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FUNCTIONAL ASSESSMENT OF LASER IRRADIATION(U) OHIO  
WESLEYAN UNIV DELAWARE DEPT OF PSYCHOLOGY D O ROBBINS  
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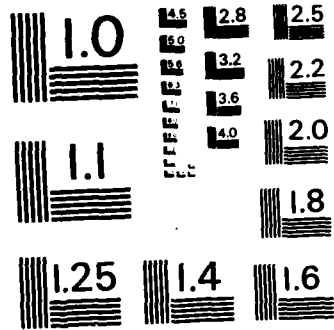
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Functional Assessment of Laser Irradiation

Annual Report

June 1978

by

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## INTRODUCTION

The adverse effects of intense incoherent irradiation on the eye have been realized for some time. Structural and functional alterations have historically been studied in experimental animals exposed to suprathreshold levels of white light and to humans either exposed accidentally or exposed as part of a therapy or research program in eyes where a severe retinal pathology already existed. Safety standards have been developed from the data collected in these studies, using primarily morphological and ophthalmological criteria, to protect an observer from accidental exposures to irradiation levels believed hazardous. In recent years these standards have been extended to include other types of visible irradiation including lasers. The optical coherency of laser light is, however, a unique characteristic of this type of light source and this factor has not been generally considered in determining ocular hazards. Recent investigations suggest that more prolonged and significant losses in visual sensitivity may result from prolonged viewing of laser light than from comparable viewing of incoherent sources at the same peak wavelength and energy (1, 2). During the current reporting period, we have compared coherent and either incoherent light or time-averaged coherent light with respect to the capability of these sources to produce prolonged changes in the visual apparatus. We have attempted to investigate the coherency factor using electrophysiological and behavioral assessments of light induced alterations of visual functioning.

During seven of the past twelve months of this contractual period of contract No. DAMD-17-C-5008, the effort was expended at Letterman Army Institute of Research (LAIR), Division of Non-Ionizing Radiation in San Francisco, CA. in accordance to the proposal submitted and approved for this contractual period. The remaining five months of effort were conducted at Ohio Wesleyan University, Delaware, OH. The collaborative effort conducted at LAIR involved developing an electrophysiological correlate to our behavioral analyses of the adverse effects of intense, laser irradiation on the visual system. In addition, technical discussions were held daily with the staff at LAIR during this time period regarding the nature and significance of past data collected in this and other research efforts. Discussions were also directed toward the most fruitful approaches to take in the future in deriving the optimal safety criteria for permissible exposure energies and durations to various lines of coherent, laser light. The remaining five months of this effort involved training new subjects at Ohio Wesleyan to perform the required discrimination task. In addition, the electrophysiological studies begun at LAIR were continued in our laboratories at Ohio Wesleyan.

In order to derive the greatest benefit from the laser in the field and at the same time protect personnel and missions against the adverse consequences of retinal alterations, an accurate assessment of the overall effects of intense irradiation on the human visual system and perceptual system must be carefully examined. It has been the goal of this research project to attempt to correlate many of the past approaches - morphological, electrophysiological, ophthalmological, and behavioral - into a more unified approach to the problem of laser safety. Obviously no experiment or series of experiments can include all of the necessary exposure parameters

to cover all of the possible situations where accidental disruptions in visual performance might occur. Rather a series of key stimulus dimensions are used to depict the underlying neurological and morphological mechanisms which can alter visual performance and from this, one can propose the modification or establishment of new laser safety standards. As this and other projects have shown, the sole use of a fundoscopic criterion, or for that matter any other single criterion, for assessing the deleterious effects of laser irradiation is inadequate in producing realistic standards which will adequately protect personnel in the field without jeopardizing the usefulness of the laser. What is needed is the correlation of data from many different approaches each with their own unique sensitivities of the damage process.

It was one of the specific intentions of the current effort to take a subject (rhesus monkey) exposed to suprathreshold levels of irradiation under the self-exposure conditions and energy levels derived at Ohio Wesleyan (3) and transfer the subject to LAIR, where continued post-exposure behavioral, electrophysiological, and ophthalmological examinations at LAIR could be made using their unique assessment conditions. Unfortunately approximately three weeks after our subject's arrival at LAIR, the animal contracted an acute, fatal gastrointestinal disorder and died before treatment could be administered. In place of this animal, parallel experiments were conducted at LAIR in behaviorally naive subjects, who were exposed to irradiation levels comparable to those experienced by our animals and ophthalmological examinations made. At the exposure levels which produced a permanent alteration in spectral acuity (6.0 mW) no distinct ophthalmologic lesion could be seen.

The electrophysiological assessment of retinal alteration consisted of both gross retinal recordings (ERG) and single cell recordings in the central nervous system. Minute morphological disruptions at the retinal level as a result of low-level, laser exposure will affect not only the organization and activity of retinal neurons as reflected in the electroretinogram (ERG) but will also affect the organization and functional properties of individual cells located more centrally in the visual system. These central cells receive their electrochemical inputs from photoreceptors each being located at slightly different positions on the retinal surface and with pigments having different absorption characteristics. Specifically, the receptive field organization of central cells is organized into spatially separate and spectrally opponent areas. The disruption of photopigments and/or electrical responsiveness of any photoreceptor cell by laser irradiation should be able to be depicted at a central level by changes in the shape of a cell's receptive field as well as shifts in the degree of antagonism between the two spatially and spectrally opponent areas. This analysis, since it is based on the electrical responses of a very limited number of spectrally opponent photoreceptors, may eventually lead to one of the most sensitive assessments of minimal laser "damage" once it becomes correlated with the other assessment techniques currently being employed. Furthermore, this electrophysiological approach should better delineate the mechanism

behind the alterations in visual performance and correlate with our behavioral data.

## METHODS

A lock-in amplifier technique was used at LAIR to measure spectral sensitivity for low voltage ERG criteria (0.5 uv rms). A conventional maxwellian viewing system was employed with a test channel subtending 42 degrees and a background channel of 55 degrees. The background channel was comprised of one of three systems: a CW coherent dye laser source, a filtered incoherent tungsten filament source, or a time averaged CW dye laser source. Peak wavelength was 620 nm for all sources and retinal irradiance was  $10^{15}$  quanta/sec/cm<sup>2</sup>. Coherent light was time-averaged by successively diffusing it through a vibrating and stationary diffuser. This procedure perceptibly eliminated the laser speckle by averaging, but did not eliminate speckle for the instantaneous case.

The technique for recording extracellular tectal activity in *Pseudemys* has previously been described (4). The receptive fields were mapped on a tangent screen, and spectral sensitivity was measured at various locations within the receptive field. Test spots used to map the receptive field were produced from an incoherent broad band source filtered with 10 nm narrow pass filters. These test spots, equated for quantal flux, subtended 3 degrees at the tangent screen and were scanned electronically across the screen. Spectral sensitivities were determined for specific loci within the field using a threshold titration procedure for each component of the response pattern. Light from a HeNe laser (633 nm) was presented either as a discrete 3 degree spot at a tangent screen, or was diffused over a large portion of the screen covering the entire receptive field of the cell. In this latter exposure condition, the diffuser could be either stationary or vibrated at 60 Hz, eliminating any perceptible laser speckle at the screen (5). The retinal irradiance produced by the laser exposure was varied from  $10^{14}$  quanta/sec/cm<sup>2</sup> to  $10^{10}$  quanta/sec/cm<sup>2</sup> ( $20\mu\text{W}/\text{cm}^2$  to  $0.02\ \mu\text{W}/\text{cm}^2$ ).

The training procedure used during the remaining period of this contractual effort has been the subject of previous reports (3,6) and will be only briefly summarized in this report. Subjects were trained, using negative reinforcement, to press a lever whenever a Landolt C was presented and not to respond when gapless rings were presented. If the subject failed to respond to a Landolt C during the 2 second presentation, he received a brief electric shock which was annoying but not highly painful or dangerous. To discourage responding indiscriminately to all rings, every third lever press during gapless ring trials was also punished. Threshold acuity measurements were obtained by a tracking method which allowed the subject to adjust in discrete steps the size of the test object about his threshold. All testing was performed under monocular viewing conditions. The test patterns were conventional black Landolt rings presented against a light background. These test objects were projected onto a high-resolution rear-projection screen that subtended  $3 \times 3^\circ$  at a distance of 1 m from the subject's pupil. The

size of the gap could be varied from 0.25' to 30' visual angle in equal steps and each test target was presented in a set of three gapless rings of equal diameter. The light background was varied in intensity and wavelength by neutral density and interference filters placed in the optical pathway. Following training, stable spectral acuity levels are established for each subject. A minimum criterion of 14 consecutive sessions of threshold measurements at each wavelength is used to establish a mean and standard deviation spectral acuity level for each subject. Once pre-exposure levels are established, the subject is exposed to brief flashes of coherent light of increasing power densities. Only one exposure is given per session and a repeated measures design is employed for both background wavelength of the test target and irradiation level.

## RESULTS

Figure 1 shows the changes in receptive field size and spectral sensitivity for on and off responses of one cell immediately following a two-minute, coherent, 3 degree exposure in the center of this cell's receptive field (retinal quantal irradiance of  $10^{14}$  quanta/sec/cm<sup>2</sup>). Spectral sensitivity was determined separately for the on and off portions of the response pattern using a one second flash presented in the center of the field. Pre-exposure sensitivity for the on portion peaked in the long wavelength region and in the intermediate and short wavelength region for the off portion. No on response could be elicited by intermediate or short wavelength light prior to exposure. An off response was elicited by all wavelengths presented. Significant depressions in sensitivity were noted throughout the visible spectrum for the off and, to a lesser extent, in the longer wavelength region for the on component following exposure. Postexposure spectral sensitivity remained depressed for the next two hours. In contrast to the pre-exposure state, the off response following exposure could only be elicited by intermediate wavelengths. Receptive fields for different wavelengths were measured using both stationary and scanning test targets. Prior to exposure the fields were rather restricted with the on field being larger for longer wavelength stimuli and the off field larger for shorter wavelength stimuli. Constriction of the receptive field for the on, off, and on-off response patterns occurred immediately after exposure and also did not recover with time. Similar depressions in sensitivity and constrictions in receptive fields were noted in other cells for quantal irradiances as low as  $10^{11}$  quanta/sec/cm<sup>2</sup> presented either as a discrete spot or diffused over the entire receptive field.

In Figure 2, the effect of a one-hour exposure diffused over a cell's entire receptive field is shown. Quantal retinal irradiance was  $10^{11}$  quanta/sec/cm<sup>2</sup>. Spectral sensitivities determined for both the on and off response patterns in the center of the field were similar prior to exposure with a  $\lambda$  max near 460 nm. On and off responses were elicited across the entire visible spectrum. Immediately following exposure the on portion was maximally affected in the intermediate and short wavelength regions while the off portion was maximally affected in the long and short regions of the visible spectrum. With time, the sensitivity of both portions of the response pattern continued to decrease until no response at all could be elicited to the maximum amount of incoherent light available in our system (two hours postexposure). The receptive field of this cell, prior to exposure, showed a nearly total overlap for the on and

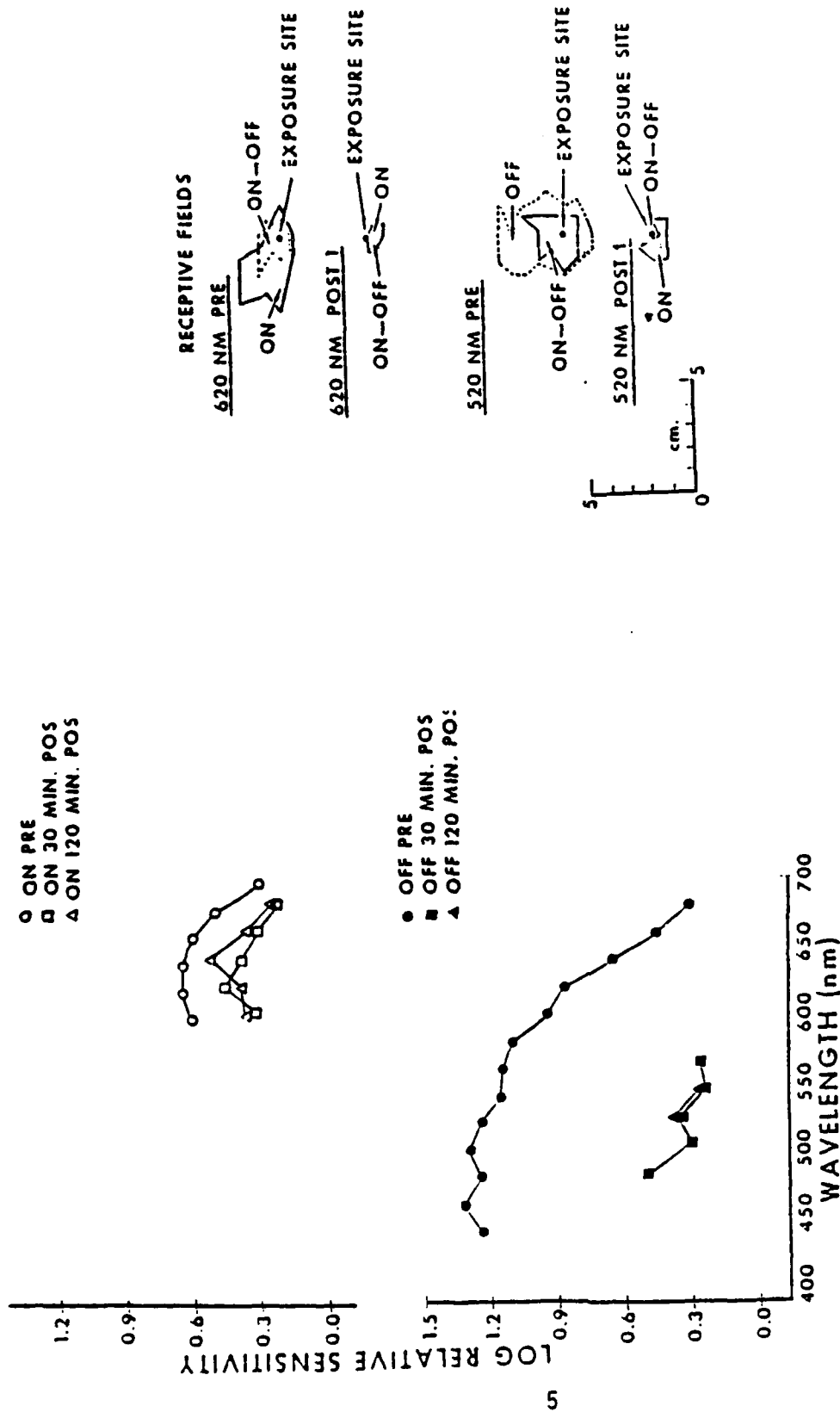


Figure 1. Comparison of pre- and postexposure spectral sensitivity and receptive field size for discrete (3 degree spot) laser exposure (2 minutes) made at the center of this cell's receptive field. Immediately following exposure at 1014 quanta/sec/cm<sup>2</sup>, spectral sensitivity for both the on and off components became significantly depressed. No recovery was obtainable in measurements made as long as 120 minute postexposure. Receptive fields measured with both the 620 and 520 nm test stimuli constricted for all response components.

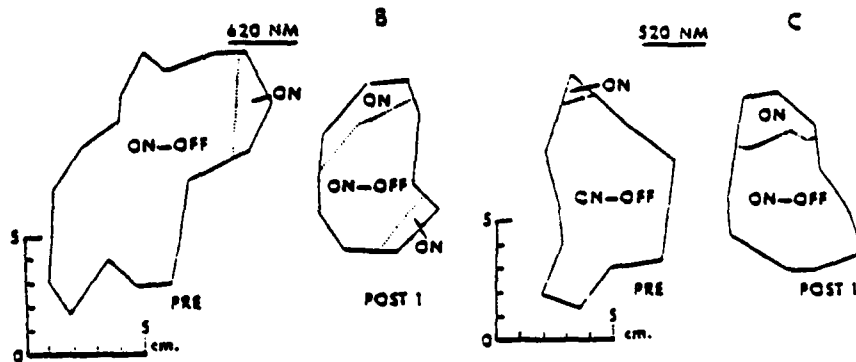
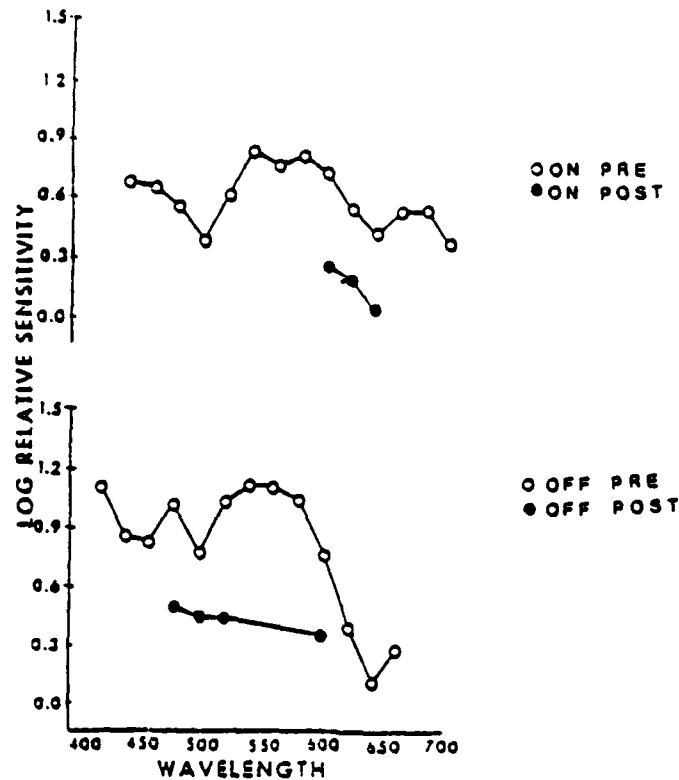


Figure 2. This cell was exposed for one hour over its entire receptive field at  $10^{11}$  quanta/sec/cm<sup>2</sup>. Post-exposure spectral sensitivities for both the on (upper portion) and off (lower portion) components decreased significantly within the first two hours postexposure and were no longer measurable after two hours. Receptive fields constricted and by the end of two hours were no longer measurable.

off regions to 620 nm and 520 nm light. Following exposure, the field to both portions of the response pattern gradually and uniformly constricted until it also was no longer measurable.

In the upper portion of Figure 3 (A), pre- and postexposure spectral sensitivity is shown for the on and off responses of a cell exposed to a 15 minute exposure diffused over the entire receptive field. Quantal retinal irradiance was  $10^{11}$  quanta/sec/cm<sup>2</sup>. Pre-exposure spectral sensitivity for the on and off response patterns of this cell was similar to that shown in Figure 1 and following a lower-energy but longer-duration exposure, a similar although not as severe a depression in spectral sensitivity was noted. The cell in the lower portion of this figure (3B) was exposed to the same retinal energy ( $10^{11}$  quanta/sec/cm<sup>2</sup>) and duration (15 minutes) as the cell in the upper portion but the exposure was time-averaged so that perceptible speckle was eliminated. The effect, if any, on the on and off spectral sensitivities of this cell and two others was significantly reduced. No continuous postexposure deterioration in either spectral sensitivity or receptive field organization was observed and in general these cells behaved much like non-irradiated ones held for comparable periods of time.

In several cells exposed over their entire receptive field to lower energy, coherent light, and actual enhancement in sensitivity was observed for either the on or off component. Such effects in one cell are shown in the upper portion of Figure 4 for the off but not the on portion of the response pattern. After a single 15 minute,  $10^{10}$  quanta/sec/cm<sup>2</sup> exposure, the sensitivity of the off component increased while that of the on component decreased. After a second exposure to the same energy one hour after the first, the enhancement effect was somewhat reduced. The sensitivity and receptive field topography was followed for another 24 hours without any additional exposures. Twenty-four hours later, the spectral sensitivity of the off component was nearly identical to its pre-exposure level while the on component was virtually lost.

Control cells which were not irradiated were readily held for periods in excess of 24 hours with no significant change in spectral sensitivity, receptive field topography, or dimensions. In Figure 5, the sensitivity of a nonexposed cell was followed for 24 hours. The sensitivity of both portions of the response pattern for this cell were similar and neither the absolute or relative sensitivity of the cell shifted significantly with time.

Electroretinograms (ERG) were measured by the staff at LAIR on Pseudemys and the comparative effects of chromatic adaptation at 620 nm is shown in Figure 6. The 620 nm chromatically adapted function for an incoherent background shows three well defined peaks in the short (470 nm), intermediate (540 nm), and long (640 nm) wavelength regions. Comparable peaks in the short and long wavelength regions are not observed for the coherent chromatically adapted function. Only a peak in the intermediate (540 nm) spectral region is present for this exposure condition.

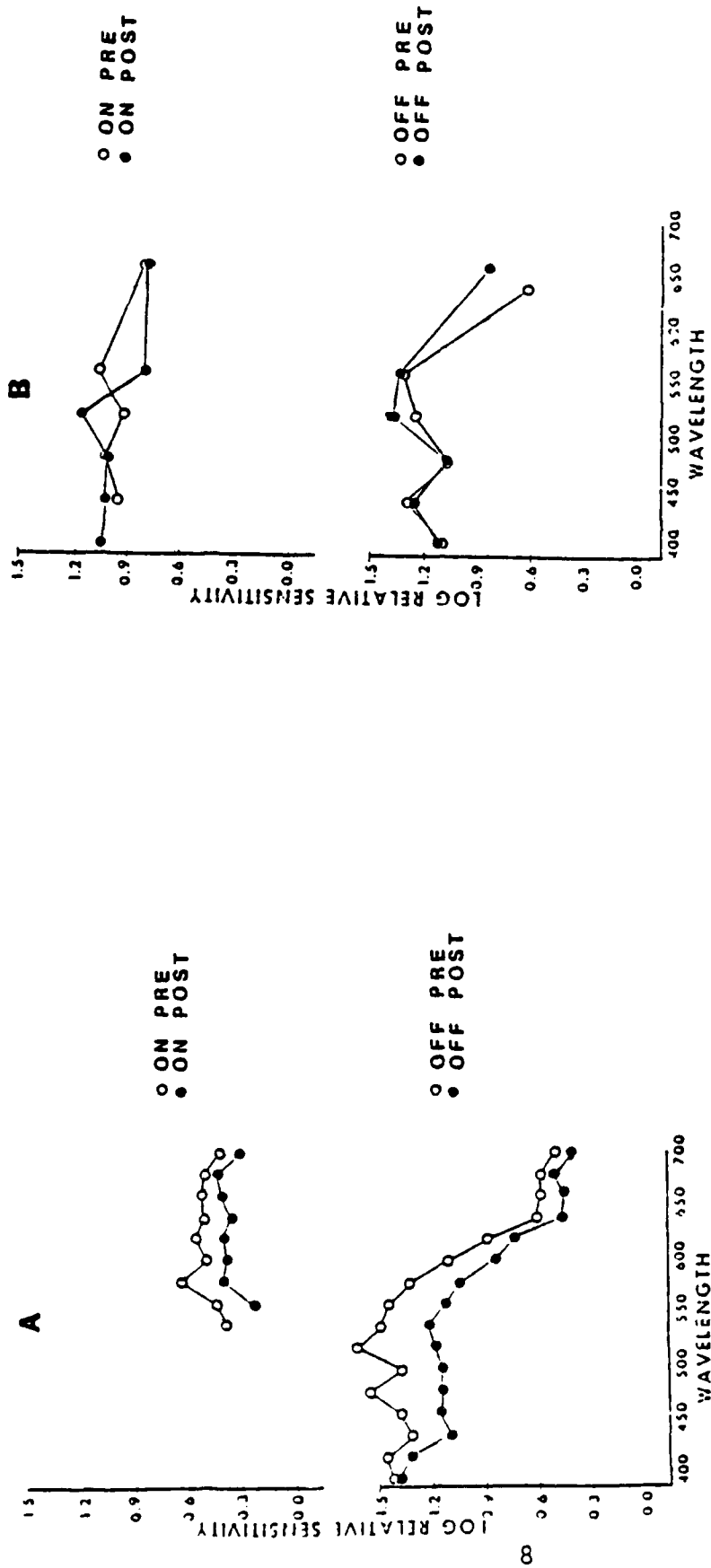


Figure 3. 3A. Comparison of pre- and postexposure spectral sensitivity for a 15-minute exposure diffused over the cell's entire receptive field at a quantal irradiance of  $10^{11}$  quanta/sec/cm<sup>2</sup>. Recovery in sensitivity was not achieved for measurements made over several hours postexposure. Constriction of receptive fields similar to that shown above was typical of this cell and others similarly exposed.

3B. This cell received time-averaged diffuse exposure over its entire receptive field at the same quantal irradiance ( $10^{11}$  quanta/sec/cm<sup>2</sup>) and exposure duration (15 minutes) as the cell shown in the upper portion of this figure (3A). Unlike changes obtained in 3A, observed changes are small with no evidence of delayed change or constriction of receptive fields.

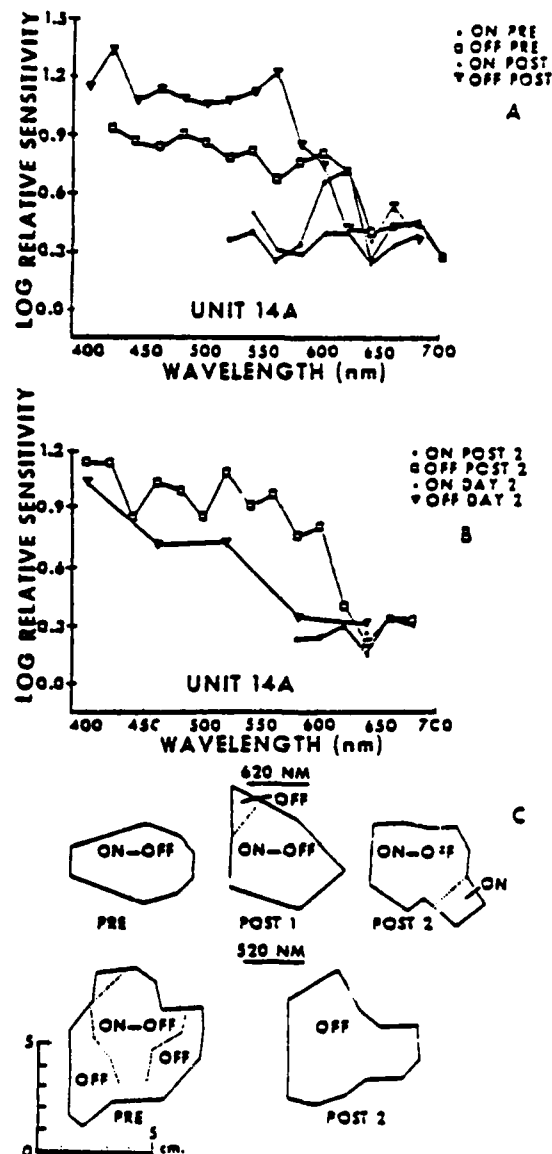


Figure 4. This cell was irradiated at  $10^{10}$  quanta/sec/cm<sup>2</sup> for two successive 15 minute exposures over its entire receptive field. After the first such exposure, sensitivity of the off component was enhanced to all wavelengths below 600 nm, while the sensitivity of the measurable on component decreased to all wavelengths beyond 560 nm. A second exposure to the same cell reduced the sensitivity of the off component to near its preexposure level while further reducing the sensitivity of the on component. The cell was kept into a second day and these measurements suggest a gradual decline in sensitivity especially for the on component, typical of other cells exposed at this level over multiple or longer exposure durations. Receptive field changes revealed little overall changes in receptive field size with the exception of the response topography for the 520 nm test field. Receptive fields eventually constricted in a manner similar to cells irradiated at higher levels.

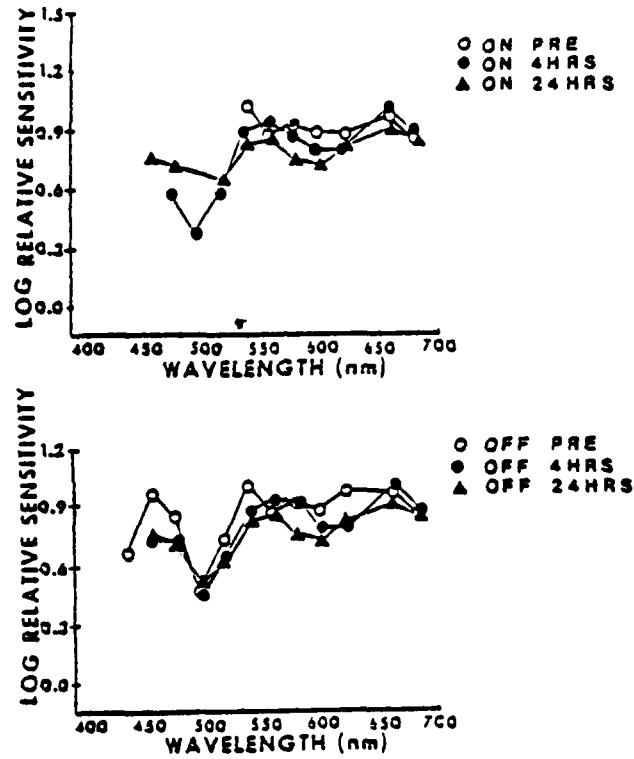


Figure 5. This cell was not exposed to laser radiation. Measurements of its spectral sensitivity over 24 hours showed no significant shifts in peak spectral sensitivity.

While these measurements were made after about 1 hour of exposure in the presence of the backgrounds, progressive loss in the short and long wavelength region were evident in the coherent function beginning after 15 minutes of total exposure. No subsequent or progressive changes were evident with the incoherent background.

When the backgrounds were terminated after 30 minutes, postexposure spectral sensitivity for the incoherently adapted function quickly returned to within the noise level of baseline preexposure measurements, but postexposure spectral sensitivity for the coherently adapted function failed to return to baseline within several hours after exposure and showed a permanent, somewhat uniform depression throughout the spectrum. (Figure 6B)

In Figure 7, the chromatically adapted ERG function and postexposure ERG function for the time-averaged coherent background are shown for an exposure equal in energy to that shown in Figure 6A. Time-averaging failed to eliminate the peak in the short wavelength region nor did it eliminate the presence of a long wavelength peak. The chromatically adapted, time-averaged function was more similar to the incoherent than coherent function shown in Figure 6B. The postexposure sensitivity function shows some loss in sensitivity through the spectrum, but not as significant a loss as that seen for coherent light exposure.

In our behavioral studies at Ohio Wesleyan we have begun training one naive rhesus monkey and one previously tested animal to perform our visual discrimination task. The new animal is quickly learning the task and his performance level on the training series of Landolt rings (all of equal size) should be complete within the next three weeks. Following this phase of the training procedure the animal will be transferred to the testing series of Landolt rings (rings of decreasing ring diameters) and baseline, preexposure spectral acuity measured. These measurements should be completed within the next several months. The animal will then be exposed to brief flashes of Argon irradiation and the recovery functions measured for energies of increasing power levels. The second animal currently has an unacceptably high false positive rate (responding to both gapless rings and Landolt rings as Landolt rings) and various reinforcement contingencies are being employed to shape this animal's behavior. Thus far, we have been unable to decrease the false positive rate without also significantly decreasing the per cent correct detection of Landolt rings.

An Argon plasma tube has been ordered for our Model 164 Ion laser (Spectra-Physics) to replace the current Krypton tube. Following its delivery, the new tube will be installed and the laser system aligned in accordance with our exposure paradigm (3,7).

## Discussion

In the electrophysiological experiments we have shown that coherent light (633 nm) can severely alter the receptive field and spectral sensitivities of optic tectal cells of *Pseudemys*. The range of retinal

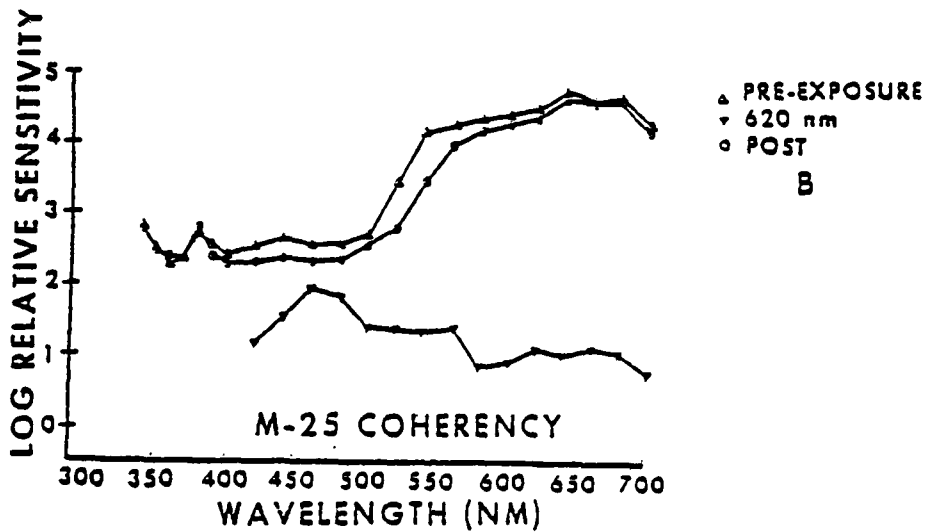
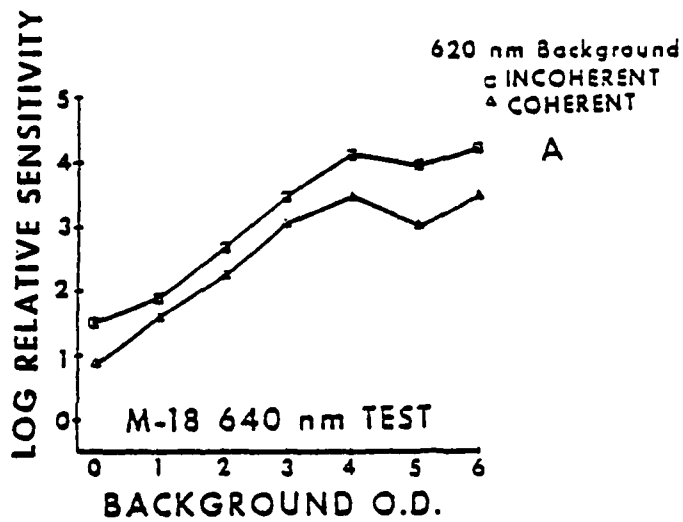


Figure 6. ERG recorded from *Pseudemys* measured for various intensity 640 nm test flashes in the presence of (upper graph) or following (lower graph) either coherent or incoherent light.

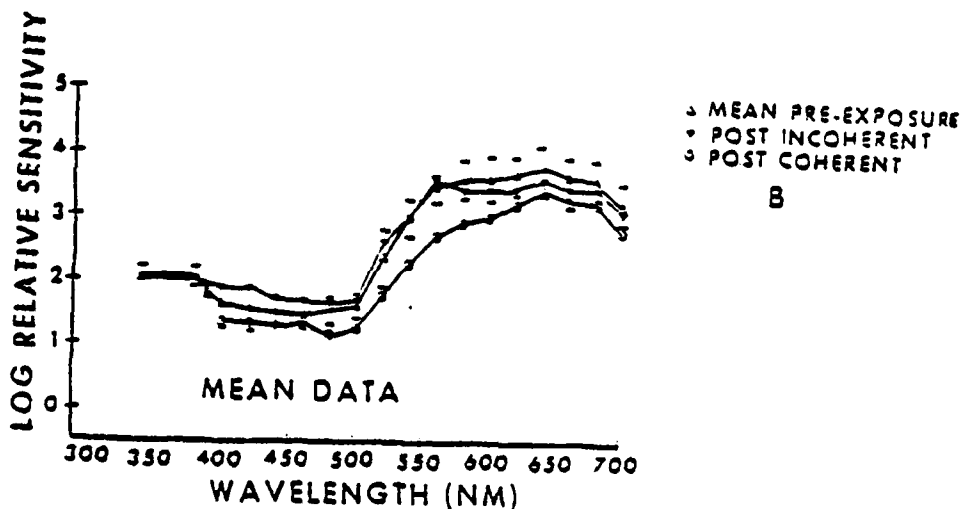
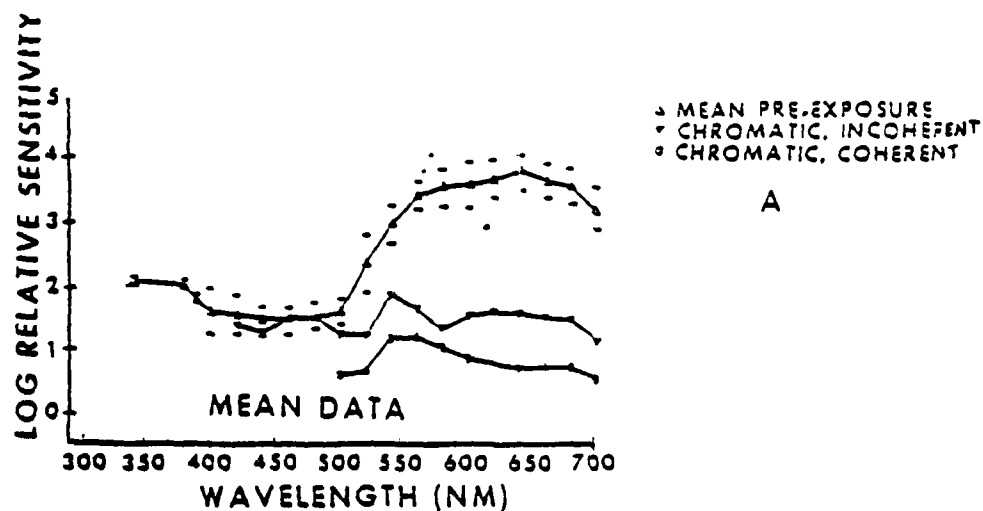


Figure 7. ERG recorded from *Pseudemys*. In the upper graph (A) mean preexposure spectral sensitivity is compared to the animal's spectral sensitivity during exposure to either 620 nm coherent or incoherent light. In the lower graph (B) the animal's postexposure spectral sensitivity is compared to its preexposure level for the two different exposure conditions.

irradiances used was well below levels where disruption of visual processes could be attributed to thermal changes at the retina. These levels were at or below the average retinal irradiance used with our primate studies (2). As in previous investigations with *Pseudemys* (5), elimination of perceptible speckle by time-averaging greatly attenuated the more permanent effects observed at equivalent irradiance levels for non-time-averaged stimuli. In our previous work, we proposed that the high spatial frequencies of speckle might be manifested at the retina by very small minimal spots of high monochromaticity and contrast subtending about the diameter of a photoreceptor. Although the occurrence of such minimal spots would not have high probability, they could occur and could have very high peak irradiances. These experiments suggest that prolonged exposure to a fairly stationary speckle pattern from a laser can pose a unique input to the visual system and increase the likelihood of seriously altering the sensitivity of the system. Whether the effects of such input are the result of an injury process or an active inhibitory process remains to be elucidated.

In our behavioral studies which have been reinstated following our return from Letterman, two animals are being trained to perform the required visual discrimination to measure the postexposure effects of coherent irradiation on spectral acuity. A third animal is being shipped to Ohio Wesleyan and training will begin on this animal following his delivery. After preliminary training and preexposure baselines are established, these animals will be exposed to Argon (514 nm) irradiation and the alterations in spectral acuity acquired with this spectral line compared to our previous subjects irradiated with 647 nm (Krypton) and 633 nm (HeNe) coherent light.

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