Comparison of macular versus paramacular retinal sensitivity to femtosecond laser pulses

Clarence P. Cain
Litton-TASC, Inc.
4241 Woodcock Drive
Suite B-100
San Antonio, Texas 78228

Cynthia A. Toth
Duke University Eye Center
Durham, North Carolina 27710

Robert J. Thomas
Gary D. Noojin
Val Carothers
David J. Stolarski
Litton-TASC, Inc.
4241 Woodcock Drive
Suite B-100
San Antonio, Texas 78228

Benjamin A. Rockwell
Air Force Research Laboratory
AFRL/HEDO
8111 18th St.
Brooks Air Force Base, Texas 78235

Abstract. Single 130 fs laser pulses in the near-IR (800 nm) were used to create ophthalmoscopically viewed minimum visible lesions (MVLs) within the macular and paramacular regions in rhesus monkey eyes. MVL thresholds at 1 and 24 h are reported as the 50% probability for damage (ED_{50}) together with their fiducial limits at the 95% confidence level. These measured thresholds are compared with previously reported thresholds for near-IR and visible wavelengths for both macular and paramacular areas. Threshold doses were lower at the 24 h reading than at the 1 h reading for both retinal regions and the $ED_{50}s$ for the macula were slightly lower than for the paramacula. We measured the 24 h MVL ED_{50} thresholds to be 0.35 and 0.55 μJ for the macular and paramacular areas, respectively. The combined data for both areas yielded a threshold of 0.45 μJ. © *2000 Society of Photo-Optical Instrumentation Engineers.* [S1083-3668(00)00803-0]

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1 Introduction

Our goal in this study was to evaluate retinal damage thresholds from single ultrashort laser pulses at 800 nm and to compare damage thresholds between macular and paramacular areas within the fundus. Pulse widths of 130 fs were utilized to determine the minimum visible lesion (MVL) thresholds (ED_{50}) within the macula and paramacula.[2] In this study we compare our results with those for both near-IR and visible wavelengths previously reported.[3–14]

Laser-ocular tissue interaction studies for pulse widths below 1 ns are critical to the development of safety standards and in identifying hazards to the human eye from those systems presently operating in the near-IR regime. An understanding of laser-tissue interactions is basic to identifying the potential for injury and to applying therapeutic medical treatments to laser injury and disease. Laser effects in the eye have been well documented for continuous wave (cw) and pulsed laser systems with pulse widths down to 90 fs for visible wavelengths and down to 150 fs[3,10] for 1060 nm. Thus we are providing the urgently needed data at 800 nm in the primate fundus to recommend new national and international laser safety standards for laser systems operating in the near IR and to assess potential human retinal hazards from these laser sources.

The maximum permissible exposure for the retina has been established by the National Laser Safety Standard,[15] ANSI

Z136.1-1993, and The International Commission on Non-Ionizing Radiation Protection (ICNIRP) Standard,[16] for visible and near-IR laser radiation at pulse widths as short as 1 ns. In the U.S., there is no set standard for pulse widths below 1 ns, only a guideline (recommending keeping a constant irradiance) which may be overly conservative. These standards are based upon retinal injury studies conducted on primate eyes for cw lasers and pulsed laser systems with laser pulse widths greater than 2 ns. We have previously reported retinal injury studies for visible wavelengths with pulse widths down to 90 fs for pigmented rabbit eyes[17] and rhesus monkey eyes.[3]

We investigate the difference in damage thresholds between the macular and paramacular areas of the fundus. Our goal is to broaden the understanding about retinal damage mechanisms gained in similar, previous studies at longer pulse widths. A secondary goal is to allow the use of the paramacular area for MVL studies, thus minimizing the numbers of *in vivo* subjects required for validated laser safety standards and to allow the comparison of macular and paramacular MVL studies.

2 Methods

2.1 Experimental Systems

The laser used in this experiment was a Ti:sapphire regenerative amplifier system. This system consisted of four major components. The first two of these components were a Ti:sapphire oscillator and its pump laser. The oscillator operated at 800 nm with a pulse width of 100 fs. The repetition rate of this oscillator was 76 MHz. This oscillator seeded the regenerative amplifier. The regenerative amplifier amplified the

seed pulse to sufficient energy to provide a large range of energies for this experiment at 130 fs. The regenerative amplifier was pumped by a Nd:yttrium–aluminum–garnet (YAG) or a Nd:yttrium lithium fluoride (Nd:YLF) laser depending on the ultimate repetition rate of the system. Both configurations were used in this study. The laser system was always operated in the single shot mode. The output pulse width of the system was monitored with a slow-scan or single shot autocorrelator. The marker lesions for this experiment were produced with a cw krypton gas laser operating at 647 nm. The krypton laser was shuttered to yield a 3–4 ms pulse and the output was adjusted to give a high-contrast, white marker lesion (see Figure 1).

In this experimental configuration the eye was positioned so that the retina was in the focal plane of the fundus camera. A beam splitter was placed approximately 1 cm from the cornea and was aligned so the reflected beam entered the eye collinear with the optical axis of the fundus camera. The transmitted portion of the beam was directed to an energy meter. The reflected/transmitted ratio for the beam splitter was measured for each set of exposures. The transmitted energy for each shot was recorded and the measured ratio was applied to obtain the actual energy delivered to the eye. Throughout this paper, laser energy delivered is the energy delivered to the corneal surface without a contact lens or other device to control the image size on the retina.

2.2   *In Vivo* Model

Mature *Macaca mulatta* subjects from 2.2 to 6.9 kg were maintained under standard laboratory conditions (12 h light, 12 h dark). All subjects were screened pre-exposure to ensure that no eye was more than one-half diopter from being emmetropic. All procedures were performed during the light cycle.

2.3   *In Vivo* Preparation

All animals were chemically restrained using 10 mg/kg ketamine hydrochloride (HCl) intramuscularly. Once restrained, 0.25 mg atropine sulfate was administered subcutaneously. Two drops of proparacaine HCl 0.5%, phenylephrine HCl 2.5%, and tropicamide 1% were each placed in both eyes. Under ketamine restraint, the subject had intravenous catheters placed for infusion of propofol and an initial induction dose of propofol (5 mg/kg) was administered to effect. The state of anesthesia was maintained using 0.2–0.5 mg/kg/min of propofol via syringe pump. The animal was intubated with a cuffed endotracheal tube. A peribulbar injection of 2% lidocaine was administered to reduce extraocular muscular movement. The subject was securely restrained in a prone position on an adjustable stage for the fundus photography, laser exposure, and fluorescein angiography (FA). Prior to FA, 0.6 ml of fluorescite 10% (Alcon Laboratories) was administered as an intravenous bolus. The subject's blood pressure, temperature, and pulse were continuously monitored throughout the experimental protocol. Normal body temperature was maintained by the use of circulating warm water blankets.

Observations of lesion formation and fundus photography (including FA) by the researchers were performed by monocular viewing through the Topcon fundus camera. Base line color fundus photographs were taken prior to laser exposures.

The eyelids were held open with a wire lid speculum, and the cornea was moistened throughout the procedures with 0.9% saline solution. The retina was viewed with a fundus camera at all times and all macular exposures (16–25) were delivered to the eye in a rectangular grid pattern centered on the fovea. The paramacular exposures (16–30) were placed no more than 10° temporal to the fovea and additional lesions within 5° below (see Figure 1). The right or left eye was selected randomly for exposures. All eyes were evaluated at 1 and 24 h postexposure and visible lesions at a given exposure site were reported when at least two examiners identified a lesion. Color fundus photographs were taken at 1 and 24 h postexposure along with black-and-white FA photographs.

Photographs of the fundus were taken immediately before the dye injection, during FA, and at intervals of a few seconds until 5 min had elapsed. This provided a sequence of photographs for the development of fluorescein leakage. After fluorescein injection and angiography, the lesions were also assessed for fluorescence through the camera system with excitation and a barrier filter in place.

2.4   Statistical Methods

Probit analysis[18] was used to determine the ED_{50} dose for creating a MVL in the retina and to estimate the 95% confidence intervals for the ED_{50} values. Enough exposures were delivered to ensure that the fiducial limits were reasonable and within the following limits at the 24 h postexposure reading for visible lesions only: the upper fiducial limit could be no larger than $1\frac{1}{2}$ times greater than the ED_{50} dosage and the lower fiducial limit could be no less than $\frac{1}{2}$ of the ED_{50} dosage.

3   Results

Visible lesion thresholds were measured for a pulse width of 130 fs at both 1 and 24 h after laser delivery. Laser pulses were delivered to 113 macular exposure sites (70 exposures delivered between the 24 h ED_{15} and ED_{85} energy values) and 122 paramacular exposure sites (86 exposures delivered between the 24 h ED_{15} and ED_{85} energy values). The macular exposure energies were between 0.01 and 3.3 μJ, with the highest energy that elicited no response to the retina at 24 h having 1.22 μJ and the lowest energy that produced a lesion at 0.107 μJ. The paramacular exposure energies were between 0.034 and 2.49 μJ, with the highest energy that elicited no response to the retina at 24 h having 1.31 μJ and the lowest energy that produced a lesion at 0.15 μJ. The paramacular exposures were placed within 10° temporal and 5° inferior to the fovea. We measured the 24 h MVL ED_{50} to be 0.35 and 0.55 μJ for the macula and paramacula, respectively. Table 1 lists results from 1 and 24 h postexposure readings with the corresponding fiducial limits in parentheses and the slopes of the probit curves at 24 h. There was not a significant difference between the thresholds obtained with the two laser configurations used (different pump lasers as described in Sec. 2). Both configurations were used to expose macular and paramacular sites. The data were combined and are reported in Table 1. Also included in the table are the combined macular–paramacular results (0.45 μJ at 24 h using 235 data points).

The laser pulse energies used varied from 0.01 to 3.3 μJ. Under direct ophthalmoscopic observations, the retinal re-
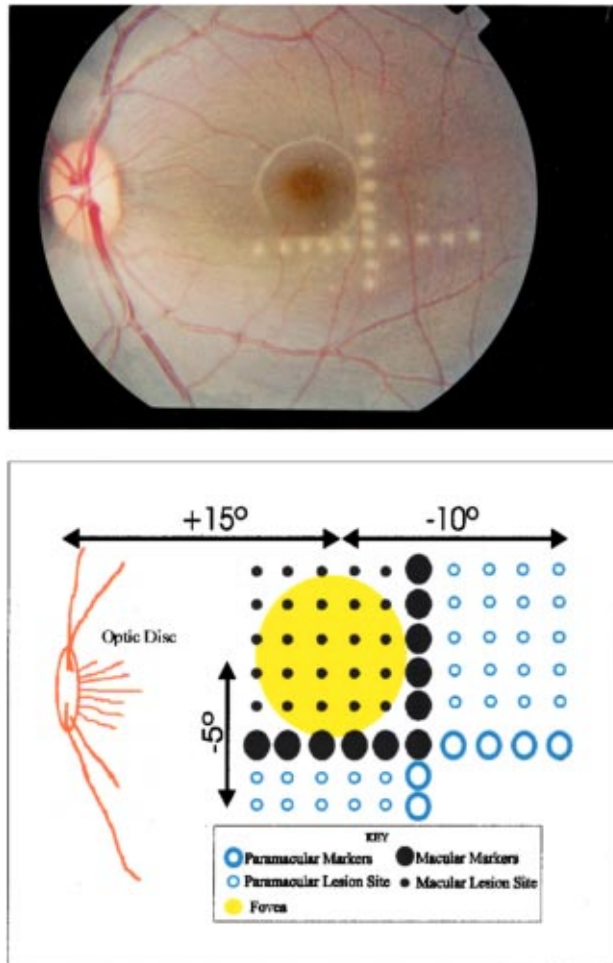
Fig. 1 Typical fundus photograph illustrating marker grid lesions (bright cross pattern) and minimum visible lesions which are difficult to detect in the photograph (top). Corresponding grid map for lesion placement in macular and paramacular regions of the retina (bottom).

Table 1 Minimum visible lesion thresholds (ED$_{50}$s) for 130 fs, 800 nm macular and paramacular exposures (fiducial limits at the 95% confidence level in parentheses).

Location	MVL-ED$_{50}$ (μJ) 1 h	MVL-ED$_{50}$ (μJ) 24 h	Slope at 24 h
Macula (113 exposures, six eyes, four subjects)	0.40 (0.30–0.53)	0.35 (0.26–0.46)	2.4
Paramacula (122 exposures, six eyes, four subjects)	0.65 (0.51–0.91)	0.55 (0.44–0.73)	2.5
Combined data (235 exposures)	0.52 (0.43–0.64)	0.45 (0.38–0.55)	2.4

sponse to minimal exposures appeared as a pale gray to white lesion increasing in whiteness and size as the energy increased. The combined data provided thresholds midway between those for the macula and paramacula as expected. For the 24 h ophthalmoscopic reading, the slopes of the probit curves were all greater than two and the fiducial limits fell within the range between $\frac{1}{2}$ and $1\frac{1}{2}$ times the ED$_{50}$ values.

As in previous reports for ultrashort laser retinal exposures,[3,10] fluorescein leakage from the smaller lesions could not be discriminated from the background choroidal flush. The FA thresholds for this pulse width were much higher than the MVL thresholds for both 1 and 24 h readings.[3,10] In order to obtain statistically valid FA thresholds, a significantly increased number of higher-energy exposures would have been required. Because of the proven reduced sensitivity and the increased number of subjects that would have been required, FA thresholds are not reported in this paper.

4 Discussion

This study documents the shortest pulse width MVL exposures reported to date in the near IR. Our macular ED$_{50}$ value was 0.35 μJ at 130 fs. This represents the lowest threshold reported for all near-IR studies.[4–14] The value was slightly less than the 0.43 μJ recorded[3] for the visible wavelength of 580 nm at 90 fs and one-third the value of 1.0 μJ measured at 1060 nm.[10] However, this 0.35 μJ was double the 0.17 μJ measured at 530 nm for 100 fs.[10] Our data[3,10] indicate that as pulse duration decreases below 1 ns, the MVL thresholds at wavelengths in the visible and near IR approach one another. Data at shorter pulse widths will allow validation of observed trends. Figure 2 summarizes all MVL threshold data for single pulse exposures shorter than 10 ns.[3–14]

Thresholds for the paramacular area were 1.6 times larger than the macular thresholds and this ratio held for both the 1 and 24 h data. Also, it is worth noting that the ED$_{50}$ thresh-
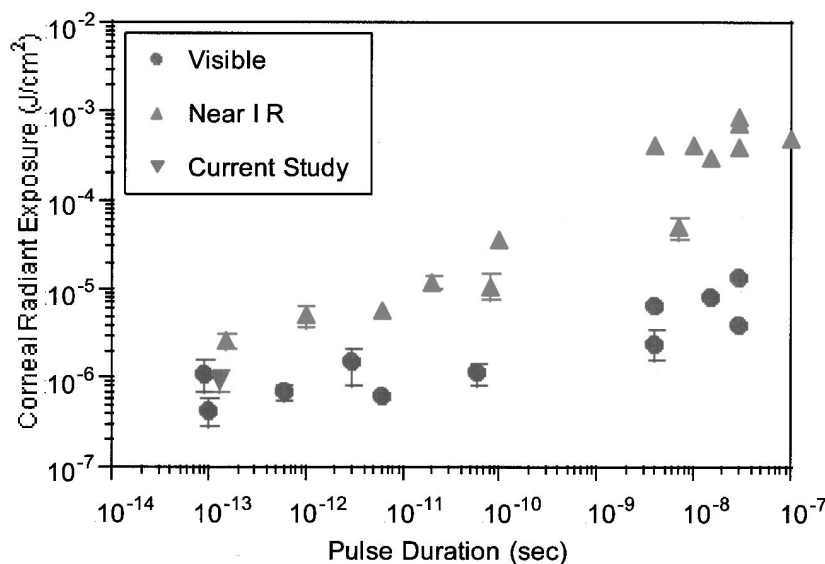

Fig. 2 Single-pulse, minimum visible lesion thresholds for pulse widths shorter than 100 ns in the rhesus monkey [data from our laboratory (see Refs. 3 and 10) are shown with error bars that represent the 95% confidence intervals]. Radiant exposures are total intraocular energy normalized to a 7 mm aperture.

old decreased by as much as 15% between the 1 and 24 h readings. We have observed similar trends in the past for other wavelengths (visible and near-IR) with pulses near 100 fs.[3,10]

This study used the paramacular area that was 10° temporal and 5° inferior to the fovea. A limited amount of data exists comparing the macular and paramacular regions of the retina.[19–22] We compare our data to previous studies that reported MVLs for the macula and the paramacula up to 30° temporal to the fovea. The reported values[19–22] indicated the paramacula was less sensitive than the macula by a factor of 1.1–2 times. These studies employed different pulse widths and wavelengths from this study. Griess, Blankenstein, and Williford[22] utilized nanosecond pulses at both longer and shorter wavelengths than 800 nm. The Griess study used a paramacular region immediately adjacent to the macula as in this study. They found macular to paramacular MVL ED_{50} ratios to be 1.47 at 1064 nm and 1.77 at 532 nm. Polhamus et al.[20] reported MVL ED_{50} values for 532 nm at a 10 ns pulse width for the macula and the paramacula at 30° temporal. The ratios of these $ED_{50}s$ had a value of 2. Lappin and Coogan[19] reported the lowest ratio (1.1), for 632 nm, 40 ms pulses, in a similar region of the retina after 5 min postexposure lesion observation. Thus this study, which reports a MVL ED_{50} ratio of 1.6, agrees with ratios measured by other investigators with various pulse widths and wavelengths.

Because of the similarity in ratios for macular versus paramacular damage measured with a large variety of laser parameters, one can conclude that the anatomical changes in the fundus and the optics of the eye are the factors responsible for this ratio. The absorptive characteristics of the retinal layers, such as the retinal pigmented epithelium, are easily implicated as key mitigating factors. The amount of pigmented melanin has been shown to appear at higher concentrations in the macular zone than other areas.[23] This is verified by clinical observation of the fundus, which has a darker appearance than adjacent retinal areas. This would imply a higher total absorption in the macular exposure area, resulting in a lower damage threshold. In addition, it is known that aberration increases with distance from the fovea.[24] This would increase the laser spot size at larger angles from the fovea, thereby increasing the energy required at the cornea to produce the same retinal fluence.

5 Conclusions

Our data show that ophthalmoscopically visible lesion thresholds at 800 nm occur at energy levels consistent with other visible and near-IR studies in the femtosecond regime. The macula is 1.6 times more sensitive to laser damage than the adjacent paramacula and therefore requires less laser energy for damage. Since our reported values are similar to studies done previously for different exposure conditions, we have concluded the existence of a common mediator for this ratio. Even with the possibility of different mechanisms for retinal damage, the differences in the optics of eye and absorption of the different regions of the fundus explicates this ratio. These data will add to our understanding of how the fundus reacts to laser insult.

We also conclude that the visible lesion thresholds measured in the paramacula are accurately scaleable to reflect the ophthalmoscopically visible lesion thresholds in the macular area. This will help in the development of future MVL protocols by enabling fewer numbers of animal subjects to be used. In addition, correlations can now be made between MVL studies which report paramacular data with those done in the macular area.

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