Action Spectrum of Retinal Light-Damage in Albino Rats

Theodore P. Williams and William L. Howell

The right eyes of anesthetized, 10-week-old albino rats were exposed to constant photon fluxes at 6 wavelengths for 6 hrs. The left eye of each animal was patched during the exposure and was used as control. Histologic examination of retinal sections disclosed a region in the superior retina that was more damaged than were other areas. Attempting to ascertain an action spectrum by measuring ONL lost in this "sensitive" region failed. However, it was shown that, when ONL thickness was integrated over the entire retinal sections, a rhodopsin action-spectrum emerged. It was concluded that (1) retinal light damage in the albino rat under these conditions was rhodopsin mediated, and (2) proper assessment of the extent of damage could only be made by some method that integrates over the entire retinal section. Invest Ophthalmol Vis Sci 24:285-287, 1983

The phenomenon of retinal light damage is rapidly becoming a subject of widespread study. This attention stems not only from an interest in how light causes the damage but also from concern that light-damage hazards may be present in the environment. Despite the considerable activity in this field (cf. refs. 3-7), the mechanism of retinal light damage is not clear. For example, there are some discrepancies among the reports of the action spectrum of damage. Noell et al., using an electroretinogram, ERG, assay, found a rhodopsin-like spectrum in albino rats. Ham et al., studying monkeys and using histologic assessment of lesions, found a monotonic function that rose continuously into the deep blue. In mid-spectral (visible) range there was no evidence of a visual pigment-like spectrum, but Ham and his colleagues called the damage, occurring at these wavelengths, "photochemical" to distinguish it from thermal damage. Anderson et al histologically examined unspecified regions of rat retinas and found a "blue light effect" when they used very broad-band colored filters. Lawwill and his co-workers, studying light damage in the rabbit, have found both rhodopsin and nonrhodopsin action spectra. The differences in the Lawwill et al results might be due to the method of assessing the damage: electroretinographic methods gave a rhodopsin-like result while ophthalmoscopic assays gave a "blue-light effect" spectrum.

Perhaps the apparent discord in the above cited results is due to the existence of at least two mechanisms of light damage, eg, a "blue-light" process and a visual pigment-mediated process. Noell has proposed that, in fact, two mechanisms do exist. On the other hand, perhaps at least part of the discord derives from a paucity of appropriate data. For example, in the studies cited above, a limited number of wavelengths have been used or very broadband filters have been employed. This could lead to poor definition of a spectrum. In addition, light damage lesions are not uniformly distributed across the retina even when uniform, diffuse illumination is used, and this fact has not been explicitly considered in the above studies.

The studies reported herein help rectify these shortcomings. We have used six interference filters of narrow (10 nm width at half-height) band-pass. Furthermore, we shall show that, because of the non-uniform distribution of damage across the retina, meaningful action spectra emerge only when an integration of the outer nuclear layer, ONL, over entire retinal slices is carried out.

Materials and Methods

Albino rats of the Sprague-Dawley strain were used at 10 weeks of age. The light histories of these animals had been controlled carefully: they were conceived, born, and raised in our colony room, which is kept at 5-10 lux with L/D cycle of 12/12. At the time of an experiment, an animal was taken from the colony, dark adapted for ca 1 hr, and then anesthetized with 12 mg Nembutal. Small booster injections of the anesthetic were administered, as needed, during the light
exposure. The rat was situated on a warming pad on a turntable, and its right eye (with lids clamped open) was aligned in the proper optical path by means of a dim red light beam. Then the alignment beam was extinguished, and the damaging beam turned on for a dim red light beam. Then the alignment beam was adjusted at each wavelength and maintained at or within 0.5°C of normal. A xenon arc was used as the damaging source. Light from it was passed first through a heat-absorbing filter and then through a preselected interference filter. The intensity of the beam was adjusted at each wavelength to a constant \((2.0 \pm 0.2) \times 10^{15}\) photons/sec \cdot cm\(^2\) with neutral filters. At the end of the exposure, the eyelids were unclamped, and the animal was put into a recovery cage in a totally darkened cubicle for four days. The only light exposure given it during this time was dim red light as needed to care for and maintain the animal. At the end of this dark period, the animals were anesthetized and perfused with glutaraldehyde-formaldehyde fixative, and the eyes were enucleated. The left eye of each rat had been patched with opaque black tape during the damaging exposure and was used as the control in each case. Orientation of the eyeballs was maintained during embedding and sectioning, and sections were taken through the vertical meridian. Details of these methods have been described elsewhere.\(^{13}\)

**Results**

The outer nuclear layer thickness was measured along the entire vertical meridian, and plots of thickness vs retinal position were generated for each of the wavelengths studied. As reported in our other studies, a region of greatest damage was observed in the superior retina.\(^{13}\) In an effort to conserve time, we, at first, attempted to generate an action spectrum by simply measuring the extent of ONL loss in that most sensitive region, ie, 1.88 mm superior to the optic disk.

No reasonable spectrum was obtained (vide infra). However, a detailed inspection of the data revealed that measuring ONL thickness in this sensitive (or any other single) region would certainly fail to reflect accurately the damaging effectiveness of the various wavelengths upon the entire retina. Some animals had severe damage in the sensitive region but not much loss of ONL elsewhere, while others had moderate damage spread over much of the retina with only a slight exaggeration of ONL loss in the sensitive region. Figure 1 shows the results of the exposure of two rats at two wavelengths (only two wavelengths are shown for the sake of clarity) compared to the average of the control eyes from these animals. Note that, while exposure to 560 nm light gives the greatest loss in the sensitive region (ca 2 mm superior to the optic nerve), there seems to be a greater overall loss of ONL when 486 nm was used. This led us to integrate the areas under such curves and to take these areas as being proportional to the ONL remaining after damage. The loss of ONL was then expressed as the difference between these areas and their respective control-eye areas. When ONL loss is calculated from such integrated areas and plotted against wavelength, a reasonably good fit to the rat rhodopsin spectrum obtains (Fig. 2). The units of ONL loss are relative due to the method of integration, and the ONL loss at 486 nm is normalized to the rhodopsin spectrum. The other points fall as shown. The insert in this figure shows what the action spectrum would have looked like if loss of ONL were measured only at the sensitive region.
Discussion

Thus, we conclude that, under these irradiation conditions, retinal light damage in the anesthetized albino rats is rhodopsin-mediated. This is significant because we have determined the action spectrum with a discrete anatomical marker: the ONL. The only other rat experiment to use such a criterion was the one by Anderson et al.9 They reported a “blue light” effect but in reality the “blue” filter they used subtended a spectral region that extensively overlapped the rhodopsin maximum. Furthermore, it seems that they may have equated their intensities for equal photopic footcandles even though it is the rod system that suffers the extensive damage in the rat.

Our result is significant for another reason: the entire rat eye was illuminated in these experiments, and the assay of damage took this into account by integrating over the entire retinal sections. We conclude from this that whenever diffuse lighting is used to cause the damage, some integrative method should probably be used for assessing the damage. The ERG, with ganzfeld stimulation, may suffice, even though much remains unknown about the mechanism of ERG generation. Of course, in those experiments in which small areas of retina are irradiated, assaying only those regions seems a reasonable approach.

The photon fluxes used in these experiments are higher than those in our ongoing studies of light damage. We shall attempt to determine the action spectrum at lower fluxes in unanesthetized, freely moving rats. Not only is such an extension of this study necessary in order to permit comparison with our results, past and future, but the anesthesia, used here, may be influencing the extent of damage.*

Key words: light-damage, action spectrum, outer nuclear layer, monochromatic light, rod cells

Acknowledgments

The authors thank B. J. Williams for drafting the figures and J. M. Lipner for preparing the manuscript.

References


* W. K. O'Steen: Personal communication.