Retinal Damage in Macaque after White Light Exposures Lasting Ten Minutes to Twelve Hours

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We induced photochemical damage in small parts of the retinas of anesthetized macaques after light exposures of varying intensity, lasting between 10 min and 12 hr. Damage was assessed both with funduscopy and densitometry at several periods after exposure. Damage was most extensive 2 days post-exposure, with similar thresholds for both methods. Reciprocity between exposure time and irradiance was found for all exposures at a threshold irradiant dose of 230 J/cm². This is in good agreement with part of the literature data on monkeys, yet contradicts another report (Sykes et al) in which a much lower threshold dose was found. The latter data probably concern a different class of damage. It remains unclear what critical factors distinguish the two classes. Observations more than 70 days post-exposure show a divergence between funduscopic and densitometric thresholds. Although the appearance of funduscopic lesions had changed, the threshold dose remained 230 J/cm². Densitometry showed full recovery of the amount of visual pigment for doses below 600 J/cm².

When the primate retina is exposed to intense light for periods varying between several seconds and 4 hr, extensive damage to the pigment epithelium and to the neural retina may be found.¹⁻⁴ In patients such damage is also reported after ocular surgery in which use was made of bright light sources.⁵⁻⁸ For periods between 10 and 1000 sec, Ham et al⁹¹⁰ verified that reciprocity holds, that is, irradiance is exchangeable for exposure time. The threshold dose for white light in Ham's experiments varied between 200 to 400 J/cm². With exposures shorter than 10 to 100 sec, depending on the size of the retinal image, reciprocity fails because the rise in retinal temperature is such that the domain of thermal damage is entered. It is unclear how far reciprocity holds for exposures beyond 1000 sec. One report is available in which macaque monkeys were exposed to 12 hr of continuous fluorescent light.¹¹ We calculated that the threshold dose in the latter experiment was about 16 J/cm², an order of magnitude lower than Ham et al’s 200–400 J/cm². The direction of the deviation from reciprocity is unexpected. Naively, it is expected that with increasing exposure time (and decreasing irradiances) repair processes gradually overtake damage induction. As a result the total dose to reach thresholds should increase, rather than decrease.

Griess and Blankenstein¹² found the time constant of the repair processes to be almost exactly 4 days. From this study alone reciprocity is expected to hold up to about 2 days. But, as Griess and Blankenstein determined the time constant in an indirect way, this is only true when no other, unexpected, processes interfere at long exposures. Sykes'¹³ experiments actually suggest such an interference.

The aim of the present study was to investigate whether or not there is a deviation from reciprocity after long exposures, as described above. Therefore, we explored the exposure range from a few hundred seconds to 12 hr. The conditions were, apart from irradiance and time, similar to those used by Ham et al,⁹ that is, anesthetized macaque monkeys and exposures restricted to small patches of retina. In addition to funduscopy, we introduced retinal densitometry as a new technique for assessing damage. This technique has the advantage of being both quantitative and noninvasive. Also, direct information is obtained on the kinetics of visual pigments after photochemical damage.

Finally, damage was also assessed between 2 and 7 months after exposure, to study the effect of repair mechanisms.

Materials and Methods

Animal Preparation

Monkeys were sedated with 25–40 mg ketamine hydrochloride (Ketalar®, Park-Davis, Barcelona, Spain) and anesthetized with an initial intravenous
dose of 20 mg/kg of pentobarbital (Nembutal®, CEVA, Paris, France), followed by a continuous infusion of 2–3 mg/kg/hr. After intubation, artificial respiration was started with a mixture of 70% nitrous oxide and 30% oxygen. To prevent eye movements an intravenous infusion of a muscle relaxant pancuronium bromide (Pavulon®, Organon, Oss, The Netherlands) was given. The initial dose was 30 µg/kg followed by a continuous infusion of 30 µg/kg/hr. Pupils were dilated with a drop of Phenytoin® (Bournonville-Pharma, Almere, The Netherlands), combined with a drop of Cyclogyrl® (Schieffelin and Co., New York, NY). The head was fixed in place with ear bars and a jaw rest. A hard contact lens was used to prevent drying of the cornea. All preparation was done in yellow light to minimize the possibility of retinal damage before exposure.

This investigation adhered to the ARVO Resolution on the Use of Animals in Research.

Irradiation

Whenever we use the term irradiation, we refer to exposures of white light to establish damage thresholds. We used a dual beam ophthalmoscopic stimulator for irradiation under Maxwellian view conditions. The light source was a 450 W xenon arc with light stabilized power supply. Infrared radiation was eliminated by a Schott KG 3 filter. UV radiation was reduced by the glass lenses. Details of this apparatus can be found in Valeton and van Norren. Four patches of retina were defined using a diaphragm with four holes imaged on the retina. The holes measured 4° diameter and were arranged in a two by two array, about 1° separated. The center of the array was positioned about 8° above the fovea. To vary the irradiance level of individual patches, neutral density filters were placed on the holes. The irradiances of the four patches were regularly calibrated with a Laser Precision RK-5100 Pyroelectric Radiometer. The spectral output of the white light at the corneal level was measured with a scanning spectroradiometer (Photo Research Spectrascn S.N. 2186). The results of the latter measurement are presented in Figure 1. The average color temperature was 5400°K. Retinal irradiance (Eret) was calculated from the measured irradiance (Eair) with:

\[ E_{\text{ret}} = E_{\text{air}} \frac{d^2 \times n^2 \times t}{f^2} \]  

in which d is the distance between focus and the irradiated plane of the measuring device of the radiometer, f the focal length of the monkey eye (about 1.35 cm), n the refractive index (about 1.33), and t the ocular transmission (about 0.9). The maximal available retinal irradiance was 400 mW/cm².

Funduscopv

The irradiated patch (seen through the ophthalmoscopic stimulator) was marked on a fundus photograph. Two days after exposure, changes were visible as white-yellowish spots.

Funduscopic observations were performed with an indirect ophthalmoscope (28 diopter lens), because the large field of view made lesions easier to recognize due to the comparison with the undamaged surroundings. We classified the observations into three categories: (1) no change, indicating subthreshold damage or absence of damage; (2) a just visible change (a patch somewhat smaller than the irradiated spot, and with fuzzy edges), as an indication for threshold damage; and (3) a distinct funduscopic change (a patch with a size comparable to the irradiated spot or somewhat larger, and with sharp edges), indicating suprathreshold damage.

Funduscopic observations were made at four different time intervals after exposure: (1) 0.5–2 hr; (2) 4–12 hr; (3) about 2 days; and (4) minimally 70 days. The most complete observations were made 2 days post-exposure because at that time funduscopic changes were most clearly present (in accordance with observations of, eg, Ham et al.10).

Densitometry

In retinal densitometry a beam of light is shone into the eye, reflected at the fundus and collected by a light-sensitive device, usually a photomultiplier (PM). Essential in the technique is: first, that measuring light bleaches only a small fraction of the photopigment. Second, measurements are done in two conditions, one with high adapting illuminance which virtually bleaches all visual pigment, resulting in a relatively high number of reflected photons, and
the density of rhodopsin was measured at minimally two of the four selected patches of retina. When these centric with the illuminated field. The bleaching light wavelengths of the measuring light amounted an anesthetized animal was placed behind the irradiating device and irradiation was started. At predetermined times subsequent holes of the diaphragm were covered to allow for different exposure times. To assess short-term damage, density was measured at a number of patches between 0.5 hr and 2 hr after irradiation was completed. On other occasions the observations and measurements were done between 4 and 12 hr post-exposure. Therefore, they were grouped into a separate category. Density was nearly always measured 2 days after irradiation. Depending on the length of the irradiation this was between 33–40 hr after completion of irradiation. Additional density measurements were obtained between 70–210 days after irradiation. Whenever density was measured, notes were made on the appearance of the fundus.

Some eyes were used more than once. Eye movements during exposure excluded some patches from evaluation.

Results

Funduscopv

Two days post-exposure, the retinal patches that underwent a funduscopical change showed a brighter appearance than undamaged retina. The size of the damaged patches varied slightly, depending on the irradiant dose.

Figure 2A through 2D summarizes the results of funduscopical observations for different irradiances and exposure times. The data are based on observations on 41 irradiated patches in ten eyes of seven animals. Up to 12 hr post-exposure not much damage was observed; only in a limited number of cases were threshold funduscopical lesions visible (half-open circles in Fig. 2A, B). In Figure 2C the results of the most extensive observations, made 2 days post-exposure, are given. In any single experiment, we noted that the transition between no damage and evident damage always was within a factor of two of irradiant dose. The spread in threshold dose between individuals was not large either: it was easy to draw a line by eye with slope −1 (constant dose) which separated sub- from suprathreshold data. This line represents a well defined and remarkably constant threshold dose of 230 J/cm² over two decades of exposure times. To enable comparisons, the threshold dose line was drawn in all plots of Figure 2. It shows that it represents a conservative estimate of a threshold course for the post-exposure observations at other times. With the longest exposure time used, 12 hr, threshold damage is found at about 5 mW/cm². For a human eye, this would correspond to about $3 \times 10^4$ td white light.
Fig. 2. Data on fundusscopic observations 0.5–2 hr (A), 4–12 hr (B), 2 days (C), and more than 70 days (D) post-exposure. Filled circles represent a clear fundusscopic lesion (suprathreshold damage), half-filled circles a just visible lesion (threshold damage), and open circles no visible lesion (subthreshold damage). The dashed line represents an irradiant dose of 230 J/cm², which is the best fit by eye for threshold damage 2 days post-exposure.

After 70 or more days, damaged patches which two days post-exposure were brighter than undamaged retina, now appeared darker and had a granular texture. They were surrounded by an annulus of about half a degree, with a somewhat brighter appearance than the rest of the retina. Recovery, in the sense that patches returned to normal, was minimal, as can be seen from Figure 2D.

Densitometry

An example of densitometric measurements at 511 nm is given in the left part of Figure 3. In the upper panel a measurement before exposure is given. The record starts with the bleaching light on; after 2 min the light was switched off and pigment regeneration was followed for 15 min in the dark. Thereafter, the bleaching light was switched on again and the trace is seen to return to its original fully bleached level. The density difference between bleached and regenerated was 0.09, fairly typical for the records obtained in these experiments. The accuracy with which the density differences could be assessed was between 10 and 20%. In the right part of Figure 3, density difference is given as function of wavelength. The difference is maximal at 511 nm, indicative for rod involvement. The middle panel of Figure 3 presents the results of measurements taken 2 days after 4800-sec exposure with an irradiance of 220 mW/cm², which was 4.6 times the funduscopic threshold dose. No changes in density are observed following an identical bleaching and regeneration sequence, which is indicative for a serious disturbance in the regeneration cycle of rhodopsin. In the lower panel of Figure 3, a measurement in the same patch 85 days after exposure is presented. Partial recovery of the density of rhodopsin was found.

In Figure 4A densitometric damage indices obtained for 2 days post-exposure are given as a function of irradiant dose. The threshold dose obtained from funduscopy, the vertical line, provides a satisfactory separation between high and low damage. In Figure 4B the same mode of presentation is chosen for densitometric changes after prolonged recovery. The threshold dose for long-term densitometric damage, though less accurately determined, lies at about 600 J/cm², which is a factor of 2.6 higher than the dose for funduscopic and densitometric threshold damage 2 days post-exposure. So, again, the fundu-
Fig. 3. Example of densitometry before exposure (top panels), 2 days after a 4800-sec exposure to 220 mW/cm² (middle panels), and 85 days after the same exposure (bottom panels). The left panels show the density traces for the measuring light of 511 nm wavelength. A trace starts with the pigment bleached. After 2 min the bleaching light is turned off, and the visual pigment is allowed to regenerate for 15 min. Then, the bleaching light is turned on again, and the density trace is seen to return to its previous level. The right panels show the difference spectrum, which resembles the rhodopsin density spectrum. Note the absence of visual pigment 2 days post-exposure and the partial recovery of visual pigment after 85 days.

scopic criterion 2 days post-exposure proves to be the most conservative damage criterion.

In Figure 5, funduscopic and densitometric results are compared for 2 days, and more than 70 days post-exposure, respectively. Correspondence is high for observations 2 days post-exposure, but much poorer after prolonged recovery. In distinct funduscopic lesions partial or even complete densitometric recovery was measured in many instances.

The data obtained between 0.5 and 2 hr post-exposure are not presented in a figure, because none of the ten measurements showed a densitometric change, despite the fact that the irradiant total dose was about 550 J/cm², and despite the fact that in one of the measured patches a vague funduscopic change was observed (after a 12 hr exposure to 11.5 mW/cm²). Only two measurements were done between 4 and 12 hr post-exposure. The irradiant doses in the two patches were 351 and 1200 J/cm², respectively, and both displayed a vague funduscopic change. Only the high dose exposure resulted in a densitometric damage (D = 1).

Discussion

Relation between Irradiance and Exposure Time at Threshold

The funduscopic threshold damages, obtained 2 days post-exposure, (Fig. 2C) occurred at a constant 230 J/cm² irradiant dose for exposure conditions of up to 12 hr. This result is interesting in two ways. In the first place it extends the domain of time-irradiance reciprocity. From Ham et al.'s data, reciprocity was clear up to at least 1000 sec. By combining data in the literature for various animal models, we could already tentatively extend that period to about 4 hr. This study now provides experimental evidence for reciprocity up to 12 hr in one animal model, the macaque.

In the second place the value of the threshold dose of 230 J/cm² merits attention. It is only slightly lower than the 410 J/cm² value derived from the data of Ham et al. This difference may have a technical explanation. Ham et al. used the integrated value from 400-800 nm, whereas ours ranged from 400-700 nm. Since all evidence suggests that wavelengths over 700 nm do not contribute to photochemical damage (provided they do not increase retinal temperature), our threshold data are comparable to Ham's data.

However, our results are clearly at variance with those of Sykes et al., who found threshold damage already at 16 J/cm² in an animal model closely related to ours. The contradiction with Ham's data was not obvious as Sykes' exposure was outside the irradiance/time domain covered by Ham. The controversy has now become much more manifest by our extension of the reciprocity domain to 12 hr.

Despite similarities in exposure time and animal model, there are differences between our and Sykes' experiments, which might explain the different threshold irradiances. These differences are the use of anesthetics, the size of the exposed retinal area,
the technique of damage assessment and the light source.

1. Anesthetics: We exposed the monkey's eye under Nembutal anesthesia, whereas the animals in Sykes' experiments were unanesthetized. There is evidence indeed that Nembutal provides protection.\(^1\) On the other hand, rough estimations of the damage dose in (unanesthetized) human sungazers are certainly not lower than our 230 J/cm\(^2\) value, because only minor damages were reported even after 1 hr of sungazing\(^1\) (retinal irradiance estimated to be about 10\(^4\) mW/cm\(^2\), no corrections for eye movements).

2. Size of exposed retinal area: Sykes et al used large-field exposure, whereas we used 4° field sizes.

As a matter of fact, Ham et al\(^1\) have suggested, but never substantiated, such an explanation.

3. Technique of damage assessment: Sykes used histologic criteria, whereas our data were based on funduscopic and densitometric observations. Both this study and our earlier literature survey have shown, however, that different techniques do not markedly differ in results. Fuller et al\(^2\) found that the irradiant dose for a funduscopic threshold was only

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**Fig. 4.** Densitometric damage index as a function of the irradiant dose, for measurements 2 days (A) and more than 70 days (B) post-exposure. The dashed lines represent the best fitting doses for threshold densitometric damage: 230 J/cm\(^2\) after 2 days, and 600 J/cm\(^2\) after more than 70 days.

**Fig. 5.** Relation between funduscopic and densitometric damage indices two days and more than 70 days after exposure. The mean densitometric damage and standard deviation are presented at three funduscopic damage indices, together with the number of observations. Correspondence between both indices is most clearly present 2 days after exposure.
about a factor of two higher than the dose for a histologic threshold.

4. Light source: We used a xenon arc lamp, whereas fluorescent bulbs were used in Sykes' experiments. However, taking account for the action spectra of photochemical damage, it is very unlikely that the difference in spectral distribution of the radiation might cause such large differences in threshold doses. Another difference might be the flicker in the fluorescent lights due to the AC power supply. We do not find any mechanistic justification for this possibility. Therefore, it seems very improbable that the differences in threshold doses would have been caused by the use of different light sources.

The importance of these possible differences may become clear when things are put in a different perspective by noting that the results of the Sykes experiments do not form just an odd point in a further consistent picture of threshold doses (Fig. 6). They seem to fit into a series of threshold data mainly found in rats (eg, 21–23) but also in pigeons and in other monkey experiments. These are the so-called Class I damages as defined by Mainster and Kremers and van Norren. Class I damage is found in experiments that have the following characteristics in common: unanesthetized animals, mainly rats, are exposed to large-field, long-term (12 hr or longer) irradiation, with retinal irradiance seldom exceeding 1 mW/cm² (data on white light sources). On histological examination of the retina, damage is mainly restricted to the photoreceptor level (for a review see Lanum). The action spectrum, found in rats, is similar to the absorption spectrum of visual pigment. Harwerth and Sperling's experiments, in which photoreceptor systems could be selectively damaged by colored light, suggest an action spectrum resembling visual pigment absorption spectrum also exists in monkeys.

The damages produced in our experiments are typically of the Class II type. Conditions in which photochemical damage of Class II is found can be characterized as follows. Small patches of retina in usually anesthetized animals were exposed, for limited periods of time, to irradiances typically exceeding 10 mW/cm² using white light sources. Damage is assessed at least 24 hr after exposure; in general the first signs of damage appear in the pigment epithelium. The action spectrum, in macaque monkeys, showed
maximum sensitivity in the UV. Animal models in these experiments involved primates and rabbits. Human subjects, about to undergo eye enucleation, were also studied. The amount of unbleached visual pigment has been suggested as crucial factor for class determination, resulting in lower threshold doses than expected for Class II damage for exposures of 12 hr or longer. Although this hypothesis seemed attractive for explaining literature data, our experiments do not support it. Possibly, a combination of some of the aforementioned factors is required for class determination. The unresolved controversy between Sykes' and our results should, therefore, not be viewed as just differences between two experiments, but should be placed in the more interesting, unresolved distinction between Class I and Class II damage.

Recovery

Two days post-exposure, damage seems to be most distinct. Thereafter, the appearance of the fundusoscopic lesions changes and the regeneration cycle of the visual pigments may recover. Two days after exposure, the damaged part of the retina appeared lighter than the rest and occasionally it seemed edematous. This is in line with reported depigmentation and mild edema. After more than 70 days, the lesion was less obvious (also observed by Ham et al.), darker than the rest of the retina, and it had a granular appearance. The granularity after prolonged recovery could be attributed to the irregular pigmentation of the RPE cells as described by Tso. We found substantial recovery from damage with densitometry. Thus, the disturbance of the visual cycle may vanish completely with the fundusscopic lesion still present. The extent of recovery was found to depend on the irradiant dose: when the dose was higher than about 600 J/cm^2, recovery was never complete. These results are in qualitative agreement with the effects reported by Moon et al. After exposing the macula of a rhesus monkey to 441 nm light, inducing a fundus omniphotic threshold lesion, they found visual performance only showed partial recovery. We found incomplete recovery only after an irradiant dose of 2.6 times threshold. This might indicate a lingering damage to the neural retina despite full recovery of the visual pigment regeneration cycle.

Key words: retina, photochemical damage, white light, irradiant dose, densitometry

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