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A STUDY OF INFORMATION PROCESSING IN THE NECTURUS RETINA
BY USING SPECIAL PATTERN STIMULUS AND DRUGS

The Ohio State University

Ph.D. 1982

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A STUDY OF INFORMATION PROCESSING IN THE NECTURUS RETINA BY USING
SPECIAL PATTERN STIMULUS AND DRUGS.

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Ricardo Sanchez, MSc.

* * * *

The Ohio State University
1932

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Adviser
Department of Electrical Engineering
To my wife and son.
ACKNOWLEDGMENTS

I would like to express my deep and loving appreciation to my wife for her constant encouragement and loving care and to my son for bringing a every day happiness to my home.

My most sincere gratitude to my adviser J. M. Jagadeesh for his continuos support during all my Ph. D program. I also would like to thanks my committee for their important suggestions and contributions to this research and to In-Ching Chen for his valious help in the hardware construction of the pattern generator system.
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FIELDS OF STUDY

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Bioengineering.

Control System

Digital System.
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CHAPTER I

INTRODUCTION

Visual information is perhaps the most important one among the sensory system for all human beings and other species as well. Considerable progress has been made in understanding the visual processes in recent years. These processes are extremely complex and require delicate and often, time consuming techniques to obtain meaningful information. It is also considered that understanding the visual processes would be extremely helpful in investigating the function of highly developed, even more complex nervous systems.

Because of the complexity of the vertebrate retina it is desirable to investigate the functioning of the retina of lower species to be able to narrow the search in higher species. The retina of Necturus Maculosus (mudpuppy) has been well studied in terms of its morphology and major neuronal functions (Dowling and Werblin, 1969). It is also known that the cells of the Necturus retina are sufficiently large, thus it is relatively easy to make intracellular recordings. Therefore in this research Necturus was choosen as the experimental species.

One approach to investigate the function of the retina is to use chemical substances with known relationship to various neurotransmitters and receptors found in the retina.
Also different patterns have been used to identify the various cells of the retina and to investigate the interactions between the cells. Most of the systems used to generate such patterns require complex set-up making it difficult and time consuming for the researchers to conduct experiments. It becomes particularly frustrating when the researcher has to fuss with many adjustments and optical set-up while he is concerned about the stability and short duration of the biological recording. With the advent of new technologies it has become feasible to construct a single system that can incorporate all the patterns used previously and to provide the flexibility to the researcher to create his own patterns.

The main objective of this dissertation is to investigate the function of horizontal, depolarizing and hyperpolarizing bipolar cells, ganglion cells and the electroretinogram to provide some insight into the information processing carried out by the retina.

To accomplish this objective a new pattern generator system is to be developed. The system should be capable of generating all the patterns previously used by the researchers and some new patterns such as rotating bar to study the symmetry of the receptive field of the retinal cells. The possible existence of nicotinic type of receptors in the outer nuclear layer is to be studied along with the response of various cells to light stimuli. The following chapters describe how the objective was accomplished.
chapter ii

review of intracellular recording on necturus maculosus.

2.1 morphology and synaptic structure of necturus retinal cells.

in 1969 john e. Dowling and frank s. werblin published a comprehensive morphology study of the synaptic structure of retinal cells of Necturus. They proposed a model for the neuronal connections in the Necturus retina which gave great insight into the function of the retina as a information processing system. Paul K. Brown, I. R. Gibbons and George Wald (1963) studied in detail the visual pigments in the visual cells of Necturus and found much similarity with visual cells of other vertebrates. After the work of Dowling and Werblin, there has been considerable activity in trying to elucidate the synaptic organization of the retinal cells of the mudpuppy (Necturus Maculosus).

The short review presented in this chapter is based on the work of Dowling & Werblin, Paul Brown et. al. and scanning electron microscopy of mudpuppy retina done by the author and John M. Hanson at the Ohio State University.

A light micrograph of Necturus retina is shown in fig 2.1.1 cones are easily distinguished by the shape of the outer segments.
The outer nuclear layer (ONL) is about 45 to 50 μm thick and contains mainly receptor cell nuclei. However, perikarya of displaced horizontal and bipolar cells are sometimes found in the outer nuclear layer. The thickness of the outer plexiform layer is very irregular and ranges between 2 to 10 μm. The inner nuclear layer contains only two to three layers of cells and its thickness varies between 50 to 60 μm.
The more distal cells are bipolar and horizontal; while the more proximal cells are mostly amacrine cells. The inner plexiform layer is about 20 - 25 μm in thickness and proximal to it are the ganglion cells. Fig 2.1.2 shows a cross sectional view of the Necturus retina. The large size of almost all the cells can be easily appreciated. Fig 2.1.3 shows a close view of the rods and cones that can be easily distinguished by the form of the outer segment. From these scanning micrographs one can see that the rods and cones have approximately a diameter of about 10 μm and can be as large as 50 μm. If we approximate the form of the cell's body as a sphere we can see from fig 2.1.4 that almost all the cells have a diameter of about 20 to 30 μm.

Fig 2.1.5 shows the structural relations between rods and cone outer segments. The lamellar particles or micelles are thought to contain porphyropsin, molecule which is involved in the receptor excitation.

In the rod all edges of the double membrane disc involve a differentiated rim structure. The discs are cut into lobules and the disc membranes contain a system of staining micelles in regular form. The lack of double layer discs is completely surrounded by a plasma membrane that extends toward the dendrites. In the cones the double layer lacks the special rim structure and lamellar micelles and is formed by repeatedly infolding the plasma membrane. The dendrites are mainly thought to be involved with the exchange of material between the outer and inner segments of the receptor cells. The pigment epithelial processes are clearly concerned with the exchange of materials between the outer segments and the pigment epithelium.
Fig 2.1.2. Scanning micrograph of Necturus retina.
The rods and cones can be easily identified by the shape of the outer segments. (Prepared by the author).
Fig 2.1.3. Rods and cones of Necturus retina.

(Scanning micrograph). (Prepared by the author).
Fig 2.1.4. Retinal cells of Necturus maculosus.
Observe the large size of these cells.
(Prepared by the author).
Fig 2.1.5. Structural relations between rod and cone outer segments in Necturus. (From Brown et al. 1963).

2.1.1. **Outer plexiform layer.**

In the outer plexiform layer the receptor terminals are grouped in the thicker portions of the layer. The grouping of receptor terminals often occurs adjacent to receptor cells that can be identified as cones but it is believed that the same type of grouping should occur adjacent to rod cells. Fig 2.16 shows some receptor terminals (RT) indicated by dark arrows.
Fig 2.1.6. Electron micrograph of outer plexiform layer.

RT: receptor terminals. Adapted from Dowling & Werblin, 1969.)
Cone terminals can often be easily identified by following the path from the cell to the outer segment. Usually the terminal portion of a cone cell is just below or adjacent to the nucleus. There are two kinds of synaptic processes at the cone terminals. They either penetrate deep portion of the terminal (fig 2.1.7). Synaptic ribbons are associated with both types of contacts as indicated by the dark arrows in fig 2.1.7. Rod terminals are often displaced laterally from the rest of the cell body and it is difficult to identify them. Fig 2.1.8 shows a case in which a rod terminal has been positively identified showing that synaptic ribbons are also associated with the superficial contacts. In this case one ribbons could have more than one contact point along the terminal.

Two types of contact processes have been found in Necturus, one type is called "invaginated process" and the other is called "superficial process". The invaginated process usually is large and goes deeply into the terminal. This type of process is shown in fig 2.1.9. In this case, one synaptic ribbon is associated with two or three processes. In Necturus, so far it has not been possible to define anatomical invagination processes for the horizontal and bipolar cells. Superficial contacts do not penetrate into the receptor terminals but just usually dent the surface of the terminal.

In the outer plexiform layer synaptic processes between receptors, horizontal and bipolar cells have been observed. Horizontal cell processes synapse mainly on bipolar cell dendrites and occasionally on other horizontal cell processes. Fig 2.1.10 shows horizontal cell making a synapse with the perikaryon of a bipolar cell.
Fig 2.1.7. Portion of a cone receptor terminal showing both
invaginated (IC) and superficial contacts (SC).
(From Dowling & Werblin, 1969).
Fig 2.1.8. Portion of a rod receptor terminal. Synaptic ribbons (filled arrows) are long, and some are arcuate.

(From Dowling & Werblin, 1969).
Fig 2.1.9. Invaginating process with one synaptic ribbon.
(From Dowling & Werblin, 1969).

Fig 2.1.10. Horizontal cell making conventional contact on the perikaryon of a bipolar cell. (From Dowling & Werblin, 1969).
There is no morphological evidence for horizontal cell processes going back into the receptors terminals. All the horizontal cell processes with the receptors are postsynaptic. Whether a horizontal bipolar cell interaction occurs in the invaginating processes has not been demonstrated yet. But it is clear that receptors make contact with both horizontal and bipolar cells mainly through invaginating processes. The horizontal cell processes into bipolar cells, seem to be all presynaptic type. Evidence has been given by Werblin & Dowling (1969) and Robert F. Miller et. al. (1976 a, b; 1978) to suggest that the synapse of horizontal cell into bipolar is negative i.e. tend to reduce the response of the bipolar cell.

2.1.2. Inner plexiform layer.

The inner plexiform layer seems to be much more complex than the outer plexiform layer since a greater variety of synaptic processes are observed in this layer. In the inner plexiform layer ribbon and conventional synapses have been observed. Ribbon synapses are characterized by a synaptic ribbon in the presynaptic site and conventional synapses are characterized by a cluster of vesicles in the presynaptic processes.

In Necturus as in other vertebrates there are many more conventional types of processes than the ribbon type. The ribbon synapses in other species (Dowling & Boycott 1966, H. Goodland 1966) are usually confined to the bipolar terminals and this seems also to be the case in Necturus. On the other hand, conventional synapses are common in amacrine cell processes in Necturus. Fig 2.1.11 shows a ribbon synaptic contact with two presumably postsynaptic amacrine cells.
Conventional contacts in the inner plexiform layer are morphologically similar to most synapses described in the nervous system. Basically they consist of a cluster of synaptic vesicles positioned close to a presumed presynaptic membrane.

Fig 2.1.11. Ribbon contact in the inner plexiform layer.

Fig 2.1.12. Shows a conventional contact in the inner plexiform layer where some amacrine cells have been presumed. The cluster of vesicles can be seen close to the presynaptic membrane.

Fig 2.1.12. Conventional contact in the inner plexiform layer.

(A) : presumed amacrine cell. (From Dowling & Werblin, 1969).
Amacrine cells make feedback synapses into bipolar terminals, feedforward synapses on ganglion cell dendrites and feedforward and lateral contacts on other amacrine cells. Some bipolar cells make no direct contact with ganglion cells but only with amacrine cells. However few bipolar cells processes have been identified making synapse with ganglion cells. Based on this morphological evidence Dowling & Werblin (1969) proposed the neural connections of the Necturus retina shown in fig 2.1.13.

![Schematic diagram proposed by Dowling & Werblin (1969)](image)

**Fig 2.1.13.** Schematic diagram proposed by Dowling & Werblin (1969) to shows the neural connection in the Necturus retina.


Dowling & Werblin proposed that at least two types of ganglion cells should be found in Necturus since some bipolar cells do not contact ganglion cells and others do contact ganglion cell.

In fact, in Necturus three types of ganglion cell have been recorded intracellularly, namely on-ganglion, off-ganglion and on-off ganglion. (Werblin & Dowling 1969; R. Miller & R. Dacheux 1976).

2.2. Intracellular recording and synaptic organization of Necturus retinal cells.

Due to the large size of Necturus retinal cells investigators have been able to make intracellular recording from all the cells. Bortoff A. (1964) was the first to succeed in recording from all the retinal cells of Necturus Maculosus. Later in 1969 Werblin & Dowling were able to identify each type of cell of the Necturus retina by using staining and intracellular recording methods. The pioneering work of Werblin & Dowling has provide an easy method for cell identification in Necturus and other species as well.

In recent years the synaptic organization of the neuronal cells of Necturus and also from others species has received a great deal of attention. (Miller & Dacheux 1976 a,b,c; Dacheux et. al. 1979; Frumkes & Miller 1979; Cunningham & Miller 1980 a,b; Fulton & Rushton 1978; Nelson 1973; Copenhagen 1975; Tuttle 1977).

The neuronal network of the Necturus retina seems to be less complex than the retina of fish and others vertebrates species. It is therefore expected that a thorough study of the organization of Necturus retina is accomplishable and could be a first step to the understanding of other more complex retinas.
2.2.1 **Receptor response.**

The receptor response to a light stimuli is a graded hyperpolarization with a rise time of about 150 to 200 msec for a step stimuli.

Fig 2.2.1. shows a typical response to a step stimuli of a Necturus receptor cell.

![Graph showing receptor response](image)

**Fig 2.2.1.** Receptor response to a spot and to a spot annulus stimuli. There is practically no difference between responses. (spot is approximately 100 μm on diameter and the annulus is 250 μm internal diameter. (From Werblin & Dowling, 1969).
The receptor response shows no antagonistic surround field. It is claimed that the receptive field of receptors could be as small as the size of one receptor cell. (Werblin & Dowling 1969). The magnitude of the response usually saturates at about 5 mv and the shape of the spectral sensitivities of rod is rather similar reaching their peaks at 525/μm and 575/μm respectively (Fig 2.2.2).

Fig 2.2.2. Spectral sensitivities of rods and cones of Necturus maculosus. The spectral sensitivity at each wavelength is the inverse of the intensity of light necessary to evoke a criterion response. The criterion response is one-half of the maximum light-evoked response. (From Fain & Dowling 1973).
2.2.2. **Horizontal cell response.**

The intracellular recordings from horizontal cells show a hyperpolarizing response which is graded and sustained with intensity. Typical magnitudes of horizontal cell responses are between 10 to 30 mV with resting potentials ranging from -30 to -40 mV. The receptive field of a horizontal cell is rather large since its response to an annulus of light is not much different from that obtained with a spot. In fact, a response to an annulus stimuli is generally greater as compared to a spot stimuli. This indicates that along with the large receptive field of horizontal cells there are also contributions of spatio-temporal summation from others horizontal cells. Fig 2.2.3 shows typical response of horizontal cell to spot and annulus stimuli.

![Diagram](Image)

**Fig 2.2.3.** Intracellular response from horizontal cell to a spot and annulus stimuli. (From Werblin & Dowling, 1969).
In Necturus there has been no observed change in polarity with different stimuli wavelength when recording horizontal cell response. An increase in the input resistance has always been observed during hyperpolarization (Miller & Dacheux 1976a; Schwartz 1976; Nelson 1973).

2.2.3. **Bipolar cell response.**

Two types of bipolar cells have been found in Necturus. One type depolarize to a light stimuli and the others hyperpolarize. Similar to receptors and horizontal cells they generate only relatively slow graded potentials to a stimuli. The typical magnitude of bipolar cell response is about 10 mV with a resting potential of about 31 mV. The receptive field is more complex as compared with that of receptors and horizontal cells. The receptive field of bipolar cells show two antagonistic zones which can be easily demonstrated by using a spot and an annulus as stimuli. A spot applied on the center field will give a response that can be changed in polarity upon the additional application of an annulus.

Fig 2.2.4 shows this effect very clearly.

![Bipolar cell response showing the antagonistic effect of an annulus upon a spot stimuli. (From Werblin & Dowling 1969).](image)
The response to central illumination always precedes the antagonism shown by peripheral illumination. The time delay between the center and peripheral response is approximately 100 msec. This characteristic behaviour of the receptive field of the bipolar cells has been demonstrated by several investigators (Shantz & Naka 1976, Miller & Dacheaux 1976 a,b,c; Frumkes & Miller 1979; Werblin 1969). Both depolarizing and hyperpolarizing bipolar cells have the peripheral antagonistic type of receptive field.

2.2.4. Amacrine cell response.

The light-evoked response of amacrine cells to a light stimulus consist typically of a few spikes superimposed on a slow graded depolarizing potential. Typical response of an amacrine cell to a diffused light stimuli is shown in fig 2.2.5.

![Amacrine cell response](image)

**Fig 2.2.5.** Amacrine cell response to diffuse light stimuli. The dark bar indicates the duration of the flash (From Miller & Dacheaux 1976 -c).
The receptive field of the amacrine cells has been difficult to determine. Some have receptive field which respond to on or off illumination of any portion of the field area and others have narrow center (100 to 200 μm) and broad peripheral field like the bipolar cells but without the antagonistic effect that characterize the bipolar cells. The latency of the spikes of the amacrine cell response changes dramatically with the intensity of the light stimuli. This effect is clearly shown in fig 2.2.6.

Fig 2.2.6. Latencies of the spike responses as a function of stimulus intensity in amacrine cells.
(From Werblin & Dowling 1969).
2.2.5. Ganglion cell response.

Three types of ganglion cells have been found in Necturus. These respond with a series of spikes to a "on", "off" and "on and off" light stimuli. Typical response of these three classes of cells are shown in fig 2.2.7.

Fig 2.2.7. Intracellular recording from on, off and on-off types of ganglion cells in Necturus. ○ - diffuse light.

○ - annulus. (From Miller & Dacheoux 1976-b).
The resting potentials of ganglion cells are quite variable but never exceed 40 mV. On and off hyperpolarization are usually not observed in on center and off center ganglion cell response. Usually it becomes somewhat difficult to differentiate between on-off ganglion cell and on-off amacrine cell. Miller & Dacheux (1976-b) have pointed out several differences between the responses of these two cells that facilitate the identification. Amacrine cells generate two types of spikes, a first large one followed by a series of short spikes, but ganglion cells give only class of continued spikes. The hyperpolarization that follow the first burst of spikes in ganglion cells is not observed in amacrine cells. A further difference is that intracellular depolarising current in ganglion cells produce a relatively sustained train of impulses while in amacrine cells it results in a single spike. On and off type of ganglion cells are very easy to differentiate from amacrine cells.

2.3. Synaptic organization of the Necturus neuronal cells.

Dowling & Werblin (1969) were the first in proposing a relatively detailed organization of the neuronal connection in the Necturus retina. (see section 2.1). This proposal was based mainly on morphological evidence and in the recent years it has been enhanced by the work of Werblin & Dowling (1969), Miller & Dacheux (1976 a,b,c), Frumkes & Miller (1979), Dacheux & Miller (1979). It is clear that a complete understanding of the retinal network of Necturus will give a great advancement in the function of the retina as a center for information processing.
2.3.1. Outer plexiform layer cell organization.

In the outer plexiform layer there are three types of neuronal cells that have synaptic processes, namely receptors, horizontal and bipolar cells (depolarizing and hyperpolarizing). Morphological evidence (Dowling & Werblin 1969) shows that receptors make contact with horizontal and bipolar cells. The horizontal and bipolar cells contact with receptors are always postsynaptic. No feedback into the receptors has been demonstrated so far, in Necturus (Dowling & Werblin 1969, Werblin 1974, Miller & Dacheux 1976–c).

The antagonistic region of the receptive field of bipolar cells has been proposed to be caused by the feedforward contact of horizontal cell to bipolar cells (Werblin & Dowling 1969, Miller & Dacheux 1976 c, Kaneko 1970). The center field of bipolar cells measure approximately 100 /im (Werblin & Dowling 1969) in diameter and the antagonistic region is best stimulated by annulus of 250 /im radius. The dendritic spread of the bipolar cell can not be accounted to cover all its receptive field (Dowling & Werblin 1969). However, horizontal cell processes extend laterally over distances of 200 to 400 /im which are very close to the dimension of the antagonistic region of bipolar cell. Thus the antagonistic effect on bipolar cell could be mediated by horizontal cell processes where their dendrites could easily reach the frontier of the bipolar receptive field. Miller & Dacheux (1976 a, b, c) have proposed that the separation of the on and off channels take place at the receptor-bipolar synaptic processes.
2.3.2. **Inner plexiform layer cell organization.**

Amacrine, ganglion and bipolar cells have synaptic processes in the inner plexiform layer. The evidence presented by Miller & Dacheux (1976 a,b,c) suggest that in the inner plexiform layer bipolar cells contacts ganglion cells in a differentiated way i.e. hyperpolarizing bipolar (HPB) cells contact off ganglion cells, depolarizing bipolar cells (DPB) contact on ganglion and on-off ganglion cells receive input from both HPB and DPB cells (fig 2.3.1). The off ganglion cell activity persists in a chloride-free environment but its surround excitation is abolished.

Since HPB do not loose activity in the same medium a direct connection between HPB and off ganglion cell is postulated (Miller & Dacheux, 1976 b,c). It is also suggested that HPB cell releases an excitatory transmitter in the dark and that the light-evoked hyperpolarization results in a reduced level of transmitter release. This is also consistent with the observation that in cat, off ganglion cells have a higher level of spontaneous activity than on-center cells (Jung 1964; Barlow & Levick 1969). It is suggested that the input from DPB cell into on-center ganglion is excitatory since depolarization of bipolar cells lead to impulse activity in the ganglion cell.

On-off ganglion cells loose their on response in a chloride-free medium which is in concordance with the fact that DPB loose their activity in this type of medium. The inhibitory surround of on-off ganglion cells are assumed to be mediated by amacrine cells since horizontal cells have no contact in this layer (Barlow 1953; Miles 1972; Schwartz 1972; Miller & Dacheoux 1976 a,b,c).
Miller & Dacheux (1976) have found that in a chloride-free environment the response of horizontal cells are abolished. However, the response of the hyperpolarizing bipolar cell (HPB) is only partially reduced since the off-ganglion cell activity still persists in this type of medium. They have postulated that a separation of off and on channels occurs at the outer plexiform layer. This channel separation is shown in fig 2.3.1. In this scheme the HPB cells contact the off-ganglion cell, the DPB cells contact the on-ganglion cell and both on and off bipolar cells contact on and off ganglion cells.

![Diagram](image)

Fig 2.3.1. On and off channels separation at the outer plexiform layer. (From Miller & Dacheux 1976 - c).
It is thought that amacrine cells have input from both DFB and HPB cells since the on response is lost in a chloride-free environment, which is very similar to on-off ganglion cells (Miller & Dacheux 1976 b-c; Werblin 1972, Toyoda 1973). Also amacrine cells may have direct input into the three types of ganglion cells and feedback into bipolar cells. (R. Cunningham & Miller 1980; Chan & Naka 1976).

2.4. Effects of several substances on the vertebrate retinal cells.

Chemical substances have become an important tool to study the functional organization of the cells of the retina. Identification and characterization of neurotransmitters in the retina and their role in the retinal function has been receiving a great deal of attention in recent years. L. T. Graham (1974), Boning (1976) & Neal (1976) have given an extensive review on the role of several chemicals (drugs, ions, amino acids etc.) as neurotransmitters in the retina. The work is extensive and hence a short summary an attempt is made to give the most important facts on the use of chemicals on Necturus retina and other vertebrates.

2.4.1. Receptors and horizontal cells.

Receptor cells responses from vertebrates retinas have been affected very little by chemicals. Murakami, Ohtsu & Ohtsuka (1972) applied atomized chemical solutions on gecko and carp retina. The chemicals were sodium L-glutamate and L-aspartate, glycine, Acetylcholine and Gaba. All these chemicals had no appreciable effect on the photoreceptor intracellular response. Unfortunately since they used atomized chemicals the exact concentration of the solutions can not be determined,
Cervetto & MacNickol (1972) superfused the eye-cup of turtle with 50 mM of glutamic acid. They found no appreciable change in the light-evoked response of receptors but the horizontal cell response was completely abolished (fig 2.4.1).

Fig 2.4.1. Effect of glutamic acid on the light-evoked response of receptor and horizontal cell of turtle. X: control solution, G: 50 mM glutamic acid (Cervetto & MacNichol, 1972).
Murakami et al. (1972) also found that L-glutamate and L-aspartate depolarized the horizontal cell response in the carp retina until the abolition of the response was almost complete. It has been postulated (Cervetto & MacNichols 1972, Murakami et al. 1972; Dowling & Ripps, 1972; Sugawara & Negishi, 1973) that glutamate and aspartate could be possible neurotransmitters in the outer plexiform layer. However questions have been raised since the concentration needed to affect the response of the horizontal cell and ERG is very high as compared to other chemicals found in the nervous system (Hanawa & Tateishi, 1970).

Dowling & Ripps (1973) demonstrated that a high concentration of magnesium chloride (100 mM) hyperpolarized the membrane potential of the skate retina until the horizontal cell response was completely abolished. They postulated that this effect is due to a decrease in the release of neurotransmitter from the receptors due to the high concentration of magnesium ions in the bath solution.

It has been shown that Cobalt ions have a relatively faster effect on horizontal cell response than other ions such as magnesium and calcium. Cervetto & Piccolino (1974) showed that in turtle retina 50 mM of CoCl$_2$ produced almost no change in the light evoked receptor response but depleted almost completely the response of horizontal cells. The same effects of CoCl$_2$ on Necturus was found by Cunningham & Miller (1980), Evans et al. (1978) found that concentration of up to 2.4 mM of CoCl do not affect the light-evoked response of receptors in frog retina but concentrations of about 5 mM or greater abolished it.

Thus the candidates for neurotransmitters in the outer plexiform layer are still glutamate and aspartate but as it was explained earlier
several others substances produce the same effects of these two amino acids which make it more difficult to accept them as the only possible neurotransmitters.

2.4.2. Bipolar cell.

Since bipolar cells have synaptic processes in both the inner and outer plexiform layers it becomes more difficult to localize the effect of chemicals substances on this cells. Murakami, Ohtsuka & Shimazaki (1975) studied the effect of glutamate and aspartate on the bipolar cells of the carp retina and found that the off-cells were depolarized and the light-evoked response was completely abolished by the action of these amino acids. Due to the small size of the bipolar cells of the carp retina they used a nebulizing system to apply the amino acid, but did not state the exact measure of the concentration of the chemicals.

Since glutamate and aspartate is found in the outer plexiform layer of frog (Kennedy & Voaden, 1974) these authors postulated that the action of these amino acids on bipolar cells could be localized at the receptors-bipolar synaptic processes.

Glycine and taurine had been found in the vertebrate retinas of rat, frog, chicken and goldfish (Kubicek & Doloneck, 1958; Brotherton, 1962; Pasantes-Morales et. al. 1972; Cohen et. al. 1973; Starr, 1973). The concentration of taurine in the whole vertebrate retina is much higher than in the CNS while glycine concentrations are similar to those found in the CNS. It has been shown that glycine depresses the b-wave of the in vitro carp ERG (Murakami et. al. 1972) and taurine depresses the b-wave of the electroretinogram of chicken (Pasantes-Morales et. al. 1973).
Cunningham & Miller (1980) studied the effect of glycine and taurine in the isolated retina of Necturus and found that these amino acids affect both hyperpolarizing and depolarizing bipolar light-evoked cell responses. 2.5 mM of taurine and glycine reduce the intracellular response of hyperpolarizing bipolar cells (HPB). Depolarizing bipolar cells (DFB) were also affected by these amino acids but less significantly as compared to HPB cells (see fig 2.4.2).

**HYPERPOLARIZING BIPOLAR CELLS**

![Hyperpolarizing Bipolar Cells](image)

**DEPOLARIZING BIPOLAR CELL**

![Depolarizing Bipolar Cell](image)

Fig 2.4.2. Glycine and taurine effects on the intracellular response of both depolarising and hyperpolarising bipolar cells. (From Cunningham & Miller, 1980).
GABA also decreased the amplitude of DPB cell response as shown in fig 2.4.1 but its action proved to be much less effective on HPB cell. They also found that CoCL has a strong depressing effect on the intracellular response of both types of bipolar cells in Necturus (Gervetto & MacNichol, 1972).

2.4.3. Amacrine and ganglion cells.

Recently, Cunningham & Miller (1980) have done a good study on the effect of glycine and taurine in amacrine and ganglion cells of Necturus. These amino acids have also been extensively studied in other species namely in rats, frog, chicken and goldfish (Graham, 1974).

In Necturus they rapidly reduce the intracellular response of amacrine and the three types of ganglion cells (see fig 2.4.3). These authors postulate that taurine or glycine could be an amacrine transmitter and that the possible taurine/glycine-sensitive neurous may be postsynaptic to amacrine cells. Since the HPB cell is much more sensitive to glycine than it is to GABA (see section 2.4.2) they presumed that a glycnergic amacrine cell may feedback onto the HPB cell while a GABAergic amacrine interacts with the DPB cells.

GABA has also been found to have inhibitory effects on the retinal light-evoked response of other species. Kishida & Naka (1968) showed that GABA inhibited spike discharges in ganglion cells of the isolated bullfrog retina in vitro and straschill (1968) showed that intracarotid injections of GABA depressed both the spontaneous and light-evoked response of cat retinal ganglion cells. Ames & Pollen (1969) also found that GABA always depressed the spontaneous activity of ganglion cells in the rabbit retina.
Fig 2.4.3. Effects of taurine and glycine on the intracellular response of amacrine and ganglion cells.

(From Cunningham & Miller, 1980).
Miller & Dacheux (1976 a,b) studied the activity of the Hecturus amacrine and ganglion cells when the retina was superfused with chloride-free ringer solution. In this environment amacrine cells always lost the light-evoked on-depolarization and off ganglion cells lack the capability of surround excitation. On-off ganglion cells lost the on-discharge but maintained the off light-evoked response. Acetylcholine (ACh) had been another frug that had been studied in the vertebrate retina (Graham, 1974). However up to now there had been no positive localization of the ACh action in the vertebrate retina. Graham & Pong (1973) studied the relative activity of cholineacetylase in pigeon, rat, frog and mupuppy. Their results are shown in fig 2.4.4. The distribution of cholineacetylase in mupuppy seems to be almost the same for all the retinal layers.

Fig 2.4.4. Relative distribution of cholineacetylase activity in the layers of several vertebrates retinas. (From Graham & Pong, 1973).
It is possible that some cholinergic type of receptors can be found in the Necturus retina but so far no study has been conducted to demonstrated this. In fact it has been extremely difficult to prove the existence of cholinergic receptors in the vertebrate retina (Bonting, 1975). Val'Teev (1966) studied atropine and hexamethonium in the isolated in vitro retina of the bullfrog and compared the effects of these drugs on the electroretinogram (ERG). He found that hexamethonium had very little effect on the isolated bullfrog ERG but that the b-wave was completely blocked by atropine. He concluded that there was a cholinergic mechanism involved in the generation of the b-wave of the ERG. However, these results are difficult to reconcile with the fact the Graham et. al. (1973) found no ChAc activity in the outer nuclear layer which is directly related with the generation of the ERG (see fig. 2.4.4). Ames & Pollen (1969) found in isolated rabbit retina that hexamethonium, a nicotinic blocker, was much more effective in depressing ganglion cell activity as compared to atropine, a muscarinic type of blocker. They postulated the possible existence of a cholinergic mechanism in the synapse of both excitatory and inhibitory circuits between the receptors and the ganglion cells. DeRobertis & Fiszer (1968) provided additional evidence to support this suggestion. They determine (in cat) in several brain areas and in the retina the AChE activity, proteolipid protein content, and the capacity to bind (C) dimethyl-d-tubocurarine. All these facts support the idea of cholinergic mechanism in the retina but still more conclusive evidence is needed.
CHAPTER III

PATTERN GENERATOR SPECIAL PURPOSE SYSTEM.

3.1. Introduction.

Investigators have used different patterns of light stimuli on the retina to understand the functional structure and behaviour of the neuronal cells of the retina. The form and characteristic of these stimuli or image pattern is important to elucidate how the information is processed in the retina. Werblin & Dowling (1969) were able to identify the neuronal cells of the retina of the Neoturus by using a spot and a ring of light. Since then, spot and rings of light have become classic types of patterns to identify the retinal cells in Neoturus and in others species as well. Barlow H. B. Hill R. M & Levick W. R. (1964) used a moving spot of light to study the response of ganglion cells in the rabbit retina to direction and speed of image motion. Several others researchers (John R. Tuttle (1977), Ursula Grusser-Cornehils et.al (1963), Daniel Finkelstein, Otto-Joachim Grusser (1965), A. L. Norton et.al (1970) and Frank S. Werblin (1969)) have used a moving spot stimuli to study different aspects of the neuronal cells of the retina. Donald R. Nelson and Samuel H. Gruber (1963) used a moving bar light stimuli to study the response of some types of ganglion cells in pigeon retina to different direction of movements.

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In 1966 Charles R. Michael also used a moving bar to study the receptive field of directionally selective cell units in the ground squirrel retina. The spot, ring, moving spot and moving bar have been the most widely used types of patterns for stimulation of the retina. However the practical implementation of these types of patterns in general is difficult and cumbersome. Frank S. Werblin in his experiments on Necturus used a CRT driven by a triangular wave generator to produce a moving spot (fig 3.1.1).

![Stimulus and recording system](image)

**Fig 3.1.1.** Stimulus and recording system for intracellular measurements in Necturus retina (Werblin, 1969).

The equipment used by Werblin & Dowling is shown in fig 3.1.2. In order to get a concentric ring with a spot they used two CRT's one with a spot and another with a ring and focused the two images on the retina by using a beam splitter and a biconvex lens. Calibration of this type of system is difficult since the images on the retina are of the order of 200 to 500 μm in diameter. Miller and Dacheoux (1976) used two light beams from separate tungsten-iodine light sources. The first beam passed through a rotating wheel to produce a spot and the second beam through a grid to produce a ring pattern.
All these patterns work.
Thus it became all the patterns needed in visu.

Fig 7.1. 

One of the research questions that should be asked is whether the results of the study can be generalized to other contexts or settings. It is important to note that the study was conducted in a specific setting and with a particular sample population. The results may not be directly applicable to other populations or settings.

The results of the study suggest that the intervention had a significant impact on the outcomes for students. The measures used to assess the outcomes were reliable and valid, and the results were consistent across different measures.

However, the study has some limitations that should be considered when interpreting the results. The sample size was relatively small, and the study was conducted in a single setting. It would be valuable to conduct further research with larger samples and in different settings to confirm the findings of the study.

Overall, the study provides useful insights into the impact of the intervention on student outcomes. The results encourage the implementation of similar interventions in other settings to support student learning and achievement.
All these different types of arrangements to generate different patterns work quite well, but are difficult to calibrate and to use. Thus it became necessary to develop an instrument that can incorporate all the patterns explained above and other novel types of patterns needed in visual experiments.

Fig 3.1.2. Apparatus for stimulation and recording from Necturus retina (From Werblin and Dowling, 1969).

One of the most desirable characteristics of this equipment is that it should be easy to change from one type of pattern to another and it should be possible to accomplish this change in a matter of seconds instead of minutes and hours. This is especially necessary since most of the intracellular recording are stable for a relatively short period of time.
After having carefully considered all the patterns and equipment other investigators have been using for the past few years it was decided to design and construct a microprocessor controlled instrument to generate the required patterns.

The basic configuration of the system is an oscilloscope interfaced with a microprocessor system. The microprocessor is used to generate the pattern and the scope to display it. Dowling (1969) proved in this experiments on Necturus retina that the intensity given by the phosphorus P31 of the screen is sufficient to elicit good responses from the neuronal cells of the retina. The author selected an oscilloscope over a raster scan television monitor (TV) because the TV has an inherent 60 Hz flickering that can affect the response of certain cells in the retina (particularly the ganglion cells). Also since it is easy to obtain dual beam oscilloscopes they could provide more versatility for the generation of different patterns as compared to a TV set which only has one beam with very little control over scanning speed. The patterns that were decided to be incorporated into the system are the following.

1. Spot
2. Rings
3. Spot and ring
4. Bar
5. Rotating Bar
6. Moving Spot
7. Store and Replay.

These patterns were implemented by using the intel 8085 microprocessor and a Tektronix model 513 dual beam scope.
The size of the oscilloscope's screen is inches. An spot displayed on the screen when focused have a diameter of about 1.5 mm. The voltage level used for the X and Y channel input are 0.5 V/inch. All the specifications of the pattern generator system are given in relation to the oscilloscope model 513 just described. The characteristic of the patterns listed above are described in the following sections.

(1) **Spot.**

Five different intensities of the light spot can be obtained on the oscilloscope screen. The spot can be moved up, down, left or right on the screen by amounts as small as 0.25 mm.

(2) **Ring.**

Fifteen rings of different diameters can be formed on the screen. The diameter of each ring can be adjusted from 1 mm through 15 mm. The user can select just one or several rings to be displayed simultaneously on the screen. The thickness of the ring is approximately 1 mm when the beam is well focused. Also the rings can be moved up, down, left or right on the screen by pressing specific keys on the system keyboard.

(3) **Spot and ring.**

This pattern consist of a ring with a spot located in the center of the ring. Fifteen spot-ring patterns of different diameters can be selected. The same characteristics given for the ring are also applicable for this pattern.

(4) **Bar.**

A bar of variable length can be displayed at any desired angle on the screen. The length of the bar can be adjusted in the range 2 to 20 mm. The bar will be moving in a direction and speed that can be selected by the user.
Four possible direction of movements can be chosen: up, down, left right. There are eight different speeds that can be selected by the user. The thickness of the bar varies between 1 to 3 mm depending on how well the beam is focused. A stationary bar on the screen can also be obtained by an appropriate selection of delay parameters by the user.

(5) Rotating Bar.

This is a special pattern created to study the behaviour of the receptive field of a neuron to flashes of a bar at different angles. The user can select either a continuously rotating bar or a flashing bar. The vector can rotate in both clockwise or counter clockwise directions. The duration of the flash time can be set up easily by the user. The length of the rotating bar can also be selected from 1 mm to 20 mm. The on time, off time and the rotational speed of the bar can be set up by the user.

(6) Moving Spot.

A spot moving in straight line at any selected angle can be generated by the system. Also, the direction of the movement can be set up by the user. Eight different speed of movement are provided and the possibility of creating a new speed is also provided.

(7) Store and Replay.

This feature of the system gives the possibility of scanning the receptive field with a spot and record its size. A spot can be moved around or across the receptive field and the exact position of the spot on the screen can be stored in the memory of the microprocessor system. After all desired positions of the spot have been stored in memory, the receptive field can be displayed on the oscilloscope screen. A diagram of each pattern is shown in fig 3.1.3.
3.2. Special characteristic of the system.

The need for a spot, ring, bar and moving spot were explained in section 3.1. The system has the flexibility of providing a ring with adjustable radius (see part 2 of section 3.1.1) with fixed thickness or a ring of a selected radius with variable thickness. It is expected that this characteristic will assist the researchers to study the receptive field of a single cell with better precision. The moving spot can be moved in any direction and is not limited to vertical or horizontal movement.

Barlow, Hill and Levick (1964) have demonstrated that the direction of the spot movement is very important to study the behaviour of ganglion cells. Also, in the system the rate of motion of the spot can be easily changed, a characteristic that is very desirable in the study of certain retinal cells (Werblin, 1969). The special characteristic of the moving bar in this system as compared with similar patterns used by others investigators (Michael, 1966) is that the length of the bar can be adjusted from 2 mm through 20 mm, such that the whole retina can be excited.

It is expected that these feature would be important in the study of the receptive field behaviour of the neuronal cells. A novel pattern incorporated into the system is the rotating bar explained in part 5 of section 3.1.1. It is expected that this pattern would help in the study of the regularity of the receptive field response when a bar is flashed and scanned through 360° around the cell (see fig 3.2.1). If a cell has a nonuniform connections with other cells, this could be detected with the rotating bar since one can expect a nonuniform type of response from the cell when the bar is flashed at different angles with respect to the center field of the cell.
3.3. Basic Hardware Requirements.

The basic configuration of the hardware to accomplish the generation and display of the patterns is shown in fig 3.3.1.

Fig 3.2.1. Rotating bar applied across a receptive field of a cell.

Fig 3.3.1. Block diagram of the basic hardware requirement for the pattern generator.
The keyboard and alphanumeric displays are used to communicate with the microprocessor system. In order to generate an image on the screen of the scope the X and Y axis input voltages should be varied in a predetermined way. For example if a circle is to be generated the X and Y input voltages should be:

\[ X = A \cos Wt, \quad Y = A \sin Wt. \]

It is the function of the microprocessor to calculate and output these X and Y inputs voltages appropriately. Since the output of a digital system is in discrete form, two digital to analog converters are used to convert the digital output into analog output. The intensity of the pattern on the screen is controlled by changing the voltage to the Z input of the scope. The circle has to be traced several times each second on the screen of the oscilloscope such that there is no flicker. It takes too long to calculate the X & Y values from \( X = A \cos Wt \) and \( Y = A \sin Wt \) by the microprocessor. It is thus necessary to store in memory (PROM) the discretized values for X and Y. Then a circle is obtained by displaying several spots whose coordinates X and Y are stroked into the D/A's converters by the microprocessor. This method permits to display a circle with uniform intensity since the points are distributed with equal arc length. The points are nearly overlapping, thus making the pattern continuosly.

To be able to display 15 rings simultaneously it would require approximately 2000 points. If the microcomputer were to read these points from PROM and output them into the D/A registers, it would take approximately 55 msec. Such a large time to display a pattern would cause flickering and hence would be unacceptable. Thus a direct memory access system is necessary.
Fig 3.3.2. Generation of a ring by displaying point by point whose coordinates have been previously stored in PROM.

The actual system takes 2.236 sec to display one point under DMA (direct memory access) operation. All the 2000 points can be displayed in 4.472 msecs. This time corresponds to a rate of 233 repetitions which is faster than the decay time of the P31 phosphorus of the scope and is quite acceptable for the intracellular experiments. Also it is desirable to load both D/A converters simultaneously to avoid some erratic movement of the beam and produce undesirable traces on the screen. This was accomplished by using a special data bus configuration which is explained in detail in section 3.4. All the patterns are generated by displaying a series of points very close to each other on the screen and requires the same hardware that is used for the ring.

The voltage levels for the X and Y input of the scope were chosen as 50 mV/mm thus, for example 50 mV applied to the X input moves the spot 1 mm on the screen.
It was found that four points in 1 mm space on the screen were sufficient to display a non interrupted line. The D/A output voltage was calibrated to give 8 mv/bit. An output of 2.048 volts is equivalent to a line of 4 cm on the screen. It was considered then that an 8 bit D/A, (256 levels) was sufficient since a 4 cm line is large enough to cover a great portion of most retinas.

Of course, the exact size of the bar on the retina depends on the optical system used to focus the scope image onto the retina.

3.4. Block Diagram of the Hardware.

The block diagram of the hardware is shown in fig 3.4.1. The CPU is an 8085 8-bit microprocessor operating at a frequency of 1.75 MHz. The system has 2 separate buses and a switch is used to separate the address bus into two buses address bus A and address bus B. There are two memory banks, one bank, consisting of 4 K of PROM (designated as MA on fig 3.4.1) is connected to address bus A and the second consisting of 2 K of RAM (designated as MB on fig 3.4.1) is connected to address bus B.

The Intel 8257 DMA controller is used to access the second memory bank through the bus B. However, when the CPU wants to access both memory banks the two buses are connected together. During DMA operation the CPU is not halted but can access all the memory banks connected to address bus A and all the I/O ports i.e. the counters, X and Y registers, 8279 keyboard/display interface chip and the two special purpose I/O ports labeled as stop DMA and request DMA. The patterns spot and rings are stored in both PROM's of the second memory bank (MB).

One PROM contains the values of the X coordinates and the second PROM stores the valued of the Y coordinates.
Fig 3.4.1 Block diagram of the Pattern System Generator. See section 3.4 for explanation.
The coordinates X and Y of the points used to generate the bar, moving spot and rotating bar are stored in both RAM's of the second memory bank. The 8279 chip is used to interface the keyboard and display with the CPU.

3.4.1. **Software Program.**

The program that controls memories and I/O ports is stored in the two PROM's of the first memory bank (MA) and the RAM is used for stack and general purpose storage.

3.4.2. **DMA Operation.**

In order to start a DMA operation the CPU should send a high level signal on the line "Request DMA". The 8257 will start the DMA operation only when the line "Stop DMA" is high. The "Stop DMA" line is controlled by the CPU. During DMA operation the four selector chips are disabled and the data from memory goes directly into the two adders and from here to the D/A's and X and Y registers.

If a ring or a spot is being displayed then the data from the first PROM goes into one adder and the data from the second PROM goes into the second adder simultaneously. If a moving spot, bar or rotating bar is being displayed the data from the RAM's connected to bus B goes to the adders. The output of the counters going into the adders is used to move the pattern on the screen. These are up/down counters that can be incremented or decremented by using special purpose key of the keyboard. The DMA operation can be put in standby state by pulling the "Stop DMA" line low. The DMA will resume execution at the same point it was stopped after the "Stop DMA" line is raised again.
3.4.3. Memory Access from Address Bus A.

The first bank of memories connected directly to the address bus A can be accessed by the CPU at any time. However, the second group of memories connected to address bus B can be accessed by CPU only when DMA operation is stopped. In order that the CPU can write on the first two RAM's connected to bus B two selectors are used. The even selector will enable the access for write into the first RAM any time the address is even. When the address is odd, the odd selector enables the second RAM for write operation. In this way the first RAM contain information that can be accessed only by even address and the information of the second RAM can be accessed only by odd address.

If the memories (RAM's and PROM's) are to be read by the CPU, then the third and fourth selector selects the proper memory according to odd or even addresses.

3.4.4. X and Y Registers.

The system has a feature in which it can read and store the coordinates of a point being displayed on the screen of a scope. The output of these two register are used for this purpose. The register are considered as I/O ports accessed by the CPU; the DMA should be stopped momentarily while the data is stored in the first two RAM's connected to bus B.

3.5. Circuit Description of the Hardware.

3.5.1. 8085, Data and Address Bus Connections.

Fig 3.5.1 shows the structure of the address buses and data bus interconnected with the CPU 8085 (IC1) IC2 (74LS244) and IC6 (74LS373)
Fig 3.5.1 8085, data and address bus connections. 1 - 8085 microprocessor. 2, 3 and 7 - octal buffers SN74LS244. 4 and 5 - 4 bit parallel bidirectional bus driver 8216.
6 - D type latch SN74LS373.
are used to buffer the address bus and the IC3 (74LS244) and IC (74LS244) are used as a tristate switches to separate the bus AA from the bus AB.

When the lines AB are being used the lines AB are disconnected from the lines AA. Signal ALE is used to latch the low address byte and the signal AEN is used to put bus AA in tristate condition. IC4 (8216) and IC5 (8216) are used to buffer the data bus in both direction in and out of the CPU.

When RD signal is low the IC's 4 & 5 are enabled to transfer data into the CPU. RST 5.5 input in the 8085 is used to interrupt the CPU and this signal comes from the 8279 keyboard encoder. IO/M output signal (from CPU) is used only when input/output operations are taking place between CPU and peripherals. RD and WR are used to read and write from memory as shown in fig 3.5.2. CLK out signal is used to clock and synchronize the direct memory access (8257) and the keyboard interface (8279). The crystal used by the 8085 has a frequency of 3.59 MHz which is internally divided by 2. Thus, the actual frequency used by the CPU is 1.795 MHz. The signal RESET OUT is used to reset all the other IC's of the system hardware. The input RESET IN is used to position the program counter of the CPU at address 0.

3.5.2. Memory Connected Only to Bus A.

The memory IC's connected to address bus AA are two 4K: PROM (2532) and one 2K RAM (6116). The diagram of these connections is shown in fig 3.5.2. The IC's 8 (2532) and 10 (6116) are enabled according to the following values for the address lines AA11, AA12 and AA13 (see table I).
TABLE I. Addresses of Bus A.

<table>
<thead>
<tr>
<th>AA11</th>
<th>AA12</th>
<th>AA13</th>
<th>IC8</th>
<th>IC9</th>
<th>IC10</th>
<th>Address Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>L</td>
<td>L</td>
<td>Enabled</td>
<td>Disabled</td>
<td>Disabled</td>
<td>0000-1FFFH</td>
</tr>
<tr>
<td>H</td>
<td>L</td>
<td>L</td>
<td>Enabled</td>
<td>Disabled</td>
<td>Disabled</td>
<td>2000-27FFH</td>
</tr>
<tr>
<td>L</td>
<td>H</td>
<td>L</td>
<td>Disabled</td>
<td>Enabled</td>
<td>Disabled</td>
<td>1000-1FFFH</td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>L</td>
<td>Disabled</td>
<td>Enabled</td>
<td>Enabled</td>
<td>2000-27FFH</td>
</tr>
<tr>
<td>L</td>
<td>L</td>
<td>H</td>
<td>Disabled</td>
<td>Disabled</td>
<td>Enabled</td>
<td>2000-27FFH</td>
</tr>
</tbody>
</table>

The signal IO/H is used to prevent access to these memories when a peripheral is being addressed. The decoding of these addresses is carried out by the IC11 (SN74LS138) and this IC is enabled by using the inputs G2B, G1 and G2A as shown in Fig 3.5.2.

3.5.3. 8257 and DMA Operation.

Fig 3.5.3 shows the circuit of the 8257 DMA chip together with the I/O ports used to stop and initiate the DMA operation. IC12 (8257) and IC13 (74LS373) are used to latch the high byte of the address. IC14 (8216) is used to drive the bidirectional input lines A0, A1, A2 and A3 of the 8257. IC15 (74LS74) and IC16 thru 19 (74LS74) configure one I/O port used to request a DMA operation from the 8257. The output of IC15 (74LS74) goes into the HLDA input of the 8257 and to a tristate switch.
Fig 3.5.2 Memory connected to address bus A. 8 and 9 - PROMs 4K 2532. 10 - RAM 2K 6116.

11 - 3 to 8 line decoder SN74LS138. See section 3.5.2 for explanation.
The outputs of IC17, 18 and 19 are connected to the DMA request lines of the 8257 as shown in fig 3.5.3. The output of IC16 goes into the 555 timer which is used to regulate the flashing time of the pattern. Also this output is used to interrupt the LS11/03 computer. IC's 17, 18, 19 can also be read by CPU.

These I/O ports can be read or written into by the CPU even when the DMA is in operation. In this system the DMA request line DRQ1 is not used. To start a DMA operation the CPU writes a 1 into flip-flop 18 and 19. The 8257 puts a high level on the HRQ line and waits until the HLDA input goes high. After the DRQ3 and DRQ2 (outputs of IC18 and 19) lines are high the CPU performs two NOP instructions and then writes a 1 into flip-flop 15 (IC15) to make the HLDA input of the 8257 go high. The NOP instructions provide the necessary delay before the HRQ line goes high.

A detailed explanation of the function of the 8257 can be found in the Intel data component manual (1979).

3.5.4. Decoding Circuit for I/O Ports.

Fig 3.5.4 shows the circuit used to decode the different input/output ports connected to the 8085 (CPU). Four counters (4 bits each), IC's 23 thru 25 (74LS193) are also shown in this figure.

The counters are not part of the decoding circuit but are used to change the value on the two D/A converters as explained in section 3.4. The signals Y-up and Y-down are used to increment or decrement the Y counter respectively. X-up and X-down serve the same function for the X counter. The output of the counters IC23 and IC24 are input to two adders shown in fig 3.5.5. These counters affect the value of the D/A converter connected to the Y axis of the oscilloscope.
Fig 3.5.3 8257 and I/O ports for DMA operation. 12 - Programmable DMA controller 8257. 15, 16, 17, 18 and 19 - D type flip-flop SN74LS74. 13 - Octal D type latch SN74LS373. 14 - 4 bit parallel bidirectional bus driver 8216.
Fig 3.5.4 Decoding circuits for I/O ports and counters 20 - 3 to 8 line decoder SN74LS138.
21 and 22 - Dual 2 to 4 lines decoders SN74LS139. 23, 24, 25 and 26 - Synchronous up/down counters SN74LS193.
The counters IC25 and IC26 are also connected to two adders but they affect only the value in the D/A converter that is connected to the X axis of the oscilloscope.

The signal "To 8257 (CE)" is used to enable the 8257 DMA chip and the signal "To 8279 (CE)" is used to enable the 8279 keyboard interface. Signals X and Y are used to enable the reading of the input to the D/A converter connected to the X axis and to the D/A converter connected to the Y axis respectively (see fig 3.5.8). RDHLA, RD-DRQ0-3, WR-DRQ0-3 and HLDA are used to read from and write into the I/O ports shown in fig 3.5.4. (IC's 15, 16, 17, 18, 19). The address mapping of the I/O ports is given in table II.

In the 8257 the "Initial DMA Address" internal register and the "Terminal Count Register" of each channel are considered as separate I/O port. More detail on these channels on its function is given in Intel manual (1979). The 8279 has only two I/O ports to be considered which are the data I/O port and the status I/O port. Each time data is to be written into an internal register of the 8279 first a command has to be issued into the status port to select the register and then the data can be sent through the data bus. A detail explanation of the function of the 8279 can be found in the Intel data component manual (1979).

3.5.5. Memory Connected to Bus B.

Fig 3.5.5 shows the schematic diagram for the memory interconnection for bus B. These are two 4K PROMS 2532 (IC's 29 and 30) and two static RAMS 2K 6116 (IC's 27 and 28). The data lines for these memory chips is rather special. From bus B all memory chips can be accessed only for read operation and the data bus in this case contain 16 lines labeled.
### TABLE II. I/O Port addresses.

<table>
<thead>
<tr>
<th>I/O Port</th>
<th>I/O Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH0 DMA address</td>
<td>$\text{OE}$ $\text{OF}$</td>
</tr>
<tr>
<td>CH0 Terminal count</td>
<td>$\text{OE}$ $\text{1F}$</td>
</tr>
<tr>
<td>CH1 DMA address</td>
<td>$\text{OE}$ $\text{2F}$</td>
</tr>
<tr>
<td>CH1 Terminal count</td>
<td>$\text{OE}$ $\text{3F}$</td>
</tr>
<tr>
<td>CH2 DMA address</td>
<td>$\text{OE}$ $\text{4F}$</td>
</tr>
<tr>
<td>CH2 Terminal count</td>
<td>$\text{OE}$ $\text{5F}$</td>
</tr>
<tr>
<td>CH3 DMA address</td>
<td>$\text{OE}$ $\text{6F}$</td>
</tr>
<tr>
<td>CH3 Terminal count</td>
<td>$\text{OE}$ $\text{7F}$</td>
</tr>
<tr>
<td>Mode set</td>
<td>$\text{OE}$ $\text{8F}$</td>
</tr>
</tbody>
</table>

#### 8279

<table>
<thead>
<tr>
<th>I/O Port</th>
<th>I/O Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>DATA</td>
<td>$\text{OF}$ $\text{0H}$</td>
</tr>
<tr>
<td>Status Command</td>
<td>$\text{OF}$ $\text{1H}$</td>
</tr>
<tr>
<td>X coordinate read</td>
<td>$\text{OF}$ $\text{4H}$</td>
</tr>
<tr>
<td>Y coordinate read</td>
<td>$\text{OF}$ $\text{5H}$</td>
</tr>
<tr>
<td>Y coordinate down</td>
<td>$\text{OF}$ $\text{6H}$</td>
</tr>
<tr>
<td>Y coordinate up</td>
<td>$\text{OF}$ $\text{9H}$</td>
</tr>
<tr>
<td>X coordinate left</td>
<td>$\text{OF}$ $\text{AH}$</td>
</tr>
<tr>
<td>X coordinate right</td>
<td>$\text{OF}$ $\text{BH}$</td>
</tr>
<tr>
<td>DRQ</td>
<td>$\text{OF}$ $\text{CH}$</td>
</tr>
<tr>
<td>HLDA' (input to 8257)</td>
<td>$\text{OF}$ $\text{DH}$</td>
</tr>
<tr>
<td>Reset counters</td>
<td>$\text{OF}$ $\text{EH}$</td>
</tr>
</tbody>
</table>
Fig 3.5.5 Memory connected to address bus B. 27 and 28 - RAMs 2K 6116. 29 and 30 - PROMs.

4K 2532. 31, 32, 33 and 34 - 4 bit binary full adders SN74LS83. 35 and 36 - octal D type flip-flop SN74LS273.
DAO...DA7DB0...DB7. The output lines of both PROMs go directly into four full 4-bit adders (IC's 31, 32, 33 and 34). The others inputs going into these adders come from the counters shown in fig 3.5.4.

IC's 35 and 36 are latches to store the D/A converters values.

The range of addrers for the memories are:
2532 PROM's (IC's 29 and 30) 8000-8FFFH (bus B).
6116 RAM's (IC's 26 and 28) 9000H-97FFH (bus B).
6116 RAM's (IC's 27 and 28) 9A00H-9A7FFH (bus A).

The address range of the static rams is different when accessed from bus B and when accessed from bus A. From bus A, the ram chips can be accessed for read or write operation and the data bus to the memory chips consist of only 8 lines. The data bus lines from IC27 are labeled DA0...DB7.

3.5.6. Memory Access From Bus A.

In order to access the static RAM's connected to address bus B from the CPU the 16 data output lines form both memories (IC's 27 and 28) are brought into the 8 line data bus by mean of the circuit shown in fig 3.1.7. IC28 can be accessed only by even addresses and IC27 only by odd addresses. Thus, when RAM 28 8s being accessed by the CPU for write operation, the octal buffer IC 37 (in fig 3.5.6) is enabled in order to connect the output lines of memory to the CPU data bus. The octal buffer IC 38 (in fig 3.5.6) is enabled only when RAM 28 is being accessed by the CPU for write. When the CPU needs to read the RAM's memory the data selector/multiplexer IC 39 and 40 (fig 3.5.6) are used. In this case, the data lines DB0...DB7 are connected to the data bus. When AA is even and the data lines DA0...DA7 are active only when AA is odd. The signal "8257 FROM I/O DECODING" is used to prevent an access of these memories when DMA is in operation.
Fig 3.5.6 Data bus selector. 37 and 38 - octal buffers SN74LS244. 39 and 40 - quad data selector SN74LS257. See section 3.5.6 for explanation.
### TABLE III. Addresses of Bus B.

<table>
<thead>
<tr>
<th>AEN</th>
<th>Address</th>
<th>RAM(27)</th>
<th>RAM(28)</th>
<th>PROM(29)</th>
<th>PROM(30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Even addr</td>
<td>$0000-0FFF$</td>
<td>Disabled</td>
<td>Enabled</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$8000-8FFF$</td>
<td>$OD_2$ inactive</td>
<td>$EV_2$ active</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactive</td>
<td>Even addr</td>
<td>Disabled</td>
<td>Disabled</td>
<td></td>
<td>$i_8$ used</td>
</tr>
<tr>
<td></td>
<td>$0000-0FFF$</td>
<td>$OD_2$ inactive</td>
<td>$EV_2$ inactive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Odd addr</td>
<td>Even addr</td>
<td>Enabled</td>
<td>Disabled</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$0000-0FFF$</td>
<td>$OD_2$ inactive</td>
<td>$EV_2$ inactive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Odd addr</td>
<td>Even addr</td>
<td>Disabled</td>
<td>Disabled</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$0000-0FFF$</td>
<td>$OD_2$ inactive</td>
<td>$EV_2$ inactive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$9000-9FFF$</td>
<td>Enabled</td>
<td>Enabled</td>
<td>Disabled</td>
<td>$OD_1$ inactive</td>
<td>$EV_1$ inactive</td>
</tr>
<tr>
<td>Active</td>
<td>$8000-8FFF$</td>
<td>Disabled</td>
<td>Disabled</td>
<td>$OD_1$ active</td>
<td>$EV_2$ active</td>
</tr>
</tbody>
</table>
3.5.7. Memory Access From Bus B.

The RAM and PROM memories connected to bus B can be accessed through bus B only for read operation. This bus is used only under the control of the 8257 i.e. when DMA is in operation. During DMA transfer the address lines labeled B1...B2 (see fig 3.5.5 and 3.5.3) are active and the data from two memories (either both RAM's or both PROM's) on 16 lines are read simultaneously into the four adders as shown in fig 3.5.5.

3.5.8. Decoding Circuit for Memories Connected to Bus B.

The decoding circuit is shown in fig 3.5.7 and it is formed by IC's 42 and 43. During DMA operation the signal AEN is active and then either lines EV1, OD1 or EV2, OD2 are active simultaneously depending on whether the PROM's or RAM's memories are being accessed by the DMA chip (8257). When AEN is inactive the memories connected to bus B can be accessed by the CPU and then one of the four output lines EV1, OD1, EV2 or OD2 is active at the time depending on which memory was accessed.

Table III explain which memory is accessed depending on the line AEN active or inactive and on the address is even or odd.

3.5.9. D/A Converter and X, Y I/O Port.

In order to read the value of the X and Y coordinates going into the scope two register are provided. These registers are numbered 44 for X coordinate and 45 for Y coordinate in fig 3.5.8. To read the value of the X coordinate the CPU should address this port by an I/O instruction by making the line X active. The same is valid for the Y coordinate. The input lines to register 44 and 45 comes from two latch registers (IC's 35 and 36 shown in fig 3.5.5).
Fig 3.5.7 Decoding circuit for memory connected to address bus B and circuit to obtain I/O port signal for read and write. 41 - quad data selector SN74LS257. 42 - 3 to 8 line decoder SN74LS138. 43 - quad 2 to 1 line data selector SN74LS157. See section 3.5.8 for explanation.
Fig 3.5.8 D/A converters and X, Y registers I/O ports. 44 and 45 - octal D type flip-flops

SN74LS273. 46 and 47 - 8-bit D/A converters LM 8214.
The input to the D/A converter comes also from the same latch registers shown in fig 3.5.5. Of course the data input to both D/A converter is from the RAM and PROM memories shown in fig 3.5.5.

3.5.10. **Keyboard Interface and Alphanumeric Display.**

Fig 3.5.9 shows the circuit interface between the keyboard, display and the CPU. The interface is primarily done by using an interface chip 8279 which has been specifically designed for this purpose. The 8279 is programable and the keyboard depression are decoded, debounced and strobed in an 8 character stack, FIFO. The 8279 provides also a programable 16 x 8 bit display RAM which is interfaced with the alphanumeric display shown in fig 3.5.9. Signals A₀ and A₁ are used to select one out of four digits on each display chip shown in fig 3.5.9.

Signals A₀ and A₁ are used to select one out of four digit on each display chip shown in fig 3.5.9. The lines CS₀, CS₁, CS₂ and CS₃ are used as chip enable for each one of the four display chips (IC's 52, 53, 54 and 55). The data input to the display chips are provided by the lines L₀ through L₆. The IC 49 in fig 3.5.9 is a 3 to 8 line decoder used in conjunction with the 8279. Each time a key is depressed the codes key values (see fig 3.5.9) is entered into the FIFO stack giving rise to the signal IRQ which is connected to the CPU via the input RST 5.5.

Thus IRQ is then used to interrupt the CPU.

3.5.11. **Time Duration and Intensity Control of the Light Pattern.**

The time during which the pattern is turned on the screen is controlled by a timer (LM 555) shown in fig 3.5.10.

The resistors connected to inputs 7 and 6 of the timer provide four different time durations, 50 msec, 300 msec and 600 msec.
Fig 3.5.9 Interface of keyboard and display to 8085 cpu. 48 - programable keyboard/display interface 8279. 50 - octal buffer SN74LS244. 49 - decoder/demultiplexer SN74LS156. 51 - dual 2 to 4 line decoder SN74LS139. See section 3.5.10 for explanation.
Fig 3.5.9 (continued) Alphanumeric display.
Fig 3.5.9 (continued). Assignations of the keys on the keyboard. The hexadecimal numbers on the circles are the code for each key.
Fig 3.5.10 Circuits for the time duration and intensity control of the light patterns.

See section 3.5.11 for explanation.
The different levels of intensity of the pattern are given by five resistors connected in series as shown in fig 3.3.10. These five resistors provide 5 levels of voltage which are applied to the Z axis of the oscilloscope. Switch 1 is used to inhibit the signal HLDA and when it is open the voltage applied to the Z axis will be high only when the output of the timer is high. When switch 1 is closed the voltage going to the Z axis will be high every time HLDA is active.

Switch 2 is used to inhibit the interrupt signal to the LS11/03 computer.

The intensities of each pattern are given in the User Manual of the Pattern Generator System. All these intensities are referred to the lowest intensity of a spot which is considered as 1.
3.6. Software.

3.6.1. Introduction.

All the software has been written in assembly language for the 8085 microprocessor. The software consists mainly of a monitor program to control the input/output data and several other routines to generate and display the different patterns. Besides of controlling the input/output data the monitor also interacts between the routines used to display the patterns. Once the user selects a pattern by pressing a key on the keyboard the monitor acknowledges the interrupt and jumps to the service routine corresponding to the selected pattern. Then the monitor will prompt the user (by using the LCD display) to enter data on the keyboard for the required parameters of that particular pattern. Soon after all the required data has been entered the monitor releases the control to the routine used to generate and display the pattern. In summary, the monitor consists of a interrupt-acknowledge subroutine called GENE and several service routines associated with each pattern and with the keyboard and display.

3.6.2. Monitor.

The monitor controls all the input and output data. All data input is done exclusively through the keyboard. The monitor outputs data also to the LCD display. When the power is turned on several register are initialized before the system waits for an interrupt. Thus, at power on the interrupt is disabled and then the following instructions are executed.
1. - Interrupt 5.5 of 8085 is unmasked.
2. - The alphanumeric display is set for right entry.
3. - The program clock of the 8279 is set up.
4. - The status register of the 8279 is cleared.
5. - The mode set register of the 8257 is cleared.
6. - All counters are reset to zero.
7. - The message "SELECT PATTERN" is displayed and the interrupt is enabled.

Following these operations the monitor waits for an interrupt. When a key on the keyboard is pressed the CPV is interrupted and the monitor jumps to the subroutine GENE (fig 3.6.1). This routine decodes which key of the keyboard has been pressed and then jumps to the corresponding service routine of that particular key. The monitor uses several routines corresponding to each basic pattern, to generate the desired pattern. Thus, what follows is a description of the routines used to generate and display each basic pattern and other routines invoked by the monitor.

3.6.3. Spot.

The size of a spot is fixed and values need to be entered by the user. In SPOT, registers B and C are used to store the initial address 876AH for DMA reading and register D,E contain the number of points to be displayed which in this case is 0. This information is used by subroutine RINN (see fig 3.6.3 for flowchart) to initialize the DMA transfer. RINN is used exclusively to load the initial address and the number of points into the registers of the 8257 DMA IC. When the loading is completed, RINN starts the DMA transfer from memory to the D/A converts.
Fig 3.6.1. GENE. After an interrupt has occurred this routine determines which key has been pressed by the user and jumps to the corresponding service routine of that particular pattern.
Fig 3.6.1. (continued)
Fig 3.6.1. (continued)
Fig 3.6.2. **SPOT.** This routine displays a spot on the oscilloscope screen. Subroutine RINN starts the DMA operation with the initial address stored in register B & C and the total number of points stored in registers D & E. 876AH: address of the spot coordinates in PROM. OAOCH: initial address of RAM to be read by DMA operation. COUNT: address where the number of points to be displayed are stored. AD1 & AD2: address where the coordinates of a spot are temporarily stored by subroutine STO.
Fig 3.6.3. RINN. It transfers the content of the register B & C to the address register of the 8257 DMA chip. It also transfers the content of the register D & E to the terminal counter of the 8257 and enables status mode register and starts DMA operation.
Fig 3.6.4. ANIL. It is used to accept the parameters "RADIUS" and "Number of circles" from the keyboard. It also decides if the "Ring" or the "Spot and Ring" key was pressed by the user. After the parameters are entered it will use subroutine RINN to start the display of the selected pattern on the screen.
Fig 3.6.4 (continued). Register B & C contain the starting address to be read by DMA and register D & E contain the number of points to be displayed. SFT address is loaded by subroutine GENE.
When the address 076 AH is read by DMA the values 36 for X coordinate and 36 for Y coordinate are brought simultaneously to the two D/A converters. These X, Y values correspond to a spot displayed at (1 cm, 1 cm) from the initial origin set by the user.

The address AD1 and AD2 shown in fig 3.1.2 are used to store temporarily the initial address 0A00H to be used by routine STO.

3.6.3. Ring and Spot-Ring Patterns.

Soon after subroutine GENE is executed the monitor jumps to subroutine ANIL (fig 3.6.4). ANIL is used to accept the parameters from the keyboard that are necessary for the display of these patterns. In fact, two parameters need to be entered by the user. The procedure that the user should follow is as follows:

a) First, a message "ENTER RADIUS" appears on the display. The user should enter a radius value from 1 through 15.

b) Next, the message "ENTER NUMBER OF CIRCLES" appears on the display. The user should enter the number of circles to be displayed simultaneously, that is any number between 1 and 15. The radius entered by the keyboard is the radius of the first ring, all the others will be adjacent to this one. Subroutine RINN (fig 3.6.3) is used to initialize the DMA and display the pattern on the oscilloscope. The flag called SFT in ANIL is used to differentiate between the patterns ring and spot-ring. SFT = 0 is used for ring and SFT = 1 is used for spot-ring. Subroutine REDO and REDA are used to store 0 or 1 into SFT respectively (see fig 3.6.5 for flowchart).
Fig 3.6.5. REDO, REDA. Depending on which key is pressed (either "Ring" or "Spot and "Ring") the flag SPT will be set or cleared by one of these routines. REDO corresponds to ring and REDA to ring and spot.
Fig 3.6.6. VECC. It accepts the parameters direction, speed, slope, number of points on and coordinates X and Y to display the bar pattern. After all the parameters are entered by the user it jump to routine VECTO. The values stored at address REC and REB are used by routine DEL2 to provide the different speeds for the bar movement. Subroutine MOD (see fig 3.6.7) is used to modify the speed and NACA accept the values of coordinates X and Y (see fig 3.6.8).
Fig 3.6.6 (continued)
Fig 3.6.6 (continued)
Fig 3.6.6 (continued)
Fig 3.6.6 (continued)
3.6.4. Bar.

When the bar pattern is selected, the monitor jumps from GENE to subroutine VECC (see fig 3.6.6). VECC is used to accept several parameters that are necessary to generate this pattern. VECC also makes use of two additional subroutines called MOD and MACA. MOD (fig 3.6.7) is used to modify the speed of the bar and MACA (fig 3.6.8) is used to accept the values of the X and Y coordinates. The user should enter the different parameters in the following order.

a) First message on the display is:

"ENTER DIRECTION"

This message refers to the four directions of movement of the bar: up (5), down (4), right (6), left (7). The user should type the respective number associated with the required direction.

b) Second message is:

"ENTER SPEED"

Eight different speeds can be selected by the user which associated with the numbers,

0. - 5.8 sec/cm
1. - 3.5 sec/cm
2. - 2.2 sec/cm
3. - 1.7 sec/cm
4. - 1.5 sec/cm
5. - 1.3 sec/cm
6. - 0.5 sec/cm
7. - 0.2 sec/cm

The user should select one of these eight numbers.
Fig 3.6.7. MOD. This routine is used to change the delay time of subroutines DEL1 and DEL2. The values stored at address TEC and TEB affect the delay time of DEL1 and those stored at address REC and REB affect the delay given by DEL2. This routine allows the user to change any of these values.
Fig 3.6.8. NACA. This routine reads the values of X and Y coordinates from the keyboard. The maximum value for X and Y is 99. The length of the bar is given by:

$$\text{length} = \sqrt{x^2 + y^2} \approx 0.25 \text{ mm}.$$
Fig 3.6.8 (continued)

c) Message on display is:

"MODIFY SPEED?"

This message refers to the possibility of creating a new speed by changing the values stored in the memory locations REC and REB, that will affect the execution time for the routine named DEL2 (fig 3.6.9). This procedure is explained in more detail in section 3.6.5. Usually the user skip this step by just pressing the key "SKIP".
d) Message on display is:

"ENTER SLOPE 0 OR 1"

This message refers to the slope of the bar, 0 and 1 correspond to positive and negative slopes respectively.
e) Message on display is:

"ENTER NUMBER OF POINTS ON"

The length of the bar is directly associated with the number of points that will be displayed on the screen. Four points are equivalent to 1 mm on the screen. The user can have from 1 to 99 points on.
f) Message on display is:

"ENTER X COORDINATE"

In order to generate a bar on the screen two coordinate values should be given. One is for X coordinate and the other for Y coordinate. The angle of the bar on the screen will depend then on the values given for X and Y coordinates. For example if X = 50, Y = 50 a bar with an angle of 45° will be generated and displayed on the screen. The maximum value for X and Y is 62.
After X has been entered a second message, 

"ENTER Y COORDINATE"

appears on the display. Once this procedure is completed the pattern will appear on the screen and the message "RUNNING" will appear on the display.

After all the parameters have been entered the monitor jumps to routine VECTO. At this points the monitor releases control to routines VECTO, VECR and RINN. The monitor will gain control only if another interrupt occurs.

Vecto (fig 3.6.10) uses an algorithm to calculate the points of a vector that should be displayed on the screen with a scope. The algorithm is described in detail in section 3.6.12. Each time a point of the vector or bar is calculated by VECTO the values of coordinates X and Y are stored starting at address $\text{PA}\$PH. Once all the points that form a bar have been calculated and stored in memory the program jumps to either subroutine VECR or VECI depending upon whether the selected pattern is a bar or a moving spot.

VECR (fig 3.6.11) is used to store the initial address $9000H$ on registers B, C from which the points are going to be read by the DMA. Also the total number of points to be read are stored in register D, E. RINN loads these values into the initial address registers and the terminal counter register respectively. The DMA operation begins after this initialization.

The movement of the bar is simulated by incrementing or decrementing the counters as shown in fig 3.6.11. A delay routine call DEL2 (fig 3.6.9) is used to change the speed of movement. The delay time provided by DEL2 depends on the value stored in registers B and C and is given by the equation shown below:
Fig 3.6.9. DEL2. This routine is used to provide a delay time given by the following formula: 
\[
[(490) * (B + 1) * (C+1) + 60 * (C + 1) + 170] * 559 * 10^{-9} \text{ sec.}
\]
Registers B and C represent the decimal content of register B and C respectively.
Fig 3.6.10. VECTO. This algorithm is used to calculate the points of the bar pattern. The angle of the bar depends on the values given for coordinates X and Y. The angle of the bar is given by \( \tan \theta = \frac{x}{y} \). AXY is the initial address to store the points of the bar.

AXY: contains initial address for coordinates X & Y.
CXX: contains update of \( X_i + 1 \).
CYY: contains update of \( Y_i + 1 \).
**Fig 3.6.10 (continued)**

COUNT: contain value of numbers of points.

F: contain value of slope.
Fig 3.6.10 (continued)
Fig 3.6.11. VECR. This routine is used to display the bar pattern. It starts the DMA operation moves the bar in the direction specified by the user. The movement is obtained by incrementing or decrementing the appropriate counter.
The values stored in registers B and C depend on the speed selected by the user.

3.6.5. Modify Speed.

If the user wished to modify the speed on the rotating bar, moving spot or bar, each time the message "MODIFY SPEED?" appears on the display he can type any character but skip (see fig 3.6.7 for flowchart of MOD routine). After this the user can modify the values stored at address TEC, TEB, REC and REB.

The first two addresses affect the delay of DELI and the others the delay of DEL2. The content of TEC and REC are stored in register B (depending on DELI or DEL2) and the content of REC and REB are stored in register C. The value for these addresses could be any value between 0 and 99. The delay obtained is given by the following formula.

\[ (490) \cdot (B + 1) \cdot (C + 1) + 60 \cdot (C + 1) + 170 \]. 559 \times 10^{-9} \text{sec.} \]

3.6.6. Algorithm to Calculate a Vector.

The routines START and VECTO use this algorithm to calculate the points of a vector to generate a bar of light in the screen. The coordinates \( x', y' \) of the vector should be given (see fig 3.6.12). An error function is defined as follow:

\[ f_i + 1 = x' (y_i + \Delta y) - y' (x_i + \Delta x) \]
\[ f_i + 1 = x' y_i - y' x_i + x' \cdot \Delta y - y' \cdot \Delta x = f_i + (x' \cdot \Delta y - y' \cdot \Delta x) \]

The variables of the error function are clearly defined in fig 3.6.12. Given the initial conditions \( (x', y') \) the steps of the algorithm are as follow:

Initially \( f(0) = 0 \)

if \( f_i \geq 0 \), then
\[ |\Delta x| = 1, |\Delta y| = 0 \quad \text{if sign}(x^* \prime) = \text{sign}(y^* \prime) \]
\[ |\Delta x| = 0, |\Delta y| = 1 \quad \text{if sign}(x^* \prime) = \text{sign}(y^* \prime) \]

if \( f_i < 0 \), then
\[ |\Delta x| = 1, |\Delta y| = 0 \quad \text{if sign}(x^* \prime) = \text{sign}(y^* \prime) \]
\[ |\Delta x| = 0, |\Delta y| = 1 \quad \text{if sign}(x^* \prime) = \text{sign}(y^* \prime) \]

\[ \text{Intermediate point} \]

Fig 3.6.12. A vector whose coordinates are \((x^* \prime, y^* \prime)\) is constructed by generating a staircase advancement of the beam.

For example, \(\Delta x, \Delta y\) steps needed for a given value of \((x^* \prime, y^* \prime) = (3, 5)\) are shown in table IV. The algorithm is terminated when \(f_i = 0 \quad (i > 0)\). The user inputs the values of the coordinates \(x^* \prime\) and \(y^* \prime\) and chooses the angle of the vector he wants to display on the scope.
The values of \( x' \) and \( y' \) entered from the keyboard need to be always positive. Thus, in order to calculate a vector with negative slope, the user has to enter the sign of the scope required.

**TABLE IV. Steps values of a vector calculation.**

<table>
<thead>
<tr>
<th>( i )</th>
<th>( f_i )</th>
<th>( \Delta x )</th>
<th>( \Delta y )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The values of the coordinates of each intermediate point are stored in memory (RAM) starting at address \( \text{FAFH} \). Then in order to display the vector the values of this intermediate coordinates are brought into the D/A converters in pairs \((x, y)\) by means of the DMA operation.

Thus the points of the vector are read successively and cyclically from memory to produce the effect of a bar being displayed on the screen. The scope has been calibrated such that four points will simulate a line of 1 mm in length.
3.6.7. Rotating Bar.

If the user selects the rotating bar pattern the monitor will jump from GENE to subroutine ROTT. ROTT (fig 3.6.13) is used to accept the parameters that are required to generate and display this pattern. The procedure to enter these parameters is the following:

a) The following message appears on the screen:

"ENTER NUMBER OF POINTS OFF"

This message refers to the number of points of the bar that the user wants not to display on the screen. The maximum number is 99. Four points are equivalent to 1 mm of the bar.

b) Second message is:

"ENTER SPEED"

Three speeds can be selected by entering either 0, 1 or 2. Speed 0 correspond to a time of 184 sec. to complete a full revolution of the bar. In the same manner speed 1 correspond to a time of 172 sec. and speed 2 to a time of 168 sec. to complete a full revolution of the bar.

c) The next message is:

"MODIFY SPEED?"

This case is explained in more detail in section 3.6.5. Usually the user skips this message by pressing the skip key.

d) The next message is:

"ENTER DIRECTION"

Two directions of rotation can be selected:

clockwise - 1

counterclockwise - 2
Fig 3.6.13. ROTT. This routine accepts all the necessary parameters to display the rotating bar. When the user types the last parameter to be entered it jumps to routine START to calculate the points of the bar and then displays it. The values stored at address TEB and REB are used by routine DEL2 and those stored at address REC and REB are used by routine DEL1 (see fig 3.6.16 and 3.6.9).
Fig 3.6.13 (continued)
Fig 3.6.13 (continued)
Display: "ENTER NC AGAIN"

Display: "ENTER NO POINTS ON"

Read Keyboard

Skip?

Yes

Display: "# POINTS ON IS NULMAN"

Delay of 2.5 sec

No

Points on = #?

Yes

Store & points on in ROM

No

Display: "ENTER"

Go to START.

Fig 3.6.13 (continued)
e) The next message is:

"ENTER INCREMENT"

This message is related to the total number of bars that will be displayed in one full turn. An increment equal to 1 is equivalent to 400 bars, increment 5 is equivalent to 80 bars and increment 10 is equivalent to 40 bars.

f) The last message requiring user input is:

"ENTER NUMBER OF POINTS ON"

The number of points on is directly related to the length of the bar as it was explained in section 3.6.7. The maximum number of points on is 99. After this parameter is entered, the message "RUNNING" appears on the LCD display and the bar pattern appears on the screen.

When all the parameters have been entered the monitor jumps to subroutine START. START (fig 3.6.14) uses the same algorithm employed by VECTO to calculate the points of the bar. When a bar has been calculated it is displayed on the screen for a certain amount of time given by the routine DEL2 (fig 3.6.9). When the display of this bar is completed a new set of values for the bar is calculated and displayed at a new position on the screen. The time off is given by DEL1 (delay routine) plus the time used by the microprocessor in calculating all the points of the bar (see fig 3.6.15). By changing the time ON and OFF, the speed of rotation of the bar can be changed.

Every time a bar is turned on a pulse is available to start an external device such as an A/D converter. The routine START is a closed loop program in which when a full rotation of the bar has been completed, automatically a new rotation begins.
Fig 3.6.14. START. This flowchart describes the algorithm to calculate and display the rotating bar. The algorithm to calculate the bar is explained in more detail in section 3.6.12. When a bar has been calculated and stored in memory it is then displayed for a certain time and then turned off. During the off time the next bar is calculated and displayed. It is a closed loop that can be stopped only by selecting another pattern or by pressing reset.
Fig 3.6.14 (continued)
Fig 3.6.14 (continued)
Fig 3.6.14 (continued)
Fig 3.6.15 Rotation of a bar.
The dark bars indicate the "ON" period of the pattern on the screen. The "ON" period is provided by a delay given by routine DEL1. $A'$ indicate the "OFF" period of the pattern and it is given by routine DEL2.
As explained in the flowchart of START the first vector displayed is the one whose coordinates are \( x' = 0, y' = 50 \). After this vector is displayed a new vector with coordinates \( x' = 0, y' = 50 \) is calculated. INC is the increment value entered by the user. When the update \( x' \) reaches the value of 50 then \( x' \) is maintained constant and \( y' \) is decreased by the amount equal to INC each time a new vector is calculated. When \( y' \) becomes 0 the cycle starts again with \( x' = 0 \) and \( y' = 50 \).

The routines, DISP (fig 3.6.17) and RINN are used to start the DMA operation. DISO loads the initial address of memory to be read by DMA operation in register B and C. It also puts the total numbers of points of the bar or vector to be read into register D and E. RINN as explained in section 3.6.3 initializes and starts the DMA operation i.e. the display of the bar.


When the moving spot pattern is selected by the user the monitor jumps from GENE to subroutine SPOM. SPOM (fig 3.6.18) accepts the following parameters, from the keyboard:

a) The first message on the display is:

"ENTER DIRECTION"

One of two directions for the moving spot can be selected by the user. Up and down directions are represented by 1 and 2 respectively.

b) The second message is:

"ENTER SPEED"

Eight different speeds can be selected. These are:

<table>
<thead>
<tr>
<th>Speed</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.4 sec/cm</td>
</tr>
<tr>
<td>1</td>
<td>6.4 sec/cm</td>
</tr>
<tr>
<td>2</td>
<td>4.4 sec/cm</td>
</tr>
<tr>
<td>3</td>
<td>2.5 sec/cm</td>
</tr>
<tr>
<td>4</td>
<td>1.7 sec/cm</td>
</tr>
</tbody>
</table>
Fig 3.6.16. DELL. This routine is used to provide a delay in the rotating bar pattern. It provides the off time of the rotational bar. The delay is given as a function of the contents of registers C and B. The actual delay is given by the following formula: 

\[(490) \times (B + 1) \times (C + 1) + 60 \times (C \cdot 1) + 170\] * 559 * 10^{-9} sec.
Fig 3,6.17. DISP. This routine loads registers B & C with initial address and registers D & E with numbers of points to be read by DMA. Actual DMA operation is started by subroutine RINN.
Fig 3.6.18. SPOM. This routine accepts all the parameters necessary to display a moving spot. After all the parameters have been entered the routine jumps to the subroutine VECTO to calculate the trajectory of the moving spot. The values stored in address REC and REB are used by the delay routine DEL2 to change the speed of the moving spot.
Fig 3.6.18 (continued)
Fig 3.6.18 (continued)
2 - 4.3 sec/cm
3 - 3.3 sec/cm
6 - 0.8 sec/cm
7 - 0.4 sec/cm

However the user has the flexibility to choose a speed different from the ones specified above, after the message "Modify Speed?" appears on the display. This procedure is explained in section 3.6.5.

c) The final message on the display is:

"ENTER SLOPE 0 OR 1"

The positive and negative slopes are represented by 0 and 1 respectively. Once these final parameters entered the message "RUNNING" appears on the screen and the pattern is displayed on the screen.

Once all the parameters have been entered the program counter jumps to subroutine VECI (fig 3.6.19). Initially VECI copies all the points of the vector in reverse order into another part of the RAM memory. Thus the memory contains two sets of points, one set for moving the spot away from the origin and the other in reverse direction.

The movement of the spot is obtained by putting a delay between successive reading of points from memory. The total procedure is shown in fig 3.6.8. Here again RINN is used to initialize and start the DMA operation.

3.6.9. Store and Replay.

If the key STORE is pressed the monitor will jump from GENE to subroutine STO. STO (fig 3.6.20) will read the current values of the x and y coordinates (i.e. the current values on the two D/A converters) and store them in successive position in memory. After this, the user can move the spot up, down, left or right and store the new position of the spot on the screen by pressing store key again.
Fig 3.6.19. VECI. All the points in the trajectory of the moving spot are rearranged in reverse order and stored in memory (RAM bus B). To produce the movement of the spot the points stored in memory are read by DMA one with a predetermined delay (given by the user). Changing the delay will change the speed of the spot. Register B, C contain the address of the initial point to be displayed. Address COUNT contains the number of points to be displayed.
Fig 3.6.20. STO. When the spot routine is already running the routine STO stores the position of the spot on the screen starting at address AD1, AD2. The number of points stored are maintained at address COUNT.
To reproduce all these stored positions of the spot the user need to press the REPLAY key once. When the Replay key is pressed the monitor jumps from GENE to subroutine REPLE (fig 3.6.21). REPLE will start the DMA using subroutine RINN and will display all the points that were previously stored in memory.

3.6.10. Read Keyboard.

In order to read the keyboard the monitor uses the following routines: CLEA, SAM and REA. CLEA (fig 3.6.22) is used to clear the FIFO (first in first out stack) of the 8279. SAM is used to sample the keyboard for a character. The program counter jumps out of SAM into routine READ any time a character is on the FIFO. The flowchart of SAM is given in fig 3.6.23. READ (fig 3.6.24) puts the first character entered in address BOX and the second character into address BLK.

Then routine DECI (fig 3.6.25) takes the two characters and converts them into one binary number which is stored in BOK. If only one character is entered the flag FL2 is set and routine DECI will transfer the contents of BOK into BLK and put a zero into BOK before starting the conversion. Thus, the read operation is completed when the character (after conversion by DECI) is stored in BOK.

3.6.11. Flashing a Pattern.

To flash a pattern for a short time on the screen the user should first press the key STOP and then the RETURN key. When the STOP key is pressed the monitor jumps from routine GENE to PIPA. PIPA (fig 3.6.26) displays the message "PRESS RETURN" and waits. When return is pressed a trigger pulse is sent out (to the A/D converter of the LS11/03 computer) and returns to the source program. The same pulse is used to trigger an internal timer (LM 555).
Fig 3.6.21. REPLE. This routine displays all the points that have been stored in memory starting at address 9ΦΦΦΗ (Bus B). The number of points displayed depends on the value stored at the address COUNT.
Fig 3.6.22. CLEA. It clears the FIFO on the 8279 chip.
Fig 3.6.23. SAM. This routine stays in a loop waiting for a character to be pressed on the keyboard.
Fig 3.6.24. READ. This routine reads the characters input by the keyboard. Flag FLO is set each time the first character is read and flag FL1 is set each time a second character is read. 03H is the code for the skip key. 0CH is the code for the return key. 05H is the code for the change key.
Fig 3.6.24. (continued). It is always possible to modify a character entered by pressing the change key. However the last character entered must be changed first. It is necessary to retype both characters if the first character has to be reentered.
Fig 3.6.24 (continued)
Fig 3.6.24 (continued)
Fig 3.6.25. DECI. This routine converts two digits stored in address HLK and BOK into its equivalent binary number and stored it at address BOK. FL2 is 1 when only one character has been input by the keyboard.
Fig 3.6.26. FIPA. When the STOP key is pressed, the program will jump to this routine. The current pattern on display will be on standby until the return key is pressed again. When this sequence happens a trigger pulse will be sent out. (to the LS11/03 computer).
The output of the timer is connected to the base of the open collector driver (fig 3.5.10) that is connected to the Z axis of the oscilloscope. The pattern is turned on for the period the timer output is high.


The user have four keys at his disposition to move the pattern on the screen. These keys are: LEFT, RIGHT, UP, DOWN. The microprocessor uses the counters to change the position of the pattern. If keys LEFT or RIGHT are pressed the monitor jumps from GENE to routines LEFT or RIGHT (fig 3.6.27) and to routines UP or DOWN (fig 3.6.28) if the keys up or down were pressed. After the movement is executed the program counter returns to the main program. The pattern can be moved by 0.2 mm or 3 mm increments on the screen. When the user presses the COARSE key before pressing any of the movement keys, the pattern moves in 3 mm increments. When the COARSE key is pressed the monitor jumps from GENE to routine CORA. CORA (fig 3.6.29) stores the number 12 on address COAR which is later used by LEFT, RIGT up or down routines.

When the coarse key is pressed twice, the pattern movement will be in 0.2 mm increments.
Fig 3.6.27. LEFT, RIGT. These routines move the current pattern on the screen left or right. COAR contains the increment by which the pattern is moved on the screen.
Fig 3.6.28. UP, DOWN. These routines are used to move the current pattern on the screen up or down. The increment moved depends on the value stored at address COAR.
Fig 3.6.29. CORA. It changes the value stored in address COAR from 1 to 12 and vice versa. The value stored in COAR is used by routines up, down, left and right.
Fig 3.6.30. LEDD. This routine is used to display a message on the alphanumeric display.
Fig 3.6.31. DEL3. It provides a fixed delay of 1.5 sec. It is used to display the message "# POINTS ON IS MAX". This occurs when by mistake the user types zero points to be displayed.
3.7. Methods.

3.7.1. Storage and preparation of the ocular sample from Necturus.

Adult mudpuppies were kept in tanks filled with water (20 to 30 gallons) maintained at 14°c. Two capsules of tetracycline HCl 250 mg were put in the tanks to prevent any possible bacterial or fungal infection on the mudpuppies. Before an experiment, a Necturus was taken out of the tank and kept in complete darkness for approximately two hours in a small container. The initial temperature of the water in the small container was about 5°c. After this dark adaptation period the animal was taken out of the container and decapitated. The head was then chopped longitudinally leaving one eye in each part of the head.

Immediately after this, one part of the head with the eye was put into a small crystallizing dish with ringer solution at 0°c. The dish was surrounded with ice and kept in dark until the eye was needed in a second experiment.

The other part of the head was placed in a homemade chamber containing ringer solution, ready for dissection (fig 3.7.1). The dissection was performed in dim red light and under a dissecting microscope. First, the skin and mucus or jelly-like material around the eye were removed. The cornea, iris and lens were dissected from the eye very carefully.

During this process some vitreous usually came out but a small quantity of the vitreous was still left inside the eye-cup. Since it is difficult to remove the vitreous completely without damaging the retina no such attempt was made. After the eye was dissected, small pieces of kimwipe paper were put around the eye-cup together with a long (16 cm long) tail of paper (see fig 3.7.1).
Fig 3.7.1 Chamber containing the eye cup surrounded by kimwipe paper.

(A) ground connector. (B) kimwipe paper. (C) chamber.

(D) eye - cup.
The purpose of this paper around the eye is to absorb and drain the ringer solution that was later perfused on the eye. At this stage, the preparation was ready for experimentation.

3.7.2. Preparation of the ringer and perfusion system.

The ringer solution used to perfuse continuously the eye-cup has the following composition:

- NaCl 6.5 gr/l
- KCl 140 mg/l
- CaCL 120 mg/l
- NaH PO 10 mg/l
- NaHCO 400 mg/l
- Glucose 2 gr/l

The pH of the solution was 7.82. Fresh solution was prepared for each experiment. The ringer was loaded into a syringe (30 c.c) which was installed in an automatic perfusion pump (see fig 3.7.2). The drug to be used was mixed with the normal ringer solution and loaded into another automatic pump. These pumps were set to provide an average continuous flow of 150 l/min. The control ringer (without drug) and the drug-ringer were brought together into a 26 gauge needle by independent plastic tubing (see fig 3.7.2). The needle was then positioned into the eye-cup by using a mechanical manipulator but without touching the eye in any place (see fig 3.7.2). The eye cup was perfused with control-ringer solution for one half hour. This initial time of perfusion with control proved to be sufficient to dilute the remaining vitreous and thus permitting the drug to diffuse into the retina without difficulty.

To select between control-ringer and drug-ringer perfusion, one or the other pump was depending on which solution was to be delivered.
Fig 3.7.2 Schematic diagram of the perfusion system.

(A) chamber. (B) mechanical manipulator. (C) gauge needle.
(D) plastic tubing. (E) toward other pump equal to I.
(F) ground with platinum. (G) syringe. (H) eye-cup.
(I) electric pump. (J) ringer solution.
3.7.3. **Light Stimuli.**

Different patterns of light were used to stimulate the retinal cells. These patterns were explained in detail in section 3.2. The intensity and duration of the light stimuli were selected according to the type of effect that was to be produced on the retinal cell. There were different intensities and different duration of the light stimuli that could be selected. These were:

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Duration of stimuli</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50 msec</td>
</tr>
<tr>
<td>2</td>
<td>150 msec</td>
</tr>
<tr>
<td>3</td>
<td>300 msec</td>
</tr>
<tr>
<td>4</td>
<td>600 msec</td>
</tr>
</tbody>
</table>

The light from the Tektronix Model 513 scope comes from a CRT with P31 green phosphor. The pattern of light generated on the scope was directed to the retina by means of a biconvex lens and a beam splitter (fig 3.7.3). The overall optical system had a gain of 0.167 i.e. a ring of radius 6 mm on the screen had a radius of 1 mm on the retina.

To have a proper focus of the light pattern on the retina the beam splitter was mounted on a dissecting microscope (see fig 3.7.3) to provide a view of the pattern on the retina. With this set up, the pattern can be focused correctly on the retina by moving the beam splitter up or down. The biconvex lens was mounted on a sliding base and the movement of this lens can also be used to focus the pattern on the retina. Scanning a spot of light over the retina could be easily accomplished by moving the spot on the screen of the scope.
Fig 3.7.3 Optical system.

(A) eye of the investigator. (B) dissecting microscope.
(C) beam splitter. (D) biconvex lens. (E) screen of the scope. (F) pattern on the scope. (G) sliding base for the biconvex lens. (H) eye-cup.
As explained in section 3.5, the pattern generator system provides four keys to do this. These keys can move the pattern up, down, left or right.

3.7.4. Microelectrodes.

The microelectrodes were made from borosilicate glass capillaries of 1.2 mm (or 1.0 mm) of outer diameter. A Frederick & CO Ultrafine Puller was used to pull the microelectrodes. The solution used to fill the microelectrodes was KCl 2 molar. The resistance of the electrodes when measured in ringer solution was between 100 to 250 MΩ. It was found that microelectrodes with higher resistance have the tendency to brake during penetration of the retina. The microelectrodes were prepared in the same day the experiments were conducted. Most often the microelectrodes were filled with KCl just before they were used. These electrodes seemed to have more strength than those left filled for two to three hours.

3.7.5. Electrical recording system.

The equipment used to record the electroretinogram and the electrical response of the retinal neurons is shown in fig 3.7.4. The piece of head of the mudpuppy containing the eye was grounded through ringer solution and a platinum wire (see fig 3.7.1). The microelectrode was connected to a high input impedance amplifier (W.P. Instrument Model 701) with gain 1. The typical input impedance of this amplifier is 20,000 MΩ. Two other amplifiers (Grass PS 16 and Textronix TM 503) are used in cascade to increase the gain to 100. Also, the PS 16 amplifier has a dial to control the dc level of the output signal. For ERG recording the cascade amplification was set to 5000. The ERG was recorded using low impedance (typical 10 or less) microelectrodes filled with 2 M KCl or 1% NaCl.
Fig 3.7.4 Circuit diagram of the electric equipment used to record from the eye-cup preparation. START is a pulse sent by the pattern system generator to start the A/D of the LS11/03 computer.
The output of the textronix amplifier was also connected to an oscilloscope (Textronic model 5113) to monitor the intracellular response or the ERG. Each time a light stimuli was applied on the retina a start pulse from the pattern generator system was sent to the LSll/03 computer to start the collection of the data. Data were collected for four seconds by the computer and stored in a disk. The sampling rate of the A/D analog converter used by the LSll/03 computer was 1KHz. When an experiment was finished all the data was transfered to an IBM 4331 computer where it was analized and processed.
CHAPTER IV

RESULTS

4.1. Scanning Micrographs of the Necturus Retina.

Small sections of different parts of the Necturus retina were photographed by using a scanning electron microscope. A view of the receptor and lateral side of the retina is shown in fig 4.1.1. Rod and cones are easy to differentiate by the form of the outer nucleus.

Rod's outer nucleus has the form of a bar while cone's outer nucleus looks like a semi-cone. The diameter of the rods and cones seem to be as large as 50 \( \mu \) m. It can be observed from fig 4.1.2 that the cross-sectional dimension of the retina is approximately 150 \( \mu \) m, where about 50 \( \mu \) m correspond to the length of rods and cones.

Fig 4.1.3 gives a close view of some rods and cones. The outer nucleus of the rods can be easily identified. The diameter of a rod seems to be typically about 10 \( \mu \) m while the thickest part of a cone is approximately 12 \( \mu \) m.

The small diameter of the receptors generally do not allow an easy penetration of their membrane by the microelectrode. Most, often it was found that an intracellular recording from receptors was of very short duration. Because of the similarity in form of the second and third order neurons of the retina, their identification can not be made
Fig 4.1.1 Top view of the Necturus retina. (Scanning micrograph prepared by the author).
Fig 4.1.2 Lateral view of Necturus retina (Scanning micrograph prepared by the author).
Fig 4.1.3 Close view of rods and cones of Necturus retina.

(Scanning micrograph prepared by the author).
with certainty. Fig 4.1.4 and 4.1.5 show the characteristics of some of the second and third order neurons. All the second and third order neurons appear to be approximately of the same size. Considering them to be shaped as spheres, their diameter varies between 20 - 30 μm. Very close views of the tip of a rod and a cone are shown in figs 4.1.6 and 4.1.7. In the rod, it can be seen that several layers folding into one another repeatedly. The cone’s tip seems to be formed by several discs piled upon top of each other.

4.2. Effects of different patterns of light on the behaviour of the retinal cells.

Intracellular and extracellular responses of different cells of the retina were measured from the retina of 60 Necturus. The recordings were very unstable in 40 experiments. There was very small ERG response from the retina of 15 Necturus, therefore these experiments were not continued. The responses from horizontal, depolarizing and hyperpolarizing bipolar cells, and ganglion cells were recorded in ten, two, two and four experiments respectively. In a majority of the experiments, spot and ring patterns were used to test and identify the cells.

4.2.1. Horizontal cell responses with spot, ring, ring spot, moving spot and bar, and rotating bar stimuli.

A typical intracellular response of a horizontal cell for a spot stimulus had 15 mV amplitude, a delay time of 100 msec and a rise time of about 400 msec. One such typical recording is shown in fig 4.2.1. About 50 recordings with spot and ring stimuli were made from horizontal cells.
Fig 4.1.4 Cells of outer nuclear layer of Necturus retina.

(Scanning micrograph prepared by the author).
Fig 4.1.5 Close view of retinal cells of Necturus. (Scanning micrograph prepared by the author).
Fig 4.1.6 Tip of a rod (Necturus). (Scanning micrograph prepared by the author).
Fig 4.1.7 Tip of a cone (Necturus). (Scanning micrograph prepared by the author).
Fig 4.2.1 Intracellular response of a horizontal cell with a spot stimulus.

Intensity = 3.3.6.
A typical intracellular response of a horizontal cell obtained with a ring stimulus had also an amplitude of about 15 mV but the delay time was about 50 msec and the rise time was usually 300 msec (fig 4.2.2). The intracellular response elicited by a moving spot does not show appreciable change when compared to the one obtained with a stationary spot. A typical response for this pattern is shown in fig 4.2.3. Different speeds of the moving spot did not produce appreciable change in the wave form of the intracellular response. About 10 recordings were made from horizontal cells with moving spot stimuli. These results are similar to the ones reported in the literature. (Dowling and Werblin, 1969; Werblin 1969).

The bar stimulus was used in five experiments with horizontal cells. The length of the bar as measured on the eye-cup was 2 mm. Fig 4.2.4. shows an intracellular response of a horizontal cell to a bar stimulus at a speed of 2.5 mm/sec. The response is similar to the one obtained with a spot stimulus applied at the center field of the cell.

One horizontal cell showed a peculiar response when a moving bar stimulus crossed its receptive field. The intracellular response of this cell to a bar (2 mm long) moving with a speed of 2.5 mm/sec. is shown in fig 4.2.5. The bar of the same length when moved across the receptive field of the same cell with a speed of 0.83 mm/sec, the response was different and it is shown in fig 4.2.6. The fast moving bar elicited two peaks while only one peak was observed with the slow moving bar. It appears that the response of this cell was dependent on the speed of the stimulus, a characteristic that was very peculiar to this particular cell.
Fig 4.2.2 Intracellular response of a horizontal cell with a ring stimulus.

radius = 660μm and thickness = 330μm. Intensity of ring = 20.
Fig 4.2.3 Intracellular response of a horizontal cell with a moving spot.

Speed of spot = 4.16 mm/sec. Intensity = 3.36.
Fig 4.2.4 Intracellular response of a horizontal cell to a bar stimulus.

Speed of bar = 2.5 mm/sec. Intensity = 15.78.
Fig 4.2.5  Intracellular response of a horizontal cell to a moving bar.

Speed of bar = 2.5 mm/sec. Intensity = 15.78.
Fig 4.2.6 Intracellular response to a moving bar.

Speed of bar = 0.83 mm/sec. Intensity = 15.78.
Fig 4.2.7 Intracellular response of a horizontal cell to a ring + spot stimulus.

radius = 660μm, thickness = 830μm. Intensity = 27.36.
Fig 4.2.13 Polar plot of the intracellular responses of a horizontal cell.

Magnitude: 2mm on polar plot 1mV of intracellular amplitude response.
Fig 4.2.9 Intracellular response of a depolarizing bipolar cell to a full field stimulus.
In several experiments on horizontal cells a ring stimulus elicited responses with two peaks as shown in fig 4.2.2. Such response was observed in 20 out of 50 cells recorded. This is a characteristic most often found in cells that have an antagonistic surround field.

Comparing the responses for a ring (fig 4.2.2), and for a spot (fig 4.2.1) it may be observed that the rise time for the spot is greater than the rise time for the ring stimulus. If the stimuli is a ring with a spot in the center (fig 4.2.7) the response resemble the one obtained with a single spot and the two peaks do not appears as was the case with just a ring stimulus. Thus it is clear that multiple peaks appear only when the surrounding receptive field alone is stimulated.

The rotating bar pattern was applied to several horizontal cells to study the symmetry of their receptive field. All the horizontal cells that were recorded intracellularly using the rotating pattern showed no significant asymmetry of their field. A polar plot of the amplitude of the cell response is shown in fig 4.2.8.

4.2.2. Bipolar cell response with full-field illumination.

All the responses measured from bipolar cells were made by using a full field stimulus. A typical response of a depolarizing bipolar cell is shown in fig 4.2.9. The response of both types of bipolar cells, were similar to the ones reported in the literature (Miller et.al. 1976).
Fig 4.2.10 Extracellular response of a ganglion cell to a spot stimulus.

Intensity = 3.36.
Fig 4.2.11 Extracellular response of a ganglion cell to a ring stimulus.

radius = 660μm and thickness = 325μm. Intensity = 20.
Fig 4.2.12 Extracellular response of a ganglion cell to a ring stimulus.

radius = 1500 \mu m and thickness = 325 \mu m. Intensity = 24.73.
4.2.3. Ganglion cell responses with spot, ring and rotating bar.

Figs 4.2.10, 4.2.11, 4.2.12 show extracellular recording from ganglion cells with spot, ring of radius = 660 μ m and thickness = 325 μ m, and ring with radius of 1500 μ m and thickness = 325 μ m.

The extracellular response to a spot stimulus had fewer spikes than the response with the ring stimulus. However when the diameter of the ring was increased the number of spikes was decreased. For example, the number of spikes/sec given by a ring of radius 660 μ m was approximately 20 spikes/sec while for a radius 1500 μ m is about 5 spikes/sec. The intensity of the ring stimulus with a radius 660 μ m was 15% less than the intensity of the ring stimuli with a radius 1500 μ m.

From the results obtained here, it was postulated that these ganglion cells had an excitatory surrounding field with a maximum excitation by a ring stimuli with a radius close to 600 μ m. The same cell when illuminated with a ring of radius 830 μ m and thickness 830 μ m increased the depolarization as shown in fig 4.2.13. When the thickness of the ring was increased to 1600 μ m the depolarization increased further and the amplitude of the spikes decreased, probably increased depolarization (fig 4.2.14). It seems then, that the depolarization showed by this ganglion cell is dependent on the area of illumination and that the size of the spikes are inversely proportional to the depolarization amplitude. Extracellular experiments on the ganglion cells revealed that the receptive field of one off-center ganglion cell was asymmetric. The asymmetry of a ganglion cell was determined by using a bar pattern, rotated three consecutive full turns around the receptive field. A polar plot of the number of spikes is shown in fig 4.2.15.
Fig 4.2.13 Intracellular response of an on-center ganglion cell to a spot stimulus.

Intensity = 3.36.
Fig 4.2.13 (continued) Intracellular response of an on-center ganglion cell to a ring stimulus.

radius = 830 μm, thickness = 830 μm. Intensity = 28.42.
Fig 4.2.14 Intracellular response of an on-center ganglion cell to a ring stimulus.

radius = 830 μm, thickness = 1600 μm. Intensity = 33.68.
Fig 4.2.15  Polar plot of rate of spikes of an off-center ganglion cell.

Magnitude: 2mm on polar plot = 1 impulse/sec.
Fig 4.3.2 Intracellular response of a depolarizing bipolar cell. Cobalt chloride 4mM.
It is clear from this figure that the receptive field is asymmetric having the highest rate of spike/sec for 180°. The polar plot magnitude is the average of three consecutive recording on the same ganglion cell.

4.3. Effects of some chemicals on the retinal cells.

Several chemical substances were applied on the Necturus retina to determine their effect either on the intracellular the electroretinogram (ERG). In all the intracellular experiments with chemicals the eye-cup was always washed out with control solution after the chemicals were applied to have a reasonable partial recovery. The chemicals applied and the responses measured on the retina are discussed in the following sections.

4.3.1. Cobalt chloride.

Cobalt chloride (4 mM) was applied on the superfused eye-cup of the Necturus. The b-wave of the electroretinogram (ERG) was completely abolished within 3 min. after the cobalt chloride was applied, leaving only the a-wave of the ERG (fig 4.3.1). However, the b-wave recovered almost completely when the eye-cup was superfused again with control ringer solution (fig 4.3.1). The same reduction of the b-wave was obtained in three different experiments conducted on the mudpuppy retina. Cobalt chloride (4 mM) also depressed the intracellular response of depolarizing bipolar cells (DPBC). The responses of DPBC at different times, before, during and after perfusion of cobalt chloride, are shown in fig 4.3.2. A decrease in the amplitude and an increase in the pulse width of the DPBC intracellular response was observed within 2-3 minutes after application of cobalt chloride.
Two experiments were conducted on DPBC and the results were same in both.

### 4.3.2. Nicotine

The application of 1 mM of nicotine on the superfused eye-cup of the Necturus, resulted in an increase of the b-wave of the electroretinogram (fig 4.3.3). Soon after nicotine was applied (about 2 min) the effect of the drug was noticed by an increased in the b-wave amplitude of the ERG. The drug was applied for about 6 minutes and then the eye-cup was superfused again with control ringer solution. In all the three experiments conducted with this dose a slow recovery of the b-wave was observed. The application of 0.1 mM of nicotine also increase the b-wave amplitude, slightly.

### 4.3.3. Hexamethonium and Hexamethonium-Nicotine

Hexamethonium, a nicotine receptor blocker was also superfused on the eye-cup, to determine its effect. A concentration of 3 mM of this drug was applied on the eye-cup and the ERG was monitored for 26 min. the drug was superfused for 16 minutes.

The b-wave amplitude is plotted in fig 4.3.4. In all the three experiments conducted with this drug no appreciable effect on the b-wave of the ERG was observed. However, when hexamethonium (3mM) mixed with nicotine (1mM) was superfused on the retina, no appreciable increase in the b-wave amplitude of the ERG was observed (fig 4.3.5). Three experiments were conducted on different eye-cups of Necturus with this combination of drugs. The results of these experiments with hexamethonium + nicotine indicate that the effects of nicotine on the ERG are blocked by the presence of hexamethonium in the bath solution.
Fig 4.3.3 b-Wave amplitude of ERG. Nicotine 1mM.
Fig 4.3.4 b-Wave amplitude of ERG. Hexamethonium 3mM.
Fig 4.3.5 b-Wave amplitude of ERG. Hexamethonium (3mM) + Nicotine (1mM).
Hexamethonium also did not have any effect on the intracellular recording of horizontal cells (fig 4.3.6).

4.3.4. d-Tubocurarine and d-Tubocurarine-Hexamethonium.

d-Tubocurarine, also a different type of nicotine receptor blocker was superfused in the isolated eye-cup of the Necturus. In three experiments a concentration of 1 mM of the drug affected the ERG amplitude, slightly. After the application of the drug, a slight increase in the b-wave of the ERG was observed (fig 4.3.7) followed by a slow return to the pre-drug value when the control ringer solution was again superfused. However, the superfusion of d-tubocurarine (1 mM) + Nicotine (1 mM) did effectively increase the b-wave amplitude of the ERG (fig 4.3.8). The result of three of these experiments using nicotine d-tubocurarine clearly indicate that d-tubocurarine did not block the effect of nicotine.

Thus, it was demonstrated that only hexamethonium was effective in blocking the effect of nicotine. Also atropine was applied in the same way on the eye-cup to determine the possibility of some muscarinic blocking activity in the retina. A concentration of (100 nM) did not change the amplitude of the b-wave of the ERG (fig 4.3.9). Three experiments with atropine gave the similar results. However, even if the negative results of atropine predict no muscarinic receptors in the retina it is not yet possible to eliminate completely the existence of those type of receptors.

4.3.5. Glutamic acid.

Glutamic acid has been postulated as a possible neurotransmitter in the vertebrate retina. However strong concentration (50 mM) have been used to see its effect on the retina (Cervetto et al. 1972).
Fig 4.2.6 Intracellular response of horizontal cell. Hexamethonium 3mM.
Fig 4.3.7  b-Wave amplitude of ERG. d-Tubocurarine 1mM.
Fig 4.3.8  b-Wave amplitude of ERG. d-Tubocurarine (1mM) & Nicotine (1mM).

Fig 4.3.9 b-Wave amplitude of ERG. Atropine 100μM.
In the experiments conducted on Necturus retina a lower concentration was used to find out if the depressing effect of glutamic acid was still present. A result showing a decrease of the b-wave amplitude of the ERG was observed with a dose of 4mM (fig 4.3.10). In two other experiments with 2mM concentration of glutamic acid a decrease in the b-wave amplitude was observed. Thus, it seems that it not necessary to use a high concentration of glutamate to affect the ERG response of the Necturus retina.

4.4. Conclusions.

A novel pattern generator system has been developed. In addition the generating commonly used patterns (spot, ring, moving bar and spot, ring + spot) it can generate a new pattern namely rotating bar.

It is very flexible system and is easy to use. It is also possible to add new patterns by simply writing a new program and loading it into memory. The rotating bar has proven to be very useful to study the symmetry of the receptive field of horizontal and ganglion cells. It was found that the receptive field of the horizontal cells is very symmetric i.e. that a bar of about 1500µM applied radially to the center of the field will give the same type of response (in wave-form and amplitude) at any angle of the bar. Also horizontal cells did not show any particular preferences when a moving bar crossed the receptive field in several different directions. However, one horizontal cell showed different responses for different speeds of a moving bar (fig 4.2.7).

It is possible that some horizontal cells may have a time-space integrative response in which the response of the cell is dependent not only on the speed but also on the relative position of the stimuli with respect to the center of the receptive field.
Fig 4.3.10  b-Wave amplitude of ERG. Glutamic acid 4mM.
This type of function of the horizontal cell could transmit some information about movement and distance of the object to the brain. Werblin and Dowling (1969) found that the response of horizontal cells is proportional to the area of illumination in Necturus. Similar results were obtained in our experiments. Another characteristic that was found in all the horizontal cells is that the rise time of the intracellular response is always faster for a ring than for a spot stimulus of the same intensity. This is probably due to the greater number of synapses excited by a ring as compared to a spot.

The fact that some (about 50% of the experiments) horizontal cells showed slight antagonistic surround field characteristic is surprising since other investigators have not reported this fact. This would indicate that all the synapses from horizontal to horizontal cells may not be of the same type, that is, there may be inhibitory and excitatory synapses between them or some feedback could occur from horizontal cell to the receptors. Piccolino et al. (1981) found a peripheral antagonism in the L-horizontal cells of the turtle retina. The results of the extracellular recordings from ganglion cells using the rotating bar throw some new light regarding the symmetry of the receptive field of the ganglion cells (off-center). The ganglion cell response is maximum for a bar at 180° position as can be seen in the polar plot of the number of spikes/sec. This would indicate that some off-center ganglion cells have some mechanism to detect the position of the object's images projected on the retina. Karwoski et al. (1976) using extracellular and Werblin (1969) using intracellular measurement from the retina of Necturus found that some ganglion cells do show directional selectivity when a moving spot crossed their receptive fields.
Valuable information about the form of the receptive field of horizontal and off-center ganglion cells of the retina of Necturus has been obtained by using the bar pattern. Intracellular recording obtained from one on-center ganglion cell showed that in this cell the amplitude of the spikes was dependent on the area of illumination and that increasing depolarization always occurred when the area of illumination was increased. It may postulated that some ganglion cells transmit information in pulse amplitude modulation. This hypothesis has to be tested further by obtaining more results.

The effect of cobalt chloride on the ERG was dramatic. Several investigators have studied the effect of cobalt magnesium on the vertebrate retina (chapter II, section 2.4) and found that these chemicals depleted completely the response of the second order neurons of the retina. Katz and Miledi (1965) found that chemical synapses require extracellular calcium for the secretion of the transmitter and Weakly (1973) showed experimentally that cobalt is a strong antagonist of the effect of calcium. Thus it is believed that in the retina, cobalt ion inhibits the release of the transmitters from the receptor terminals.

The experiments done on the Necturus retina confirm this assumption since the ERG was completely abolished by the cobalt chloride leaving only the a-wave intact. Another effect noticed was an increase in the latency of the a-wave after the superfusion of the solution containing cobalt. It is possible then that cobalt ions affect not only the release of the neurotransmitters but also change the dynamic characteristic of the receptor membranes. Evans et.al. (1978) suggested that for concentrations greater than 2.4 mM cobalt may affect the function of the outer segments which could be related to the change in the latency of
the a-wave. Cobalt chloride changed not only the amplitude but also the
time response of the intracellular recording from depolarizing bipolar
cells (fig 4.3.2). The time expansion showed in fig (4.3.2) may be
due to slow elimination of the feedback from some of the horizontal cells
in the bipolar cell terminals.

Several nicotinic related drugs were used to study their effects
on the electroretinogram of the Necturus retina. A review on the
possible existence of nicotinic receptors in the retina was given in
chapter II, section 2.4. Nicotine increased the b-wave amplitude of
the ERG considerably but its effect was blocked by hexamethonium.
These two facts alone support the idea of the existence of some nicotinic
receptors on the Necturus retina. These nicotinic receptors appears to
be similar to the well known receptors found in peripheral autonomic
ganglia. Since atropine did not have any effect on the ERG it indicates
the absence of muscarinic receptors. d-Tubocurarine, also a nicotine
blocker in the skeletal muscle, did not block the effect of nicotine on
the ERG. However tubocurarine do show some agonist effect on the
denervated muscle (Bowman, 1980), so its slight agonist effect on the
retina is not completely unexpected.

From the experiment on these nicotinic related drugs it is possible
to postulate the existence of nicotinic receptors in the Necturus retina.
Unfortunately since the ERG is an ensemble response from all the neuronal
cells it is not adequate to state with certainty the localization of
these types of receptors.
BIBLIOGRAPHY

1.- Ames A, Pollen D. A.

Neurotransmission in central nervous tissue: a study of isolated rabbit retina.

The visual cells and visual pigment of the mudpuppy Necturus.

3.- Brotherton J.

Studies on the metabolism of the rat retina with special reference to retinitis pigmentosa, II. Amino acid content as shown by chromatography.

4.- Bonting S. L.

Transmitters in the visual process.

5.- Cohen A. I, Mc Daniel M, Orr H.

Absolute levels of some free amino acids in normal and biologically fractionated retinas.
6. - Cervetto L., Mac-Nichol F. E.

Inactivation of horizontal cells in turtle retina by glutamate
and aspartate.

7. - Chan Y. Raymond and Naka Ken-Ichi.

The amacrine cell.

8. - Copenhagen R. David.

Time course of threshold elevation in on-off ganglion cells of
Necturus retina: Effects of lateral interactions.

9. - Dick Evan, Miller Robert F.

Light-evoked potassium activity in mudpuppy retina.

10.- Dowling E. John and Werblin S. Frank.

Organization of retina of the mudpuppy, Necturus maculosus.

11.- Dacheux F. Ramon, Frunkes E. Thomas and Miller F. Robert.

Pathways and polarities of synaptic interactions in the inner
retina of the mudpuppy.

12.- Dowling E. John and Ripps Harris.

Effect on magnesium on horizontal cell activity on the skate retina.
13.- Data component manual.
      Intel (1979).
14.- Dowling J. E, Boycott B. B.
      Organization of the primate retina; electron microscopy.
      Differential effects on cobalt ions on rod and cones synaptic
      Activity in the isolated frog retina.
16.- Fain L. Gordon, Dowling E. John.
      Intracellular recording from single rods and cones in the
      Mudpuppy retina.
17.- Fulton B. Anne and Rushton H. A William.
      Rod ERG of the mudpuppy: Effect on dim red backgrounds.
18.- Finkelsteind D. Otto-Joachim Grusser.
      Frog retina: Detection of movement.
19.- Grusser-Cornehls V., Grusser O., Bullock T. H.
      Unit responses in the frog's tectum to moving and nonmoving
      visual stimuli.
20.- Graham T. L. JR.
      Comparative aspects of neurotransmitters in the retina.
21.- Hagins A. W and Yoshikami S.
A role for Ca** in excitation of retinal rods and cones.

22.- Karwocki J. Chester.
Ganglion cell responses of the mudpuppy retina to sinusoidal flicker.

Effects of aspartate and glutamate on the bipolar cells in the
carp retina.

24.- Goodland H.
The ultrastructure of the inner plexiform layer of the retina of
cotus hubalis.

Effects of chemicals on receptors and horizontal cells in the retina.

26.- Michael M. R.
Receptive fields of directionally selective units in the optic
nerve of the ground squirrel.

27.- Miller Robert F and Dacheux Ramon F. (a,b,c).
Synaptic organization and ionic basis of on and off channels in
mudpuppy retina, I, II, III.
28.- Neal J. Michael.

*Amino acid transmitter substances in the vertebrate retina.*


*Responses to directional stimuli in retinal preganglionic units.*


30.- Nelson D. R, Gruber S. H.

*Contour interaction and visual resolution: contralateral effects.*


31.- Pasantes-Morales H, Ledig J.K, Mandel P.

*Free amino acids of chicken and rat retina.*


32.- Schwartz A.E.

*Electrical properties of the rod syncytium in the retina of the turtle.*


33.- Straschill M.

*Action of drugs on single neurones in the cat's retina.*


34.- Starve M. S.

*Effect of dark adaptation on the GABA system in the retina.*


35.- Sugawara K. and Negishi K.

*Effects on some amino acids on light-induced responses in the isolated carp retina.*


36.- Tuttle JR.

Comparison of the responses of Necturus retinal ganglion cells to stationary and moving stimuli.

37.- Werblin F. S, Dowling J. E.

Organization of the retina of the mudpuppy, Necturus maculosus.

38.- Werblin F. S.


39.- Val'Tsev, V. B.

Role of cholinergic structures in outer plexiform layer in the electrical activity of frog retina.

40.- User manual pattern generator system

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