AD-A151 109 THE EFFECTS OF COHERENT AND INCOHERENT LIGHT ON OCULAR TISSUES AND VISUAL..(U) PACIFIC MEDICAL CENTER SAN FRANCISCO CA W H SPENCER OCT 80 DAMD17-79-C-9132 1/1

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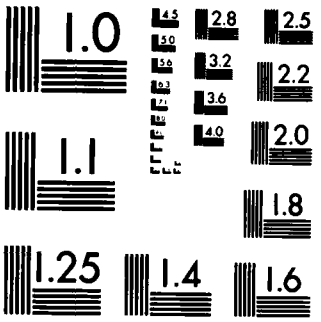

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THE EFFECTS OF COHERENT AND INCOHERENT LIGHT ON OCULAR TISSUES AND VISUAL FUNCTION IN NON-HUMAN SUBJECTS

Annual Progress Report

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October 1980

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-79-C-9132

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A151 109	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) THE EFFECTS OF COHERENT AND INCOHERENT LIGHT ON OCULAR TISSUES AND VISUAL FUNCTION IN NON-HUMAN SUBJECTS		5. TYPE OF REPORT & PERIOD COVERED Annual 1 October 1979 30 September 1980
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) William H. Spencer, M.D.		8. CONTRACT OR GRANT NUMBER(s) DAMD17-79-C-9132
9. PERFORMING ORGANIZATION NAME AND ADDRESS Pacific Medical Center 2340 Clay Street and Webster San Francisco, California 94115		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62773A.3E162773A819.00.067
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND Fort Detrick Frederick, Maryland 21701-5012		12. REPORT DATE October 1980
		13. NUMBER OF PAGES 6
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Ultrastructural study
Low levels of coherent and incoherent light
Retina

20. ABSTRACT (Continue on reverse side if necessary and identify by block number) This joint investigation by personnel at the Pacific Medical Center (PMC) Eye Pathology Laboratory and the Division of Biorheology at the Letterman Army INstitute of Research (LAIR) is designed to evaluate the functional and ultrastructural effects upon the retinas of non-human subjects of chronic repeated exposure to low levels of coherent and incoherent light.

The eyes of 3 Rhesus monkeys were exposed to diffuse coherent light at 2 levels of intensity and duration. (20 microwatts /cm^2 for 20 accumulated hours and 40 microwatts /cm^2 for 40 accumulated hours) (OVER)

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20) ABSTRACT: Following sacrifice, enucleation and fixation, selected areas of the retina, retinal pigment epithelium and choroid were dissected from both eyes of each animal and embedded in araldite. All blocks were sectioned at 1.0 micron and stained with toluidine blue. Parallel studies were performed at LAIR and PMC upon the same blocks. Each block was analyzed histopathologically and light photomicrographs obtained of segments from blocks with optimal orientation and fixation. Thin sectioning was then performed on these blocks and appropriate EM photomicrographs prepared.

No artefact free ultrastructural abnormalities attributable to exposure to coherent light were observed. No differences in retinal or retinal pigment epithelial structures were found when the exposed and the non-exposed (patched control) eyes of each animal were compared.

On the basis of the tissues studied to date we are unable to detect histopathological evidence of adverse effects caused by diffusely applied coherent light at the exposure levels and durations utilized.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No.(NIH) 78-23, Revised 1978).

Report

This is a report of an investigation performed at the Eye Pathology Laboratory of the Pacific Medical Center (PMC) as a joint effort with personnel of the Division of Biorheology at the Letterman Army Institute of Research (LAIR). The study is designed to evaluate the functional and ultrastructural effects in the retinas of non-human subjects produced by chronic exposure to low levels of coherent and incoherent light.

Background

For the past decade an interdisciplinary group now located at LAIR has explored the effects of coherent and incoherent light on ocular tissue and visual function in non-human subjects in order to provide a base upon which safety standards may be established. Mechanical, thermal and photochemical retinal damage is known to be produced by exposure to optical sources which produce a discrete image on the retina at wave lengths between 400 and 1400 nm (Footnote #1). The type of damage produced depends upon the power level and exposure time. Emphasis in the present study is directed toward evaluating the effects upon the retina of chronic or repeated low-level laser exposure of the diffuse rather than discrete type. Functional alterations in the form of electro retinographic spectral sensitivity depression have been detected following exposure to diffusely applied coherent light at a level of 20 microwatts per square centimeter for a total of 20 accumulated hours over a period of three weeks. (Footnote #2)

The contractor has been requested to assist in a search for histopathological evidence of tissue alteration in the exposed retinas that might be correlated with the observed psychophysical alterations.

Scope of Work

Commencing 1 October 1979 personnel from PMC Eye Pathology Laboratory

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Footnote:#1 Ham, WT; Mueller, H.A.; Ruffolo, J.J.; Clark, A.M.: Sensitivity of the Retina to Radiation Damage as a Function of Wavelength. Photochemistry and Photobiology. Vol.29, pages 735-743, 1979

Footnote:#2 Zwick, H; Beatrice, E.S.; Garcia, T.A.: Low Level Coherent Light Effects on Rhesus - Long Term and Progressive Changes. Color Vision Deficiencies. Vol.5, 1980

Footnote:#3 Zwick, H; Beatrice, E.S.: Long-Term Changes in Spectral Sensitivity After Low Level Laser (514 nm) Exposure. Modern Problems in Ophthal. Vol.19, pp 319-325. 1978.

have met on many occasions with LAIR personnel to discuss details of laser exposure, tissue sampling techniques and histopathologic analysis. Blocks of retina, pigment epithelium, and choroid from the eyes of a non-exposed monkey were provided to the contractor by LAIR in order to standardize tissue sampling techniques. This animal had been sacrificed for other purposes and served as an internal control.

Laser exposures to the eyes of 3 Rhesus monkeys (N-515, N-403, N-239) were performed at LAIR by in-house personnel. Prior to exposure the animals had ophthalmoscopic examination of their fundi. No fundus photographs were taken because it was felt that the bright light used at photography had the potential to modify the experiment. Between exposures all three animals were maintained in an environment illuminated by fluorescent light at 90 foot candles on a 12 hour day-night cycle.

Animal N-515 was exposed to coherent light (Argon) at 20 micro watts per square centimeter measured through the diffuser (Ganz feld). Both eyes were exposed for two hours per setting for a total of twenty accumulated hours over a period of three weeks. This exposure was identical to that utilized by Zwick. (Footnote #3). Subsequent ophthalmoscopic examination revealed no evidence of retinal or choroidal lesions. The animal was sacrificed and both eyes perfused before enucleation with a combination of glutaraldehyde and formalin fixative. Several areas from each retina and choroid were excised, and after appropriate post-fixation, embedded in araldite by LAIR in-house personnel. Selected blocks were then submitted to this contractor. Adjacent blocks were retained by LAIR for light and electron microscopic studies to be performed at LAIR.

The left eye of animal N-403 was exposed to coherent light (Argon) at 20 micro watts per square centimeter for two hours at a time for a total exposure of 20 accumulated hours over a period of three weeks. The right eye was patched only during the period of exposure to coherent light.

The left eye of animal N-239 was exposed to coherent light (Argon) at 40 micro watts per square centimeter for two hours at a time for a total exposure of 40 accumulated hours over a period of five weeks. The right eye was patched only during the period of exposure to coherent light.

Animals N-403 and N-239 were sacrificed and the eyes enucleated immediately. Each eye was then opened in cross section through the frontal plane at the equator and their posterior portions were flooded with a combination of glutaraldehyde and formalin fixative as soon thereafter as possible. Several areas from each retina and choroid were then excised and after post-fixation they were embedded in araldite by LAIR in-house personnel. Selected blocks from each eye were then submitted to this contractor and adjacent blocks retained by LAIR for studies to be performed at LAIR.

One micron sections were prepared by the contractor from each block and these were stained with toluidine blue for light microscopic observations. Subsequently, thin sections for electron microscopic observation were prepared from each block. Photomicrographs of the one micron light microscopy sections, and of thin sections studied with the electron microscope were prepared and examined. Black and white prints, and our interpretation of their content were prepared from each block from both retinas of all three animals. These were placed in four loose-leaf books and are available at the office of Dr. Ryan Neville, LAIR, ATTN: SGRD-ULZ=RCM/Building 1110, Presidio of San Francisco, California 94129.

Our observations of the retinas of animal N-515 suggested that considerable variation from normal anatomy was present and that this could be attributed to imperfect perfusion of the retinas and attendant autolysis. There was marked vacuolization of all retinal layers which also showed evidence of autolytic change within intracellular organelles (mitochondria). It could not be determined whether any of the observed changes could be attributed to exposure to coherent light. Subsequent to the examination of these tissues consultation was sought with Dr. Toichiro Kuwabara at the National Eye Institute, who visited LAIR and instructed in-house personnel in the technique of enucleation and rapid fixation of the ocular tissues immediately after enucleation. It was suggested that this technique be utilized in studying the retinas of animals N-403 and N-239. The technique described by Dr. Kuwabara was carried out in preparing these tissues for study.

Examination of one micron and thin sections prepared from the left and right eyes of animals N-403 and N-239 showed no artefact free ultrastructural abnormalities that could be attributed to exposure to coherent light. These tissues showed much less artefact than the tissues of animal N-515. However, there was still evidence of vacuolization of the inner retinal layers particularly the nerve fiber layer and of portions of the outer retinal layers and the pigment epithelium. Embedding artefact was manifested by tissue distortion causing several sections to contain both a tangential and a cross sectional view of the retina.

Discussion of Results

Studies on tissues obtained from the retina and choroid of the exposed animals have not to date, demonstrated histopathologic evidence of tissue alteration attributable to exposure to diffuse coherent light (Argon) at the intensity and duration utilized. The intensity and duration levels used in animals N-515 and N-403 are similar to those used by Zwick et al and found to cause longterm depression of ERG spectral sensitivity. Increasing the exposure to 40 micro watts per square centimeter and 40 accumulated hours of exposure has not to date,

produced a demonstrable morphologic alteration in the retina or choroid. It is to be noted, however, that spectral sensitivity studies were not performed on animals N-515, N-403, and N-239 at the beginning and completion of the exposures to coherent light. The absence of histopathologic change following this exposure (which in the past has been sufficient to produce functional depression) may be attributed either to the absence of a functional and anatomic effect or to modification of electrical conductivity occurring at a level that does not produce demonstrable ultrastructural alteration.

Recommendations

1) It is suggested that in future investigations the controls utilized be somewhat more rigid. For example, all animals should be age matched and have a known light exposure history.

2) An attempt should then be made to produce a demonstrable morphologic alteration in the retina at a much higher level of intensity and duration of exposure than has been utilized in the present experiment. This could be attempted by greatly increasing the level of both coherent and non-coherent light exposure and duration with and without pupillary dilatation. Similar exposure could be tried in both the light adapted and dark adapted animal.

3) ERG spectral sensitivities and other psychophysical behavioral studies should be performed and recorded before and after exposure as well as immediately pre-sacrifice.

In this investigator's opinion, it is imperative that it first be demonstrated that diffusely applied coherent light can produce morphologic alteration in the retina of these subjects. Following this demonstration the minimal level at which damage can be produced should be sought.

An attempt could be made to augment the behavioral psychophysical and morphological studies by also investigating possible modifications in the enzyme histochemistry of the retina and retinal pigment epithelium (for example, tetrazolium blue precipitate studies could be performed).

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