Acute Laser Lesion Effects on Acuity Sweep VEPs

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Q-switched neodymium-YAG (infrared) laser lesions at energies up to and including retinal hemorrhages were placed under visual control in the parafovea and the fovea of anesthetized monkeys. Visual-evoked potential (VEP) data were obtained by parallel analog (vector voltmeter) techniques from scalp electrodes in response to high luminance counterphasing sine wave gratings. The gratings were swept downward in spatial frequency to determine an acuity estimate by recording of the VEP magnitude increase onset. Acuity estimates were determined immediately post-exposure and at 15 sec intervals up to 12 min. These were analyzed as a function of laser exposure site and retinal lesion produced. Significant delays in VEP lock-in were demonstrated in subjects that had parafoveal burns or parafoveal subretinal hemorrhages. Foveal burns caused severe short-term fluctuations before a sustained decrease in acuity. Contained foveal hemorrhages produced sustained acuity losses. Foveal exposures that did not produce an immediately visible lesion did not produce measurable changes in VEP response lock-in time. These results probably are independent of visible flash effects and indicate that there may be a transient neural shock effect from parafoveal lesions that can affect the fovea.


Long-term visual compromise resulting from retinal laser burns is well documented in the clinical literature and in accident reports. These have been summarized, and their effects on visual acuity have been tabulated. Although this tabulation is useful, the range of visual deficits for a particular estimated exposure type are extremely wide and therefore have limited predictive value. Clinical treatment texts focus on how laser treatment affects diseased tissues; the effects of this treatment on the remaining normal retina usually are relegated to a small chapter on complications. These complications usually are dominated by a discussion of hemorrhage, with a reference to foveal coagulation. Admittedly, the clinical end point is the arrest of a retinal disease effect and the retention of useful vision. However, even the treating physician cannot know whether the final best-corrected visual acuity of the patient will be 20/40, with which the patient can drive a car, or 20/200, in which case the patient fulfills one of the criteria for legal blindness.

Histologic and anatomic studies predominantly in primates have related laser wavelength, pulse duration, and energy to retinal lesion size and depth. Based on these changes and on maps of visual acuity versus retinal eccentricity, inferences can be made about the functional defects of detectable lesions. There is general agreement about the histology of laser lesions in the retina, but the visual correlates of these lesions still are unclear. Behavioral assays in primates have shown clear losses in fine target detection under high luminance conditions with large, obvious foveal burns. However, as soon as the laser exposure approaches the minimal irradiance diameters produced by the optics of the eye with a collimated beam or the dose becomes less severe—ie, near the ED50 level for visible retinal alteration—the behavioral assays show conflicting results that may include substantial startle components. In addition, there may be significant low-level effects from exposures extended across longer time spans or greater retinal extents that complicate the overall picture of the interaction of the retina, visual function, and laser light.

The visual system of the monkey has been shown to histologically, anatomically, electrophysiologically, and, within certain constraints, psychophysically parallel the human system. Thus, if certain psychophysical values can be predicted from physiologic experiments, an alternative approach to the behavioral assays is possible. To circumvent the problems of psychophysical research in animals, more direct assays of neural function have been attempted. However, be-
cause laser lesions are inherently irreversible, the usual averaging techniques (eg, using repeating laser doses to the same retinal area in the same animal) can not be employed. The result has been that retinal burns have produced effects on recorded signals that could not be related easily to visual function. Because the VEP has been correlated to perception in humans by averaging and vector-voltmeter techniques, the vector-voltmeter may permit a rapid estimation of visual function in animals as well.

Previous studies have examined the effects of laser exposures on the electrophysiologic function of circumscribed areas of the retina. This was accomplished by recording the visual-evoked potential (VEP) from scalp electrodes. Although this montage samples the entire central retina, averaging was required to extract the low signal, limiting the exposure doses to “safe” levels. The use of implanted bipolar cortical electrodes has allowed single exposure evaluation at higher doses. However, these electrodes have “receptive fields” that are only 1°–2° in diameter. The pattern electroretinogram also has been recorded, thus sampling the entire macular area, but the signal-to-noise level and the standard television stimulus systems now employed do not permit determination of what the animal can “see” at high photopic (daylight) levels. The contrast sensitivity functions can describe accurately the threshold sensitivity of the visual system and can be measured by the vector-voltmeter technique, but they do not describe the visual system’s suprathreshold response characteristics and cannot measure changes in suprathreshold response. In contrast, the sweep VEP can objectively estimate visual acuity rapidly and repeatably. Therefore, it seems well suited to measuring transient laser bioeffects on visual function.

This report examines how retinal laser exposures (from minimally visible retinal lesions to supra-clinical treatment-level burns) near and on the fovea affect acute visual acuity. To determine this, I used a counterphasing high-contrast grating swept in spatial frequency as a stimulus and a vector-voltmeter as an electroencephalogram (EEG) analyzer to obtain acuity estimates at 15 sec intervals in the immediate time interval around the placement of superimposed single laser exposures.

Subjects and Methods

Lasers

The laser lesion source was a 10 Hz repetitively pulsed Q-switched neodymium laser (1064 nm, near infrared, 300 mJ per pulse maximum energy, 8 nsec nominal pulse width; Spectra Physics, Piscataway, NJ). The optics were installed for a near Gaussian beam profile, and the laser was tuned by setting the Q-switch delay longer than optimal. This reduced the beam diameter to approximately 4 mm and set the output energy to about 20 mJ per pulse. The laser was made collinear, with a low-power helium-neon alignment laser used as a “spotting” beam to direct the neodymium laser exposures. Lasers were calibrated and monitored on-line with an RT10 energy/power meter (Laser Precision Corp., Utica, NY).

The combined laser beams were inserted with an infra-red mirror into the optical path between the stimulus system and the cornea (Fig. 1). Final beam energy was controlled by neutral density-absorbing filters. Graded retinal laser lesions were placed in separate sessions using the native collimated beam from the pulsed laser without expansion or focusing by any lens system other than the eye itself. Alignment was ensured by double projecting the alignment laser and superpositioning the corneal and fundus reflexes. The alignments were checked with an artificial (glass) eye that contained a frosted glass posterior pole. During the exposure and VEP recording, the alignment laser and the fundus camera illumination were turned off to prevent glare. Placement and severity of lesions was governed by attempts to produce three separable classes of retinal effects ranging from no visible change through contained retinal hemorrhage with a single Q-switched exposure (“Wolfe grade” equivalent effects).

Stimulus

The stimuli were produced by a custom-built high-luminance, high resolution computer-driven monochrome video monitor system (VisionProbe; Krug Int., San Antonio, TX). The stimulus display was always centered on the fovea for all laser exposure locations and subtended 11.3° square. The screen was modulated under computer control to produce high mean illuminance (1.62 log-foot-lamberts) 99.3% contrast vertical sine-wave achromatic gratings on the retina. These were counterphased at 7.2 Hz (14.4 reversals/sec) to evoke the VEP. Simultaneously, the spatial frequency of the grating was stepped down in spatial frequency from 41 to 4.1 cycles/degree until the visual system responded. This “acuity sweep” was repeated every 15 sec, and the signal onset point was noted. The spatial frequency steps were not equally spaced in time, but were biased toward the higher (earlier) spatial frequency steps (Fig. 2). This permitted the threshold determination to be combined with a response speed determination, thus increasing sensitivity to small disturbances in the visual system’s ability to detect and “lock onto” the grating. In addition, because the visual system dynamically changes after the exposure is placed, remaining at the
higher spatial frequencies longer allows this response onset point to be determined more precisely.

Subjects

Choosing a suitable species was driven by three considerations: (1) the animal must be a primate; (2) it must have color vision and show good acuity under high luminance conditions; and (3) its retina must have a fovea. These requirements arise from the need to model the human visual system organization (anatomy and function) retinally and cortically so the results of this study can be generalized to human visual performance. Therefore, we selected the lowest species that could be used, the cynomolgus monkey (Macaca fascicularis). Only the right eye was used for the laser exposures. The animal’s other eye remained unexposed. All procedures adhered to the Institutional Animal Care and Use Committee, the ARVO Resolution on the Use of Animals in Research, and the National Institutes of Health guidelines. All procedures were performed with the assistance of the veterinary staff.

Recording

Steady-state pattern VEPs were recorded from gold cup scalp electrodes in response to the counterphasing grating and stored as digitized records (Brainwave Systems, Denver, CO). A vector-voltmeter (Stanford Research Instruments, Palo Alto, CA) was synchronized with the counterphase driver reference signal; it analyzed the EEG response derived from electrodes located over the primary visual cortex (the equivalent to T5 and T6 locations in humans) referenced to a platinum subdermal needle electrode in the brow. The meter’s magnitude output tracked the response power as the stimulus swept through spatial frequencies. The onset of a strong magnitude response coupled with a phase transient was used to define the electrophysiologic acuity of the animal for each sweep (see below).

Procedure

The animal was sedated with ketamine (10 mg/kg) and acepromazine (1 mg/kg) intramuscularly, intubated, and wrapped in a circulating hot water warming blanket. The pupils were dilated and accommodation inhibited with 2.5% phenylephrine HCl and 1% tropicamide. The corneas were protected with contact lenses individually fitted and optically corrected for the stimulus distance. The animal then was further anesthetized with pentobarbital (10 mg/kg) intrave-
nously and muscularly relaxed with a bolus of pancuronium bromide (0.14 mg) intravenously. This level of barbiturate anesthesia is sufficient to avoid the perception of discomfort by the animal while not significantly depressing the amplitude of the VEP. Rectal temperature, electrocardiogram (ECG; cardiograph), and end-tidal Pco2 were monitored. Ventilation (depth and rate) and blanket temperature were adjusted to maintain physiologic values. EEG and ECG were monitored for signs of discomfort, and the anesthetic level was adjusted accordingly. The animal was placed in front of a fundus camera on a rotating stage padded with absorbent quilted paper pads, and the retina was visualized and aligned. The laser exposure was placed under visual control and the EEG was monitored on-line. Acuity estimates were made from the magnitude traces produced by the vector voltmeter. The criterion for the estimated acuity was the existence of an inflection point in the magnitude trace at which the signal rose to a peak (or series of peaks) with each succeeding step in grating size, coupled with an inflection of the phase trace just before this peak, followed by a period of relatively constant phase, which indicated EEG “lock on” to the stimulus (Fig. 2, upper panel).

In some cases, an actual pattern onset VEP could be seen in the simultaneously acquired EEG. When available, this also was used to help determine the magnitude inflection point. When no such inflection point could be determined, the VEP onset time was coded as the total sweep length of 15 sec (Fig. 2, bottom panel). The data produced were a set of curves relating acuity to time. A baseline series of 10 sweeps was acquired to ensure optical alignment and signal integrity. During the subsequent 400 sec experimental period, the preselected laser exposure was triggered at between sweeps 6 and 7, and data collection continued at least through sweep 24 (12 min total).

### Table 1. Retinal lesion pattern

<table>
<thead>
<tr>
<th>Locus</th>
<th>INF</th>
<th>TEMP</th>
<th>SUP</th>
<th>FOV</th>
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<tr>
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<td>IA</td>
<td>IIIA</td>
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<td>IB</td>
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<tr>
<td>TEMP</td>
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<td>IA</td>
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<td>SUP</td>
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<td>IIIA</td>
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<tr>
<td>FOV</td>
<td>IIIB</td>
<td>IIA</td>
<td>IIA</td>
<td>IIB</td>
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</tbody>
</table>


### Table 2. Distribution of doses (μJ)

<table>
<thead>
<tr>
<th></th>
<th>No dot</th>
<th>White dot</th>
<th>Red dot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parafoveal</td>
<td>mean</td>
<td>602.07</td>
<td>1470.69</td>
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<tr>
<td>SD</td>
<td>451.21</td>
<td>906.78</td>
<td>717.49</td>
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<tr>
<td>low</td>
<td>171.70</td>
<td>224.00</td>
<td>1195.00</td>
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<tr>
<td>high</td>
<td>1506.20</td>
<td>3003.00</td>
<td>3101.00</td>
</tr>
<tr>
<td>Foveal</td>
<td>mean</td>
<td>168.50</td>
<td>422.75</td>
</tr>
<tr>
<td>SD</td>
<td>42.50</td>
<td>146.03</td>
<td>428.35</td>
</tr>
<tr>
<td>low</td>
<td>126.00</td>
<td>174.00</td>
<td>1611.00</td>
</tr>
<tr>
<td>high</td>
<td>211.00</td>
<td>548.00</td>
<td>2631.00</td>
</tr>
</tbody>
</table>

SD, standard deviation.

On separate occasions, four single Q-switched laser exposures were placed in the right eye, one per recording session. To minimize the animal's physiologic stress, each animal was scheduled for recording (with or without laser exposures) no more frequently than once every 10 days. As a result, acuities 1-10 days after an exposure were not recorded in this study. The exposures were placed in the order and at the grades as indicated in Table 1 on separate days. Energies were adjusted based on experience to attain the lesion grades desired. The final distribution of energies and resulting retinal effects are shown in Table 2.

### Recovery

After the 2–3 hr experimental session, the animal was allowed to recover from the muscular immobilizing agent and the anesthetic. When it could breathe unassisted, it was extubated and moved to the transfer cage. When it could sit unassisted, it was returned to the colony under the supervision of the veterinary staff.

### Design and Analysis

The design of this set of experiments was necessarily constrained by the need to conserve animals. Therefore, a fully randomized factorial design is impossible, and animals had to have more than one exposure in the experimental eye. Therefore, the exposures were placed in a graded sequence with the most benign 5° eccentricity locations first, but with the intensities counterbalanced in order. The nine animals were run in three cohorts, as shown in Table 1. The data from one animal (no. 6, a foveal 2B exposure) were lost because of computer malfunction. This reduced the number of replications from 3 to 2 for this condition in the statistical analysis.

The series of curves relating acuity estimate (in milliseconds during the sweep) to time (in seconds during the run) as a function of lesion location (foveal versus parafoveal) and type were produced. The lesion types...
(retinal effect) were graded as follows: no immediately visible retinal change in pigmentation, “no dot”; an immediately visible gray or white lesion, “white dot”; and an immediately visible red, dark, or hemorrhagic lesion, “red dot.” As a comparison, “white dot” lesions are approximately equivalent to therapeutic retinal treatment doses, while “red dot” lesions are more severe and are avoided clinically. The data in these curves were further reduced to five variables for statistical analysis by the CSS Statistica program (Statsoft, Inc., Tulsa, OK). Two of these variables combined the acuity data of all the animals over all sweeps into a set of averages and standard deviations for five time segments (baseline, pre-exposure, and three sequential post-exposure periods, each the same length as the pre-exposure period, labeled “post1,” “post2,” and “post3”). These two variables (average acuity and acuity variability in each time segment) were calculated for each run and the data set was analyzed by analysis of variance (lesion site by retinal effect by time segment).

Although further data (ie, more sweeps post-exposure) were recorded for the higher retinal doses, these were not analyzed for significance because they did not have matching comparison periods from the lower-dose exposures. Qualitatively, these later periods simply continued the trends seen in the post1, post2, and post3 time segments. The three remaining data points abstracted were: (1) immediate post-exposure acuity estimate, coded as “IMMED”; (2) worst acuity demonstrated during the post-exposure period, coded as “WORST”; and (3) the time at which that worst acuity first was recorded during the run, coded as “WHEN.” These three single measurement point variables also were analyzed by analysis of variance (lesion site by retinal effect). Further analyses for significant differences were made by post-hoc Scheffe test or by the embedded specific comparisons (contrast analysis) from within the same program.

Results

Results from the 27 successful parafoveal exposure data runs are graphed in Figures 3 and 4 for the time segment data and in the upper panel of Figure 5 for the single measurement point data. The traces in Figure 3 show an increasing effect on post-exposure VEP magnitude onset time with increasing retinal effect. In addition, an apparent trend to recovery can be seen even at the “red dot” lesion level. In all cases, the parafoveally exposed animals returned to baseline acuities by the next exposure. Results from the eight parafoveally exposed animals returned to baseline acuities by the next exposure. Results from the eight parafoveally exposed animals returned to baseline acuities by the next exposure. Results from the eight parafoveally exposed animals returned to baseline acuities by the next exposure. Results from the eight parafoveally exposed animals returned to baseline acuities by the next exposure.

In addition, significant interaction effects were seen between the three post-exposure time segments and the baseline time segment demonstrated that all three main effects (retinal location, retinal effect, and measurement time segment) were significant at $P < 0.001$ when viewed over the entire data set. Specifically, foveal exposures more effectively delayed the VEP onset than did parafoveal exposures, and the more severe the lesion, the greater the effect. In addition, significant interaction effects were seen between location and lesion ($P < 0.01$) and between lesion and measurement time segment ($P < 0.05$).

For the parafoveal exposure sites, specific comparisons between the three post-exposure time segments and the baseline time segment demonstrated that the lock-in delay was significant for red dot lesions for the first 3 min (post1 and post2 time segments; $P < 0.04$ for each) and for white dot lesions for the post1 ($P < 0.01$) and post3 time segments ($P < 0.04$). No significant relationship was found in the measures of the time segment standard deviation for these parafoveal exposures—the acuity estimates within the time segment did not change their sweep-to-sweep variability. In contrast, the foveal exposure results were less clear-cut statistically because of the small number of samples and the resulting greater variance in each cell.
Nevertheless, the post2 and post3 time segments were significantly elevated (delayed) compared to the baseline for the red dot lesions ($P < 0.01$ for each), as was the post3 time segment for the white dot lesions ($P < 0.05$). The variability of the post1 time segment also was significantly elevated for the white dot foveal lesion only ($P < 0.01$). The remaining data points had too great a range to attain significance.

The overall analysis of the three single measurement point data showed significant effects for location ($P < 0.05$) and retinal effect ($P < 0.01$), but no interaction effects. Specific comparisons (contrast analysis) on the immediate effect for the parafoveal and the foveal exposures showed the same results: Effects produced by either retinal lesion type (white or red dot) were significantly worse than effects produced by no dot exposures ($P < 0.05$ for parafoveal no dot vs red dot; all other comparisons to no dot for each location were $P < 0.01$). Overall, red dot and white dot immediate lesion effects were not statistically different. Directly comparing the most severe effect by contrast analysis of the no dot and red dot

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**Fig. 3.** Averaged acuity estimate data from all parafoveal exposures by retinal effect—no dot, white dot, and red dot. Boxes indicate the data points that were combined for summary statistics. The discrete spatial frequency transition points are given on the ruler to the right (horizontal short lines) and are matched to the time into sweep axis on the left.
parafoveal data showed a significant difference ($P < 0.05$) and accounted for the significant difference between the overall foveal and parafoveal data sets as shown by the analysis of variance ($P < 0.05$). The onset time of greatest acuity deficit showed no effect at $P < 0.05$, although the trend suggests an increasing effect—ie, earlier onset of worst acuity with increasing retinal effect and with the foveal (versus parafoveal) location.

**Discussion**

In general, the data sets show clear results and expected trends. The loss of statistical significance for certain comparisons can be ascribed to a small "n" effect or a "floor" effect in that the acuity could get no worse than a nominal 0 cycles/degree, coded in this experiment as an acuity onset (ceiling) of 15,000 msec (the end of each sweep). In contrast, the unexpected result that localized single parafoveal burns can have a significant effect on immediate post-exposure (foveal) response—ie, an effect on retina 5° away may have broad implications in treatment and accident scenarios. This response time shift was not sufficient to drop the visual system out of the spatial frequency time segment in the time allotted. Specifically in this experiment, a factor of 1.5 in angle of resolution for 5 sec would have been required and would have moved the average acuity estimate from 19.9 cycles/degree (1.5 arcmin or 20/30 equivalent) to 13.2 cycles/degree (2.27 arcmin or 20/45 equivalent). This change may not be considered a clinically significant change in "vision," but it is enough to show a delay in acquiring the stimulus and implies that the effect produced was sufficient to require a recovery time from the exposure. This effect may be analogous to an induced threshold shift that can produce a delay in perception.

**Fig. 4.** Means and standard errors of the mean (upper panel) and standard deviation of the means (lower panel) derived from the combined data points of the parafoveal exposures. Spatial frequency steps are given in the upper panel, as in Figure 3.

**Fig. 5.** Point values taken from the acuity estimate traces for parafoveal (upper panel) and foveal (lower panel) exposures. The left two sets of bars are the mean acuity variables "IMMED" and "WORST" taken from each sweep for the three retinal effects. The spatial frequency steps are shown, as in Figure 2. The right-most set of bars is the variable "WHEN" extracted from the time into the experimental session.
Fig. 6. Averaged acuity estimate data from all foveal exposures graphed, as in Figure 3.

or reaction time.33-34 Alternatively, the effect might be modeled as the induction of an "amblyopia"35 or the superposition of an equivalent background mask.36

Of further interest is that foveal burns produced a severe initial short-term fluctuation of acuity and a permanent decrease in final acuity. The mechanism for this remains obscure but might be conceptually similar to the induction of fluctuating photic afterimages by direct changes to neurons or the neuronal environment,37 rather than by photopigment bleaching. Although second harmonic generation by rhodopsin to infrared laser flashes has been reported (ie, perception of 532 nm from a 1064 nm pulsed laser38), the intensity of this flash, if present, probably is sufficiently weak to explain only a small portion of the response changes seen here. However, this cannot not be ruled out as a contributing factor. The infrared laser wavelengths used here by themselves do not produce a "flashblinding" effect, as might be seen with a visible wavelength laser exposure. These wavelengths provide evidence of an effect that is not restricted to the irradiance diameter of the exposure. The existence of an induced elevated short-term response variability and the extent of the retinal spread of these effects may have functional implications for time-sensitive visual tasks.

Clinically, laser burns are used to seal off leaking retinal vessels, tack down detached retinas, and ablate tissue in the eye. Many patients develop conditions that require laser treatment in the macula. A concern of the patient and the treating physician is the risk to visual acuity from contiguous injury to retinal tissue near a treatment site. Such damage spread may adversely threaten vision to the extent that the treatment could be contraindicated. Until now, there has been no parametric study on the effect of such treatment in an animal model, so clinicians have based their judgments on experience and the risk of disease progression, with minimal help from case histories. The work
reported here indicates that, at least transiently, laser injury has a nonlocal effect on the retina that may affect visual acuity. The long-term visual effects from such laser injury await VEP microperimetry of the lesion sites in these animals longer term follow-up VEP acuity measurements.

Key words: acuity, laser, lesion, monkey, retina, VEP

References