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Functional and Cellular Responses to Laser Injury in the Rat Snake Retina

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ABSTRACT

Acute (1-hr, 6-hr) and longer term (24-hr) effects of laser injury on retinal function and cellular responses have been studied in the Great Plains rat snake, *Elaphe guttata emoryi*. This animal is of interest for vision research because its eye has an all-cone retina. A linear array of 5 thermal lesions was placed in the retina of anesthetized animals, near the *area centralis*, using a Nd:VO₄ laser (532 nm), that delivered 50 mW per 10-msec pulse. Retinal function was assessed with the pattern electroretinogram (PERG), recorded before and after the placement of the lesions. PERGs were elicited with counterphased square-wave gratings, and were analyzed by Fourier analysis. The fate of lesioned cells was assessed by immunohistological staining for the transcription factor, NF- κ B (which is activated by ionizing and non-ionizing radiation), as well as for the apoptosis marker, caspase-9. The normal snake PERG had the maximum, real amplitude frequency component, determined by Fourier analysis, at the reversal frequency of the grating (i.e. shifts/sec). In the hour following the lesion-producing laser exposures, the PERG response exhibited frequency doubling, i.e. a new response waveform appeared at twice the reversal frequency. By 24-hr post exposure, many lesioned photoreceptors stained positively for both NF- κ B and caspase 9. Because the PERG largely reflects retinal ganglion cell activity, the appearance of frequency doubling in the PERG suggests that complementary (push-pull) inputs to ganglion cells are disrupted by the laser lesions. The immunohistological results indicate that activation of NF- κ B is not necessarily associated with photoreceptor survival after a laser injury.

Keywords: apoptosis, electroretinogram, injury, laser, NF- κ B, photoreceptor, retina, snake

1. INTRODUCTION

1.1 Functional investigation of laser eye injuries

Historically, many ocular laser bioeffects studies have been carried out in non-human primate subjects, or in smaller mammalian models, especially rabbits.¹⁻⁸ Non-mammalian species typically have not been used for these types of studies, although many members of the reptile and amphibian classes have excellent visual function, and would be suitable for research into the ocular effects of laser exposure. One promising reptilian candidate is the Great Plains rat snake, *Elaphe guttata emoryi*. The small size, high numerical aperture, all cone retina, and transparent spectacle (i.e. the specialized scale covering the eye) characteristic of the snake eye combine to form a unique suite of ocular properties. These features enable prolonged imaging through the natural pupil of cellular detail in distinct layers of retinal tissue, using the confocal scanning laser ophthalmoscope (cSLO). This animal model has been used previously to examine, *in vivo*, the temporal development of laser-induced retinal photoreceptor damage.^{9, 10} In addition to its imaging capabilities, the cSLO can also be modulated externally to produce stimulus patterns directly on the subject's retina for psychophysical or electrophysiological testing, in order to achieve functional evaluation of laser eye injuries. The advantage of this method is that the region of retina under investigation may be imaged and stimulated simultaneously in order to evaluate visual function. In the present investigation, we have demonstrated that the pattern electroretinogram (PERG) can be recorded from the snake's eye by direct stimulation of the retina with counterphased square wave gratings produced by the cSLO.¹¹ This evoked response has been shown to be due to the mass response of

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retinal ganglion cells,¹²⁻¹⁴ and also reflects the functional integrity of the central retina, including the fovea in primates.¹⁵ Although the snake retina lacks a foveal region, it has an *area centralis*, which may subserve visually-intensive tasks such as prey catching, etc., and, therefore, laser-induced perturbations in the PERG provide a useful indication of the effects of laser retinal injuries on overall visual function.

1.2 Cellular investigations of laser eye injuries

In addition to light and laser injuries that affect retinal function, the cellular responses to photic injuries, such as cellular disruption, denaturation, and photochemical damage, have also been investigated.¹⁷⁻¹⁹ For exposures in which the irradiance is low enough to avoid inducing acute cellular damage through thermal or stress-related mechanisms, photochemical damage may result through oxidative stress. In the snake retina, previous studies have documented the role of photo-oxidative stress in inducing photoreceptor damage.²⁰ The present research was designed to characterize the laser-induced response of intracellular signaling pathways, in particular, the transcription factor, NF- κ B, that are known to be responsive to oxidative stress. Many external stressors activate NF- κ B, including toxins, pathogens (including bacteria and viruses), physiological stress (e.g. ischemia or shear stress), oxidative stress, and physical stress (e.g. hyperthermia), as reviewed by Pahl.²¹ Other specific activators include inflammatory cytokines^{22, 23} and ionizing radiation.²⁴⁻²⁶ Photo-oxidative stress, such as that produced by UV exposure, has been shown to induce NF- κ B activation in cultured cells, primarily through the generation of reactive oxygen species.²⁷⁻²⁹ Moreover, UV exposure can activate NF- κ B in enucleated cells, indicating that the sensitive receptor element mediating the activation is in the cytoplasmic compartment, most likely in the plasma membrane.^{27, 30} Consistent with the interaction of oxidative stress with NF- κ B, treatment with antioxidants usually reduces the activation of the transcription factor.³¹⁻³³ In our previous research, we have demonstrated that laser exposure induces NF- κ B nuclear translocation in cultured human retinal pigment epithelial (RPE) cells, and this activation can be reduced by ascorbic acid (vitamin C), but not by thiol antioxidants.³⁴

1.3 Consequences of NF- κ B activation in injured cells

The genes activated by NF- κ B are involved in a wide range of cellular activities and function. About 150 genes are known to be activated by NF- κ B/Rel proteins.²¹ These genes are involved in cellular repair, restoration and proliferation, as well as production of pro- and anti-apoptotic factors. Many of these genes have contrasting actions on the cell; not all the genes are activated by every NF- κ B/Rel family member. Particularly noteworthy is that NF- κ B activation is capable of modulating immune responses and mediating inflammatory reactions, as evidenced by its effect on the expression of pro-inflammatory cytokines, such as TNF- α and $-\beta$, IL-1 α and $-\beta$, IL-6, and IFN α .²¹ Recognition of the role of NF- κ B in promoting or mediating an inflammatory response after retinal laser injuries would be an important advance in understanding the cellular responses to - and possibly developing an effective therapy for - such injuries. A additional question is whether NF- κ B activation represents a death (apoptotic) signal, or the initiation of a repair response. The answer to this question appears to be complex, and highly cell type-dependent. Depending on the cellular system, and the type of activating stress involved, NF- κ B may either promote or prevent apoptosis.³⁵ Although NF- κ B has been associated with both cell survival and apoptosis, depending on the particular cell line or tissue,³⁶ in ocular light damage it has been associated with survival, i.e. light-damaged retinal photoreceptors *in vitro*, that failed to activate NF- κ B, did not survive, undergoing cell death by either apoptosis or necrosis.^{37, 38} Therefore, characterization of NF- κ B activation following laser injury to the retina and retinal pigment epithelium (RPE) may be a useful way to assess the severity of the laser injury, as well as the efficacy of experimental therapies. In the current research, NF- κ B activation has been examined in the snake retina following suprathreshold, visible light laser injuries, and the association of the transcription factor with cell survival or apoptosis has been determined.

2. METHODS

2.1 Subjects

Great Plains rat snakes (*Elaphe guttata emoryi*) were studied. Animals were anesthetized with an intramuscular injection of ketamine/zylazine in a ratio of 3.5:1, respectively, at an approximate dose of the combined drugs of 75 mg/kg. During the recording sessions, the animals' body temperature remained at the ambient (room) temperature.

2.2 Retinal imaging, stimulation, and laser exposure

A Rodenstock cSLO was used to image the snake retina. Animals were placed in a custom-made holder that allowed their eyes to be positioned at the aperture of the cSLO. This arrangement permitted imaging the fundus of the eye,

revealing aspects of retinal structure such as the photoreceptor matrix, retinal vessels, and optic nerve head. The PERG was elicited with high contrast, counterphased square wave gratings generated directly on the snake's retina, by modulating the HeNe laser (632.8 nm) of the cSLO. The pattern modulation was produced with a computer-based visual stimulator (VisionProbe®, San Antonio, TX) driving the acoustic-optical modulator of the cSLO, which in turn actively modulated the cSLO's HeNe laser to produce the grating stimuli on the animal's retina. During visual stimulation, the retina was simultaneously imaged with the cSLO to ensure that the eye was stable throughout the electrophysiological recording period. The experimental arrangement for visual stimulation and recording of the PERG is shown in Figure 1. Laser exposures to the retina were made with a shuttered CW, 532 nm, Nd:VO₄ laser (Intellite, Inc., Albuquerque, NM), that was aligned co-axially with the cSLO. Each exposure delivered 50 mW in a 10-ms pulse, resulting in a 75-100 μ m diameter retinal lesion.

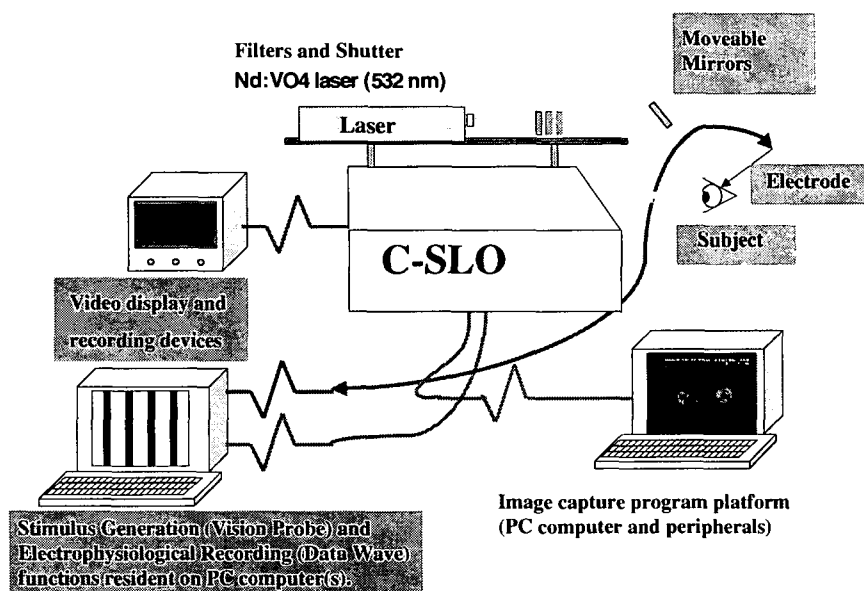


Figure 1. Schematic of cSLO, laser, and electrophysiological recording apparatus.

2.3 Electrophysiology

The snake's eye is covered by a specialized scale called the spectacle that is electrically nonconductive. In order to record the PERG from the eye, a small triangular flap was cut in the spectacle near the limbus of the eye, and reflected to expose the cornea. This was accomplished without injuring the cornea itself. The tip of a blunted, Grass needle electrode was placed through the incision so that it contacted (but did not penetrate) the corneal surface. Electrical contact was improved by occasionally applying a drop of Ringer's solution onto the electrode tip-tissue junction. Following the recording session, the spectacle flap was replaced, to prevent corneal dessication, and the animal was allowed to recover. At the animal's next skin-shed cycle, the entire spectacle was replaced normally.

2.4 Signal Acquisition and Data Processing

Electrophysiological responses were amplified using a Grass Neurodata 12 amplifier at 10,000 times gain with frequency bandpass limits set from 1 Hz to 3 KHz. Amplified signals were acquired with the Experimenter's Workbench® system (DataWave, Longmont, CO). Conventional signal averaging was performed to acquire the PERG. The PERG responses to sixty-four iterations of a complete cycle of the pattern reversal were recorded and averaged. The averaged waveform was exported as an ASCII data file, and imported into the ProStat statistical analysis software package (PolySoftware, Pearl River, NY), in which it was digitally filtered to remove noise and then subjected to FFT, in order to extract the real amplitude of frequency components at the stimulus counterphase rate and its even harmonics.

2.5 Immunohistology

The cellular responses of snake retinal neurons were studied using immunohistology. Animals were euthanized at

various times after placement of laser lesions in the retina. The eyes were quickly dissected and fixed in 10% neutral buffered formalin. Following dehydration, the tissue was embedded in paraffin and histological sections were cut at 6 micron thickness and mounted on glass slides. Activation of the transcription factor, NF- κ B, in retinal cells was detected by staining with a rabbit antibody against the p65 monomer. Binding of the anti-p65 primary antibody was determined with an anti-rabbit detection antibody conjugated to the fluorophore, Texas Red. Cells undergoing apoptosis were detected by staining with a rabbit anti-caspase-9 antibody. Caspase-9 is a protease and a component of the apoptotic signal cascade in many cells. Binding of the caspase-9 antibody was detected with an anti-rabbit secondary, fluorescein-conjugated antibody. All of the antibodies were purchased from Abcam (Cambridge, MA; www.abcam.com), and were used as directed by the manufacturer's protocol. The stained sections were imaged with conventional epifluorescence microscopy, using excitation and emission filters appropriate for the fluorophores conjugated to the secondary antibodies.

3. RESULTS

3.1 The rat snake PERG: dependence on stimulus spatial and temporal frequency

The pattern ERG (PERG) response is formally elicited by retinal stimuli producing a contrast change, but no net change in luminance. These stimulus conditions were met by generating counterphasing, high-contrast, square wave gratings with the cSLO, and projecting these grating patterns directly onto the snake's retina. The PERG response consisted of an electrical potential generated across the eye by each reversal of the square wave grating. The spatial frequency of the stimulus is defined as the number of light/dark bars per degree of visual angle. The temporal frequency of the stimulus is defined as the number of complete cycles of pattern reversals per second; for example, a 1 Hz grating undergoes two pattern reversals per second, i.e. has a counterphase rate of 2 shifts/sec. The stimulus spatial and temporal parameters were varied in order to characterize the response characteristics of the snake PERG, as shown in Figures 2 and 3. In terms of the optimal stimulus conditions, the maximum amplitude PERG was produced by square wave gratings of 0.5-1.0 cycles per degree (cpd) spatial frequency (see Figure 2), and 2 Hz temporal frequency (see Figure 3).

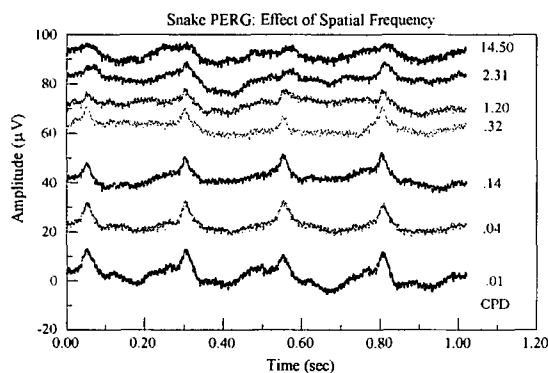


Figure 2. Snake PERG as function of stimulus spatial frequency.

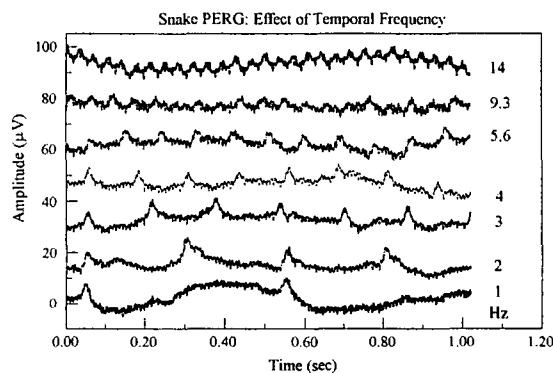


Figure 3. Snake PERG as function of stimulus temporal frequency.

3.2 Effect of defocus on the PERG

In order to demonstrate that the PERG was evoked by a change of contrast (i.e. the stimulus pattern) and not by an overall luminance change (e.g. caused by an artifact in the stimulus presentation), an experiment was done in which the grating pattern produced by the cSLO on the snake's retina was defocused by moving the confocal plane of the instrument away from the retinal photoreceptor layer. This caused a decrease in the stimulus contrast (which is focus-dependent), but not in the overall stimulus luminance (which is focus-independent), and resulted in loss of the PERG amplitude and synchrony, proving that the recorded response was pattern-dependent. The results of this experiment are illustrated in Figure 4.

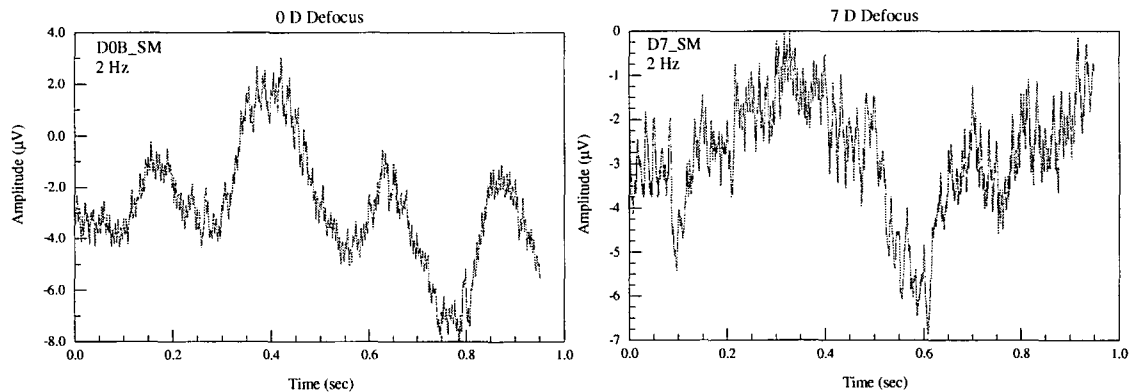


Figure 4. Effect of defocus on the snake PERG. Defocus was induced by changing the confocal plane of the cSLO. The response to a focused grating is shown on the left, while the effect of 7 diopters of defocus is shown on the right.

3.3 Histological appearance of laser lesions in the snake retina

Exposure of the snake retina to the visible laser emissions of the Nd:VO₄ laser produced lesions that appeared almost immediately, and were easily imaged with the cSLO. The lesions appeared as whitish rings that became more intense in the minutes following the exposure (Figure 5, upper panel, showing fresh appearance of laser lesions in the cSLO), and then reached a stable intensity that lasted for several days to weeks, after which time their appearance faded slowly without entirely disappearing over follow-up times as long as 2.5 yrs. Histological analysis of the lesioned retinas revealed that the laser-induced damage was confined to the photoreceptor and retinal pigment epithelial layers (Figure 5; lower panel, showing hematoxylin and eosin stained tissue).

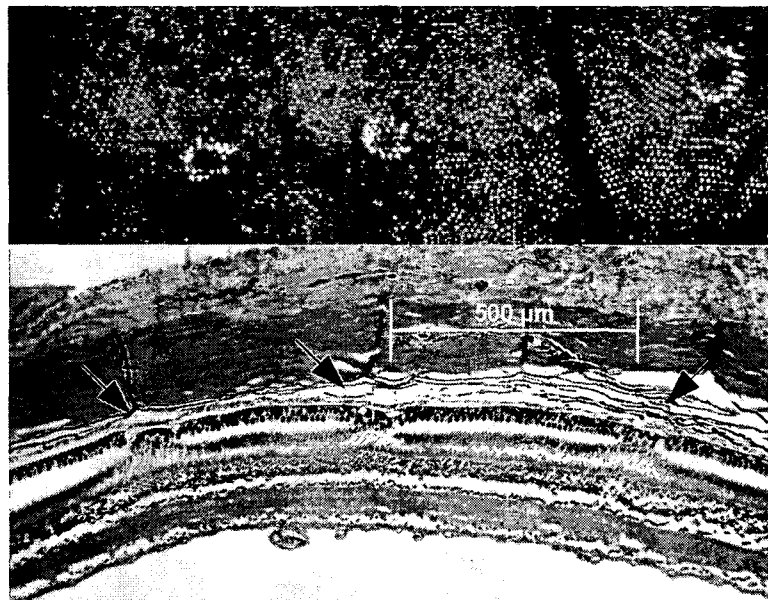


Figure 5. Upper panel: cSLO image showing appearance of linear array of 5 laser lesions. Lower panel: hematoxylin and eosin stained section of snake retina; arrows point to 3 lesions visible in the outer retina and RPE layer.

3.4 Perturbations in the snake PERG following suprathreshold laser lesions

Due to technical limitations, the PERG could not be recorded during the actual placement of the laser lesions, but it was recorded prior to and after the exposure. The effect of the laser exposure was not to suppress the PERG, but, rather, to induce a peculiar change in its response characteristic. The typical PERG response consists of a positive potential produced by each contrast-shift of the stimulus pattern, as noted above in section 3.1. Following the completion of the laser lesions, however, the response of the PERG was notably changed, even in the first sample epoch, i.e. about 1.5 min after the laser exposure. Instead of the peak response at a frequency component corresponding to the counterphase rate of the stimulus, a frequency-doubled response was recorded, in which the peak response occurred at frequency component at twice the counterphase rate. In some stimulus conditions, there was even frequency-quadrupling observed in the PERG. These response perturbations are illustrated in the following figures. As noted in the Methods, the waveforms shown in these figures are the result of averaging 64 "sweeps", i.e. each sweep contains a complete back-and-forth alternation of the stimulus pattern.

In Figure 6, the PERG responses to a 3 cpd ($\approx$ cycle per degree), 4 Hz ($\approx$ cycle per second, cps), square-wave grating are shown before and after the placement of a linear array of five lesions in the *area centralis*. Before the laser lesion, the PERG response is composed primarily of a waveform with eight peaks, although some higher frequency components are clearly visible. The eight peaks correspond to each shift of the stimulus pattern (4 Hz temporal frequency has 8 shifts/second). After the laser exposures, however, the PERG waveform is clearly dominated by a response at twice the counterphase rate (~ 16 Hz). This response perturbation is called "frequency doubling"

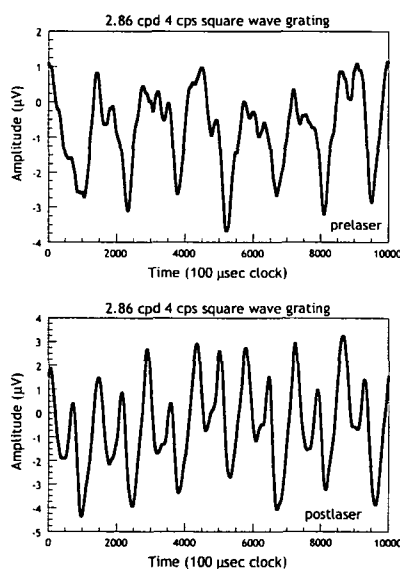


Figure 6. Frequency doubling in the snake PERG after retinal laser lesion; example 1

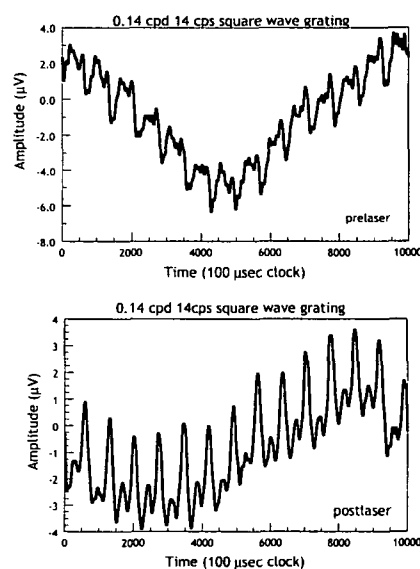


Figure 7. Frequency doubling in the snake PERG after retinal laser lesion; example 2.

Another example of frequency doubling is shown in Figure 7. In this case, the stimulus grating was presented at a lower spatial frequency, 0.14 cpd, but a higher temporal frequency, 14 Hz. After the laser exposure, frequency doubling is obvious, but the frequency components of the response are asymmetric, with the peak corresponding to the onset of the pattern shift having a larger amplitude than the induced high frequency component. The response perturbation in this example differs from the previous one, in which the frequency components, although not completely equal, were more similar in amplitude.

The degree of frequency doubling may be appreciated by the ratio of the F_2 to F_1 components, as determined by FFT analysis. " F_1 " refers to the real amplitude of the frequency component corresponding to the counterphase rate, while " F_2 " refers to the real amplitude of the component corresponding to twice the counterphase rate. A summary of results is shown in Figure 8; frequency doubling is most pronounced at higher spatial frequencies, i.e. those above 1 cpd,

possibly because the lower spatial frequencies present too few contrast boundaries across the damaged photoreceptors to produce the abnormal response.³⁹

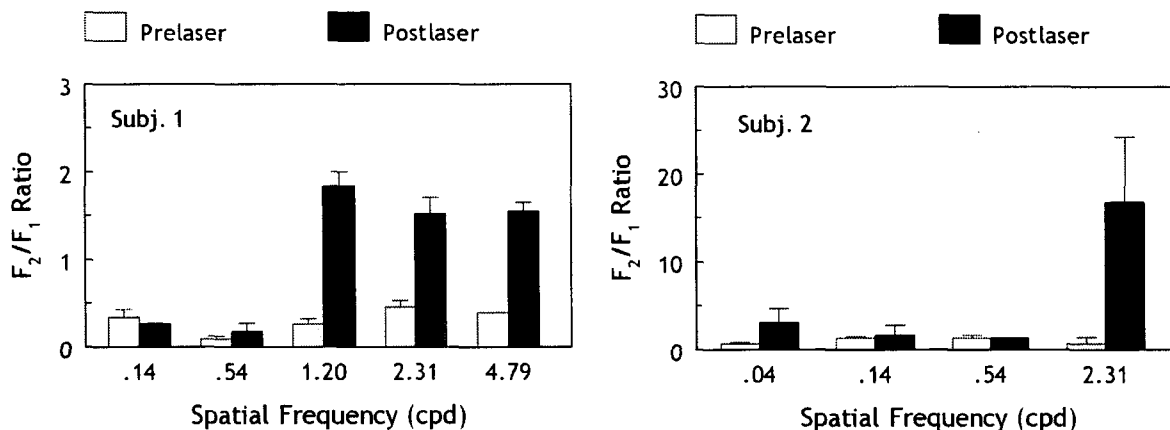


Figure 8. Summary of laser-induced frequency doubling in the PERG (F_2/F_1 ratio from FFT analysis) for two snake subjects, data are shown for various stimulus spatial frequencies.

Finally, the frequency doubling appears to be a permanent change in the retinal response. Over the course of one hour following the laser exposure, the PERG continuously exhibited frequency doubling. This perturbed response was still present at subsequent recording sessions, even as late as 1.5 years following exposure. Thus, the change in the PERG response likely reflects permanent tissue damage affecting the retinal circuitry.

3.5 Immunohistological analysis of retinal laser lesions

A linear array of laser lesions, made with the same exposure parameters as for the PERG sessions, were made in three snakes. One animal each was sacrificed at 1, 6, and 24 hrs post-exposure, and the eyes were prepared for immunohistological analysis as described in Section 2.5. The tissue was probed with antibodies against the p65 component of NF- κ B, and against caspase-9. In this series of studies, the laser lesions were clearly defined histologically by 24 hrs (as shown in Figure 5). In tissue from the 24-hr post-exposure animal, the immunohistology revealed co-localization of the two markers in damaged photoreceptors (the structures showing yellow fluorescence in Figure 9), indicating that both NF- κ B and caspase-9 are expressed in the same cells after laser injury, and that expression of NF- κ B does not necessarily protect or prevent these cells from undergoing apoptosis.

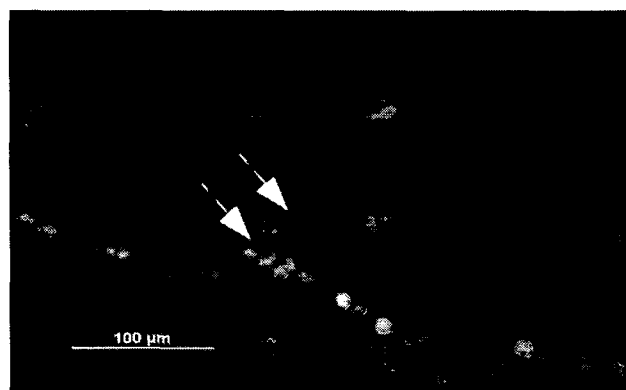


Figure 9. Immunohistological section of snake retina with laser lesion. Cellular damage occurred in photoreceptor and RPE cells. The white arrows indicate sites of NF- κ B and caspase-9 co-localization in the photoreceptor cell bodies and terminals.

4. DISCUSSION

4.1 Spectral sensitivity and the PERG in the rat snake retina

Previous work characterized the spectral sensitivity of the flash ERG in the garter snake, which has an all-cone retina and is also a colubrid snake closely related to the rat snake^{40, 41}, but to our knowledge this is the first report of a pattern-evoked electroretinographic response recorded from the rat snake's eye. It is also the first reported use of the cSLO for electrophysiological testing in a reptile model. Based on the observations reported here, the snake eye has a relatively low acuity function, exhibiting a maximum PERG response with square wave gratings of approximately 1 cpd, and at the relatively low temporal frequency of 2 Hz. According to the study of Sillman et al.,⁴¹ the colubrid all-cone retinas have mid-wavelength (554 nm), short-wavelength (482 nm), and UV (360 nm) sensitive cones. Our cSLO was only configured with a HeNe laser for production of the visual stimuli; the 633 nm emission of this laser represented a wavelength at which the mid-wavelength cone has about 20% of its maximum sensitivity. Although the red stimuli were suboptimal for this animal's retina, there was sufficient sensitivity in the mid-wavelength cone system to mediate a robust PERG response.

4.2 Proposed mechanism for laser-induced frequency doubling

Because the histologically demonstrated damage inflicted by the laser exposure is restricted to the photoreceptors and RPE cells, it is likely that frequency-doubling results from the loss of particular inputs to ganglion cells that generate the PERG. The neural information from photoreceptors is transmitted to the ganglion cells through a highly interconnected neural circuit comprised of the bipolar and amacrine cells.⁴²⁻⁴⁴ The bipolar to ganglion cell synapse is rectifying and its transfer function is highly nonlinear. In order to linearize the ganglion cell response, additional input is required from other neurons. Such linearizing input is provided by "push-pull" inputs mediated by laterally-connecting amacrine cells. Recently, the narrow-field glycinergic amacrine cells have been shown to carry such push-pull input to the ganglion cell (Figure 10).⁴⁵ We hypothesize that the multiple laser lesions placed in this study disrupt push-pull inputs, possibly by disabling photoreceptors generating the input to the narrow-field amacrine cells, and thus introduce harmonic distortion in retinal ganglion cell response. The frequency doubling in the PERG, that we have observed in the present investigations, typify the nonlinear distortion expected to result from loss of complementary inputs. In order to test this hypothesis, it is possible that similar distortion could be introduced into the response of ganglion cells, and hence, into the PERG, by the introduction of a glycine inhibitor, such as strychnine, into the retinal neuronal circuits (thereby removing the contribution of the narrow-field amacrine cells).

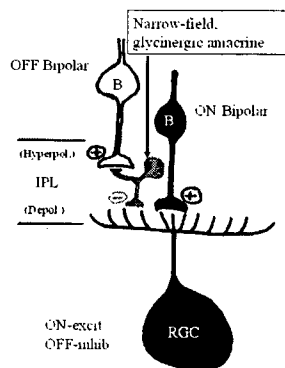


Figure 10. Schematic showing narrow field amacrine cells thought to carry push-pull input to ganglion cells. Laser damage to these circuits is hypothesized to be the source of frequency doubling in the PERG.

4.3 NF- κ B activation does not necessarily signal survival in laser-injured cells in the snake retina

The CW laser used to produce the retinal lesions in this study delivered exposures sufficient to produce thermal stress in the retina, based on the damage mechanisms underlying the ANSI laser safety standards. Moderate thermal stress has been shown to activate the transcription factor, NF- κ B.⁴⁶⁻⁴⁸ Suprathreshold laser lesions producing thermal stress lead to apoptosis in damaged cells, although the higher the laser irradiance, the more likely the cells will undergo immediate necrotic death, rather than apoptosis.⁴⁹ Nevertheless, previous work with the snake eye model revealed that laser exposures of the magnitude used in the current research also induce oxidative stresses.²⁰ Moreover, visible laser exposure produces photo-oxidative stress through the interaction of light with susceptible chromophores, such as melanin in the RPE cells, resulting in the formation of reactive free radicals.³⁴ This process does not involve additional energy sources, or other enzymatic pathways (such as oxidases), although various enzyme-catalyzed reactions may also be involved.⁵⁰ Thus, the laser-induced change in the cell's redox state may itself induce NF- κ B upregulation and nuclear translocation.^{31, 51} In this context, our observation that both NF- κ B and caspase-9 are activated in laser-damaged photoreceptors shows that NF- κ B upregulation is not necessarily a survival factor in these cells after light damage. This differs from the findings of others,^{37, 38} who reported that light-damaged photoreceptors (in cell culture) insufficiently expressing NF- κ B did not survive. Our results may differ, not only because of possible differences between *in vivo* and *in vitro* models with respect to their response to a given stressor, but also because the light injury in the present study was more severe than in the earlier work (the *in vitro* study used an irradiance of 4.5 mW/cm²).³⁸ Whether the NF- κ B activation is actually pro-apoptotic in these damaged photoreceptors has yet to be proven. Nevertheless, the present observations suggest that pharmacological intervention in the NF- κ B pathway may not be a productive approach for the development of novel therapies for laser eye injuries, and other strategies must be pursued.

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REFERENCES

1. R.D. Glickman, "Prolonged activity of retinal ganglion cells following intense, nanosecond laser flashes," *Clin. Vision Sci.*, **4**, pp. 1-18, 1989.
2. D.H. Sliney, J. Mellerio, V.P. Gabel, and K. Schulmeister, "What is the meaning of threshold in laser injury experiments? Implications for human exposure limits," *Health Phys.*, **82**, pp. 335-347, Mar. 2002.
3. J.A. Zuclich, P.R. Edsall, and P.K. Kennedy, "Variation of laser induced retinal-damage threshold with retinal image size," *J. Laser Applications*, **12**, pp. 74, 2000.
4. R. Birngruber, V.P. Gabel, and F. Hillenkamp, "Experimental studies of laser thermal retinal injury," *Health Phys.*, **44**, pp. 519-531, 1983.
5. C.P. Cain, C.D. DiCarlo, B.A. Rockwell, P.K. Kennedy, G.D. Noojin, D.J. Stolarski, D.X. Hammer, C.A. Toth, and W.P. Roach, "Retinal damage and laser-induced breakdown produced by ultrashort-pulse lasers," *Graefes. Arch. Clin. Exp. Ophthalmol.*, **234 Suppl 1**, pp. S28-S37, 1996.
6. D. McHugh, C. England, E. van der Zypen, J. Marshall, F. Fankhauser, and S. Fankhauser-Kwasnieska, "Irradiation of rabbit retina with diode and Nd:YAG lasers," *Br. J. Ophthalmol.*, **79**, pp. 672-677, 1995.
7. W.P. Roach, M.E. Rogers, B.A. Rockwell, S.A. Boppart, C.D. Stein, and C.M. Bramlette, "Ultrashort laser pulse effects in ocular and related media," *Aviat. Space. Environ. Med.*, **65**, pp. A100-A107, 1994.
8. S.T. Schuschereba, P.D. Bowman, R.E. Ferrando, D.J. Lund, J.A. Quong, and J.A. Vargas, "Accelerated healing of laser-injured rabbit retina by basic fibroblast growth factor," *Invest. Ophthalmol. Visual. Sci.*, **35**, pp. 945-954, 1994.
9. H. Zwick, B.E. Stuck, P. Edsal, R. Elliott III, H. Hacker, E. Wood, and R. Cheraimie, "Photoreceptor damage and recovery in the high numerical aperture eye of the snake," in *Ophthalmic Technologies XV*, F. Manns, P.G. Söderberg, A. Ho, B.E. Stuck, M. Belkin Eds., Proc. SPIE **5688**, pp. 320-328, SPIE, Bellingham, WA, 2005.
10. H. Zwick, R. Elliott, G. Li, A. Akers, P. Edsall, and M.E. Stuck, "In-vivo imaging of photoreceptor structure and laser injury pathophysiology in the snake eye," in *Ophthalmic Technologies IX*, P.O. Rol Ed., Proc. SPIE **3591**, pp. 368-374, SPIE, Bellingham, WA, 1999.
11. R.D. Glickman, W.R. Elliott III, H. Zwick and H. Rentmeister-Bryant, "The pattern ERG in the rat snake

- exhibits frequency-doubling as an acute response to laser retinal injury," *Invest. Ophthalmol. Vis. Sci.*, **46**, E-Abstract 5691, 2005.
12. R.P. Schuurmans and T. Berninger, "Luminance and contrast responses recorded in man and cat," *Doc. Ophthalmol.*, **59**, pp. 187-197, 1985.
13. J.B. Troy, G. Einstein, R.P. Schuurmans, J.G. Robson, and C. Enroth-Cugell, "Responses to sinusoidal gratings of two types of very nonlinear retinal ganglion cells of cat," *Vis. Neurosci.*, **3**, pp. 213-223, 1989.
14. G. Dagnelie, M.J. de Vries, J. Maier, and H. Spekreijse, "Pattern reversal stimuli: motion or contrast?" *Doc. Ophthalmol.*, **61**, pp. 343-349, Jan 15, 1986.
15. L. Maffei, A. Fiorentini, S. Bisti, and H. Hollander, "Pattern ERG in the monkey after section of the optic nerve," *Exp. Brain Res.*, **59**, pp. 423-425, 1985.
16. A. Fiorentini, L. Maffei, M. Pirchio, D. Spinelli, and V. Porciatti, "The ERG in response to alternating gratings in patients with diseases of the peripheral visual pathway," *Invest. Ophthalmol. Visual. Sci.*, **21**, pp. 490-493, 1981.
17. R.D. Glickman, "Phototoxicity to the retina: Mechanisms of damage," *Int. J. Toxicol.*, **21**, pp. 473-490, 2002.
18. D.T. Organisciak, R.A. Darrow, L. Barsalou, R.M. Darrow, and L.A. Lininger, "Light-induced damage in the retina: differential effects of dimethylthiourea on photoreceptor survival, apoptosis and DNA oxidation," *Photochem. Photobiol.*, **70**, pp. 261-268, 1999.
19. J.E. Roberts, "Ocular phototoxicity," in *Dermatotoxicology*, F.N. Marzuli Ed., pp. 307-313, Taylor and Francis, Washington, D.C., 1996.
20. W.R. Elliott III, H.K. Rentmeister-Bryant, N. Barsalou, J. Beer, and H. Zwick, "N-acetylcysteine and acute retinal laser lesions in the colubrid snake eye," in *Laser Interaction with Tissue and Cells XV*, S.L. Jacques, W.P. Roach Eds., Proc. SPIE **5319**, pp. 267-273, SPIE, Bellingham, WA, 2004.
21. H.L. Pahl, "Activators and target genes of Rel/NF-kappaB transcription factors," *Oncogene*, **18**, pp. 6853-6866, 1999.
22. A.A. Beg and D. Baltimore, "An essential role for NF-kappaB in preventing TNF-alpha-induced cell death," *Science*, **274**, pp. 782-784, 1996.
23. X. Hu, M. Tang, A.B. Fisher, N. Olashaw, and K.S. Zuckerman, "TNF-alpha-induced growth suppression of CD34+ myeloid leukemic cell lines signals through TNF receptor type I and is associated with NF-kappa B activation," *J. Immunol.*, **163**, pp. 3106-3115, 1999.
24. N. Mohan and M.L. Meltz, "Induction of nuclear factor kappa B after low-dose ionizing radiation involves a reactive oxygen intermediate signaling pathway," *Radiation Research*, **140**, pp. 97-104, 1994.
25. S.J. Lee, A. Dimtchev, M.F. Lavin, A. Dritschilo, and M. Jung, "A novel ionizing radiation-induced signaling pathway that activates the transcription factor NF-kappaB," *Oncogene*, **17**, pp. 1821-1826, 1998.
26. U. Raju, G.J. Gumin, and P.J. Tofilon, "Radiation-induced transcription factor activation in the rat cerebral cortex," *Int. J. Radiat. Biol.*, **76**, pp. 1045-1053, 2000.
27. M.M. Simon, Y. Aragane, A. Schwarz, T.A. Luger, and T. Schwarz, "UVB light induces nuclear factor kappa B (NF kappa B) activity independently from chromosomal DNA damage in cell-free cytosolic extracts," *J. Invest. Dermatol.*, **102**, pp. 422-427, 1994.
28. G.F. Vile, A. Tanew-Ilitschew, and R.M. Tyrrell, "Activation of NF-kappa B in human skin fibroblasts by the oxidative stress generated by UVA radiation," *Photochem. Photobiol.*, **62**, pp. 463-468, 1995.
29. N. Li and M. Karin, "Ionizing radiation and short wavelength UV activate NF-kappaB through two distinct mechanisms," *Proc. Natl. Acad. Sci. U. S. A.*, **95**, pp. 13012-13017, 1998.
30. Y. Devary, C. Rosette, J. DiDonato, and M. Karin, "NF-kB activation by ultraviolet light not dependent on a nuclear signal," *Science*, **261**, pp. 1442-1445, 1993.
31. T. Hayashi, Y. Ueno, and T. Okamoto, "Oxidoreductive regulation of nuclear factor kappa B. Involvement of a cellular reducing catalyst thioredoxin," *J. Biol. Chem.*, **268**, pp. 11380-11388, 1993.
32. H. Schenk, M. Klein, W. Erdrugger, W. Droge, and K. Schulze-Osthoff, "Distinct effects of thioredoxin and antioxidants on the activation of transcription factors NF-kappa B and AP-1," *Proc. Natl. Acad. Sci. U. S. A.*, **91**, pp. 1672-1676, 1994.
33. K. Schulze-Osthoff, H. Schenk, and W. Droge, "Effects of thioredoxin on activation of transcription factor NF-kappa B," *Methods. Enzymol.*, **252**, pp. 253-264, 1995.
34. R.D. Glickman, M. Natarajan, B.A. Rockwell, M. Denton, S. Maswadi, N. Kumar, and F. Nieves-Roldan, "Intracellular signaling mechanisms responsive to laser-induced photochemical and thermal stress," in *Optical Interactions with Tissue and Cells XVI*, S.L. Jacques, W.P. Roach Eds., Proc. SPIE **5695**, pp. 260-269, SPIE, Bellingham, WA, 2005.
35. M. Barkett and T.D. Gilmore, "Control of apoptosis by Rel/NF-kappaB transcription factors," *Oncogene*, **18**,

pp. 6910-6924, 1999.

36. V.R. Baichwal and P.A. Baeuerle, "Activate NF-kappa B or die?" *Curr. Biol.*, **7**, pp. R94-R96, 1997.
37. M.J. Crawford, R.R. Krishnamoorthy, V.L. Rudick, R.J. Collier, M. Kapin, B.B. Aggarwal, M.R. Al-Ubaidi, and N. Agarwal, "Bcl-2 overexpression protects photooxidative stress-induced apoptosis of photoreceptor cells via NF-kappaB preservation," *Biochem. Biophys. Res. Commun.*, **281**, pp. 1304-1312, 2001.
38. R.R. Krishnamoorthy, M.J. Crawford, M.M. Chaturvedi, S.K. Jain, B.B. Aggarwal, M.R. Al-Ubaidi, and N. Agarwal, "Photo-oxidative stress down-modulates the activity of nuclear factor-kappaB via involvement of caspase-1, leading to apoptosis of photoreceptor cells," *J. Biol. Chem.*, **274**, pp. 3734-3743, 1999.
39. J. Hoekstra, D.P.J. van der Goot, G. van den Brink, and F.A. Bilsen, "The influence of the number of cycles upon the visual contrast threshold for spatial sine wave patterns," *Vision Res.*, **14**, pp. 365-368, 1974.
40. G.H. Jacobs, J.A. Penwick, M.A. Crognale, and J.F.I.I. Deegan, "The all-cone retina of the garter snake: spectral mechanisms and photopigment," *J. Comp. Physiol. A*, **170**, pp. 701-707, 1992.
41. A.J. Sillman, V.I. Govardovskii, P. Rohlich, J.A. Southard, and E.R. Loew, "The photoreceptors and visual pigments of the garter snake (*Thamnophis sirtalis*): a microspectrophotometric, scanning electron microscopic and immunocytochemical study," *J. Comp. Physiol. A*, **181**, pp. 89-101, 1997.
42. P.D. Lukasiewicz, "Synaptic mechanisms that shape visual signaling at the inner retina," *Prog. Brain Res.*, **147**, pp. 205-218, 2005.
43. H. Wässle, "Parallel processing in the mammalian retina," *Nat. Rev. Neurosci.*, **5**, pp. 747-757, Oct. 2004.
44. R. Heidelberger, W.B. Thoreson, and P. Witkovsky, "Synaptic transmission at retinal ribbon synapses," *Prog. Retina Eye Res.*, **24**, pp. 682-720, Nov. 2005.
45. B. Roska, A. Molnar, and F.S. Werblin, "Parallel processing in retinal ganglion cells: how integration of space-time patterns of excitation and inhibition form the spiking output," *J. Neurophysiol.*, **95**, pp. 3810-3822, 2006.
46. C.L. Li, X.Y. Wang, J. Shao, J.S. Zhang, W.G. Feng, Y.B. Wang, and Z.L. Chang, "Heat shock inhibits IL-12 p40 expression through NF-kappa B signalling pathway in murine macrophages," *Cytokine*, **16**, pp. 153-159, Nov 21. 2001.
47. H.R. Wong, M.A. Ryan, I.Y. Menendez, and J.R. Wispe, "Heat shock activates the I-kappaBalpha promoter and increases I-kappaBalpha mRNA expression," *Cell Stress Chaperones*, **4**, pp. 1-7, Mar. 1999.
48. P. Maroni, P. Bendinelli, L. Tiberio, F. Rovetta, R. Piccoletti, and L. Schiaffonati, "In vivo heat-shock response in the brain: signalling pathway and transcription factor activation," *Brain Res. Mol. Brain Res.*, **119**, pp. 90-99, Nov 6. 2003.
49. N.P. Matylevitch, S.T. Schuschereba, J.R. Mata, G.R. Gilligan, D.F. Lawlor, C.W. Goodwin, and P.D. Bowman, "Apoptosis and accidental cell death in cultured human keratinocytes after thermal injury," *Am. J. Pathol.*, **153**, pp. 567-577, 1998.
50. A.E. Dontsov, R.D. Glickman, and M.A. Ostrovsky, "Retinal pigment epithelium pigment granules stimulate the photo-oxidation of unsaturated fatty acids," *Free Radic. Biol. Med.*, **26**, pp. 1436-1446, 1999.
51. L. Flohe, R. Brigelius-Flohe, C. Saliou, M.G. Traber, and L. Packer, "Redox regulation of NF-Kappa B," *Free Radic. Biol. Med.*, **22**, pp. 1115-1126, 1997.