EVOKED POTENTIALS FROM THE LASER-IRRADIATED RETINA*

By

Franklin G. Hempel and Ashley J. Welch
Department of Electrical Engineering

Technical Report No. 83

March 20, 1970

BIO-MEDICAL ENGINEERING RESEARCH LABORATORY

ELECTRONICS RESEARCH CENTER
THE UNIVERSITY OF TEXAS AT AUSTIN
Austin, Texas 78712

*Research sponsored in part by USAECOM, Department of the Army under Contract DAAB07-68-C0319 and the Joint Services Electronics Research Program under Research Grant AFOSR 69-1792.

ABSTRACT

Few investigations have been made of the effect of retinal photocoagulation on the light evoked electrical responses of the visual system. This research was therefore conducted to examine the electroretinogram (ERG) and the lateral geniculate nucleus (LGN) and striate cortex potentials of the rabbit before and after irradiation of the retina with a high-energy ruby laser. Dark-adapted, curare-paralyzed rabbits were secured in a stereotaxic apparatus where concentric needle electrodes were inserted into the contralateral LGN and cortex. A corneal ring electrode detected the ERG. Protocol consisted of (1) recording the three potentials while stimulating the eye with an argon laser operated at several flash intensities, (2) exposing the retina to a calculated ruby laser radiation dosage, and (3) recording again the responses to photic stimulation.

The data show that mild 6 mm lesions inflicted by laser radiation pulses with retinal energy densities of 1.25 to 3.2 J/cm^2 apparently act to uncouple the tonic inhibition normally imposed on the lateral geniculate nucleus and visual cortex by the retina. Such disengaged inhibition is observable as an augmentation of light evoked geniculate and cortical population responses, usually by low level stimuli and less frequently by bright stimuli. The ERG a to b wave amplitude is reduced as much as 20% by these irradiances, and recovery processes associated with late PIII activity are disrupted. Maximum ERG amplitude loss is 30% following 5.1 or 6.3 J/cm^2 pulses, while LGN and cortex potentials are diminished or even extinguished by the same insult. Lesions increase the postirradiation latent time centrally by removing functional tissue from the retinal path traversed by impulses leading to the LGN and cortex signals. ERG latencies decrease under these conditions, perhaps because of diminished PIII activity. On gross examination, the 6 mm injuries appear as deep retinal plications of a type uncharacteristic of the smaller lesions produced during therapeutic photocoagulation.

TABLE OF CONTENTS

Page

CHAPTER I - INTRODUCTION 1

CHAPTER II - RETINAL THERMAL INJURY 3

A. Historical Review of Light-Induced Eye Injury 3

B. The Minimal Retinal Burn 5

C. Lesion Classification 9

D. Retinal Light Absorption and Ensuing Thermal Reactions 11

CHAPTER III - METHODS AND MATERIALS 13

A. Equipment 13

B. Electrodes 21

C. Experimental Protocol and Preparation of the Rabbit 22

D. Data Processing 28

CHAPTER IV - WAVEFORM CHARACTERISTICS OF PHOTICALLY EVOKED POTENTIALS OF THE RABBIT VISUAL SYSTEM 29

A. The Laser-evoked Rabbit Electroretinogram 29

B. Discussion of the Electroretinogram 38

Historical introduction 38

Origins of the ERG waves 39

Neural processes in the retina affecting the retinographic response 42

Factors influencing the ERG waveform 43

C. Waveform of the Laser Evoked Responses of the Rabbit Lateral Geniculate Nucleus 47

D. Discussion of the Electrophysiology of the Lateral Geniculate Nucleus 50

The light evoked LGN response 50

Electrically evoked activity and neural processes in the geniculate body 58

E. The Laser Evoked Response of the Rabbit Striate Cortex . . . 60
F. Discussion of the Electrical Activity of the Visual Cortex . . 61
Factors which influence the waveform of the cortex response 61
Electrically evoked cortical potentials 68
Spectral sensitivity of the striate cortex 70
CHAPTER V - RESULTS 71
A. Retinal Injury Produced by the Ruby Laser Operated in the Q-switched Mode 71
B. Laser Radiation Injuries with 6 mm Spot Sizes 76
Behavior of the c wave of the ERG from the irradiated retina 96
Latent time for the evoked responses 101
Gross appearance of the irradiated retina 111
CHAPTER VI - DISCUSSION, SUMMARY AND CONCLUSIONS 114
A. Discussion and Comment 114
The retinal lesion 114
The electroretinogram 116
Stray light and the ERG 119
Lateral geniculate and cortex potentials 121
Q-switched lesions 125
Latent time 127
B. Summary and Conclusions 128
APPENDIX - GROSS, MICROSCOPIC, AND ARCHITECTONIC ANATOMY OF THE RABBIT VISUAL SYSTEM 130
A. Gross Anatomy of the Visual Apparatus 130
B. Fine Structure of the Rabbit Retina 136
C. Cytoarchitectonic Structure of the Rabbit Lateral Geniculate Body 139
Projections of the retina on the LGN 140

D. Cytoarchitecture of the Rabbit Striate Cortex 140

Projections of the retina and lateral geniculate on the cortex 143

Projections of the retina and cortex onto the superior colliculus and pretectal nuclei 147

REFERENCES 150

CHAPTER I

INTRODUCTION

The widespread use of laboratory and operational lasers has caused increased concern for eye safety. Radiation dosages which damage the eye have been determined by, among others, investigators at the Stanford Research Institute [1,2], Medical College of Virginia [3], and the EG & G Company [4] for several laser devices. At each laboratory, the basis for detecting a retinal burn has been the post-irradiation appearance of an ophthalmoscopically or histologically visible lesion.

Although the energy threshold for producing changes in retinal tissue has been determined for a variety of animal species, a correlation has not been established between subjective visual loss and the intensity of the radiation administered to the eye. That is, we do not know how laser irradiation may affect the visual performance of a highly trained animal such as the monkey. Only preliminary results have been published relating the loss in visual performance to the laser irradiation of the eye. [5]

As well, little information about the irradiated retina's functional status has been obtained from the electroretinogram or the electrical activity of the visual stations in the brain. Only a few publications deal with this area of research. For example, reduction of the rabbit ERG amplitude was shown by McNeer et al [6] to occur following xenon arc exposures one-half the intensity required to produce a threshold burn, provided a retinal area of 40 mm^2 was involved. Destruction of the macula of the monkey with xenon photocoagulation reduced the ERG temporarily due to the loss of dark adaptation, but other than this, there was no permanent

difference demonstrated between the responses of the coagulated and the normal monkey eye.[7] Another study involving 58 monkeys showed that the loss of the ERG was linearly related to the fraction of the retina damaged, including eight cases where retinographic activity was completely eliminated by destruction of 12 to 47 percent of the retinal area.[8]

Laser-induced lesions have been seen to depress the ERG a and b waves of the cat immediately following the insult, but the a wave recovers its control amplitude shortly afterward.[9] The b wave progressively declines. Potentials of the optic tract fibers arising in the damaged area range from 18 per cent of control to complete disappearance.

In order to understand more fully the effect that laser radiation may have on the light evoked responses of the eye and brain, a pilot study was carried out in the Biomedical Electronics Laboratory of the University of Texas. These experiments, involving more than fifty rabbits, were designed to record electroretinographic, lateral geniculate nucleus, and striate cortex potentials before and after ruby laser irradiation.

This report, taken from the senior author's doctoral dissertation, summarizes the important findings from this work. Chapter II is devoted to the thermal reactions of chorioretinal tissue, and the succeeding chapters describe in detail the equipment, experimental protocol, light evoked responses of the rabbit visual system, and results of the study. A review of the gross, microscopic, and architectonic anatomy of the rabbit follows in the appendix.

C H A P T E R II

RETINAL THERMAL INJURY

A. Historical Review of Light-Induced Eye Injury

Visual disturbances caused by looking at an eclipse of the sun have been known since Socrates' time. The Pergamonian ocular anatomist and physiologist Galen (129 - 200 AD) was aware of solar burns, and Galileo is said to have received an eye injury by gazing at the sun through his famous telescope.[10,11] Attempts to cause deliberate retinal burns are reported in 1867 by Czerny, who focused sunlight into a rabbit's eye.[12]

A classic paper by Verhoef and his associates in 1916 described at length the nature of solar burns.[13] They called particular attention to the injury of the pigment epithelium (PE) and choroid and were the first to report that solar burns were due to a temperature increase and not simply an overstimulation of the photoreceptors with light. They furthermore duplicated the previous work of Birch-Hirschfeld, who in 1912 employed a carbon arc lamp as a light device to injure the retinae of both pigmented and albino rabbits. Data showing similarities between solar injury and carbon arc burns were given by both these groups, and the pigmented rabbit's greater susceptibility to burning than his albino counterpart was demonstrated. These studies marked the beginning of the rabbit's role as the animal chosen for the study of retinal burns.

By the mid 1940's, both the atomic bomb and the xenon lamp appeared as new powerful sources of light. Byrnes, Buettner, Rose, et al.,[14-16] emphasized the vulnerability of the retina and vision to an atomic bomb flash. In an extensive study by Byrnes and his colleagues,

Schawlow and Townes[20] developed the principle of the laser in 1958, and Maiman[21] successfully tested their theory in 1960 by achieving stimulated emission from a chromium-doped aluminum oxide rod. Physicians especially were quick to use this promising new source of intense light and in 1961 publications began appearing in which laser photocoagulation of the retina was described.[22,23] Several significant advantages of laser over xenon arc photocoagulation of the human retina were noticed:[24,25]

1. More accurate focusing and aiming of the beam was possible because of its collimation and coherence.

2. Uncomfortable dazzle associated white xenon light was no longer a problem, and irritation resulting from stray flux on the iris was avoided.

3. Transmission by the ocular media and absorption by the pigmented tissues of laser light were more efficient since a large part of the xenon source is infrared and not utilized.

4. Short pulses obviated the use of retro-orbital anesthesia or immobilization of the subject eye, and at the same time the brief heating phase on the retina meant little dissipation of heat peripheral to the lesion occurred. (Lesions could now be placed in the area between the disk and macula, or in the perimacular regions, for example.) Histologically, the burn fused neuroepithelium and pigment epithelium of the retina with the superficial choroid in a firm cicatrix.

A new area of research, the study of the interactions of laser light with ocular tissues, began. This research was directed at the development of lasers as therapeutic tools in the treatment of various retinal maladies, and to devise protective devices and measures for the investigator using the laser. As a result, the greater part of laser burn research literature has come from clinical laboratories.

B. The Minimal Retinal Burn

Several factors are involved in producing a radiation injury of the retina, including the exposure duration and the spectral distribution and power of the beam, and its diameter at the retinal plane. Within the eye, the effect of fundus pigment density, absorption characteristics, and properties of conductance must be considered. Most research by groups studying retinal burns has been directed at understanding the mechanisms which account for the minimal or threshold burn, and at correlating the findings in the threshold burn with what is known about the radiating beam and its target.[26]

The rabbit's eye has been used to establish standards, and to obtain physical data pertinent to retinal thermal injury, particularly threshold damage. In calculating energy levels which produce comparable degrees of retinal burns from subject to subject and with various light sources, it is necessary to take into account the spectral transmission, absorption, and reflection by the transparent ocular media, pigment epithelium and choroid.[27,28] Figures 1 a,b,c, show these values for enucleated and dissected rabbit eyes. Approximately 95 per cent of the ruby light incident on the rabbit cornea reaches the retina and choroid where 50 per cent is absorbed in a medium pigmented fundus and an additional 10 per cent reflected. In such a case, the remaining 35 per cent apparently goes into the sclera and retro orbital fat. There are many similarities in light absorption in the ocular media and fundus of medium to darkly pigmented rabbit compared to the same tissues in the human eye. To a great extent, the rabbit eye can justifiably substitute for the human eye.

"Minimal" or "threshold" retinal damage has its characteristic appearance, but its detection depends on the method chosen to find it. In early studies,[19] threshold burns of the rabbit retina were

Figure 1a

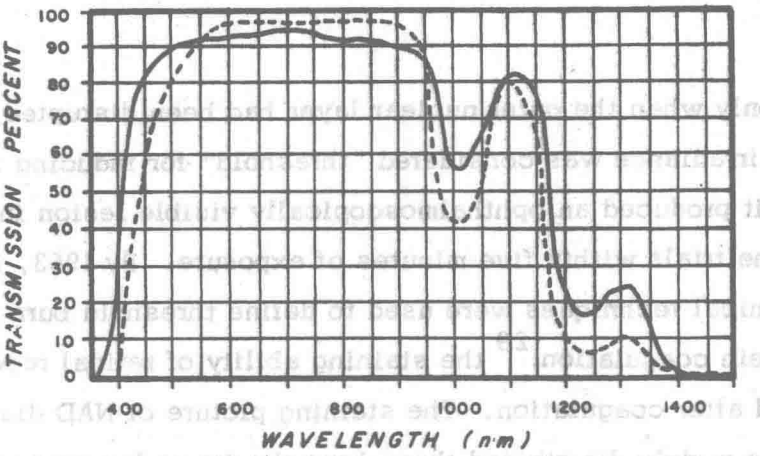
The mean transmission of the ocular media to light incident on the cornea of the rabbit (solid line) and human (dashed line). At the ruby wavelength of 694 nm, only 5% of the radiation is absorbed before reaching the retina. After Geeraets, et al.[27]

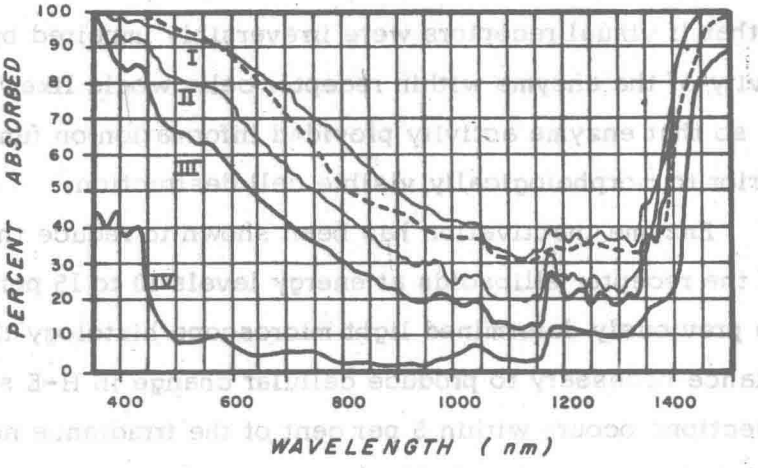
Figure 1b

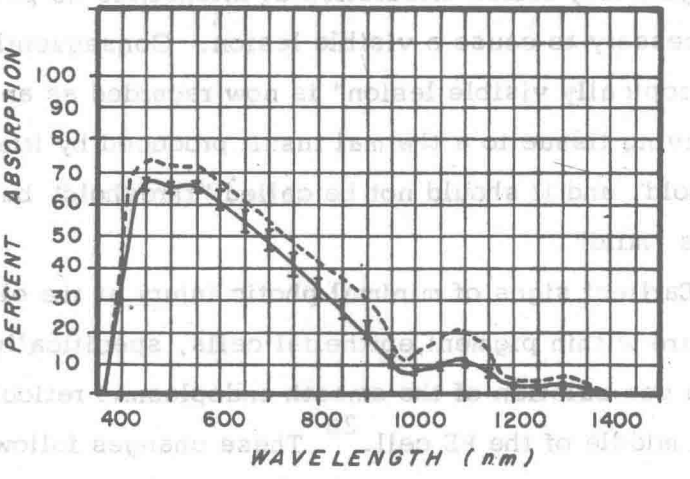
The mean percent absorption of light incident on the fundus by the retina (including pigment epithelium) and choroid. Four degrees of pigmentation of the rabbit (I - IV) and human (dashed line) fundi are presented. Group I is dark, II is medium, III is light, and IV is albino in pigment density.[27]

Fibure 1c

Mean percent absorption in the medium rabbit retina and choroid for light incident on the cornea. Dashed lines show these data uncorrected for reflection by the fundus. The solid line is corrected for reflection. Redrawn from Geeraets and Beery.[28]


TRANSMISSION PERCENT
100
90
80
70
60
50
40
30
20
10
0
400
600
800
1000
1200
1400
WAVELENGTH (nm)


PERCENT ABSORBED
100
90
80
70
60
50
40
30
20
10
0
I
II
III
IV
400
600
800
1000
1200
1400
WAVELENGTH (nm)


PERCENT ABSORPTION
100
90
80
70
60
50
40
30
20
10
400
600
800
1000
1200
1400
WAVELENGTH (nm)

noticed only when the outer nuclear layer had been disrupted. In this case the irradiance was considered "threshold" for inducing injury because it produced an ophthalmoscopically visible lesion in 50 per cent of the trials within five minutes of exposure. By 1963, however, histochemical techniques were used to define threshold burn by its mild protein coagulation;[29] the staining ability of retinal receptors increased after coagulation. The staining picture of NAD diaphorase, an enzyme widely distributed throughout the retina but concentrated in receptor ellipsoids, was examined in irradiated retinae. This technique assumed that if visual receptors were irreversibly impaired by irradiation, then activity of the enzyme within receptor cells would likewise be affected, so that enzyme activity provided information on functional damage prior to morphologically visible cell destruction.

Enzyme inactivation has been shown to reduce the staining ability of the receptor ellipsoids at energy levels 10 to 15 per cent below the previously determined light microscope histology threshold.[29] The irradiance necessary to produce cellular change in H-E stained paraffin sections occurs within 5 per cent of the irradiance necessary for the ophthalmoscopic threshold. It is likely, therefore, that irreparable functional injury may follow irradiation at intensities 20 per cent less than that necessary to cause a visible lesion. Consequently, the "ophthalmoscopically visible lesion" is now regarded as an edematous reaction of living tissue to a thermal insult produced by irradiance <u>above</u> threshold, and it should not be called "threshold" but is best referred to as "mild".

Earliest signs of minimal photic injury at the electron microscope level are within pigment epithelial cells, specifically present are fractures and vesiculation of the smooth endoplasmic reticulum lying in the apex and middle of the PE cell.[26] These changes follow a 175 micro-

second dose of 0.7 J/cm^2 on an 0.8 mm image. As the lesion becomes clinically visible (dose - 1.0 J/cm^2) a non-specific "granulation effect" in PE villi and mitochondria and in receptor cells is seen. The granules are made up of swollen and densified cell membranes and cytoplasmic ground substance. Immediate damage in these mild burns extends to the lamellated outer segments of the photoreceptors, but innermost segments vitread to the middle limiting membrane degenerate later. Choroidal layers beneath the mildly damaged zone appear morphologically normal.

C. Lesion Classification

Laser-induced suprathreshold rabbit retinal burns are roughly assigned to four categories on the basis of clinical appearance and light microscope examination.[25,30,31] A grade 1 category burn is a small grayish lesion representing the opthalmoscopically determined threshold burn of older literature. An occasional central charred point is found, surrounded by large pale area and pigmented rim. Histologically, there is dislocation of PE cells and pigment, resulting in a clumpy and uneven distribution of pigmentation. The outline of PE cell membrance and Bruch's membrane are lost, but photoreceptors may retain their structural integrity. A retinal exudate extends into the retina proper. The flow of fluorescein into the injured area is rapid, due to breakdown of capillary structure and increased capillary permeability.[32] About thirty days later, pigment from the posterior of the eye migrates into the retina, and a chorioretinitic adhesion forms.

A grade 2 injury is defined by blanching of the retina and formation of intraretinal bubbles, some of which escape into the vitreous. Flecks of pigment are accumulated in the burn, and a faint gray corona encircles the irradiated area. A microscopic section shows

marked distortion and necrosis of nuclear layers along with greater PE cell and choroid disruption and retinal exudate. Fractures through part or the entire thickness of the retina are evident. Fluorescein angiography demonstrates a slow diffusion of this indicator into the area,[32] presumably because heat coagulation occludes much of the choroidal capillary bed. Pigment cell proliferation and pigment migration proceed in the weeks that follow the insult, and a cicatricial band replaces the normal retinal structure, providing a firm chorio-retinal adhesion. A grade 2 lesion represents the optimal therapeutic coagulation.

Grade 3 lesions, too severe for clinical application, are manifest as blanching and excavation of the irradiated area. A sharp corona and vitreous gas bubbles are common and small retinal hemorrhages may occur. Pathologic findings are similar to Grade 2 but greater in magnitude. A sub-retinal exudate elevates the retina and throws it into folds extending to the retina- vitreous interface and blood and vapor bubbles enter the vitreous freely through penetrating cracks of the retina. The postinsult history follows the history of a grade 2 burn.

Grade 4 burns are similar to 3 plus gross vitreal hemorrhage and bubble formation. The lesion size is larger than the irradiated image and implosive tears are plainly evident. Choroidal blood flows through the retina into the vitreous along with retinal debris.

These lesion grades apply to both ruby and short xenon pulses of a few hundred microseconds length. Lesions produced by both sources correspond closely in gross and histopathologic features except when low ruby irradiances are applied. In this case, "hot spots" in the non-uniform ruby beam produce scattered visible pinpoint lesions.[33] Each of these resembles the usual mild lesion in histologic appearance.

Longer exposures to radiation, not possible with conventional pulsed ruby lasers but available with xenon lamp systems, produce significantly different effects on the retina than short exposures.[33] Longer pulses involve more retinal layers, and with increasing energy densities even the sclera is involved. Minimal or mild lesion sizes are equal to or slightly smaller than the beam retinal image size in microsecond exposures because the lesion is produced before peripheral heat dissipation becomes a significant factor. Clinically, longer exposure lesions are darker in color; the rim of the lesion is blurred and extends beyond the diameter of the beam image. Mild microsecond (< 1 msec) lesions cause a temperature rise in areas immediately adjacent to the PE and the diaphorase staining image is sharply defined especially in the ellipsoids of the photoreceptor. In contrast, a 30 millisecond pulse produces a less distinct picture, having blurred and poorly defined diaphorase denaturation boundaries. The differences between histologic and funduscopic features for the microsecond and millisecond exposures are explained by the increased conductance of heat peripherally from the irradiated site during longer exposures.

Retinal blood vessels of rabbits appear unaffected by grade 2 and 3 coagulation intensities, but the white medullated fibers on which they rest show slight cellular edema.[31] The vasculature is seemingly not affected by any heat produced in irradiated hemoglobin. Choroidal circulation is, as noted before, largely obliterated by heat rise in the pigmented tissues.

D. Retinal Light Absorption and Ensuing Thermal Reactions

Pigment epithelial and choroidal melanin granules become black body radiators when they absorb sufficient light energy, and transfer heat from themselves by conduction, convection, and radiation

to the nearby cellular constituents. The result is a thermal insult to the cell, and it is morphologically and functionally altered because of it.

It is a generally accepted thesis that thermal injury results from denaturation of cellular protein. Many proteins have demonstrated in vitro that their degradation by heat is a first order reaction with each protein governed by its characteristic energy of activation.[34] The degree of injury within a cell is not necessarily a function of maximum temperature attained by the cell but on how long elevated temperatures prevail, that is, the effect which temperature has on the physical or chemical properties of a protein molecule is dependent on time. This is called a rate process.[35] In any attempt to correlate the retinal histochemical, microscopic or funduscopic burn data with theories of thermal injury based on threshold temperatures for protein denaturation, the time-temperature history in the retina must be considered. This history must either be calculated in terms of a mathematical model such as the differential equation of heat flow[34-41] or it must be measured experimentally.[42-48] Then the principles of chemical kinetics and thermodynamics on which the rate process is based can be applied to describe thermal degradation of the protein molecule.