The Reduction of Retinal Autofluorescence Caused by Light Exposure

Jessica I. W. Morgan, Jennifer J. Hunter, William H. Merigan, and David R. Williams

Purpose. A prior study showed that long exposure to 568-nm light at levels below the maximum permissible exposure safety limit produces retinal damage preceded by a transient reduction in the autofluorescence of retinal pigment epithelial (RPE) cells in vivo. The present study shows how the effects of exposure power and duration combine to produce this autofluorescence reduction and find the minimum exposure causing a detectable autofluorescence reduction.

Methods. Macaque retinas were imaged using a fluorescence adaptive optics scanning laser ophthalmoscope to resolve individual RPE cells in vivo. The retina was exposed to 568-nm light over a square subtending 0.5° with energies ranging from 1 to 788 J/cm^2, where power and duration were independently varied.

Results. In vivo exposures of 5 J/cm^2 and higher caused an immediate decrease in autofluorescence followed by either full autofluorescence recovery (exposures $\leq$ 210 J/cm^2) or permanent RPE cell damage (exposures $\geq$ 247 J/cm^2). No significant autofluorescence reduction was observed for exposures of 2 J/cm^2 and lower. Reciprocity of exposure power and duration held for the exposures tested, implying that the total energy delivered to the retina, rather than its distribution in time, determines the amount of autofluorescence reduction.

Conclusions. That reciprocity held is consistent with a photochemical origin, which may or may not cause retinal degeneration. The implementation of safe methods for delivering light to the retina requires a better understanding of the mechanism causing autofluorescence reduction. Finally, RPE imaging was demonstrated using light levels that do not cause a detectable reduction in autofluorescence. (*Invest Ophthalmol Vis Sci.* 2009;50:6015–6022) DOI:10.1167/iovs.09-3643

It has long been known that the retina can be harmed by bright light and that it is important to understand the limits and mechanisms of retinal phototoxicity to incorporate safe practices of retinal light exposures. In particular, phototoxicity is important because several clinical procedures (such as slit-lamp examination, fundus photography, fluorescein angiography, and retinal surgery) are often performed at light levels close to the limits imposed by current safety standards.[1–3] In addition, those procedures are used to examine retinas affected by eye diseases, and the extent to which patients have increased susceptibility to light-induced retinal damage remains unknown. Indeed, it has been shown that certain genetic mutations can cause higher susceptibility to light in animal models,[4] and that phototoxicity increases with age, even in the normal human retina,[5] as is observed by the accumulation of lipofuscin in the retinal pigment epithelial (RPE) cells.[6] Previous studies have shown that A2E, a primary component of lipofuscin, is one of the phototoxic materials in the retina. In cultured ARPE-19 cells, the combination of A2E and blue light resulted in cell death[7] by a photooxidative mechanism.[8] The role of phototoxicity and photooxidation in the retina has led to the theory that light exposure plays a role in some retinal diseases including age-related macular degeneration,[5,9–12] although this association is controversial.[13,14] Regardless, until phototoxic mechanisms are fully understood, studies of retinal toxicity remain important for the implementation of safe practices in ophthalmic procedures such as retinal surgery and ophthalmic imaging applications including fundus photography, fluorescein angiography, and lipofuscin autofluorescence imaging.

Lipofuscin autofluorescence imaging has been used to observe features of the RPE layer in both normal and diseased retinas in vivo.[15–20] This imaging modality takes advantage of the autofluorescent properties of lipofuscin, a conglomeration of materials that accumulate in the cytoplasm of the RPE cells as byproducts of the visual cycle and phagocytosis.[21–23]

There has been considerable effort to develop in vivo retinal imaging modalities with cellular resolution. The use of adaptive optics (AO), which involves measuring the higher-order optical aberrations of the eye with a wavefront sensor and correcting these aberrations with a wavefront corrector—usually a deformable mirror—has allowed improvements in contrast and resolution such that it has become possible to routinely characterize the photoreceptor mosaic in both normal and diseased eyes in vivo.[24–31] Recently, the combination of autofluorescence and AO with the scanning laser ophthalmoscope (SLO) has allowed the visualization of the individual cells of the RPE mosaic in human and macaque retina in vivo.[32,33]

With the ability to image the RPE mosaic in vivo, we reported observations of retinal phototoxicity in macaque retina caused by exposure to the 568-nm light that was used for autofluorescence excitation.[34] Indeed, this study led to the unexpected finding that exposures to 568-nm light at levels below the American National Standards Institute (ANSI) Safe Use of Lasers[35] maximum permissible exposure (MPE) produced retinal damage. In all cases, this retinal damage was preceded by a reduction in the autofluorescence intensity from the RPE cells. In addition, dependent on the retinal radiant exposure, some exposures caused the immediate reduction in autofluorescence, but subsequently the autofluorescence recovered in full, and no long-term retinal damage was detected in the RPE and cone mosaics at the site of the exposure.[34]

It is known that retinal damage can occur by either thermal or photochemical mechanisms, depending on exposure conditions such as wavelength and duration.[36–38] Thermal damage is thought to occur when the temperature of the retina rises 10°

From the Center for Visual Science, University of Rochester, Rochester, New York.

Supported by the National Institutes of Health Grants EY014375, EY01319, and EY07125, and Research to Prevent Blindness, the National Science Foundation Science and Technology Center for Adaptive Optics (Santa Cruz, CA), managed by the University of California at Santa Cruz (Cooperative Agreement AST-9876783).

Submitted for publication February 27, 2009; revised June 11, 2009; accepted September 8, 2009.

Disclosure: **J.I.W. Morgan**, None; **J.J. Hunter**, None; **W.H. Merigan**, None; **D.R. Williams**, None

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Corresponding author: Jessica I. W. Morgan, 309 Stellar Chance Lab, 422 Curie Boulevard, University of Pennsylvania, Philadelphia, PA 19104; jwmorgan@mail.med.upenn.edu.

Table 1. Macaque Parameters

Macaque Number	Species	Age (y)	Sex	Eye	Axial Length (mm)
320	*Macaca fascicularis*	8	M	OD	17.85
				OS	17.59
526	*Macaca mulatta*	7	F	OS	20.22
620	*Macaca fascicularis*	3	M	OS	18.07
903	*Macaca nemestrina*	11	M	OD	20.97
				OS	20.95

above its ambient temperature,[36,39,40] whereas photochemical effects cause retinal damage when the incoming light interacts with molecules to cause a chemical change.[36,38,40–42] Retinal lesions exhibit different characteristics depending on whether they are caused by thermal or photochemical mechanisms. For example, thermal lesions are in general immediately visible, whereas photochemical lesions can take up to 48 hours to appear.[38,43] In addition, thermal lesions can spatially expand beyond the exposed retinal region because of lateral heat diffusion, whereas photochemical lesions are usually confined to the irradiated retinal area.[36] Also, photochemical lesions will exhibit reciprocity of time and irradiance, whereas thermal lesions do not.[42] The concept of reciprocity is that exposures of equivalent energies cause equivalent effects regardless of how the total exposure is distributed in time.[42]

Morgan et al.[34] showed that the retinal damage observed with exposures to 568-nm light is caused by a photochemical mechanism. However, it is currently unknown whether the observed reduction in lipofuscin autofluorescence and long-term retinal damage are caused by the same mechanism and represent stages of a phototoxic continuum or whether the two phenomena are caused by separate mechanisms. It is important to understand the mechanism causing the reduction in autofluorescence to determine whether this phenomenon is detrimental to retinal health, and whether reduced autofluorescence represents a pathway to phototoxicity in the retina. In the present study, we tested the mechanism that causes reduced autofluorescence by examining reciprocity of time and irradiance for 568-nm light exposures.

Regardless of the mechanism causing retinal damage and reduction of autofluorescence, it is important to determine the limits of phototoxicity to establish exposure guidelines that allow the safe practice of procedures requiring retinal illumination such as retinal imaging. In the present study, the effects of exposures over a range of almost 4 log units of radiant exposure were measured to determine the boundaries between permanent retinal damage, reduced autofluorescence, and no detectable change. After establishing those limits, we demonstrate successful imaging of the RPE cell mosaic using illumination conditions more than an order of magnitude below the exposure level causing a detectable reduction in lipofuscin autofluorescence.

Methods

Macaque Preparation

Six eyes of four macaques were used for these experiments. Table 1 characterizes the parameters of each macaque. During each imaging session, macaques were anesthetized with isofluorane (1.0%–3.0%), body temperature was monitored, and pupils were dilated and cyclopleged with one drop each of phenylephrine hydrochloride (2.5%) and tropicamide (1%). A lid speculum held the eye open for imaging, and a rigid gas permeable contact lens was used to protect the cornea.[44] The animal's head and pupil of the eye were aligned with the imaging system by a head-post rotation mount and a three-axis translation stage. Axial lengths were used to determine the dimensions of the retinal images by linearly scaling the LeGrand model eye.[45] In the macaques, axial length was measured by averaging 10 B-scan ultrasound measurements. The University of Rochester review board approved all experiments and the study adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Autofluorescence Imaging of the RPE Cells

The fluorescence adaptive optics scanning laser ophthalmoscope (AOSLO) used in this experiment has been previously described.[32,33] Three lasers were simultaneously scanned across the retinal area, 568-nm light for the autofluorescence excitation, 830- or 794-nm radiation for reflectance imaging, and 904-nm radiation for wavefront sensing. Autofluorescence images were taken by exciting the retina with 568-nm light and collecting the emission over a 40-nm bandwidth centered at 624 nm. The simultaneous imaging and registration scheme was used as previously described.[32] In this scheme, the reflectance video frames were registered to determine interframe eye motion, the motion was corrected by shifting the frames of the simultaneously recorded autofluorescence video, and the shifted autofluorescence frames were averaged to give the final RPE mosaic image.

Laser Modulation

As previously described,[34] the 568-nm light was modulated with an acousto-optic modulator relative to the position of the two scanners. Under normal conditions of operation, the 568-nm laser light illuminated the retina 37% of the time (ON ratio = 0.37). To increase the average power for some exposures, the 568-nm laser light remained on continuously (ON ratio = 1.00). The 794-nm SLD and the 830- and 904-nm lasers always had an ON ratio of 1.00.

Exposures

Exposures were delivered to the retina by using the same general method as previously described.[34] Pre-exposure images of the RPE cells and cone photoreceptors were taken of a square 2° in visual angle per side using an average power of 20 μW (ON ratio = 0.37, instantaneous power of 55 μW) of 568 nm for autofluorescence imaging, 50 μW of 904 nm for wavefront sensing, and approximately 300 μW of 794 nm for reflectance imaging. Some exposures used 830-nm radiation for reflectance imaging, but the majority of exposures performed in this study used 794 nm for reflectance imaging. No differences were observed between exposures using the two different reflectance sources. The same powers were used for wavefront sensing and for reflectance imaging during the exposures. The power of all radiation entering the cornea was measured using a power meter with a silicon detector (power meter model 1930-C, detector model Phto-918-SL; Newport Corp., Irvine, CA). The power meter and detector have an accuracy of ±1% and were calibrated before the experiments. Retinal radiant exposure was then calculated from the measured power by assuming an ocular focal length of 15 mm.

Exposures of 568-nm light were delivered to the retina over a square 0.5° in visual angle per side (~120 μm per side) by the AOSLO. Radiant exposures ranged from 1 to 788 J/cm^2 of the 568-nm light. All exposures were obtained with the normal mode of operation for the AOSLO (ON ratio = 0.37), except for the 788 J/cm^2 exposures, which used an ON ratio of 1.00. The exposure power and duration were independently varied to test whether reciprocity of radiant exposure holds for those exposure conditions. Tables 2 and 3 provide the exposure powers and durations that were tested as well as the number of trials tested for each radiant exposure and each combination of exposure power and duration.

During the exposures, eye motion was monitored, and the retina was stabilized as previously described.[34] If the eye motion could not be stabilized within approximately 0.1° in any direction, the exposure location was abandoned. Immediately after each exposure, a postex-

TABLE 2. AF Ratio and Radiant Exposure

Radiant Exposure (J/cm²)	Number of Trials	AF Ratio (Mean ± SE)	
		Immed. Post-expo	Days Post-expo
1	16	0.987 ± 0.007	1.024 ± 0.008
2	4	0.984 ± 0.009	0.992 ± 0.013
5	9	0.922 ± 0.006*	1.000 ± 0.017
14	13	0.903 ± 0.011*	0.996 ± 0.007
39	16	0.829 ± 0.011*	1.003 ± 0.014
105	4	0.765 ± 0.004*	0.993 ± 0.051†
289	3	0.709 ± 0.022*	N/A§
788‡	4	0.577 ± 0.028*	N/A§

Immed. Post-expo, immediately after exposure.

* The AF ratio was significantly reduced (paired *t*-test, $P < 0.05$).

† Only three of the four exposures were followed up several days after exposure.

‡ Exposures of 788 J/cm² used the 568 nm light with an ON ratio of 1.00. All other exposures used the normal mode of AOSLO operation with an ON ratio of 0.37.

§ The AF ratio was not measured days after exposure for those locations because the RPE exhibited permanent damage at the site of the exposures.

posure image was taken of the RPE cells and cone photoreceptors over the same 2° square area and under the same conditions as the pre-exposure image. Thus, the immediately postexposure image contains retinal area both exposed and unexposed. Additional images of the RPE cells and cone photoreceptors were taken at the same location between 5 and 63 days after exposure (average ± SD, 33 ± 13 days). Preimages for each exposure did not overlap with each other, and each exposure location was used only once. All exposures were contained within the vascular arcade. Results from some exposures previously published are included in the current analysis.[34]

Quantification of the Autofluorescence Intensity Decrease

The amount of autofluorescence reduction was quantified by the AF ratio as previously described.[34] The AF ratio is the ratio of autofluorescence intensity inside to outside the exposure area for a postexposure image normalized by the ratio of the autofluorescence intensity inside to outside the exposure area for the pre-exposure image:

$$\text{AF ratio} = \frac{\left(\frac{\text{mean (autofluorescence}_{\text{inside-post}})}{\text{mean (autofluorescence}_{\text{outside-post}})}\right)}{\left(\frac{\text{mean (autofluorescence}_{\text{inside-pre}})}{\text{mean (autofluorescence}_{\text{outside-pre}})}\right)}$$

AF ratios were averaged for exposures of the same radiant exposure. Paired *t*-tests between the pre-, immediately post-, and several days postexposure AF ratios were performed. Those differences with $P < 0.05$ were considered significant. To test the reciprocity of radiant exposure, we averaged the AF ratios for exposures of the same retinal irradiance and conducted an analysis of variance (ANOVA) between the power of the exposures and the AF ratios immediately after exposure. Again, the results were considered significant when $P < 0.05$.

RESULTS

Effect of Radiant Exposure on Autofluorescence Reduction

Figure 1 shows three series of images for exposures of 788, 39, and 1 J/cm². With the 788- and 39-J/cm² exposures, a reduction in lipofuscin autofluorescence was observed immediately after the exposure. Long-term imaging showed disruption in the RPE mosaic at the site of the 788 J/cm². No long-term disruption was observed at the site of the 39-J/cm² exposure, and instead the autofluorescence intensity fully recovered. No changes were observed in the autofluorescence intensity immediately after exposure or in long-term follow-up imaging for the 1-J/cm² exposure.

Figure 2 is a graph of the mean and SE of the AF ratios immediately after exposure versus the radiant exposures. Exposures of 247 J/cm² and higher caused permanent retinal damage.[34] Exposures of 5 J/cm² and higher caused a significant immediate reduction in autofluorescence at the site of the exposure, where the magnitude of autofluorescence reduction increased as radiant exposure increased (ANOVA, $P < 0.001$; observed power = 1). No significant autofluorescence reduction was observed immediately after exposure for exposures of 1 J/cm² (paired *t*-test, $P = 0.074$) or 2 J/cm² (paired *t*-test, $P = 0.177$).

Table 2 shows the mean and SE of the AF ratios immediately after and several days after exposure for various radiant exposures. Autofluorescence images several days after exposure showed either full recovery of autofluorescence or permanent

TABLE 3. AF Ratios for Exposures of Various Combinations of Power and Duration

Energy (J/cm²)	Exposure Average Power (μW)	Exposure Duration (sec)	Number of Trials	AF Ratio (mean ± SE)	
				Immed. Post-expo	Days Post-expo
1	1.9	90	4	0.987 ± 0.017	1.034 ± 0.018
	2.9	60	4	0.968 ± 0.008	1.040 ± 0.016
	5.7	30	4	1.000 ± 0.019	1.002 ± 0.015
	11.0	15	4	0.992 ± 0.004	1.018 ± 0.017
5	7.4	120	4	0.920 ± 0.013	0.996 ± 0.009
	20.0	45	5	0.924 ± 0.004	1.003 ± 0.017
14	7.4	330	4	0.894 ± 0.016	1.003 ± 0.011
	20.0	120	4	0.921 ± 0.033	0.978 ± 0.016
	45.0	53	5	0.896 ± 0.009	1.005 ± 0.006
39	7.4	900	4	0.863 ± 0.013*	1.004 ± 0.012
	20.0	330	4	0.847 ± 0.014*	0.997 ± 0.028
	36.0	184	4	0.810 ± 0.005*	1.031 ± 0.020
	45.0	147	4	0.795 ± 0.032*	0.978 ± 0.045

Immed. Post-expo, immediately after exposure.

* Although the AF ratio appears to decrease with exposure power for the 39 J/cm² trials, this was not a significant trend.

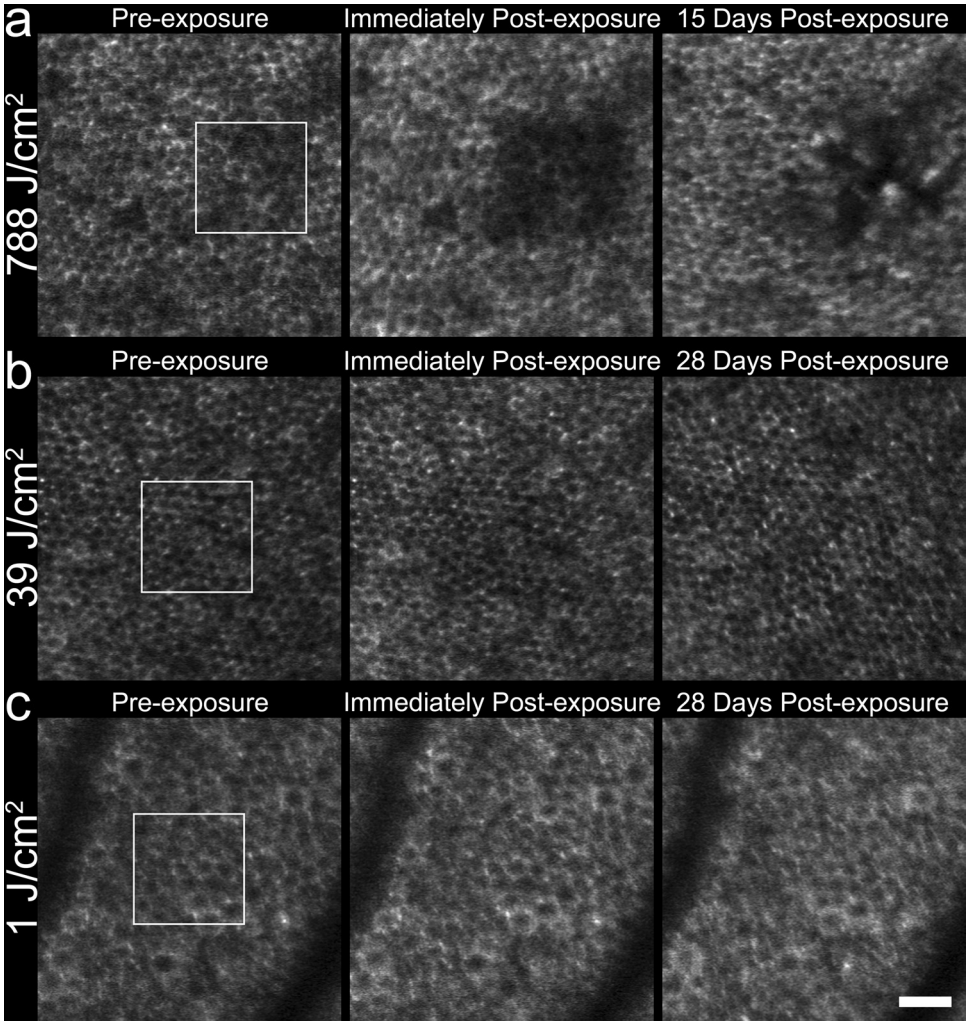

FIGURE 1. Time series of autofluorescence images of the RPE mosaic for locations exposed to (**a**) 788, (**b**) 39 and (**c**) 1 J/cm^2. The 788-J/cm^2 exposure (**a**) caused an immediate decrease in autofluorescence intensity followed by long-term disruption in the RPE mosaic at the site of the exposure. The 39-J/cm^2 exposure (**b**) caused an immediate decrease in autofluorescence intensity at the site of the exposure followed by long-term full recovery of the autofluorescence. The 1-J/cm^2 exposure (**c**) caused no change in autofluorescence intensity. *Boxes*: the exposure locations. Scale bar: 50 μm.

RPE cell damage at the site of the exposure. At exposures of 210 J/cm^2 and lower,[34] the several days after exposure AF ratio was not significantly different from the pre-exposure AF ratio, (paired *t*-test, $P = 0.233$) indicating full recovery of autofluorescence.

Reciprocity over Radiant Exposure

Table 3 shows the various combinations of exposure power and duration tested for four different radiant exposures, and the resulting mean and SE of the AF ratios for those exposures. No significant changes between the pre-exposure and the immediately postexposure AF ratio were observed at any of the four combinations of exposure powers and durations yielding a radiant exposure of 1 J/cm^2 (ANOVA, $P = 0.407$, observed power = 0.217). In Figure 3, AF ratio immediately after exposure is plotted for the combinations of exposure power and duration tested for the four radiant exposures. The two combinations of exposure power and duration tested for exposures of 5 J/cm^2 were not significantly different from each other (ANOVA, $P = 0.752$, observed power = 0.059), but did cause a significant decrease in the AF ratio immediately after exposure (paired *t*-test, $P < 0.001$). Likewise, the three combinations of exposure power and duration tested for 14 J/cm^2, and the four combinations tested for 39 J/cm^2 were not significantly different from one another (ANOVA, $P = 0.727$, observed power = 0.110, $P = 0.081$, observed power = 0.536, respectively). Overall, reciprocity of exposure power and duration held for the exposures tested; however, there is a possibility that the variability and small sample size of this study masked an effect of radiant exposure on the AF ratio.

DISCUSSION

The two primary objectives of this study were to determine the maximum exposure that does not produce a detectable reduction in autofluorescence and to establish the nature of the reduction in autofluorescence. No measurable change in the autofluorescence was detected after exposures to 2 J/cm^2 or less, but a reduction in autofluorescence was observed after radiant exposures of 5 J/cm^2 and higher. This range (2–5 J/cm^2) represents the sensitivity limit for detecting the autofluorescence reduction with the AOSLO imaging technique rather than defining a true threshold for the phenomenon. The photochemical nature of this phenomenon leads to the expectation that any light exposure would result in some amount of autofluorescence reduction.

Varying the exposure duration and power while maintaining a constant radiant exposure produced the same amount of autofluorescence reduction, demonstrating that the total radiant exposure delivered to the retina, rather than its distribution in time, determined the amount of autofluorescence reduction. That result implies that reciprocity of radiant exposure held over the tested exposure durations of 15 to 900 seconds. However, there is a possibility that the variability and small

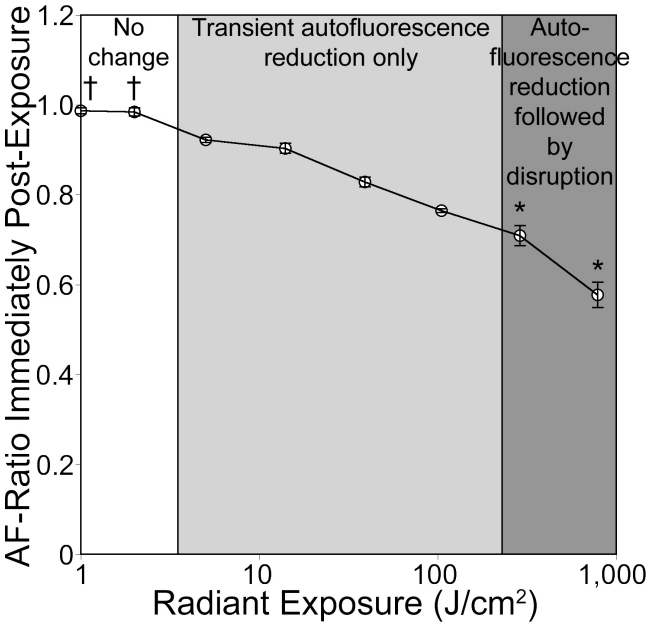

FIGURE 2. Mean and SE of AF ratios immediately after exposure at a range of radiant exposures. Data are shown for *exposures that resulted in permanent damage and †no significant reduction in autofluorescence. Ranges of radiant exposures causing no change (*unshaded*), transient autofluorescence reduction (*light gray*), or permanent damage (*dark gray*) are indicated.

sample size of this study masked an effect of radiant exposure on AF ratio and thus did not permit the finding of a breakdown in reciprocity for these exposures.

Our previous study describing the long-term retinal damage[34] detailed some reasons supporting the idea that the retinal damage was caused by a photochemical mechanism. These reasons include the delayed appearance of structural damage in the RPE cell mosaic and the lack of a damaging temperature increase in the retina. In addition, and as expected for a photochemical mechanism, the autofluorescence reduction is confined to a square area of the retina limited by the spatial extent of the radiant exposure (Figs. 1a, 1b). Likewise, the observation from the results in this study that reciprocity holds is consistent with a photochemical origin[40,43,46] for the autofluorescence reduction phenomenon as well.

In the present study, we tested and found reciprocity of radiant exposure at exposure durations from 15 to 900 seconds. However, reciprocity is expected to fail under certain conditions on both ends of the time scale. For example, Ham et al.[40] found that reciprocity held down to 10 seconds, below which the exposures did not show reciprocity and the observed retinal lesions were thus presumed to be caused by thermal rather than photochemical mechanisms. A study by Lund et al.[43] shows conditions in which reciprocity held down to 5 seconds. On the other side of the scale, Ham et al.[40] found that reciprocity broke down after extremely long durations. After finding the radiant exposure that produced a minimal lesion, Ham et al.[40] exposed the retina four separate times to one fourth of the radiant exposure causing a minimal lesion, where each exposure was separated by 48 hours. In this case, no lesion was observed, and thus those exposure conditions exemplified a failure of reciprocity. A study of the additivity of radiant exposure by Griess and Blankenstein[46] found that additivity could be described by an exponential function with a time constant of 4 days.

As previously discussed by Morgan et al.,[34] possible mechanisms for the autofluorescence reduction include A2E photoisomerization[47,48] or photooxidation.[8,49] Understanding the mechanism causing the decrease in autofluorescence remains an important question because it would help to determine whether the observed reduction in autofluorescence is detrimental to retinal health. For example, if the autofluorescence reduction is caused by the photooxidation of A2E or another component of lipofuscin, the phenomenon may be toxic to the retina. However, if the autofluorescence reduction is caused by the photoisomerization of one or more of the components of lipofuscin, the observed retinal changes may be benign. Regardless, the long-term full recovery of the autofluorescence and the breakdown of reciprocity described by Ham et al.[40] and Griess and Blankenstein[46] point to the existence of at least one retinal repair mechanism, by which the retina can to some extent counterbalance the deleterious effects of light exposures. Given that no long-term changes can be detected in the AOSLO images of the RPE cells or cone photoreceptors at exposure sites after autofluorescence recovery in this study, it is likely that the autofluorescence reduction phenomenon is either intrinsically benign or is counteracted by a retinal repair mechanism that happens to restore autofluorescence. Of course, we cannot eliminate the possibility that retinal damage is caused by the process of autofluorescence reduction and recovery and that the permanent damage just remains undetected by the AOSLO imaging methods used in this study. Histologic studies including electron microscopy may help determine whether any toxic retinal changes after autofluorescence reduction and recovery exist.

Effect of Multiple Wavelengths

In this study, the retina was simultaneously exposed to multiple wavelengths for wavefront sensing (904 nm), reflectance imaging (830 or 794 nm), and autofluorescence imaging or test exposures (568 nm). We have shown in a prior study that the

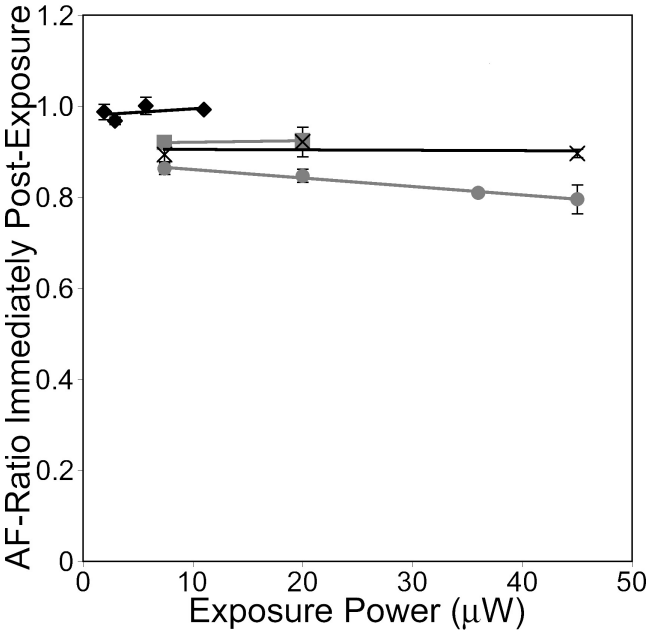

FIGURE 3. Data points plot the mean and SE of the AF ratio immediately after exposure for various combinations of exposure power and duration resulting in radiant exposures of 1, 5, 14, and 39 J/cm^2. Although the AF ratio appeared to decrease with exposure power for the 39 J/cm^2 trials, the trend was not significant. No significant reduction in autofluorescence was observed at exposures of 1 J/cm^2. *Filled diamonds*: 1 J/cm^2; *gray squares*: 5 J/cm^2; *X*: 14 J/cm^2; *gray circles*: 39 J/cm^2.

expected temperature increase in the retina from the infrared (IR) and 568-nm exposures is minimal.[34] In addition, the photochemical mechanism of autofluorescence reduction is probably caused by a molecular change in one or more components of lipofuscin, which minimally absorbs IR radiation.[50] In current safety standards, photochemical and thermal mechanisms are treated independently,[51] and thus the minimal temperature rise and absorption by lipofuscin of the IR radiation used in this study would have no effect on the observation of autofluorescence reduction. However, we cannot rule out the possibility that thermal and photochemical mechanisms interact in the retina. If this is the case, then theoretically, a minimal rise in retinal temperature would lead to enhanced photochemical effects, including the long-term retinal damage and autofluorescence reduction phenomena in this study. Indeed Han et al.[52] show that multiple photon absorption in synthesized melanin does not follow an independent model of thermal and photochemical effects and therefore that thresholds for damage in multiple-wavelength exposures may be underestimated. Further experiments are necessary to determine the full effect of multiple-wavelength simultaneous exposures and any resulting interactions between thermal and photochemical effects in the retina.

Light Exposures and Safety Standards

Because the photochemical molecular mechanism causing the reduction and recovery of autofluorescence is unresolved, it is important to examine the exposures in the context of light safety standards.[35,53–55] Figure 4 shows the tested exposures in comparison with the ANSI standard[35] MPE for exposures of 568 nm over a square 0.5° in visual angle per side in the monkey eye of 15 mm focal length. As described in the present study and previously by Morgan et al.,[34] we have found long-term damage at light levels below the ANSI standard and previously thought to be safe. As well, we have observed

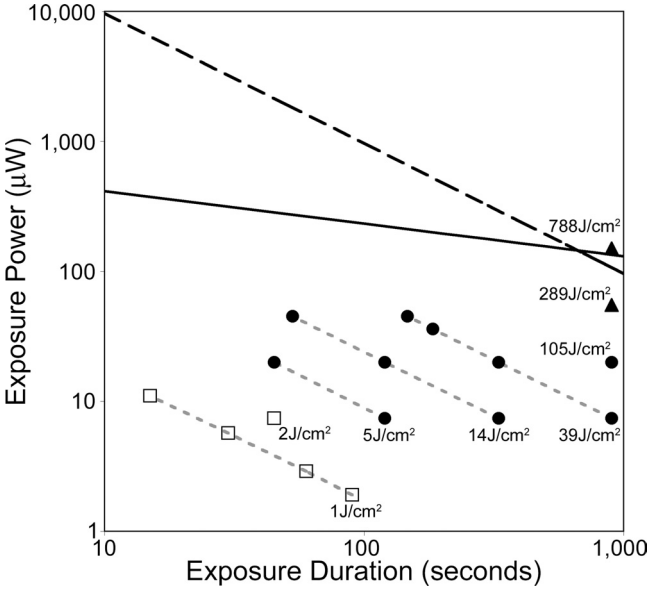

FIGURE 4. The exposures tested in this study are compared with the ANSI standard.[35] *Solid line*: thermal MPE exposure limit calculated from the ANSI standard for exposures to 568-nm light over a square 0.5° in visual angle per side for an eye with a focal length of 15 mm. *Dashed line*: photochemical MPE for the same conditions. (▲) Exposures that caused long-term permanent damage to the RPE; (●) exposures that caused a significant reduction in autofluorescence; (□) exposures in which no significant change in the RPE autofluorescence was observed. The radiant exposures tested in this study are labeled.

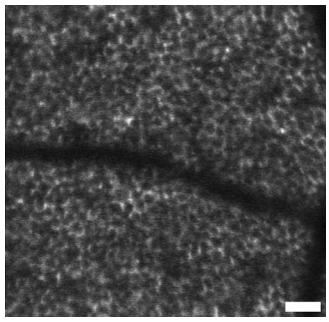

FIGURE 5. Images of the RPE mosaic taken with an exposure of only 0.15 J/cm^2 of 568-nm light. A 90-nm band-pass emission filter centered at 630 nm was used for the detection of autofluorescence emission. Scale bar, 50 μm.

autofluorescence reduction with exposures (5 J/cm^2) 31 times less than the ANSI MPE (155 J/cm^2, thermal limit). In light of the new data, the ANSI standard[35] and other light safety standards[53–55] should be modified to ensure adequate retinal protection from light exposures. However, the extent to which those standards must be decreased will depend on whether the mechanism causing the reduction in autofluorescence is found to be harmful.

Safe RPE Mosaic Imaging

The ability to image the RPE cells on an individual cellular level in vivo provides numerous advantages for studying the normal and diseased retina because quantitative metrics such as cell density, size, and regularity can be applied to advancing the understanding of retinal disease progression and treatment efficacy.[32] However, the imaging techniques used on the living human eye need to be completely noninvasive. Until it is known whether or not autofluorescence reduction is benign, light exposures causing a reduction in autofluorescence should be avoided whenever possible. Figure 5 shows a sample image of the macaque RPE mosaic taken with an exposure of only 0.15 J/cm^2 of 568 nm and using a broader emission filter for autofluorescence detection. This exposure is more than an order of magnitude below the 2-J/cm^2 exposure that caused no detectable autofluorescence reduction. Thus, we have demonstrated safe and successful imaging of the individual cells in the RPE mosaic despite the reduced light intensity for autofluorescence excitation.

CONCLUSIONS

We investigated the nature of and limits to the reduction and recovery of autofluorescence after exposure to light as was described by Morgan et al.[34] The sensitivity of this method yielded a limit to detecting reduced autofluorescence with the AOSLO after a light exposure between 2 and 5 J/cm^2. The magnitude of the autofluorescence reduction increased as the radiant exposure increased, and reciprocity held for all exposures tested from 15 to 900 seconds, showing that the phenomenon is a result of photochemical changes in the retina. It is still unknown whether autofluorescence reduction poses a safety risk to the retina. Future studies to describe the action spectrum of autofluorescence reduction and histologic characteristics of retinal locations experiencing both autofluorescence reduction with recovery or long-term disruption could help elucidate the retinal health risk and the mechanisms involved. Until these phenomena are completely understood, it is prudent to ensure exposures to the retina remain below those that cause any retinal changes, including the reduction in

autofluorescence, unless absolutely necessary. To meet this goal, we demonstrated RPE cellular imaging using light exposures more than an order of magnitude below the autofluorescence reduction detection limit. In general, the implementation of safe methods, not only to image the RPE cell mosaic in vivo, but also to deliver high-intensity light to the retina, requires a better understanding of the cause and effects of autofluorescence reduction.

Acknowledgments

The authors thank Robert Wolfe and Benjamin Masella for assistance in the work.

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