SOME UNRESOLVED MYSTERIES IN THE ANATOMY OF THE VISUAL SYSTEM

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I am indeed most grateful to the Singapore Academy of Medicine for having honoured me with the invitation to deliver the Galloway Memorial Lecture for this year. However, after having accepted the invitation, I have had several anxious moments because I was never sure whether I would be able to do full justice to the ideas and ideals of this great gentleman (Dr. Galloway) who by transcending all prejudices of race, colour and creed had revealed to us the rich and noble heritage and tradition of our medical profession.

It was, no doubt, a happy coincidence that while on May 8, 1858, Dr. Galloway's parents were celebrating the birth of their beloved son, on the very same day Singaporeans were celebrating the birth of their first dispensary. Dr. Galloway arrived in Singapore in 1885 at the age of 27 and took over the medical practice from the old family physician, Dr. Robertson. In 1908, Dr. Galloway was appointed lecturer at Singapore's new Medical College where he undoubtedly distinguished himself in teaching the Principles and Practice of Medicine to the students of his time. Dr. Galloway, who was himself a Scotsman, was a contemporary of other famous Scotsmen, such as Patrick Manson, Ronald Ross and Malcom Watson, names which are all too familiar to you. Everyone of them, including Dr. Galloway, served in Asia and each one distinguished himself not only by discovering something new in the field of medicine but also re-discovered that universal truth which despite some apparent contradictions exemplifies the principle of unity in diversity and the brotherhood of all mankind.

INTERDISCIPLINARY NATURE OF NEUROLOGICAL RESEARCH

The structural and functional mechanisms concerning the various organs of the body are fairly well known whereas those concerned with the nervous system are the least understood despite the tremendous outburst of neurological researches during the past two centuries. Neurological researches are often most difficult to perform, follow, observe and interpret since they involve a sound knowledge of not one, but several disciplines. For example, when we consider the visual system, the passage of the light rays through the eye ball is a physical phenomenon. At the retina the light energy is converted in some mysterious way into electrical impulses through the intermediary of some biochemical processes which involve the bleaching of certain pigments resident in the rods and cones of the retina. The electrical impulses which are carried by the optic nerve fibres to the visual cortex are translated once again in some mysterious manner into sensory perception. Thus in the understanding of the visual function, we pass from the physical to the biochemical and then to the physiological and finally to the psychological processes of vision. Where psychology is unable to explain the various visual phenomena we even enter into the realms of speculation of the philosopher! Thus, the physicist, the biochemist, the physiologist, the psychologist and even the philosopher are mentioned as contributing to our knowledge about visual function. What about the role of the anatomist? Indeed, it is he who provides the sound foundation of the structural basis on which the edifice of visual function has to be erected if this edifice were to stand the test of time.

EYE AND CAMERA

The eye is of great interest not only to the poets, artists and lovers, but also the medical man, the anatomist and the physiologist. The eye is one of nature's greatly perfected instruments through which we receive the maximum amount of sensory information about our environment. It is also an instrument whose resemblance to the camera is indeed astonishing. Both the eye and the camera are independent evolutions; one biological and the other technological. While the technological advances have given us a clear insight into the physical aspects of the camera, the functional aspects of the eye remain mostly a mystery to us. Perhaps we may find an excuse for our incapacity in not having understood the functional and structural mechanisms of the eye. The eye is, after all, an end product of a biological evolution which has

taken millions of years whereas technological advances have occupied only the last few hundred years which is merely a tick of time of the evolutionary clock.

The lens of the camera and the lens of the eye, the diaphragm of the camera and the iris of the eye, the sensitive film of the camera and the retina, the fine and rough grain of the film and the rods and cones of the retina, oxidation of the metallic silver of the film and the bleaching of the pigments within the rods and cones no doubt reveal a close resemblance between the eye and the camera. Nevertheless, while the most important refracting medium in the camera is the lens, the lens of the eye is by no means the most significant structure concerned with the refraction of the light rays in the eye of the land living vertebrates. It is the cornea that performs this function in this group of animals although in some aquatic organisms the lens is once again the chief refracting medium.

VISUAL PATHWAYS

The visual pathways are similar to other sensory pathways in that both have peripheral receptors from which impulses are conducted by the first, second and third order of neurons to the sensory cortex. While the peripheral receptors of the skin are made up of Meissner's corpuscles and other specializations, rods and cones form the receptor apparatus of the retina. The rods and cones are the structures concerned in converting the light energy into electrical pulses which form the language understood by the brain. Thus rods and cones are biological transducers. But how the light energy is converted into electrical energy is a mystery to us although we know that in this process of conversion certain pigments contained within the rods and cones are bleached. While the rods which are concerned with vision in dim light is concentrated in the periphery of the retina, cones functioning in daylight and giving colour vision predominate in the central part of the eye. Thus periphery to centre is the evolutionary sequence.

The first order of neurons carrying skin sensation is represented by the dorsal root ganglion cells (or their homologues) while the bipolar cells of the retina perform a similar function. The second order of neurons with reference to skin sensation is represented by either the posterior horn cells of the spinal cord, the gracile and cuneate nuclei, or the sensory nucleus of the Vth cranial nerve whereas these are represented in the retina by the ganglion cells. The third order of neurons are situated in the dorsal thalamus where nerve fibres carrying skin sensation converge. Similarly, the optic nerve fibres from the ganglion cells of the retina end in the lateral geniculate body which is also a part of the thalamus. In the case of both sensory mechanisms i.e. the skin as well as the eye the final pathway is from the thalamus to the cortex.

It is known that about one million nerve fibres emerge from the ganglion cells of the retina. Such a large number of fibres is not without significance in that only 650,000 fibres carry various types of cutaneous and deep sensibility from the body parts and a mere 30,000 fibres issue from the internal ear. Even fewer fibres subserve olfcation and taste sensation. The number of fibres subserving a particular sensation appears to determine the extent of its cortical representation so that the visual input with a million fibres has the largest cortical area while that for somatic sensations subserved by 650,000 fibres is smaller than the visual area despite the fact that the skin area is several hundred folds larger than the retinal surface.

Of the million fibres that emerge from the retina, about half of them (i.e. those from the nasal half of the retina) decussate in the optic chiasma to get to the opposite side. Caudal to the chiasma the retinal fibres are carried in the optic tract, a large proportion of which terminates in the lateral geniculate body, while a small group of fibres subserving reflexes terminates in the superior colliculi, pretectal nuclei and in some other small groups of cells situated in the brain stem. From the lateral geniculate body, fibres forming the geniculo-calcarine tract pass through the optic radiation situated in the posterior limb of the internal capsule and reach the occipital cortex where they are distributed in an orderly fashion in the visual area of the brain.

LAMINAR PATTERNS IN THE LATERAL GENICULATE BODY

Some of my colleagues and myself have, for the past few years, been interested in the anatomical correlates concerning both peripheral and central sensory mechanisms. In the course of our investigations on the structure of the thalamus in various primates our attention was drawn to a paper in which was described the significance of the lamination in the lateral geniculate nucleus of primates. In that paper, it was contended that the number of laminae in this nucleus is usually six and that the layers are paired in all primates for the reception of contralateral and ipsilateral op-

tic fibres respectively. We therefore, proceeded to examine the pattern of lamination in various groups of primates. In the prosimian primate, *Galago senegalensis* only 5 layers were generally recognisable (Fig. 1), although in some sagittal and horizontal series, a few sections showed traces of subdivision of layer 4 thus giving an appearance of 6 layers (Fig. 2). There was never a complete subdivision of layer 4 in any series. However, in the slow loris *(Nycticebus coucang)* an allied primate belonging to the same suborder as the Galago, 6 layers were clearly recognisable in portions of the lateral geniculate nucleus (Fig. 3). Moreover, there was an attempted subdivision of layer 1 and if this was considered as a separate layer then there would be altogether 7 layers recognisable in the lateral geniculate body of slow loris. When the lateral geniculate body of simian primates was examined it was observed that there were 2 magnocellular and 4 parvocellular layers in a group of monkeys known as *Macaca fascicularis* (Fig. 4). Moreover, the centre of the nucleus in this primate showed a further subdivision of layers 3 and 4 thus resulting in an 8-layered pattern. On careful scrutiny one might even see traces of sublayers lying on either side of layer 1 thus making a fantastic total of 10 layers for this primate. In the gibbon *(H. lar and H. agilis)* which stands on a higher evolutionary pedestal than the monkeys, there were 2 magnocellular and 2 parvocellular layers (Fig. 5). In a few sections there was an extra band of cells lying outside layer 4. Degeneration experiments proved that this extra layer was not a part of layer 4 but an attempt towards the formation of a separate layer 5. In the Siamang gibbon *(Symphalangus syndactylus)* the 4-layered pattern was again predominant. However, the posterior portion of the nucleus showed a subdivision of layers 1 and 2 thus resulting in a 6-layered pattern. The division into sublayers is more prominent towards the periphery of the nucleus and is in sharp contrast to the arrangement in the monkey in which the sublayers are found towards the centre of the nucleus. In another specimen of Siamang gibbon only 4 layers could be observed. It may, therefore, be concluded from our observations that the laminar pattern in the lateral geniculate body of primates may vary between species of the same genus, family or suborder and that the laminae need not necessarily be paired in all primates. This may therefore imply that there is a possibility of both ipsi—and contralateral optic fibres terminating in what could anatomically be discerned in Nissl preparations as a single layer. Moreover the contention that there are 6 layers

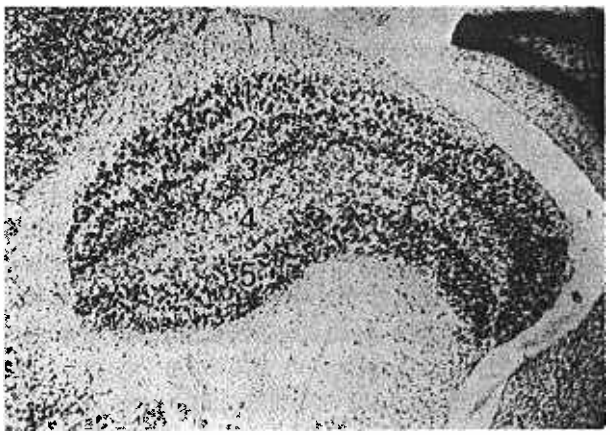

Fig. 1. Lateral geniculate nucleus of *Galago senegalensis* showing 5 layers. Layers 1, 2, 3 and 5 are dark stained while layer 4 is paler than the other layers. × 34.

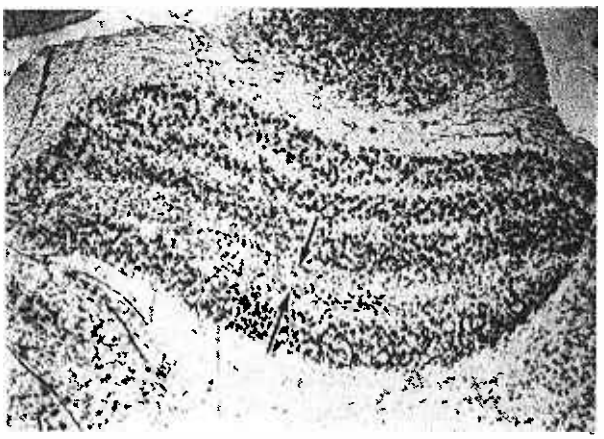

Fig. 2. Lateral geniculate nucleus of *Galago senegalensis* showing a trace of subdivision of layer 4 (arrows). × 34.

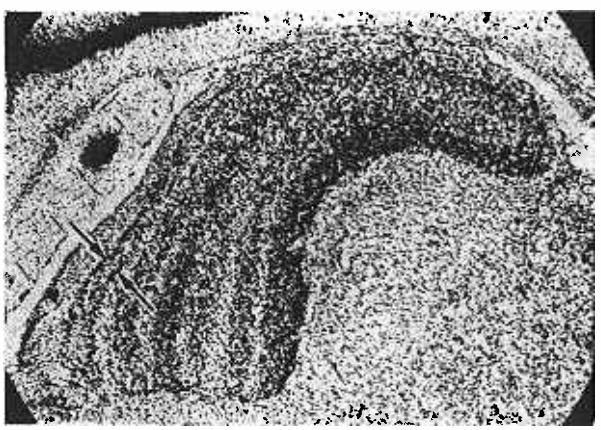

Fig. 3. Lateral geniculate nucleus of slow loris *(Nycticebus coucang)* showing 6 layers. Note subdivision of layer 1 (arrows). × 24.

Fig. 4. Lateral geniculate nucleus of Monkey *(Macaca fascicularis)* showing 6 layers. Note sublayers on either side of layer 1 containing somewhat smaller cells (arrows). × 17.

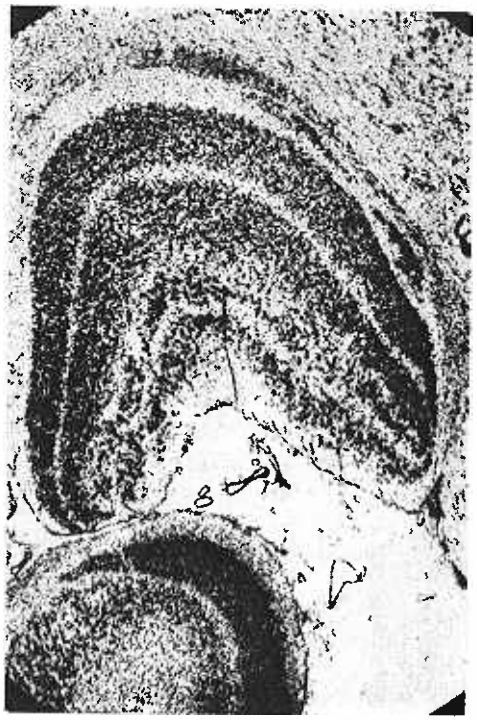

Fig. 5. Lateral geniculate nucleus of Gibbon *(Hylobates lar)* showing 4 layers and an incipient 5th layer (arrow). × 13½.

Fig. 6. Right lateral geniculate nucleus of grivet monkey *(Cercopithecus aethiops)* with long term blindness of right eye showing degeneration of layers 2, 3, 5. Note intact cells in layer 2 (arrow). × 19.

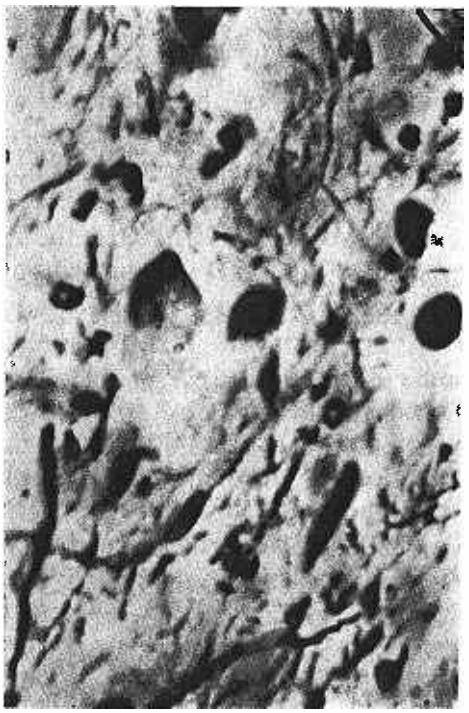

Fig. 7. Glees preparations to show Boutons in degenerating layers after removal of one eye. × 1280.

in all groups of primates is indeed misleading and that the number of layers in the various groups of primates is as divergent as the various primates themselves. The 6-layered pattern of which 3 layers receive fibres from the contralateral and 3 from the ipsilateral eye was once thought to represent a mechanism for the reception of 3 primary colour signals from each eye and account for the trichromatic theory of vision. However, such a view may now be abandoned not only because of the variability of the number of layers in the lateral geniculate body of primates but also because 6 layers are present even in some nocturnal prosimian primates such as the slow loris in which colour vision is completely absent.

PROJECTION OF THE RETINA IN THE LATERAL GENICULATE NUCLEUS

Experiments with focal lesions in the retina reveal that there is an orderly point for point projection of the retina on the lateral geniculate body. Such results have been obtained chiefly by the Nissl method which shows the transynaptic or transneuronal atrophy in certain layers of the lateral geniculate nucleus depending on whether the lesions are placed on the ipsilateral or the contralateral eye. Studies of this nature have shown that in both man and monkeys layers 1, 4 and 6 receive contralateral and layers 2, 3 and 5 receive ipsilateral optic fibres. Moreover, it is known that the dorsal part of the retina projects onto the medial portion of the lateral geniculate body and the ventral part of the retina to the lateral part while the macular area projects onto the intermediate portion of the nucleus. Furthermore, there is stated to be an increase in the central mass of the lateral geniculate nucleus in an ascending phylogenetic sequence among the primates. We have investigated the results of unilateral and bilateral eye enucleations and have also observed the results of long term degenerative changes occurring in the lateral geniculate body following accidental injury to the eye. In the monkey with long term blindness of one eye we noticed the presence of some normal looking cells in layers 1 and 2 of the nucleus while in other layers remnants of cells in an atrophic condition were observed (Fig. 6). This meant that cells which were intact must be receiving afferents from sources other than those issuing from the optic nerve of the affected eye. Such sources of afferents may be from the normal eye, visual cortex or may even be situated within the nucleus itself. In order to test whether the afferents were coming from the opposite (normal) eye we devised an experiment in which we enucleated one eye in a monkey and allowed preterminal and terminal degenerations to proceed for a period of nearly 6 weeks. By the end of this period the second eye was enucleated and the monkey was allowed to live for a further period of 7 days. If in this experiment both preterminal and terminal degenerations could be shown to be present in all layers, it was felt that their presence could only be due to the effects of the removal of the second eye. Indeed our material confirmed that preterminal degeneration as revealed by the Nauta method was present in all layers. Similarly, boutons (Fig. 7) stained by Glees method were also seen in all layers. Thus we were forced to conclude that there might be a projection from each retina to all layers of the lateral geniculate nucleus. However, it must be noted that the intensity of degenerative changes varied not only in the various layers but also within a single layer itself. Thus preterminal degeneration was more massive in layers 1, 4 and 6 of the contralateral and 2, 3 and 5 of the ipsilateral nucleus of the monkey. Moreover preterminal degeneration was densest in the rostral and peripheral portions of the nucleus while the central portion revealed only slight changes. Furthermore, layers 1 and 2 showed more drastic changes than the parvocellular layers 3, 4, 5 and 6. Similarly, Glees method revealed that the distribution of boutons was once again somewhat similar to what we have observed by the Nauta method in that a large number of boutons was present only in the anterior and peripheral portions of the nucleus. The picture presented in the gibbon material after unilateral eye enucleation was also essentially similar to that observed in the monkey with bilateral eye enucleation. Although the evidence thus far presented had confirmed our earlier suspicion that optic fibres terminate in all layers of the lateral geniculate nucleus, we have to emphasize the limitations and uncertainties of light microscopic methods. Consequently, we proceeded to examine similar material under the electron microscope.

ELECTRON MICROSCOPY OF OPTIC TERMINALS AFTER EYE ENUCLEATION

It is known from experimental studies that optic fibres terminate chiefly in relation to the dendrites of the geniculate neurons where they establish mostly axo-dendritic contacts known as synapses. Axo-somatic and axo-axonal are

also seen occasionally (Fig. 8). These synapses are characterised by an accumulation of vesicles within the presynaptic component and there is usually a thickening of the postsynaptic membrane. The synapses were distributed not only within the various laminae but also in the interlaminar zones. Changes at the synaptic regions of the optic nerve terminals would naturally be expected to occur following eye enucleation since optic nerve fibres are severed from their cells of origin by the operative procedure. In a monkey which was sacrificed 5 days after removal of one eye, changes were chiefly confined to the presynaptic regions within the lateral geniculate nucleus. However, such changes were not uniform since some of the affected synapses showed a complete accumulation of neurofilaments with only a few synaptic vesicles and mitochondria within the presynaptic bag (Fig. 9) while in others, there was only a ring of neurofilaments surrounding an inner core of mitochondria. In a third category, the filamentous change was minimal while the mitochondria and vesicles which were more abundant occupied the periphery rather than the centre of the presynaptic bag. It is therefore clear that significant differences exist in the rate of filamentous changes occuring at the optic nerve terminals. Whether such differences are a reflection of the differences in the size of the optic nerve terminals or their interconnections are yet unknown.

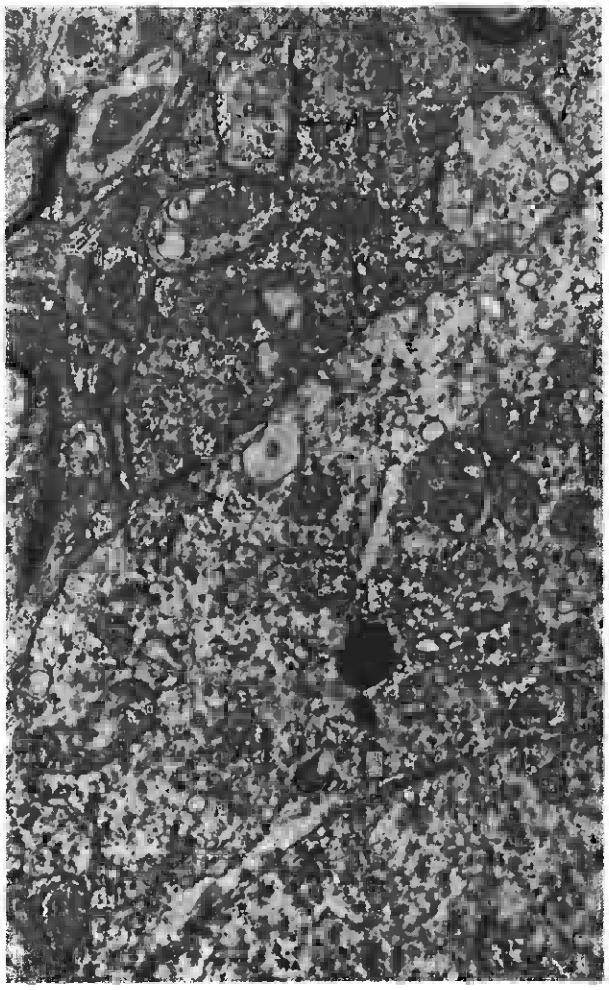

Fig. 8. To show axosomatic (AS), axo-axonal (AA) and axodendritic (AD) synapses in lateral geniculate nucleus of monkey. × 24000.

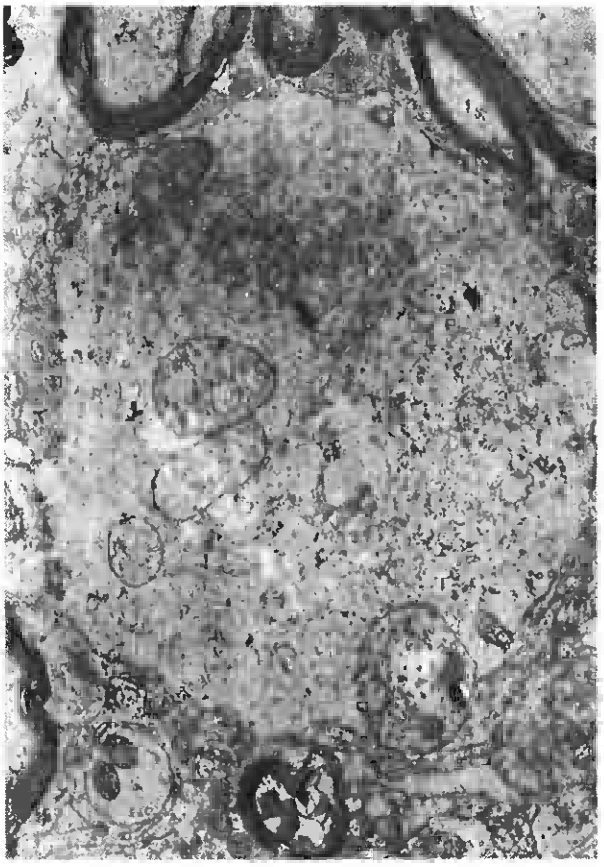

Fig. 9. Filamentous change in the optic terminal. The size of this enlarged terminal is about 5u. × 33000.

BOUTONS AND NEUROFILAMENTS

In the central part of the nucleus subserving macular vision the distribution of the degenerating optic nerve terminals as indicated by filamentous changes are usually found within layers 1, 4 and 6 of the contralateral and 2, 3, 5 of the ipsilateral nucleus, whereas in the more peripheral portions of the nucleus they are distributed among all layers although only one eye had been removed. These results confirm our earlier light microscopic observations on the distribution of boutons among all layers even after single eye enucleation. It is therefore tempting to suggest that the boutons of the light microscope may correspond to the neurofilamentous accumulations within the terminals seen under the electron microscope. Indeed such a concept is now generally accepted. Nevertheless, the size and variability in structure

of the filamentous accumulations after eye enucleation seem to throw some doubt on the validity of this concept. The size of boutons usually ranges from ½ to 2 u. whereas some of the filamentous accumulations reach a fantastic size of 10-15 u. Moreover, the bouton has a dense periphery and a central lighter zone. Such a picture is seldom presented by the neurofilamentous changes in the optic terminals of the monkey.

We have considered whether myelinated preterminal axons in their early stages of degeneration could represent the boutons. Some of our photographs, both under light and electron microscope seem to favour such a possibility. Nevertheless there are arguments against such a view. For instance, changes in the myelin sheath are seen to extend for a period as long as 2-3 months whereas boutons are visible for a period ranging from 3 days to 3 weeks following eye enucleation. Lastly, a third view has been suggested that the filamentous changes within the dendritic knobs may be the representatives of the boutons seen during degeneration. The size, distribution and structure of the dendritic knobs seem to favour such a view. However, the lack of consistency of neurofilamentous changes within the dendrites receiving the optic nerve terminals seems to contradict such a view. Thus there is still no unanimity of opinion as to what corresponds to the bouton under the electron microscope. In addition to what I have described there are also junctional zones known as filamentous and dendro-dendritic contacts. Their functional significance is also not yet understood.

NONOPTIC TERMINALS WITHIN THE LATERAL GENICULATE NUCLEUS

In contrast to the degenerating nerve terminals, there were a number of synapses which seem to persist in a normal condition following the removal of one eye. These must obviously be the nonoptic afferents to the lateral geniculate nucleus. In fact, corticogeniculate and intrageniculate fibre connections have been described. But the exact way of distinguishing which is which remains to be resolved. However, we know that there are at least two morphological types of synapses that persist after eye enucleation. In one type, the vesicles are round (Fig. 10), the terminals small and the mitochondria when present are dark while in the second variety the vesicles are oval (Fig. 10), and the mitochondria which are more often present are dark in appearance. Of the

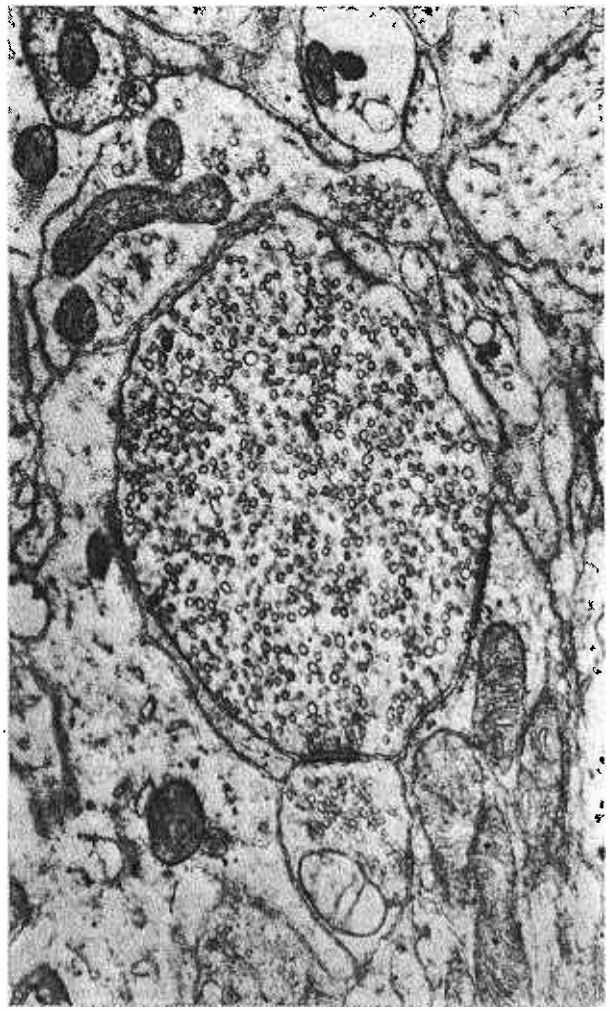

Fig. 10. Axo-axonal synapse to show large rounded vesicles in one axon and predominantly oval vesicles in the smaller axon. × 33000.

two types just described, the terminals with round vesicles are usually presynaptic whereas those with oval vesicles are almost invariably post-synaptic. In addition to these types of terminals there are others which do not fall into either of the categories. Thus the origin of a large number of nonoptic fibres terminating within the lateral geniculate nucleus is still a mystery to us.

EPILOGUE

We have selected for our studies the lateral geniculate nucleus since this has long been regarded as a simple relay station for transmission of visual impulses from the retina to the visual cortex. It is also known that in its anatomical structure the lateral geniculate nucleus is much less complex than either the retina or the visual cortex. Despite such simplicity the lateral geniculate nucleus shows extreme variability among primates in that the number of layers may range anything from 4 to 10. However, the exact significance of these

variations is not clear. Moreover, the central part of the nucleus shows the maximum number of layers in *Macaca fascicularis* whereas this feature is more prominent in the peripheral part of the nucleus in the gibbon. Does this mean a greater specialisation of the central field of vision in the monkey and the peripheral field in the gibbon? Again, the reason for such divergent specialisations is not obvious since both the monkey and the gibbon are arboreal primates. Our long term degeneration material *(C. aethiops)* clearly shows the presence of intact cells within the degenerating layers indicating the possibility of both ipsi- and contralateral optic fibres terminating in what can anatomically be discerned as a single layer in Nissl preparation. We are encouraged in these conclusions by similar findings in both Nauta and Glees preparations after unilateral and bilateral eye enucleation experiments in the monkey. Electron microscopy has provided further confirmation of these results.

Our electron microscopic investigations have revealed the presence of various types of synapses not only within the cell laminae but also within the interlaminar zones. Moreover, these synapses themselves show such variations in their morphological patterns that the concept that the impulses from the retina may pass through this nucleus with little or no modification is no longer tenable. The various cell types found within the nucleus and their dendritic patterns will certainly add another dimension to the complexity of its structure and function so that the impulse when it leaves the lateral geniculate nucleus may be profoundly transformed from its original character.

While we begin to recognise something of the complexity in the structure of the lateral geniculate nucleus, we do not know the exact pattern of organisation within the various cell laminae or the interlaminar zones; e.g. we do not know the origin of the various types of afferents reaching the lateral geniculate nucleus; the way these afferents terminate; how many of them arrive from the cortex and how many are derived from sources outside the eye and the cortex; how many interneurons are found within the nucleus and just what they do. We do not even know how many dendritic knobs there are in most of the lateral geniculate cells nor do we know the functional significance of the filamentous contacts—whether they may be electrical synapses or not. Finally, we do not even know what exactly the boutons look like under the electron microscope. If there are so many details we do not know about the simplest organ of the visual system namely the lateral geniculate nucleus which has only 1 million cells, one can then understand the magnitude of the complexity in the organisation of the visual cortex with its approximate population of a billion cells.

Some think that the visual system can be understood in terms of comparatively simple concepts such as nerve impulse, convergence, excitation and inhibition and that it may not be necessary to resort to higher mathematics, computers and network theories. However, there are others who believe that equations and not circuit diagrams as in a telephone switchboard could unravel the mysteries in the design of the visual system. In any case, what do all these teach us? They certainly drive home the point that what we know is precious little and what we do not know is enormous. This is, no doubt, a frightening reality but I hope that it brings along with it a sobering influence which should make us all humble. Has it not been said that humility is the beginning of wisdom? Is it not also true that service can best be done in a spirit of humility? There is little doubt that it is in this spirit of humility that Dr. Galloway dedicated his services to the people of this country. By his selfless service and noble example Dr. Galloway has given us a new experience, an experience which is richer and deeper and which is truly a reflection of the grace and goodness of the human spirit.