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The Ultrastructure of the Optic Lobes of Octopus Vulgaris

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THE ULTRASTRUCTURE OF THE
OPTIC LOBES OF OCTOPUS VULGARIS

by

Donald B. Newman

A Thesis Submitted to the Faculty of the
Graduate School of Loyola University
in Partial Fulfillment of the
Requirements for the
Degree of
Master of Science

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BIOGRAPHY

Donald B. Newman was born in Chicago, Illinois, on January 1, 1948.

He attended Brother Rice High School in Chicago, and graduated in June of 1965. He entered Loyola University of Chicago, Illinois, where he majored in Biology and graduated with a Bachelor of Science degree in June of 1969.

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ABSTRACT

A study was undertaken for the purpose of identifying various neuronal and glial cell types in the optic lobe of the octopus brain with the use of the electron microscope. Neuronal and glial cell types of the optic lobe have been observed by previous workers, using the light microscope, and their possible roles in the visual memory system of the octopus have been considered.

Several medium-sized (36 cm.) female specimens of Octopus vulgaris were obtained and their optic lobes removed. These were prepared for study with both the light and electron microscope.

The results indicate that certain neuronal types described by previous workers with the light microscope have been tentatively identified with the electron microscope. Bipolar neurons thought by earlier workers to classify and channel visual input to motor centers in the optic lobe have been observed at the ultrastructural level. Axo-axonic and axo-somatic synapses observed around these neurons lends credence to speculation regarding their function.

Upper motor neurons in the optic lobe medulla have been tentatively identified; synaptic relationships observed here also dovetails with postulations by previous workers regarding their functions.

A unique neuronal synaptic relationship was observed in the
medullary cell islands of the octopus optic lobe, in that a post-synaptic thorn was observed arising directly from a neuronal perikaryon.

Important relationships between glial cells and neurons of the optic lobe have been observed. Glia have been seen on numerous occasions to completely encircle neurons of the optic lobe. Glia bearing vesicle-containing swellings along their processes have been observed.

Glia have been observed actually invaginating the cytoplasm of neurons to the extent that glial processes abutted against the neurons nuclear membrane.

The observed phenomena lends support to claims by previous workers that neuroglia play an important trophic role in the functional economy of the nervous system.
I. INTRODUCTION

The optic lobe of the brain of Octopus vulgaris is a mass of nervous tissue which is situated between the octopus' eyeball and the parts of its brain surrounding the esophagus. It is concerned with processing visual input from the octopus' retina and mediating behavior patterns in response to that information (Young, 1962a).

The fact that the optic lobe of Octopus apparently stores visual information and initiates stereotyped responses on the part of the animal (e.g. feeding and swimming motions) implies a fairly sophisticated neuronal organization within the optic lobe. As such, the optic lobe of the octopus has been studied by various workers for several decades in an attempt to uncover a relationship between the morphology of various neuronal and glial types and their possible function (Cajal, 1917; Young, 1962a).

To date, the majority of studies on the octopus' optic lobe have been done with the light microscope. Young and others have published studies in which the various neuronal and glial types of the octopus' optic lobe were identified at the light microscope's level and catalogued as to their possible function (Young, 1962a). However, the light microscope's limits of the resolution precluded close scrutiny of the cytological details.
of the various neuronal and glial cells. Also, synaptic interconnections between neurons were too small to be observed with the light microscope. Thus, the fine details of the interrelationships between the neurons and glia of the octopus' optic lobe proved to be beyond the scope of light microscope, yet, no one to date has undertaken a comprehensive study of the octopus' optic lobe using the electron microscope.

The purpose of this investigation, therefore, was to examine the optic lobes of Octopus vulgaris, using the electron microscope, with three objectives in mind: (1) to describe the appearance of the various types of neurons and to correlate them with their counterparts as observed with light microscopic observations; (2) to describe the appearance of the various types of glial cells and to compare them with their counterparts as observed with the light microscope; (3) to tentatively corroborate the appearance of various types of interconnections, postulated by earlier workers to occur among the neurons of the optic lobe of the octopus but not observed for the lack of adequate resolution of the light microscope.
II. LITERATURE REVIEW

The Cephalopoda are a class of molluscs which have far surpassed other members of their phylum in neural development. They are among the most active invertebrates, living a predatory life aided by their keen vision and complex behavioral repertoire (Lane, 1960).

Among the cephalopods, the octopi manifest invertebrate nervous development at its zenith. The brain of the octopus rivals that of fishes and amphibians in its complexity. In addition, the octopus has demonstrated a capability for learning, unparalleled among other invertebrates (Bullock & Horridge, 1965). As such, the octopus brain has been a focus of investigation by neuroanatomists for several decades (Young, 1971). Much has already been learned concerning the cytology of the octopus brain, but much remains to be discovered (Young, 1971).

As in most invertebrate brains, the brain of the octopus surrounds the esophagus. The subesophageal lobes mediate reflex movements of the octopus' mantle and tentacles and also control the autonomic functioning of the viscera (Bullock & Horridge, 1965). The supraesophageal lobes are concerned with processing sensory information and initiating modifiable reactions to the varied situations in which the animal finds itself (Bullock &
Horridge, 1965). Connected to the supraesophageal lobes are the optic lobes, which, in synergy with the supraesophageal lobes, transduce visual information into appropriate behavioral responses. The supraesophageal lobes (fig. 1) are analogous to the human brain in that higher centers handle increasingly complex functions.

Immediately above the esophagus one finds the basal lobes which initiate motor responses. Above them are the superior and posterior buccal lobes, which mediate movements of the octopus' mouth and tentacles involved in eating (Bullock & Horridge, 1965). Above these lobes are two sets of centers, each consisting of four lobes, which are strikingly similar in their connection patterns, although serving entirely different functions. The lower set of centers consists of four lobes; the lateral inferior frontal, the median inferior frontal, the subfrontal, and the posterior buccal (Young, 1970). These lobes process chemotactile information. The posterior buccal lobe is of particular importance, since this is thought to be the seat of chemotactile memory enabling the octopus to respond in a suitable manner to events involving chemical and tactile stimuli (Wells, 1959).

The upper four lobes (the lateral superior frontal, the medial superior frontal, the vertical, and the subvertical) together with the optic lobes, have aroused most of the interest on the part of neuroanatomists. These four centers, together
with the optic lobes, subserve the octopus' visual memory system (Young 1963). Although this paper deals solely with the optic lobe, brief mention must be made of research efforts involving these upper four supraesophageal lobes as these were long thought to be the sole seat of learning in the octopus' brain (Young, 1958 & 1961). Only recently has it been determined that the optic lobes are also significantly involved in the learning process (Young, 1962 & 1971).

Evidence that the upper lobes of the octopus brain were essential for visual learning first came to light in 1950. It was known that the octopus could learn to avoid rectangles and squares of various sizes after negative conditioning techniques. After interruptions of the upper visual circuit, however, the octopus' inductive capacity was found to be impaired (Boycott & Young, 1950). It was observed that after removal of the vertical lobe the octopus exhibits an excessive proclivity to attack, inspite of indications that it is likely to be unrewarding or actually traumatic (Boycott & Young, 1950). Conversely, learning to form an association between a given figure and rewards of food proceeds slowly in the absence of the vertical lobes (Young, 1958; Maldonado, 1963).

Therefore, it was concluded that the vertical lobe was the ultimate site of the octopus' visual memory while the superior frontal lobes and subvertical lobes served as input and output
amplifiers (Young, 1965).

The vertical lobe was assumed to be concerned mainly with setting up representations and processing visual and tactile information to initiate a generalized positive or negative reinforcement to a response initiated in the optic lobe (Young, 1961).

Gray and Young (1964) published a paper which described the light and electron microscopical appearance of the vertical lobe. They indicated that the vertical lobe had a cortex and medulla. The cortex, they reported, contained two distinct classes of neuronal perikarya. The majority of the cells were seen to be small amacrine cells with vesicle-filled trunks which extended into the medulla of the lobe. Other cell types were described as large neurons possessing axons which led towards the sub-vertical lobe. Entering into the vertical lobe medulla, Gray and Young showed, were axon tracts from the median superior frontal lobe and from the median superior frontal lobe and from the brachial lobes (Gray & Young, 1964).

The significance of these fibers leading into the vertical lobe was made clear in a study by Gray (1970) dealing entirely with the electron microscopy of the vertical lobe. In this work Gray demonstrated that incoming fibers from the median superior frontal lobe (conveying tactile and visual information from other centers) made cruciform (en passant) connections with the trunks of the small amacrine cells of the vertical lobe cortex. These
trunks, in turn, synapsed with dendrites of the large cortical neurons, whose axons in turn lead toward the subvertical lobe. Other axons (of presumably inhibitory nature) were seen to synapse on other dendritic collaterals of the large neurons (Gray, 1970a).

Instrumental as the vertical lobe appeared to the process of learning in the octopus, it became apparent that the optic lobe played an even more fundamental role in the memory process (Young, 1962a), since records of visual events are also adequate under some circumstances, even in the absence of the vertical lobe.

It has been shown that animals without a vertical lobe can learn to attack unfamiliar figures for a reward although they do so more slowly (Muntz, Sutherland & Young, 1962). In light of the fact that the chief output of the vertical lobe is to the optic lobe and that the optic lobe effects motor and visceral responses to various stimuli, it became obvious that the optic lobe may contribute to the octopus' memory system.

The optic lobe of cephalopods have been intensively studied by various researchers for decades (Kopsch, 1899), (Cajal, 1917), (v. Lenhossek, 1896). Young (1962a) published the most comprehensive work to date on the light microscopy of the octopus optic lobe. Employing the Kopsch modification of Golgi technique (Kopsch, 1899), he has identified numerous cell types and neuron-
connections in the various parts of the octopus brain.

In light of the fact that this treatise represents an ultrastructural expansion of Young's efforts, brief mention is made at this point of some of the more pertinent findings as given in Young's 1962 paper.

The optic lobe in the *Octopus vulgaris* is a complex neural center containing at least $60 \times 10^6$ neurons (Bullock & Horridge, 1969). It has a cortex and a medulla (Fig. 1a) which consists of irregular islands of cells separated by tracts of neuropil and nerve fibers. Converging on the cortex of the optic lobe, which lies immediately behind the octopus' eyeball, are the optic nerves. Leading away from the center of the optic lobe is the optic tract, which joins the supraesophageal lobe on either side and carries afferent and efferent impulses to and from the optic lobe.

The cortex of the optic lobe is subdivided into three layers; (1) an external granule layer, (2) a plexiform layer and (3) an inner granule layer.

The cells of the external granule layer are strikingly similar in their external configuration. Although they vary considerably in size (the largest having nuclei up to $10\mu$ in diameter), they represent a homogenous population of round, closely packed perikarya. The external granule cells are all amacrine cells—no one process can be identified as an axon (Young, 1962a).
They are round unipolar neurons whose single process ramifies into numerous smaller processes among the optic nerve axon terminals within the plexiform layer. Small dendritic collaterals can be seen coming directly off the main trunk or one of its rather numerous branches.

The plexiform layer consists entirely of nerve fibers (i.e., axons and dendrites) of various types. No neuronal perikarya are found in this layer. In this layer incoming retinal axons bear dilatations which were thought by Young (1962a) to be axon terminals. Also found in this layer are numerous dendrites belonging to neurons in both the inner and external granule cell layers.

The inner granule cell layer presents a more variegated appearance than the external granule cell layer. Young (1962a) listed four cell types found in the inner granule cell layer: (1) centrifugal cells, with branches in the plexiform zone and an axon proceeding to the optic nerves, (2) centripetal cells, with branches in the plexiform zone and axons that proceed inward to the medulla of the optic lobe, (3) small multipolar cells (4) inner amacrine cells, with branches restricted to the plexiform layer.

The centrifugal cells are rather large neurons with irregularly shaped perikarya. Although they may have basal dendrites, their most striking feature is a large dendrite-bearing
trunk which sends its efferent axon to the optic nerves. These axons are thought to end in the retina of the octopus' eye (Young, 1962b).

The centripetal cells of the inner granule layer are either bipolar or unipolar neurons (whose axons and dendrites split off a short common trunk). Some have short basal dendrites stemming directly off the cell body. The dendrites of all of the inner granule centripetal (bipolar and unipolar) neurons ramify in the plexiform layer, whereas their axons proceed among the cell islands of the optic lobe medulla to the center of the lobe. The unipolar cells of the inner granule layer sometimes give off a long process which bears dendritic branches in the plexiform zone and then returns through the inner granule cell layer to end among the cell islands of the optic lobe medulla (Young, 1962a).

According to Sutherland (1961), both unipolar and bipolar centripetal cells exhibit dendritic branching patterns of amazing regularity; their dendrites may ramify in either horizontal or vertical directions with respect to a tangential section of the optic lobe. As a tangential plane of the optic lobe approximates the plane in which the octopus holds its eye, it can be seen that the centripetal cell dendrites relay incoming light impulses as horizontal or vertical projections from the retina. As Young (1962a) pointed out, it is no small coincidence that the recog-
nition of visual patterns is done by an octopus in part at least by measuring the vertical and horizontal extent of any figure (Sutherland, 1961).

Young, thus designated the inner granule centripetal cells as "classifying cells", since they process visual information into an encoded output. Their significance and electron microscopic appearance appears to be germane to the functioning of the octopus' visual memory system. Young's classifier cells will be considered extensively in subsequent portions of this paper.

The inner granule layer gives way to clusters of multipolar cells at the border of the medulla whose axons course chiefly in a tangential direction. Thus the outer part of the medulla is referred to as the tangential cell layer. This gives way to the deeper medulla, consisting of widely separated cell islands consisting of small multipolar as well as large unipolar neurons. The axons of these cells converge centripetally to form the optic tract. The dendrites of these neurons, Young presumed, are contacted by the centripetal neurons of the inner granule layer.

In summary, Young's 1962 paper revealed the following concerning the probable pathway of light impulses perceived on the octopus retina. (1) Retinal neuron axons run in the optic nerves and terminate in the plexiform layer of the optic lobe cortex. (2) External granule amacrine synapse with the retinal axons and relay the visual information to (3) the dendrites of
inner granule layer centripetal neurons. (4) These cells in
turn relay impulses to the large neurons in the medullary cell
islands. (5) Axons from these cells converge to form the optic
tracts which convey the optic lobe output to the supraesophageal
lobes.

Young (1962a) failed to mention the possible connections
that the inner granule amacrine cells may make. The significance
of these cells, and verification of the optic lobe connections
given above became evident only after applying electron micro­
scopical techniques to the study of the optic lobes.

In 1963, the first electron microscope study of the octopus
optic lobe appeared. Dilly, Gray and Young (1963) published a
paper dealing with the ultrastructure of optic nerve synapses in
the plexiform layer of the optic lobe. This work confirmed
earlier speculations by Young in that optic lobe axon terminals
containing many small vesicles were seen synapsing with dendritic
spines of what were thought to be external granule cells. Un-
explained phenomenon observed at this time was the presence of
axons tunneling through the optic nerve axon terminals.

Gray, (1970b), showed that the retinal axon terminals con-
tained only agranular vesicles.

The latest ultrastructural study of neurons in the optic
lobe was Gray's 1972 paper elucidating the function of the tunnel
fibers observed earlier in retinal axon terminals. The tunnel
fibers were shown to be the trunks of the amacrine cells of the external granule layer passing through the optic nerve dilatations (Gray, 1972).

The only other ultrastructural study of the octopus optic lobe at this writing consists of a study by Gray in 1969 of the gliovascular organization of the octopus optic lobe. In this work Gray made tentative steps towards identifying the various types of glial cells extent in the optic lobe. His suggestions as to their possible functions were mainly in the realm of nutritive and supportive concepts (Gray, 1969).

The preceding review of the literature has shown that relatively little is known concerning the complete ultrastructural aspects of the octopus optic lobe. Gray's papers (1963 & 1972) deal solely with the external granule layer and plexiform layer. No work has been done previous to this study to corroborate or disprove Young's hypothesized neuronal pathways, yet the optic lobe is of paramount importance in the functioning of the octopus visual memory system. In addition, as it will be subsequently demonstrated in this thesis, Gray's initial study of octopus glia barely scratches the surface of the subject; octopus glial cells might be of a greater variety and have a more intricate role in neuronal functioning than anyone has previously suspected. The point is that the ultrastructure of the octopus optic lobe is virtually a virgin field for exploration. This paper, hopefully, will prove to be a small initial step in that exploration.
III. Materials and Methods

Three (female) medium-sized specimens of *Octopus vulgaris* (with a tentacle span of about 2-3 feet) were obtained alive from the Caribbean Sea. The octopi were anesthetized with 3% urethane in sea water, and the fibrous tissue surrounding the orbit was incised with a small sharp scissors. The optic lobe, which lies immediately behind the eyeball within the orbit, was readily exposed.

Immediately after the optic lobe was exposed, cold phosphate-buffered glutaraldehyde (3.5%) was poured on it and surrounding tissues. Subsequently, the optic lobe was removed, placed in a Petri dish containing cold phosphate-buffered glutaraldehyde, and cut into small pieces. Pieces of known orientation were then placed in small Wheaton jars containing 3.5% phosphate-buffered glutaraldehyde at 4°C (Sabatini et al., 1963), and fixed for two hours. The tissue was rinsed after fixation for three hours in three changes of Sorensen's phosphate buffer (pH 7.4) and post-fixed for one hour in 1% phosphate buffered osmium tetroxide at 4°C (Millonig, 1961). The tissues were then rapidly dehydrated in a graded series of ethanol baths at 4°C, and embedded in Epon 812 (Luft, 1961) using Beem capsules.

Tissue to be examined with the light microscope were cut
with glass knives on a Servall MT-1 (Porter-Blum) ultramicrotome at a thickness of 0.5μm. The sections were mounted on glass slides, stained with Toluidene blue, and coverslipped. The sections were then photographed with a Zeiss Photomicroscope II apparatus.

Tissue to be examined with the electron microscope was sectioned with glass knives on a Servall MT-1 (Porter-Blum) ultramicrotome at a thickness of 90-120μm and mounted on 200-mesh copper grids.

The grids were stained with uranyl acetate (Swift & Rasch, 1958) and counterstained for 1-1/2 to 3 minutes with lead citrate (Reynolds, 1963). The tissues were then examined with an RCA-EMU 3F-2 electron microscope at an accelerating voltage of 50 KV.
IV. OBSERVATIONS

I. Cortex of the Optic Lobe.

A. Neurons of the external granule cell layer of the cortex:

Light microscopic study of the external granule layer showed that the neurons in this layer are largely uniform in their appearance. They are fairly large neurons (about 25μ long) - the largest have nuclei which are 10μ in diameter. They possess pale cytoplasm and a large, round nucleus with vesicular clumps of chromatin (Fig. 2). Fibrous glia possessing dark, irregular nuclei can be seen interspersed among the neurons of the external granule layer (Fig. 2).

As observed with the electron microscope, the external granule cell neurons present an appearance similar to that observed with the light microscope. The neurons possess a pale cytoplasm filled with glycogen granules. Mitochondria can occasionally be observed in the cytoplasm, although as a whole the latter is rather devoid of organelles (Fig. 3). Lamellated bodies are sometimes seen in the cytoplasm of the external granule neurons (Fig. 4). Occasionally one observes an amacrine trunk branching from the perikaryon of an external granule neuron (Fig. 3). These neurons are tightly packed against one another, and occasional puncta adherentia can be observed between two adjacent external granule neurons (Fig. 4).
B. Plexiform layer of the optic lobe cortex:

The plexiform layer consists entirely of axons and dendrites of various neurons contacting one another. No cell bodies are present in this layer. Figure five shows an axon terminal contacting a dendritic spine. The axon terminal in this picture (approximately 1μ wide) contains small, agranular vesicles, and several mitochondria. The dendritic thorn being contacted by it is clear and contains a postsynaptic density.

Figure six shows an axon terminal contacting a dendrite which is itself being contacted by a dendrite containing small agranular vesicles. The axon terminal contains agranular vesicles and numerous mitochondria. The dendrite it is contacting can be seen to have a postsynaptic density. This dendrite is apparently also post-synaptic to the dendrite containing the clear vesicles.

A similar triad configuration can be seen in Fig. 7, where a dendrite is post-synaptic to an axon terminal with agranular vesicles and a large dendrite also containing agranular vesicles.

C. Neurons of the inner granule cell layer of the cortex:

Light microscopy of half-micron thick sections of the inner granule cell layer reveals that it consists of radially arranged (to the surface of the optic lobe) rows
of neurons separated by areas of neuropil. The rows of neurons are approximately 10 cells deep. Figure eight shows that the majority of the neurons observed possess either round or oval nuclei ranging in diameter from 5-10\(\mu\). These neurons have sparse cytoplasm, and their nuclei contain dense clumps of chromatin. Occasionally, large pale nuclei with irregular outlines can be observed among the round or oval nuclei.

Low power (4,000 X) electron micrographs of the inner granule cell layer exhibit clusters of small neurons (with a nuclear diameter of 5-7\(\mu\) ) with round nuclei containing dense clumps of chromatin and sparse cytoplasm (Fig. 9 & 10) labelled as amacrines. Interspersed among these neurons are larger neurons with oval nuclei. The chromatin of these nerve cells is usually less densely clumped than the nuclei of the small, round neurons. One of these neurons may be seen in Fig. 10 (labelled as a bipolar).

Higher power electron micrographs (eg. 14,000X) of the inner granule neurons with round nuclei (amacrines) reveal that their cytoplasmic border has a rather regular configuration, with no appreciable number of protruberances. The cytoplasm of these neurons is rather sparse, and filled with glycogen granules (Fig. 11). Occasionally, a mitochondrion can be in their cytoplasm (Fig. 13). The larger, oval
neurons labelled as "bipolar" also have a rather regular profile. They possess a sparse, clear cytoplasm villed with glycogen granules and an occasional mitochondrion (Fig. 12). A classic neuron of this variety can also be seen in Fig. 14 lying next to an amacrine neuron. The difference between the two cell types can clearly be appreciated; the amacrine neuron being smaller than the bipolar and possessing a round nucleus and denser chromatin clumps. The nucleus of the bipolar neuron is oval and it's karyoplasm is lighter than the amacrine's karyoplasm.

Occasionally within the inner granule layer one observes large neurons (with a nuclear diameter of about 12μ) with irregular nuclei containing very pale karyoplasm but dark, densely-clumped chromatin. The nuclei of these neurons (as seen in Fig. 13, labelled as centrifugal cell) contrasts greatly to the darker nuclei of the smaller amacrine surrounding it.

Higher magnification electron micrographs of inner granule neurons (30,000X - 48,000X) reveal that they receive a fairly large number of axon terminals upon their perikarya. Figure fifteen shows an axosomatic synapse upon an oval (bipolar) neuron of the inner granule layer. The axon terminal is about two microns in diameter, and contains small (about 400 Å in diameter) agranular vesicles and two mitochondria.
The synaptic cleft between the axon terminal and the bipolar neuron is narrow (about 200 Å wide) and no appreciable postsynaptic thickening can be seen. What might possibly be a sub-synaptic cistern, however, can be seen in the bipolar neuron's cytoplasm immediately below the postsynaptic membrane.

An axosomatic synapse similar to the one in Fig. 15 can be seen in Fig. 16. An axon terminal containing small (400 Å) agranular vesicles is contacting an oval (bipolar) neuron. The synaptic cleft between the two elements is rather narrow (200 Å) and no pre or postsynaptic thickenings can be observed. Several mitochondria can be observed within the axon terminal.

An axon terminal can be seen in Fig. 17 wedged between two oval neurons, and it appears to be synapsing with one of them. This axon terminal also contains small agranular vesicles; in addition, the synaptic cleft between it and the perikaryon to which it is pre-synaptic is rather narrow (200 Å).

Axosomatic synapses were mostly observed upon the larger oval neurons of the inner granule layer. The small, round neurons exhibited relatively few axosomatic synapses.

A rather interesting phenomenon observed concerning the oval neurons was that the axon terminals contacting their
perikarya often were themselves contacted by other axon terminals. Figure eighteen, for example, shows a large (about $3\mu$ wide) axon terminal containing round agranular vesicles contacting an oval (bipolar) neuron. The axon terminal is post-synaptic to a second axon terminal which is rather dark and which contains closely-packed small round agranular vesicles.

Figure nineteen shows a large (about $3\mu$ wide) axon terminal with agranular vesicles contacting a bipolar neuron. Another axon terminal (also with agranular round vesicles) is effecting an axo-axonic synapse with it, as in Fig. 18.

Figure twenty presents a similar configuration to that observed in Fig. 18 in that a large ($3\mu$ wide) axon terminal contacting a bipolar neuron is itself post-synaptic to a second axon terminal containing round, agranular vesicles.

A phenomenon observed among the small, round inner granule amacrine cells was the presence of possible puncta adherentia between adjacent neuronal perikarya (Fig. 21).

II. Medulla of the Optic Lobe

A. Neurons of the tangential cell layer:

As observed with light microscopy, $0.5\mu$ thick sections of the tangential layer reveal that it contains many large,
oval neurons with round nuclei containing extremely dark, dense chromatin, which contrasts sharply with the pale karyoplasm of these cells (Fig. 26 & 27, labelled bipolar). The tangential layer also contains other neurons with round nuclei containing evenly dispersed chromatin (Fig. 26 & 27, labelled multipolar). The nuclei of these cells appear a uniform shade as observed with light microscopy.

The appearance of the tangential neurons as observed with the electron microscope matches their appearance as observed with the light microscope.

As was observed with the light microscope, the electron microscope reveals tangential neurons to be of two major types—large neurons with pale nuclei (i.e. pale karyoplasm) and dense clumps of chromatin and neurons with more or less round, gray nuclei containing evenly dispersed chromatin. (Figs. 28 & 29; the pale neurons are labelled bipolar; the darker, smaller neurons are labelled multipolar).

Both these neuronal types exhibit fairly large cytoplasmic areas. Their cytoplasm appears watery, with very few organelles visible (Figs. 28 & 29).

This paucity of cytoplasmic organelles in these neurons is especially apparent in Fig. 30, which shows two of each type of tangential neuron lying alongside a small venule. A classic example of the tangential neuron with the pale
karyoplasm (a bipolar) can also been seen in Fig. 31.

B. Neurons of the deep medullary cell islands:

The center of the optic lobe consists of cell islands surrounded by neuropil. The cell islands contain two types of neurons; large unipolar neurons and smaller multipolar neurons.

The electron microscope reveals that the large unipolar neurons (with a nuclear diameter of about 10 microns) in the deep medulla are similar to those in the tangential layer in that they possess oval nuclei with pale karyoplasm and dense clumps of chromatin. Such a neuron (labelled as a command cell) can be seen in Fig. 39. It can be seen to have a rather prominent nucleolus. Another unipolar neuron can be seen in Fig. 40 (also labelled as a command cell). The cytoplasm is pale and contains glycogen granules. Several mitochondria can be seen within the neuron's cytoplasm. Figure forty-one also shows a unipolar neuron. Its one large process can be seen emerging from its perikaryon.

Figure forty-nine shows several unipolar neurons of the deep medulla, as well as what appears to be a smaller multipolar neuron. The latter exhibits a nucleus about five microns wide with pale karyoplasm and densely-clumped chromatin.

A unique feature of the deep medulla is the rich variety
of synapses associated with the large unipolar cells. Dark, vesicle-filled trunks occur commonly. One can be seen in Fig. 42 synapsing on the perikaryon of a unipolar neuron. Another such trunk can be seen in Fig. 43. It is apparently pre-synaptic to a large axon terminal contacting a unipolar neuron. Other axosomatic terminals can be seen filled with dense cored vesicles (Fig. 44).

Observed once or twice were axon terminals filled with giant (600Å) round vesicles of a type previously unreported in ultrastructural literature (Figs. 41 & 45).

On some occasions, medullary unipolar neurons exhibited post-synaptic thorns arising directly from the perikaryon. Such a thorn can be seen in Fig. 46. It is being contacted by an axon terminal containing round, agranular vesicles.

III. Glial cells of the Optic Lobe.

A. Fibrous glia:

Fibrous glia are found in both the external and internal granule layer of the optic lobe cortex, as well as the optic lobe medulla. Light microscopy shows them to have dark nuclei with dense chromatin clumps, and dark strands of cytoplasm radiating away from the nucleus (Fig. 2) in a highly irregular pattern. Electron microscopy shows them to have a similar appearance. Fibrous glia of the inner granule cell layer can be seen in Figure 10 & 14. They have
a dark nucleus with an irregular outline and densely-clumped chromatin. Similar fibrous glia are seen in the tangential layer (Fig. 30) of the medulla. They are seen in Fig. 31 to completely encircle a tangential bipolar neuron.

Fibrous glia are especially numerous in the deep medullary cell islands. They usually surround the unipolar neurons of the deep medulla in large numbers (Figs. 39 & 40). Long fibrous processes of fibrous glia can be seen streaming through the neuropil in figure 39 and 47.

B. Protoplasmic glia:

Protoplasmic glia occur less frequently in the optic lobe than fibrous glia. One can be seen among neurons of the inner granule cell layer in Fig. 14. It has an elongated, irregularly-shaped nucleus with dense karyoplasm. Its cytoplasm is rather watery. It appears to have been in an ameboid state when fixed. Another such protoplasmic glia can be seen in Fig. 31. A lamellated body can be seen in its cytoplasm.

C. "Dark" glia:

A third type of glial cell is found in the inner granule cell layer of the optic lobe cortex, as well as the medulla. These dark glia are seen in a half-micron section of the inner granule layer to be interspersed among the neurons of this region. As stained with Toluidene blue, they appear
extremely dark (Fig. 8).

Lower power (4,000X) electron micrographs of the inner granule layer also shows these "dark" glia. That they stain very darkly with Uranyl Acetate is readily apparent. They have an irregular outline, and stain so darkly that their nucleus is often indistinguishable from their cytoplasm (Figs. 9 & 10).

Higher power (14,000X) micrographs of the inner granule "dark" glia shows that they often abutt directly against the cytoplasm of the neurons in this region. Figure twenty-two shows these dark glia surrounding an amacrine neuron of the inner granule layer, and Fig. 24 shows one applied to the surface of a bipolar neuron. It can be seen giving off a long process which at high magnification (30,000X) appears to be a mitochondrion (Fig. 25). Dark glial processes of the inner granule layer are seen occasionally to bear swellings filled with vesicles. Such a vesicle-laden glial process appears to be contacting a neuronal perikaryon in Fig. 23.

Dark glia are also present in the tangential layer of the optic lobe medulla, and exhibit a variety of peculiar configurations. A light micrograph of the tangential region of the optic lobe medulla shows several dark cells, one of which is completely encircling a multipolar neuron (Fig. 27).
Low power (4,000X - 8,000X) electron micrographs of the tangential region show numerous dark glia (Fig. 28 & 29). One can be seen to completely encircle a bipolar neuron in Fig. 32. It bears clear swellings along its processes.

Another dark glia can be seen to completely encircle a tangential multipolar neuron in Fig. 33. Its processes also bear clear swellings. A high power (30,000X) electron micrograph of a dark glial process shows that these clear swellings might be mitochondria (Fig. 34).

Dark glia of the tangential layer, like those of the inner granule layer, have cytoplasm so dark that it is difficult to distinguish the outline of the nucleus. Figure thirty-five shows a high power (30,000X) view of a tangential dark glia. The dark cytoplasm appears filled with granules. The outline of the nucleus can be distinguished from the cytoplasm in this picture.

As in the inner granule layer, tangential dark cells sometimes are seen to bear swellings containing round, agranular vesicles (Fig. 36 & 37). The dark cell swelling in Fig. 37 appears to be contacting an axon terminal containing round, agranular vesicles about 400 A wide.

Perhaps the most remarkable observation concerning dark glia can be seen in Fig. 38. A dark glia is seen invaginating the cytoplasm of a tangential bipolar neuron
to the extent that a glial process is apparently abutting against the neuron's nuclear membrane.
One of the goals of this thesis was to study the ultrastructure of neurons in the optic lobe that have been described by Young (1962a) and others (Cajal, 1917). Reasons will now be set forth why I feel various neurons I have observed with the electron microscope correspond to the neuronal types catalogued by Young and others by dint of light microscopic studies. In addition, it will be speculated as to what various synaptic types observed among neurons in the optic lobe signify in terms of interconnections between these neurons. Finally, the significance of the various glial types found in the optic lobe will be discussed.

A. External Granule Neurons:

Young (1962a) stated that the external granule layer was composed exclusively of round, unipolar amacrine neurons whose processes ended in the plexiform layer. The light microscope showed that each neuronal perikaryon gave off a single trunk which ramified into numerous dendritic branches within the plexiform layer. Electron microscopic studies by Dilly, Gray and Young (1963) and Gray (1972) of external granule amacrines revealed that at high (4,000X) magnification these neurons demonstrated a round nucleus, sparse cytoplasm, and amacrine trunks. The external granule neurons observed in this study (Figs. 3 & 4) correspond
closely to electron micrographs of the external granule amacrines taken by previous workers. One, in fact, was seen giving off an amacrine trunk. The fact that these neurons are tightly packed together, and are joined at points by puncta adherentia, points toward their depolarizing together as functional units.

B. Plexiform Layer:

This layer, as previously stated, consists entirely of neuropil-tracts of axons and dendrites from various neuronal groups synapsing with one another.

Dilly, Gray and Young (1963) described one type of synapse found in the plexiform layer—the axon terminals of retinal neurons synapsing with the dendrites of external granule neurons. The retinal neuron axon terminals, Gray et al. reported, were carrot-shaped bags tightly packed with small (400 Å) agranular round vesicles. These axon terminals were seen to synapse with dendritic thorns which, by using serial section techniques, Gray (1972) determined belonged to external granule amacrine neurons. The axon terminal in Fig. 5 fits the description of a retinal cell terminal bouton given by Gray, and so is deemed to be one.

Dilly, Gray and Young (1963) described a second type of axon terminal in the plexiform layer. It was a large (2μ) terminal containing loosely-packed agranular vesicles. It
was seen to contact dendritic thorns. Dilly et al. showed that this axon terminal arose from external granule amacrines and synapsed upon the dendrites of inner granule bipolar neurons. I believe that in this study an example of such an external granule amacrine-bipolar synapse is shown in Figure 6, as it conforms to the description of the one given in Dill et al. (1963). An unexpected finding regarding these synapses was the presence of dendrites containing synaptic vesicles contacting the supposed inner granule bipolar dendrites. Figures six and seven both show external granule axon terminals contacting bipolar dendritic thorns which are in turn contacted by dendritic thorns containing synaptic vesicles. To my knowledge, no one has previously described dendro-dendritic synapses in the plexiform layer of the optic lobe cortex.

It is postulated that these dendrites with synaptic vesicles contacting the bipolar dendrites are arising from inner granule amacrine neurons. Young (1962a) described with the light microscope inner granule amacrines whose processes ramified among inner granule bipolar dendrites. Amacrines, it is known, exist in the inner nuclear layer of the primate retina. Their trunks make dendro-dendritic synapses with the dendrites of the ganglion cells (whose dendrites also synapse with bipolar cell axons) and these
synapses are of a reciprocal nature, in that there are synaptic vesicles on either side of the synaptic cleft (Dowling & Boycott, 1969). As the retinal-external granule cell synapses and external granule-bipolar cell synapses have already been characterized by electron microscopy (Dilly, Gray and Young, 1963), these dendro-dendritic synapses, by process of elimination, might stem from inner granule amacrines.

With regard to the role of the inner amacrine neurons, one can only postulate at this time, as no physiological studies have been performed on optic lobe neurons. Dowling and Boycott (1969) allude to amacrines in the primate retina as playing a role in neuronal adaptation phenomena. Dendro-dendritic synapses have also been observed in the olfactory bulb in the rat (Rall et. al. 1966), (Hinds, 1970), between granule cell dendrites and mitral cell dendrites. These synapses resemble those I have observed in that in both cases, the pre- and post synaptic dendrites contain agranular vesicles, (see Figs. 6 & 7) and the synaptic cleft is narrow (about 200 Å wide). Here too, the granule cells are thought to play a damping role, habituating olfactory mitral cells to a given impulse frequency (Rall & Shepard, 1968).

Perhaps inner amacrines play a similar role in the optic lobes of the octopus, inhibiting bipolar dendrites to
dampen the spread of neurons excitation triggered by continuous light impulses of a given frequency hitting the retina.

C. Inner Granule Layer Neurons:

Young (1962a) identified five neuronal types within the inner granule layer of the optic lobe cortex; (1) bipolar centripetal neurons, (2) unipolar centripetal neurons, (3) amacrines, (4) centrifugal neurons, and (5) multipolar neurons. These were identified by use of light microscopic studies on golgi preparations.

I believe I have tentatively identified with the electron microscope certain neuronal counterparts to Young's neuronal types:

(1) Inner granule amacrines -

Young characterized these neurons as being small (compared to other neuronal types) round neurons closely packed together and giving off only one process—an amacrine trunk. Numerous small (having a nuclear diameter of 5 μ or more) round neurons with sparse cytoplasm can be seen in Fig. 9 & 10. A trunk-like process can be seen arising from one in Fig. 11; this phenomena plus the fact that these small neurons crowd together lends credence to the idea that they might be inner granule amacrines.

(2) Inner granule bipolar centripetal cells-
Young described these cells as being good-sized neurons with a fusiform perikaryon and a process emerging from either end. Such a neuron can be seen in Fig. 11. It can be seen to be appreciably larger (having a nuclear diameter of 9 μ) than the neighboring amacrine neurons, and is definitely fusiform shaped.

Another such fusiform neuron can be seen in Fig. 12. Strong evidence for concluding that this oval neuron is indeed a different type than the small round amacrine type is seen in Fig. 14. The larger, oval neuron is strongly suggestive of Young's fusiform bipolar, and is obviously a different cell type from the amacrine neuron lying next to it. These bipolar neurons, as was previously mentioned, Young referred to as classifier cells, as they are thought to react as units to various degrees of retinal stimulation.

I have not as yet encountered any large unipolar neurons which might correspond to Young's unipolar centrifugal neuron's, and, therefore, would not venture to guess as to their appearance.

(3) Centrifugal neurons -

Young described these neurons as being large neurons with irregular outlines. They are, he reported, much less numerous than the centripetal neurons. A neuron fitting Young's description of a centrifugal neuron can be seen in
Fig. 13. A large (nuclear diameter of 10μ) neuron with irregular outlines can be seen lying next to some smaller amacrine neurons.

(4) Small multipolar neurons - Although Young claimed to find many of these small neurons among the inner granule layer cells, his observation was not reflected in this study.

With regard to the types of synapses present in the inner granule layer, very few axon terminals were found in the vicinity of the small round amacrine neurons, or the centrifugal neurons. Numerous axon terminals were seen in the immediate vicinity of the oval, bipolar classifier neurons, however.

These neurons are often seen to have axon terminals synapse on their perikarya (Figs. 15-17). As was pointed out earlier in this thesis, these axon terminals possess small, agranule synaptic vesicles and a narrow, synaptic cleft about 200 Å wide, with no pre or post-synaptic thickenings discernible. In this sense these axosomatic synapses seem to possess characteristics of both Gray type I synapses and Gray type II synapses. Gray type I synapses are characterized by axon terminals with round, agranular vesicles and a wide (400 Å) synaptic cleft with pre and post-synaptic thickenings visible. This type of terminal is usually
associated with cholinergic excitatory neurons (Gray, 1962b, Bodian, 1968).

Gray type II synapses are characterized by axon terminals with flattened vesicles, narrow (200 Å) synaptic clefts, and little or no pre or post-synaptic thickening present. These synapses were originally associated with inhibitory neurons (Gray, 1962b).

It can be seen that the axosomatic synapses found on the inner granule bipolar neurons possess properties of both type I and type II synapses, in that they contain round, not flattened, vesicles, and yet a narrow, not wide synaptic cleft. Thus, ventures as to the physiological function of these axosomatic synapses is beyond the scope of this thesis.

The rashness of assigning a physiological role to a synapse on the basis of its morphology has become increasingly clear to other workers, in light of the spectrum of synaptic types recently discovered which are neither distinct type I or type II synapses. In the words of Peters, et al. (1970), "It is now clear that Gray's classification of synapses into types I and II is too rigid. Certainly, these two types of synapse may be readily identified, but they seem to represent extremes in a continuous series of morphological variations. It must unfortunately be concluded that in the present state of our knowledge it is premature to
attempt a classification of chemical synapses, let alone to draw conclusions about their functions."

In spite of the dearth of supportive physiological evidence regarding their function, I would like to speculate that these axosomatic synapses upon the bipolar classifiers are excitatory to these neurons. It is postulated that these terminals represent the endings of vertical-subvertical efferent fibers — those which maintain the classifiers at a lowered threshold for future quick responses.

It was previously mentioned that axon terminals contacting the mostly bipolar, oval classifiers were themselves sometimes contacted by axon terminals (Figs. 19, 20 and 18).

Numerous cases of axo-axonic synapses have been reported in the literature (Gray, 1962; Gabel and Dubner, 1969; Pappas, et.al. 1966). Eccles (1961) proposed that these axoaxonic synaptic complexes form the morphological basis for pre-synaptic inhibition. The proposed mechanism involves depolarization of the post-synaptic axon, which is pre-synaptic to a cell body or dendrite. It is postulated that the axon terminals inhibiting the axosomatic terminals of the classifier cells are from the small multipolar cells theorized by Young to be activated by pain fibers projected to the optic lobe.

D. Tangential Cell Layer of the Medulla of the Optic Lobe:
Young described this layer as being composed of large bipolar neurons and smaller multipolar neurons. Their axons and dendrites were to be running horizontally across axons of the classifier cells (Young, 1962). Ultra-thin sections precluded the observance of many cells with dendrites or axons continuous with the perikaryon. Thus, it can only be postulated that the large, pale neurons seen in the tangential cell layer (Figs. 26-32) correspond to the large bipolar tangential cells. By process of elimination, the smaller neurons with round, gray nuclei (Figs. 26, 27, 28, 30, 33) would of necessity be the tangential multipolar cells.

Tangential cells are known to exist in the primate retina (Dowling and Boycott, 1969) and are thought to be responsible for "pooling" of retinal fields. Perhaps the tangential cells of the octopus optic lobe perform the same functions: recruiting distant groups of classifier cells for propagation of a localized retinal stimulation.

E. Cell Islands in the Deep Medulla of the Optic Lobe:

The deep medulla consists of islands of large, pale, neurons, surrounded by fibrous glia (Figs. 39 & 40). It is postulated that these large pale neurons are Young's attack-retract "command cells."

These large, pale neurons often receive axon terminals upon their perikarya (Figs. 46, 45, 44). It is postulated
that these axon terminals (since they contain round, agran-
ular vesicles) are excitatory axons from classifier cells.
Occasionally, axo-somatic synapses on the command cells are
themselves contacted by vesicle-packed axon terminals, as
when observed among the classifier cells. It is postulated
these are inhibitory fibers from small multipolar neurons.
That these small multipolar cells occur among the large pale
command neurons can be inferred from several photomicro-
graphs, such as Fig. 49, showing a very small neuron situa-
ted among the larger command cells in a medullary cell
island.

It would be in order at this time, to consider Young's
1969 schema for the octopus' visual memory system, and
attempt to correlate his neuronal components (as observed
with light microscopy) with their counterparts as observed
with electron microscopy by myself.

Figure forty-eight shows a diagram of the octopus
visual memory system. It is based on a theory by Young
(1969) but modified at key places to conform to what I feel
are pertinent data concerning the system. Briefly, refer-
ring to the diagram, the visual memory system as theorized
by Young and myself works as follows: Light impulses are
relayed via the retinal cells to the external granule cells,
which relay them to the dendrites of the bipolar or unipolar
classifying cells in the inner granule layer. The classifying cells, with bifurcated axons, relay the visual input to "command cells" in the optic lobe medullary cell islands. The command cells in the medulla initiate an attack by the animal upon whatever object was perceived by a given set of classifying cells. The classifying cells also relay visual information to the four lobes of the vertical lobe circuit. These process visual, tactile and gustatory sensations from the object consumed and relay signals back to the classifiers to keep them at a lower threshold. Then when the octopus perceives a similar object, that particular set of classifying cells, being held at a lower threshold by the vertical lobe output, will readily initiate an attack response again.

On the other hand, let us suppose the octopus attacked a victim which retaliated with a response painful to the octopus. Pain impulses from the octopus' tentacles (which are known to project to the vertical lobe and back to lower centers along the same circuits) would be amplified in the vertical lobe system and project into the optic lobe. There they could initiate a dual response. (i) They are thought to stimulate certain command cells in the medullary cell islands into a retreat response. (ii) They are thought to stimulate small multipolar
cells into inhibiting the excitation of the "attack" command cells by the bipolar classifiers. These small cells occur at two sites -- among the classifier perikarya, and around the command cell perikarya. I think I found evidence which points to their existence in both places. In addition, pain fibers are thought by Young to block the outflow of impulses from the vertical lobe to the classifying cells.

F. Glial Cells:

As discussed in the Observations section, three types of glia can be discerned in the optic lobe of the octopus-fibrous glia, protoplasmic glia and "dark" cells. Identification of the glial types is based on Gray's (1969) article on glia in the octopus brain; and Young's (1969) article on the same subject. Fibrous glia are ubiquitous in the optic lobe. Their fibrous process can be seen radiating away from their cell bodies in Fig. 47.

Protoplasmic glia were identified on the basis of Gray's electromicrographs and Young's descriptions (Figs. 14 & 31).

The enigmatic "dark" cells, first described by Gray (1969) remain a mystery. Gray theorized they were a form of glia, but did not attempt to categorize them. As previously shown, these cells exist in large numbers in the inner granule cell layer and tangential layer. They are often wrapped around inner granule bipolar cells (Figs. 9,
10, 22, 23, 24) and the tangential neurons (Figs. 28, 29, 32 & 33).

Their role in the functioning of the optic lobe circuits remains a mystery, but some speculations may be offered:

A. The way the dark cells wrap around neuronal perikarya suggests they may have a tropic influence upon these neuron's excitability. Recent reports indicate glia may be able to influence neuron excitability by altering potassium levels in the neighboring extracellular fluid. Baylor and Nicholls (1969) speculated that a long-term signalling mechanism mediated by extracellular potassium could operate in some neural systems, and that glial structures might segregate neuronal processes into groups which could interact by way of extracellular potassium. Recent studies by Trachtenberg and Pollen (1970) demonstrate that astrocytic processes in cat cortex can take up excess extracellular potassium; giving further weight to the hypothesis that glial cells maintain or modify potassium concentrations in the extracellular milieu (Kuffler & Nicholls, 1966; Rosenbluth, 1968).

B. The way the dark cells wrap around the classifiers and tangential cells points to a possible role for them as part of a memory storage system involving the classifying
cells. Egyházi and Hyden (1962) maintain that glial cells in the surround of active neurons release RNA. Concurrently active neurons are assumed to establish a potential gradient resulting in electrophoretic migration of glial RNA into the simultaneously active neurons. This invasion is analogous to a viral RNA infection, which results in modification of the invaded neurons so as to produce proteins specified by the invading RNA. The newly synthesized protein is assumed to "tune" cellular membranes to a frequency-specific sensitivity corresponding to the temporal pattern of stimulation caused by the represented event (John, 1967). Evidence that glial do exchange RNA with neurons can be seen in Fig. 38 where a "dark" glial cell can be seen invaginating a tangential neuron to the point where the glial cell is almost touching the neuron's nuclear membrane.

C. Recent evidence points to the fact that glia may interact with neurons via electrochemical means. Pru and Briceno (1972) reported observing processes of astrocytes in apparent synaptic contact with dendrites of mitral cells in the rat olfactory bulb. Kemp and Powell (1971) reported observing synaptic vesicles in glial processes in the caudate nucleus of the cat. As previously mentioned, swellings were observed along dark cell processes, some of which can be seen to contain small round vesicles (Figs. 23, 36 & 37). Thus,
the "dark" cells may be glia modifying the electrical output of the closely attached classifying and tangential cells.
VI. CONCLUSIONS

In light of the preceding evidence, I feel the following statements can be made concerning the optic lobe of the octopus:

(1) External granule amacrine neurons, inner granule amacrine and bipolar neurons, tangential neurons and deep medullary neurons have all been tentatively identified in electron micrographs on the basis of their morphology and location.

(2) The arrangement of glial cells among the neurons in the optic lobe points to their playing a significant role in the economy of the octopus visual memory system.

(3) Certain synaptic arrangements seen in certain neurons in the optic lobe (i.e. classifying cells and command cells) lend evidence to the belief that they play integral roles in the octopus visual memory system.
Literature Cited


Lane, F. 1960, Kingdom of the Octopus. Dell, New York.


Figure 1. Schematic drawings of the lobes of the octopus brain.
Figure 1a. A diagram of a transverse section through the optic lobe of the octopus brain.
Figure 2. Amacrine cells of external granule layer. X1,600.
Figure 3. Amacrine cells of the external granule layer. One can be seen giving off a trunk (AT) towards the plexiform layer. X14,000.
Figure 4. Higher magnification of external granule amacrine. One contains a lamellated body (LB), and appears to contact another amacrine via a puncta adhaeren-tia (PA). X30,000.
Figure 5. An axon terminal (AT) of a retinal cell contacting an external granule cell dendrite (DEN). X 48,000.
Figure 6. Presumed inner granule amacrine dendrite (DEN 1) contacting bipolar cell dendrite (DEN 2) also being contacted by external granule cell axon terminal (AT). X48,000.
Figure 7. Dendrite of inner granule amacrine (DEN 1) contacting dendrite of bipolar cell (DEN 2) which is also receiving synapse from external granule cell axon terminal (AT). X48,000.
Figure 8. Cells of inner granule layer. Many small amacrine (AM) can be seen, as well as a centrifugal cell (CF). X1,600.
Figure 9. Amacrine (AM) of Inner granule layer. Several "dark" cells can be seen (DC). X4,000.
PLATE X

EXPLANATION OF FIGURE

Figure 10. Inner granule cell layer. Several amacrine (AN) can be observed, as well as bipolar cell (BI) "dark" cells (DC) and a fibrous glia (FG) can also be observed. X4,000.
Figure 11. Several Inner granule amacrine (AM) and a fibrous glia (FG) can be seen. X14,000.
Figure 12. An inner granule bipolar cell (BI) can be seen. X14,000.
Figure 13. Several inner granule amacrine (AM) and a possible centrifugal cell (CF) can be seen. X14,000.
Figure 14. Cells of Inner granule layer. An amacrine (AM) lies next to a bipolar neuron (BI). A protoplasmic glia (PG), a fibrous glia (FG) and a "dark" cell (DC) can also be observed. X8,000.
Figure 15. An axon terminal (AT) synapsing on an inner granule bipolar neuron (BI). X48,000.
Figure 16. An axon terminal (AT) synapsing on an inner granule bipolar neuron (BI). X14,000.
Figure 17. An axon terminal (AT) synapsing on an inner granule bipolar neuron (BI). X30,000.
Figure 18. An axon terminal (AT₁) synapsing on an inner granule bipolar neuron (BI) being contacted by another axon terminal (AT₂). X48,000.
Figure 19. An axon terminal (AT₁) synapsing on a bipolar neuron of the inner granule layer (BI) and being contacted itself by an axon terminal (AT₂). X30,000.
Figure 20. An axon terminal (AT₁) synapsing on a bipolar neuron (BI) and being contacted itself by an axon terminal (AT₂). X30,000.
Figure 21. Possible puncta adhearentia between two inner granule amacrine cells (PA). X30,000.
Figure 22. "Dark" cells (DC) among inner granule amacrine (AM). X14,000.
Figure 23. "Dark" cell swelling (DCS) in inner granule layer synapsing on neuron. (Notice synaptic vesicles) x22,000.
Figure 24. Inner granule cell bipolar (BI) being surrounded by "dark" cell (DC). X14,000.
Figure 25. End process of same "dark" cell as in Fig. 26. Note mitochondrion (MIT). X48,000.
Figure 26. Neurons of tangential layer. Bipolar (BI) and multipolar (MP) neurons can be seen. X1,600.
Figure 27. Neurons of tangential cell layer. Bipolar (BI) and multipolar (MP) neurons can be seen. Note "dark" cell (DC) surrounding multipolar neuron. X1,600.
Figure 28. Neurons of tangential cell layer. Bipolar (BI) and multipolar (MP) can be seen. Notice "dark" cell surrounding bipolar neuron. X4,000.
Figure 29. Neurons of tangential layer. Bipolar (BI), multipolar (MP) neurons can be seen, along with several "dark" cells (DC). X4,000.
Figure 30. Cells of tangential layer. Bipolar neurons and multipolar neurons (BI) and (MP) can be seen alongside a small vesicle (V). A fibrous glia (FG) can also be seen. X5,000.
Figure 31. A tangential bipolar neuron (BI) surrounded by fibrous glia (FG). Two protoplasmic glia (PG) can also be seen. X48,000.
Figure 32. A tangential bipolar neuron (BI) surrounded by "dark" cell. X8,000.
Figure 33. A tangential multipolar cell (MP) being surrounded by a "dark" cell (DC). X14,000.
Figure 34. A "dark" cell process (DCP) alongside a tangential bipolar (BI) neuron. X48,000.
Figure 35. A "dark" cell lying against a tangential bipolar. Its cytoplasm (C) is almost as basophilic as is nucleus (NuC). X48,000.
Figure 36. A "dark" cell against a tangential multipo lar cell. Notice swelling dark cell (DCS) filled with vesicles. X8,000.
Figure 37. "Dark" cell (DC) contacting large axon terminal (AT) in tangential layer via swelling with vesicles (DCS). X14,000.
Figure 38. Dark cell (DC) invaginating cytoplasm of Bipolar cell (BI). X22,000.
Figure 39. Cell island in deep medulla. Notice command neuron (CO) surrounded by fibrous glia (FG). Some of their glial process (GP) can be seen. X8,000.
Figure 40. Cell island in deep medulla. Command neuron (CO) surrounded by fibrous glia (FG). X8,000.
Figure 41. Large pale command neuron (CO) in deep medulla. Notice adjacent axoaxonic synapse (AA); the same one as in Fig. 43. X12,000.
Figure 42. Axon terminal (AT₁) against command neuron (CO) being contacted by another axon terminal (AT₂). X48,000
PLATE XLIII
EXPLANATION OF FIGURE

Figure 43. Same command cell (CO) as in Fig. 41 being contacted by axon terminal (AT₁) which in turn is being contacted by another axon terminal (AT₂). X68,000.
Figure 44. Command cell (CO) of deep medulla being contacted by axon terminal (AT). X48,000.
Figure 45. Command cell of deep medulla (CO) being contacted by axon terminal (AT) containing giant vesicles. X48,000.
Figure 46. Command cell (CO) of deep medulla bearing thorn (T) which is receiving synapse from an axon terminal (AT). X48,000.
Figure 47. Fibrous glia (FG) in deep medulla. X3,000.
Figure 48. Diagram modified from Young (1965) showing interrelationship between the four lobes of the vertical lobe circuit and the optic lobe neurons participating in the octopus visual memory system.
Figure 49. Cell island in deep medulla. Several command neurons (CO) can be seen as well as a small multipolar neuron (SM). X8,000.
The thesis submitted by Donald B. Newman has been read and approved by members of the faculty of the Graduate School of Loyola University of Chicago.

The final copies have been examined by the chairman of the thesis committee and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form and accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

04/15/73
Signature of Advisor

Date