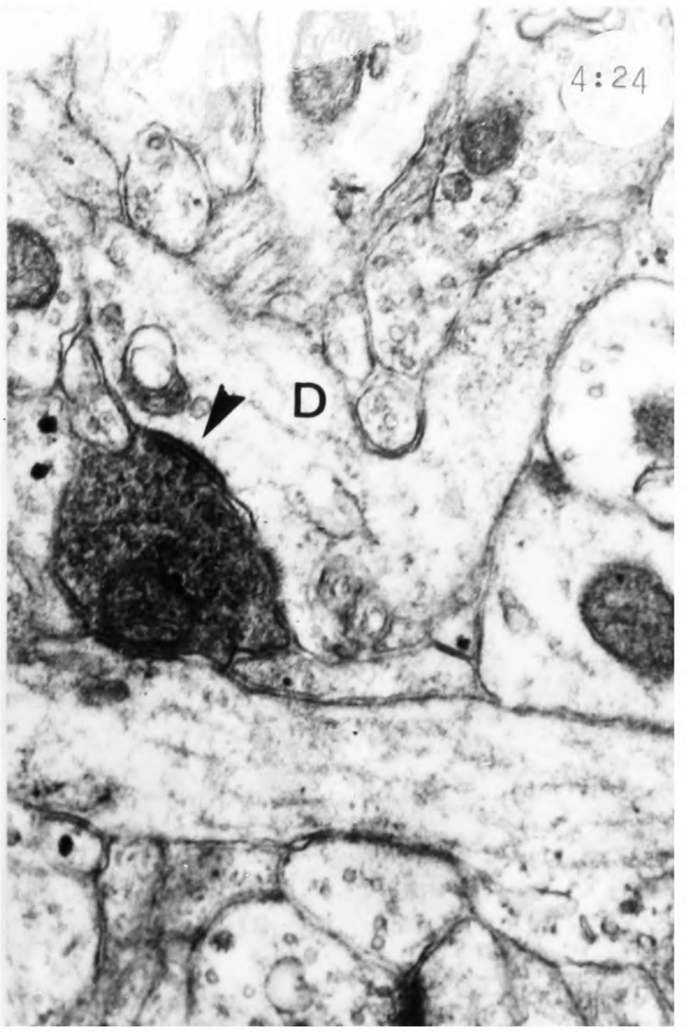
A degenerating terminal in layer V making a symmetrical synapse (arrowhead) upon a dendrite (D). This synapse is opposite to the origin of a spine (S), which can be seen receiving an asymmetrical synapse (arrow) from a terminal (T). X 42,000

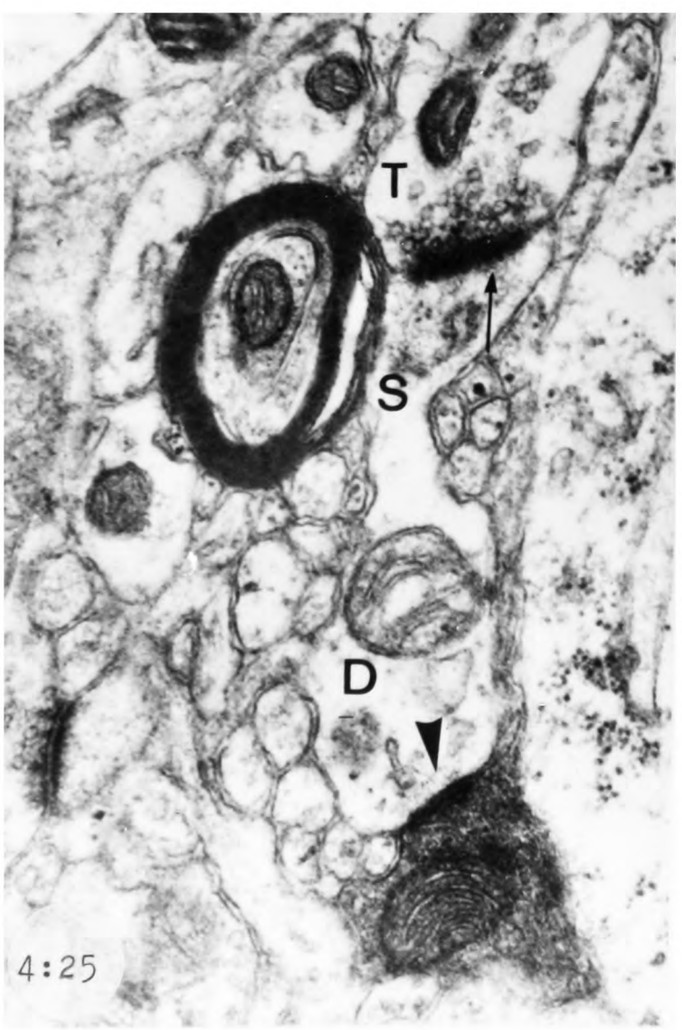
Fig. 4:26

A dark dendrite (D) in layer IV close to the lesion receiving a symmetrical synapse (arrowhead) from a degenerating axon terminal (T); the dendrite also receives a synapse (arrow) from a normal terminal. Glial processes (G) were common in this area. X 42,000

Plate 12.







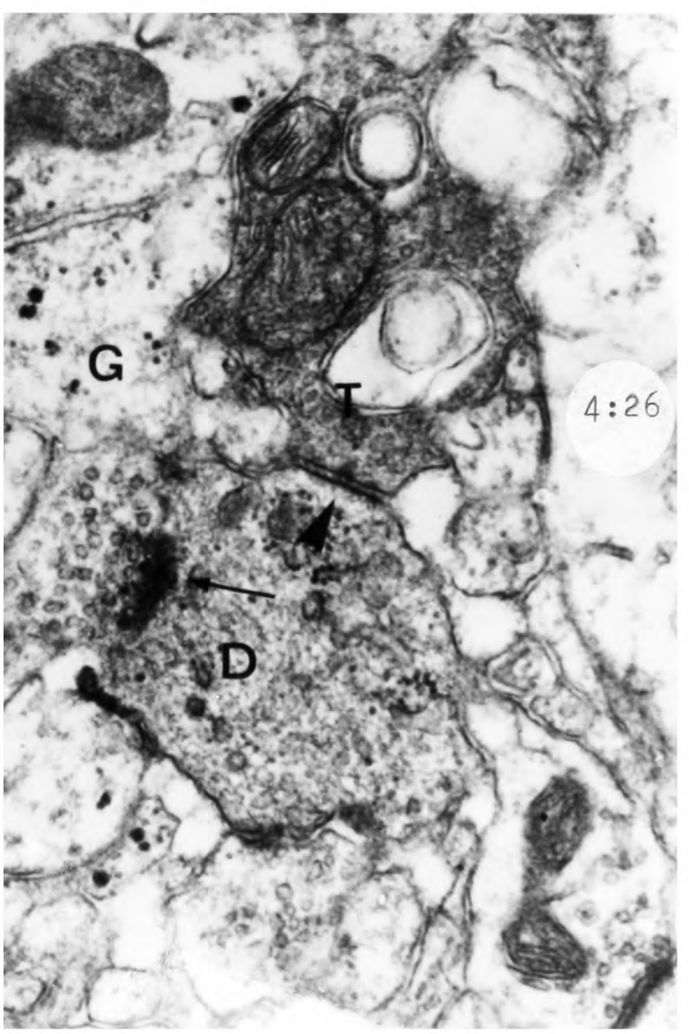


PLATE 13

Fig. 4:27

A large dendrite (D) in layer III, almost certainly belonging to a pyramidal cell, receiving a symmetrical synapse (arrowhead) from a degenerating axon terminal. X 35,000

Fig. 4:28

The degenerating terminal in Fig. 4:27 at higher magnification. X 48,000

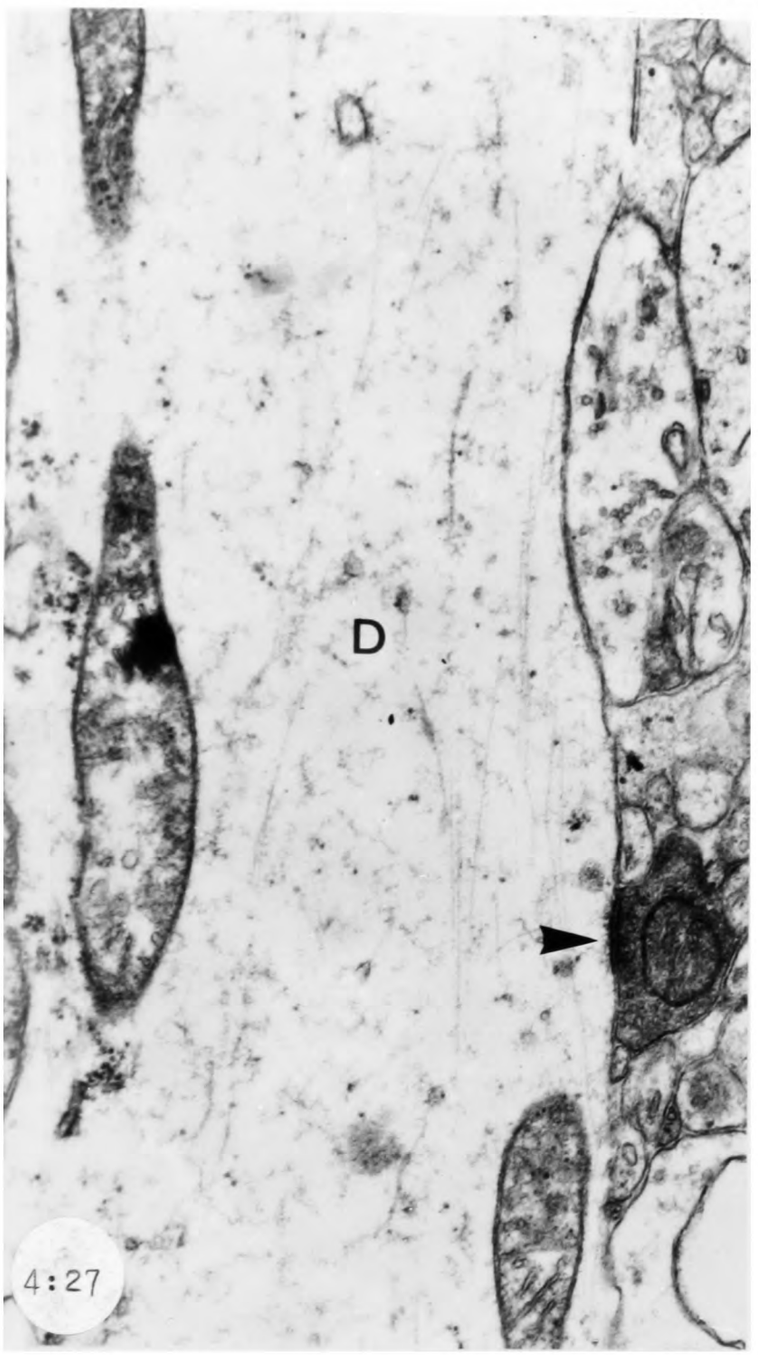
Fig. 4:29

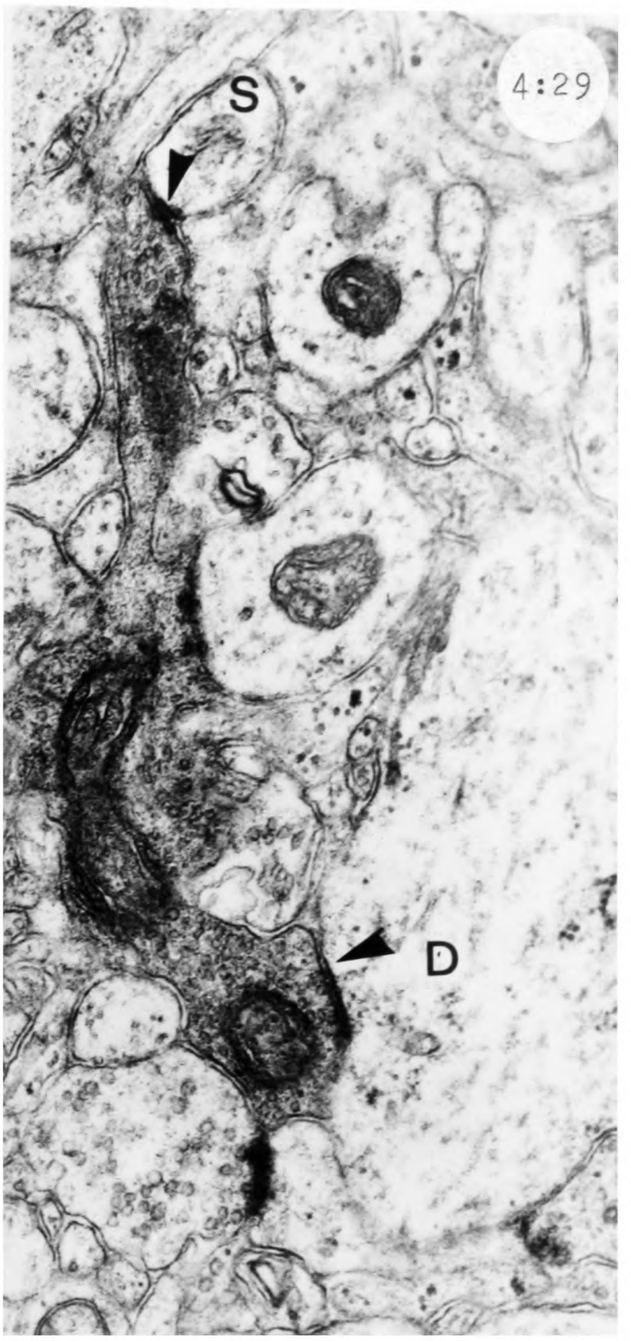
A degenerating axon in layer III making multiple symmetrical contacts (arrowheads), including one upon a dendritic spine (S) and one upon a large dendrite (D). X 28,000

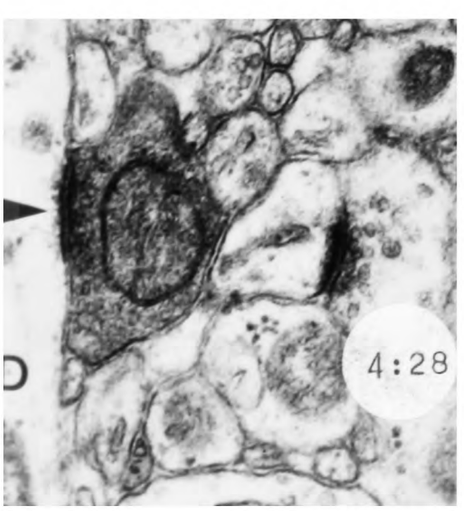
Fig. 4:30

A degenerating terminal (T) making a symmetrical synapse upon a cell soma (C) in layer III which is probably of the small stellate type. N, nucleus. X 55,000

Plate 13.







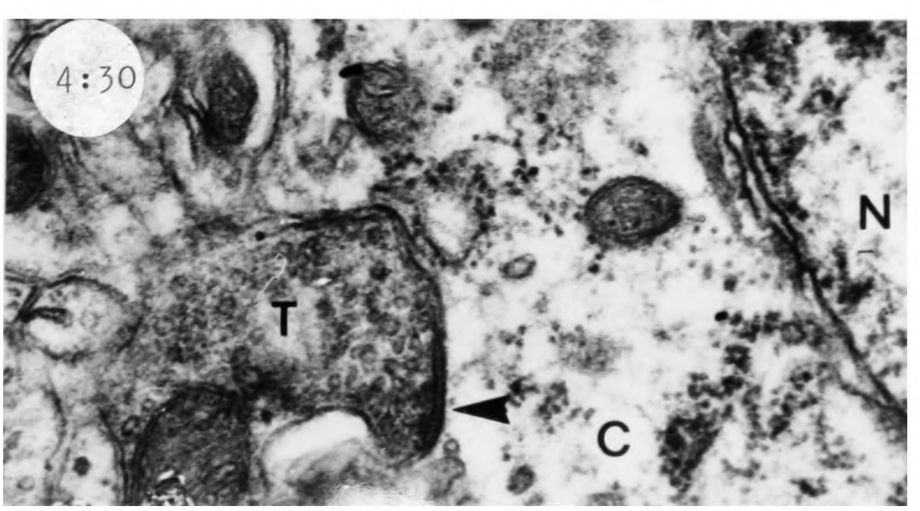


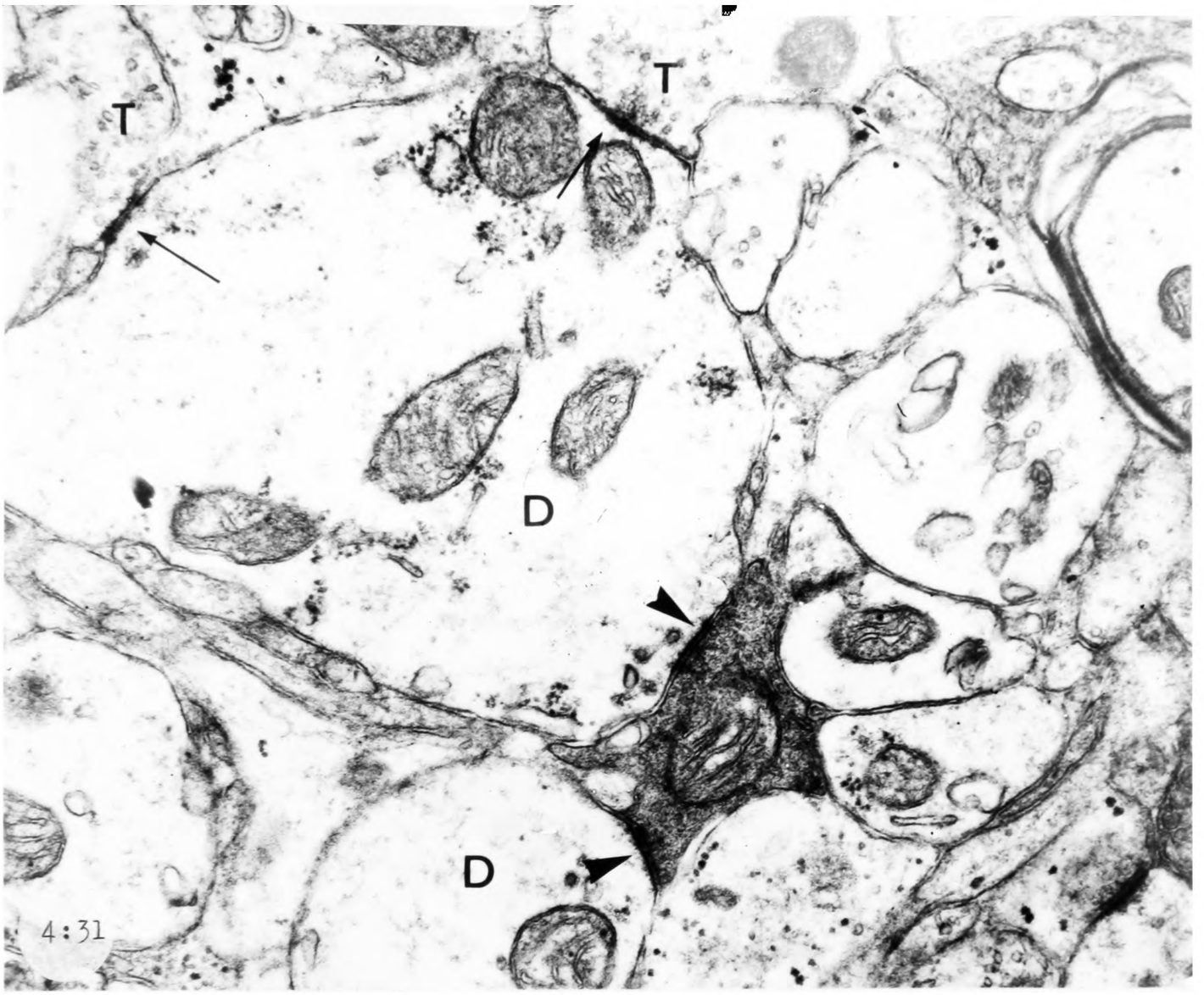
PLATE 14

Fig. 4:31

A degenerating terminal in layer III making symmetrical synapses (arrowheads) with two dendrites (D), the larger of which receives other synapses (arrows) from normal axon terminals (T). X 35,000

Fig. 4:32

A degenerating terminal in layer IV making a symmetrical synapse (arrowhead) upon a large dendrite, which also receives a synapse (arrow) from a normal terminal (T). X 32,000


T
T
D
D
4:31

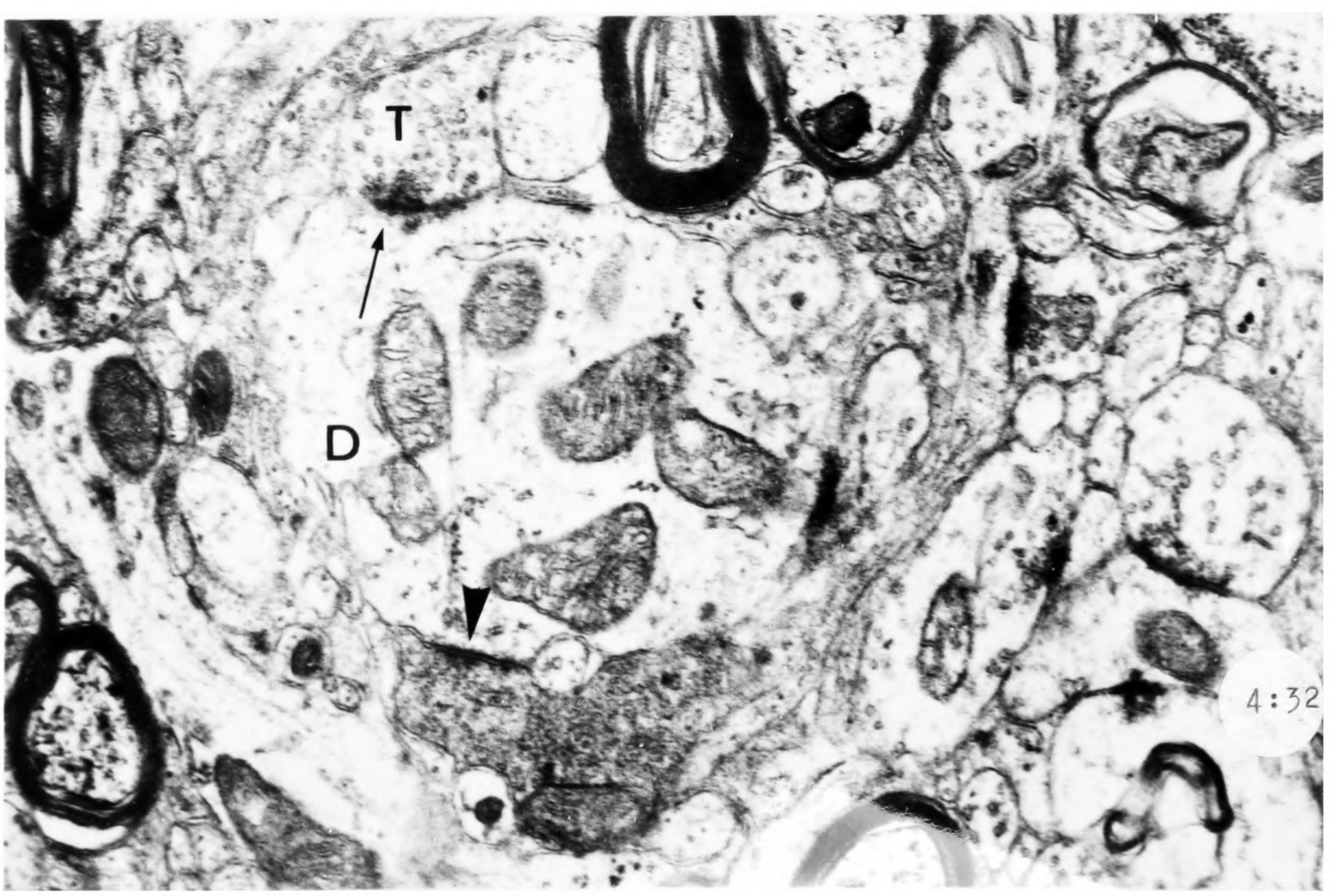

T
D
4:32

PLATE 15

Fig. 4:33

A large cell in layer V, which may be a Meynert cell on account of the appearance of its nucleus (N) which shows the typical pallor of a pyramidal cell nucleus, with absence of chromatin clumps under the nuclear membrane, and because of its cytoplasm (C) which, though containing numerous mitochondria, does not show the high degree of diffuse granularity normally seen in the cytoplasm of large stellate cells. The appearance of the cell is similar to that of Betz cells in area 4 of the motor cortex. A basal dendrite of this cell (D) receives a symmetrical synapse (arrowhead) from a degenerating axon terminal (T). X 8,400

Insert: The degenerating terminal and its synapse at higher magnification. D, dendrite. X 16,800

Plate 15.

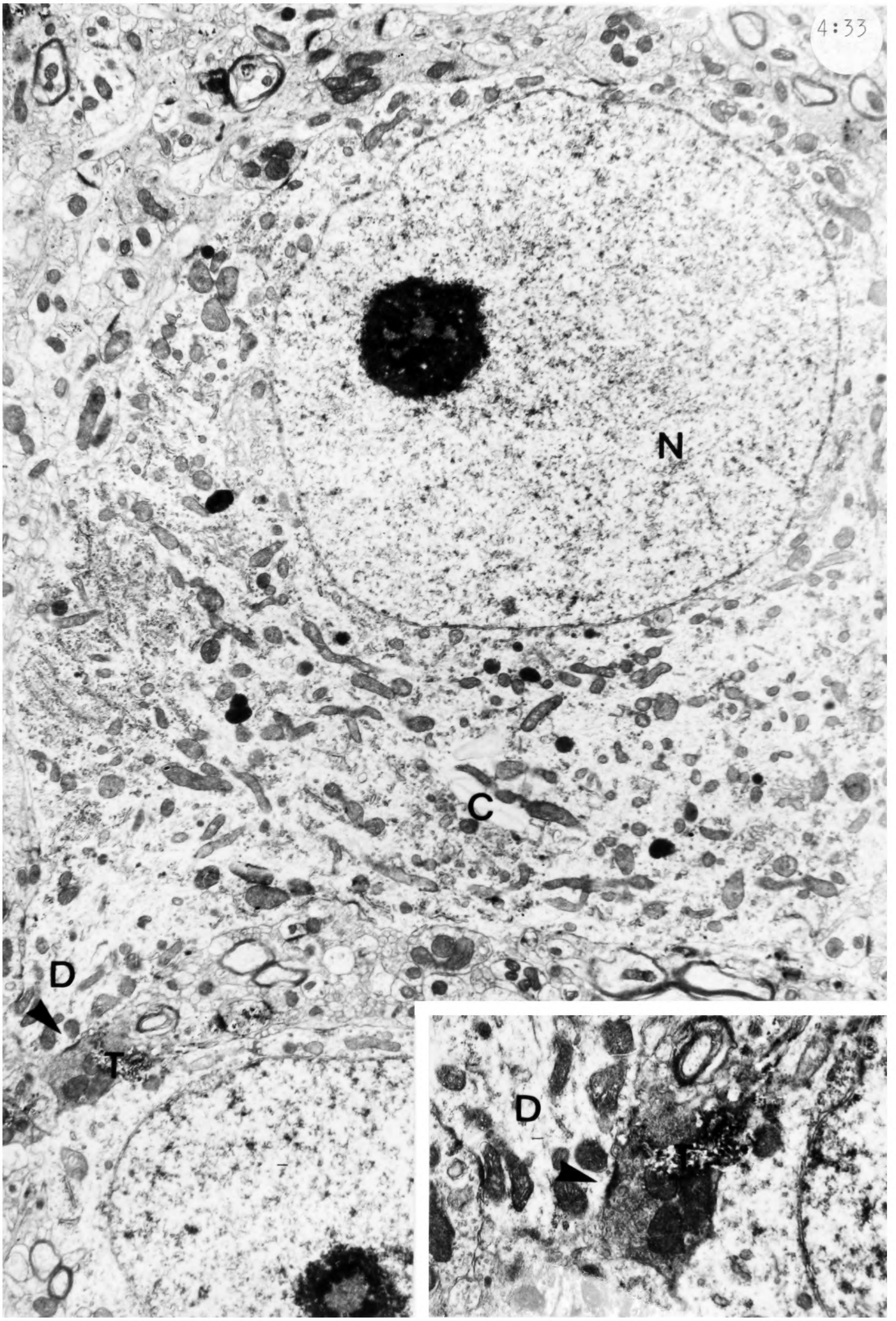


PLATE 16

Fig. 4:34

A pyramidal cell in layer V, showing the typical appearance of a pale nucleus (N) with diffuse chromatin and pale cytoplasm (C), with relatively few organelles. The cell receives a symmetrical synapse (arrowhead) from a degenerating axon terminal. X 8,500

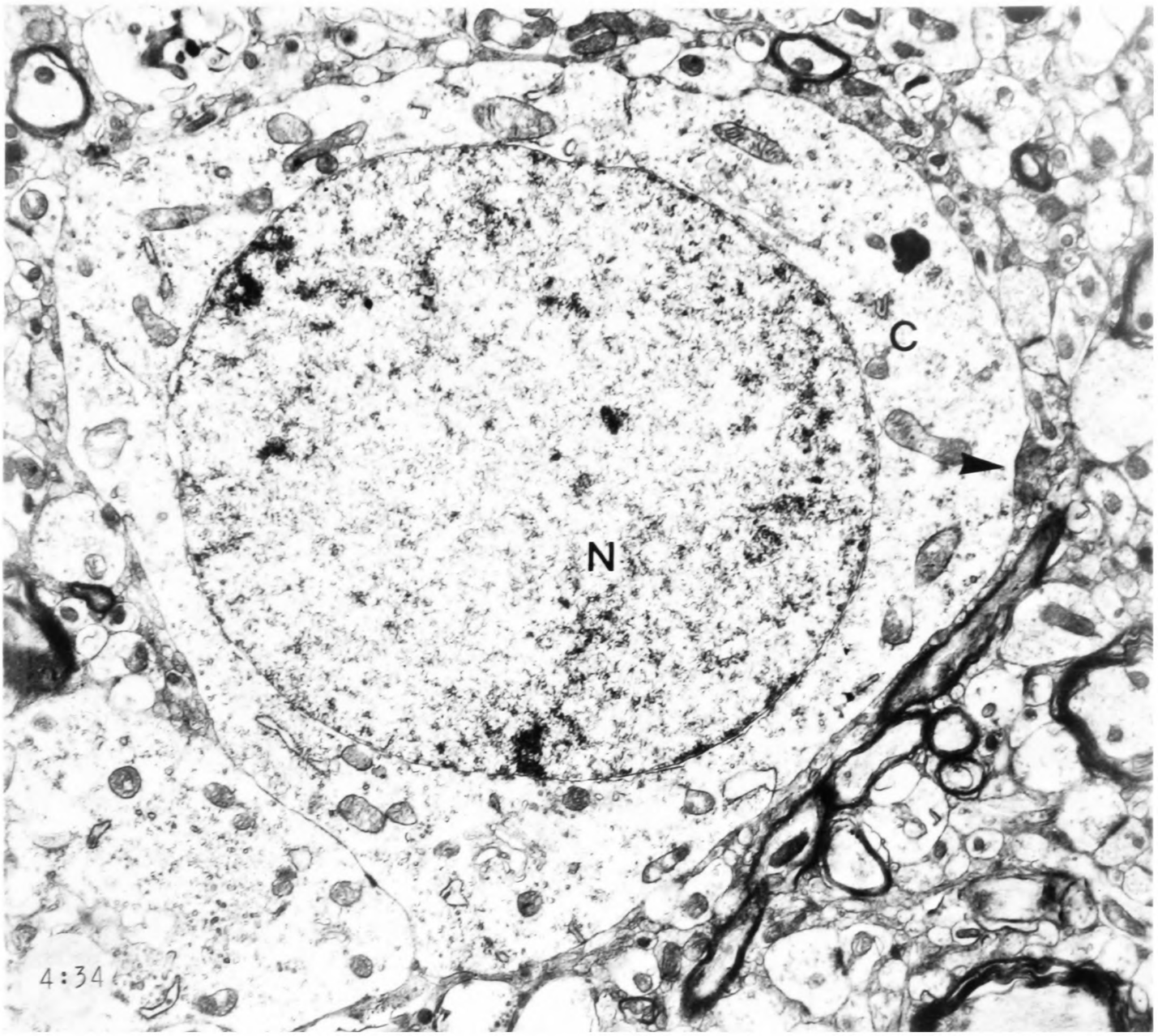
Fig. 4:35

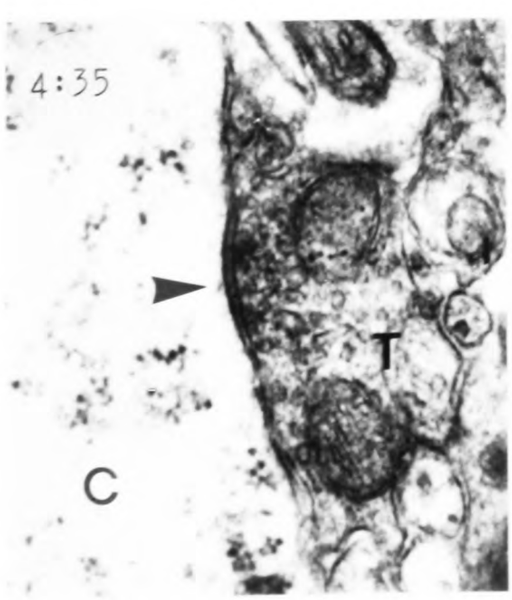
The degenerating terminal (T) and the axo-somatic synapse shown in Fig. 4:34 at higher magnification. X 52,000

Fig. 4:36

A degenerating terminal (T) in layer V making a symmetrical synapse (arrowhead) with a large dendrite (D). X 52,000

Plate 16.





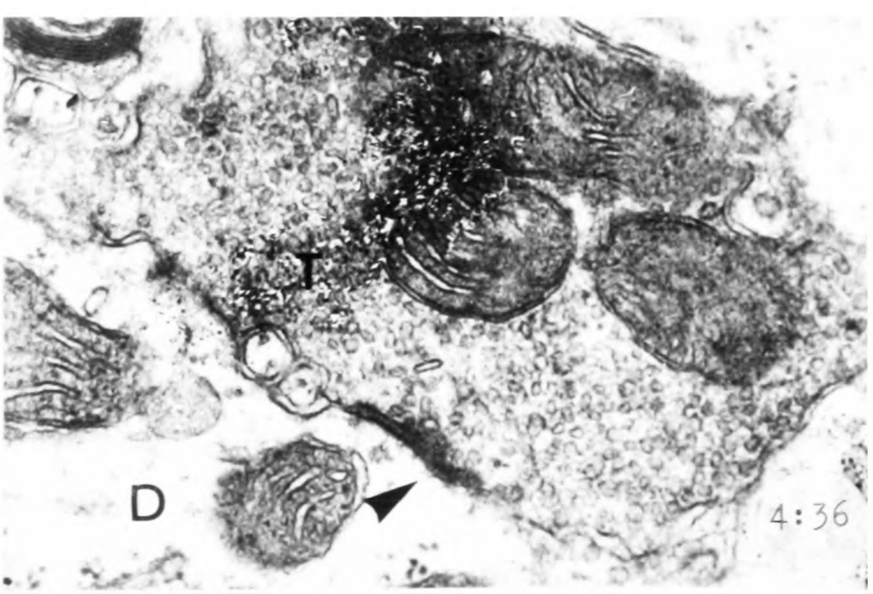


PLATE 17

Fig. 4:37

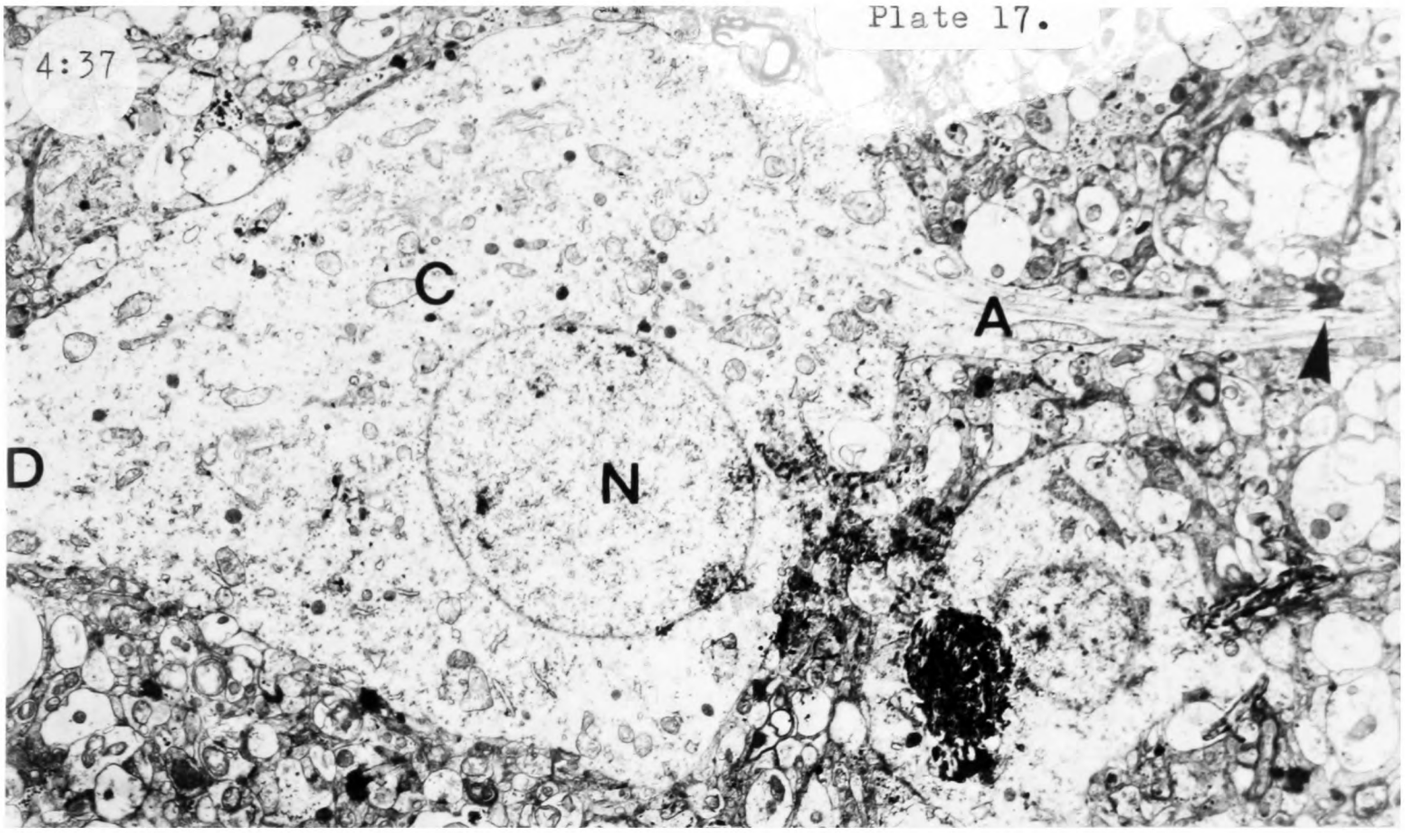
A small pyramidal cell in layer II with its apical dendrite (D) at the left of the figure. The axon initial segment (A) can be seen at the right of the figure; it receives a synapse (arrowhead) from a degenerating axon terminal. N, nucleus; C, cytoplasm. X 3,500

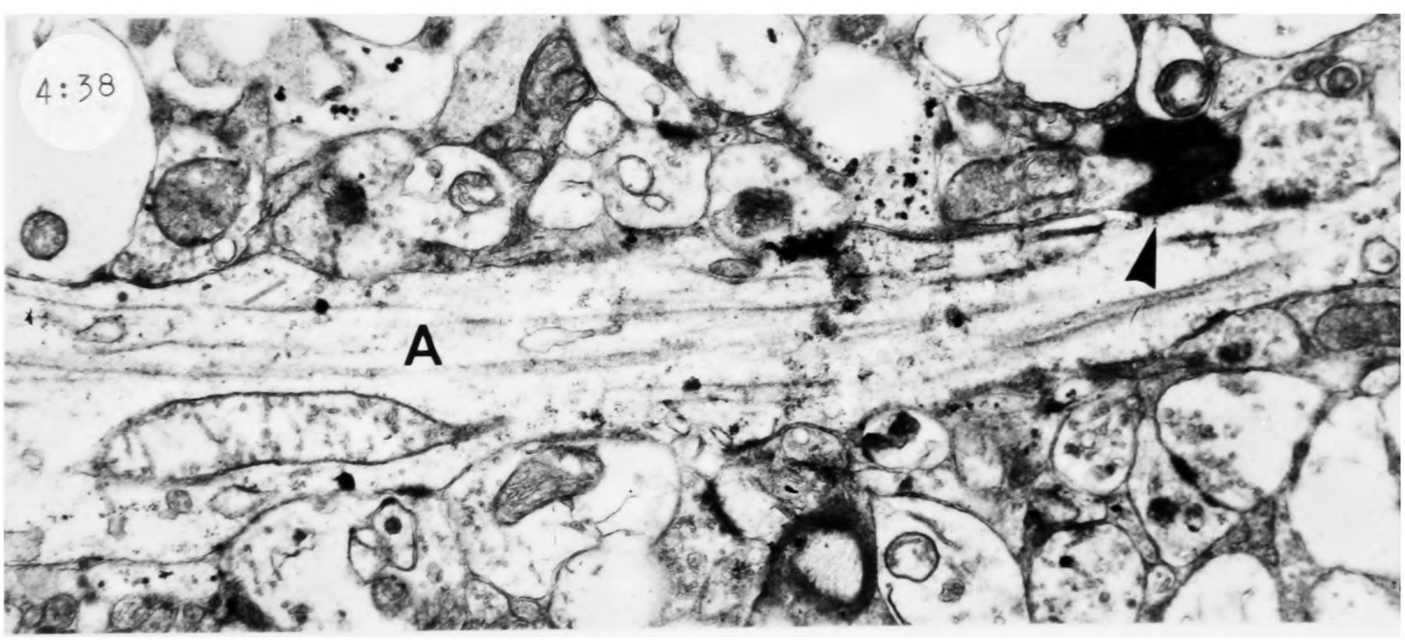
Fig. 4:38

The axon initial segment (A) of the cell in Fig. 4:37, with the synapse made by the degenerating axon terminal (arrowhead). X 14,000

Fig. 4:39

The same degenerating axon terminal and axo-axonic synapse shown in the previous two figures. The axon terminal (T) and initial segment (A) make synaptic contact (arrowhead) with one another, the synapse having symmetrical membrane thickenings. X 70,000


4:37
C
A
D
N


4:38
A

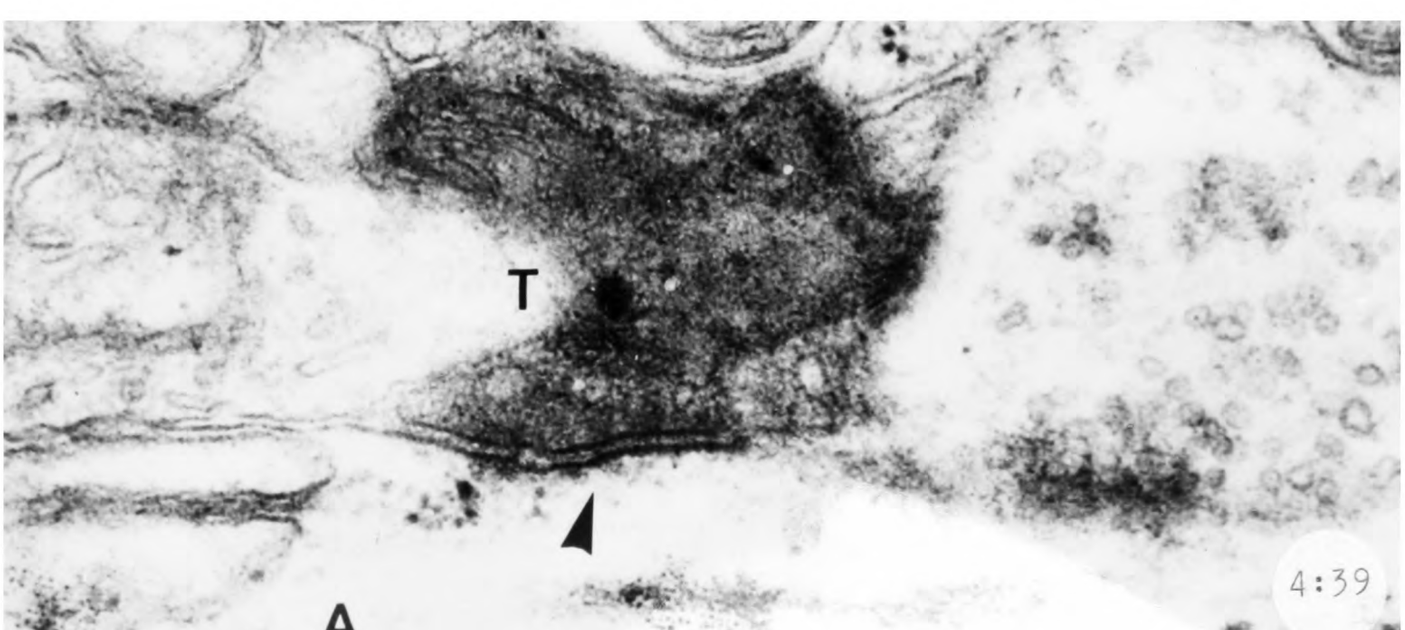

T
A
4:39

Fig. 4:40

An example of a systematic map of vertical sections of the whole depth of the cortex, showing the distribution of degenerating terminals in the different laminae at different distances from the lesion (indicated by the hatching at the left-hand edge of the figure). White squares indicate asymmetrical terminals, black squares indicate symmetrical terminals. Laminae of the cortex are indicated by Roman numerals.

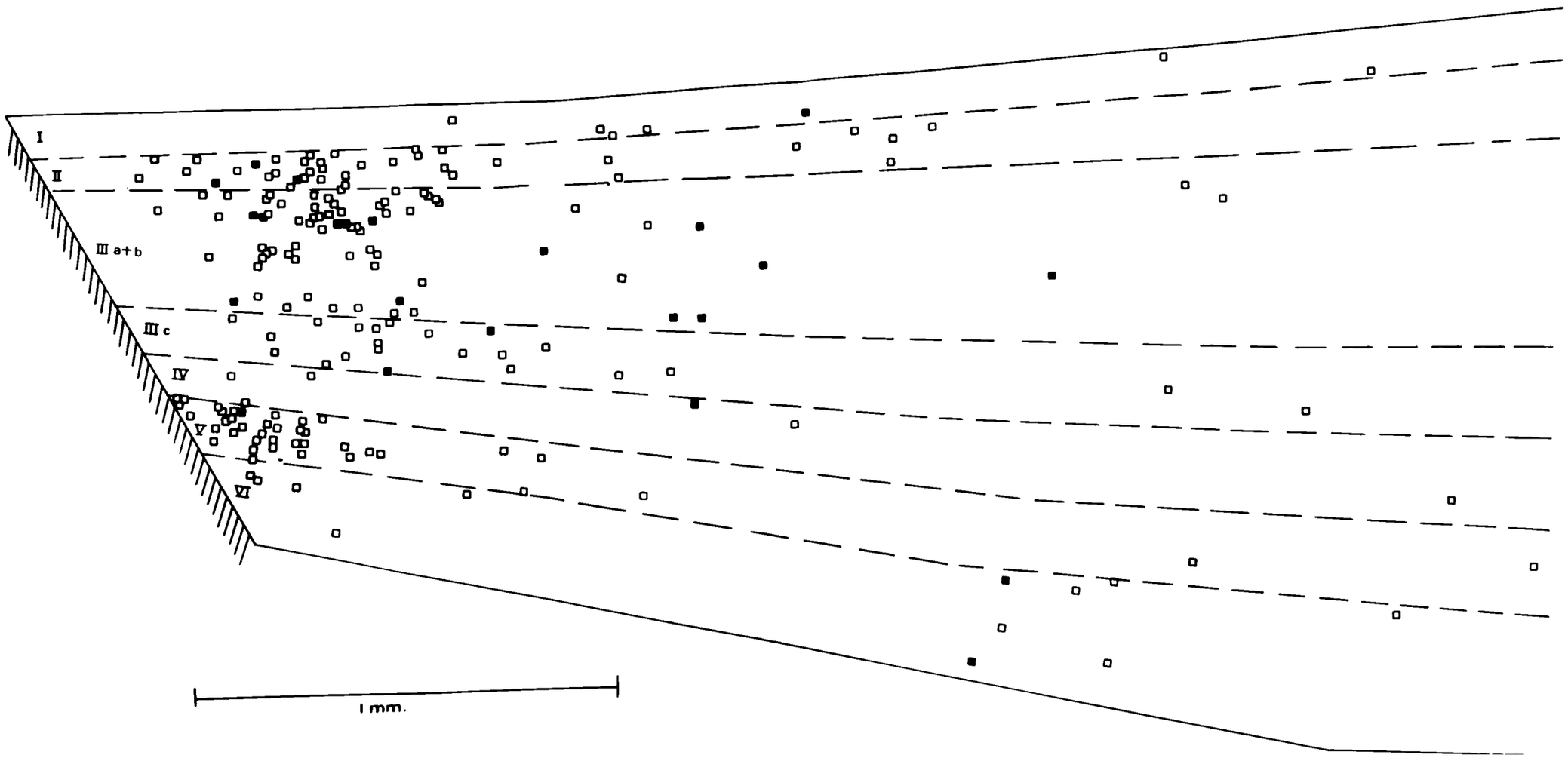

I
II
III a+b
III c
IV
V
VI
1 mm.

Fig. 4:41

A histogram showing the total numbers of degenerating terminals seen in three systematic mapping studies of the cortex alongside a small lesion. White columns represent asymmetrical terminals and shaded columns represent symmetrical ones. Each column represents the total number of degenerating terminals, of the appropriate type, which were found in a 0.5 mm-wide area of cortex at a known distance from the lesion.

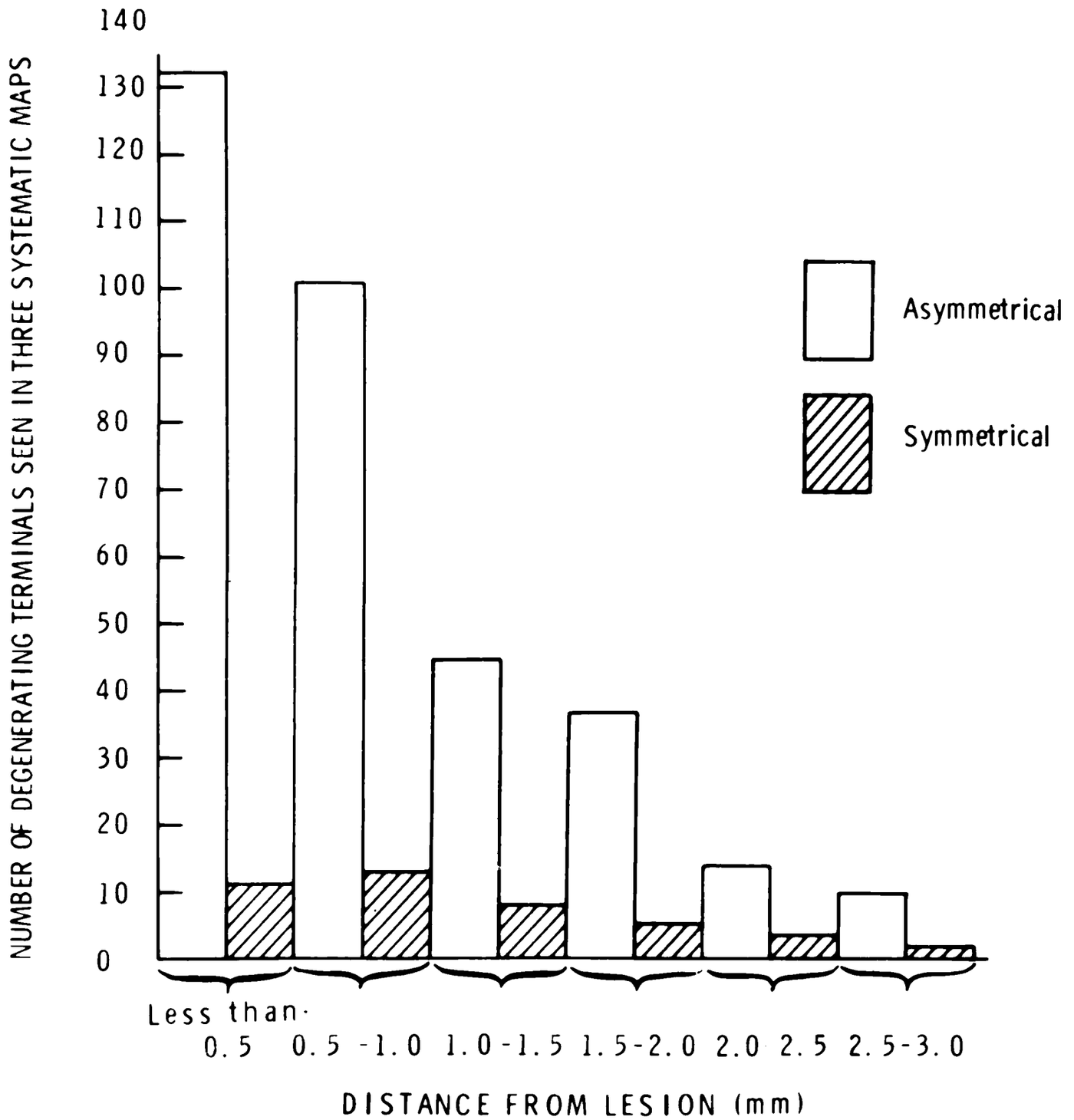

NUMBER OF DEGENERATING TERMINALS SEEN IN THREE SYSTEMATIC MAPS
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Asymmetrical
Symmetrical
Less than 0.5
0.5 -1.0
1.0-1.5
1.5-2.0
2.0 2.5
2.5-3.0
DISTANCE FROM LESION (mm)

TABLE 1

This table gives the total numbers of asymmetrical and symmetrical degenerating terminals at different distances from the lesion; this information was obtained from three systematic maps of the cortex and is represented on the histogram of Fig. 4:41 Each figure in the percentage columns gives the proportion of the total number of terminals of a certain type which was present at a given distance from the lesion.

Distance from lesion (mm)	No. (%) of asymmetrical terminals	No. (%) of symmetrical terminals
< 0.5	132 (38.9%)	11 (26.2%)
0.5 - 1.0	101 (29.8%)	13 (31.0%)
1.0 - 1.5	45 (13.3%)	8 (19.1%)
1.5 - 2.0	37 (10.9%)	5 (11.9%)
2.0 - 2.5	14 (4.1%)	3 (7.1%)
2.5 - 3.0	10 (3.0%)	2 (4.7%)
Total	339 (100.0%)	42 (100.0%)

TABLE 2

A total of 769 degenerating terminals, found in quantitative studies, were classified according to nature of postsynaptic thickening, as follows:

Lamina	No.of terminals with asymmetrical thickening	No.of terminals with symmetrical thickening	No.of terminals with unidentifiable thickening
Total	574 (91.4%)	54 (8.6%)	141
I	17 (94.4%)	1 (5.6%)	4
II	74 (97.3%)	2 (2.7%)	6
IIIa+b	226 (91.9%)	20 (8.1%)	64
IIIc	59 (92.2%)	5 (7.8%)	22
IV	45 (90%)	5 (10%)	9
V	117 (90%)	13 (10%)	27
VI	36 (82%)	8 (18%)	9

.....

TABLE 3

The nature of the postsynaptic profile contacted by the two main types (asymmetrical and symmetrical) of degenerating terminal was analysed as follows:

	Spines	Dendrites	Cell Somata	Unidentified profiles
Total	307 (66.7%)	149 (32.4%)	3 (<1%)	319
Asymmetrical	276 (75%)	88 (24%)	1 (< 1%)	219
Symmetrical	5 (13%)	29 (78%)	2 (~5%)	17

In addition, one degenerating terminal with a symmetrical thickening was found to contact the axon initial segment of a small pyramidal cell.

TABLE 4

The mode of termination (asymmetrical or symmetrical membrane thickening) of degenerating terminals on to the different types of postsynaptic profiles was analysed as follows:

Profile	Asymmetrical terminals	Symmetrical terminals	Membrane thickening unidentifiable
Spine	276 (98%)	5 (2%)	26
Dendrite	88 (75%)	29 (25%)	32
Cell Soma	1	2	-

.....

CHAPTER 5

A light and electron microscope study of the connections between area 17 and certain other cortical areas in the cat and monkey

Section A: Commissural Connections

Introduction

The material to be described in this study was obtained from the brains of four monkeys and five cats which had sustained lesions of the visual cortex of one side. In the monkey, lesions were made by the removal by subpial suction of the cortex of both walls of the lunate sulcus and of the crown of the prelunate gyrus, over most of the extent of the sulcus. In the case of two cats, areas 17, 18 and 19 on one side were entirely removed by subpial suction; in the remaining three, needle lesions were placed in area 18 on the lateral gyrus without involvement of adjoining areas. For light microscopy, blocks some 2-3 mm thick and oriented in the sagittal plane (monkeys) or the coronal plane (cats) were taken from the opposite hemisphere from sites corresponding to the position of the lesion, and 25 μm sections of such blocks were stained by reduced silver methods. For electron microscopy, material was obtained from the same brains as those used to provide material for light microscopy. In each case, one or more slices of tissue some 1.0 mm thick were taken immediately alongside the light microscopy material. Each slice was cut into blocks

approximately 1 mm wide and reaching through the full depth of the cortex, which were bevelled and separately numbered so that the position and orientation of each block with respect to the important nearby sulci were known. Blocks were chosen for systematic study by reference to adjoining light microscopic material, and maps were made of the chosen blocks by selecting 0.5 mm-wide strips reaching through the depth of the cortex.

Results - Light Microscopy

In the monkey, several areas of clearly localised terminal degeneration could be seen in the sections; one of these is at the junction of areas 17 and 18, while others are in regions of the peristriate cortex which fit closely in position and extent with those described by Zeki (1970) in horizontal sections. However, as the electron microscopic study has been concerned only with the region at the junction of areas 17 and 18, a detailed description will be given of degeneration in this region only.

The terminal and fibre degeneration is clearly restricted to a narrow region some 2 mm wide, the centre of which is formed by the junction of architectonic areas 17 and 18 (Figs. 5:3, 5:4 and 5:5); the latter is marked by a group of half a dozen or so conspicuous, large pyramidal cells situated exactly at the junction of layers III and IV (Figs. 5:1 and 5:2) (cf. von Bonin and Bailey, 1947). In area 18 degeneration is found throughout all layers of the cortex; in the layers superficial to layer V it is mainly

granular, terminal degeneration, while in layers V and VI there are in addition a number of degenerating fibres, some of which undoubtedly run obliquely, though the majority are arranged perpendicular to the cortical surface. The density of the fine terminal degeneration in layer IV is slightly greater than that in the layers superficial and deep to it. As one traces the degeneration towards area 17 a definite change occurs, in that the degeneration in layer IV suddenly diminishes and, within a few hundred microns of the large cells, disappears completely. The degeneration in the layers superficial and deep to layer IV continues, however, the maximum extent being seen in the most superficial and deep parts of the cortex, so that the distribution of the degeneration, which has a surprisingly sharp edge, leaves a clear zone in the peripheral part of area 17 which is wedge-shaped with its apex at the large cells situated at the 17/18 boundary; in particular, the spread of degeneration towards area 17 in the superficial layers is very striking. An exactly similar distribution of commissural degeneration has been described recently at the striate/peristriate boundary in the Virginia opossum (Benevento and Ebner, 1971). These features have been presented in some detail as they are important for the interpretation of the electron microscopic findings both of ourselves and of earlier workers (see Chapter 6).

So far as the cat is concerned, the distribution of

fibre and terminal degeneration in terms of the architectonic subdivisions of the visual cortex has already been described (Wilson, 1967; Garey et al. 1968; Heath and Jones, 1971), so that the present description will be concerned with the distribution at the boundary of areas 17 and 18 only; this boundary region is also the subject of the accompanying electron microscopic study. Commissural degeneration is restricted to a narrow zone, which is mainly in area 18 but extends for a short distance into area 17 (Fig. 5:6). There is fibre and terminal degeneration throughout the depth of the cortex, but it is less dense in layers I and II and the superficial part of layer III. In the deep half of layer III and the superficial part of layer IV there is a prominent band of dense, fine granular terminal degeneration; the density of the granules diminishes in the deep part of IV, and in layers V and VI there are many degenerating fibres, with a moderate amount of terminal degeneration scattered throughout. At about the boundary of areas 17 and 18 the dense degeneration in layers III and IV stops abruptly, and medial to this in area 17 the degeneration is largely restricted to layers V and VI, with a very much smaller amount in the superficial part of layer III. It is well known that it is difficult to delimit precisely the boundary between areas 17 and 18 in the cat, and because of the curvature of the cortex and the change in thickness in the laminae in this region it is also difficult to be certain of the precise limits of the individual laminae. For these reasons

the allocation of the band of dense degeneration must be considered provisional; nevertheless, it is striking how closely this pattern resembles that already described in the monkey, in that degeneration in layer IV stops abruptly at the boundary of areas 17 and 18.

Results - Electron Microscopy

Degenerating terminals seen in this material showed various stages of dark degeneration; no examples were seen of terminals showing a filamentous type of reaction. The appearance of these stages of degeneration was essentially the same as that described previously in the cortex, in that the terminals showed progressive darkening, followed by distortion and disruption of vesicles, shrinkage and crenation of the terminal outline and engulfment by reactive glial processes. Only those terminals were accepted for further consideration which could clearly be seen to be in one of these phases of degeneration and which were definitely associated with a synaptic contact showing distinct membrane specialisations.

All of the degenerating terminals seen in this study ended with asymmetrical membrane thickenings; this finding is in agreement with those of workers in other cortical areas. Most of the degenerating terminals in both the cat (76%) and the monkey (72%) ended on dendritic spines (see Table 5). A few examples have been seen, in both species, of degenerating terminals contacting more than one spine - Fig. 5:9, for example, shows a

terminal contacting two spines; examples may also be found of a single degenerating terminal ending upon a spine and an adjoining dendrite (Figs. 5:10 and 5:11). Some, at least, of the spines which receive commissural afferents appear to receive other synapses as well; Fig. 5:8 shows a spine receiving a degenerating callosal terminal together with a normal, symmetrical terminal. Unfortunately it has not proved possible to identify the type of cell to which these spines belong. The remaining callosal terminals end on dendritic shafts, and where it has been possible to identify them, these have been found to be of the varicose type normally believed to belong to stellate cells, in that they bore a large number of normal synapses, many of which were of the asymmetrical type (Fig. 5:7).

In the deeper layers of the cortex a certain number of degenerating myelinated fibres were seen; most of these were vertically oriented and of fine to medium calibre (1-2 μm). The number of such fibres decreased progressively as one approcahed the more superficial layers.

Although, as has been mentioned, blocks for electron microscopic study were taken only from the sites at which degeneration was seen in sections of an immediately adjoining slice of tissue including area 17 and peristriate cortex, the distribution of the degeneration on maps of the thin sections showed definite variation from block to block, particularly in

regard to degeneration in the middle layers (compare, for example, the maps of Figs. 5:12 and 5:13). A possible explanation for this variability was found on a detailed re-examination of the Nauta-stained sections, when it was observed that the distribution of the degeneration in the different laminae changed suddenly at the 17/18 boundary (vide sup.). As has been described, the degeneration in the deep part of layer III and layer IV diminishes within a few hundred microns as one moves towards area 17, and as the whole band of degeneration is only a few millimetres wide, it is more or less inevitable that a variation of only a few hundred microns in the precise position of the electron microscope blocks would result in changes in the amount of degeneration in the middle layers of the cortex. Blocks of tissue, approximately 4 mm wide and 0.5 mm or so thick, were therefore taken from a representative monkey brain such that the 17/18 boundary fell roughly in the middle of each block. In the "thick" sections of these, the large cells at the 17/18 boundary were identified and thin sections were taken from the area 18 side. In these sections degeneration was found throughout the layers of the cortex, including layer IV, on qualitative survey. Sections taken from the area 17 side of such blocks tended to show very little degeneration in layer IV. It is considered therefore that the earlier variations are explicable on the basis of the differences in laminar distribution on either side of the 17/18

boundary together with the difficulty in determining the precise site of the ultrathin sections. Similar variability in the distribution of degeneration on electron microscope sections was seen in the cat.

Section B: Association Connections

Introduction

The present results are based on observations made in those cat brains which had needle lesions made in area 18 on the lateral gyrus without involvement of underlying white matter or of adjoining cortical areas; these three brains were also used in the above study of commissural connections. In each case, a coronal cut was made through the hemisphere at the level of the lesion and a block of tissue some 2-3 mm thick was taken out immediately in front of or behind this cut. Sections of this block (25 μm) were stained by reduced silver methods for light microscopy. A 1 mm-thick coronal slice was taken from the other face of the original cut and was divided into 1 mm-wide blocks. As before, appropriate blocks for systematic study were chosen by noting the position of maximum degeneration with the light microscope, and 0.5 mm-wide strips, reaching through the depth of the cortex, were studied in these blocks.

Results

The terminal and fibre degeneration seen with the light microscope was heavy in area 18 but decreased quite suddenly at the boundary with area 17. In the latter, marked degeneration of fine fibres in layer I extended down almost to the level of the suprasplenial sulcus; in addition, degenerating fibres could be seen entering layer VI from the white matter, passing obliquely downwards and medially to ramify mainly in layers V and VI. Distributed throughout these layers and extending up into layer III was a little fine granular degeneration. This degeneration in the deeper layers was restricted to the lateral part of area 17 and, in contrast to that in layer I, did not extend down to the level of the suprasplenial sulcus.

Under the electron microscope degenerating axon terminals were again seen to be undergoing various stages of dark degeneration, and again no examples of filamentous degeneration have been found. Degenerating terminals are found in appreciable numbers only in the lateral part of area 17 near the boundary with area 18. They are present in all layers of the cortex, but seem to be more numerous in the middle layers and rather less so in the superficial layers and in layer VI; a representative map showing this distribution of degeneration is given in Fig. 5:18. All of the degenerating terminals seen ended with asymmetrical membrane thickenings. Most (some 62%) ended on dendritic spines (see Table 6); in one case, such a spine was seen in continuity with its parent dendrite, which seemed to be of the pyramidal type

(Fig. 5:15), but in the remaining cases it was not possible to identify the dendrite of origin. A few examples were seen of a degenerating bouton contacting two spines (Fig. 5:17); in other cases, a spine received a degenerating terminal and a normal, asymmetrical terminal. Finally, an occasional example has been seen (Fig. 5:14) of a degenerating terminal ending upon a spine and a nearby dendrite. The remaining 38% of degenerating terminals ended upon the shafts of dendrites. Many of these were difficult to classify, but a number seemed to be of the stellate type (Fig. 5:16).

PLATE 18

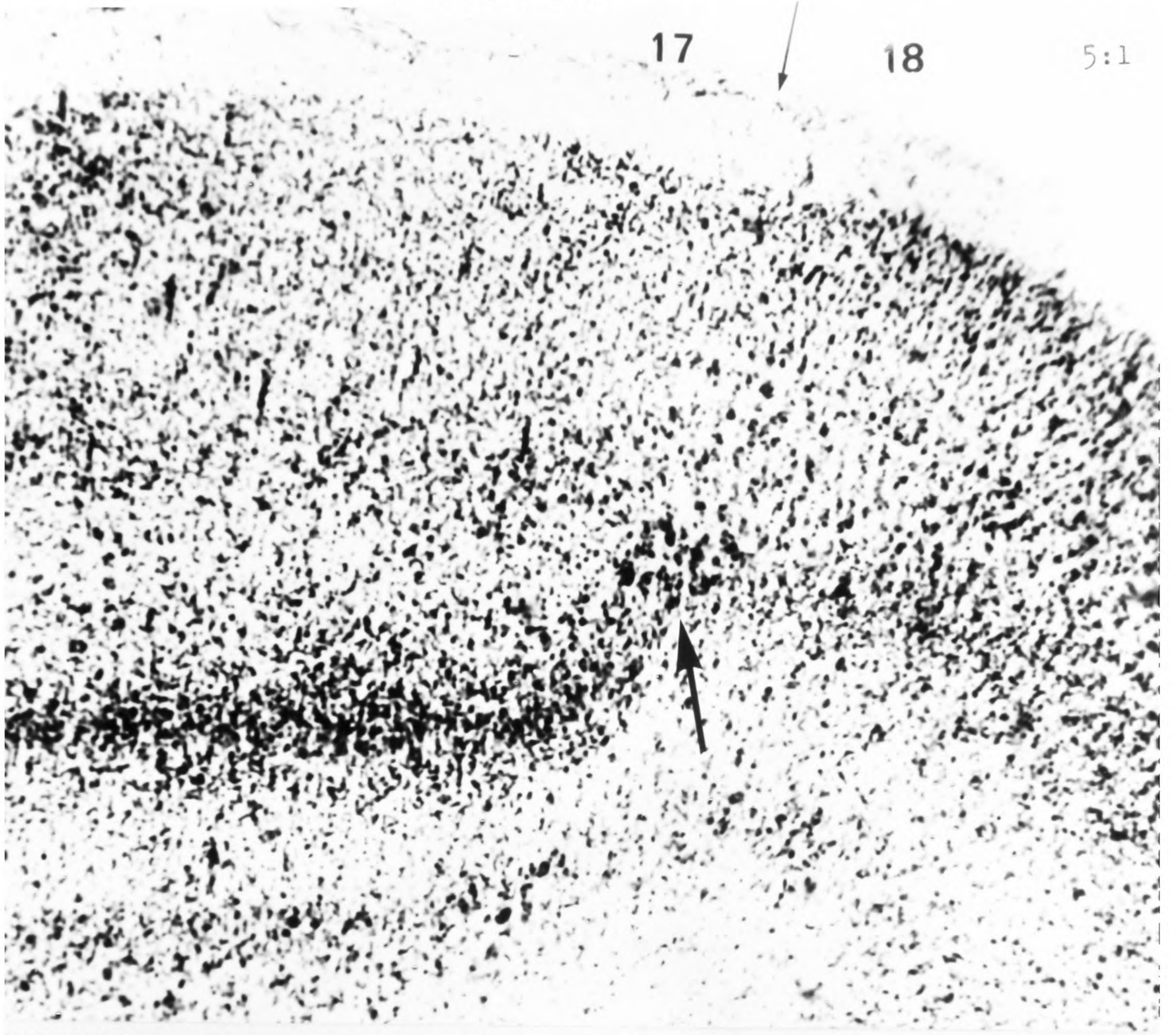
Fig. 5:1

The cortex at the boundary of areas 17 and 18 in the monkey, to show the large cells in the deep part of layer III (arrow). Thionin stain. X 90.

Fig. 5:2

The deep part of layer III and layer IV of the previous figure, at a higher magnification. The large cells are indicated by the arrow. Thionin stain. X 220.

Plate 18.



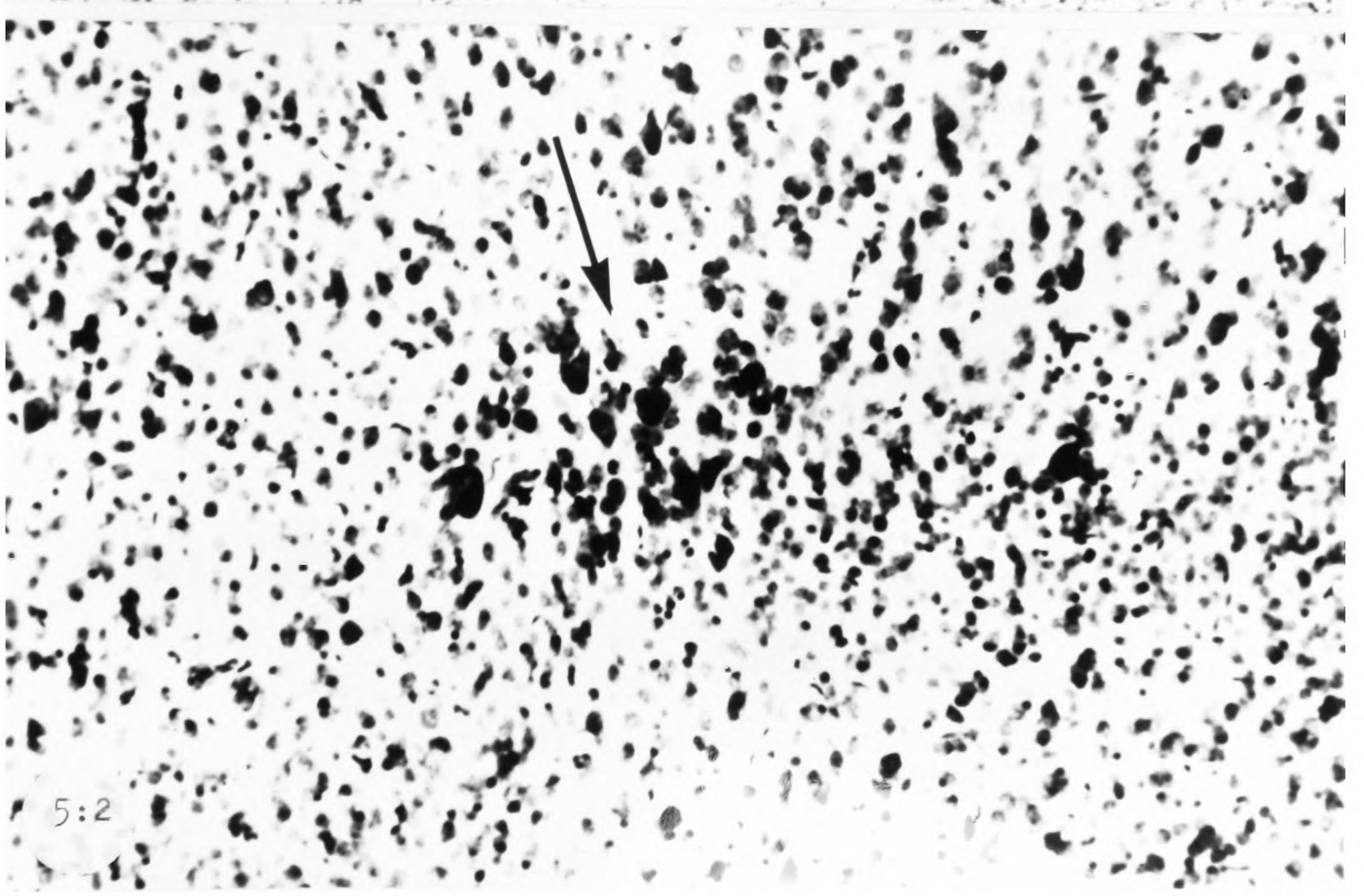


PLATE 19

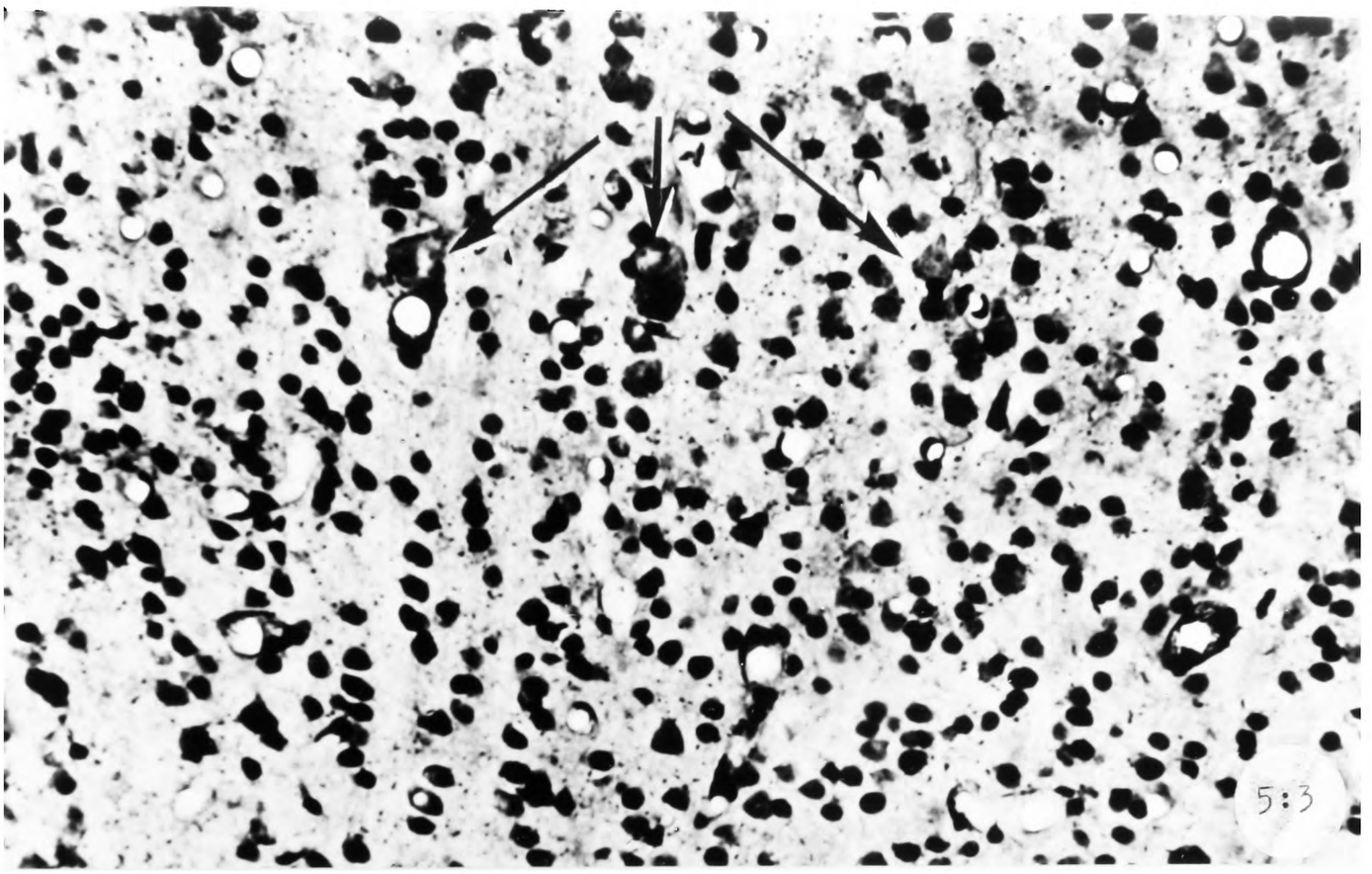
Fig. 5:3

The deep part of layer III and layer IV at the boundary of areas 17 and 18 in a Fink-Heimer stained section of the cortex of the monkey, to show the fine degeneration of commissural fibres in the region of the large cells in layer III. The large cells are indicated by arrows. X 300.

Fig. 5:4

The part of the previous figure with large cells, at higher magnification. X 550.

Plate 19.



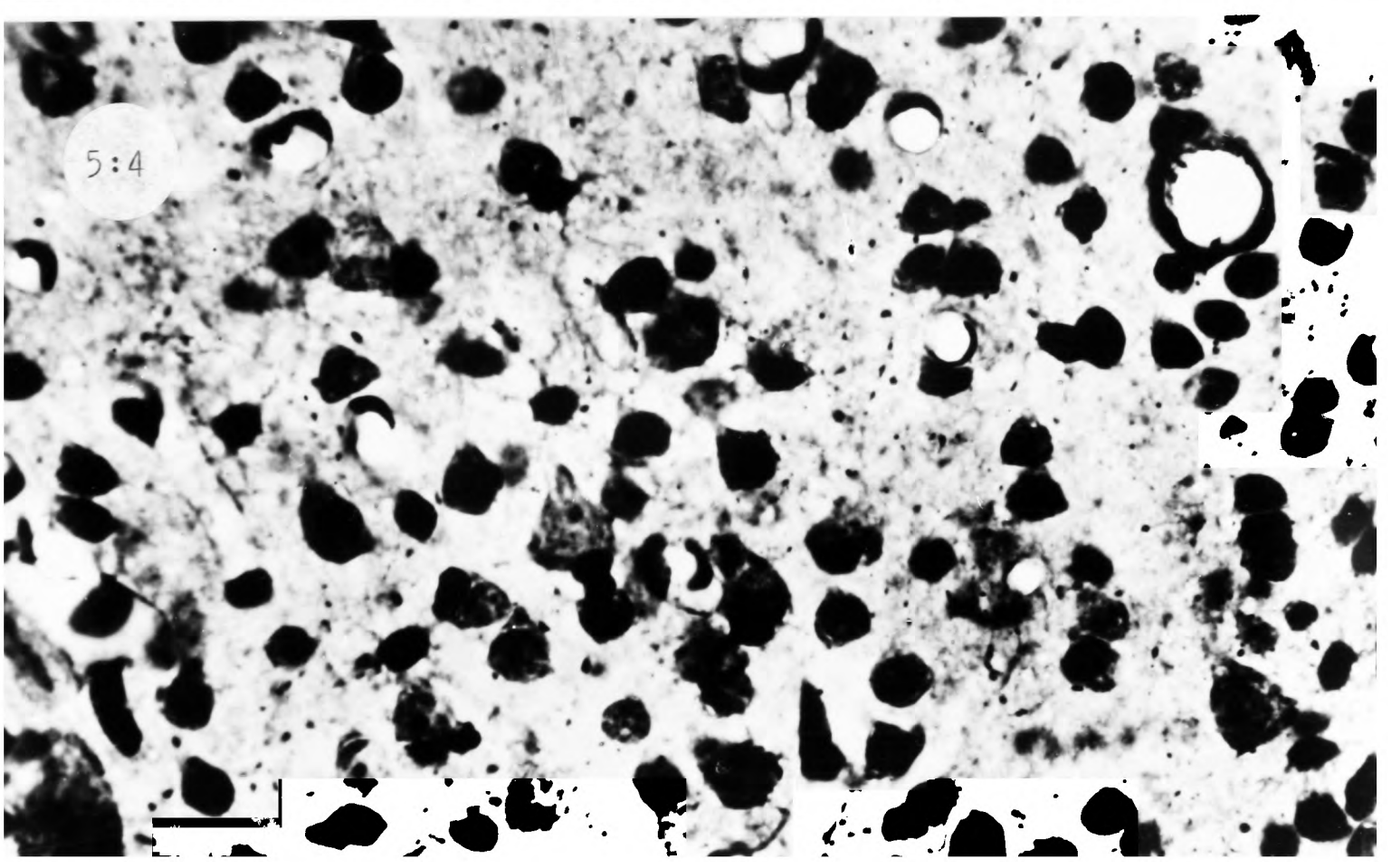


Fig. 5:5

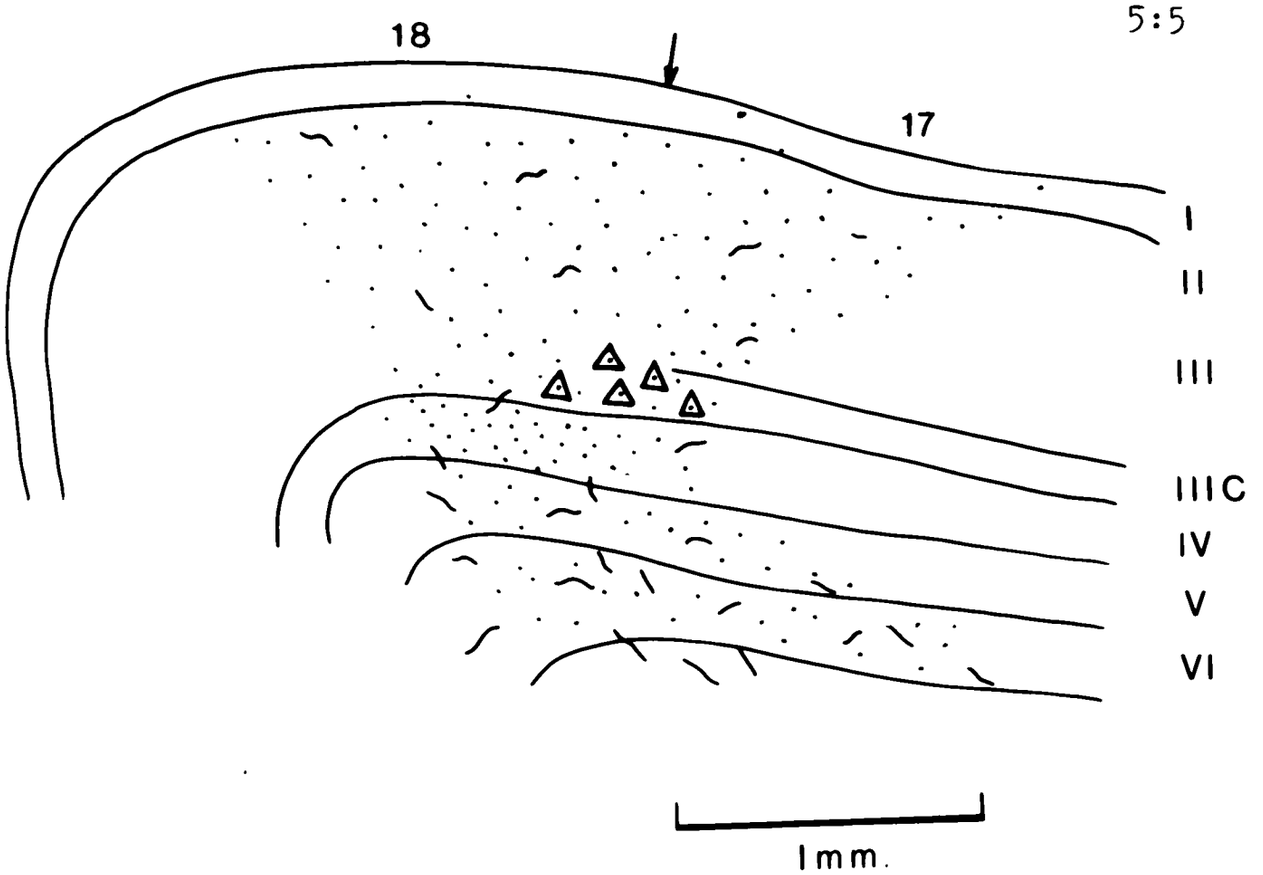
Diagrammatic representation of a parasagittal section of the visual cortex of the monkey at the boundary of areas 17 and 18, to show the fibre (dashes) and terminal (dots)degeneration seen in Nauta-stained material after a lesion of the corresponding region of the opposite side. The exact boundary of areas 17 and 18 is marked by the presence in the deep part of layer III of a group of conspicuous, large pyramidal cells (triangles). Laminae of the cortex are indicated by Roman numerals.

Fig. 5:6

Diagrammatic representation of a coronal section of the visual cortex (17/18 boundary region) of the cat, to show the fibre and terminal degeneration seen in Nauta-stained material after a lesion of the visual areas of the opposite side. Laminae of the cortex are indicated by Roman numerals.

MONKEY

5:5



CAT

5:6

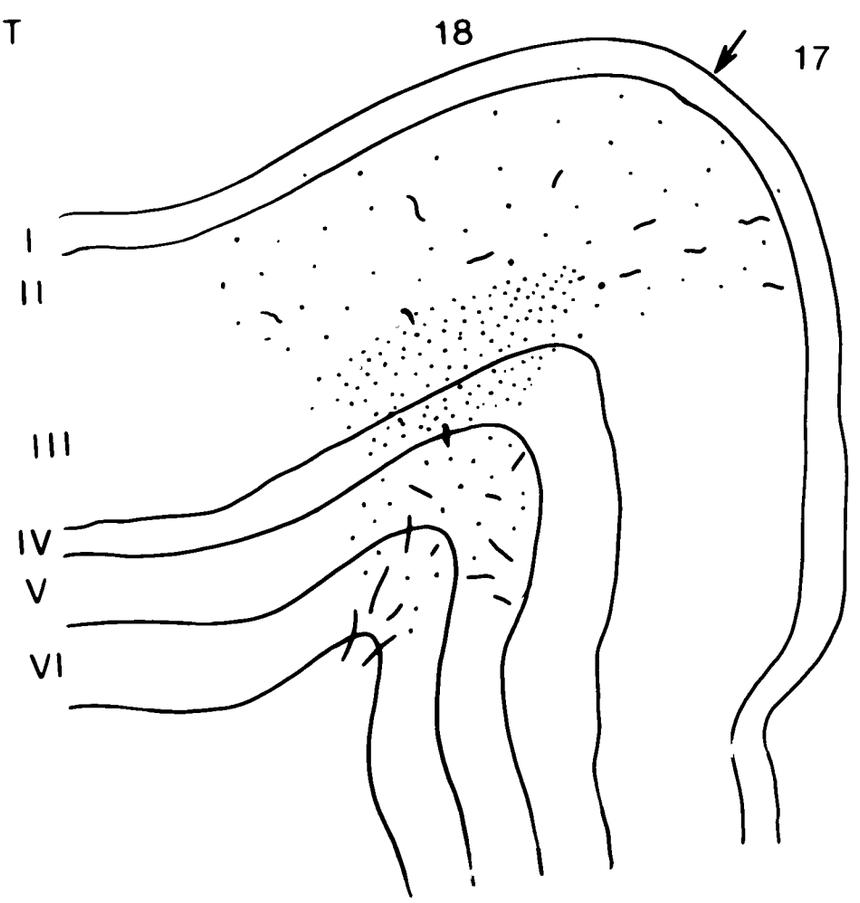


PLATE 20

Fig. 5:7

A large dendrite (D) in layer III of the visual cortex of the cat which receives an asymmetrical synapse from an axon terminal which is degenerating (arrow head) following a lesion of the visual cortex of the opposite side. The dendrite receives three other synapses (arrows) from normal axon terminals, one of which is asymmetrical. The dendrite is presumed to be one of a stellate cell. X 52,000

Fig. 5:8

A degenerating commissural terminal in layer III of the visual cortex of the cat, contacting a small spine with an asymmetrical synapse (arrowhead); the spine also receives a normal, symmetrical terminal (arrow). X 44,000

Fig. 5:9

A degenerating commissural terminal in layer V of the cat's visual cortex making asymmetrical synaptic contacts (arrowheads) upon two spines, one of which (S) has a spine apparatus. Reactive glial processes with glycogen (G) can be seen nearby. X 42,000

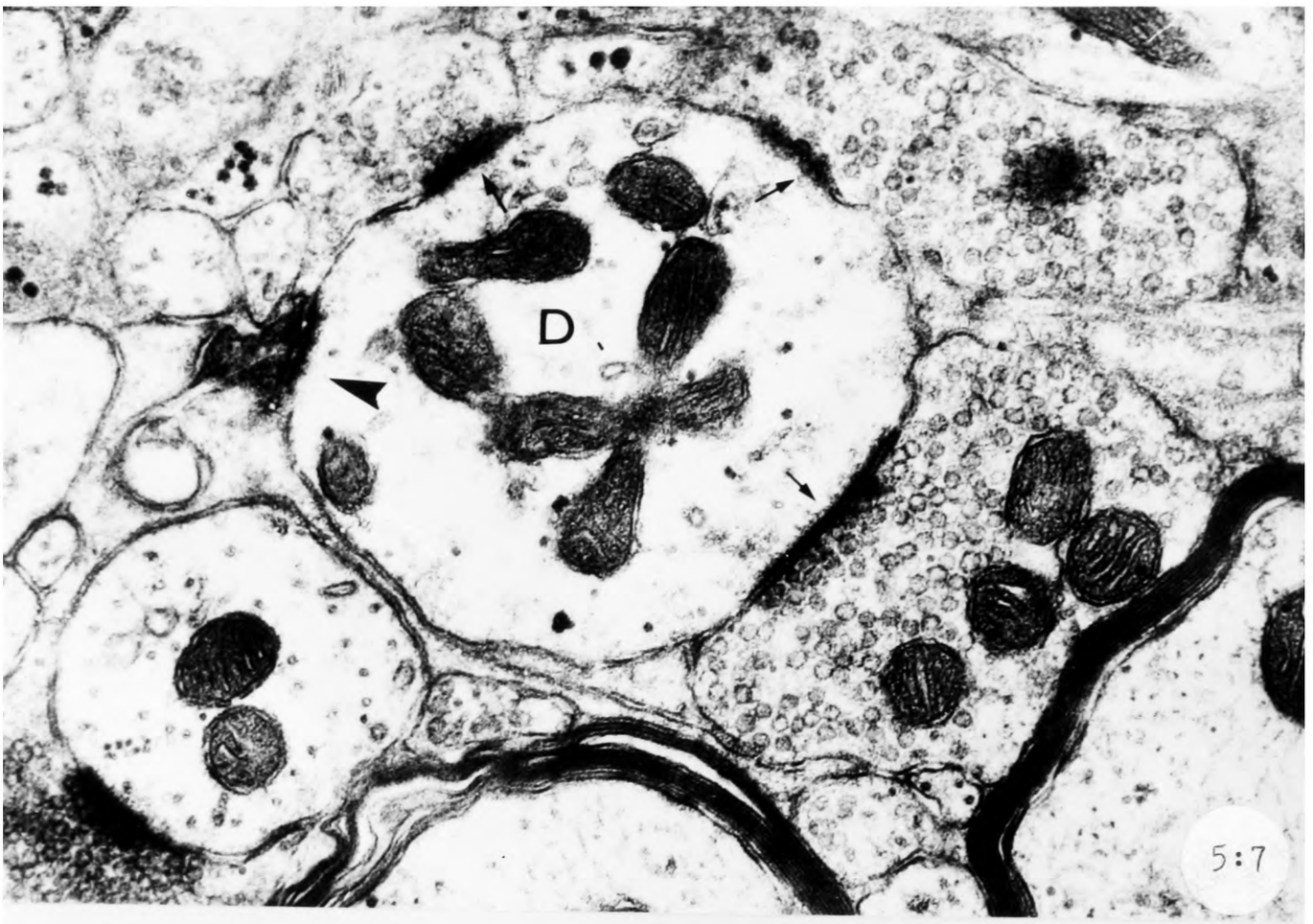
Fig. 5:10

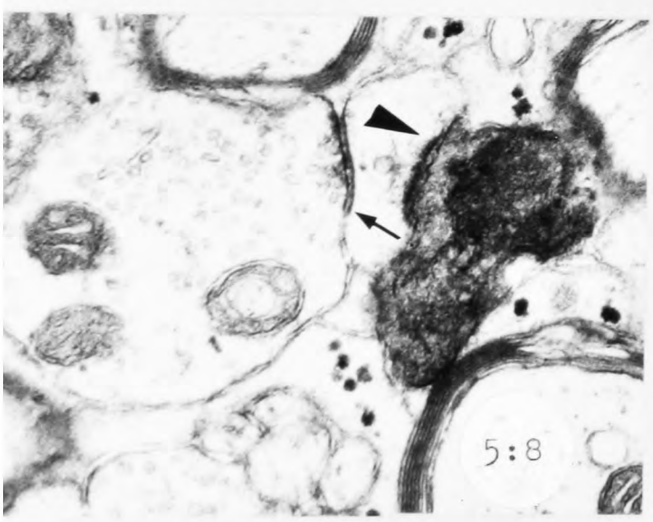
An axon terminal at a relatively early stage of degeneration in layer VI of the visual cortex of the monkey after a lesion of the opposite visual cortex. The terminal makes asymmetrical synaptic contacts (arrowheads) with a dendrite (D) and a small spine. X 44,000

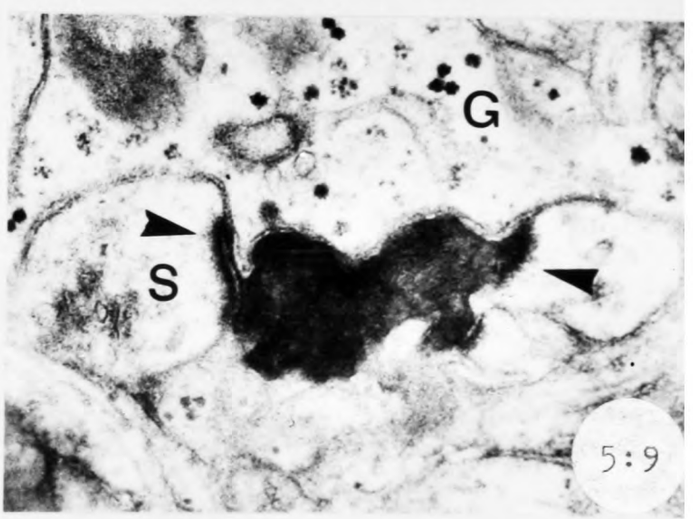
Fig. 5:11

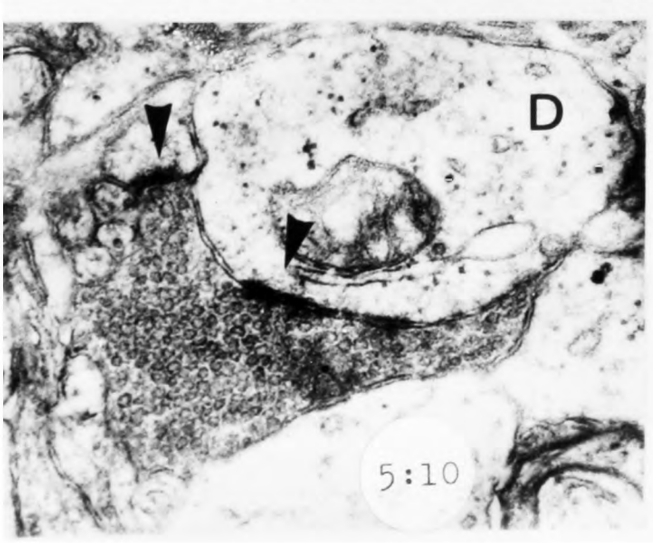
An axon terminal (T) in layer II of the cat's visual cortex, at an early stage of degeneration after a lesion of the opposite visual cortex. The terminal makes asymmetrical synapses (arrowheads) upon a small spine and a small dendrite (D). X 44,000

Plate 20.









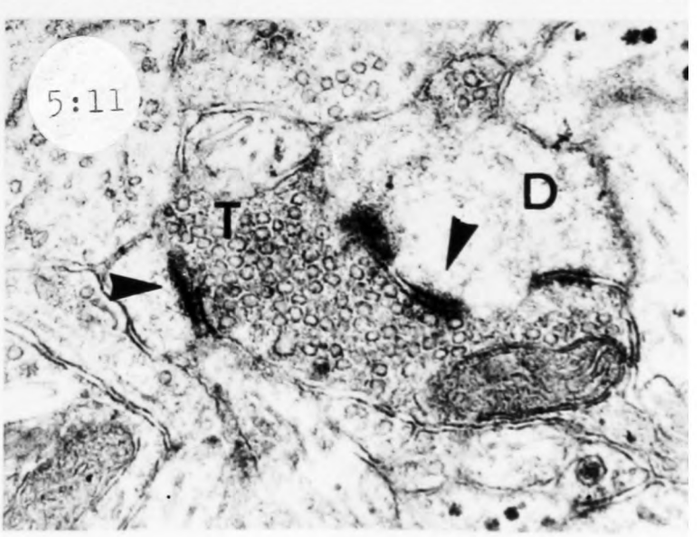


Fig. 5:12

A map of vertical sections of the area 17/18 boundary of the monkey, made with the electron microscope, showing the distribution of degenerating terminals (dots) seen after a lesion of the peristriate cortex of the opposite hemisphere. It will be noted that the distribution of terminals is reasonably even throughout the superficial two-thirds of the cortex, falling off in density somewhat in the deep layers.

COMMISSURAL TERMINALS IN THE VISUAL CORTEX (17/18 OF THE MONKEY.

5:12

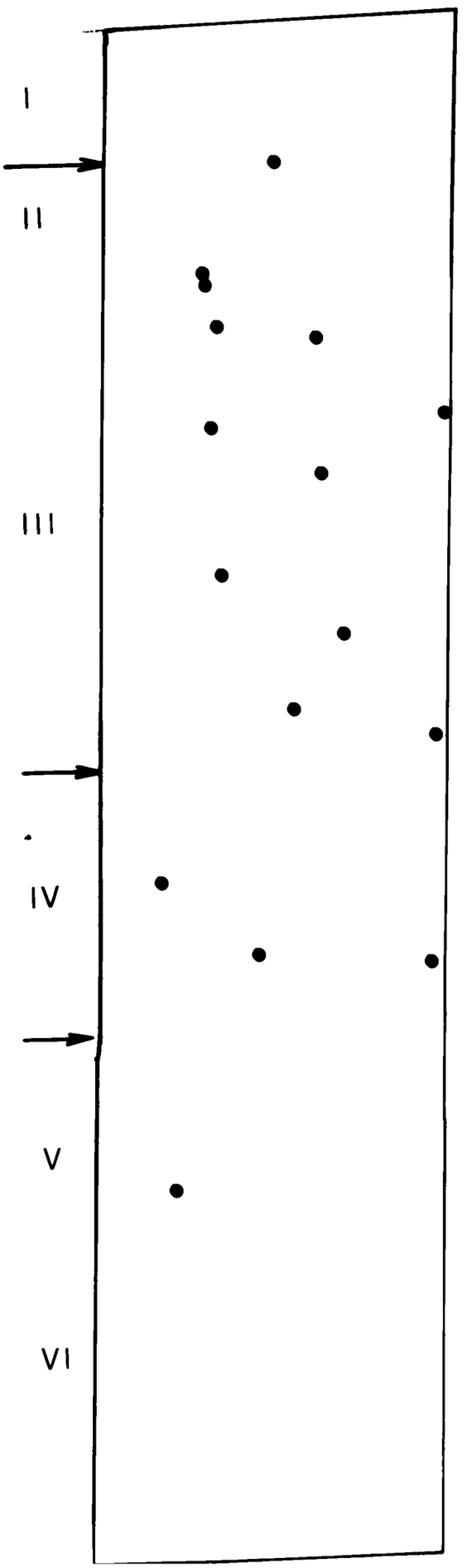


Fig. 5:13

A similar map to that of Fig. 5:12. In this example, however, degeneration is unevenly distributed, with no degenerating terminals seen in layer IV.

COMMISSURAL TERMINALS IN THE VISUAL CORTEX (17/18) OF THE MONKEY

5:13

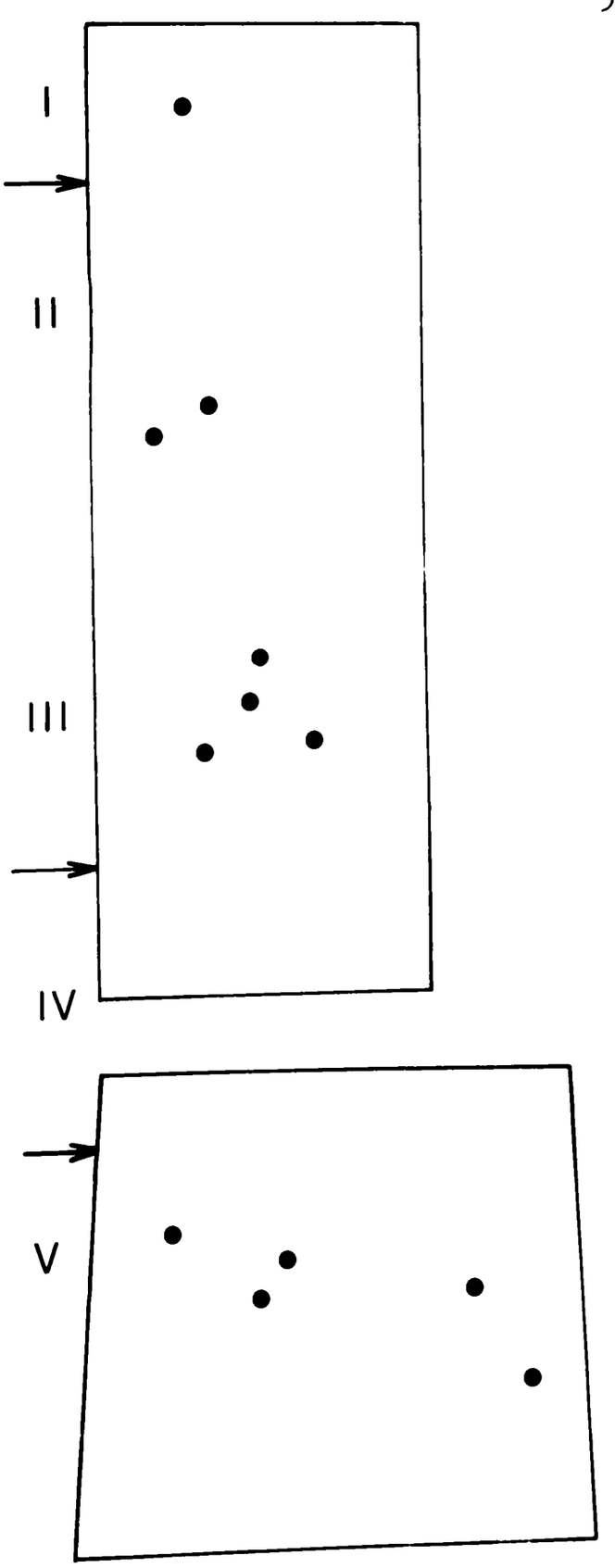


PLATE 21

Fig. 5:14

A degenerating terminal in layer IV of area 17 of the cat after a lesion of the adjoining part of area 18 of the same side. The terminal makes asymmetrical synapses (arrowheads) with a spine (S) and a dendrite (D); the latter also receives a synapse from a normal terminal (arrow). X 42,000

Fig. 5:15

A degenerating terminal in layer III of area 17 of the cat following a lesion of area 18 of the same side. The terminal makes an asymmetrical synapse (arrowhead) upon a spine (S), which contains a spine apparatus and can be seen arising by a short pedicle from its parent dendrite (D); the latter receives a normal, symmetrical synapse (arrow). X 48,000

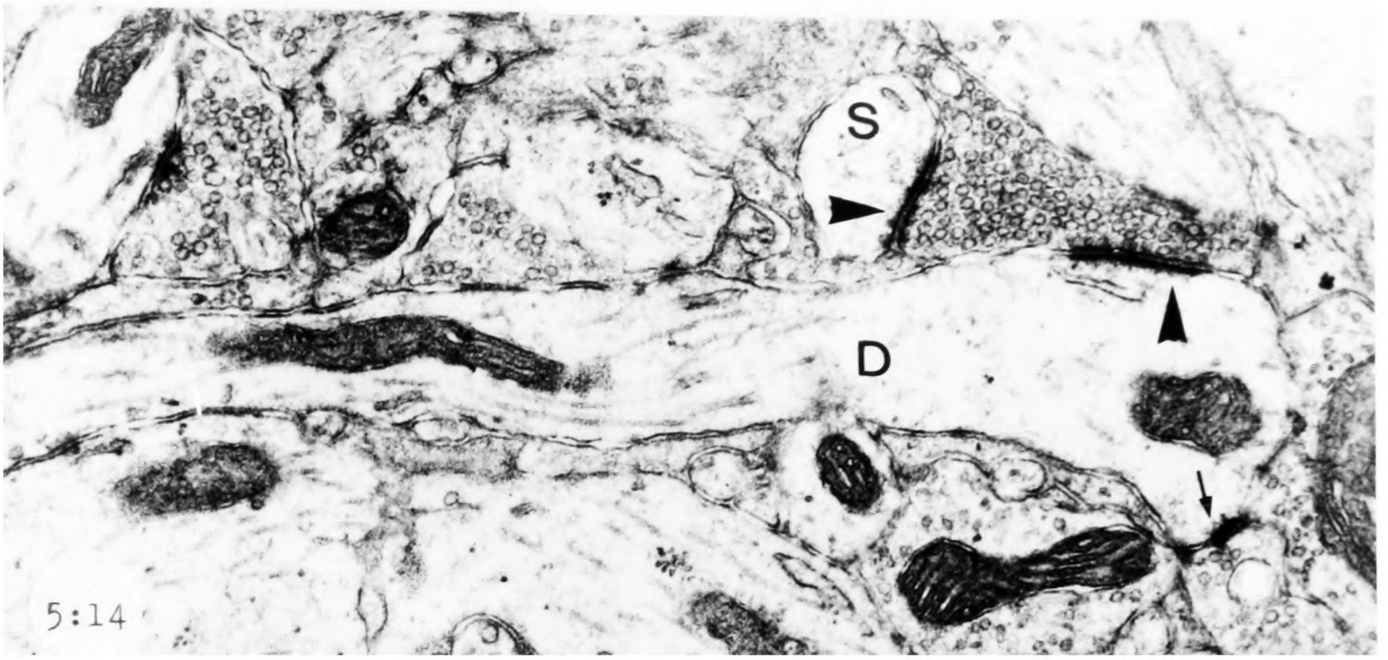
Fig. 5:16

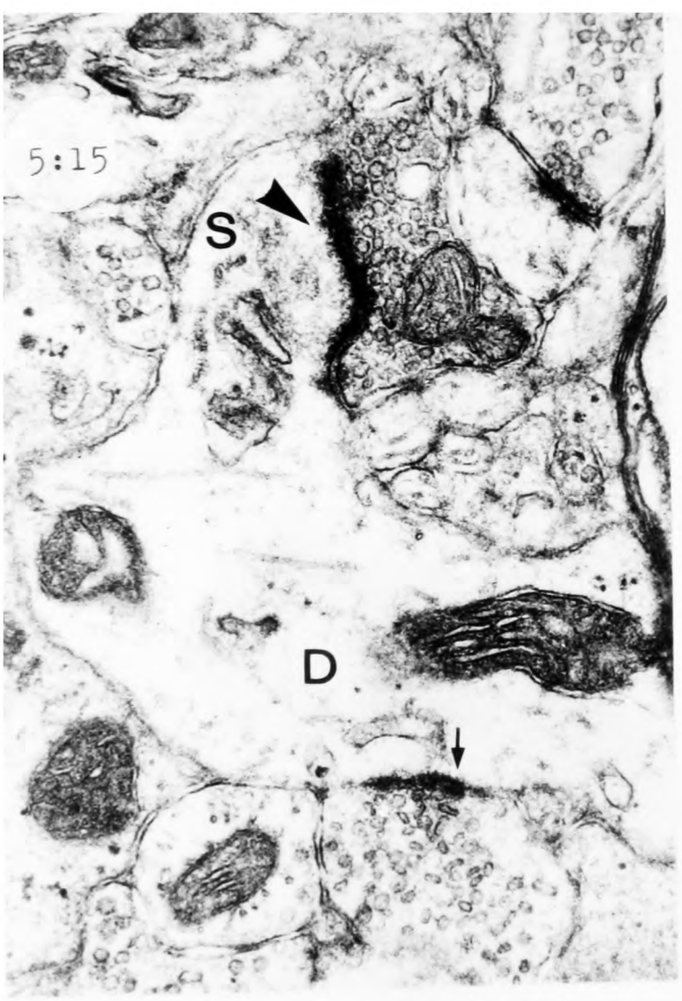
A dendrite (D) in layer IV of area 17 of the cat after a lesion in area 18. One of the axon terminals which makes an asymmetrical synapse (arrowhead) with the dendrite is at an early stage of degeneration. The dendrite receives several other synapses (arrows) and is probably one of a stellate cell. X 52,000

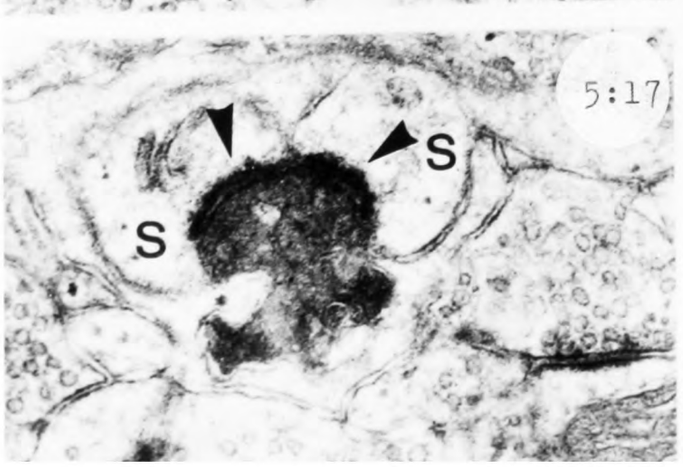
Fig. 5:17

A degenerating terminal in layer III of the cat's visual cortex (area 17) after a lesion in area 18. Asymmetrical synapses (arrowheads) are made with two small spines (S), one of which contains a spine apparatus. X 52,000

Plate 21.







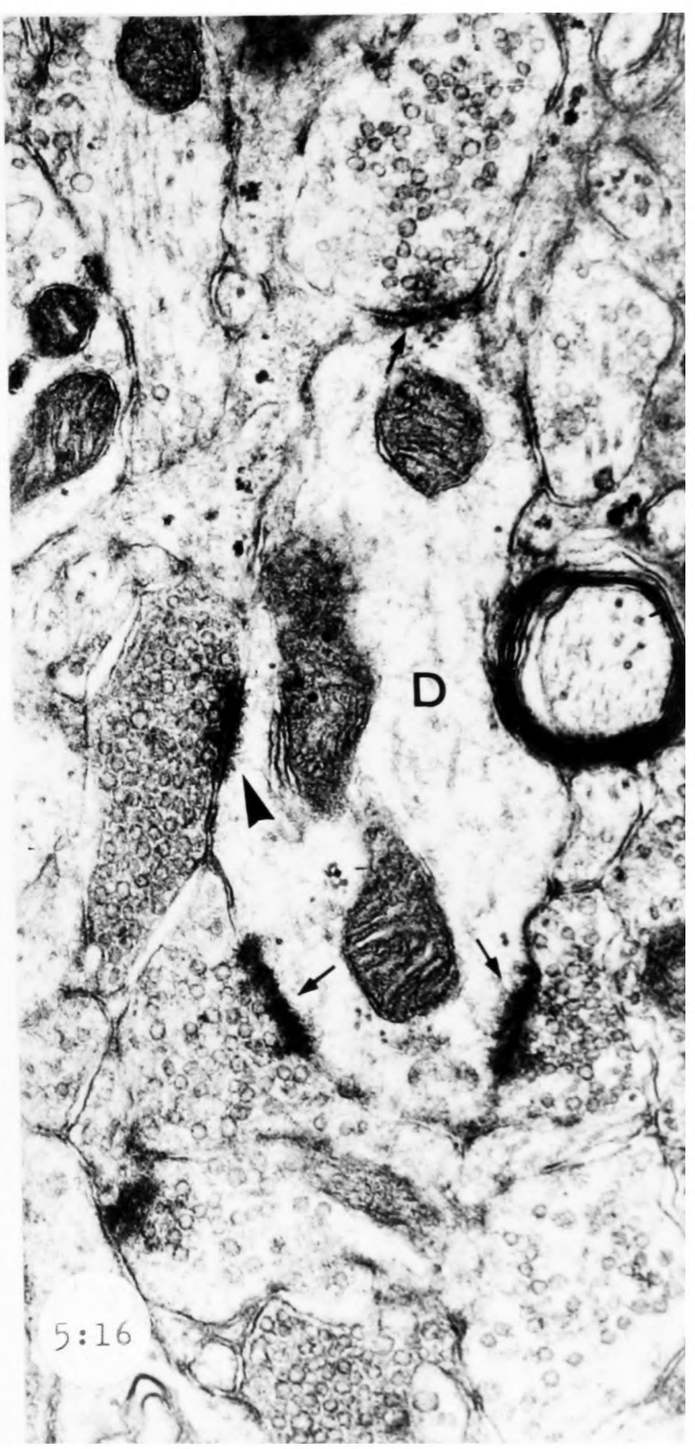


Fig. 5:18

A map of a vertical section of the cat's area 17, made with the electron microscope, showing the distribution of degenerating terminals seen after a lesion of the adjoining area 18; degenerating terminals are indicated by dots. The distribution of the degeneration is reasonably even throughout the middle layers of the cortex, but layers I and II and layer VI show very few terminals.

DEGENERATING TERMINALS IN AREA 17 OF THE CAT AFTER A LESION OF AREA 18.

5:18

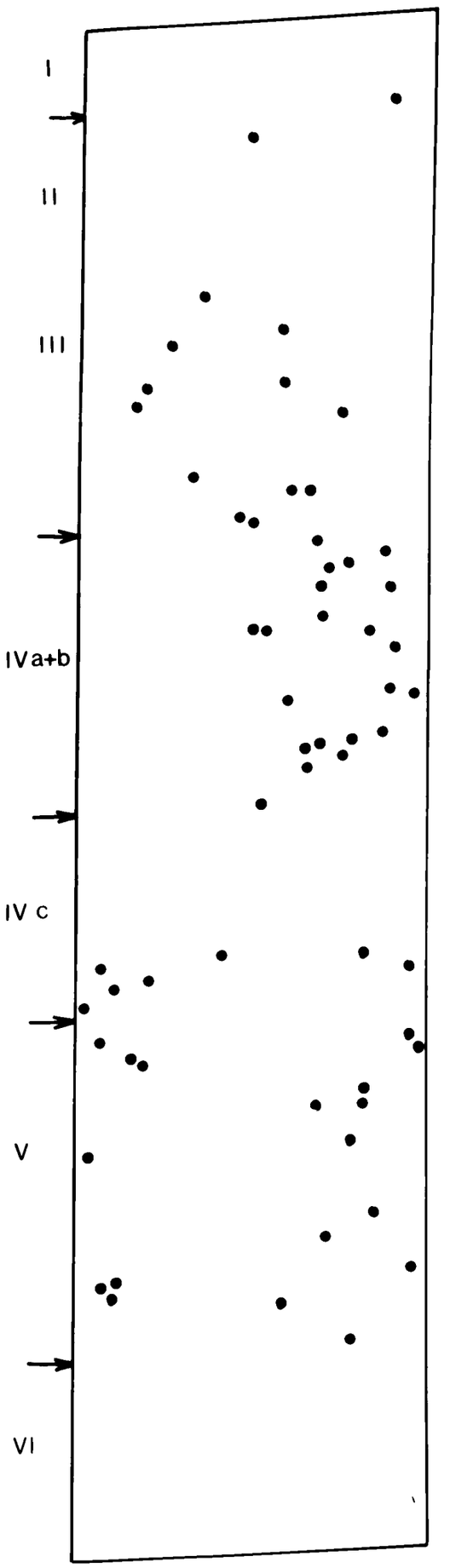


TABLE 5

Numbers of degenerating axon terminals on to dendritic spines and dendritic shafts as seen at the area 17/18 boundary on one side after a lesion of the corresponding region on the other side.

Cat		Monkey	
Spines	99 (76%)	Spines	31 (72%)
Dendrites	32 (24%)	Dendrites	12 (28%)
Unclassified	71	Unclassified	28

TABLE 6

Numbers of degenerating axon terminals on to dendritic spines and dendritic shafts as seen in area 17 after a lesion in the ipsilateral area 18 (Cat).

Spines	74 (62%)
Dendrites	46 (38%)
Unclassified	54

CHAPTER 6

General Discussion

It appears from the present study that experimental neurohistological techniques are capable of providing a good deal of information about the details of intrinsic as well as extrinsic cortical connections. In order to allow for a satisfactory discussion of the points raised by our findings, it may, perhaps, be valuable at this point to make a brief restatement of our observations. It is worth stressing again that the great majority of our results were obtained within the part of area 17 of the cortex representing the central ten degrees of the visual field, but that, as far as they go, these observations were made in such a way as to try to minimise the risk of missing or misinterpreting features of the degeneration: all sections of the occipital lobes were kept and several series of sections were stained, using two or three different techniques. Also, attempts were made to correlate as far as possible the appearances of lesions in the sections with the records made at operation of their site and depth, and sections were examined carefully for evidence of incidental lesions or vascular damage.

Intrinsic connections of area 17 of monkey

The most striking feature of the horizontal intrinsic connections of area 17 is their restricted extent: in both light and electron microscopic material there is dense granular

degeneration for only 200 μm or so from a lesion. Even though degeneration extends *in toto* for a good deal further than this, it is nevertheless restricted to a region some 6 mm in diameter. In broad terms it is true to say that horizontal degeneration in a lamina appears in significant amounts only when that lamina is damaged, so that the pattern of degeneration seen with a lesion of the whole depth of the cortex is essentially the sum of the individual laminar patterns. With a lesion confined to layers I and II, granular degeneration, with a few fibres, is seen extending for approximately 1 mm on either side, tailing off in a semicircular pattern as one passes deeply (Fig. 3:3). Vertically, a few fibres pass down into the upper part of layer III. When the lesion extends into layer III, the degeneration in layer I is approximately as before but, as would be expected, local degeneration appears in layer III and is comparable in appearance and extent with that in layer II. This degeneration is noteworthy for two reasons: first, it is almost entirely granular and contains very few fibres, and secondly it displays a feature which is common to many intrinsic connections, viz. it is asymmetrical, extending for 1.5 to 2 mm on one side of the lesion but only 0.5 to 1 mm on the other. At each side, its disappearance is comparatively sudden. Provided that the lesion does not involve layer IIIc, degeneration in this sublamina is no greater in quantity or extent than that in the rest of layer III, i.e., there does not appear to be any special projection into the stria

of Gennari from the more superficial layers. Degeneration in the suprastriate layers is less in quantity after lesions restricted to those layers than after lesions extending through the full depth of the cortex, i.e., there does seem to be some projection into these layers from infrastriate cells. Also, lesions of layers I, II and III give rise to a small amount of vertical degeneration, with a small patch of granularity in layer V and a few horizontal fibres in the inner band of Baillarger. Thus the cells of the superficial layers do seem to project into layer V, though by no means as massively as has been reported in Saimiri (Spatz et al., 1970). Lesions of the supragranular layers give rise to a few degenerating fibres in layer VI which pass out into the white matter.

As soon as the lesion comes down to involve layer IIIc an important new feature appears: dense, horizontal fibre degeneration is seen in the stria of Gennari. This is more extensive than the degeneration in the more superficial layers and is again asymmetrical, extending some 3.5 mm in one direction and 2 mm in the other. Some degenerating fibres appear to leave the stria to enter layer IIIb and the superficial part of layer IV; there is thus a difference in connections between the superficial and deep parts of layer IV. The view that horizontal fibres in the stria arise from cells within and below it, rather than from those above, is supported by an experiment in which a microelectrode lesion involved layer IIIc alone; this lesion

gave rise to extensive degeneration in the stria. It also appears that degeneration in the stria after a lesion of layers I, II and III, including IIIc, is virtually as dense as after lesions of the full depth of the cortex, which means that fibres reaching the stria from below must travel almost exactly vertically. A small microelectrode lesion in layer IV provides further evidence (Fig.3:7), for this lesion resulted in considerable degeneration in the stria, with an appearance highly suggestive of fibres arising within the focus of degeneration in layer IV and spreading obliquely upwards and outwards to enter the stria, i.e., it may well be that a high proportion of the intrinsic fibres in the stria arises from cells of layer IV. It should be pointed out, moreover, that degeneration in layer IV itself is very restricted, being essentially confined to the 200 μm or so immediately adjoining the lesion. What is more, this observation is constant for all intrinsic lesions restricted to the cortex, even those involving all the layers from I to VI. This finding is very striking in view of the fact that layer IV receives the great bulk of the thalamo-cortical projection to area 17, and the contrast between the clear appearance of layer IV and the dense degeneration in layers IIIc and V above and below it is easily seen on light microscope sections. Degeneration in layer V after lesions of layers I to IIIc is less than after lesions of the whole depth of the cortex, i.e., the intrinsic connections of layer V must arise mainly in layers IV, V and VI. The idea that the

intrinsic connections of layer V arise predominantly within that layer is supported by the appearance of degeneration after lesions of layers I to V, when fibre and terminal degeneration in layer V is greater in quantity and extent than after lesions of the more superficial layers. Degenerating fibres are seen to extend further in the inner band of Baillarger; even with these lesions, however, degeneration in this band is appreciable for only about 1 mm, becoming very sparse thereafter, though it appears that degeneration extends overall for about the same distance as that in the stria of Gennari. As has been mentioned, lesions involving layer V give rise to more degeneration in layers II and III than do supragranular lesions, indicating some degree of upward projection. They also give rise to more degeneration in layer VI and the white matter, i.e., as might be expected, it appears that the basal layers are mainly responsible for the efferent projection from area 17 (however, see Spatz et al., 1970). It is interesting to note that these efferent fibres pass out in a narrow, vertical column lying strictly below the line of this lesion. (A schematic summary of the progressive increase in quantity of degeneration when a lesion is present at progressively greater depths is given in Fig. 6:1).

When the lesion involves layer VI the number of degenerating fibres in layers V and VI increases, and a number of obliquely arranged fibres are seen; however, the extent of degeneration in layer V is not increased, and degeneration in the layers above V is unaffected. Even when white matter is involved the pattern of degeneration does not change greatly, the only differences being that the number of obliquely arranged fibres

in layers V and VI increases and terminal degeneration becomes more extensive in layer IV on one side of the lesion; this degeneration is presumably due to the interruption of the thalamocortical fibres in the white matter, as it was always found on the distal side of the lesion, as it were, from the point of view of these fibres.

The notable features of the intrinsic degeneration in area 17, as seen with the light microscope, are, therefore, four. First, a number of lesions, of different depths, give rise to degeneration which is asymmetrical, i.e., extends further on one side of the lesion than the other. This asymmetry is puzzling, especially as we could not, despite several attempts, discover any pattern which would link the findings of the various experiments with either the gross structural or the retinotopic features of the cortex. Nevertheless, these findings confirm the impression gained from the reports of earlier workers (e.g., Hubel and Wiesel, 1968, 1969, 1972) that the visual cortex is not homogeneous but contains specific patterns of connections at a multicellular as well as a cellular level.

The second finding of note relates to the question of the origin of fibres in the stria of Gennari. Although it was widely believed by the older neuroanatomists (Cajal, 1911) that the stria was made up of the terminal ramifications of incoming thalamocortical fibres, more recent work has tended to favour the idea that its fibres are of intrinsic origin, as chronic isolation

of the cortex by undercutting has no significant effect on the staining properties of the fibres of the stria (Clark and Sunderland, 1939). This view is supported by the present work, as the critical factor in producing degeneration in the stria appeared to be the entry of the lesion into layer IIIc, not its entry into the white matter. Moreover, degeneration in the stria appeared relatively suddenly as the lesion entered IIIc and did not increase significantly in quantity as the lesion passed through layers V and VI and the white matter. This, together with the appearance of a small lesion in layer IV, strongly implies that the fibres of the stria arise predominantly from cells of layers IIIc and IV.

The third notable finding is that this strial degeneration contrasted sharply with the lack of degeneration in layer IV, which, with lesions confined to the cortex, was invariably clear beyond the first 200 μm or so. Thus the cells of layer IV do not send intrinsic connections within their own layer for more than a very short distance, if at all.

Fourthly, it is surprising to find that, in this material, degenerating fibres in layer I did not extend for more than a millimetre or so, whereas in other studies (Jones and Powell, 1969a) such fibres have been found to extend for about 5 millimetres on either side of the lesion, irrespective of whether they cross a cytoarchitectonic boundary in doing so. It may be that this represents a genuine difference between area 17 of the monkey

and other cortical areas in other species, or it may be that these fibres in layer I are, for some reason, more difficult to stain in the monkey than in the cat or rabbit.

In the present work it has not been possible to gather much information about the vertical intrinsic connections present in area 17, except to say that some degree of reciprocal connection between layers II and III, on the one hand, and layer V, on the other, is present. It has been reported recently (Spatz et al. 1970) that lesions of the supragranular layers of the visual cortex of the squirrel monkey (Saimiri sciureus) produce massive degeneration in layer V. Our experiments show slight to moderate degeneration in layer V after superficial lesions, and in no case has its density approached that reported by Spatz et al. It is possible that this represents a genuine difference between the squirrel monkey and the macaque. Another possibility, however, arises from the fact that Spatz et al. used rather bigger lesions than ourselves: from 4 mm in diameter upwards. It could be argued that such lesions might appreciably compromise the blood supply to the deeper cortical layers, since the greater part of the flow to these layers comes from the pia; in this case, a good deal of nonspecific degeneration might be seen as the result of ischaemia, and it is felt that a small lesion would have a better chance of producing degeneration which reflected the true density of the projection from superficial to deeper layers. Recent work by Nauta, Butler and Jane (1973) tends to confirm this view. They reported that a band of fairly dense degeneration

appeared in layer V following a heat lesion applied to the skull or to the intact dura; this degeneration was co-extensive with the lesion and was denser than any seen in our experiments but not so dense as that reported by Spatz et al. , a finding which is consistent with the fact that the lesions of Nauta et al. were not so directly damaging to the cortex as those of Spatz et al., but were still likely to cause a certain amount of nonspecific vascular damage.

The observations with the electron microscope are in good agreement with the light microscope findings; in particular, the rather restricted extent of the degeneration and the variations in density and extent of degeneration between the different laminae are shown (Fig.4:40). Some clustering of degenerating terminals was apparent, though it was not very marked. It would be fair to say that the terminals which degenerate after an intrinsic lesion of the cortex form only a small proportion of the total number of terminals present. This may at first seem surprising, but it is the product of a number of quite readily intelligible factors. If it is assumed that a given cell in the cortex can receive horizontal connections from any cell within a radius of 3 mm of it in any direction, then it is clear that a given slit lesion will destroy a maximum of only 50% of the connections to that cell, since connections from cells on the side away from the lesion will be preserved. Secondly, a slit lesion will interrupt a progressively smaller proportion of the connections to a given point the further away it is from the point being studied, and as

these connections are relatively short, one would have to study material very close to the lesion in order to see a high proportion of degenerating terminals. Thirdly, a given cell will receive vertical and oblique connections, which will not be affected by a nearby slit lesion, as well as horizontal ones, which will. Fourthly, degenerating terminals could only be positively identified if certain morphological criteria were met, so that a number of degenerating terminals would be missed by this study.

It is interesting to note that the majority (approximately 90%) of all terminals ended with asymmetrical membrane thickenings (type I of Gray). This proportion was even higher in layers I and II (94.4% and 97.3% respectively). This finding might result from the fact that the peripheral parts of pyramidal cell dendrites, which are numerous in these layers, bear a larger proportion of dendritic spines than the more proximal parts (Valverde, 1967; Jones and Powell, 1969 d), and it is known that the overwhelming majority of synapses on spines are asymmetrical (Jones and Powell, 1970 e; see also Chapter 4). The majority (75%) of these asymmetrical intrinsic terminals end on dendritic spines, which tend to be small, with indistinct spine apparatus. It is noteworthy, however, that a significant proportion (some 24%) of asymmetrical intrinsic terminals end on dendrites, whereas only 14% of thalamocortical terminals will do so. Those dendrites which were contacted by asymmetrical terminals were stellate in certain cases, though many could not be identified. No examples were seen ending on identifiable pyramidal cell dendrites, though the

possibility must be admitted that they could contact the peripheral parts of such dendrites. A few examples were observed of asymmetrical terminals contacting the cell somata of large stellate cells, but no such terminals were seen on the somata of small stellate or pyramidal cells.

Intrinsic terminals ending with symmetrical membrane thickenings form only about 10% of the total. Their distribution with respect to the constituent parts of neurones is complementary to that of asymmetrical terminals, as 78% end on dendrites and only 13% on spines. Both pyramidal and nonpyramidal-type dendrites are contacted. On a few occasions a symmetrical intrinsic terminal has been seen ending opposite the origin of a dendritic spine (Fig.4:25), suggesting that some specific blocking of the excitatory effect of that spine might be occurring. Symmetrical terminals also contact the somata of cells, both pyramidal and large and small stellate; in addition, one example has been seen of a symmetrical intrinsic terminal contacting the basal dendrite of a Meynert cell in layer V (Fig.4:33). Degenerating non-myelinated axons have been found to make symmetrical contacts "en passage". One example has been seen of a degenerating symmetrical terminal ending on the initial axon segment of a small pyramidal cell in layer II; this finding tends to confirm earlier suggestions (Jones and Powell, 1969c) that synapses on initial segments are made by axons which are intrinsic in origin. The fact that no more than this one example were found may suggest that such terminals arise from

axons which run for a very short distance, so that most of their terminals would lie within the 200 μm-wide region of cortical damage alongside the lesion. This suggestion is supported by the fact that the one example found was only some 250 μm from the lesion.

One interesting feature of the degeneration seen with the electron microscope was that the asymmetrical terminals, though greatly in the majority, were numerous only within the first millimetre from the lesion, falling off in density thereafter, while the symmetrical terminals, though never so dense as the asymmetrical, were much more evenly spread throughout the 3 mm or so from the lesion which they occupied. The possible significance of this distribution can only be guessed at, though it has been pointed out by Bishop et al. 1971 b, (vide inf.) that the cells of area 17 appear to be under an appreciable degree of tonic inhibition, which may serve as a subtractive mechanism for increasing the specificity of excitatory inputs by removing background activity; it may also have a significance in relation to the cortical mechanisms postulated by Marr (1970; vide inf.).

In one experiment after a lesion of the peristriate cortex an unusually large number of axon terminals with symmetrical membrane thickenings were found to be degenerate; they were most numerous in layer III and were found to end predominantly upon large dendrites. In view of the possible importance of this observation, other brains in which lesions had been placed in

either the peristriate cortex or in the peripheral part of area 17 were re-examined. In addition, further experiments were done and lesions placed in the same site as in the experiment with numerous degenerating symmetrical terminals. In none of these experiments, however, were there more degenerating symmetrical terminals than was found in the other experiments with small lesions in area 17.

In the ultra-thin sections of the brain with an unusually large number of degenerating symmetrical terminals, dark cells and dark profiles were also present. A careful study of the 'thick' sections of the blocks of this material also showed small foci of necrosis in the superficial layers of the cortex. These two findings suggest that the explanation of the large number of degenerating symmetrical axon terminals in this experiment was due to the fact that the cortex had suffered slight ischaemia which resulted in damage to, or even death of, a large number of neurons over quite a large area. This interpretation is in accord with that of Sloper (1973) in relation to similar findings in the motor cortex in one experiment.

As has been pointed out in Chapter 1, it is hoped that the present study may shed some light on the problem of the anatomical pathways used for the processing of visual information within area 17. It is worth remembering, first of all, that area 17 of the monkey probably receives no extrinsic connections except those from the lateral geniculate nucleus: the major part

of area 17 certainly receives no commissural connections, while it is very likely (Kuypers, Szwarcbart, Mishkin and Rosvold, 1965) that it does not receive any association cortical connections either (see also Chapter 5, pp 86-87). Thus, information reaches area 17 from the lateral geniculate and terminates especially in layer IV and also in layer IIIb. The results of the present work would indicate that, from layer IV information is passed, via the stria, to cells of layers IIIb, IIIc and the superficial part of IV; in this way, the two receptive zones for thalamic information, layers IV and IIIb, would be connected together, and, perhaps more importantly, information could be passed on to units in layers IIIb and IIIc for further processing. This proposal carries with it the implication that the cells of layer IV themselves receive a major part of the thalamic input to area 17, a proposition which is by no means established, although it would seem reasonable on a priori grounds since the majority of thalamocortical terminals occur in this layer. The objection that most geniculocortical terminals end on dendritic spines, thought to be a feature especially of pyramidal cells, is not very important, as has been pointed out by Garey and Powell (1971), since the stellate cells which largely make up layer IV do, in fact, bear spines (Cajal, 1911; Valverde, 1968; Garey and Powell, 1971; Lund, 1973), often (Lund, 1973) in quite considerable numbers. With regard to the course of the axons of layer IV stellate cells, it is interesting to note that Cajal (1911) describes these cells as giving off "an axon which ... is very

variable in direction but which seems, despite that, to most often adopt an ascending course." Lund (1973) described the axons of these cells as projecting to layers IIIb and IIIc. Thus it does seem as if there is evidence for a pathway: lateral geniculate-stellate cells of IV - cells of layers IIIb and IIIc, although this is almost certainly not the only pathway within the cortex. Fig. 6:2 shows a diagrammatic representation of such a possible scheme of connections.

It now becomes necessary to enquire as to what function such a pathway might be serving. As has been mentioned in Chapter 1, layer IV contains a high proportion of simple cells (Hubel and Wiesel, 1968) which are almost exclusively monocularly driven, while layers III and V contain high proportions of complex cells, which are mostly binocularly driven. Also, layer IV contains a mosaic of patches receiving alternately from the two eyes, these patches, which may be part of ocular dominance columns, being some 300 μm wide (Hubel and Wiesel, 1969, 1972). Hubel and Wiesel therefore suggested that simple, monocular cells of layer IV project to complex, binocular cells in layers III and V; this would involve some degree of oblique lateral projection from cells in one eye-preference column to the adjoining columns. However, as eye-preference columns are only 300 μm or so wide, such a pathway would not require projections of 3 - 3.5 mm extent from any given point such as have been observed in our material, but it is quite possible that such longer connections could subserve some other integrative function such as linking up distant columns with the same preference for stimulus orientation, for stimulus type (slit, edge or dark bar) or for degree of

symmetry of response to stimulus movement (see Hubel and Wiesel, 1968, 1972).

An alternative view of the projection into and through layer IV has been put forward recently by Bishop et al. (1971b). These authors took note of the fact that 53% of the units recorded in layer IV by Hubel and Wiesel (1968) have no preference for a particular stimulus orientation (non-oriented units). Hubel and Wiesel believed that these units were incoming geniculate fibres, but Joshua and Bishop (1970) report that 71% of such units in the cat can be driven by both eyes, suggesting that they are cortical cells. Bishop et al.(1971b) also point out that a number of the cortical units excited monosynaptically by afferent stimulation can be discharged antidromically by stimulation of the optic radiation (Toyama and Matsunami, 1968), i.e. are projection cells. They therefore proposed that simple cells are pyramidal cells (they could also be large stellate cells in layers IIIc and IV) and that the granule cells of layer IV are non-oriented, inhibitory interneurones which establish the inhibitory side-bands of simple cells and provide for their asymmetrical responses to movement. If such a pattern of connections is the true one, then the horizontal projection of several millimetres length, which we have suggested arises in layer IV, becomes a little more difficult to place in a functional scheme, especially since our findings suggest that the great majority of horizontal connections are excitatory rather than inhibitory. However, our material does not allow us to discriminate effectively

between a projection arising in layer IV and one arising in layer IIIc, so that, if Bishop's model is correct, the projection in the stria may be arising from simple cells in layer IIIc (such cells undoubtedly exist in appreciable numbers in IIIc; indeed, only about a third of the simple cells in area 17 of the monkey are confined within granular layer IV itself, the remainder lying above it in the deep part of layer III - see Hubel and Wiesel, 1968). In this case, the projection in the stria might still be subserving the functions suggested above (p. 102). What does seem quite clear is that an important part of the pathway into and through area 17 consists of a thalamocortical projection to first-order cells of layer IV, which project, in turn, on to second-order cells in layer III. It is interesting to note that Whitsel, Roppolo and Werner have recently (1972) proposed a similar type of connection for area 1 of the somatic sensory cortex of the monkey. They found that a number of cells in this area respond asymmetrically to movement of tactile stimuli on the skin, i.e. movement in one direction evokes a greater response than movement in the opposite direction. Such directionally selective cells form about 50% of the population of layer III, but are much less numerous in layer IV; Whitsel *et al.* feel that this situation is consistent with a projection from non-selective cells of layer IV, which receives the bulk of the thalamocortical input, to directionally selective cells in layer III. Such a projection would also be consistent with the finding that the cells of layer IV of area 1 have a high level

of spontaneous activity, which might result from rather unselected input, while those of layer III have a low frequency of spontaneous discharge, as would be expected if the connections to them were fairly specific, or if they were under a high level of tonic inhibition; such a possibility has been suggested for simple cells of the visual cortex by Bishop et al. (1971b) to account for the fact that they have a low rate of spontaneous firing despite a resting EPSP frequency of the order of 150-300 per second.

Commissural connections at the area 17/18 boundary in the cat and monkey

With the light microscope it can be seen that commissural degeneration at the 17/18 boundary occupies a very limited zone only 2 mm or so in width; this finding is in agreement with earlier workers (see Chapter 1). It is very interesting to note that, in the monkey, all layers in area 18 are involved by degeneration, but that, as one moves towards area 17, the degeneration in layer IV ceases abruptly so that the degeneration at the transition zone is bounded by a narrow parabola. An identical finding has been reported in the Virginia opossum (Benevento and Ebner, 1971). A rather similar situation obtains in the cat, in that the dense band of degeneration in the deep part of layer III and layer IV which is present in area 18 ceases abruptly at the 17/18 boundary. Thus the

laminar distribution of commissural degeneration in area 18 agrees with that described by Jones and Powell (1970 e) in the somatic sensory cortex in the cat. Two points may usefully be made: first, it is hardly surprising that difficulties should arise in the description of the laminar pattern of commissural degeneration, especially as it is seen with the electron microscope, in view of the fact that the zone of degeneration is so narrow and the pattern of degeneration changes abruptly in the middle. The present study was complicated by this very problem, which was only resolved by more detailed study of the light microscopic material. It may be the case, also, that this difficulty underlies the apparent discrepancy between the findings of Jones and Powell (1970 e) and ourselves on the one hand and those of Lund and Lund (1970) on the other. The finding of the latter workers that layer IV was free of degenerating commissural fibres in the rat brain may have been due to studying the part of the zone containing degeneration which was close to area 17, whereas the distribution of the commissural fibre degeneration in the somatic sensory cortex is similar to that in area 18, where all layers contain degeneration. Secondly, it is known that the architectonic structure of area 17 differs between the cat and the monkey (see, for example, Garey, 1971), especially in relation to layer IV, so that in terms of cortical connections the difference described here between the two species may be more apparent than real. One striking feature of this distribution of degeneration

is that, in area 17, the primary thalamic receiving area (at least in the monkey), that layer which receives the major part of the thalamic input is devoid of commissural connections, while all other layers receive some; a similar situation obtains, it will be recalled, with regard to intrinsic connections. Thus it seems as if layer IV of area 17, especially in the monkey, is really a pure processing mechanism for primary afferent information, with negligible modification from regions or laminae dealing with other, possibly more complex, information.

It has already been mentioned that the zone at the 17/18 boundary where commissural degeneration is found corresponds closely with the architectonic boundary itself as marked by the presence of half a dozen or so conspicuous, large pyramidal cells situated at the junction of layers III and IV. The significance of such groups of cells is not yet clear but they may be important as cells of origin of commissural fibres (D. Whitteridge, personal communication). The appearances of degeneration seen with the electron microscope are not greatly different from what one would expect: all degenerating terminals ended with asymmetrical membrane thickenings. It is, perhaps, of interest that the proportion of terminals contacting dendritic spines (about 75%) is lower than those described by other workers in the sensorimotor cortex (Jones and Powell, 1970 e ; Sloper, 1973 b). One cannot be sure that this is a real difference between the visual and the sensorimotor areas; however, it may be the case,

especially since a number of the axodendritic synapses seen in our material ended on what appeared to be stellate cell dendrites, that this finding is related to the somewhat higher number of stellate cells in the visual area as compared with other areas.

Connections from area 18 to area 17 in the cat

In view of the demonstration that the visual areas I, II and III as mapped electrophysiologically are coextensive with the architectonic areas 17, 18 and 19 (Hubel and Wiesel, 1965), it would be very valuable to have some information on the interconnections between these areas. At present, however, little such information exists. With the Nauta method, Wilson (1967) described sparse degeneration in all parts of area 17 following lesions in the homotopic parts of area 18; however, this author also described sparse but definite degeneration in all parts of area 17 after a lesion of the opposite side and concluded that the whole of area 17 is commissurally connected, a view which is not generally accepted. Garey, Jones and Powell (1968) describe a projection into the lateral part of area 17 from the medial part of 18, but emphasise that their material is insufficient for a complete study of the problem. The present experiments also suggest that the lateral part of 17 receives from 18 and suggest further that the greater part of 17, except for the lateral part, does not. From our observations it seems that all of the cortical layers below the middle of layer III may receive some of this association projection, with an additional

connection through fibres in layer I which, indeed, extends further into 17 than the projection to the deeper layers.

With the electron microscope, degenerating terminals were again seen to be of the asymmetrical (presumed excitatory) type. The majority (62%) of these ended on dendritic spines, some of which (Fig.5:15) apparently belong to pyramidal cells, while the remainder end on dendrites, some of which belong to stellate cells (Fig.5:16). Thus the distribution of association connections on the various cellular processes resembles that of commissural connections.

It seems, therefore, that the connections from area 18 to area 17 are essentially restricted to the lateral part of 17 but that, in other respects, their pattern of distribution does not display any very unusual features. However, the distribution is interesting in that again, as with the commissural connections, it seems to leave most of area 17 "pure" for the analysis of thalamocortical information. Clearly, further work will be necessary both to establish firmly the present position and to answer further questions, such as whether there is any particular laminar pattern of association connections, and, if so, what its significance might be.

Conclusions

It is easily seen that the unravelling of the neocortex to the level which has been achieved in work on the cerebellar cortex (as summarised in Eccles, Ito and Szentágothai, 1967) will

be a long process, especially since the neocortex is not uniform in structure over its extent and because the laminae of the neocortex, in contrast to those of the cerebellar cortex, are, to a varying degree, heterogeneous in their cellular composition. Nevertheless, powerful techniques exist which should allow a good deal more information to be obtained: these techniques, both physiological (single unit extracellular and intracellular recording) and anatomical (light microscopy, including the Golgi technique, electron microscopy, autoradiography, etc.) have advanced our knowledge of the cortex greatly in the last decade or so. First, a great deal is now known about the origin and termination of afferent and efferent pathways within the cortex, not only connections from the thalamus (recent contributions in this field being those of Wilson and Cragg, 1967; Jones and Powell, 1970 e ; Garey and Powell, 1971) but also from other cortical areas (e.g. Choudhury, Whitteridge and Wilson, 1965; Kuypers et al. 1965; Diamond, Jones and Powell, 1968; Jones and Powell, 1968, 1969a,b ; Cragg and Ainsworth, 1969; Zeki, 1969, 1970; Sloper, 1973 b). In addition, the functional significance of certain of these cortico-cortical connections is beginning to be understood, notably in regard to commissural connections (e.g., Choudhury et al. 1965; Hubel and Wiesel, 1967). Secondly, the concept of functional specialisation of architectonic areas has been advanced to include the demonstration that the response properties of cells change systematically as

one moves from one cortical area to another (Powell and Mountcastle, 1959; Hubel and Wiesel, 1965). Thirdly, the concept of columnar organisation according to functional properties has become well established, first in the somatic sensory cortex (Mountcastle, 1957) and then in the visual cortex (Hubel and Wiesel, 1962, 1963, 1965, 1968). Fourthly, as a result of the work of Powell and Mountcastle, of Hubel and Wiesel and of others (Barlow, Blakemore and Pettigrew, 1965; Pettigrew, Nikara and Bishop, 1968; Bishop et al. 1971a, b; Whitsel et al. 1972) a picture is beginning to emerge of which stimulus parameters are "important" for cortical cells and of how they are transformed at successive levels of processing. The implications of this last development are considerable, as it is now becoming possible to conceive of perception not as a sudden event occurring when a group of lowly relay cells present their information to some kind of "master cells" but as a process of continuous evolution of images (or, if it be preferred, adequate stimuli) through (in the case of visual perception) slits, moving slits of particular size, corners, etc. to progressively more sophisticated moving shapes and contours leading up to the familar patterns and objects of the visual world. At present, it must be admitted, our knowledge of this process is very basic, being only at the level of the rather specialised stimuli of the neurophysiological laboratory. Nevertheless, there are some grounds for confidence that our knowledge of such mechanisms will continue to advance. For example, Gross, Rocha-Miranda and Bender (1972) have described

a few units in the inferotemporal cortex of the monkey whose responses to stimuli increased progressively as the shape of the stimulus approximated more and more the shape of the upright shadow of a monkey's hand, i.e. they appeared to be "hand detectors".

One further piece of work which is of interest is that of Campbell, Cooper and Enroth-Cugell (1969). They reported that cells of the visual cortex in the cat are especially sensitive to stimuli of certain spatial frequencies (i.e. gratings where light and dark bands alternate with a particular frequency of bands per degree of visual angle). Whether these sensitivities to particular spatial frequencies reflect anything more than the arrangement of the moving bands relative to the excitatory and inhibitory zones of the receptive fields is not clear, nor is there any anatomical model of organisation available which might subserve this frequency selection. Nevertheless, since patterned visual stimuli consist, in a sense, of light and dark areas arranged in various positions with various spatial frequencies, and since the relative spatial frequency pattern of an object will remain unaltered irrespective of how far away it is from the observer, it seems that the concept of spatial frequency as a psychophysical parameter may be valuable in the consideration of future findings.

As regards the question of what arrangements of cortical connections subserve the processing of sensory stimuli

in the ways described above, it must be admitted that little can be said at present. Schemes have been elaborated by Hubel and Wiesel (1962, 1965, 1968) and also by Bishop et al. (1971b) on the basis of their respective physiological observations. In addition, it may be of interest to mention another scheme which was put forward on the basis of information theory considerations by Marr (1970). This author, believing that there may be certain analogies between cerebellar and cerebral cortical mechanisms and noticing the fact that patterned stimuli, selected for properties of length, movement, orientation etc. are important for driving cortical cells, proposes that afferent information coming to the cortex is handled in small packets (these must be small for information theoretical reasons) by "codon cells" analogous to granule cells in the cerebellum, which select from the rather random mass of incoming data those basic features of the stimuli which occur frequently (position, length, continuity, orientation and arrangements of light and dark areas, for example) and feed them on to cells which are able to recognise a stimulus as belonging to any one of a number of different classes of patterned stimuli, the nature of the class being determined by the "experience" of the second-order cell of the various patterned stimuli offered to it by the codon cells and being "learned" by the second-order cell by means of modifiable codon-cell synapses. So far as the selection of information is concerned, this model has obvious analogies with that of Hubel and Wiesel, the codon cells corresponding to

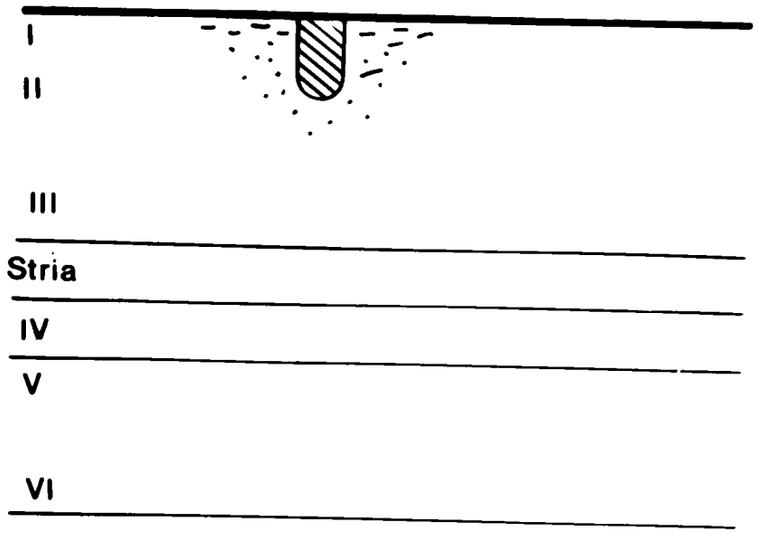
simple cells in layer IV and the second-order, etc. cells being complex and hypercomplex cells in layer III and possibly layer V. If, therefore, it turns out that the projection to cells of layer IV and then to layer III is of primary importance in the handing-on of information rather than being a parallel pathway concerned with inhibitory shaping and control of the primary message, as suggested by Bishop et al. (1971b), then it could be that the models of Hubel and Wiesel and of Marr reflect two different views of the same underlying plan. With regard to the rather diffuse, low-level inhibiti[illegible]hose presence we have inferred from electron microscopic studies (p. 73), it is interesting to note that Marr has postulated such a pattern of inhibition acting on codon cells (and possibly on other sorts of interneurones) so as to keep constant the number of codon cells, or of other sorts of relay cells, active at any given moment - this constancy is necessary in order that the cell on to which a group of relay cells project shall be able to recognise its particular "learned event" even when provided with incomplete information.

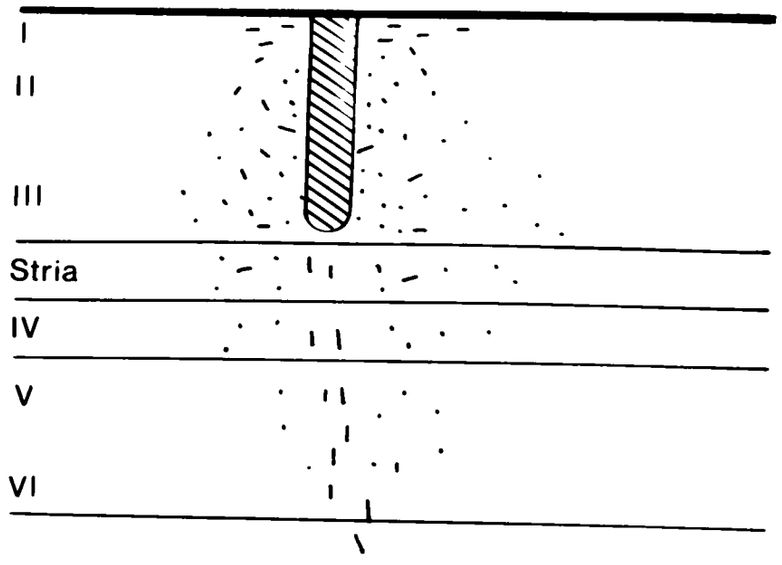
The known anatomical basis for the integration discussed in this chapter is very limited at present. However, it does seem that layer IV of area 17 has a special role to play in the early handling of afferent information; this is suggested by the experiments of Hubel and Wiesel (1968) and of Whitsel et al. (1972), and also by the findings that most of area 17, and all of

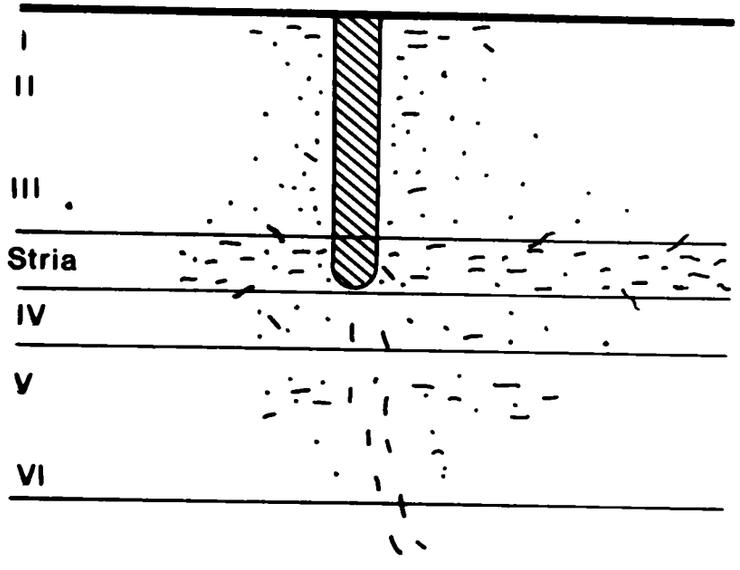
layer IV, are devoid of commissural (see Chapter 5) and possibly of association connections. What is more, a good deal of work has been reported recently concerning the appearance of cells in the visual cortex in Golgi material (Valverde, 1971; Garey, 1971; Szentágothai, 1973; Lund, 1973); such work will, it may be hoped, provide information about the neuronal substrate for any future schemes of cortical connections. Finally, it is hoped that the present work has shed a little light on the subject, as we have been able to show that the stria of Gennari is, as had been suspected, the most important pathway for tangential intrinsic connections within the cortex and that the great majority of its fibres arise from cells in the region of layers IIIc and IV. Future work, one may reasonably hope, will provide further information about the details of intrinsic connections, including vertical (intracolumnar) connections, and including further data about the parts of neurones and the types of neurones contacted by the axons of the various cell types, so that it may be possible, in the not-too-distant future, to speak of "The Cerebrum as a Neuronal Machine".

Fig. 6:1

Schematic representation of the changes in the amount and pattern of degeneration seen with the light microscope with a lesion (shaded) at progressively greater depths in area 17 of the monkey. Terminal degeneration is represented by dots and fibre degeneration by dashes. An account of these progressive changes is given on page 89 *et seq*.


I
II
III
Stria
IV
V
VI


I
II
III
Stria
IV
V
VI


I
II
III
Stria
IV
V
VI

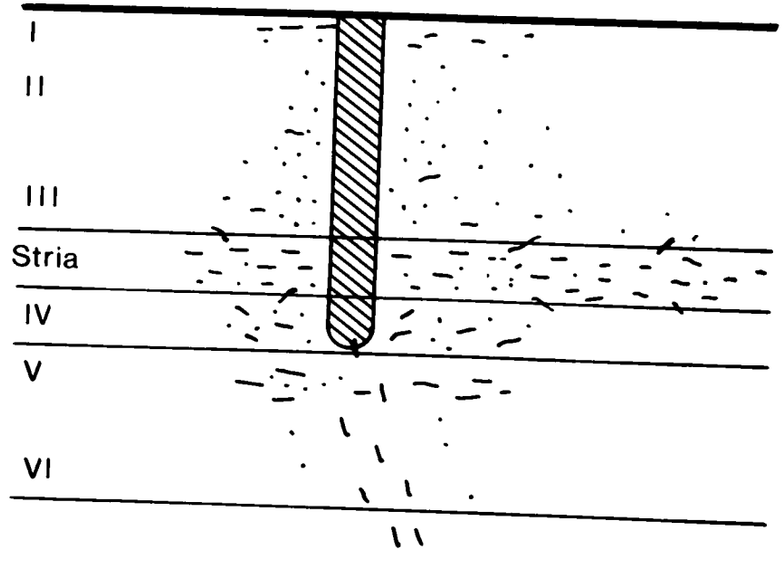
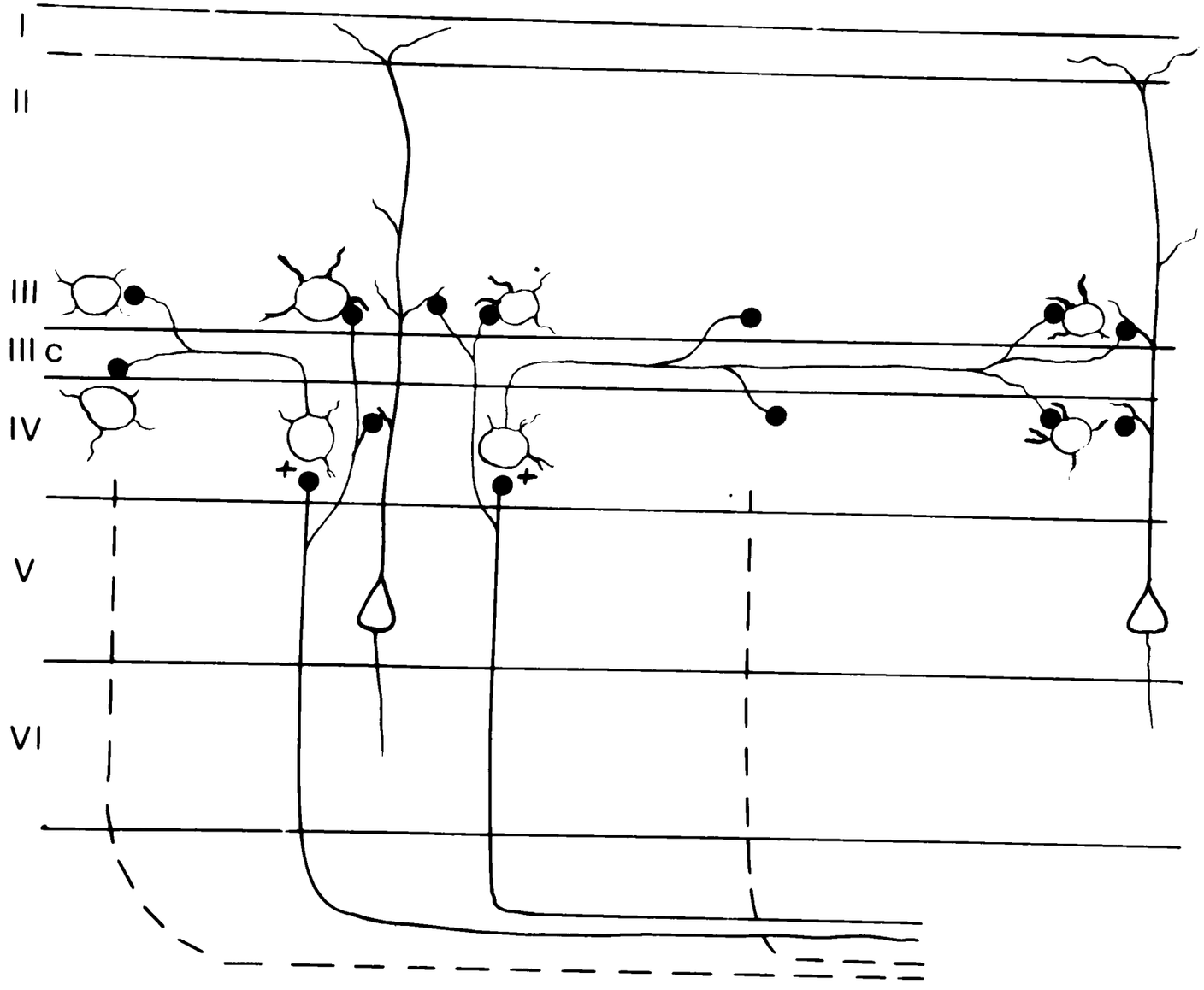

I
II
III
Stria
IV
V
VI

Fig. 6:2

Diagrammatic representation of a possible scheme of intrinsic connections in area 17 of the monkey. Thalamocortical fibres are seen terminating in layer IV and IIIb, by means of excitatory terminals (black knobs with plus signs) on stellate cells (white circles) and on dendritic spines belonging to the apical dendrites of pyramidal cells. Stellate cells are seen sending their axons horizontally in the stria of Gennari in layer IIIc, to end in layers IIIb, IIIc and IV. Other possible inputs to area 17 are indicated by dotted lines.

MONKEY AREA 17



REFERENCES

ADRIAN, E. D. (1941) Afferent discharges to the cerebral cortex from peripheral sense organs. J. Physiol. 100: 159-191.

BARLOW, H. B., BLAKEMORE, C. and PETTIGREW, J. D. (1967) The neural mechanism of binocular depth discrimination. J. Physiol. 193: 327-342.

BENEVENTO, L. A. and EBNER, F. F. (1971) The areas and layers of cortico-cortical terminations in the visual cortex of the Virginia opossum. J. comp. Neurol. 141: 157-190.

BILGE, M., BINGLE, A., SENEVIRATNE, K. N. and WHITTERIDGE, D. (1967) A map of the visual cortex in the cat. J. Physiol. 191: 116P.

BISHOP, P. O., COOMBS, J. S. and HENRY, G. H. (1971 a) Responses to visual contours: spatio-temporal aspects of excitation in the receptive fields of simple striate neurones. J. Physiol. 219: 625-657.

BISHOP, P. O., COOMBS, J. S. and HENRY, G. H. (1971 b) Interaction effects of visual contours on the discharge frequency of simple striate neurones. J. Physiol. 219: 659-687.

BONIN, G. von (1942) The striate area of primates. J. comp. Neurol 77: 405-429.

BONIN, G. von and BAILEY, P. (1947) The neocortex of Macaca mulatta. University of Illinois Press, Urbana, Ill.

BRODMANN, K. (1903) Beiträge zur histologischen Lokalisation der Grosshirnrinde. 1 Mitteilung: die Regio rolandica. J. Psychol. Neurol. (Leipzig) 2: 79-107.

BRODMANN, K. (1909) Vergleichende Lokalisationslehre der Grosshirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenhaues. Leipzig: J. A. Barth.

CAJAL, S. Ramon y (1911) Histologie du système nerveux de l'homme et des vertébrés, Tome II. Paris: Maloine.

CAMPBELL, A. W. (1903) Histological studies on cerebral localisation. Proc. Roy. Soc. 72: 488-492.

CAMPBELL, A. W. (1905) Histological studies on the localisation of cerebral function. Cambridge University Press, Cambridge.

CAMPBELL, F. W., COOPER, G. F. and ENROTH-CUGELL, C. (1969) The spatial selectivity of the visual cells of the cat. J. Physiol 203: 223-235.

CHOUDHURY, B.P., WHITTERIDGE, D. and WILSON, M. E. (1965) The function of the callosal connections of the visual cortex. Quart. J. exp. Physiol. 50: 214-219.

CLARK, W. E. Le Gros and BOGGON, R. H. (1935) The thalamic connections of the parietal and frontal lobes of the brain in the monkey. Phil. Trans. Roy. Soc. B. 224: 313-359.

CLARK, W. E. Le Gros and POWELL, T. P. S. (1953) On the thalamo-cortical connections of the general sensory cortex of Macaca. Proc. Roy. Soc. B. 141: 467-487.

CLARK, W. E. Le Gros and SUNDERLAND, S. (1939) Structural changes in the isolated visual cortex. J. Anat. 73: 563-574.

COLONNIER, M. (1966) The structural design of the neocortex. In: "Brain and Conscious Experience", ed. J. C. Eccles. pp 1-23. New York : Springer.

COLONNIER, M. (1968) Synaptic patterns on different cell types in the different laminae of the cat visual cortex. An electron microscope study. Brain Res. 9: 268-287.

COWAN, W. M., GOTTLIEB, D. I., HENDRICKSON, A. E., PRICE, J. L. and WOOLSEY, T. A. (1972) The autoradiographic demonstration of axonal connections in the central nervous system. Brain Res. 37: 21-51.

COWEY, A. (1964) Projection of the retina on to striate and ~~peristriate~~ prestriate cortex in the squirrel monkey, Saimiri sciureus. J. Neurophysiol. 27: 366-393.

COWEY, A. and ELLIS, C. M. (1969) The cortical representation of the retina in squirrel and rhesus monkeys and its relation to visual acuity. Exp. Neurol. 24: 374-385.

CRAGG, B. G. and AINSWORTH, A. (1969) The topography of the afferent projections in the circumstriate visual cortex of the monkey, studied by the Nauta method. Vision Res. 9: 733-747.

CURTIS, H. J. (1940) Intercortical connections of corpus callosum as indicated by evoked potentials. J. Neurophysiol. 3: 407-413.

DANIEL, P. M. and WHITTERIDGE, D. (1961) The representation of the visual field on the cerebral cortex in monkeys. J. Physiol. 159: 203-221.

DIAMOND, I. T., JONES, E. G. and POWELL, T. P. S. (1968) The association connections of the auditory cortex of the cat. Brain Res. 11: 560-579.

EBNER, F. F. and MYERS, R. E. (1965) Distribution of corpus callosum and anterior commissure in cat and racoon. J. comp. Neurol. 124: 353-366.

ECCLES, J. C., ITO, M. and SZENTÁGOTHAI, J. (1967) "The Cerebellum as a Neuronal Machine." Berlin : Springer-Verlag.

FINK, R. P. and HEIMER, L. (1967) Two methods for selective silver impregnation of degenerating axons and their synaptic endings in the central nervous system. Brain Res. 4: 369-374.

FRITSCH, G. and HITZIG, E. (1870) Über die elektrioche Erregbarkeit des Grosshirns. Arch. F. Anat. Physiol. u. wissench. Med. 37: 300.

GAREY, L. J. (1971) A light and electron microscopic study of the visual cortex of the cat and monkey. Proc. Roy. Soc. B. 179: 21-40.

GAREY, L. J. and POWELL, T. P. S. (1968) The projection of the retina in the cat. J. Anat. 102: 189-222.

GAREY, L. J. and POWELL, T. P. S. (1971) An experimental study of the termination of the lateral geniculo-cortical pathway in the cat and monkey. Proc. Roy. Soc. B. 179: 41-63.

GAREY, L. J., JONES, E. G. and POWELL, T. P. S. (1968) Inter-relationships of striate and extrastriate cortex with the primary relay sites of the visual pathway. J. Neurol. Neurosurg. Psychiat. 31: 135-157.

GAROL, H. W. (1942) Cortical origin and distribution of corpus callosum and anterior commissure in the cat. III. J. Neuropath. Exp. Neurol. 1: 422-429.

GLOBUS, A. and SCHEIBEL, A. B. (1967) Synaptic loci on visual cortical neurons of the rabbit: the specific afferent radiation. Exp. Neurol. 18: 116-131.

GRAY, E. G. (1959) Axo-somatic and axo-dendritic synapses of the cerebral cortex: an electron microscope study. J. Anat. 93: 420-433.

GROSS, C. G., ROCHA-MIRANDA, C. E. and BENDER, D. B. (1972) Visual properties of neurons in inferotemporal cortex of the macaque. J. Neurophysiol. 35: 96-111.

GUILLERY, R. W. (1967) Patterns of fiber degeneration in the dorsal lateral geniculate nucleus of the cat following lesions in the visual cortex. J. comp. Neurol. 130: 197-222.

HASSLER, R. and WAGNER, A. (1965) Experimentelle und morphologische Befunde über die vierfache corticale Projektion des visuellen Systems. 8th Int. Cong. Neurol. 3: 77-96.

HEATH, C. J. and JONES, E. G. (1970) Connexions of area 19 and the lateral suprasylvian area of the visual cortex of the cat. Brain Res. 19: 302-305.

HEATH, C. J. and JONES, E. G. (1971) The anatomical organisation of the suprasylvian gyrus of the cat. Ergbn. Anat. EntwGesch. 45(3) : 1-64.

HUBEL, D. H. and WIESEL, T. N. (1959) Receptive fields of single neurones in the cat's striate cortex. J. Physiol. 148: 574-591.

HUBEL, D. H. and WIESEL, T. N. (1962) Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. J. Physiol. 160: 106-154.

HUBEL, D. H. and WIESEL, T. N. (1963) Shape and arrangement of columns in cat's striate cortex. J. Physiol. 165: 559-568.

HUBEL, D. H. and WIESEL, T. N. (1965) Receptive fields and functional architecture in two nonstriate visual areas (18 and 19) of the cat. J. Neurophysiol. 28: 229-289.

HUBEL, D. H. and WIESEL, T. N. (1967) Cortical and callosal connections concerned with the vertical meridian of visual fields in the cat. J. Neurophysiol. 30: 1561-1573.

HUBEL, D. H. and WIESEL, T. N. (1968) Receptive fields and functional architecture of monkey striate cortex. J. Physiol. 195: 215-243.

HUBEL, D. H. and WIESEL, T. N. (1969) Anatomical demonstration of columns in the monkey striate cortex. Nature 221: 747-750.

HUBEL, D. H. and WIESEL, T. N. (1972) Laminar and columnar distribution of geniculo-cortical fibres in the macaque monkey. J. comp. Neurol. 146: 421-450.

JONES, E. G. and POWELL, T. P. S. (1968) The commissural connexions of the somatic sensory cortex in the cat. J. Anat. 103: 433-455.

JONES, E. G. and POWELL, T. P. S. (1969 a) Connexions of the somatic sensory cortex of the rhesus monkey. I. Ipsilateral cortical connexions. Brain 92: 477-502.

JONES, E. G. and POWELL, T. P. S. (1969 b) Connexions of the somatic sensory cortex of the rhesus monkey. II. Contralateral cortical connexions. Brain 92: 717-730.

JONES, E. G. and POWELL, T. P. S. (1969 c) Synapses on the axon hillocks and initial segments of pyramidal cell axons in the cerebral cortex. J. Cell Sci. 5: 495-507.

JONES, E. G. and POWELL, T. P. S. (1969 d) Morphological variations in the dendritic spines of the neocortex. J. Cell Sci. 5: 509-529.

JONES, E. G. and POWELL, T. P. S. (1970 a) Electron microscopy of the somatic sensory cortex of the cat: I. Cell types and synaptic organisation. Phil. Trans. Roy. Soc. B. 257: 1-11

JONES, E. G. and POWELL, T. P. S. (1970 b) Electron microscopy of the somatic sensory cortex of the cat: II. The fine structure of layers I and II. Phil. Trans. Roy. Soc. B. 257: 13-21.

JONES, E. G. and POWELL, T. P. S. (1970 c) Electron microscopy of the somatic sensory cortex of the cat: III. The fine structure of layers III to VI. Phil. Trans. Roy. Soc. B. 257: 23-28.

JONES, E. G. and POWELL, T. P. S. (1970 d) An electron microscopic study of terminal degeneration in the neocortex of the cat. Phil. Trans. Roy. Soc. B. 257: 29-43.

JONES, E. G. and POWELL, T. P. S. (1970 e) An electron microscopic study of the laminar pattern and mode of termination of afferent fibre pathways in the somatic sensory cortex of the cat. Phil. Trans. Roy. Soc. B. 257: 45-62.

JONES, E. G. and POWELL, T. P. S. (1970 f) An anatomical study of converging sensory pathways within the cerebral cortex of the monkey. Brain 93: 793-820.

JOSHUA, D. E. and BISHOP, P. O. (1970) Binocular single vision and depth discrimination. Receptive field disparities for central and peripheral vision and binocular interaction on peripheral single units in cat striate cortex. Exp. Brain Res. 10: 389-416.

KARNOVSKY, M. J. (1965) A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron micrsocopy. J. Cell Biol. 27: 137A.

KUYPERS, H. G. J. M., SZWARCBART, M. K., MISHKIN, M. and ROSVOLD, H. E. (1965) Occipitotemporal cortico-cortical connexions in the rhesus monkey. Exp. Neurol. 11: 245-262.

LUND, J. S. (1973) Organisation of neurons in the visual cortex, area 17, of the monkey (Macaca mulatta). J. comp. Neurol. 147: 455-496.

LUND, J. S. and LUND, R. D. (1970) The termination of callosal fibres in the paravisual cortex of the rat. Brain Res. 17: 25-45.

MARR, D. (1970) A theory for cerebral neocortex. Proc. Roy. Soc. B. 176: 161-234.

MARSHALL, W. H., WOOLSEY, C. N. and BARD, P. (1941) Observations on cortical somatic sensory mechanisms of cat and monkey. J. Neurophysiol. 4: 1-24.

METTLER, F. A. (1935) Corticofugal fibre connections of the cortex of Macaca mulatta. The occipital region. J. comp. Neurol. 61: 221-256.

MOUNTCASTLE, V. B. (1957) Modality and topographic properties of single neurons of cat's somatic sensory cortex. J. Neurophysiol. 20: 408-434.

MYERS, R. E. (1962) Commissural connections between occipital lobes of the monkey. J. comp. Neurol. 118: 1-16.

NAUTA, H. J. W., BUTLER, A. B. and JANE, J. A. (1973) Some observations on axonal degeneration resulting from superficial lesions of the cerebral cortex. J. comp. Neurol. 150: 349-360.

NAUTA, W. J. H. and GYGAX, P. A. (1954) Silver impregnation of degenerating axons in the central nervous system: a modified technique. Stain Technol. 29: 91-93.

OTSUKA, R. and HASSLER, R. (1962) Über Aufbau und Gliederung der corticalen Sehsphäre bei der Katze. Arch. Psychiat. Nervenkr. 203: 212-234.

PALAY, S. L., SOTELO, C., PETERS, A. and ORKAND, P. M. (1968) The axon hillock and the initial segment. J. Cell Biol. 38: 193-20

PETERS, A., PALAY, S. L. and WEBSTER, H. de F. (1970) "The Fine Structure of the Nervous System". New York: Harper & Row.

PETERS, A., PROSKAUER, C. C. and KAISERMAN-ABRAMOF, I. R. (1968) The small pyramidal neuron of the rat cerebral cortex. The axon hillock and initial segment. J. Cell Biol. 39: 604-619.

PETTIGREW, J. D., NIKARA, T. and BISHOP, P. O. (1968) Responses to moving slits by single units in cat striate cortex. Exp. Brain Res. 6: 373-390.

POLLEY, E. H. and DIRKES, J. M. (1963) The visual cortical (genicul cortical) area of cat brain and its projections. Anat. Rec. 145: 345.

POLYAK, S. (1927) An experimental study of the association, callosal and projection fibres of the cerebral cortex of the cat. J. comp. Neurol. 44: 197-258.

POLYAK, S. (1932) The Main Afferent Fiber Systems of the Cerebral Cortex in Primates. University of California Press, Berkeley, Cal.

POWELL, T. P. S. and MOUNTCASTLE, V. B. (1959) Some aspects of the functional organisation of the cortex of the postcentral gyrus of the monkey: a correlation of findings obtained in a single unit analysis with cytoarchitecture. Bull. Johns Hopkins Hosp. 105: 133-162.

REYNOLDS, E. S. (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17: 208-212.

RICHARDSON, K. C., JARETT, L. and FINKE, E. H. (1960) Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Tech. 35: 313-323.

ROSE, J. E. (1949) The cellular structure of the auditory region of the cat. J. comp. Neurol. 91: 409-440.

ROSE, J. E. and WOOLSEY, C. N. (1949) The relations of thalamic connections, cellular structure and evocable electrical activity in the auditory region of the cat. J. comp. Neurol. 91: 441-466.

ROSSIGNOL, S. and COLONNIER, M. (1971) A light microscope study of degeneration patterns in cat cortex after lesions of the

lateral geniculate nucleus. Vision Res., suppl. No.3: 329-338.

SLOPER, J. J. (1973 a) An electron microscopic study of the neurons of the primate motor and somatic sensory cortices. J. Neurocytol. 2: 351-359.

SLOPER, J. J. (1973 b) An electron microscope study of the termination of afferent connections to the primate motor cortex. J. Neurocytol. 2: 361-368.

SPATZ, W. B., TIGGES, J. and TIGGES, M. (1970) Subcortical projections, cortical associations and some intrinsic interlaminar connections of the striate cortex in the squirrel monkey (Saimiri). J. comp. Neurol. 140: 155-174.

SZENTÁGOTHAI, J. (1973) Synaptology of the visual cortex. In "Handbook of Sensory Physiology" Vol. VII/3, part B., Ed. R. Jung, pp 269-324. Berlin : Springer-Verlag.

TALBOT, S. A. and MARSHALL, W. H. (1941) Physiological studies on neural mechanisms of visual localisation and discrimination. Amer. J. Ophthalmol. 24: 1255-1264.

TOYAMA, K. and MATSUNAMI, K. (1968) Synaptic action of specific visual impulses upon cat's parastriate cortex. Brain Res. 10: 473-476.

UCHIZONO, K. (1965) Characteristics of excitatory and inhibitory synapses in the central nervous system of the cat. Nature, 207: 642-643.

UCHIZONO, K. (1968) Inhibitory and excitatory synapses in vertebrate and invertebrate animals. In "Structure and

Function of Inhibitory Neuronal Mechanisms", eds. C. von Euler, S. Skoglund and U. Söderberg, pp 33-60. Oxford: Pergamon.

VALVERDE, F. (1967) Apical dendritic spines of the visual cortex and light deprivation in the mouse. Exp. Brain Res. 3: 337-352.

VALVERDE, F. (1968) Structural changes in the area striata of the mouse after enucleation. Exp. Brain Res. 5: 274-292.

VALVERDE, F. (1971) Short axon neuronal subsystems in the visual cortex of the monkey. Internat. J. Neurosci. 1: 181-197.

VOGT, O. and VOGT, C. (1903) Zur anatomischen Gliederung des Cortex cerebri. J. Psychol. Neurol. 2: 160-180.

WALBERG, F. (1968) Morphological correlates of the postsynaptic inhibitory process. In "Structure and Function of Inhibitory Neuronal Mechanisms", eds. C. von Euler, S. Skoglund and U. Söderberg, pp 5-14. Oxford : Pergamon.

WALKER, A. E. (1938) The Primate Thalamus. Chicago : Chicago University Press.

WHITSEL, B. L., ROPPOLO, J. R. and WERNER, G. (1972) Cortical information processing of stimulus motion on primate skin. J. Neurophysiol. 35: 691-717.

WIITANEN, J. T. (1969) Selective silver impregnation of degenerating axons and axon terminals in the central nervous system of the monkey (Macaca mulatta). Brain Res. 14: 546-548

WILSON, M. E. (1967) Cortico-cortical connexions of the cat visual areas. J. Anat. 102: 375-386.

WILSON, M. E. and CRAGG, B. G. (1967) Projections from the lateral geniculate nucleus in the cat and monkey. J. Anat. 101: 677-692.

ZEKI, S. M. (1969) Representation of central visual fields in prestriate cortex of monkey. Brain Res. 14: 271-291.

ZEKI, S. M. (1970) Interhemispheric connections of prestriate cortex in monkey. Brain Res. 19: 63-75.

APPENDIX

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

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

ABSTRACT

In recent years our understanding of the functional properties of mammalian sensory cortex has advanced considerably, and it has been possible to correlate some of the physiological findings with certain anatomical features. At present, however, there is little anatomical evidence available concerning the columnar arrangemnt of cortical cells and the apparently high degree of specificity of connections between them. In an attempt to elucidate certain of these problems, material has been studied from the visual cortex (area 17) of rhesus monkeys. In addition, observations have been made on the commissural connections to the cortex at the boundary of areas 17 and 18 in the cat and monkey and on the association fibre projection from area 18 to area 17 in the cat.

Intrinsic connections within area 17 of the monkey

Small lesions were placed in the cortex of area 17, either with a needle or fine scalpel (slit lesions) or with a tungsten microelectrode, and the animals were allowed to survive for periods of 1-6 days. For light microscopy the occipital lobes were sectioned, in either the coronal or sagittal plane, at 25 μm. Sections were stained by the Nauta-Gygax, Fink-Heimer or Wiitanen method. For electron microscopy, small blocks of tissue of 1-2 cu. mm volume were taken and embedded in Epon-Araldite. One μm 'thick' sections, perpendicular to the pial

surface, were cut from the whole block face. Areas for thin sections were chosen so that, when maps of several areas were fitted together, they would form an almost complete representation of the whole depth of the cortex extending out from the lesion for 3 mm.

With the light microscope, axonal degeneration after lesions through the depth of the cortex is seen to extend for only a few millimetres from the site of the lesion, and in almost all cases this extent is asymmetrical, being 1.0-1.5 mm further on one side of the lesion than the other. Except for approximately 200 μm on each side, where there is dense, fine terminal degeneration throughout all layers, there are distinct differences both in the appearance and the extent of the degeneration in different laminae. In layer I there is a little fine granularity and a few degenerating fibres distributed over a few millimetres from the lesion. Layers II and III resemble each other in showing an even distribution of fine, granular degeneration which is dense for only up to 200 μm on either side, after which it remains more or less uniform for a distance of about 1.5 mm, when it stops rather suddenly. On its deep aspect this degeneration is continuous with marked horizontal fibre and terminal degeneration in the stria of Gennari in layer IIIc, and the extent of this axonal degeneration is the greatest of all, reaching up to 4 mm away and apparently terminating in layer IIIc and the adjacent parts of layers IIIb and IV. Layer IV shows no degeneration except for a zone of some 200 μm

immediately adjacent to the lesion, where there is dense granularity. Layer V shows an appearance similar to that of the stria, except that the fibre and terminal degeneration is coarser in V and extends in any quantity for only 1 mm or so from the lesion, being very sparse thereafter. Layer VI is again similar but shows many more obliquely arranged fibres so that there is no appearance of a horizontal fibre band comparable to those in IIIc and V (bands of Baillarger). The pattern of degeneration after lesions which extend into the underlying white matter differs from the above description only in that there is more fibre degeneration in the deep layers and the terminal degeneration in layer IV is more widespread on the side of the lesion away from the direction from which the thalamo-cortical fibres approach.

The laminar origin of intrinsic axons was determined by lesions which involved only some of the layers of the cortex or which were in the form of discrete microelectrode lesions in individual laminae. Lesions restricted to layers I and II produced only local degeneration which was mainly granular and reached no deeper than the bottom of layer IIIa. Extension of the lesion down to the bottom of layer IIIb produced only a modest increase in the horizontal extent of the degeneration, which now reached to about 1.5 mm from the lesion. Deep to the lesion, degeneration was seen in layers IIIc, IV and V; however, that in IIIc, in the stria, was only of the same extent and density as in the rest of layer III, while that in layer IV

took the form of vertically oriented fibres going down to a patch of granularity in layer V. A few degenerating fibres were seen to leave the cortex. Thus it seems that cells in the supragranular layers give off intrinsic projections only to the immediately adjoining region for a distance of about 1.5 mm, and there is no evidence that the projection of supragranular cells into the stria of Gennari is anything more than small in amount and rather restricted in extent. Some cells in layer III project to layer V, though this projection does not appear to be particularly dense. Only a few degenerating axons descend into the white matter.

When the lesion reached the stria of Gennari there was a sudden increase in both the density and extent of degeneration in that layer, and this degeneration was very similar to that seen after a lesion through the whole depth of the cortex. Involvement of layer IV gave rise to a pattern which was essentially the same as that resulting from lesions of the stria, and even a small focal lesion of layer IV produced fairly dense, extensive degeneration in layer IIIc. These results suggest that much of the stria is made up of the horizontal ramifications of ascending axons, and that many of these fibres arise from granule cells in layer IV; some may arise from cells of layer IIIc itself or may be axon collaterals of cells in layers V and VI.

When the lesion reached down to layer V there was an increase in the amount of terminal degeneration in layer IV and

in the density and extent of degeneration in the inner band of Baillarger in layer V. In addition, more degenerating fibres could be seen entering the white matter. Thus the cells of layer V appear to send axonal branches into the inner band of Baillarger for about 1 mm; since the apical dendrites of these cells may receive a thalamic input, this projection may be related to the further processing of information by infragranular complex and hypercomplex cells. The cells of layers V and VI also project to the white matter and give rise to ascending collaterals to more superficial layers.

The observations with the electron microscope were in good agreement with the light microscope findings; in particular, the rather restricted extent of the degeneration and the variations in density and extent of degeneration between the different laminae were shown. Some clustering of degenerating terminals was apparent, though it was not very marked. It is worthy of note that the terminals which degenerate after an intrinsic lesion of the cortex form only a small proportion of the total number of terminals present. This may seem surprising, but several important considerations apply: the cells projecting horizontally to a given point will be distributed throughout a very restricted region around it, so that a lesion would have to be very close to that point in order to destroy more than a small fraction of all of its horizontal connections. When it is also considered that many connections to a point may travel vertically and that degenerating terminals could be identified with the electron

microscope only if certain criteria were satisfied, the apparently low yield of terminals in our material becomes readily understandable. The majority (some 90%) of all terminals seen with the electron microscope ended with asymmetrical membrane thickenings (type 1 of Gray); this proportion was even higher in layers I and II (94.4% and 97.3% respectively), possibly correlated with the relatively high concentration of spine-rich apical dendrites in these layers. The majority (75%) of the asymmetrical intrinsic terminals ended on dendritic spines, which tended to be small, with an indistinct spine apparatus. Almost all of the remainder ended on dendrites, which were of stellate origin in some cases, though a number could not be identified. No asymmetrical terminals ended on identifiable pyramidal cell dendrites. A few examples were observed of asymmetrical terminals contacting the cell somata of large stellate cells, but no such terminals were seen on the somata of small stellate or pyramidal cells.

Intrinsic terminals ending with symmetrical membrane thickenings (Type 2 of Gray) form only about 10% of the total. Their distribution with respect to the constituent parts of neurones is complementary to that of asymmetrical terminals, as 78% end on dendrites and only 13% on spines. Both pyramidal and non-pyramidal type dendrites are contacted. Symmetrical terminals also terminate upon the somata of cells, both pyramidal and large and small stellate; in addition, one example has been seen of a degenerating symmetrical terminal ending on

the initial axon segment of a small pyramidal cell in layer II.

It is interesting to note that the asymmetrical terminals, though greatly in the majority, were numerous only within the first millimetre from the lesion, falling off in density thereafter, while the symmetrical terminals were much more evenly spread throughout the distance which they occupied (some 3 mm from the lesion).

The results are consistent with the view that information reaching area 17 from the lateral geniculate nucleus and terminating predominantly upon cells in layer IV (and also layer IIIb) is passed out via the stria to cells of layers IIIb, IIIc and the superficial part of layer IV, further processing being performed by the cells of these layers. This concept is consistent with the fact that layer IV contains a high proportion of what have been defined functionally as 'simple' cells, which are monocularly driven, while layers III and V contain a high proportion of 'complex' and 'hypercomplex' cells, which are binocularly activated. It is possible that the longer horizontal connections, whose existence may be inferred from our material, might subserve other integrative functions, such as linking up distant columns with the same preference for stimulus orientation, for stimulus type or for movement response pattern. So far as the observations with the electron microscope are concerned, our findings would seem to suggest that excitation plays a more important role in the functioning of horizontal intrinsic connections than does inhibition, as judged by the relative

numbers of asymmetrical and symmetrical terminals.

Commissural connections at the area 17/18 boundary in the cat and monkey

In the cat, small lesions (a single lesion in area 18) or large lesions (removal of the cortex of areas 17, 18 and 19 on one side) were made. In the monkey, lesions were made by the removal of the cortex of both walls of the lunate sulcus and of the crown of the prelunate gyrus, over most of the extent of the sulcus. For light microscopy, blocks oriented in the coronal plane (cats) or the sagittal plane (monkeys) were taken from the opposite hemisphere and frozen sections of these blocks were stained by reduced silver methods. Small blocks for electron microscopy were taken from immediately alongside the light microscopic material.

With the light microscope it was seen that commissural degeneration at the 17/18 boundary occupies a very limited zone only some 2 mm in width. In the monkey, area 18 showed degeneration in all layers, but as one approaches area 17 the degeneration in layer IV ceases abruptly at the boundary. A similar situation obtained in the cat: a dense band of degeneration in the deep part of layer III and layer IV was present in area 18 but ceased abruptly at the 17/18 boundary. Thus it seems as if layer IV of area 17, especially in the monkey, is really a pure processing mechanism for primary afferent information with negligible input from other laminae or from the opposite side.

With the electron microscope, all degenerating terminals were seen to end with asymmetrical thickenings. Approximately 75% of terminals ended on spines, while the remainder ended on dendrites, some of which were of the stellate type.

Connections from area 18 to area 17 in the cat

Material for this study was taken from those cat brains which had been subjected to needle lesions in area 18 on the lateral gyrus without involvement of adjoining areas. Blocks of tissue were taken in the coronal plane and processed for light microscopy by the techniques described above. Small blocks for electron microscopy were also taken.

With the light microscope, degeneration was found only in the extreme lateral part of area 17; this degeneration took the form of fine granules disposed throughout the deeper layers and reaching up to layer III. Fibre degeneration in layer I did, however, extend to the level of the suprasplenial sulcus. With the electron microscope, all degenerating terminals were seen to be of the asymmetrical type; 62% ended on dendritic spines, while the remainder ended on dendrites, some of which belonged to stellate cells.

Thus the greater part of area 17 of the cat appears to be unaffected by information coming directly from adjacent cortical areas or from the opposite hemisphere, being left 'pure' for the processing of information from the lateral geniculate nucleus of the thalamus.