ORIGIN OF THE OCULAR LIGHT-MODULATED STANDING POTENTIAL IN CAT

By

HARRIS R. LIEBERMAN

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1977

ACKNOWLEDGEMENTS

I would like to thank the Chairman of my committee, Dr. William W. Dawson, for his guidance. He generously shared his time providing intellectual stimulation and advice and assistance that were invaluable to me. I would also like to thank the other members of my committee, Dr. Calvin K. Adams, Dr. Jay M. Enoch, Dr. Merle E. Meyer, and Dr. Horst O. Schwassmann, for their thoughtful criticism and constructive advice. I am also very grateful to Dr. Constance Fitzgerald who photocoagulated the discs of the subjects used in the vascular lesion experiments. I would also like to thank Minnie Hawthorne who did the histology and Carl Litzkow who constructed the high impedence DC amplifier. I wish to thank the Center for Neurobiological Sciences for financial support.

Finally, my Ellice provided invaluable help actively assisting throughout.

TABLE OF CONTENTS

Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

ORIGIN OF THE OCULAR LIGHT-MODULATED STANDING POTENTIAL IN CAT

By

Harris R. Lieberman

August, 1977

Chairman: William W. Dawson
Major Department: Psychology

In many vertebrates a direct current (DC) potential or standing potential (SP) exists across the eye with the cornea being positive relative to the posterior pole. After the onset of a light preceded by a period of dark adaptation a slow damped oscillation of the potential is observed. The initial peak of this oscillation occurs at about 6 minutes after light onset. At light cessation a similar damped oscillation of opposite polarity and lower amplitude results. These modulations in the standing potential induced by light onset and cessation are termed the light modulated standing potential (LMSP). To directly record the LMSP in animals a highly DC stable recording system was developed. It was employed to localize the origin of the ocular LMSP in the cat. The precise origin of the potential is controversial although the PE and receptors are generally

considered the source. Three experiments were performed to determine if other retinal structures participate. In one experiment the LMSPs elicited by the onset or cessation of a steady or flickering light of equal time averaged irradiance were compared. The neural elements in the retina are known to be responsive to transient changes in irradiance. Therefore large differences in the LMSPs should be elicited by the paired flickering and steady stimuli if these neural elements or an associated process participate in generation of the LMSP. No differences were found. In two other experiments the inner retina (all retinal elements except the receptors) was lesioned either chemically with sodium aspartate or by occlusion of the retinal vasculature at the optic disc. After either type of lesion the LMSP could no longer be elicited or was reduced significantly. The b-wave of the electroretinogram (ERG) which originates in the inner retina was also abolished or similarly reduced while the a-wave arising mainly in the receptors was preserved. It was concluded an inner retinal component is essential for generation of the LMSP. Since the LMSP did not respond differentially to flickering vs. steady illumination, as expected if it was of neural origin, it was hypothesized that the Muller cells (retinal glia) are responsible for its generation.

CHAPTER 1

INTRODUCTION

Since the mid-nineteenth century it has been known that there is a potential difference between the anterior and posterior pole of the eye (Skoog, 1975). In the vertebrate eye the cornea is positive with respect to the fundus (Skoog, 1975). This standing potential (SP) across the eye varies with changes in retinal illumination. Specifically, the SP voltage increases when illuminance is increased (Fig. 1a) and decreases with dark adaptation (Fig. 1b). In the human these changes resemble damped oscillations and have a period of approximately 25 minutes (Arden and Kelsey, 1962a,b; Skoog, 1975; Taumer, et al., 1975).

Until recently, the human SP as influenced by changes in retinal illumination had been measured only by the indirect electrooculogram technique because of the technical difficulties associated with direct current (DC) recording. For this indirect method electrodes are placed at the inner and outer canthus of each eye and, because the eye is a dipole, horizontal eye movements of a standard amplitude indirectly monitor standing potential. As the eye rhythmically moves left and right a potential resembling a triangular waveform is generated. The peak to trough amplitude

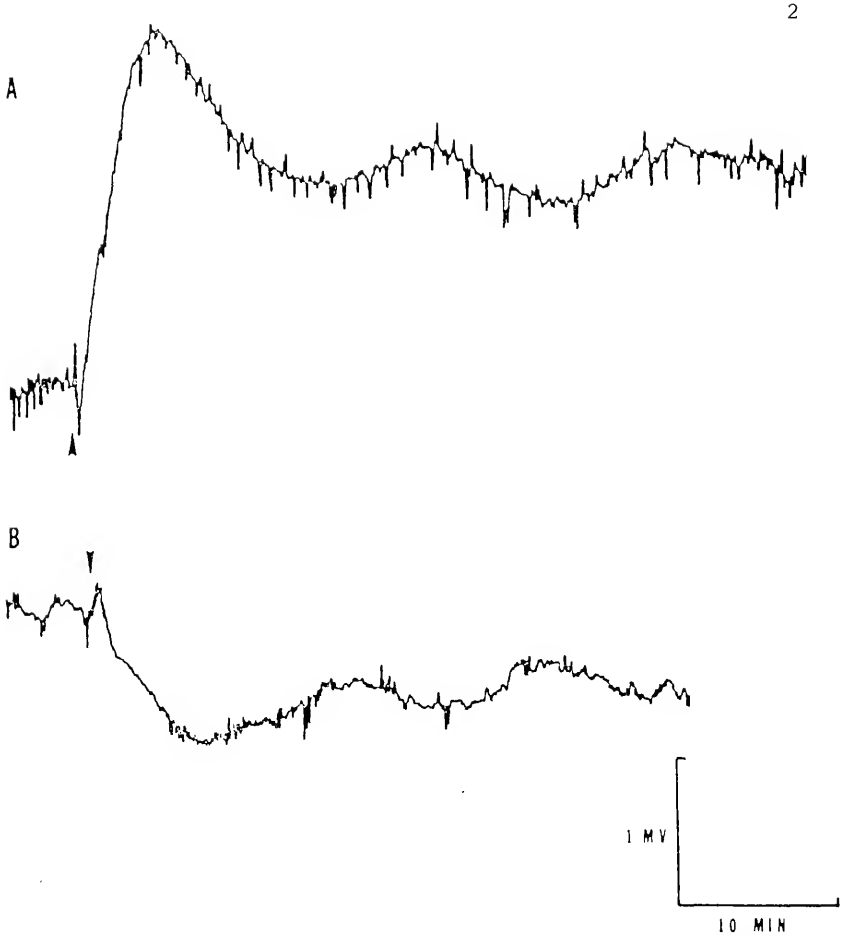


Figure 1. The light-on (A) and light-off (B) light modulated standing potentials (LMSPs) elicited by the onset or cessation of the steady unattenuated light source. Light-on and off are indicated by arrows. Note the long time course of the changes. In this and all other illustrations of electrophysiological signals an upward deflection is a cornea-positive response.

of this potential is an indirect measure of the SP across the eye.

A number of problems, however, are associated with this technique. The subject must be cooperative and able to make the necessary eye movements. Therefore it is not readily applicable to children and animals. Even fully cooperative subjects fatigue rapidly and any variance in the extent of eye movements may be an artifact.

To avoid confusion the following terminology will be adapted:

SP - the D.C. voltage across the eye

LMSP - the change in SP induced by an increment or decrement in retinal illumination

EOG - indirect measure of the light modulated SP as described above

Recently, however, it has been demonstrated that high quality direct recordings of the light modulated SP in the human are possible (Nilsson and Skoog, 1975; Skoog, 1975). Directly recording the SP is difficult because of its DC nature. Direct current amplifiers must be substituted for conventional capacitively coupled amplifiers and specially constructed non-polarizing and highly stable electrodes must be employed.

Both direct and indirect methods of recording SP have been attempted in non-human subjects by a few investigators. For indirect recordings mechanical devices have been em-

ployed to physically move the eyes of animals (Foulds and Ikeda, 1966; Heck and Papst, 1957).

Both direct and indirect recordings have shown that like the human SP, the SPs of many vertebrates respond to changes in retinal illumination and these changes resemble damped oscillations (Kikwada, 1968).

Due perhaps to the severe technical problems there has been little basic research on the LMSP and SP in spite of the wide acceptance of the EOG as a clinically useful diagnostic test. We have been successful, however, in developing a satisfactory system for directly recording the standing potential. It has been employed here to localize the LMSP in the cat.

The precise origins of the SP and the LMSP are controversial. Generally it is believed they originate from a pigment epithelium (PE) and receptor interaction (Arden and Kelsey, 1962b; Arden, et al., 1962; Noell, 1954; and Taumer, et al., 1974). It was Noell (1954) using rabbits, who documented the importance of the PE in generating the SP (but not the LMSP). His research was largely based on his accidental discovery of SP responsiveness to a chemical, sodium azide. When small amounts of this drug are introduced into the circulation, a transient increase in SP voltage results. Noell found that when the retina was severely lesioned with iodoacetic acid the azide response of the SP was preserved although somewhat reduced in amplitude. However in animals with an intact retina but a PE lesioned

by another chemical (iodate), the SP responsiveness to azide was not present. Noell concluded that the PE was an essential element in the generation of the SP across the eye.

The importance of the retinal receptors in the generation of the SP is evident from the human action spectrum of the light modulated EOG. It closely resembles the C.I.E. scotopic sensitivity curve (Arden and Kelsey, 1962b). Also in the rabbit the LMSP is totally abolished after retinal detachment (Arden and Kelsey, 1962b; Foulds and Ikeda, 1966).

Gouras and Carr (1965) and Gouras (1969) while conceding the importance of the PE and photoreceptor in the generation of the SP have stated that the LMSP is influenced by retinal structures other than the receptors. This conclusion was based on studies of monkeys with occluded central retinal arteries and humans with similar lesions. Succeeding this occlusion the inner retina degenerates but the photoreceptors and PE remain intact (Brown, 1968; Gouras and Carr, 1965). In monkeys Gouras and Carr (1965) report that one to five weeks following surgery the LMSP response to "light on" was absent or severely attenuated. In humans with similar lesions it has been reported that the light onset rise of the EOG is abolished (Nagaya, 1964) but the light-off response initially disappears but eventually returns. Gouras (1969) concluded that the light rise and dark trough of the SP arise at different loci and that the light rise requires the presence of a functioning inner

retina. Arden and Kelsey (1962a,b) also suggest that the light peak and dark trough of the EOG have fundamentally different properties. They based this conclusion on apparent differences between the two EOG components. Other investigators, notably Taumer et al. (1974) believe that the light rise and dark trough of the LMSP represent the same process.

Since we have developed a method of recording LMSP in animals, it was employed to begin to localize the LMSP. The overall approach taken was to determine if certain retinal layers or processes were necessary for the generation of the LMSP. Since the retina transmits information serially for the most part, it seemed advisable to begin at the earliest stages of this process and move inward.

Four experiments make up the body of this dissertation. The first characterizes the LMSP of the cat. Specifically a quantitative description of the time course of the light-on and light-off LMSP is presented. In addition amplitude by intensity functions were generated for both the light-on and off LMSP. This function could then be compared with amplitude by intensity functions at certain stages in the retinal visual process. A second experiment compared LMSPs elicited by steady and flickering lights of equal time averaged irradiance. This experiment was designed to determine the contribution of retinal neural activity to the LMSP as compared to photopigment related processes. The neural retina is known to be highly sensitive to time varying stimuli

(as reviewed by Brown, 1965) while the photopigment in the receptors and related metabolic processes are not sensitive to such stimuli (Campbell and Rushton, 1955).

The third and fourth experiments determined if the LMSP is generated in the receptors and PE alone or if neural and/or glial inner retinal components are involved. To functionally separate the inner and outer retina two techniques, one chemical and one surgical, were applied. The chemical approach used sodium aspartate which by activity on the horizontal and bipolar cells prevents conduction of impulses beyond the receptors (Cervetto and MacNichol, 1972; Murakami et al., 1972; Murakami et al., 1975). The other technique consists of surgically occluding the blood supply to the inner retina by lesioning the retinal vessels as they enter the eye at the optic disc. The receptors and PE are supported by the choroidal circulation so they are not affected. This is a standard technique (Brown, 1968; Maffei and Poppele, 1968). The effectiveness of both types of inner retinal lesions were confirmed by recording the electroretinogram (ERG). The a-wave is a measure of outer retina viability while the b-wave has an inner retinal site of origin (Brown, 1968).

In all experiments the primary dependent variable was the LMSP. It was examined as a function of the various experimental conditions described individually below. In some experiments ERGs were also recorded.

Significance

Research on various field potentials in the eye has a long history. The major components of the electroretinogram have been localized and this has led to a number of basic and applied contributions. For example, because the a-wave has been shown to reflect the activity of the rods and cones various researchers and clinicians have used it to study the receptors. For example, Boynton and his associates (Baron and Boynton, 1974; Boynton and Whitten, 1970, 1972) have made important basic contributions by using the a-wave to study receptor information coding in primates. Their findings concur with intracellular recordings of Normann and Werblin (1974) in the mudpuppy.

It is not currently feasible to record from mammalian receptors and even in other animals it is extremely difficult. Field potentials are presently the only available technique for directly studying the electrophysiology of receptors.

It is generally believed that the LMSP and SP are a measure of receptor and PE metabolic activity (Arden and Kelsey, 1962b; Arden, et al., 1962; Noell, 1954; Taumer, et al., 1974). This viewpoint has therefore channeled basic research and clinical application regarding the SP and LMSP in a particular direction. As discussed above this may not be a correct formulation since Gouras and Carr (1965) have apparently demonstrated an inner retinal contribution to the light modulated SP. Recent basic research of Skoog (1975),

Nilsson and Skoog (1975) and Taumer, et al., (1974), for example, are based on the assumption the LMSP is a measure of PE and receptor activity. Similarly the clinical community has considered the EOG as useful only for diagnosing PE related lesions (Arden et al., 1962).

A more definite localization of the origin of the SP should be an important step in successfully utilizing it for basic and applied purposes. In the LMSP is a measure of inner retinal metabolic activity a powerful new tool may be available for studying the metabolic concomitants of retinal activity in normal and pathological states. The potential importance of such readily recordable, non-invasive measures of retinal metabolic activity should not be underestimated. Very little is known about how retinal neural activity is related to retina and PE metabolism. It is hoped that localization of the LMSP will provide a new advance in studying such relationships.

CHAPTER 2

LITERATURE REVIEW

In this section a thorough review of the classical and modern literature on the SP and LMSP is presented.

The existence of a standing potential across the eye was first documented by Du Bois Reymond in 1849. Using a freshwater fish (the tench), he found that a potential difference existed between the front and the back of the eye. During the late 19th and early 20th century other investigators (Dewar and M'Kendrick, 1876; Kuhne and Steiner, 1881) extended this observation to a variety of species and it became apparent that this potential was cornea positive for vertebrates and cornea negative for invertebrates (Arden and Kelsey, 1962a; Marg, 1951; cited in Miles, 1940). This polarity difference has been attributed to the reversed position of the receptor cell layers in the vertebrates and invertebrates (Arden and Kelsey, 1962a). It was also demonstrated that this SP was modulated by light (cited in Arden and Kelsey, 1962a; Himstedt and Nagel, 1902; Kuhne and Steiner, 1881). The early investigators also generated data which suggested that the SP originated in the retina and/or PE. In 1903 DeHaas found that the potential changed dramatically at the ora serrata. Additionally it was demon-

strated that large potential differences exist across the isolated retina (cited in Arden and Kelsey, 1962a; DeHaas, 1903; Dewar and M'Kendrick, 1876; Kuhne and Steiner, 1881). Dewar (1877) was the first to record the human SP (Miles, 1939). All of these early investigators were recording the SP directly and because of the difficulties associated with such DC recordings and discovery of the more easily recorded ERG, a decline in interest in the SP occurred (Ten Doesschate and Ten Doesschate, 1956).

Interest in the SP was reawakened in the 1930's in connection with attempts to monitor human eye movements (Jacobsen, 1930; Meyers, 1929). Electrodes were placed at the inner and outer canthus of each eye and when horizontal eye movements were made voltage changes resulted. These investigators believed that these electrical changes were due to activity of the extraocular muscles. However, Mowrer, et al. (1936) disproved this hypothesis. They demonstrated that passive movements of the eyes of anesthetized cats led to similar potentials and injection of chromic acid into the eye abolished these potentials. After Mowrer et al. (1936) established the intraocular origin of the SP, interest in it increased.

Miles (1939) was one of the first to apply this new technique for other than eye movement research. He demonstrated that the EOG in humans is modulated by changes in retinal illuminance confirming the classical work on the SP. Additionally he determined that a variety of other parame-

ters influenced the EOG, including pressure on the eye and changes in blood pressure.

In the middle and late fifties a number of papers appeared which investigated the effects of various visual parameters on the EOG and attempted to develop it as a clinical test of visual function (Aserinsky, 1955; Francois, et al., 1955, 1956; Kris, 1958; Ten Doesschate and Ten Doesschate, 1956). This activity culminated with important papers by Arden and his associates which established the EOG as a useful clinical test (Arden and Kelsey, 1962a,b; Arden, et al., 1962).

Aserinsky (1955) evaluated the effect of illumination and sleep on the amplitude of the EOG. He found that it was significantly elevated by light and depressed by dark. Additionally he found that a long period of dark adaptation and sleep did not further reduce the amplitude of the SP beyond the trough reached during a period of dark adaptation. Instead the EOG on awakening was considerably greater in amplitude than the pre-sleep dark adapted voltage. This may reflect diurnal variations in EOG which have been reported but not quantified by Kris (1958).

Ten Doesschate and Ten Doesschate (1956), confirmed the findings of Aserinsky (1955) with respect to the light peak and dark trough of the human EOG. Additionally they stated that the dark trough was followed by a rise in EOG potential. Kris (1958) and Kolder (1959) confirmed these observations and concluded that both light on and off responses

resembled damped oscillations. Kris noted that the first peak of the light adapted EOG occurred at the 10th minute plus or minus 2 minutes after the change in illumination, the first trough at 25th minute plus or minus 4 minutes and the second peak at the 36th minute plus or minus 4 minutes. She also found that the dark response reached an initial trough at 8-10 minutes after light off and, in agreement with Ten Doesschate and Ten Doesschate (1956), observed that it returned to a steady state level below the preceding light adapted baseline value.

The first studies on clinical application of the EOG appeared in 1955 and 1956. Francois et al. (1955, 1956a,b) studied the effect of light on the EOG. In general their findings are in agreement with the other studies mentioned above. They also found abnormal EOG Ss with various retinal ocular pathologies. The EOG sensitivity to light onset or cessation was abnormal in cases of retinal detachment, central retinal artery occlusion and glaucoma but normal in cases of papillitis, retrobulbar neuritis, and lesions of the visual cortex. Additionally they noted a dissociation of the SP and the light modulated SP. For example, in central retinal artery occlusion the SP was intact but could not be modulated by light.

These papers, published in the mid and late 1950's laid the groundwork for the pivotal works of Arden and associates on the EOG and its clinical application (Arden and Barrada, 1962; Arden and Kelsey, 1962a,b; Arden, et al., 1962).

Arden's group published the first detailed parameteric study of the EOG. The parameters explored included light intensity, length of period of illumination, the relationship between the light peak and dark trough and the wave length of the light stimulus employed. In addition, they proposed a standardized clinical EOG testing procedure which has been widely accepted.

Arden and Kelsey (1962a), based on a variety of experimental results, suggest that both the light peak and dark trough components of the EOG are generated in one structure. However, they also conclude the dark trough and light rise differ fundamentally in their properties.

Support of a common structural site for the origin of the SP comes from their discovery that the two opposite waves interact rather than sum algebraically in the manner that the a- and b-waves of the ERG, generated at different loci, summate. They also found what they considered to be two fundamental differences between the light peak and dark trough. The dark trough had an all-or-nothing nature with respect to the brightness of the preceding light period. Its amplitude was independent of the duration to the preceding illumination. The light rise, however, was graded in amplitude depending on the brightness and duration of the evoking stimulus. They concluded that light peak and dark trough are the result of processes acting upon a single generator of the LMSP.

In a second paper, Arden and Kelsey (1962b) concentrated on exploring various aspects of the light peak.

Preliminary to determining the action spectrum of the light rise they did an amplitude by intensity function over a 7 log unit range on the light peak. They found the amplitude of the light rise to vary linearly with the logarithm of the light intensity from 20 to 10,000 trolands (td). Above that range the light rise was saturated. They then went on to measure the EOG action spectrum and found it was nearly identical to the C.I.E. scotopic visibility curve (the average dark adapted spectral sensitivity of the human visual system). They concluded that the first step in the generation of the SP light rise was the rhodopsin in the rods of the retina.

Arden and Kelsey (1962b) also investigated in some detail the influence of the length of the dark period on the succeeding light rise. With increasingly long periods of dark adaptation (up to 20 minutes) the light rise increased in amplitude. When these data were compared to estimates of the percent of rhodopsin regenerated in the human following a total bleach (Rushton, 1961) the correspondence was fairly good.

Because Arden and Kelsey (1962b) hypothesized that the light rise was, in a very general sense, due to an increase in metabolic activity, they inferred that it would be very sensitive to changes in blood supply to the eye. Therefore, another portion of the Arden and Kelsey (1962b) paper is devoted to measuring the effects of decreased blood supply to the eye created by pressure exerted on the globe. The

resulting ischemia caused a fall in SP. In addition, when pressure was applied at various times during the light-dark cycle, the magnitude of the fall but not the final resting value varied. Regardless of whether pressure was applied to the eye during a light peak or a dark trough the SP fell to the same level which was about equal to the dark trough value. They concluded that the light sensitive and pressure sensitive portions of the SP were similar.

Arden and Kelsey (1962b) and Arden et al. (1962) also presented data from various patients with ocular lesions to further examine the effect of metabolic changes on the SP and to localize the site of its generation. The light rise was severely reduced in cases of central retinal vein occlusion which they thought indicated the importance of vascular factors. It was also observed, in early cases of trauma induced retinal detachment where light perception and small ERGs were still present, that the EOG was absent. In cases of choroiditis where the ERG remained normal the light rise of the EOG was abnormally small. It was concluded that the light rise of the SP requires a functional PE and PE-retina interface and the primary event eliciting the light rise occurred in the pigment epithelium.

Since the appearance of the papers by Arden and his co-workers in 1962, there have only been a few non-clinical studies on the light modulated SP in spite of the wide acceptance of the EOG as a clinically useful procedure. Some of these papers have contradicted data and interpre-

tation of the studies by Arden and associates in 1962. For example, in 1965, Gouras and Carr demonstrated the light rise was abolished by CRA occlusion in the rhesus monkey. In a later paper Gouras (1969) suggested, based mainly on clinical data, that the light rise and dark trough arise in different structures. Taumer et al. (1974) argue against Arden and Kelsey's (1962b) conclusion that the light rise and dark trough differ fundamentally. They found that the two light modulated components of the human EOG are similar, both being damped oscillations with a common cycle time of about 25 minutes.

Foulds and Ikeda (1966) examined the EOG of the rabbit following experimentally induced retinal detachment. They noted a decrease in SP amplitude and the complete absence of the light peak if the detachment was complete. This is in agreement with what Arden and Kelsey (1962b) found on humans with retinal detachments.

Some of the most recent basic research on the SP demonstrated that it could be recorded directly from the human by using a highly stable calomel half-cell electrode system (Skoog, 1975). In agreement with other investigators, notably Taumer et al. (1974), he found the light on and off components of the SP to be similar in time course, to be damped oscillations with a period of about 30 minutes, and to be of opposite polarity.

CHAPTER 3

GENERAL METHODOLOGY

Subjects

The thirteen subjects in all experiments on the LMSP were adult cats of either sex weighing 2.5-4.0 kilograms. The cats were housed in individual cages and were maintained on a cycle consisting of 12 hours of light and 12 hours of dark. Appendix A lists the various experiments for which each cat was used.

Electrode Fabrication

The single greatest obstacle to high quality DC recording was construction of high stability electrodes with no DC drift. DC drift is a slow constant change in potential seen across electrodes when their stability is measured in physiological saline. This type of drift is not normally a problem for electrophysiological recordings because the potentials usually measured last no more than a few seconds. However for the measurement of the LMSP, which has a time course many minutes long, such instability is unacceptable. Therefore high DC stability Ag-AgCl electrodes were successfully fabricated. The method used in their construction, as described below, was derived in part from Geddes

(1972). Ten mil platinum wires about 4 cm long with a small loop at one end were first cleaned by sonication in Freon 12. In order to avoid the deposition of organic materials on the electrodes they were only handled with jeweler's forceps once this initial cleaning was completed. Following the cleaning the loops on the end of the wires were immersed in a mixture of pure AgO and a small amount of distilled water. This mixture adhered to the loop and formed an irregularly shaped cylinder which was approximately 4 mm long and approximately 1.5 mm in diameter. The electrode was then placed in a 500°C oven for 1 hour. This converted the AgO to pure silver. The next process was to coat the platinum but not the silver cylinder with an insulator, Robert's epoxy. Finally the silver was chlorided by using a constant current DC power supply. To accomplish this the electrode was placed in a bath of physiological saline and connected to the positive terminal of the power supply. A platinum plate also immersed in the saline was connected to the negative pole of the power supply. A current of 10 milliamps at 10 volts was passed across the electrode for 5 minutes. The electrodes were then stored in a bath of physiological saline and grounded together until used. Prior to an experiment they were checked for DC stability and noise level in a physiological saline bath. DC drift of the complete recording system and electrode measured across a saline bridge was less than 65 uV/hr. These same elec-

trodes were also used to record the ERGs. Their low noise characteristics proved excellent for this purpose.

Generation and Presentation of Visual Stimuli

A Maxwellian view stimulator with a Sylvania R1131C glow tube (Buchmann-Olsen and Rosenfalck, 1957) was the source that provided the steady or flashing visual stimulus used to modulate the SP. This stimulus subtended 27.2° of visual angle. The total irradiance of the stimulus when unattenuated was 5.4 mW/steradian as measured by an Epply thermopile. The spectral emission of the R1131C glow tube at 30 mA, the current at which it was always run, is presented in Appendix B. When necessary the stimulus was attenuated with Kodak #96 wratten neutral density filters. The unattenuated irradiance will be referred to throughout as the 0.0 intensity level and all other irradiance values are calculated with reference to this value.

Since a human dark adaptation experiment was performed the stimulus was also calibrated in photometric units. The retinal illuminance of the stimulator was 57,000 photopic tds when unattenuated. This was calculated by the method described by Westheimer (1966). A Salford Electrical Industries photometer was used for the necessary luminance measurements. The glow tube was driven by a Roush Corporation timer and flash generator. Current was continuously monitored. A Beckman Universal timer and counter Model 5510-11 monitored flicker rate.

In all cases at least twenty for dark adaptation and twenty-five minutes for light adaptation were necessary between stimulus alterations, i.e. turning the light on or off. Such long intervals of adaptation were mandatory because of the oscillation of the LMSP. Taumer et al. (1974) have shown that the oscillatory rises and troughs of the LMSP will interact with a subsequently elicited change in illumination unless a sufficient time period is allowed for the SP to reach an equilibrium. The time period for light adaptation was longer than for dark adaptation because light adaptation produced greater amplitude oscillations which required a longer time period to reach an equilibrium. As a rule an LMSP eliciting stimulus was not presented unless a stable baseline, defined as less than 0.2 mV of DC drift over a 4 minute period was obtained.

ERGs were generated by placing a Grass PS-2 xenon photostimulator approximately 25 cm from the eye. The flash had a 20 μ second duration and was operated at the W-16 (brightest) setting. An interflash interval (IFI) of 10 seconds was generated by the Roush timer and monitored on the Beckman timer. In all cases ERGs were recorded when the S was dark adapted for at least 10 minutes, the opposite eye was always patched to prevent the recording of spurious signals.

Signal Amplification and Processing

A locally designed and constructed high input impedance DC differential amplifier provided the first stage of amplification. Its gain was set at 10 and the bandpass was 0-14 Khz at the 3 db point. The input impedence was 10^{12} ohms. The signal was further amplified by a Redcor Corp 371 differential DC amplifier. Depending on whether SPs or ERGs were being recorded its gain was set respectively at 10 or 20 and the upper bandpass was 0.1 or 1.0 khz at the 3 db point.

The SP and ERG signals were processed on a Nicolet Nic-80 minicomputer, displayed on a monitor oscilloscope and digitally stored on disc for subsequent analysis. SP data were also recorded on an Ominiscribe strip recorder. ERGs were summed sixteen times using the Nic-80.

Preparation and Maintenance of Animal

All Ss were deprived of food for at least twelve hours prior to the experiment. Anesthesia was initiated by an intramuscular (IM) injection of 8-10 mg/kg of Ketamine HCl (Bristol). Also at this time the S's pupil was dilated and accommodation paralysed with a few drops of 1% cyclopentolate HCl instilled in the conjunctival sac. Occasionally it was also necessary to use a similar amount of viscous 10% phenylephrine HCl to retract the nicitating membrane. The S was also given an IM injection of 0.1 mg/kg of atropine sulfate to suppress salivation. When the S was sufficiently

anesthetized (five to ten minutes after the initial injection) a catheter was inserted in the femoral vein and the cat was intubated with an appropriately sized trachea tube. The outside of the tube was coated with 2% lidocaine jelly to prevent discomfort after the anesthetic wore off. Other necessary minor invasive procedures were all performed while the cat was still anesthetized. A small (3-4 mm.) incision was made above the supraorbital ridge of each eye for insertion of the indifferent and ground electrodes. These areas were infiltrated with 2% lidocaine HCl to prevent any subsequent discomfort. On occasions when an intraocular injection was planned a small incision was made at the external canthus of the eye to be injected. This area was also infiltrated with lidocaine. When these procedures were completed the animal was placed in a Baltimore stereotaxic holder and points of pressure, notably the region of the external auditory meatus, were covered with 2% lidocaine jelly. Then an intravenous (IV) infusion of a tubocurarine-gallamine mixture was initiated (See Appendix C for formula). After an initial dose of 1 cc/kg a slow infusion of the paralytic agent delivered via a persitaltic pump (Extracorporeal Inc. Model 907) was begun. During the first 30 minutes a dose equivalent to the initial dose of the paralytic agent was infused. After that time the rate of delivery was reduced by a factor of 1/2 by dilution of the mixture. Repeat doses of atropine (0.07 mg/kg) were administered IM every four or five hours.

Artificial respiration using a Harvard apparatus small animal respirator was begun when the initial dose of tubocurarine-gallamine had caused a marked decrease in voluntary respiration. Percent expired CO_2 was continuously monitored by a Beckman LB-1 medical gas analyser and was maintained at 4.3 $\pm$ 0.2 by varying the respiratory rate and volume. To prevent atelectasis the S was hyperventilated occasionally.

While the S's physiological condition was stabilizing, the electrodes were placed on the cat. The active (non-inverting) electrode was placed in one end of an agar-saline filled bridge in a 3 mm inner diameter (ID) polyethylene tube. The other end of the agar-saline bridge was connected to a specially constructed transparent contact lens. The lens was moistened with a 2.5% hydroxypropy methylcellulose solution. The indifferent and ground electrodes (Ag-AgCl) were in 4 mm ID polyethylene tubes filled with agar saline bridges. They were also placed in the incisions made above the S's orbital ridges. The indifferent electrode (inverting input) was located above the eye to be recorded from. To insure a low impedance, and a high stability interface, the indifferent and ground electrode tubes had agar saline soaked cotton wicks inserted in the ends which were in contact with the cat. Body temperature was monitored with an electronic thermometer via a rectal probe and was maintained at 38 $\pm$ 2°C with a fluid filled heating pad.

Once the electrodes were in place and the animal was stable, the Maxwellian view stimulator was aligned. The

focal point of the stimulator was located at the center of the entrance pupil of the S's eye approximately aligned on the visual axis.

CHAPTER 4

EXPERIMENT 1: CHARACTERISTICS OF THE LMSP OF THE CAT

Rationale and Methodology

The objective of this experiment was to characterize the directly recorded LMSP of the cat. After a thorough literature search no data on the LMSP of the cat were found. In fact no quantitative data on directly recorded LMSP in any species were found although the characteristics of the human EOG have been adequately described (Arden and Kelsey, 1962a,b; Kris, 1958; Taumer, et al., 1974).

In pilot experiments the light-on and light-off LMSPs of the cat were observed to be damped oscillations. To quantify the time course of these oscillations and to permit comparison with human LMSP oscillations the latencies of the various peaks and troughs of the cat were measured. In addition to quantifying the latencies of the peaks and troughs of the light-on and light-off LMSP, amplitude by intensity functions were generated over a 3-5 log unit range of irradiance for the first peak of the on LMSP and the first trough of the off LMSP.

The LMSP eliciting stimulus used in this experiment was always the onset or cessation of a steady light. The stimulus intervals were as described in Chapter 3.

Results

The waveforms of both the on and off LMSP of the cat resembled damped oscillations although they had opposite initial polarities (Figure 2). The time course of both types of responses were extremely long compared to other electrophysiological responses.

Table 1 summarizes the mean latencies, standard deviations, ranges, and sample sizes of the various peaks and troughs of the on and off LMSP. In all cases a peak refers to a cornea-positive inversion point and a trough refers to a cornea-negative inversion. Responses were pooled for 8 cats. The steady stimulus which elicited these light-on or light-off LMSPs ranged from 5.4-.0054 mW/steradian in irradiance. The responses elicited by the various intensities were pooled since no consistent differences in latency as a function of stimulus intensity were observed. There were some consistent intersubject differences in latency however. Sample size decreases with each successive peak or trough because in many cases, especially when less intense stimuli were presented, peaks and troughs were not observed. Typically the light-on response of the SP (Figure 2a) was a cornea-positive signal which reached a first mean peak at 6.4 minutes after light onset (Table 1). Following this initial peak the potential dropped for a few minutes until the first trough was reached at a mean of 17.4 minutes. Generally the rising phase of the first peak had a steeper slope than the subsequent falling phase. After this first

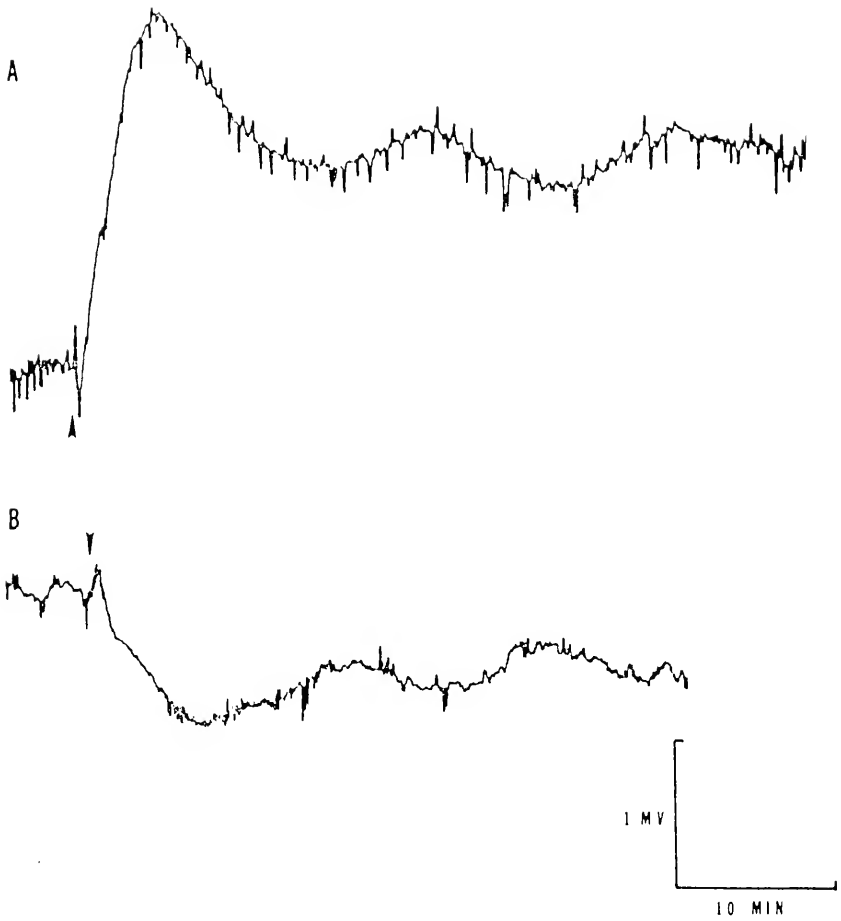


Figure 2. The light-on (A) and light-off (B) light modulated standing potentials (LMSPs) elicited by the onset or cessation of the steady unattenuated light source. Light-on and off are indicated by arrows. Note the long time course of the changes. In this and all other illustrations of electrophysiological signals an upward deflection is a cornea-positive response.

A - Light-On

	Peak 1	Trough 1	Peak 2	Trough 2	Peak 3
mean latency (min)	6.44	17.45	26.39	33.31	42.30
standard deviation (min)	.71	2.43	3.99	4.24	6.84
range (min)	2.8	8.8	10.7	10.3	11.99
sample size	24	13	9	5	3

B - Light-Off

	Trough 1	Peak 1	Trough 2	Peak 2	Trough 3
mean latency (min)	8.09	16.60	23.11	30.97	40.70
standard deviation (min)	1.76	.88	1.6	1.79	
range (min)	6.6	2.5	4.5	3.4	
sample size	18	9	6	3	1

Table 1. Mean latencies, standard deviations, ranges and sample sizes for the various peaks and troughs of the light-on LMSP (A) and light-off LMSP (B).

trough was reached the SP rose to a second peak at a mean of 26.4 minutes after light onset. This peak was always of smaller amplitude than the first peak. This second peak was followed by another trough at a mean of 33.3 minutes after light onset. By this time in most recordings it became difficult to distinguish further oscillations. When present a third peak occurred at a mean of 42.3 minutes after light onset (Figure 2a) and appeared to be smaller in amplitude than the second peak. The troughs of the light-on LMSP rarely fell to a value which was as low as the SP value preceding the light presentation (Figure 2a).

The light-off response was of opposite polarity than the light-on response. It began with a trough and then rose to a peak. The light-off response (Figure 2b), like the light-on response, consisted of oscillations of decreasing amplitude though their time course and waveform differed somewhat from the light-on LMSP. The light-off response reached a first trough at a mean of 8.1 minutes after light onset (Table 1). This was followed by a first peak at a mean of 16.6 minutes and then a second trough at a mean of 23.1 minutes. The initial off response rose to a first peak more gradually than the corresponding fall of the first peak of the light-on response making it more difficult to score the off response for amplitude and latency. It was also more difficult to observe the succeeding oscillations of the light-off LMSP because the total off response was of a smaller amplitude than the light-on response elicited by the

same irradiance. Nevertheless in one case when the signal was cessation of the brightest light and the recordings were of exceptional stability a third oscillation could be plainly observed at 41 minutes after onset (Table 1). Generally no portion of an off response reached a voltage which was as great as the light adapted SP amplitude which immediately preceded cessation of the light.

In addition to the major components of the light-on and light-off responses, certain early minor components of the light-on and off LMSP are also consistently present. These components can be observed in Figure 2a but are better seen when displayed on an expanded time scale as in Figure 3. The early minor components of the light-on LMSP (Figure 3a) consist of an early cornea-positive component (P1) followed by a slower, cornea-negative component (N1). The N1 component is followed by the first major cornea-positive component of the light-on LMSP. The early minor components of the off response tend to be difficult to observe. They are present in Figure 2b though they are more easily observed on an expanded time scale (Figure 3b). They consist of an early cornea-negative component (N1) followed by a slower cornea-positive wave (P1) which in turn is followed by the first major component of the off response.

A third minor component in the light-on SP is a small irregularity in the first peak at 1.6 minutes after light onset. This component is indicated by the curved arrows in Figure 4a and was elicited by the highest levels of illumin-

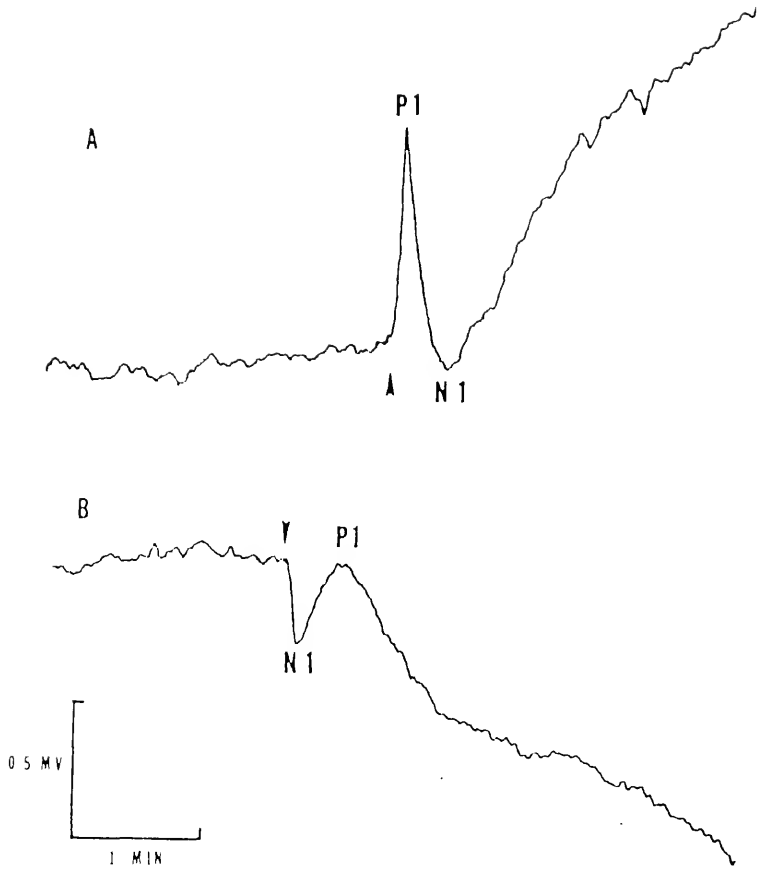
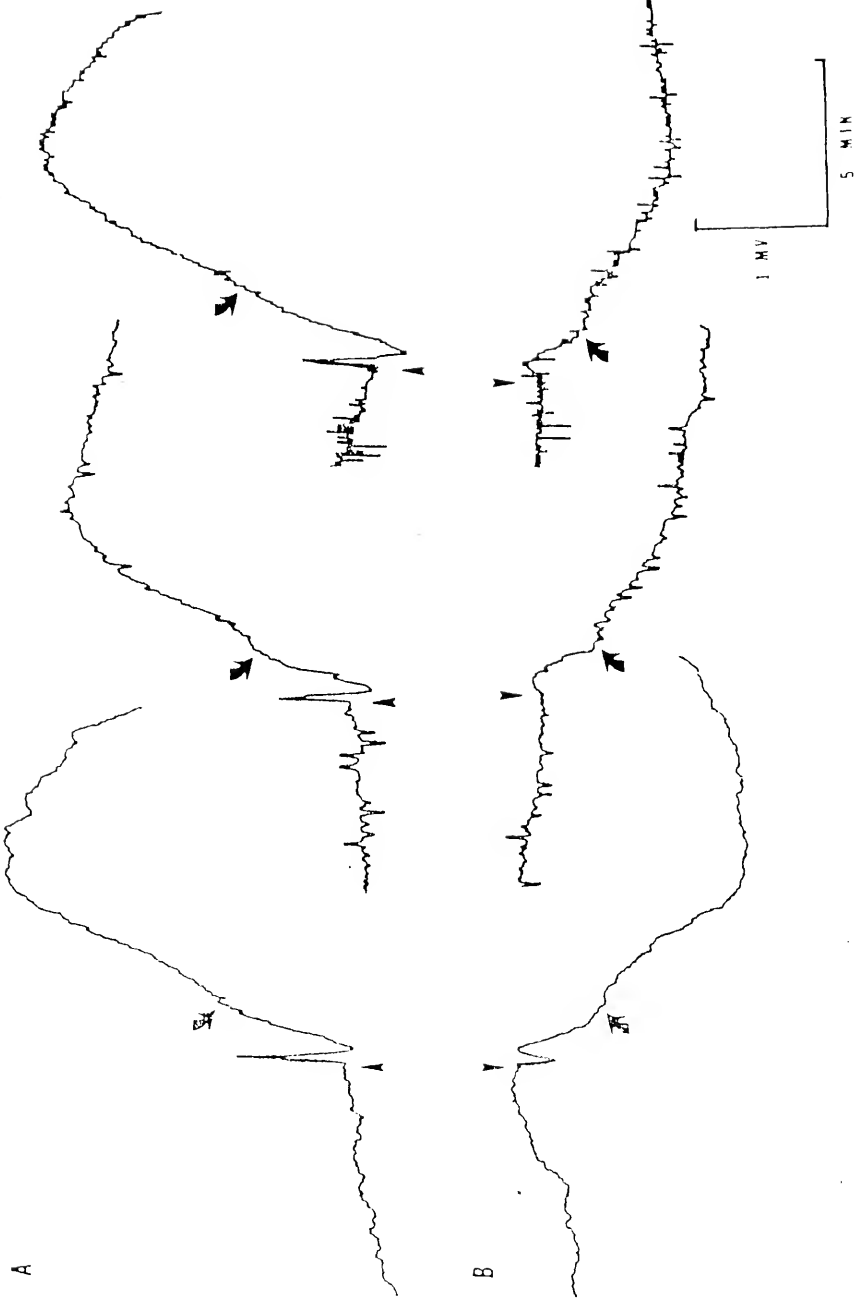


Figure 3. The early minor components of the LMSP. Waveform A is the response to light onset. The two early components are identified as positive one (P1) and negative one (N1). Waveform B is the response to light cessation. The nomenclature is the same as waveform A. Note the time scale is more expanded than the previous figure. Both the on and off responses were elicited by the onset or cessation of a steady light (as indicated by the straight arrows) with a log relative irradiance of $\bar{1}.3$.

Figure 4. Illustration of another minor component seen in the LMSP. Figure 4A is the light-on response and Figure 4B is the light-off response to steady light with a log relative irradiance of $\bar{1}.3$. The records are the same as Figure 2; however, the time scale has been lengthened. The curved arrows indicate the location of these additional early components. This third minor component is seen in both on and off response. The straight arrows indicate light onset and cessation.

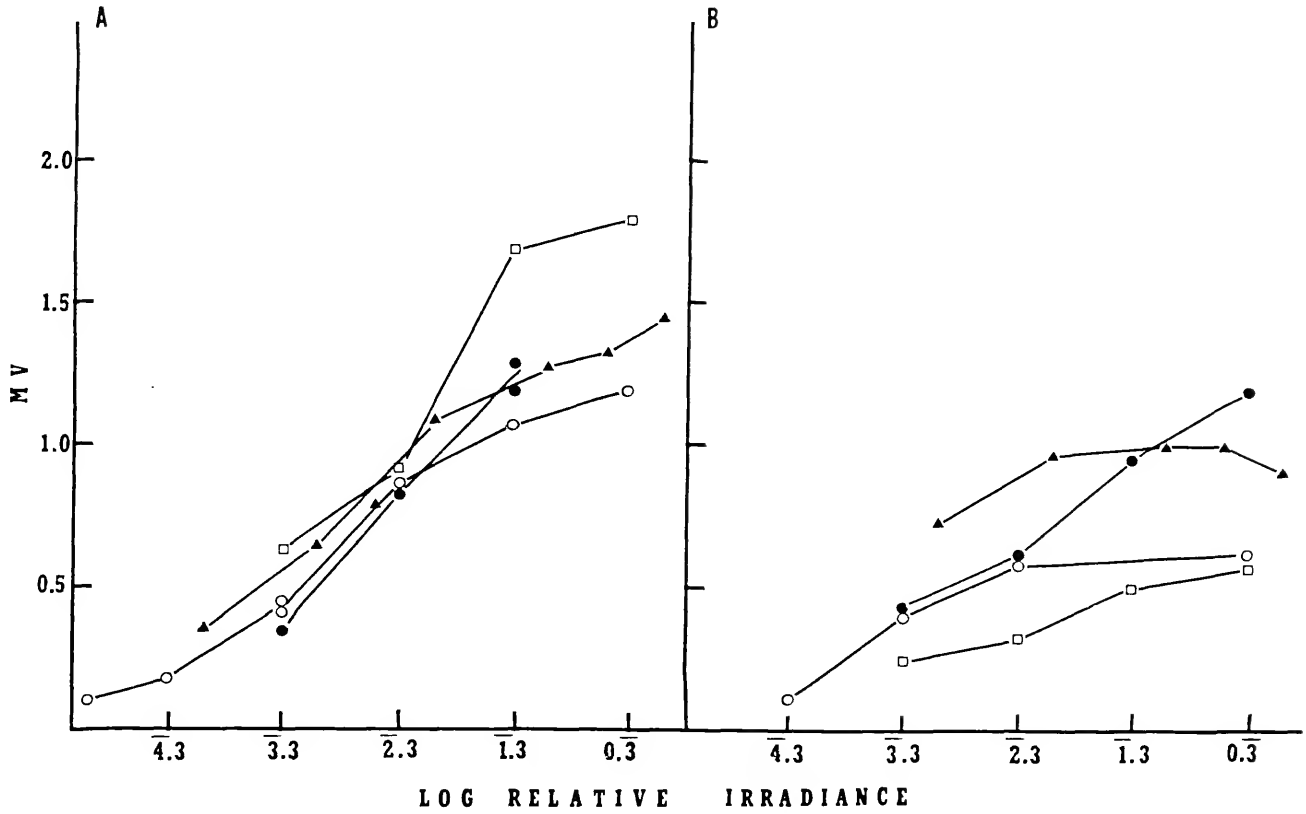

A
B
1 MV
5 MIN

ation presented. It was observed consistently in most light-on LMSPs. A similar component can occasionally be seen in light-off LMSPs. It is illustrated in Figure 4b as indicated by the curved arrows.

To further characterize the LMSP of the cat, amplitude by intensity functions for the first major peak of the light-on LMSP of 4 cats are presented in Figure 5a. Over this range the light-on response varies in an essentially linear fashion with the logarithm of stimulus intensity.

Figure 5b presents amplitude by intensity functions for the first major trough of the light-off response of the LMSP for the same eyes as Figure 5a. The off response functions are of a lower amplitude and not as orderly as the light-on LMSP data. In one case, cat 46, the response appears saturated at higher intensities. In most cases, however, a clear off response could not be recorded below $\bar{3}.3$ log units. This is partly attributable to longer time course of the first oscillation of the off LMSP which made it difficult to be certain a response had occurred when scoring data. The overall lower amplitude of the off response also contributed to the problem of measuring the off response. It does appear, however, that the off LMSP responds in a graded fashion to decrements in irradiance. In the 2 cases, cats 22 and 216, where clear cut graded changes occur, the decrement in amplitude appears to be linear with respect to the logarithm of stimulus intensity.

Figure 5. Amplitude by intensity function for the first major peak of the light-on (Figure 5A) and first major trough light-off (Figure 5B) LMSP for 4 Ss. Amplitude in mV is on the Y- axis and it is plotted as a function to log relative irradiance on the X- axis. Each function represents data from a single S. Each point is the amplitude of a single response. The stimulus was always the onset or cessation of a steady light.


A
B
2.0
1.5
1.0
0.5
M V
4.3
3.3
2.3
1.3
0.3
LOG RELATIVE IRRADIANCE

Discussion

As illustrated by Figure 2 the light-on LMSP and light-off LMSP of the cat are of opposite initial polarities and are damped oscillations with similar time courses (Table 1). The first major component of the light-on response is cornea-positive and the first major component of the light-off response is cornea-negative. Similar findings with respect to waveform have been reported in all vertebrate species studies (Gouras, 1969; Kikawada, 1968; Skoog, 1975). The initial major peak of the light-on LMSP of the cat occurs at 6-7 minutes after light onset. The first major trough of the light-off response occurs at 8 minutes after light cessation. In the human Kris (1958) reported the first light-on peak occurs at 10 plus or minus 2 minutes and the first light-off trough occurs at 8-10 minutes when measured indirectly by the EOG. Skoog (1975) recording the LMSP of the human directly, shows records where the first light peak occurs about 6-10 minutes after light onset and the first dark trough occurs 8-12 minutes after light cessation. The amplitude of the off response in the cat was lower than the on response elicited by the same stimulus. The same holds true for the directly recorded human LMSP and EOG (Arden and Kelsey, 1962a; Skoog, 1975; Taumer, et al., 1974). The human on and off LMSP takes 36 plus or minus 4 minutes to reach the second light-on peak or light-off trough (Kris, 1958). This is longer than the cat LMSP which required 10-13 minutes less to reach the equivalent inversion. It is

important to note that in the cat, like the human (Skoog, 1975; Taumer et al., 1974), the on and off responses are similar in waveform and time course but are opposite in polarity. Gouras and Carr (1965) found oscillations with a similar time course in the rhesus monkey eye. Kikawada (1968) studied the first peak and trough of the light-on and off LMSP of a variety of species from all major vertebrate classes. He found considerable variability within and across the major classes of vertebrates (3-13 minutes for the first light peak and 7-20 minutes for the first dark trough). The similar period of the on and off LMSP of the cat and other species indicates that pigment bleaching or a directly related metabolic process is probably not the basis for the on and off LMSP. Pigment bleaching is a very rapid process while pigment regeneration requires many minutes of time. Therefore if the on and off LMSP are of similar origin it is unlikely they arise from pigment regeneration or a directly related process.

The two early minor components of the cat light-on LMSP (Figure 3) are also present in the human EOG and are similar in waveform and time course (Taumer et al., 1974). The third component which occurs riding on the major initial component of the on and off LMSP about 1.6 minutes after light onset also has been observed in the human LMSP (Skoog, 1975).

It is well established that the amplitude of the first major light-on peak of the human EOG varies linearly with

the logarithm of stimulus intensity (Arden and Kelsey, 1962b; Taumer, et al., 1974). This also is the case for the cat light-on LMSP as shown in Figure 5a. The log-linear relationship between stimulus intensity and light-on LMSP amplitude is another indication that the photopigment in the receptors or a directly related process is not the source of the light-on LMSP. As noted above the extent of pigment bleached by a stimulus is linearly related to the stimulus intensity not the logarithm of the stimulus intensity (Dowling and Ripps, 1970). If the light-on LMSP is directly related to visual pigment kinetics then it also should be directly related in a linear fashion to stimulus intensity.

There is some controversy about the graded nature of the light-off response of the LMSP. Arden and Kelsey (1962a) found it to be an all or none response, always falling to the same initial trough regardless of the stimulus intensity that evoked it. They also found a great deal of response variability in the light-off EOG amplitude unrelated to stimulus variations. Taumer et al. (1974) found graded changes in light-off EOG linearly related to the logarithm of stimulus intensity and dispute the findings of Arden and Kelsey (1962a). The results of this study on the cat LMSP tend to support the findings of Taumer et al. (1974) since graded changes in the amplitude of the off response were recorded (Figure 5). There was a great deal of variability however, as Arden and Kelsey (1962a) found for the light-off EOG.

CHAPTER 5

EXPERIMENT 2: STEADY VERSUS FLICKERING STIMULI

Rationale

This experiment was designed to determine if the LMSP elicited by steady as opposed to flickering stimuli of equal time average irradiance (quanta) are equivalent. The neural components of the retina are highly sensitive to time varying stimuli while other retinal processes such as visual pigment related metabolism and glial cell activity are not as sensitive (Campbell and Rushton, 1955; Miller and Dowling, 1970). Therefore, by studying LMSP responsiveness to flickering vs equivalent steady stimuli it is possible to indirectly determine the importance of various elements in generating the LMSP.

For example the first component of the visual process is the photochemical response of the rods and cones. One part of this process is pigment regeneration which is fairly well understood. Since the PE is known to participate in pigment regeneration, a comparatively slow process (Dowling and Gibbons, 1961), it is possible the LMSP, which has a long time course reflects related metabolic processes (Arden and Kelsey, 1962b; Taumer et al., 1974). One purpose of this experiment will be to compare the LMSP elicited by

stimuli which bleach equal overall amounts of pigment but effect the rest of the neural components of the retina differentially. It is apparent from intracellular recording in many lower vertebrates that neural cells in the retina respond differentially to steady and flickering light (Rodieck, 1973; Werblin and Dowling, 1969). The stimuli therefore were a steady light and flickering light of twice the luminance and half the duration (50% duty cycle). It has been demonstrated that the fraction of pigment bleached is proportional to the product of the intensity of a light and its duration. This relationship is termed the Bunsen-Roscoe Law. Campbell and Rushton (1955) demonstrated its holds for human rod pigment for durations ranging from 0.3 to 48 seconds. They did not investigate shorter durations. It is known that at microsecond flash durations and/or high intensities the Bunsen-Roscoe Law does not hold (Hagins, 1957; Pugh, 1975). For this reason very short durations or high intensities were not employed in this experiment.

A variety of light intensities and rates of flicker were employed in order to be certain that any flicker vs. steady differences or similarities were of a general nature and not unique to particular stimulus situations. Additionally the equivalence of the bleach produced by the flickering and steady light was confirmed by presenting each type of stimulus to a human observer and measuring the subsequent dark adaptation function.

Methodology

Cat Experiments

The object of this experiment was to investigate the effects of square wave flicker versus an equivalent steady retinal irradiance. The flicker and steady stimuli were equivalent with respect to averaged irradiance and therefore at the durations employed bleached equivalent amounts of pigment (Rushton and Campbell, 1955). The flickering stimulus had a square wave configuration with a 50% duty cycle. Since it was on one half as long as the equivalent steady state stimulus, its irradiance was increased by 0.3 log units. This increased the irradiance by a factor of two. The equivalence of the two types of stimuli with respect to extent of photopigment bleached was empirically confirmed by a human psychophysical dark adaptation experiment which is described separately.

In addition to varying flicker rates from 0.5 to 30 Hz, certain flicker rates were tested over a wide range of retinal illuminations (3-4 log units). Therefore, there were two basic protocols for the flicker versus steady experiments. In one type of experiment a particular rate of flicker was tested over a 3 or 4 log unit range of irradiances. In this type of experiment, a flickering stimulus of a particular irradiance was always preceded or succeeded by a steady stimulus of the equivalent time averaged irradiance.

The other type of flicker versus steady experiment was designed to explore a large range of flicker rates. In

these experiments different rates of flicker were presented at one irradiance. The equivalent steady irradiance condition was also presented periodically throughout the experiment for comparison purposes and to monitor any change in overall responsiveness. The interstimulus intervals of both types of experiments followed the guidelines described in Chapter 3.

Human Dark Adaptation Experiment

The object of this experiment was to test the equivalence of the bleach produced by the steady and flickering stimuli. The S in the human psychophysical dark adaptation study was a 25 year old female who had normal vision. She had little previous psychophysical experience. The observer was first light adapted with the same Maxwellian view stimulator used to generate the LMSP in the cat experiments. Then immediately after light adaptation dark adaptation functions were measured on a dark adaptometer.

For the preliminary light adaptation the observer was instructed in and then practiced proper alignment of the Maxwellian view stimulator. She was instructed to fixate the center of the field with her right eye. Stable head position was maintained by a head and chin rest. The left eye was patched. The S was light adapted by either a steady light attenuated by 0.3 log units or an unattenuated light flickering at 2 cps. After 5 minutes of light adaptation the S moved to the dark adaptometer. The adaptometer was constructed locally and was based on a design described by

Hecht and Shlear (1936). The observer fixated a red light emitting diode. The test stimulus was a 12° diameter disc of unfiltered tungsten light. It was centered 14° nasal to the point of fixation on the horizontal meridian of the right eye. Since the adapting stimulus subtended 27.2°, the dark adaptation test stimulus fell fully within the adapted area. The maximum luminance of the test stimulus was set at 1.65 log foot lamberts as measured with a Salford Electrical Industries photometer. It could be attenuated in 0.1 log unit steps with Kodak #96 wratten neutral density filters and had a duration of approximately 200 msec. For 35 minutes dark adaptation thresholds were determined with this test stimulus. The ascending method of limits was employed and approximately 3 measurements were made each minute.

Results

For two cats amplitude by intensity functions were generated at 2 and 8 Hz respectively. For three cats flicker rates ranging from 0.5-32 Hz were tested individually. In both types of experiment responses to the equivalent steady light conditions were also recorded. Figures 6a and b present the on and off LMSP to steady and flickering light (2 Hz) of equivalent time averaged irradiance (equal total quantal flux). The waveforms are similar especially the first major component of the on and off response. Second peaks and troughs are present in the flicker on and off response and their time course is similar to the oscillations elicited by steady stimuli.

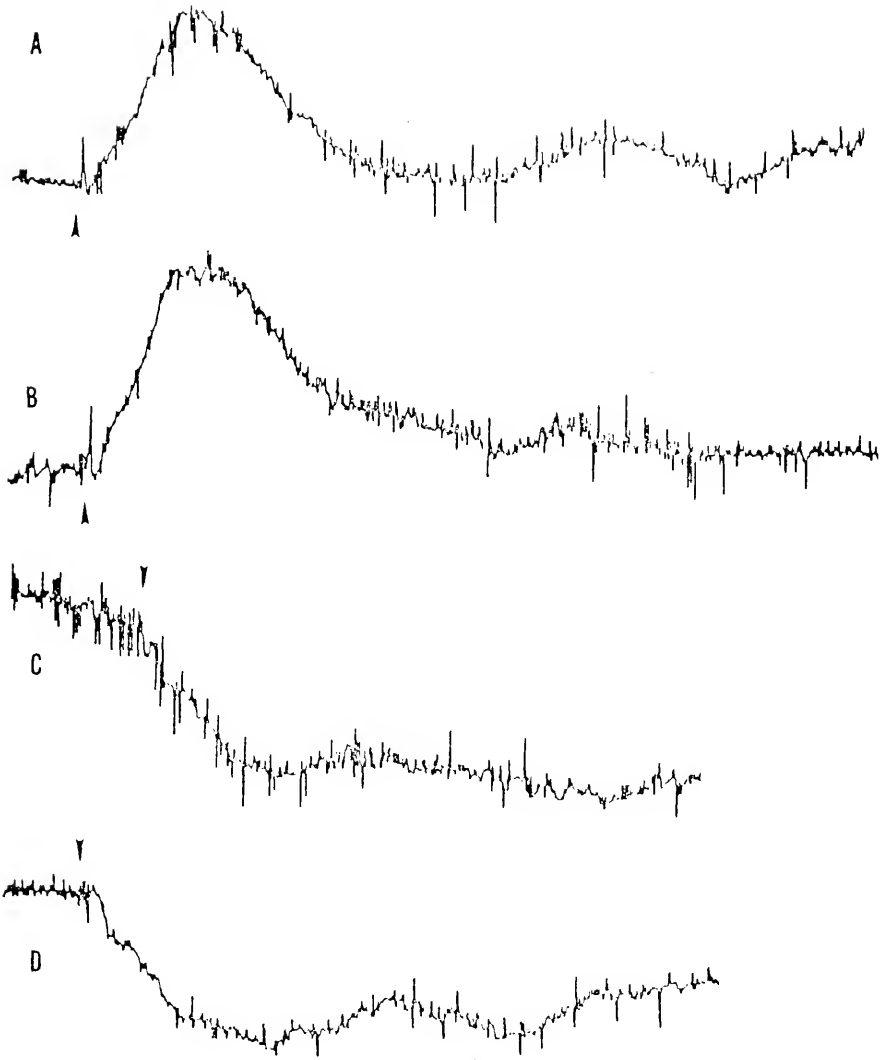


Figure 6. The light-on (Figure 6A and B) and light-off, (Figure 6C and D) LMSP elicited by the onset and cessation of a steady light (A and C) or a flickering light (B and D) of a corresponding time averaged irradiance.

Figure 7 presents the data from the experiment where on and off LMSP amplitude by intensity functions were generated over a 3 log unit range for a stimulus flickering at 8 Hz and a steady light of equivalent time averaged irradiance. In Figure 7a the light-on data demonstrate that over this 3 log unit range of stimuli there were no consistent differences between equivalent steady and flickering stimuli. The light-off steady vs flicker data recorded during the same experiment are presented in Figure 7b. There are no consistent differences across the adaptation levels for the off response to flicker vs steady stimulation.

Figure 8a graphically presents a comparison of the amplitudes of the first peaks of the light-on LMSP elicited by the equivalent steady and flicker light conditions. The data from five Ss were pooled. At each flicker rate the response has been compared to the steady stimulus of an equivalent time averaged irradiance recorded from that S. The difference in mV between the two was calculated by subtracting the appropriate steady response amplitude from the corresponding flicker amplitude. For each square wave flicker rate tested and presented on the abscissa the steady vs flicker difference in amplitude has been calculated and is presented on the ordinate. The number of observations that each point is based upon is presented above each point. The error bars represent $\pm$ 1 standard deviation. To determine if there was a significant difference overall between the steady and flicker conditions, the light-on data were

Figure 7. Amplitude by intensity function for the first major peak of the light-on (Figure 7A) and first major trough of the light-off (Figure 7B) LMSP for a single S. The functions represent: a steady illumination ( ) and a square wave flickering stimulus of 8 Hz of equivalent time averaged irradiance (o). The S was cat 216.

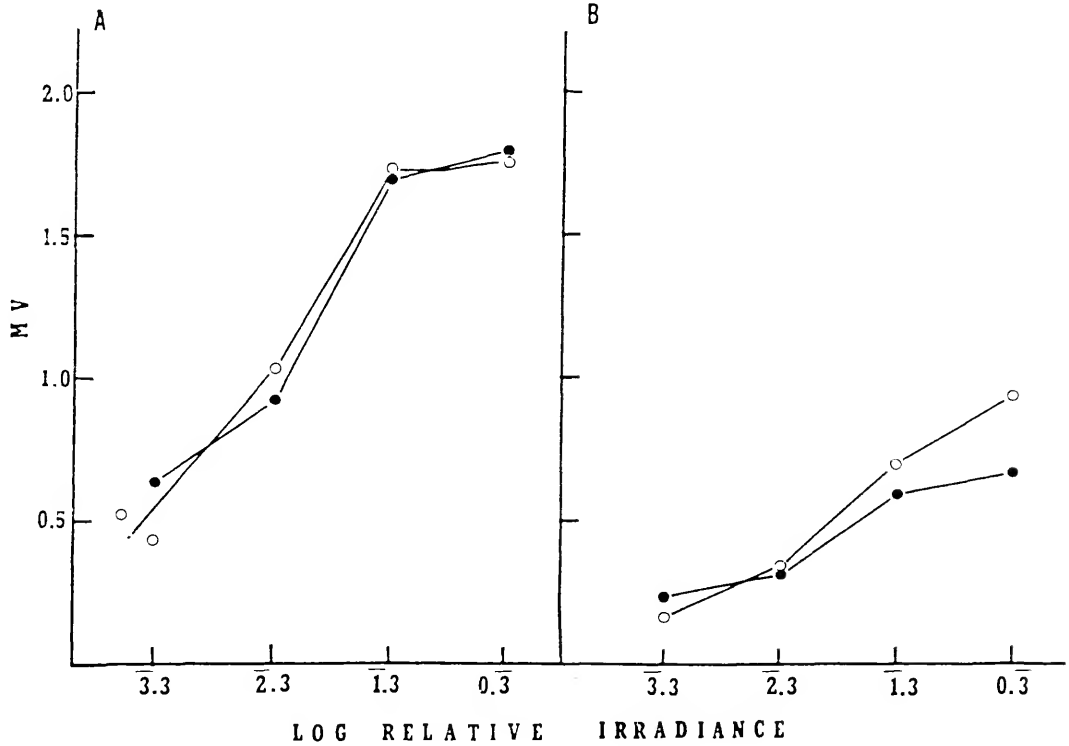
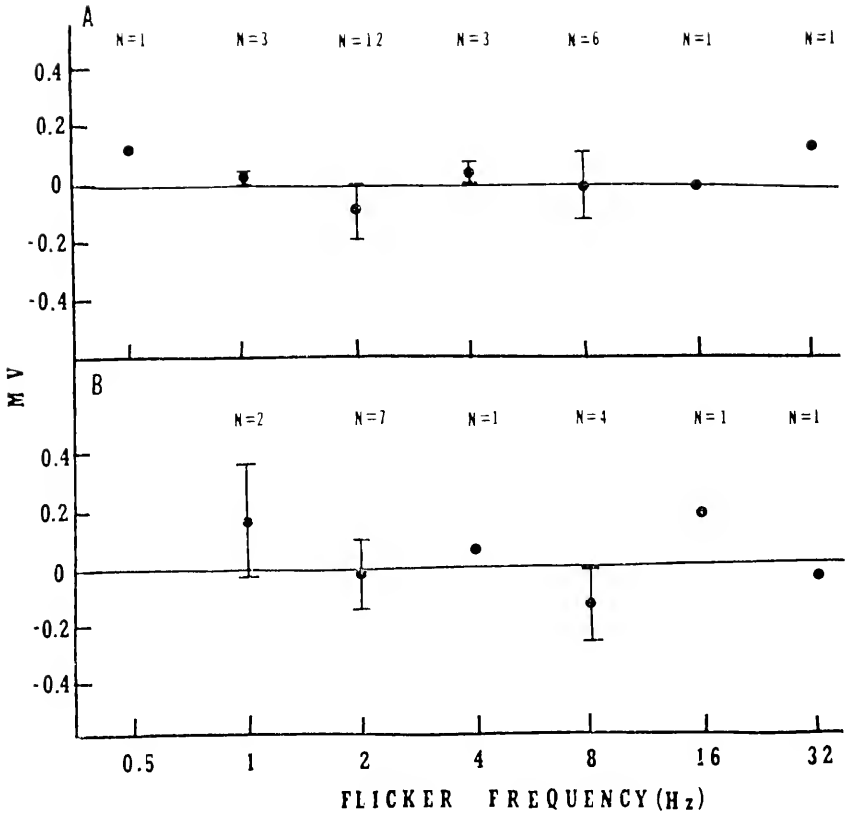

A
B
2.0
1.5
1.0
0.5
MV
$\bar{3}.3$
$\bar{2}.3$
$\bar{1}.3$
$0.\bar{3}$
LOG RELATIVE IRRADIANCE

Figure 8. The differences in amplitudes between the first major peak of the light-on LMSP (Figure 8A) elicited by a steady light and the equivalent flickering light stimulus. To arrive at a value in mV plotted on the X axis the steady amplitude in mV was subtracted from the equivalent flicker amplitude. Flicker rate is plotted on the y-axis and each point is a mean with the sample size indicated directly above the point. The bars represent $\pm$ 1 standard deviation. Figure 8B presents the difference in amplitude between the off LMSP elicited by equivalent steady and flickering stimuli.

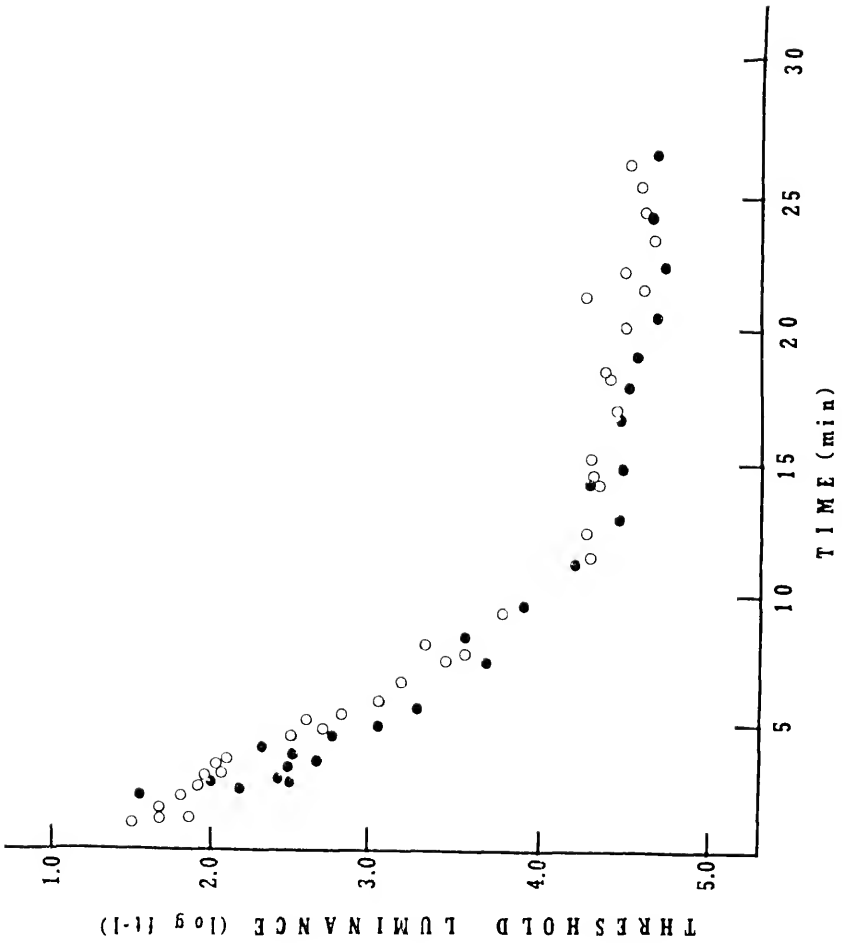

A
N=1
N=3
N=12
N=3
N=6
N=1
N=1
0.4
0.2
0
-0.2
-0.4
B
N=2
N=7
N=1
N=4
N=1
N=1
0.4
0.2
0
-0.2
-0.4
M V
0.5
1
2
4
8
16
32
FLICKER FREQUENCY (Hz)

pooled for all flicker rates and a two tailed student's t test was performed. There was no a significant difference between the steady and flicker conditions ($t = 0.76$, $df = 41$, $p > 0.25$).

Figure 8b presents the same data for the off steady vs off flicker conditions. Although the variation in amplitude is greater as noted previously for the off response of the LMSP there was no consistent effect of a steady vs a flickering stimulus on the amplitude of the off LMSP from 1-32 Hz ($t = 0.58$, $df = 25$, $p > 0.25$).

The flicker versus steady experiments are to a certain extent based on a corollary of the Ferry-Porter law e.g. that a flickering light with the same equivalent time averaged irradiance as a steady light will bleach an equivalent amount of photopigment (Campbell and Rushton, 1955). This assumption was tested for the specific stimuli presented in this experiment in a dark adaptation experiment on a human observer. Figure 9 represents the two dark adaptation curves generated for the human observer preadapted to either a steady or flickering stimulus of equivalent time averaged luminance (38,500 photopic trolands). Threshold luminance in log foot lamberts is plotted as a function of time after cessation of the preadaptation stimulus. There was no major difference between the two functions.

Figure 9. Dark adaptation curves following preadaptation to either a steady (solid circles) or flickering stimulus (open circles) of equal time averaged luminance. Threshold luminance in log foot lamberts is plotted as a function of time in minutes after the preadapting light cessation.


THRESHOLD LUMINANCE (log I[-1])
1.0
2.0
3.0
4.0
5.0
5
10
15
20
25
30
TIME (min)

Discussion

The results of these experiments demonstrate that there are no major significant differences, over the range of flicker rates tested, in both the light-on and light-off LMSP elicited by steady and flickering stimuli equated for time averaged irradiance. This was true for a wide range of flicker rates and over a range of intensities. The equivalence of these two stimulus conditions was confirmed in the human dark adaptation experiment. The similarity of the on and off LMSP to stimuli which produce extremely dissimilar responses of retinal neural elements indicates that the LMSP is not a measure of retinal neural activity. There are considerable electrophysiological and psychophysical data on the cat and many other vertebrate species which demonstrates that the retina is highly sensitive to the flicker rates employed in this study as reviewed by Brown (1965). Dodt and Enroth (1954) report that the cat ERG and retinal ganglion cell responses follow square wave flickering stimuli up to 50 Hz. In a behavioral study Kappauf (1936, cited in Brown, 1965) reported cats could distinguish stimuli flickering at up to 50 Hz. When the large differences in the responses of the neural elements of the retina to flickering as opposed to steady light are compared to the similarity of the LMSP elicited by these stimuli, it seems likely these components do not make a significant contribution to either the light-on or light-off LMSP.

The equivalence of LMSPs produced by stimuli which produce an equivalent bleach suggests the LMSP may in some way be related to this phenomenon. However some results of experiment 1 (ie. the amplitude by intensity functions for the LMSP) argue against a direct relationship between bleaching and the LMSP. Also the differences in time course for bleaching and regeneration when compared to the similarity seen in the light-on and light-off LMSP time course is, as noted in experiment 1, further evidence that neither pigment nor pigment related processes are the source of the LMSP.

CHAPTER 6

EXPERIMENT 3: ASPARTATE

Rationale

Both experiments 3 and 4 were designed to determine if the LMSP is generated in the receptors and PE alone or if neural and glial retinal components are involved. In order to make this distinction two techniques, one chemical and one surgical, have been applied. The chemical approach (this experiment) is based on a number of recent papers which have shown that sodium aspartate isolates the receptors from the rest of the retina by acting on the horizontal and bipolar cells. This has been determined in fish and amphibians (Cervetto and MacNichol, 1972; Murakami et al., 1975; Murakami et al., 1972) via intracellular recordings. When sodium aspartate is applied to the retina in minimal concentrations the horizontal cells depolarize and their response to light stimuli is reduced (Cervetto and MacNichol, 1972). Bipolar cells also are sensitive to aspartate. The nature of the response varies with the type of bipolar cell but photic sensitivity is completely suppressed (Murakami et al., 1975). To account for these effects it has been postulated that aspartate or a similar substance is the transmitter released by the photoreceptors (Murakami et al.,

1975). Whatever the mechanism, aspartate appears to isolate the receptors from the rest of the retina. These observations have been extended to the mammalian retina by Honda and Dawson (1975) and Hanitzsch (1973) in the rabbit and Baron and Boynton (1974) in the monkey. Their studies have shown that the a-wave of the ERG is not effected by aspartate but that the b-wave is extinguished. The a-wave of the ERG has been shown to be a measure of receptor activity while the b-wave has an inner retinal origin (Brown, 1968; Miller and Dowling, 1970).

Therefore, sodium aspartate was injected in a single, or in one case multiple, dose into the vitreous of the cat. Prior to and after the injection(s) the LMSP of that eye was examined. To confirm that aspartate was effecting the inner retina but not the receptors simultaneous ERG recording before and after the aspartate injection were made. It has been noted that aspartate has other ocular effects in the monkey, notably constriction of the retinal vasculature and formation of cataracts (Baron, 1974). All Ss were examined for such effects.

Methodology

In these acute experiments the S was set up and stabilized as described above. Preliminary to the injection(s) of aspartate the LMSP and dark adapted ERGs were recorded. After satisfactory records were obtained an intravitreous injection of sodium aspartate was given via a tuberculin

syringe with a 27 guage needle. Dosages of sodium aspartate ranged from 3.27 mg. to 33.0. For most experiments an intermediate dosage of 16.4 mg. in 0.04 cc of sterile water was employed. The sodium aspartate was prepared from aspartic acid (Sigma). To achieve the desired concentration at a pH of 7.3 the aspartic acid was added to sterile water for injection and the pH was increased by addition of concentrated sodium hydroxide.

Before and after injection of the sodium aspartate the dark adapted ERG was monitored. Since aspartate causes a decrement in the b-wave of the mammalian ERG (Hanitzsch, 1973; Honda and Dawson, 1975) this provided the necessary indication of aspartate effectiveness. At various times following the injection the light on and off components of the LMSP were recorded. Interstimulus intervals for both LMSPs and ERGs were as described in Chapter 3.

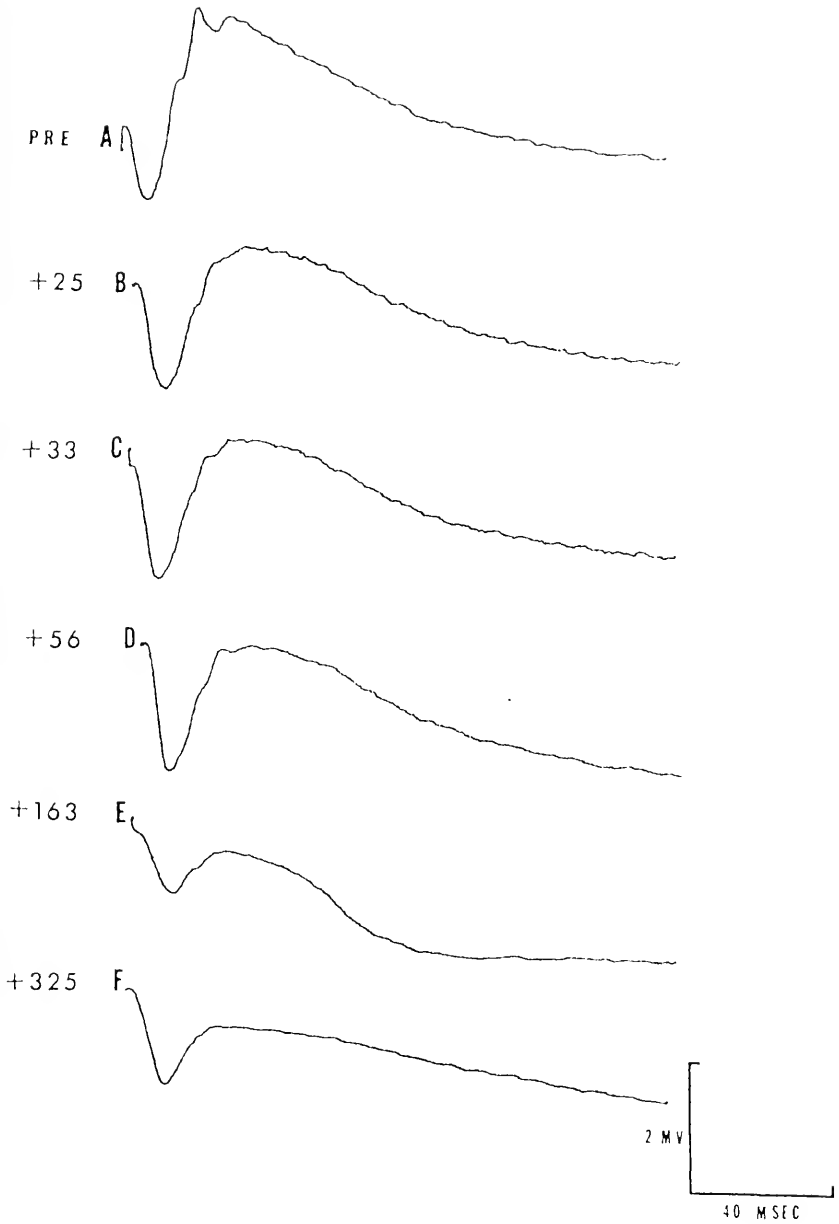
Results

The dosage of sodium aspartate employed in this experiment was arrived at in a pilot experiment (data not presented). An initial dosage of 4.1 mgs, the same dose as Baron (1974) administered per hour in the macaque monkey, was initially injected into the vitreous. Following this injection the b-wave of the ERG was monitored since its decrease in amplitude is indicative of aspartate effectiveness. When no significant effect on the b-wave was observed, increasing dosages of aspartate were administered at

1 hour intervals until a significant effect on the b-wave (defined as a reduction in b-wave amplitude of at least 50%) was noted. This effect occurred after a total dosage of 16.4 mg. Therefore a single injection of 16.4 mg of sodium aspartate was administered in one eye of 3 cats. In all cases this single injection of 16.4 mg of sodium aspartate had a profound effect on the ERG and LMSP. In a fourth cat, to induce a larger effect, a larger dose of 49.4 mg was administered in 2 intravitreous injections separated by 1 hour in time. This larger dose had an even greater effect on both dependent variables. In all 3 cases where 16.4 mg of aspartate was injected, the b-wave of the ERG was reduced in amplitude by at least 70% while the a-wave remained largely intact. This is illustrated in Figure 10 which presents the change in ERG due to a single injection of 16.4 mg of sodium aspartate in the left eye of cat 421. Figure 10a is the ERG recorded from the same eye immediately preceding the injection of aspartate. A normal a- and b-wave are present and are so labeled. Records b through f illustrate a large decline in b-wave due to the aspartate injection.

Figure 11 presents some LMSP traces recorded from cat 421 during the same experiment presented in Figure 10. Figure 11a is the light-on LMSP recorded in response to the onset of the unattenuated light. It was recorded preceding the aspartate injection. The response appears normal. Figure 11b is the response to the same level of irradiance

Figure 10. The effect of sodium aspartate (16.4 mg) on the electroretinogram (ERG). Record A is the ERG recorded prior to the aspartate injection. Record B through F demonstrate the change in the ERG as a function of time after the injection indicated in minutes with each record. Each record is the sum of 16 signals.


PRE A
+25 B
+33 C
+56 D
+163 E
+325 F
2 MV
40 MSEC

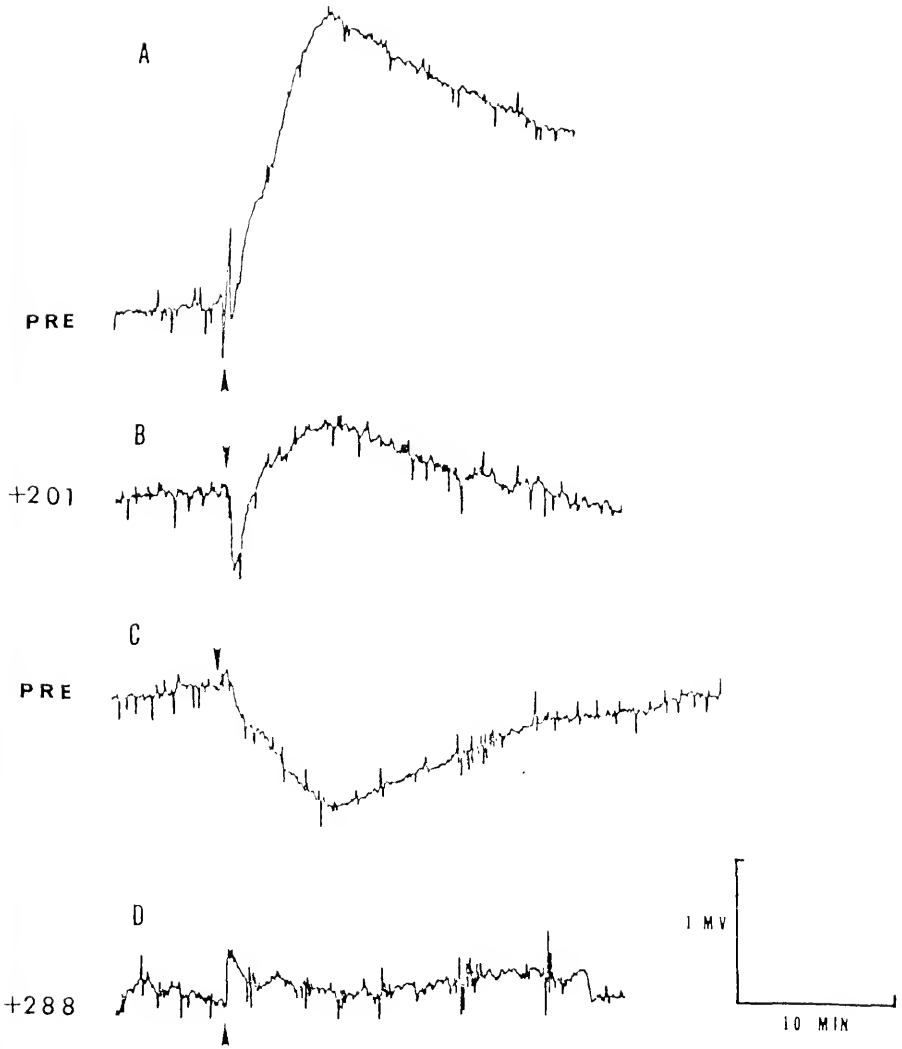


Figure 11. Presents the light-on and off LMSP recorded before and after aspartate. Records A and B are the pre and postaspartate on responses respectively. Record C and D are the pre and postaspartate off responses. The times of the post aspartate measurements in minutes after injection are indicated with each record.

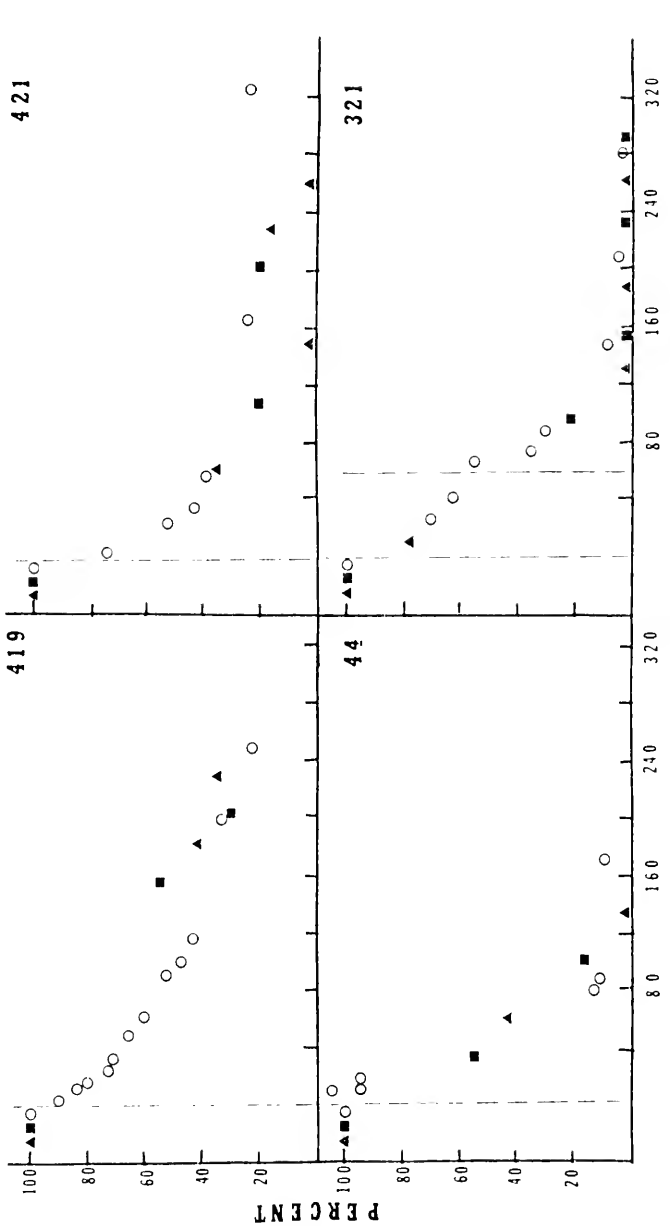
201 minutes after aspartate injection. The SP light-on response is severely attenuated. The ERG b-wave was also severely attenuated by that time after the injection as illustrated in Figure 10.

Figure 11c presents the light-off LMSP recorded prior to aspartate injection. It too is large and appears normal with respect to waveform. Figure 11d presents the off response 255 minutes after aspartate injection. A cornea-negative off response is no longer present. The b-wave is also severely reduced by this time as demonstrated by Figure 10.

A summary graph (Figure 12) presents both the ERG and LMSP data from four aspartate experiments. Since the relevant parameter with respect to ERG is the decline in b-wave relative to a-wave amplitude, the b- to a-wave ratio was calculated for the pre and postaspartate ERGs. The data were then normalized by using the preaspartate b-wave to a-wave ratio as 100%. This permitted more direct comparison with the decline in the on and off LMSP which were also normalized. This was accomplished by employing the preaspartate on and off responses as the baseline 100% level. Figure 12 therefore represents the effect with time of an injection of sodium aspartate on the b- to a-wave ratio of the ERG, the light-on LMSP, and light-off LMSP, all normalized to percent of preaspartate amplitude.

The data in Figure 12 are from animals which, with the exception of S 321, received a single ocular injection of

Figure 12. The effect of sodium aspartate on the b- to a-wave ratio of the ERG, light-on LMSP and light-off LMSP for 4 Ss as a function of time in minutes after an injection. For comparison purposes each dependent variable has been normalized with the preaspartate value equaling 100%. The dashed line(s) in each figure indicate an injection of aspartate. Note that S 321 had a second injection of aspartate. Each symbol represents on of the dependent variables; ERG, , light-on LMSP, , light-off LMSP, .


421
321
419
44
PERCENT
100
80
60
40
20
80
160
240
320
TIME (min)

16.4 mgs of aspartate in 0.5 ml of solution at time 0. Some of the data in Figure 12 from cat 421 are presented pictorially as Figures 10 and 11. Cat 321 had 2 injections of sodium aspartate. The first was 16.4 mg at time 0; the second given 62 minutes later was 32.8 mg in 0.1 cc of solution.

The ERG data from cat 419 best illustrate the orderly and gradual decline in b-wave amplitude relative to a-wave amplitude following a single injection of sodium aspartate. In this cat by 120 minutes after the injection the b- to a-wave ratio has declined from the preaspartate value over 50%. The large gaps in time for ERG records are unavoidable since it is not possible to simultaneously record ERGs and LMSP without the stimuli which elicit each interacting and contaminating the results of both. The ERGs of the other 3 cats follow the same pattern with the time course and the ultimate reduction in b- to a-wave ratio varying somewhat. The greatest decrement in ERG occurred for cat 321 who received the second injection of aspartate.

In all cats (Figure 12) the decrease in both the light-on and off LMSP consistently occurred in tandem with the decrease in b- to a-wave ratio. In cat 44 where a light-on and off response were collected in the initial 80 minutes after the injection the decrement is moderate (40-60%) as would be expected from the reduction in b- to a-wave ratio usually seen at these times after a single aspartate injection and the ERGs recorded from this cat. In cat 419 the

decrease in both LMSP and b- to a-wave ratio was moderate and of approximately the same value, both reaching about 30% at 200 min (Figure 12). In cat 421 and 44 (Figure 12) the b-wave reduction after 120 minutes is greater than cat 419 and the LMSP values are also somewhat lower (0-25%). These intersubject variations in the time course of the effect are probably related to the precise place of the injection in the vitreous and the associated diffusion gradient. In cat 321 where a second injection of aspartate was administered to induce a larger decrement in b-wave than seen in the other experiments the ERG b-wave and LMSP were both essentially abolished by 120 minutes after the initial injection (60 minutes after the second injection). It can be concluded that sodium aspartate severely reduces the b-wave of the ERG relative to the a-wave and that the LMSP is similarly reduced.

As a control experiment a cat was injected with 0.5 ml of sterile water in the vitreous. The pH was adjusted to 7.3 to equal the pH of the aspartate solution. The ERG and LMSP were recorded prior to and at various times after the injection. No effects of the control injection were observed on either the ERG or LMSP, up to 150 minutes after the injection.

At the conclusion of all experiments the cat was examined ophthalmoscopically for retinal vascular constriction and cataracts which have been reported to occur after aspartate injection (Baron, 1974). In cat 321, which re-

ceived a double injection of aspartate, a cataract was present. Due to the reduced visibility of the fundus of this cat it was impossible to determine if the retinal vasculature had constricted. Neither cataracts nor vasoconstriction were noted in the other cats.

Discussion

The effects of sodium aspartate on the ERG and LMSP were unequivocal. Following an injection of aspartate into the vitreous the b-wave began to decline steadily. A simultaneous decrement in on and off LMSPs also occurred. Most importantly when the b-wave was reduced to about 20% or less of its preinjection amplitude relative to the a-wave, the light-on and off LMSPs were similarly reduced or absent. The near abolishment of the b-wave and retention of the a-wave is a characteristic effect of sodium aspartate on the retina of vertebrates and invertebrates (Baron and Boynton, 1974; Cervetto and MacNichol, 1972; Hanitzsch, 1973; Honda and Dawson, 1975; Murakami, 1972; Murakami, et al., 1975). Based on intracellular recording in invertebrates it has been concluded that sodium aspartate isolates the receptors from the rest of the retina by saturating the horizontal and bipolar cells (Cervetto and MacNichol, 1972; Murakami , 1972; Murakami, et al., 1975). The precise mechanism of this effect is not known although it has been postulated that aspartate or a similar substance is the transmitter released by the photoreceptors. In mammals, where intracellular

recording techniques have not yet been perfected, the effect of aspartate on the ERG is the same as seen in invertebrates. Furthermore, a large body of literature exists which demonstrates that in mammals the a-wave is a receptor based phenomenon while the locus of the b-wave is the inner retina (Brown, 1968; Granit, 1933). It is clear, therefore, that when an ERG is recorded with a severely reduced or absent b-wave but a remaining a-wave, the inner and outer retina have effectively been separated. The presence of an a-wave in such a preparation indicates that the receptors are still functional and that the preparation has not yet generally deteriorated. Additionally the control experiments, with an injection of an equivalent volume of a control solution instead of sodium aspartate, had no effect on the ERG or LMSP indicating that the aspartate effects were not artifactual. If intraocular pressure changes or other injection induced trauma induced the alterations in the dependent variables then similar effects would result following the control injection. It is concluded, therefore, that some postreceptor components of the retina either neural or glial must be functioning normally for the generation of LMSPs. This conclusion is further reinforced by the results of the retinal vascular occlusion experiments.

In the only other in vivo experiments on the effect of sodium aspartate, Baron (1974) and Baron and Boynton (1974) reported that in monkeys certain other ocular effects of the chemical were observed. Specifically by 30 minutes after

the start of infusion of sodium aspartate into the vitreous vasoconstriction of the retinal vasculature was noted. In addition within 3 hours they noted the formation of a posterior cataract in the eye being infused. No such effects were observed in these experiments with the exception of cat 321 which received a second injection of sodium aspartate. It is difficult to account for this species difference since the dosage here was larger than Baron's (1974). However, the effect of aspartate on the ERG is constant across all vertebrates examined (frog, rabbit, monkey, and cat) whether the preparation is in vivo or in vitro (Baron and Boynton, 1974; Cervetto and MacNichol, 1972; Hanitzsch, 1973; Honda and Dawson, 1975; Murakami, et al., 1972; Murakami, et al., 1975). It is therefore unlikely that the in vivo effect is due to retinal vasoconstriction although this cannot be ruled out since such vasoconstriction would at least partially lesion the inner retina while leaving the receptors intact.

CHAPTER 7

EXPERIMENT 4: VASCULAR LESION

Rationale

To surgically lesion the inner retina, the retinal blood supply was interrupted thereby destroying the inner retina while leaving the receptors intact. The receptors are preserved because they are nourished by the choroidal circulation (Brown, 1968). The effectiveness of such a lesion has been confirmed histologically in the macaque monkey by Brown (1968) and squirrel monkey by Kroll (1968). Using electron microscopic techniques Kroll (1968) found that by 16 hours after occlusion all neural and glial cells vitread to the outer nuclear layer were severely autolyzed. The receptors were spared. This technique therefore has been employed to isolate receptor driven electrical potentials in the cat and other species (Brown, 1968). When the retinal blood supply is occluded in cat and monkey, the b-wave of the ERG is eliminated very rapidly but the a-wave remains intact (Brown, 1968; Maffei and Poppele, 1968). After 20 days Brown (1968) using light microscopy, found the inner layers to have degenerated with the exception of only a few cells while the photoreceptors appeared intact.

This technique was therefore applied in the cat to create the same type of lesion. At various times after the lesion (ranging from 1-55 days) the LMSP and ERG were examined. The extent of damage was verified histologically at various times after the lesion.

Experiment 4, like experiment 3, therefore determined using a completely different technique if the inner retina was participating in the generation of the LMSP.

Methodology

A variety of techniques were tested before a successful lesion of the inner retina could be generated. The retinal blood supply of the cat is unlike that of monkey or human in that there is no single central retinal artery in the optic nerve where it enters the eye (Wong and Macri, 1964). Rather a number of arteries (3-4) enter the eye between the optic nerve and adjacent sclera. Because of the complex nature of the retinal vasculature first attempts to surgically tie off or cut the vessels were unsuccessful. At this point an attempt was made to electrocoagulate the retinal vessels using a specially designed probe inserted into the eye and placed above the major retinal vessels as they left the disc. This invariably resulted in severe bleeding into the vitreous and the procedure was discontinued. Finally, an attempt was made to lesion the retinal vasculature with a xenon arc photocoagulator. In this procedure brief flashes of high intensity light were di-

rected at the optic disc successfully sealing the retinal blood supply of one eye of the cat.

Three cats were the Ss for this experiment. The lesions were all made in a single experimental session. At various times after the lesion was made, ranging from one to fifty-five days, the LMSP was measured in response to the onset and cessation of the unattenuated stimulus. The general methodology followed is described above in Chapter 3. For control purposes the LMSP of the other eye was also recorded. In addition dark adapted ERGs were recorded from both eyes to confirm the extent and nature of the lesion.

After each S had been tested in this manner on at least one occasion following the photocoagulation of the optic disc, it was sacrificed. The 3 subjects were sacrificed at 12, 21 and 55 days after the initial lesion was made. The eyes were immediately removed, a slit was made at the ora serrata and they were placed in Karnovsky's fixative (Karnovsky, 1965). After at least 24 hours of fixation at 4°C the anterior chambers were removed and portions of the posterior pole were embedded in celloidin. Various regions of peripheral and central retina of each eye were sampled for all eyes. Tangential sections were cut at 20 μ, stained, examined, and photographed. The stain employed was cresyl violet. Some tissue was also embedded in plastic, sectioned at 2 μ, and stained with toluidine blue.

Results

Immediately after the lesion the disc region appeared very pale. It was apparent that the retinal vasculature was lesioned since blood was no longer flowing in the large vessels. In one of the cats, 6H44, a small amount of bleeding into the vitreous occurred. After an hour it appeared to be about 3 disc diameters wide. Subsequently it was largely reabsorbed. At various times after the lesion the ERG and LMSP of both eyes of each cat were recorded. Cat 6H44 and 2B9 were each recorded from on two separate occasions.

Figure 13 presents the ERGs recorded from cat 7B9 one day after the lesion. Figure 13a recorded from the control eye is a normal ERG. Figure 13b is the ERG recorded from the lesioned eye. The b-wave is severely depressed but a small component is still present. The a-wave is intact. The on and off LMSP recorded from this eye during the same session were also reduced as illustrated by Figure 14. Figure 14a is the on LMSP from the control eye and Figure 14b is the light-on LMSP recorded from the lesioned eye. The amplitude of the response of the normal eye is 2.1 mV while the amplitude of the lesioned eye's response is 1.6 mV. The off LMSP recorded from the control and experimental eyes are presented in Figure 14c and d respectively. The control eye amplitude is 0.6 mV and the lesioned eye amplitude is 0.35 mV.

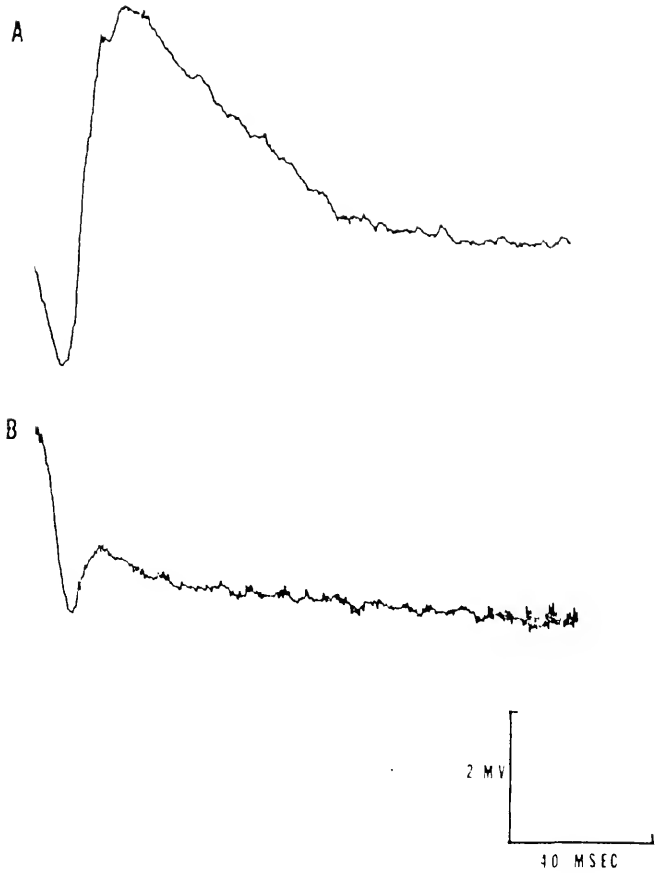


Figure 13. ERG recorded from the control eye (Figure 13A) and from the lesioned eye (Figure 13B) of cat 7B9 one day after the optic disc was photocoagulated.

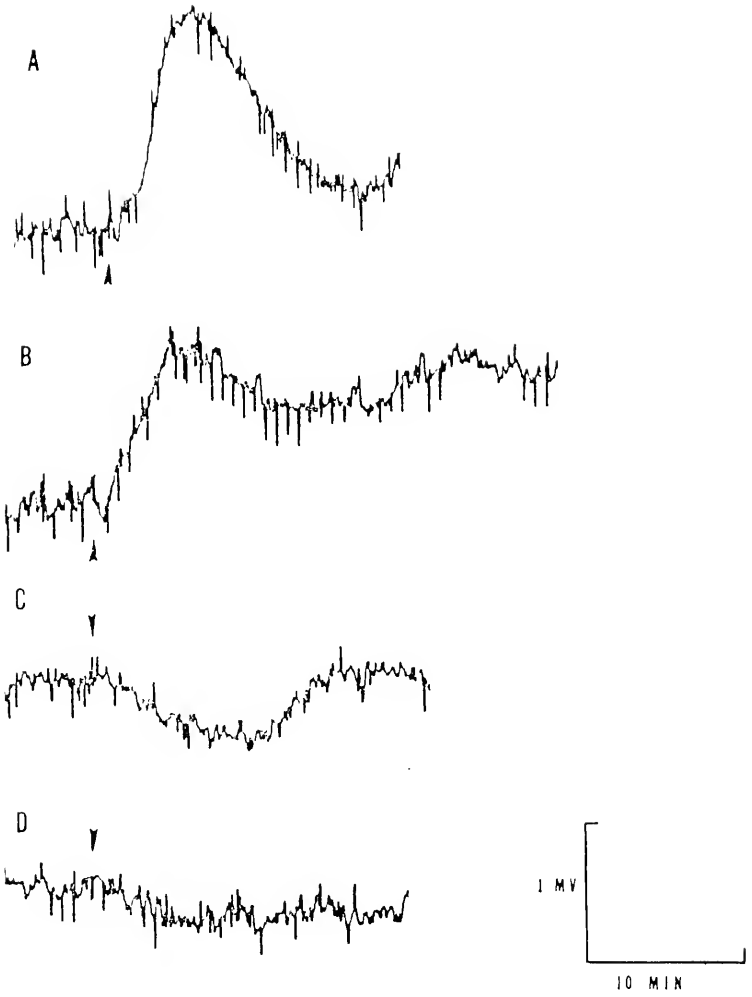


Figure 14. A, B, is the light-on LMSP recorded respectively from the control eye and experimental eyes of cat 7B9 one day after the optic disc was photocoagulated. Traces C and D present the light-off response of the control and experimental eyes.

The LMSP and ERG data collected from all the Ss in the vascular lesion experiments are summarized in Figure 15. Figure 15a,b,c represent respectively the b- to a-wave ratio, the light-on LMSP and the light-off LMSP recorded in the control and experimental eyes as a function of time in days secceeding the lesion. Since like the aspartate experiments, the relevant parameter for the ERG is the decline in b-wave relative to the a-wave amplitude, the b- to a-wave ratio was calculated for the control and experimental eyes. The data were then normalized by using the mean of the control eye value as 100%. This permits more direct comparison with the on and off LMSP data which were also normalized in the same way i.e. the mean amplitude recorded from the control eye equaling 100%. On day 1, cat 7B9, a small b-wave was present and the on and off LMSP of the lesioned eye had declined to about 50 or 60% of the control eye amplitude. These data were displayed in part in Figures 13 and 14. On day 6, cat 6H44 was the S and his b-wave and both the on and off LMSP were absent (Figure 15). Twelve days after the lesion cat 1CH was run. The b-wave was absent, the off LMSP was not present but a small on response, 20% or less than the control eye, was observed (Figure 15). Twenty one days after the initial lesion when 6H44 was again the S all measures in the experimental eye were still absent. Cat 7B9 was recorded from again 55 days after the lesion. The on and off LMSP were severely reduced and the b- to a-wave ratio was still at about 10% as seen in

Figure 15. Vascular lesion experiment summary figure. The effect of lesioning the retinal vascular supply on the b- to a-wave ratio of the ERG, Figure 15A, the light-on LMSP, Figure 15B and light-off LMSP, Figure 15C as a function of time after the lesion in days. For comparison purposes each of these dependent variables has been normalized with the control eye value recorded in the same session equaling 100% (if more than one response was collected the mean was used as 100%). Recordings from control eyes are indicated by an and from experimental eyes with a . The data presented for each day was recorded from a single subject. The S for each experiment is indicated above each day.

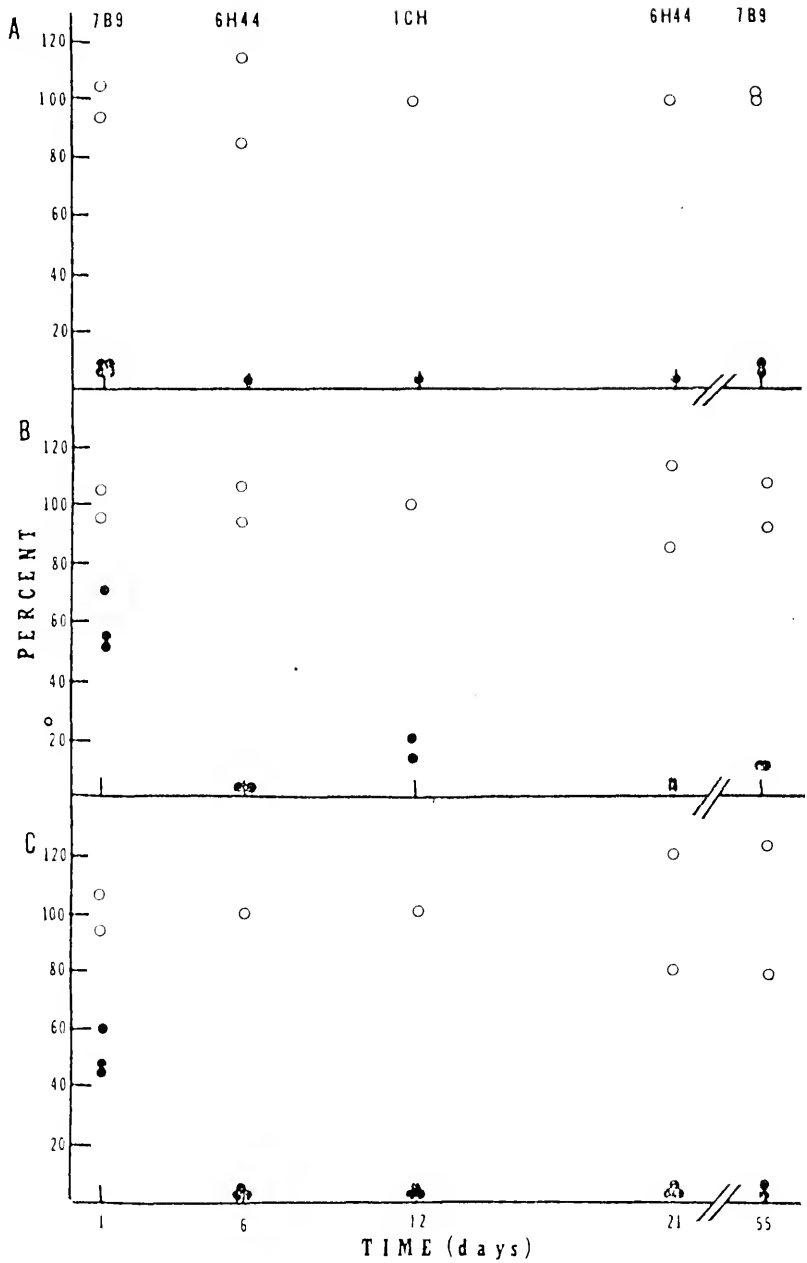

A
B
C
7B9
6H44
1CH
6H44
7B9
120
100
80
60
40
20
PERCENT
1
6
12
21
55
TIME (days)

Figure 15. The records from this session are presented in Figure 16 and 17. Figure 16 presents the on and off LMSPs and Figure 17 presents the ERGs.

As a further summary means, standard deviations and sample sizes were calculated for each dependent variable i.e. b/a wave ratio of the ERG, the on LMSP, and the off LMSP (Table 2). Data from all the cats recorded from after day 1 were pooled (Table 2b); the data from 7B9 recorded from on day one are presented separately in Table 2a since the LMSPs of that experiment were clearly different from other postlesion LMSPs. From the data recorded from all the animals it is apparent that by 6 days after the occlusion of the retinal blood supply the LMSP is absent or tremendously reduced. The greatest reduction in b-wave occurs by one day after the lesion and at this time the LMSP is reduced by about 50%. By 6 days post-lesion the on and off LMSPs are further reduced or absent.

Figure 18 presents photographs of the normal and lesioned retina embedded in celloidin. Figure 18a presents a photograph of a section from the control eye of cat 7B9 cut at 20μ and stained with cresyl violet. All layers of the retina are present and labeled. Figure 18b presents the retina of the experimental eye which was lesioned 55 days before. Only the receptors remain. Their nuclei are normal in appearance and number. All central and peripheral areas surveyed in this cat (7B9) had receptors present except for the disc region. The PE was normal throughout.

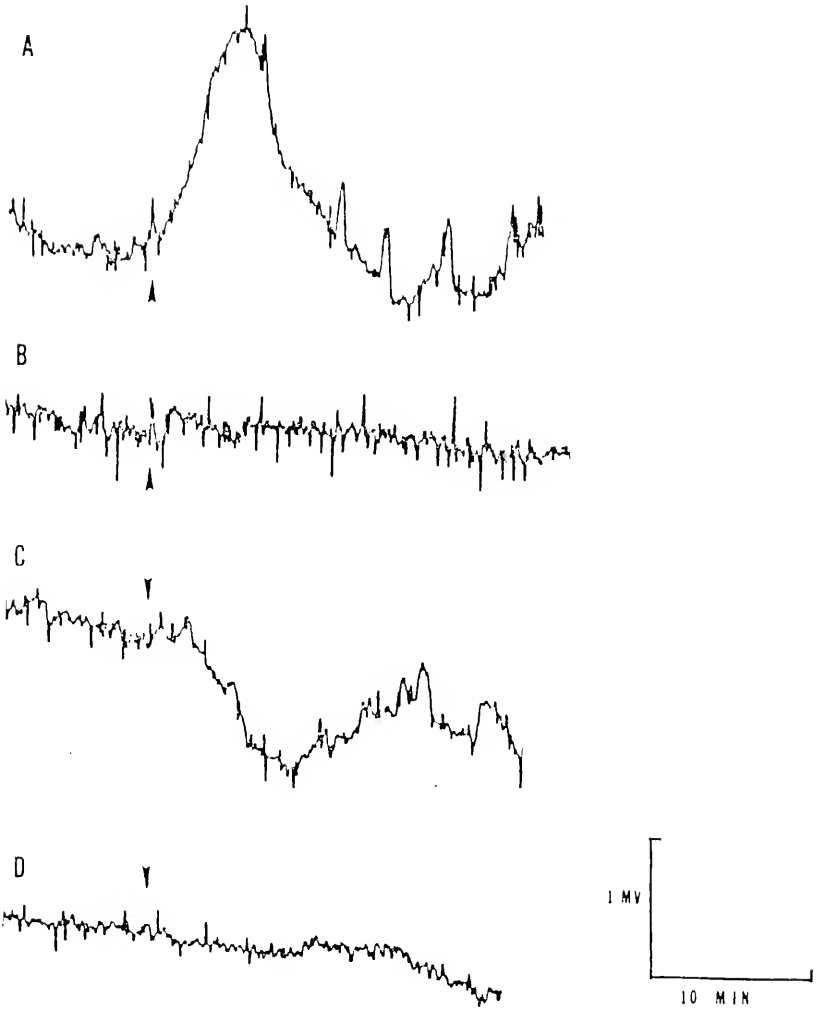


Figure 16. Figure 16A, B, is the light-on IMSP recorded respectively from the control eye and experimental eyes of cat 7B9 55 days after the retinal vasculature was photocoagulated. Traces C and D, present the light-off response of control and experimental eyes.

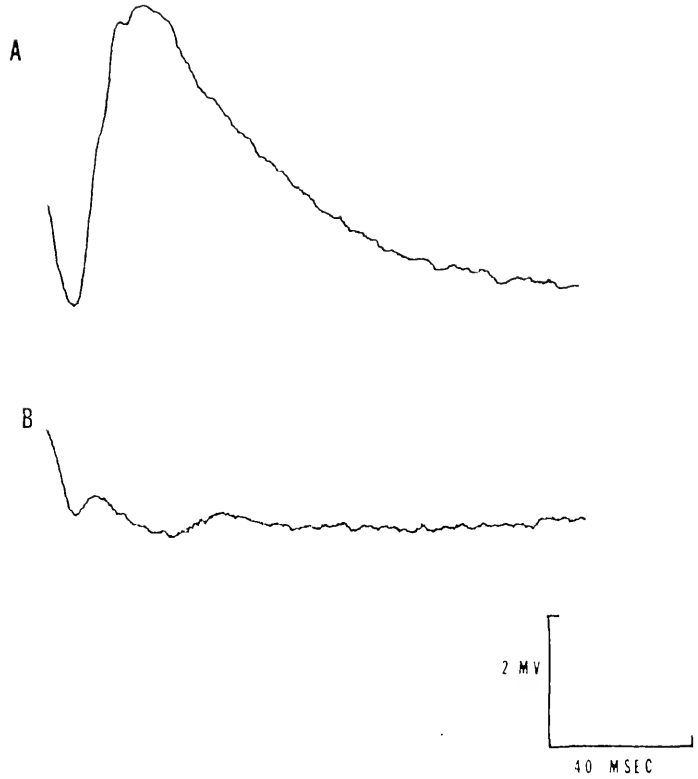


Figure 17. ERG recorded from the control (A) and experimental (B) eyes of cat 7B9 51 days after lesioning the optic disc.

Table 2: Means, standard deviations and sample sizes for the B/A-wave ratio of the ERG, the light-on and the light-off LMSP in the control and experimental eyes. Table 2A is the data recorded from cat 7B9 on day 1. Table 2B is the pooled data recorded from all cats run on day 6 through day 55.

A - Cat 7B9, Day 1

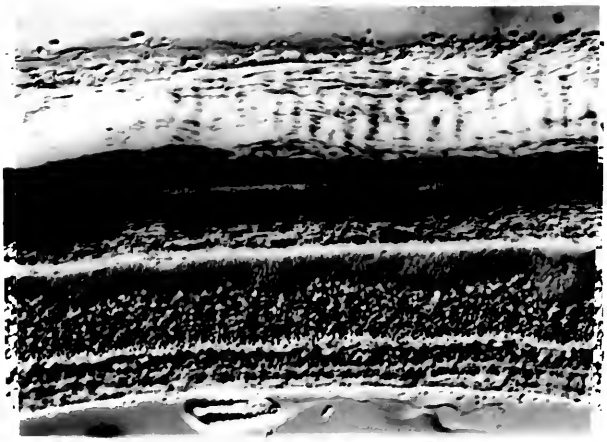
	B/A Ratio	Light-On LMSP	Light-Off LMSP
mean-control eye	3.8	1.52 mV	.63 mV
standard deviation-control eye	.28	.12	.06
sample size-control eye	2	2	2
mean-lesioned eye	.33	.91	.32
standard deviation-lesioned eye	.01	.16	.05
sample size-lesioned eye	4	3	3

B - All Other Ss, Days 6 to 55

	B/A Ratio	Light-On LMSP	Light-Off LMSP
mean-control eye	2.93	1.51 mV	.84 mV
standard deviation-control eye	.87	.29	.26
sample size-control eye	7	7	6
mean-lesioned eye	.14	.10	.01
standard deviation-lesioned eye	.10	.14	.02
sample size-lesioned eye	8	8	10

Figure 18. Tangential sections from the normal (A) and experimental (B) retinas of cat 7B9. The tissue was embedded on celloden, cut at 20μ and stained with cresyl violet.

A


sclera
tapetum
PE
outer segments
receptor nuclei
outer plexiform
inner/nuclear l.
ganglion cell layer

B

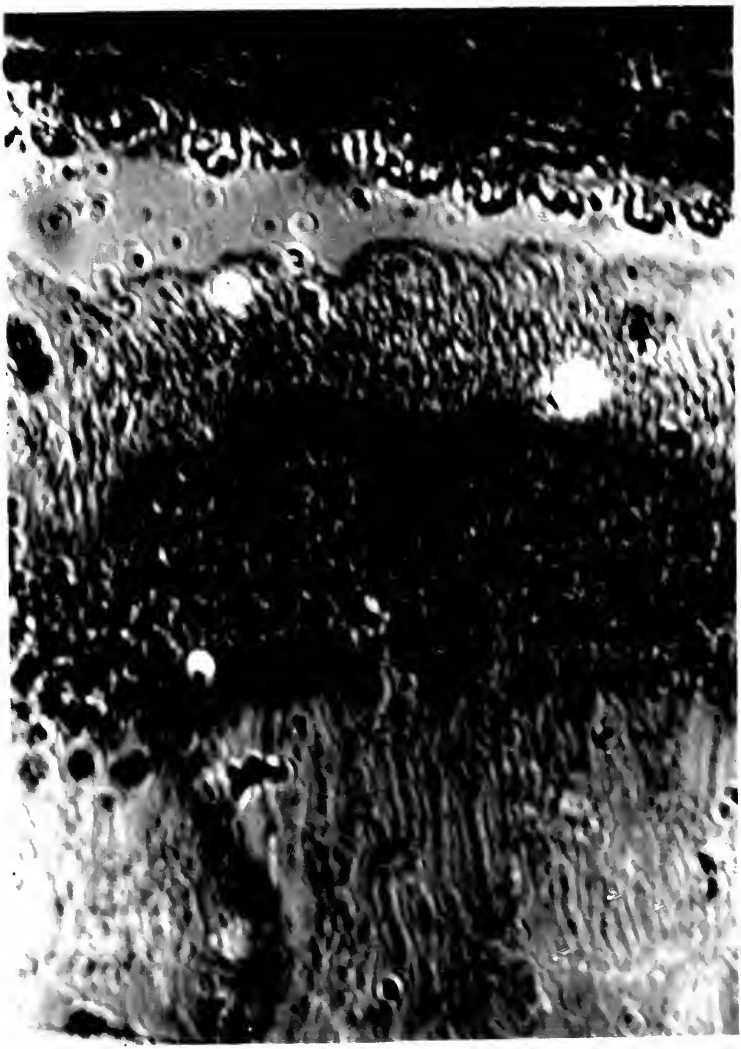

sclera
tapetum
PE
outer segments
receptor nuclei
remnants of inner
layers

To better demonstrate that the outer and inner segments of the receptors were present in the lesioned eyes tissue from the lesioned eye of cat 6H44 embedded in plastic is shown in Figure 19. The receptor inner and outer segments are present although the retina became detached during processing. It should also be noted that the major nonneural glial cell of the retina, the Muller cell, also appeared to be absent. The Muller cells were probably not present since their nuclei and most of their cytoplasm lies in the inner retina (Miller and Dowling, 1970).

When S 1CH's experimental eye was examined most areas of the retina had normal appearing receptors but no inner layers. An area of approximately 10% of the retina, however, was largely degenerated with only a few receptors intact. Some small areas of reduced receptor density were also observed. The PE appeared normal throughout. When the retina from the experimental eye of S 6H44 was examined a fairly large receptorless area was observed (40%). Some areas of thinned receptors were also present. Areas of normal appearing receptors without inner retinal layers were also present. All areas of the PE examined appeared normal.

It should be noted that in all cats there were always a considerable number of normal appearing receptors remaining. Additionally an a-wave, indicative of functional receptors, was also always present. There were however some small differences across cats with respect to the ERGs. For cat 7B9 where all the receptors were present except

Figure 19: Tangential section from the lesioned eye of cat 6H44. The tissue was embedded in plastic and sectioned at 2 μ and stained with toluidine blue.


Tapetum
PE
outer segments
inner segments
receptor nuclei
degenerated remnants of inner layers

those around the optic disc the a-wave was only 14% lower on day 55 in the experimental eye, than in the control eye (Figure 16). Cat 6H44 and 1CH had a-waves which were reduced by approximately 25% (measured at the last experimental session for each cat) which is surprising considering the extent of the receptorless area in cat 6H44.

In cat 7B9 a larger b-wave was preserved also indicating that the lesion was not as severe. Only a miniscule b-wave or none at all was observed in the ERGs of the experimental eyes of the other two cats indicating a more severe lesion than in 7B9.

Discussion

Lesioning the retinal blood supply eliminating the inner retina but leaving the receptors intact is a standard technique in cat and monkey (Brown, 1968; Maffei and Poppele, 1968). Like aspartate, lesioning the inner retina eliminates the b-wave yet leaves the a-wave intact.

As expected photocoagulating the optic disc of the cat had the predicted effect on the ERG, reducing or eliminating the b-wave although the a-wave was still present. As in the aspartate experiments severe reduction in ERG b-wave to a-wave ratio occurred in tandem with severe reduction in the LMSP responses in all lesioned eyes. On the first day after the lesion the decrement in ERG was more severe than the reduction in LMSPs. Between the first and sixth days after the photocoagulator lesion the LMSP is reduced by 80% or

more as is the b- to a-wave ratio of the ERG. Both signals remain depressed up to 55 days after the initial lesion, the longest time at which a measurement was made. The nature of the lesion was confirmed histologically demonstrating that the photoreceptors were present but the rest of the neural retina was absent.

In two cats the lesion was more severe and some photoreceptor change was observed. This was surprising since interrupting the retinal circulation of the cat has been used by other investigators to reportedly produce the same lesion. Brown (1965) reported the photoreceptors were intact but the inner retina was absent in the cat following occlusion of a branch vessel of the retinal arteries. Maffei and Poppele (1968) photocoagulated the optic disc of cats and report only the a-wave of the ERG remained but presented no histological data to confirm the extent of the lesion. There are two interpretations of the receptor damage found in two of the Ss in this experiment. It could be either that the retinal circulation in some cats supplies parts of the inner retina or that the choroidal circulation was damaged by the photocoagulation. With respect to the first hypothesis it should be pointed out that although the retinal vasculature is reported to supply only the inner retina in the cat, variability in vascular patterns across individual Ss is a well known occurrence. The second hypothesis also cannot be ruled out. Choroidal arteries lie directly around the disc region of the cat (Wong and Macri,

1964) and either through direct damage or edema may have been temporarily or permanently occluded. Fortunately in two of the cats the damage to the receptors was either nil or not severe. It can be concluded therefore that the inner retina is necessary for generation of the LMSP. This experiment therefore confirms the findings of the aspartate experiment via another totally different technique.

CHAPTER 8

ANALYSIS AND CONCLUSIONS

The major finding of this study is that the inner retina is involved in an essential fashion in the generation of the LMSP. In addition it was demonstrated that the LMSPs elicited by a flickering light are not readily distinguishable from the LMSPs elicited by a steady light of equal time averaged irradiance. When this result is considered within the context of the aspartate and vascular lesion experiments one must conclude that the LMSP is probably generated by a nonneural element of the inner retina. This element(s) must meet at least two requirements. First, it must be situated at least in part vitread to the receptors on the inner retina. Second, it must be relatively insensitive to the rates of flicker tested in this experiment which are known to be psychophysically and physiologically distinguishable by the cat (Dodt and Enroth, 1954; Kappauf, 1936 cited in Brown, 1965).

A number of authors have suggested that the PE modulated by the receptors is the site of the LMSP (Arden et al., 1962; Nilsson and Skoog, 1975; Skoog, 1975; Taumer et al., 1974). The PE obviously does not meet the first requirement stated above i.e. it is not located in the inner retina and there is no evidence that it is in any way re-

lated to the inner retinal functions. This PE hypothesis can probably be attributed to the work of Noell (1954) on the SP in the rabbit. He demonstrated that a chemical lesion of the PE induced by sodium iodate and confirmed histologically reduces or eliminate the large positive response of the SP to sodium azide. He concluded that the PE must "participate in the production of the normal direct current potential across the eye (Noell, 1954, p. 80)." Heck and Papst (1957) confirmed Noell's findings. Neither Noell (1954) nor Heck and Papst (1957) measured the LMSP. The dependent variable they measured was the chemical responsiveness of the SP. Noell (1954) attributes the constant DC voltage across the eye to the PE; he does not attribute the LMSP to the PE as he never recorded this variable. Heck and Papst (1957) thought that the SP and LMSP both originated in the PE driven by the receptors. However there is no a priori reason why the DC potential across the eye should originate at the same locus as the light induced changes in DC level (LMSP) and the results of the aspartate and vascular lesion experiments rule out this hypothesis. However, this PE localized DC potential Noell localized could be the current source for the LMSP. It is possible that the LMSP results when the conductance of the retina changes due to the effect of light thereby changing the current pathways of the PE produced SP. All neural activity induces associated extracellular ion flux and these must alter the resistance of the retina. This hypothesis is perhaps more parsimonious

than a dual source of DC potentials in the eye but considering the number of sites for the various components of the ERG it is not a compelling argument.

Whenever considering the origin of the LMSP two of its characteristics must be recalled: a) its long time course and b) the fact that it is a damped oscillation. Various authors have stated these characteristics indicate the LMSP is directly related to certain PE and receptor or inner retinal metabolic functions (Arden and Kelsey, 1962b; Arden et al., 1962; Taumer et al., 1974).

Arden et al. (1962) and Arden and Kelsey (1962a) have suggested the LMSP is an indication of rhodopsin renewal. The time course of rhodopsin renewal is of the same order of magnitude as the LMSP and has been shown to involve the PE (Dowling and Gibbons, 1961). In addition, Arden and Kelsey (1962b) have demonstrated the amplitude of the first major peak of the light-on EOG succeeding a complete bleach is highly correlated with the percent of rhodopsin regenerated. The results of experiment 1 (where the characteristics of the cat LMSP were described) and the aspartate and vascular lesion experiments demonstrate, however, that a purely receptor-PE based explanation of the LMSP is untenable.

Taumer et al. (1974) have proposed the retinal ADP/ATP quotient is responsible for the LMSP although no specific mechanism is suggested. In this scheme the initial peak or trough of the LMSP and the oscillations are supposedly signs

of an incoming energy overshoot or undershoot. The LMSP and the associated oscillations thus would be representative of an oscillating feedback process which is providing the necessary supply of energy to the retina. In support of this hypothesis they cite the work of Sickel (1972) who found long lasting variations in ADP/ATP quotient in an *in vitro* isolated frog retina preparation without a PE. However, other findings of Sickel (1972) considered with regard to the flicker vs. steady experiments described here would seem to weaken this hypothesis. Sickel (1972) using a variety of techniques measured retinal metabolism under various light, dark and flickering light conditions. The preparation was an *in vitro*, perfused frog retina with the PE removed. He found that CO_2 production from ^{14}C labeled glucose, a measure of retinal respiration, declined when a steady light was presented to a dark adapted retina but increased when a 1 Hz square wave flickering light was substituted for an equivalent time average luminance steady light. He also measured retinal 0_2 uptake in light and dark and found that in light it decreased. If the differential effects Sickel (1972) found for steady and flickering light hold true for cat retinal metabolism as well, then the absence of a significant difference in the steady vs. flicker experiment indicates that the LMSP is not a measure of retinal energy consumption.

If the LMSP is not generated by the receptors and PE alone as demonstrated by the basic characteristics of the

LMSP (experiment 1) and the aspartate and vascular lesion experiments and it is not generated by purely neural or energy related metabolic activity in the retina, as the steady vs. flicker experiments indicate, what does generate it? As discussed above it is unlikely that a neural component of the retina other than the receptors or retinal energy metabolism per se is the source of the LMSP. One obvious possibility remains, the glial cells of the retina, specifically the Muller cells. Processes of these cells are closely associated with the nuclear region and synaptic terminals of receptors although no synaptic contacts can be seen at the EM level (Miller and Dowling, 1970). The Muller cells could be intimately related to receptor metabolic activity. In addition Muller cells have been shown via intracellular recordings in mudpuppy to be relatively insensitive to flickering stimuli compared to the neural cells of the mudpuppy retina (Miller and Dowling, 1970). Muller cell bodies proper are in the inner retina and at least in the monkey they are known to degenerate after occlusion of the retinal vasculature (Kroll, 1968). Finally, if sodium aspartate acts like a neurotransmitter saturating the horizontal and bipolar cells it may also interfere with normal Muller cell activity. Some glial cells in the mammalian nervous system have been shown to preferentially accumulate certain neurotransmitters (Kuffler and Nicholls, 1976). If Muller cells normally accumulate the neurotransmitter released by the receptors an excess of

the transmitter might act to saturate their normal electrical responsiveness. It is therefore hypothesized that the LMSP is the result of some metabolic interactions between the receptors and Muller cells of the retina. Glial cells have been shown to have a number of properties which distinguish them from neurons. Some of these properties resemble the kinds of changes seen in the LMSP. For example, mammalian cortical glial cells respond to ongoing activity in surrounding neurons with sustained and increasingly large depolarizations (Kelly and Van Essen, 1974). The ability of glial cells to integrate activity over a period of time many seconds long demonstrated both in the mudpuppy Muller cells and mammalian cortical glia is the key characteristic implicating the Muller cells as the origin of the LMSP. In the mudpuppy optic nerve depolarizations of up to 140 mV over a resting level have been seen. At the end of the train of stimuli inducing these large potentials it may take over 30 seconds for the cells to return to their normal resting state (Kuffler and Nicholls, 1966). Miller and Dowling (1970) who recorded intracellularly from Muller cells in the necturus retina state that in response to flashes of light these cells tended to stay depolarized unlike the ERG b-wave and responses recorded from inner retina neural cells in the same preparation. Mammalian cortical glia also have long time constants responding with sustained depolarization to activity in surrounding neurons (Kelly and Van Essen, 1974). In the retina a sustained depolarization of Muller cells

would result in a positive response recorded with a corneal electrode. This is the same polarity as the light-on LMSP as noted above.

The Muller cell therefore directly influenced by the photoreceptors would seem to be the most likely candidate for generating the LMSP. They are situated mainly in the inner retina yet contact the receptors. They are comparatively unresponsive to flicker unlike the neural components of the inner retina of the mudpuppy (Miller and Dowling, 1970). Finally intracellular recording of their light induced activity indicates they generate sustained potentials, the most distinctive characteristic of the LMSP. The polarity of these potentials, both light-on and light-off components are in the correct direction to be the initial peak or trough of the LMSP. It should also be noted the spatial orientation of the Muller cells in the retina is compatible with production of large field potentials such as the LMSP's measured at the cornea. In the retina extracellular currents add in the radial direction (the orientation of the Muller cells) while currents parallel to the retinal surface tend to cancel (Rodieck, 1973). Finally, it is worth noting that the long time course of the LMSP has been considered by many authors (Arden et al., 1962; Arden and Kelsey, 1962b; Taumer, 1974; Skoog, 1975) to indicate it is related to supportive metabolic functions of the retina. This has classically been a function attributed to glial cells in the nervous system (Kuffler and Nicholls, 1976).

Another controversial question concerning the LMSP is the relationship between the on and off responses. The data presented here indicate that the two components have a common site of origin. The common insensitivity of the on and off responses to steady vs. flickering stimuli indicates both are of non-neural origin. The severe and correlated reduction in both components due to inner retinal lesions is another important similarity. If these findings are considered in conjunction with the similarity in waveform and time course of the oscillations shown in this experiment for cat and by Skoog (1975) in the human a strong case is made for a common site of origin. The increased variability seen in off responses and the difference in waveform shown here of the initial peak and trough may be indicative of some basic differences but these are subtle when compared to the noted similarities (Table 1). In the literature the strongest argument for a different site of origin of the on and off LMSP has been made by Gouras (1969). His argument is based in part on a clinical case of central retinal artery occlusion reported by Nagaya (1964). Nagaya presented data recorded on the fourth and sixtieth days after a patient suffered a complete obstruction of the central retinal artery. On the fourth day neither the light-on nor the light-off response was present. On day 60 a smaller than normal light-off response was present but the on response was still absent. In the cat I tested 55 days after vascular occlusion neither response was present. In addition

at all other times when cats were tested, except day 1 after vascular lesion, both on and off responses were absent or severely reduced in cats. Gouras (1969) also noted that the dark trough remained normal in certain other pathologies that eliminate or reduce the light peak such as chloroquine retinopathy and all but severe cases of retinitis pigmentosa.

Clinically the EOG has been used for many years as an aid in diagnosis of various ocular lesions especially hereditary disorders of the eye such as vitelliform macular degeneration, fundus flavimaculatus, and the various forms of retinitis pigmentosa and toxic lesions (Deutman, 1971; Gouras 1969). Generally clinicians have assumed it is a measure of retina-PE and especially PE activity (Arden et al., 1962; Skoog, 1975). This is certainly not the whole story. It would be useful to reevaluate the EOG as a clinical test. It may be more widely applicable as a diagnostic and especially prognostic test in a variety of retinal lesions since it measures the viability of the inner retina not just the PE and receptors. For example the EOG might be a valuable diagnostic and prognostic test in diabetic retinopathy or glaucoma.

One disease the EOG is usually very effective in diagnosing is vitelliform macular degeneration (VMD) (described in detail by Deutman, 1971). Ophthalmoscopically the disease usually presents as a purely macular lesion. In its early stages visual acuity is usually normal or slightly

subnormal. Dark adaptation functions and visual fields also appear normal. The foveal ERG and visually evoked response are also usually normal. The light rise and dark trough of the EOG are typically absent however Deutman (1971) suggests that this EOG finding is indicative of a diffuse disorder which appears ophthalmoscopically only at the macular region due to its unusual characteristics. Specifically Deutman (1971) proposes a diffuse metabolic lesion of the PE as the cause of the abnormal EOG. It is difficult to reconcile the abnormal EOG seen in VMD with the findings of this study. It may be that when the SP, arising in the PE (Noell, 1953), is abolished, an LMSP cannot be generated. It also may be that a diffuse retinal lesion is present in cases of VMD but that such a lesion is too subtle to cause a major decrement in visual function as measured in the clinic.

Additional work to precisely specify the neural origin of the LMSP is still needed. The first experiment to perform might be intracellular recording from Muller cells. If they could be held for ten minutes it would be possible to determine if they were the source of the LMSP. Other experiments relating retinal metabolism and photopigment regeneration directly to the LMSP would also be extremely interesting. If better understood the LMSP could provide a new tool for studying retinal metabolism, and perhaps glial-neural interactions.

Conclusions

In one experiment it was demonstrated that the light-on and off LMSP of the cat does not respond differentially to steady vs. flickering light of equal time averaged irradiance. Also it was demonstrated, using two totally different techniques, that to elicit a light-on and light-off LMSP a functional inner retina must be present. Based on these findings it was hypothesized the LMSP originates in the Muller cells of the retina. They are located mainly in the inner retina, are relatively insensitive to flickering light unlike the neural cells of the inner retina and tend to integrate even low frequency flickering stimuli (Miller and Dowling, 1970). Based on these findings it was suggested that the clinical EOG be reevaluated since it may be of more general applicability.

APPENDIX A

Subjects Used in Each Experiment

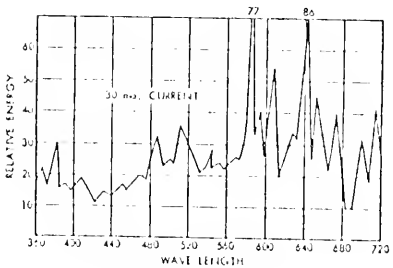
Experiment #1	Experiment #2
119	215
215	315
315	411
329	413
411	419
413	
419	
1CH	

Experiment #3	Experiment #4
419	7B9
421	6H44
44	1CH
321	

APPENDIX B

Sylvania R1131C Glow Tube Spectral Emission

Data from Manufacturer



RELATIVE ENERGY VS WAVE LENGTH

APPENDIX C

Formula for Paralytic Agent

29.9 cc	0.021% NACL in sterile water with 5% Dextrose
4.5 cc	Gallamine triethiodide (20 mg/ml)
0.6 cc	Tubocurarine Chloride (3 mg/ml)

REFERENCES

Arden, B.G. & Barrada, A. (1962). Analysis of the electro-oculograms of a series of normal subjects. British Journal of Ophthalmology 46, 468-482.

Arden, G.B., Barrada, A. & Kelsey, J.H. (1962). New clinical test of retinal function based upon the standing potential of the eye. British Journal of Ophthalmology 46, 449-467.

Arden, G.B. & Kelsey, J.H. (1962a). Changes produced by light in the standing potential of the human eye. Journal of Physiology (London) 161, 189-204.

Arden, G.B. & Kelsey, J.H. (1962b). Some observations on the relationship between the standing potential of the human eye and the bleaching and regeneration of visual purple. Journal of Physiology (London) 161, 205-226.

Aserinsky, E. (1955). Effect of illumination and sleep on the amplitude of the electro-oculogram. Archives of Ophthalmology 53, 542.

Baron, W. (1974). Lenticular and fundus changes induced by intraocular infusion of sodium aspartate. Investigative Ophthalmology 13, 459-462.

Baron, W.S. & Boynton, R.M. (1974). The primate foveal local electroretinogram: An indicator of photoreceptor activity. Vision Research 14, 495-501.

Baron, W.S. & Boynton, R.M. (1975). Primate cone - Sinisod flicker. Journal of Physiology (London) 246, 311-331.

Boynton, R.M. & Whitten, D.N. (1970). Visual adaptation in monkey cones: Recordings of late receptor potentials. Science 170, 1423-1426.

Boynton, R.M. & Whitten, D.N. (1972). Selective chromatic adaptation in primate photoreceptors. Vision Research 12, 855-874.

Brown, J.L. (1965). Flicker and intermittent stimulation. In Vision and Visual Perception (edited by Graham,

C.H.) pp. 251-321, John Wiley and Sons, Inc., New York.

Brown, K.T. (1968). The electroretinogram: Its components and their origins. Vision Research 8, 633-677.

Buchmann-Olsen, B. & Rosenfalck, A.M. (1957). Spectral energy calibration of a light flash used in physiological experiments. Journal of the Optical Society of America 47, 30-34.

Campbell, F.W. & Rushton, W.A.H. (1955). Measurement of the scotopic pigment in the living human eye. Journal of Physiology (London) 130, 131-147.

Cervetto, L. & MacNichol, E.F., Jr. (1972). Inactivation of horizontal cells in turtle retina by glutamate and aspartate. Science 178, 767-768.

DeHaas, H.K. (1903). Lichtprikkels en retinastroomen in hun quantitatief verband. In avg. Dissert. Leiden. (Quoted by Arden & Kelsey, 1962a).

Deutman, A.F. (1971). The hereditary dystrophies of the posterior pole of the eye. Van Gorcum, Assen, Netherlands.

Dewar, J. (1877). The physiological action of light. Nature 15, 433. (Quoted by Miles, W., 1939).

Dewar, J. & M'Kendrick, J.G. (1876). On the physiological action of light. Transactions of the Royal Society of Edinburgh 27, 141-166. (Quoted by Arden & Kelsey, 1962a).

Dodt, E. & Enroth, C. (1954). Retinal flicker response in cat. Acta Physiologica Scandinavica 30, 375-390.

Dowling, J.E. (1960). Chemistry of visual adaptation in the rat. Nature 188, 114-118.

Dowling, J.E. & Gibbons, I.R. (1961). The effect of vitamin A deficiency on the fine structure of the retina. In Structure of the Eye (edited by Smelser, G.K.) pp. 85-99, Academic Press, New York.

Dowling, J.E. & Ripps, H. (1970). Visual adaptation in the retina of the skate. Journal of General Physiology 56, 491-520.

Du Bois Reymond, E. (1849). Untersuchungen uber die thierische Elektrizitat. Band II, pp. 256-257. Verlag von G. Reimer, Berlin (Quoted by Arden & Kelsey, 1962a).

Fenn, W.O., Galambos, R., Otis, A.B. & Rahn, H. (1948-9). Corneo-retinal potential in anoxia and acapnia. Journal of Applied Physiology 1, 710-716.

Foulds, W.S. & Ikeda, H. (1966). The effects of detachment of the retina on the induced and resting ocular potentials of the rabbit. Investigative Ophthalmology 5, 93-108.

Francois, J., Verriest, G. & de Rouck, A. (1955). Modification of the amplitude of the human electro-oculogram by light and dark adaptation. British Journal of Ophthalmology 39, 398-408.

Francois, J.G., Verriest, G. & de Rouck, A. (1956a). Electro-oculography as a functional test and pathological conditions of the fundus. I. First results. British Journal of Ophthalmology 40, 108-112.

Francois, J., Verriest, G. & de Rouck, A. (1956b). Electro-oculography as a functional test in pathological conditions of the fundus. II. Base value and drop during dark adaptation. British Journal of Ophthalmology 40, 309-312.

Geddes, L.A. (1972). Electrodes and the Measurement of Bioelectric events. John Wiley and Sons, Inc., New York.

Gouras, P. (1969). Clinical electro-oculography. In The Retina (edited by Straatsma, B.R.) pp. 565-581, University of California Press, Berkeley and Los Angeles.

Gouras, P. & Carr, R.E. (1965). Cone activity in the light-induced DC response of monkey retina. Investigative Ophthalmology 4, 318-321.

Granit, R. (1933). The components of the retinal action potential in mammals and their relation to the discharge in the optic nerve. Journal of Physiology (London) 77, 207-239.

Hagins, W.A. (1955). The quantum efficiency of bleaching rhodopsin in situ. Journal of Physiology (London) 129, 22P-23P.

Hanitzsch, R. (1973). Intraretinal isolation of PIII subcomponents in the isolated rabbit retina after treatment with sodium aspartate. Vision Research 13, 2093-2102.

Hecht, S. & Shlaer, S. (1936). Intermittent stimulation by light: V. The relationship between intensity and critical frequency for different parts of the spectrum. Journal of General Physiology 19, 965-979.

Heck, J. & Papst, W. (1957). Uber den Ursprung des corneo-retinalen Ruhepotentials. Bibliotheca Ophthalmologica (Basel) 48, 96-107.

Himstedt, F. & Nagel, W.A. (1902). Festschr. d. Univ. Freiburg z. 50 jahr. Reg.-Jubil Sr. Kgl. Hoheit d. Grossherzogs Freidrich von Baden, pp. 262-263. (Quoted by Arden & Kelsey, 1962a).

Honda, Y. & Dawson, W.W. (1975). Botulinum intoxication of isolated retinas. Ophthalmological Research 7, 108-117.

Jacobsen, E. (1930). Electrical measurements of neuro-muscular states during mental activities. III. Visual imagination and recollection. American Journal of Physiology 95, 694. (Quoted by Miles, W., 1939).

Kappauf, W.E. (1936). Flicker discrimination in the cat. Psychological Bulletin 33, 597-598.

Karnovsky, M.J. (1965). A formaldehyde glutaraldehyde fixative of high osmolarity for use in electron microscopy. Journal of Cellular Biology 27, 137A.

Kelly, J.P. & Van Essen, D.C. (1974). Cell structure and function in the visual cortex of the cat. Journal of Physiology (London) 238, 515-547.

Kikawada, N. (1968). Variations in the corneo-retinal standing potential of the vertebrate eye during light and dark adaptations. Japanese Journal of Physiology 18, 687-702.

Knave, B., Persson, H.E. & Nilsson, S.E.G. (1974). The effect of barbiturate on retinal functions. II. Effects on the c-wave of the electroretinogram and the standing potential of the sheep eye. Acta Physiologica Scandinavica 91, 180-186.

Kolder, H. (1959). Spontane und experimentelle Anderungen des Bestandpotentials des menschlichen Auges. Pflugers Archiv fuer die gesamte Physiologie 268, 258-272.

Kris, C. (1958). Corneo-fundal potential variations during light and dark adaptation. Nature 182, 1027-1028.

Kroll, A.J. (1968). Experimental central retinal artery occlusion. Archives of Ophthalmology 77, 453-469.

Kuffler, S.W. & Nichols, J.G. (1966). The physiology of neuroglial cells. Ergebnisse der Physiologie 57, 1-90.

Kuffler, S.W. & Nichols, J.G. (1976). From Neuron to Brain Sinauer Asso., Inc., Sunderland, Mass.

Kuhne, W. & Steiner, J. (1881). Ueber electrische Vorgange im Sehorgane. Unters. Physiol. Inst. Univ. Heidelberg 4, 64-160. (Quoted by Arden & Kelsey, 1962a).

Maffei, L. & Poppele, E. (1968). Transient and steady state electroretinal responses. Vision Research 8, 229-246.

Marg, E. (1951). Development of electro-oculography. Archives of Ophthalmology 45, 169.

Meyers, I.L. (1929). Electronystagmography--A graphic study of the action currents in nystagmus. Archives of Neurology and Psychiatry 21, 901. (Quoted in Miles, W., 1939).

Miles, W.R. (1939). Experimental modification of the polarity potential of the human eye. Yale Journal of Biology and Medicine 12, 161-183.

Miles, W.R. (1940). Modification of the human eye potential by dark and light adaptation. Science 91, 456.

Miller, R.F. & Dowling, J.E. (1970). Intracellular responses of the Muller (glial) cells of mudpuppy retina: Their relation to b-wave of electroretinogram. Journal of Neurophysiology 33, 323-341.

Mowrer, O.H., Ruch, T.C. & Miller, N.E. (1936). The corneo-retinal potential difference as the basis of the galvanometric method of recording eye movements. American Journal of Physiology 114, 423-428.

Murakami, M., Ohtsu, K. & Ohtsuka, T. (1972). Effects of chemicals on receptors and horizontal cells in the retina. Journal of Physiology (London) 227, 899-913.

Murakami, M., Ohtsuka, T. & Shimazaki. (1975). Effects of aspartate and glutamate on the bipolar cells in the carp retina. Vision Research 15, 456-458.

Nagaya, T. (1964). The standing potential of the eye in vascular and degenerative disease of the retina. Bulletin of Yamaguchi Medical School 11, 187-201.

Nilsson, S.E.G. & Skoog, K.O. (1975). Covariation of c-wave and standing potential of the human eye. Acta Ophthalmologica 53, 721-730.

Noell, W.K. (1953). Studies on the electrophysiology and the metabolism of the retina. U.S.A.F. School of Aviation Medicine, Project No. 21-1201-0004, Report No. 1, p. 122.

Noell, W.K. (1954). The origin of the electroretinogram. American Journal of Ophthalmology 38, 78-90.

Noell, W.K. (1963). Cellular physiology of the retina. Journal of the Optical Society of America 53, 36-48.

Normann, R.A. & Werblen, F.S. (1974). Control of retinal sensitivity. I. Light and dark adaptation of rods and cones. Journal of General Physiology 63, 37-61.

Pugh, E.N., Jr. (1975). Rhodopsin flash photolysis in man. Journal of Physiology (London) 248, 393-412.

Rodiek, R.W. (1973). The Vertebrate Retina: Principles of Structure and Function. W.H. Freeman & Company, San Francisco.

Rushton, W.A.H. (1961). Dark-adaptation and the regeneration of rhodopsin. Journal of Physiology (London) 156, 166-178.

Sickel, W. (1972). Retinal Metabolism in Dark and Light In Handbook of Sensory Physiology (edited by Fuortes, M.G.F.) Vol. VII/2: Physiology of Photoreceptor Organs. pp. 667-729, Springer-Verlag, Berlin.

Skoog, K.O. (1975). The directly recorded standing potential of the human eye. Acta Ophthalmologica 53, 120-132.

Skoog, K.O., Texterius, O. & Nilsson, S.E.G. (1975). Ethyl alcohol effects on the directly recorded standing potential of the human eye. Acta Ophthalmologica 53, 710-720.

Taumer, R., Hennig, J. & Pernice, D. (1974). The ocular dipole - A damped oscillator stimulated by the speed of change in illumination. Vision Research 14, 637-645.

Ten Doesschate, G. & Ten Doesschate, J. (1956). The influence of the state of adaptation on the resting potential of the human eye. Ophthalmologica 132, 308-320.

Von Norren, D. & Padmos, P. (1977). Influence of anesthetics ethyl alcohol and freon on dark adaptation of monkey cone ERG. Investigative Ophthalmology 16, 80-83.

Werblin, F.S. & Dowling, J.E. (1969). Organization of the retina of the mudpuppy, Necturus maculosus. II. Intracellular recording. Journal of Neurophysiology 32, 339-355.

Westheimer, G. (1966). The Maxwellian view. Vision Research 6, 669-682.

Wong, V. & Macri, F. (1964). Vasculature of the cat eye. Archives of Ophthalmology 72, 351-358.

BIOGRAPHICAL SKETCH

Harris R. Lieberman was born in Philadelphia, Pennsylvania, and attended Central High School there. Upon graduation he attended Franklin and Marshall College in Lancaster, Pennsylvania, where he majored in psychology and was a teaching assistant in that department. He graduated in 1972 and in that year began graduate school in the Psychology Department of the University of Florida. At Florida he was a research assistant in the Visual Sciences Laboratory and in July, 1974, received a Center for Neurobiological Sciences Fellowship. He was awarded the Master of Arts degree in December, 1974. His major areas of interest are the neurophysiological and behavioral aspects of vision and their clinical application.

In June, 1973, he married Ellice S. Silver. She attended the University of Florida College of Medicine and graduated in June, 1977.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

William W. Dawson, Chairman
Professor of Psychology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Calvin K. Adams
Assistant Professor of
Psychology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Jay M. Enoch
Graduate Research Professor
of Psychology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Merle E. Meyer
Professor and Chairman of
Psychology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Horst O. Schwassmann
Associate Professor of
Zoology

This dissertation was submitted to the Graduate Faculty of the Department of Psychology in the College of Arts and Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1977

_____

Dean, Graduate School