FUNCTIONAL ASSESSMENT OF LASER IRRADIATION

ANNUAL PROGRESS REPORT

JULY 1979

BY

David O. Robbins, Ph.D.
Department of Psychology
Ohio Wesleyan University
Delaware, OH 43015

SUPPORTED BY

US Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

CONTRACT No. DAMD17-75-C-5008

Approved for public release; distribution unlimited

The views, opinions, and/or findings contained in this report are those of the author and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.
FUNCTIONAL ASSESSMENT OF LASER IRRADIATION

David O. Robbins, Ph.D.

Ohio Wesleyan University
Department of Psychology
Delaware, OH 43015

US Army Medical Research & Development Command
Fort Detrick, Frederick, MD 21701

Approved for public release; distribution unlimited

The use of high-resolution, chromatic targets to assess the adverse effects of laser irradiation on rhesus visual acuity provides a much more sensitive measure of the acquired visual deficits than can be observed with achromatic targets. The use of chromatic targets, however, does not appear to indicate a high degree of selectivity in terms of the visible spectrum most affected by laser exposure. This nonselective nature of the acquired deficit was also observed in our electrophysiological studies of changes in spectral sensitivity and receptive field organization following brief and prolonged laser exposures.
## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>SECTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>METHODS</td>
<td>6</td>
</tr>
<tr>
<td>RESULTS</td>
<td>11</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>21</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>22</td>
</tr>
</tbody>
</table>

## LIST OF ILLUSTRATIONS

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>Illustration Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGURE 1</td>
<td>Optical system for the presentation of visual discriminanda and laser flashes in the behavioral studies</td>
<td>7</td>
</tr>
<tr>
<td>FIGURE 2</td>
<td>Electrical and optical system used to isolate and stimulate single neurons in the turtle optic tectum</td>
<td>10</td>
</tr>
<tr>
<td>FIGURE 3</td>
<td>Sample data of threshold acuity under maximum photopic viewing conditions using a tracking technique</td>
<td>11</td>
</tr>
<tr>
<td>FIGURE 4</td>
<td>Recovery functions derived from chromatic test targets following relatively low energy flashes from an Argon laser</td>
<td>12</td>
</tr>
<tr>
<td>FIGURE 5</td>
<td>Photographs of the receptive fields of a single optic tectal neuron to slowly moving red and green spots of light</td>
<td>14</td>
</tr>
<tr>
<td>FIGURE 6</td>
<td>Exposure of a long wavelength sensitive cell to HeNe (633 nm) laser light</td>
<td>15</td>
</tr>
</tbody>
</table>
FIGURE 7  Comparisons of the pre- and postexposure spectral sensitivity for the on-off responses of a cell following exposure to combined 514 and 633 nm coherent light ........................... 16

FIGURE 8  Receptive fields of a cell following multiple exposures to the combined outputs from an Argon (514 nm) and HeNe (633 nm) laser............ 18

FIGURE 9  Spectral sensitivity of two neurons as a function of velocity ................................. 19

FIGURE 10  Effects of exposure on the spectral sensitivity of a neuron using at various velocities .......... 20
INTRODUCTION

The applications of lasers in the military, medicine and industry have grown significantly over the last decade and further proliferation is almost certain. Associated with this increased usage is the threat of ocular damage as the result of accidental or intentional exposure to laser sources. Of equal concern is the resultant momentary or permanent change in visual sensitivity and the effects that this might have on visual performance. The goal of most of the studies on light-induced retinal changes has been to establish accurate retinal damage thresholds for types and conditions of light exposure so that necessary precautional measures can be made to protect those subject to exposure. It is important to realize, however, that any thresholds defined need to consider the quantal efficiencies of different wavelengths on the eye.

When a laser beam is incident on biological tissue, a variety of physical reactions may take place depending upon the wavelength, energy and duration of the exposure. For wavelengths in the longer region of the light spectrum, the principal effect is the absorption of incident energy and its conversion to heat. The biological organ most vulnerable to injury from the conversion of light energy to thermal energy is the eye (1). Most lasers currently being employed in the field are transmitted by the ocular media of the eye and are focussed onto the highly pigmented retina. The absorption of this energy by either the outer segments of the receptor cells or the pigment epithelium can cause an elevation in retinal temperature which often leads to morphological damage. A damage model based purely on thermal dynamics would predict that a pathological condition would occur within the retina when a given amount of heat was generated following light absorption. Studies supporting this notion have typically employed extremely high energy, short duration exposures to coherent light which lead to significant elevations of retinal temperatures over relatively short periods of time. The adverse effects of this type of intense irradiation on the eye have been realized for some time. The original studies (2) were based on solar retinitis, sun-gazing during an eclipse, although more recent studies have shown that exposure to much less intense light sources can also produce changes in retinal morphology (3,4,5). It has been demonstrated, however, that repeated low energy exposures likewise produce changes in retinal pathology and the power densities have been showed to significantly below those predicted purely from a thermal model (6). As a consequence the thermal model may not be the sole predictor of retinal alteration especially when more chronic low energy exposures are employed. Rather under these conditions, light induced effects may be nothing more than a series of cumulative alterations in the natural cyclic changes occurring within the cell to the point where the cell is no longer capable of carrying out its normal function within the system.

Retinal pathology as a result of laser and incoherent irradiation has been extensively studied in the rhesus. Using suprathreshold dosage levels, gross pathological damage has been found to exist in the cornea, pigment epithelium and in the outer segments of the photoreceptors (7-11). The
primary mechanism for this type of damage has traditionally been considered to be thermal. As the morphological techniques for detecting minimal retinal alterations have been refined, the threshold power density for such alterations has decreased. Associated with these decreases in exposure power levels is a shift in the site of maximum alteration from the pigment epithelial layer to the outer segments of the photoreceptors and the possibility for other mechanisms for damage besides the elevation of basal retinal temperature. Since the site of anatomical alteration is at the location where the initial transduction of light energy to electrochemical energy occurs, it has become important to examine not only the nature of any morphological disruptions but also the functional and electrophysiological consequences of the induced alteration as well.

In the past, three different approaches have been used to examine light-induced changes in the visual system. Historically, a structural approach was exclusively used to the MPE (minimal permissible energy) for laser exposures. Two different methods have been used to measure any structural changes; ophthalmoscopic and morphological techniques. These techniques have produced varied results ranging from the demonstration of an observable retinal opacity to more subtle disturbances in the fine appearance of the photoreceptor. While these techniques have demonstrated their sensitivity with respect to detecting thermal as well as mechanical damage mechanisms for exposures to intense light of short durations, they have not generally provided information regarding the immediate or long term changes in vision associated with exposure to moderate levels of laser light or to changes associated with repeated exposures at relatively low energies densities. Even higher resolution examination of the retina with the newer techniques of electromicroscopy have failed to reveal definite results since it has been difficult in these studies to differentiate the normal cyclic regeneration process from that induced by light stimulation beyond the physiological operating range. While the morphological approach has greatly contributed to the development of the ANSI guidelines, they have not been the most sensitive criteria to use since they are not generally sensitive to actinic insult and to subtle changes in visual performance.

A second approach has been to examine electrophysiological changes in the visual system following laser exposure. Morphological changes in the retina should be accompanied by changes in the electrical properties of visual neurons throughout the visual system. The laser as a research tool would appear especially suited for exploring the spatial and chromatic organization of visual cells. Its spectral purity and coherency should provide an especially ideal method for isolating the neural interconnections from receptors with different underlying photopigments. Laser irradiation of photoreceptors in the roach, for example, produce specific and predictable suppressions in the excitability of horizontal cell slow potentials (12,13). Furthermore, these studies suggest that the inputs of rods and cones on other retinal neurons can be differentiated following laser irradiation. At a more central level, where the receptive field geometry and mixture of receptor systems become more complex, the underlying receptor inputs may become more obscured by the spatial, temporal and chromatic channels developing later in the transmission network. The unique characteristics of laser light may interact with such higher order neurons in a more global and less spectrally specific manner. For example, measurements of visual functioning in rhesus following laser
irradiation have not generally revealed the strict wavelength specificity noted at the receptor level (14-16). Electrophysiological assessments of light-induced changes can be performed at either the cellular level (single cell recordings) or from a more global level (EEG's or ERG's). The electrophysiological approach should depict the underlying morphological and biochemical structure of the visual system and depending upon the sensitivity of the measuring devise the operation of the visual system.

Functional determinations of laser irradiation, however, are of prime concern in determining safety standards for several reasons. First, this approach may provide a more sensitive criteria for determining laser safety thresholds. Minute enzyme changes in the photoreceptors associated with low level laser exposures may not be revealed by more conventional morphological examinations but such alterations may greatly affect the overall functioning of the visual system. Second, neither morphological nor electrophysiological criteria alone tell us much about the degree or type of degradation in visual performance. Of course, changes in the ability of observers to perform visually would be of prime concern in missions where successful completion is dependent upon visual and/or visual-motor behavior.

In order to derive the greatest benefit from the laser beam and at the same time develop realistic standards for safety, accurate assessments of its adverse reaction on the human eye must be carefully examined from all three approaches. Human experimentation in the area of suprathreshold retinal burns, though, is virtually impossible, since intentional burns can only be performed on eyes that suffer from severe retinopathies or eyes which are slated for early enucleation. Since enucleations are rarely performed until substantial loss of vision has occurred, it is virtually impossible to do complete functional or electrophysiological studies on these patients (17-18). As an alternative, the rhesus has most often been chosen as the experimental model upon which human standards are to be set. The selection of the rhesus as the experimental subject stems from numerous anatomical investigations that have shown that the retinal pathology and physiology of the visual pathways in man and rhesus are quite similar. These facts suggest that the rhesus would make an excellent model for morphological investigations of laser alterations.

In previous studies, we have compared the visual functioning in rhesus and humans. This data was necessary before rhesus behavioral data could be directly employed in determining the functional consequences of laser exposures in humans. Since there is a good degree of similarity in structure between the rhesus and human visual system, there is to be expected similarities in function as well. The ability of the rhesus to resolve spatial detail and their sensitivity to color have been shown in the past to resemble that of humans in many ways. Although they are similar, recently some minor differences in the ability of the rhesus to resolve detail under different achromatic luminance conditions have been reported (19). At high luminance levels the acuity of the human observer is superior to that of the rhesus, while the rhesus has slightly better spatial resolution at lower luminance levels. These differences in effective luminances have been attributed to optical rather than to physiological factors. In a previous paper we have reported that the spectral sensitivity of more central regions in the rhesus retina resemble
more the sensitivity of the human protanomalous observer than normal human trichromats (20). In these studies spectral sensitivity was measured in both humans and rhesus monkeys using a Landolt ring acuity task with the target being presented in the center of a small screen which the subject was trained to fixate on. Using a chromatic grating pattern which did not require central fixation, Behar and Bock (21) have also reported that the long wavelength sensitivity of the rhesus is reduced relative to that of the normal human observer. Similar evidence suggestive of weak rhesus dichromacy can be observed in the data found in the literature although the magnitude of the long wavelength insensitivity was usually not great and in all cases optical rather than physiological factors have been used to explain this phenomenon. By restricting chromatic visual functioning to the central most regions of the retina rather than presenting targets spread across the entire retinal mosaic, we have represented stronger evidence for photopic protanomalogy which is more difficult to explain purely optically. Such a condition will obviously affect the light absorbing properties of the photoreceptors and, given a thermal model for retinal damage, the tolerance of the retina to long wavelength laser irradiation. Hence, corrections for species differences in terms optical as well as retinal physiology and photochemistry must be made before direct comparisons can be made of the rhesus morphological and functional laser exposure data to predicted human consequences of accidental exposures.

After a correction factor is applied to the rhesus data, minimum functional safety standards can be established for laser irradiation under different laser as well as visual performance conditions. The use of these functional assessments will then provide information not only on the morphological mechanisms but also the adverse effects of laser irradiation on performance criteria of specific missions where eye damage to personnel may occur. This deleterious effect on performance should occur at both threshold and subthreshold power densities for distinct morphological or fundoscopic damage. This functionally oriented injury criteria should also provide a sound base for the understanding of laser eye effects and the establishment of proper guidelines for human eye safety which reflect considerations of the interplay between transitory functional deficits (flash blindness) and the safety and performance criteria of specific missions. The electrophysiological data should provide additional information regarding the mechanisms behind the functional alterations and furthermore, provide an additional parameter upon which to judge both laser safety standards and evaluations of structural alterations resulting from exposure.

In the past, functional studies concerned with the effects of intense irradiation on the retina have been restricted, however, to the evaluation of severe morphological disruptions of the rhesus fovea or parafovea (22, 23). The effects of these foveal irradiation levels are usually permanent, producing impairments in visual acuity ranging from 40% to 80% of pre-exposure acuity levels. Virtually no exploration of the exposure levels at or below the transition from temporary to permanent visual losses has been conducted, since no technique has been available to expose an awake, task-oriented animal. Immediate acuity effects following intense irradiation are critical, though, in the exploration of thresholds for functional alterations and in determining the consequences of immediate degradation of visual performance on a specific mission. In all previous
functional studies, anesthesia was required for placement of retinal lesions, thereby eliminating the possibility of immediate postexposure acuity measurements for at least 24 hours. The inability to measure transient changes in acuity at threshold and subthreshold power levels for permanent alterations, as well as a means to follow the initial phases of the visual deficits elicited by suprathreshold power levels, has been a serious limitation in previous studies dealing with a functional approach to laser safety. In the initial phases of this contractual effort, a procedure was developed for producing consistent foveal exposures in awake, task-oriented animals. This procedure, along with a modification of a rapid method to measure rhesus visual acuity, has been used to determine the magnitude of the immediate deficit in acuity following laser exposure. It also has provided information regarding the time course of the recovery process for energy levels up to the transition level between temporary and permanent functional alterations (23).

The objectives of the present research program were two-fold. First, we have continued to measure the immediate deficits in visual acuity and the long term recovery processes immediately following exposure to laser irradiation in additional animals. The primary effort during the current period was to train and derive baseline measurements of pre-exposure visual acuity and contrast sensitivity in two new animals. Some preliminary exposures were begun to examine the effects of relatively large diameter spots on the retina of various power densities on the spectral acuity of the subjects immediately following exposure. Second, we have continued to measure the electrophysiological changes in the responsiveness of retinal and more central neurons following brief and prolonged exposure to laser stimulation. This research is a continuation of the efforts begun in collaboration with LAIR and involved examination of the spectral sensitivity and receptive field organization of neurons prior to and immediately following exposure.

In the behavioral phase of the protocol, initial exposures were relatively low in power density and below the threshold for either permanent functional or morphological alterations. The power of the exposure is then systematically increased over a period of time, usually a minimum of six to eight months, until recovery is no longer complete. That is, until recovery to pre-exposure baseline acuity no longer occurs either within the test session or the days which follow. Thresholds for permanent functional deficits are designated at this exposure power level, exposure duration and retinal position. Recovery functions were derived using a continuous assessment of the subject's acuity level immediately prior to and following laser irradiation. This assessment was continued after exposure until total recovery was maintained or, if above threshold for functional alteration, until the recovery process stabilized. In previous efforts in our laboratory, the size of the exposure on the retinal surface was approximately 150 microns in diameter. In the current study a significantly larger diameter exposure of 323 microns was used. The rationale for the use of larger areas of involvement was to make the results from these functional explorations more compatible with those of morphological studies. In the latter studies, large areas of involvement were employed to facilitate histological exploration. In addition, the use of larger exposure diameters in our functional studies will allow for histopathological examination of our subjects' retinas by LAIR should that prove beneficial.
METHODS

A detailed description of the behavioral paradigm and experimental facilities has been reported elsewhere \((20,23)\). Behavioral assessments of visual performance were measured in a light-tight, primate cubicle isolated from the programming equipment. Two such cubicles were used; one for training and the second for baseline sensitivity measures and exposure to the coherent light source. Both chambers were identical with exception to the addition of optics necessary to present the laser beam coaxial with the discrimination image on the screen. Except for the screen the entire test chamber was dark and all test sessions were preceded by at least 15 minutes of dark adaptation.

A rear projection screen mounted on the far wall of each cubicle subtended 3 degrees at a distance of 1 m from the subject's pupil. Dark Landolt rings against a light background were projected onto the screen using a conventional carousel projector. Background luminance and wavelength were determined by neural density and interference filters placed in the path of the light. Monochromatic backgrounds varied from 420 nm to 700 nm and all filters were calibrated on a Carey spectrophotometer. All monochromatic backgrounds were standardized in terms of quantal irradiance at the 580 nm level. The test patterns were conventional black Landolt rings. The thickness of the rings and the width of the gap that formed the critical detail was always 1/5 of the diameter of the ring. The size of gap was varied from 0.25' to 30' visual angle in 20% steps.

The animal was aligned through a physical restraint device with the center of the viewing screen. The subject's head was kept stationary during testing and exposure by four plexiglas head restraints mounted on the top of a standard primate chair. These restraints temporarily prevented movement in any direction. An opaque facemask and two 5.0 mm monocular iris diaphragms were aligned with the subject's pupils and viewing screen so that eye position could be well controlled during actual exposure to laser irradiation. All testing was done with monocular viewing.

Discrimination Task. The subjects were trained, using an avoidance paradigm, to depress a lever whenever a Landolt C was presented. Failure to depress the lever during the 3 second Landolt C trial was punished by a brief electrical shock. Lever responses during trials with completed rings also initiated a brief shock on a fixed ratio reinforcement schedule. The test objects were presented in sets of four rings that were of equal diameter. Three rings in each set were gapless, while the fourth was a Landolt C that appeared in a random position within the set.

Threshold acuity measurements were obtained by an up and down, tracking method which allowed the animal to adjust in discrete steps the size of the test target about his own threshold level. The size of the gap in the Landolt C increased following incorrect "C" responses and decreased by correct "C" detections. Lever responses on completed ring trials did not affect the size of the gap to be presented on the next series of trials. Means and standard deviations of threshold visual acuity were obtained by the use of Dixon and Massey's \((24)\) statistics for the up and down method.
During recovery from laser irradiation, the average number of completed rings relative to Landolt C's was reduced from an average of four to two in order to more rapidly track transient changes in visual acuity as a function of time after exposure. Baseline mean levels or variability have not been affected by changes in the ratio of Landolt C's to completed ring trials. Also unaffected were the number of lever responses during completed ring trials which was always very small in our subjects.

Laser System. In the previous projects, a standard 2 W Argon laser with a Krypton plasma tube was used to produce a larger diameter spot of essentially the same output wavelength as the original HeNe laser produced with a 150 micron diameter spot. During the current period an Argon plasma tube with a maximum output wavelength of 514 nm was installed in place of the Krypton tube. The major difference between the two sources was the higher output energy of the Argon tube in the intermediate wavelength region of the visible spectrum. The higher output energy in this spectral region was necessary when expanded beams were used for larger exposure sites on the retina (323 microns as opposed to 150 microns). A diagram of the entire optical system used to present the image targets and the adapting laser flash is shown in Figure 1.

Figure 1. Optical system for the presentation of visual discrimnanda and laser flashes.

The entire laser system, with exception of a focusing lens and beam splitter was mounted outside the experimental chamber. The "raw" beam passed first through a manual safety shutter and then a electronic shutter. The shutter was preprogrammed to produced a calibrated exposure
duration of 100 msec. The beam was then attenuated by neutral density filters before being diverted by a 4.5 cm diameter front surface mirror. The diverted beam entered a beam expanding telescope which produced a collimated beam of adjustable size. The expanded beam then passed into the experimental chamber and through a 1.25 diopter lens placed 85 cm in front of the subject's pupil. A 5 x 10 cm coated pellicle beam splitter was placed 5 cm in front of the 1.25 diopter lens and at the intersection of the diverging laser beam and the image beam from the carousel projector. Coaxial alignment with the line of sight was verified by noting that the reflected beam also passed through a 2 mm aperture and onto the critical feature of the target on the screen. Mounted on the opposite size of the beam splitter was a diffuser and ultrafast photodiode (HPA-4203). The output of this detector was displayed on a memory oscilloscope and was regularly calibrated against an EFF Model 580 Radiometer placed at the corneal plane. The power and pulse width of each irradiation was measured and recorded. Exposures of 100 msec duration were made at various corneal power levels, beginning with the lowest power level.

Laser exposure. Prior to any laser exposures, stable baseline acuity levels were established for each subject using both monochromatic and white light targets. Initially, a criterion of, at minimum, 14 consecutive sessions of white light threshold measurements were used to establish a baseline mean and standard deviation for each subject. Following the determination of a stable, white light threshold, the animal's spectral sensitivity (using an acuity criteria) was determined before exposure sessions began. Prior to each exposure, a 15 minute baseline session was completed and the mean for this pre-exposure testing was determined. The number of completed rings relative to incomplete rings (Landolt "C") was then reduced and comparisons made to assure a stable baseline. Failure of the subject to obtain mean acuity within one standard deviation of his pre-determined baseline level on either reinforcement schedule, aborted the session. Session variability which exceeded baseline variability also aborted the session.

Exposures were made during threshold measurements after the above performance criteria were met. The laser flash (100 msec duration) was triggered by the animal's correct detection of his threshold Landolt ring which most often corresponded to gap sizes of between 1.0 and 0.5 minutes of visual angle. The electronic shutter was immediately triggered by a microswitch on the response key. Somewhat causal observations of numerous animals working under these conditions imply that subjects maintain fixation during their response period. The results of this study thus far have shown that triggering the onset of the exposure to the subject's response elicited significant functional deficits in visual characteristic of inactivation of more central regions of the retina. Voluntary eye movements or blink during exposure were eliminated by the use of a 100 msec exposure duration. No more than one exposure was made per day and exposures were never made either following incorrect detections of Landolt C's or following correct detection during the last 1 second of the trial. Immediately after exposure, recovery was measured until the subject returned to baseline acuity levels or in the case of a permanent or semipermanent alteration until either the degree of deficit stabilized or 45 minutes whichever came first. The session was always terminated after a 15 minute stable was achieved. The entire test session lased approximately 2 hours. If acuity did not recover within a given session,
exposure was discontinued on subsequent sessions until evidence of recovery at all spectral points was achieved. The laser exposure level at which this recovery criterion failed to be achieved defined the transition zone between temporary and permanent acuity loss measured in these experiments.

Electrophysiological recording and optical system. The subjects used in this phase of the study were adult, female turtles, *Pseudemys scripta elegans*, measuring 20-25 cm in carapace length and 2-4 kg in body weight. Complete flaccid paralysis during surgery and recording of single cell potentials was induced by intramuscular injections of 1 ml tubocurarine chloride (15 mg/ml) and the animals were maintained by periodic artificial respiration. Topical anesthesia (pontocaine, 2% solution) was applied to exposed muscle tissue. The animal's head was held rigidly in a Horsley-Clark small animal stereotaxic instrument adapted for the turtle. The cranium was exposed by a mid-sagittal incision and the overlying optic tectum was exposed after retraction of the dura.

Extracellular action potentials were recorded by tungsten microelectrodes prepared according to the method of Hubel (25). Tip diameters of the sharpened tungsten electrodes were approximately 2-4 \( \mu \)m, with a passive resistance of 10-50 M ohms. Responses from isolated neurons were led a Bak unity-gain electrometer, amplified several thousand-fold by a Princeton pre-amplifier, passed through a window discriminator and spike analyzer being being displayed on an oscilloscope. The amplified signal was also fed into an Ampex analog tape recorder and to the Z axis of an X-Y plotter (pen up/down position controller) and storage oscilloscope (beam intensifier). A diagram of the electrical and optical recording system is shown in Figure 2.

The receptive fields of optic tectal neurons were mapped on a tangent, rear-projection screen representing 20 by 20 degrees visual field at a distance of 15-20 cm from the eye. Test spots used to map the receptive fields were produced by a conventional incoherent, broad-band source (6V, 18A tungsten lamp) and were scanned electronically across the screen using two mirror galvanometer for X and Y movements. Two triangular waveforms from a signal generator resulted in stimulus movements of different orientations, speeds and extents across the screen. The signals used to drive the mirror galvanometers also controlled the X and Y axes of the X-Y plotter and beam of a standard storage oscilloscope. Thus, the position of the beam on the oscilloscope and the pen on a sheet of graph paper always corresponded to the position of the stimulus on the screen. Both the intensity of the oscilloscope trace (Z axis) and the up/down position of the pen on the X-Y plotter carriage was modulated by cell activity. When the cell was responsive to light the trace brightened and the pen was automatically position downward in the writing position. Since most neurons showed little or no spontaneous activity in the dark it was not necessary to eliminate background activity and the presence of neural activity typically constituted a response of the cell to light. The result of this type of recording system was a spatial picture of the neural activity of a neuron as a function of position of the stimulus in the visual field. A Med60 signal averager was used to analyze the temporal patterning of nerve impulses for different types of visual stimulations.
The wavelength and intensity of the test spot could be varied by interference filters and a motor-driven neutral density wedge. Wavelengths across the visible spectrum in 20 nm steps were selected to measure spectral sensitivity and isolated wavelengths in the short, intermediate and long wavelength regions of the spectrum were used to plot the cell's receptive field characteristics. Laser exposure from either of two coherent sources (Argon at 514 nm or HeNe at 633 nm) or their combination was presented as a discrete 3 deg spot at the tangent screen and within various portions of the visual receptive field or was diffused over a large portion of the screen covering the entire receptive field of the neuron. Repeated exposures of either 30 or 60 minutes in duration were made allowing for measurements of the cell's spectral sensitivity and receptive field geometry between such exposures. If the cell was still responsive to light stimulation following postexposure testing, the cell was again exposed and the process repeated until the cell was either lost or no longer responsive. Control cells were isolated and held for similar periods of time without being exposed. In these cells no significant changes in spectral sensitivity, receptive field geometry, or in the strength or topography of the response pattern were noted during the recording period.

Figure 2. Electrical and optical system used to isolate and stimulate single neurons in the turtle optic tectum
Sample data of threshold acuity using the tracking technique is shown in Figure 3. The left-hand ordinate indicates the various sizes of the gaps in presented Landolt rings and is plotted in reciprocal minutes of arc; the right hand ordinate indicating Snellen acuity. The order of presentation of the Landolt rings was dependent upon the subject's response on Landolt ring trials. Incorrect detection of the Landolt C caused the recorder to plot downward and corresponded to the presentation of larger ring diameters while correct detections of the Landolt ring caused the recorder to plot upward and corresponded to the presentation of smaller acuity targets. The accuracy of detection of gapless rings had no effect on the order of presentation or the size of the acuity targets. Vertical excursions in this figure represent the occurrence of the gapless rings and the length of the excursions correspond to the number of gapless rings within the series. The abscissa, or the short horizontal excursions represents the presentation of the Landolt rings; reversals in the graph at this point represent errors in detection. In this particular situation, the animal's mean acuity was 1.25 (min of arc)-1 or approximately 20/16 Snellen acuity. The dotted lines represent one standard deviation on either side of the mean. The background of the dark Landolt ring was white and the overall illumination for this maximum contrast target was within the photopic operating level. Similar to the records from other animals the within session variability was quite low as was the between session variability for each animal. Mean visual acuity was monotonically related to the light intensity of the background over a range of six log units of intensity. When chromatic backgrounds were used, similar monotonic functions were derived although, depending upon the particular wavelength used, the slopes of the functions varied. Spectral sensitivity curves were derived from least squares lines calculated from these individual intensity-acuity functions at each spectral point. Typically, the correlation between acuity and intensity ranged from 0.95 to 0.98 except at the extremes of the visible spectrum where acuity decreased very rapidly for high background intensities and then stabilized at extremely low acuity levels for further decreases in background intensity.

Figure 3. Sample raw acuity data from one subject measured under maximum photopic viewing conditions.
Figure 4. Derived recovery functions for one animal exposed to two relatively low energy flashes from an Argon laser (0.05 and 0.10 mW). Recovery was followed using different chromatic backgrounds for the test targets.
In this phase of our project, recovery from laser exposures were typically followed using chromatic in addition to achromatic backgrounds. For each stimulus condition at least four exposures were made at each exposure power level, beginning with the lowest power densities. Three different chromatic backgrounds all equated for brightness were used to derive spectral acuity before and immediately following exposure. Several different overall brightness levels were also employed. The addition of these additional stimulus parameters for each power density greatly reduced the speed at which the power densities were systematically increased. Figure 4 represents for one animal the derived recovery functions using both chromatic and achromatic backgrounds all equated in terms of brightness. Similar to what was previously observed for achromatic backgrounds, immediately following exposure, acuity decreased and then gradually increased to its pre-exposure level when laser exposures were made with low power densities. The duration of the initial maximum deficit immediately following exposure was independent of both exposure power and the background wavelength used to measure visual acuity. The duration of both the initial deficit as well as the total time required for full recovery, however, was systematically related to the energy of the exposure and the type of background wavelength used to follow visual recovery. The more intense the laser exposure, the longer the time the animal's acuity remained depressed before the recovery process began. Although the difference was not significant, generally it took longer for the animal to regain his pre-exposure acuity level when long wavelength backgrounds were used to measure visual acuity then when intermediate or short wavelengths were used. In the examples presented in Figure 4 (A and B) two different exposure power densities are shown for an Argon (514 nm) exposure which produced a 323 micron diameter spot on the central fovea. In both cases recovery was typically longer for the 640 nm background then for the achromatic (000), 560 nm or 480 nm even though all chromatic backgrounds were equated for equal brightness.

Thus far we have examined the spectral sensitivity and receptive field organization of 137 neurons in the superficial layers of the turtle optic tectum. The primary purpose of these recordings was to investigate what effects laser light had on photopigments and the generation of retinal potentials. The absolute levels of irradiation over which changes in cellular activity could be elicited were from $10^{-10}$ to $10^{-8} \text{ log quanta/sec/cm}^2$ for durations from 2 minutes to 2 hours. At the light microscopy level, no alteration of retinal tissue was observed with the maximum dosage level used.

In the majority of cells isolated, both the on and off portions of the response pattern were maximally sensitive to the long wavelength region of the spectrum. The receptive field organization of these cells prior to exposure demonstrated no clear center-surround relationship. Most fields were dominated in or near the center by areas which produced vigorous on-off response patterns regardless of the wavelength of the test target. On-off regions were often not continuous but were separated from one another by either pure on or off regions and hence appeared more complex than the simple center-surround, spectrally opponent receptive fields reported elsewhere. One cell that did demonstrate a clear center-surround organization with spectrally opponency is shown in Figure 5. The upper photograph taken from a storage oscilloscope is a plot of the receptive field of a tectal neuron to a slowly moving 10 spot of red light. The
The procedure used to plot this receptive field is similar to that described in the methods sections. Each light spot on the photograph represents a discharge of the cell to light presented on a corresponding point on the screen in front of the animal. Multiple discharges to such stimulation are shown as continuous lines the length and brightness of which provided some indication of response magnitude. In the lower photograph, the receptive field of this same cell is represented by a slowly moving green spot of light equated for equal energy. In these examples the screen provided a visual field of approximately 70 x 60 deg while the size of the receptive field shown here was 10 deg for the center and 16 deg across the surround. In this particular figure the light was scanned only in the horizontal direction and cellular responses occurred only when the light entered or left a responsive zone. Vertical scanning across this same field with a slowly moving green spot created an area of responsiveness shifted 90 deg from that shown in the lower portion of the picture. As previously mentioned, the majority of cells did not demonstrate zones of differentiated sensitivity and response patterns.

The overall effect of laser irradiation on tectal neurons was to uniformly reduce the cell's sensitivity across its entire visible spectrum. Generally, the more intense the exposure, regardless of its wavelength,
the stronger its effect on the responsiveness of the cell. Comparable effects could be produced by increasing the duration of lesser intense exposures or by presenting the less intense exposures multiple times separated by periods in excess of 60 minutes. An example of the nonselective effects of laser irradiation on spectral sensitivity is shown in Figure 6. This cell was exposed three times to $10^{-4}$ log/quanta/sec/cm$^2$ for 60 minutes each. Following the third and final exposure, the cell's receptive field shrunk considerably as did the cell's overall sensitivity and eventually became totally unresponsive to any form of light stimulation. The preexposure spectral sensitivity as well as the sensitivity following the first and third 60 minute exposure are shown in this figure. The HeNe (633 nm) exposures covered the entire receptive

![Figure 6](image-url)

**Figure 6.** Exposure of a long wavelength sensitive cell to 633 nm laser light. The upper graph represents the spectral sensitivity of the on portion and the lower graph the sensitivity of the off portion of the response.
field and produced vigorous activity when it was first presented and later terminated. In the interim the cell was inactive. In this cell the on portion of the response pattern peaked in two different regions of the spectrum, 500 nm and 600 nm. Following 633 nm laser exposure, a rather uniform 0.5 log unit loss in sensitivity was observed across the visible

![Graph showing ON and OFF responses for a cell following exposure to combined 514 and 633 nm coherent light.](image)

**Figure 7.** Comparison of the pre- and postexposure spectral sensitivity for the on-off responses of a cell following exposure to combined 514 and 633 nm coherent light. The combined outputs from the two separate lasers were equated in terms of energy and were equal to each of the single exposures shown in the previous figure (10 quanta/sec/cm²).
spectrum. For the off portion of the response pattern, initial deficits were maximum in the long wavelength region but then this portion of the response pattern was relatively insensitive to short and intermediate wavelengths prior to any exposure. Had the off response been as sensitive to these spectral regions as the on response was, similar uniform reductions across the visible spectrum may have also been elicited. This was certainly true in those cells where the on and off responses were more equally sensitive to different spectral regions. Cells with peak sensitivities in regions of the spectrum other than the long wavelength region also were affected by long wavelength laser exposure. Here again, the exposures did little to shift the cell's preexposure sensitivity. Rather, these exposures outside the cell's maximum sensitive area produced only uniform depressions in the cell's overall spectral sensitivity.

Exposure of cells to 514 nm laser light produced somewhat more selective effects in the intermediate spectral region but here again depressions across the entire spectrum were obvious. Initial losses in sensitivity to low level Argon light were primarily produced in the intermediate and short wavelength regions of the spectrum. With repeated exposures these losses became less spectrally specific.

When combined exposures were made at 633 nm and 514 nm at relatively low power levels, the effects on the cell were much more dramatic than when either of the two wavelengths, equated in energy, were presented alone. In Figure 7 a cell was exposed to the combined outputs of an Argon and HeNe laser for 60 minutes. The exposure was uniformly spread across the cell's receptive field. After the first exposure, the cell's receptive field shrunk significantly as did its overall responsiveness to any type of light stimulation. As seen in Figure 6, the reductions were not limited or necessarily maximum in those regions of the spectrum corresponding to the peak output of the exposure sources or the maximum absorption of the underlying photopigments.

The effects of laser irradiation of the center only, periphery only, or the entire receptive field produced a similar overall constriction of the field. Most often, immediately following exposure, the overall size of the field was reduced from the periphery inward with the loss of either on or off regions accounting for the shrinkage. Plots of the receptive field of a cell following each of four combined 514 and 633 nm laser exposures are shown in Figure 8. Each exposure was for 30 minutes at a power level of 10 \textit{llog} quanta/sec/cm\textsuperscript{2}. The cell was exposed four separate times. Initially, exposures produced no reduction in the size of the plotted field with perhaps even some expansion of the field diameter to some stimulus wavelengths. With subsequent exposures, however, the dimensions of the field to all wavelengths shrunk until the cell was no longer responsive to light stimulation anywhere within the visual field. The enhancement in the size of the receptive field did not correspond to any general increase in sensitivity or spike activity anywhere within the receptive field. The extent and direction of either the expansion or the eventual collapse of the field also did not appear systematically related either to the type of cell isolated or to the nature of the conditions surrounding the exposure.

In collaboration with Dr. Harry Zwick at Letterman Army Institute of Research in San Francisco, we have also begun to study the effects of
Figure 8. Receptive fields of a cell following multiple exposures to the combined outputs from an Argon (514 nm) and a HeNe (633 nm) laser. Each receptive field plot was derived with a different wavelength target scanned slowly across the tangent screen in various orientations. The response pattern for various portions within the field was determined by a stationary flash presented on the tangent screen. Posts 1, 2, 3, and 4 represent the 4 different 30 minute exposures which were separated from each other by 60 minutes.
movement (velocity) on the responsive and sensitivity of neurons in the turtle optic tectum. Movement sensitivity would appear to be a very appropriate measure since the majority of cells at this level of the nervous system are highly sensitive to the direction of light movement. In some cells, the directional sensitivity is complete; that is, these cells will respond only to movement in the preferred direction and not in the null direction. Spectral sensitivity curves for two neurons derived using velocity as the criterion are shown in Figure 9. For the cell in

![Graph showing spectral sensitivity as a function of velocity]

**Figure 9.** Spectral sensitivity of two neurons as a function of velocity.
the upper portion of the figure, derived spectral sensitivity using relatively slow moving stimulus was reminiscent of those derived using a static target. This neuron showed maximum spectral sensitivity in the long wavelength region of the spectrum with a possibly secondary peak in the region of 480 to 520 nm. As the velocity of the target was increased, the overall sensitivity decreased with somewhat greater loss in the intermediate and short wavelength regions of the spectrum. The loss in the short end of the spectrum was more pronounced for the cell in the lower portion of the figure when velocity was increased. In this neuron, peak spectral sensitivity shifted from the short wavelength region to the intermediate and long wavelength with increasing velocity.

The effects of laser exposure on moving targets of various velocities is shown for one cell in Figure 10. For both static and slower velocities, spectral sensitivity in the long end of the spectrum was still measurable after 2 exposures. For the faster velocities, post-exposure measurements after the second exposure was not possible.

Figure 10. Effects of laser exposure on the spectral sensitivity of a neuron for various velocity targets.
DISCUSSION

In these studies we have shown that chromatic acuity targets provide a much lower threshold value for functional alteration than do achromatic targets. However, the use of chromatic targets do not appear to indicate a high degree of selectivity in terms of the visible spectrum most affected by the monochromatic laser exposure. For example, exposure of the retina to Argon (514 nm) irradiation does not necessarily increase the recovery time for targets of intermediate wavelengths as opposed to targets of short and long wavelengths. Recovery times were more dependent upon the energy densities of the exposure then the type of acuity target used to measure visual performance. Likewise, the degree of decrement in visual acuity was more a function the size of the exposure on the retina (perhaps area of involvement) than the type of stimulus used to explore visual sensitivities.

The nonselective nature of the deficit elicited by laser irradiation of various portions of a neuron's receptive field might be expected if a single type of receptor input could be postulated. Three different types of cone photopigments with peak absorptions at 440, 518 and 620 nm, however, are known to exist in the turtle (26). Evidence for the neural convergence of these separate cone processes beginning at the retinal level has also been reported (27). Furthermore, the shape and breadth of our spectral sensitivity curves suggest a convergence rather than direct transmission of the separate cone mechanisms. Little difference was noted in the type of deficit in spectral sensitivity elicited by laser irradiation for different output wavelengths or power densities, although when multiple output spectral lines were combined, the effect on the cell was much more dramatic than when either spectral line alone was presented.

Changes in receptive field configurations following laser irradiation also demonstrated a somewhat non-selective effect. Typically exposure anywhere within the field produced either an expansion or constriction depending upon the energy and duration of the exposure. As reported, with less intense exposures, the initial response of the cell was an expansion of the field diameter followed subsequently by a gradual reduction of the field diameter with further or prolonged exposures. These studies would suggest that the value of the laser as an analytical tool for the separation of neural channels may be no greater than that of any other intense monochromatic source. In fact, our results suggest that either the coherency or the narrow bandwidth of laser light may artificially elicit activity not commonly observed with more traditional incoherent light sources and may overdrive the neural transmission system. Combined coherent light from different spectral regions decreased the energy necessary for such an overload, suggesting that the addition of a second channel is more effective than a single channel alone. These results might suggest that the potential value of coherent light for visual system analysis is in the elicitation and possible unraveling of complex neural integrations resident in the higher visual pathways. They also raise caution for persons working around lasers with multiple wavelength outputs and the possibility that in these cases the MPE developed for a single laser system based totally on power density may be inadequate.
REFERENCES


END

FILMED

DTIC