An investigation of the role of macular pigment in attenuating photostress through comparison between blue and green photostress recovery times

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ABSTRACT

Purpose: Photostress recovery time (PSRT) is the time required for the macula to return to its normal functioning after the bleaching of cone photopigments due to light exposure, usually white. This work investigates the role of macular pigment (MP) as an optical filter which attenuates photostress by analyses of PSRT at different wavelengths.

Methods: Thirty-nine subjects (19-28 years) were exposed to blue/green photostress varying in irradiance. During photostress, pupil constriction (C_p) was measured. Twenty-seven subjects (20-27 years) were exposed to white photostress. After 25 s of photostress, the time (PSRT) required to read correctly a 0.2 logMAR letter was measured. Correlation was studied between PSRT, C_P and irradiance. Statistical significance of differences between PSRTs was evaluated at Log(irradiance(quanta s^{-1} cm^{-2}))=14 by Student's t statistics.

Results: C_p and PSRT were found linearly correlated to Log(irradiance) for blue, green, and white. At Log(irradiance(quanta s^{-1} cm^{-2}))=14, blue and green mean PSRTs resulted different ($p < 0.001$) with 3.8 ± 0.8 s and 6.7 ± 1.7 s respectively. After correcting irradiance for the optical absorption of MP, mean blue PSRT became 6.6 ± 0.8 s, at the logarithm of MP-corrected irradiance in quanta s^{-1} cm^{-2} equal to 14 ($p = 0.571$ compared to green PSRT). For white light, at the logarithm of MP-corrected irradiance in quanta s^{-1} cm^{-2} equal to 14, mean PSRT was 7.5 ± 2.2 s, not significantly different from blue and green PSRT ($p > 0.05$).

Conclusions: MP plays the role of an optical filter attenuating photostress. PSRT was substantially proportional to the number of incident photons corrected for the MP optical absorption, regardless of their wavelength.

Key words: photostress, colour, macular pigment, photons, Retinal Cone Photoreceptor Cells.

Introduction

Photostress is the bleaching of the retinal foveal cone photopigments due to light exposure resulting in a temporary scotoma. Recovering the normal retinal photosensitivity depends on the synthesis of these cone photopigments in the outer retinal segments. The photostress recovery test consists in the measurement of the time required for the macula to return to its normal functioning after transient insensitivity due to photostress.[1] The test was used to distinguish between macular and optic nerve diseases because recovery does not depend on neural mechanisms.[2,3] In order to determine the photostress recovery time (PSRT), different paradigms were used to measure the time needed to recover to a predetermined level of some functional parameters assessed subjectively, such as visual acuity[4,5] and foveal sensitivity.[6]. Alternatively this can be done by objectively measuring the time needed to return to a certain level of a baseline parameter measured by visual evoked response.[7] Several factors can affect PSRT, such as age,[6] retina diseases,[8,9] glaucoma,[10] and use of drugs.[11,12]

In an experimental setting or in clinical practice, PSRT is typically measured inducing photostress with white light, such as light emitted by an ophthalmoscope or other sources, regardless of the strong differences in terms of spectral distributions between LEDs, incandescent filaments, fluorescent lamps, etc.

Approximately ten years ago, Stringham et al. started to discuss the dependence of PSRT on the presence of macular pigment (MP) in the fovea.[13,14] Higher MP densities resulted in faster PSRTs, lower disability glare contrast thresholds, and lower visual discomfort.[13-15] The relationship between the optical density spatial profile of MP and the measures of glare disability across the macula was also explored. Stringham et al.[16] found MP density to be related to improvements in glare disability and PSRT, consistently with its spatial profile. Putnam and Bassi[17] evaluated glare disability as the difference in contrast sensitivity

between glare and no-glare conditions. These results were also found to be consistent with the glare attenuation effects of MP. The relationship between MP and visual performance was also studied by Loughman et al.[18] They found that best corrected visual acuity and contrast sensitivity were positively associated with MP optical density, in contrast to PSRT and glare sensitivity. This work aimed to investigate the role of MP as an optical filter which attenuates photostress by measuring possible differences of PSRT after photostress at different wavelengths. Pupil constriction (C_p) was also measured during photostress. It is known that PSRT is independent of pupil size.[4] However, C_p was measured as a reference parameter. With the same number of incident photons on the eye per unit time and unit area, C_p is expected to be equal for blue and green.[19]

Methods

Subjects and procedure

In the first phase of the study, thirty-nine healthy subjects (19-28 years) wearing their habitual ophthalmic correction, took part in this study. Inclusion criteria included the absence of any ocular pathology, the absence of any medical therapy, and a monocular best-corrected visual acuity (BCVA) not lower than 0.0 logarithm of the minimum angle of resolution (logMAR) in each eye. The study was conducted following the Declaration of Helsinki: after the explanation of the procedures, consent was obtained from all subjects.

The Optics and Optometry Board of the Materials Science Department of the University of Milano Bicocca granted approval for the study. High contrast monocular BCVA (100% nominal contrast) was measured at a distance of 4.3 meters using Sloan letters displayed on a LCD optotype system (Visionix L4024P) with room lighting of 500±50 lux.

After BCVA measurement, and after five minutes of full dark adaptation, subjects placed their chin on a chinrest and aligned perpendicularly to the LED (bleaching source). The LED was displayed on the midline at 45±1 cm from the eyes (Fig. 1). Subjects were asked to look at the LED keeping both their eyes open during the 25 s exposure, taking their optical correction off, if normally used. The LED was integrated with a magnifying lens which generated a wide 30 mm diameter homogeneous source of light. The retinal stimulation size was worked out for a standard emmetropic reduced eye, resulting in 1.2 mm that is equivalent to an angle subtend of about 4 deg.

After turning the LED off and putting the subjects own glasses, on if used, utilizing a stopwatch the photostress recovery was evaluated by measuring the time required to correctly read at least one letter of the suprathreshold line of 0.2 logMAR.

In order to evaluate the effect of photostress at different wavelengths on PSRT, a repeated-measures design was used. Two LEDs, 450 nm (blue) and 523 nm (green) with *full width half maximum* of 20 nm, were used together with four different neutral-density filters on the optical path, to vary the irradiance on the eye. Irradiance L was measured by a common power meter in the position of the eye of the subject. Irradiance changed, depending on the optical density of the neutral filter, from 0.32 to 1.56 W/m^2 for blue and from 0.14 to 0.77 W/m^2 for green within an error of approximately 10% of the measured value. In each condition, the number of photons N_{ph} per unit area and unit time, was calculated from the measured irradiance L in W/m^2 taking into consideration that

$$N_{ph} = \frac{L\lambda}{hc}, \quad (1)$$

where λ is the wavelength of light, *h* is the Planck's constant and *c* is the speed of light in vacuum. To summarize, in each subject the PSRT was measured for eight different repeated conditions (Table I), in a random order. Between one condition and another, each subject was allowed to rest for five minutes in the dark.

During each experimental condition, pupil size was measured. At about 15 cm from the eye, a CCD was placed to take pupil images in the near infrared (Fig. 1). Attention was paid to place the CCD out of the optical path of the blue/green LED light. In order to illuminate the pupil with near infrared light, an additional LED (880 nm) was placed close to the CCD. Before turning the blue/green LED on, and during the 25 s of glare, the pupil size was recorded by the infrared CCD. Pupil data reported here refers to one of the two eyes (dominant one), having verified that the pupillary response was not significantly different in the two eyes. Pupil constriction C_p was defined as

$$C_p = \frac{(D_{max} - D_{min})}{D_{max}}, \quad (2)$$

where D_{max} is the pupil diameter in dark condition (before turning the blue/green LED on) and D_{min} is the pupil diameter after 5 s of glare. During the following 25 s of glare, pupil constriction was observed to hold reasonably steady, as also reported in other studies and under continuous illumination.[19-23]

In a second phase, PSRT was measured on twenty-seven subjects (20-27 years) by using a white LED. Experimental setting, inclusion criteria, and general procedures were the same as described in the first experiment, except for the pupil measurement (not recorded in this second experiment). White LED emission spectrum was measured by a spectrophotometer Hamamatsu TM-UV/VIS C10082CA-2200. Also in the case of white light glare, measurement was carried out by adding four different grey filters on the optical path to vary the incident irradiance on the eye from 0.12 to 0.61 W/m^2 corresponding to logarithm of irradiance of 13,5, 13.8, 14.0 and 14.2 quanta s^{-1} cm^{-2} for the four steps respectively (Table I). In this case, the number of photons N_{ph} per unit area and unit time was calculated as the integral between 400 nm and 750 nm of the measured emission spectrum.

Statistical analysis

The Kolmogorov–Smirnov test was used to evaluate the results for a normal distribution of pupil size and PSRT data.

The relationship between pupil constriction and level of irradiance (log Irradiance) for blue/green LEDs, as well as the relationship between PSRT and level of irradiance (log Irradiance) for blue/green/white LEDs, was evaluated using correlation analysis (Pearson or Spearman coefficients).

A one-way ANOVA for repeated measures was used to determine the difference in pupil diameter and PSRT between the four levels of irradiance for each single LED color. Therefore irradiance is the independent variable, with four different levels.

Statistically significant differences between blue and green for both pupil constriction and PSRT at a fixed irradiance level were evaluated by paired Student's t statistics (threshold of significance $p < 0.05$). Differences between white and blue/green for PSRT at a fixed irradiance level were evaluated by unpaired Student's t statistics (threshold of significance $p < 0.05$).

Results

First phase

All the distributions of C_p for each level of irradiance of blue and green were normal. For the PSRT the distributions were all normal except for the second level of irradiance of green.

Mean C_p and mean PSRT of the subjects are reported as a function of incident irradiance in Fig. 2. Irradiance (number of photons per unit area and unit time) is reported in logarithmic scale, and data is well described by linear function. A significant correlation was found between mean C_p and the level of irradiance with Pearson coefficients $r = 0.995$ for blue ($P<0.01$) and $r = 0.998$ for green ($P<0.01$) respectively. A significant correlation was also

found between mean PSRT and the level of irradiance for blue and green light with Spearman coefficients r = 0.998 (P<0.01) and r = 0.998 (P<0.01) respectively.

CP differences between the four levels of irradiance were statistically significant for both green (ANOVA; F=526.47 P<0.001) and blue (AVOVA; F=246.81 P<0.001). Also PSRT differences between the four levels of irradiance were statistically significant for both green (ANOVA; F=130.23 P<0.001) and blue (AVOVA; F=217.27 P<0.001).

As far as the comparison between blue and green is concerned, no evidence of differences was observed between the mean C_p for blue and green, as it can be inferred by the overlap between the two lines in Fig. 2a and by statistical analysis of data. Cp values for a specific irradiance value ((i.e. Log(irradiance(quanta s-1 cm-2))=14) were determined from linear regression of data for each subject. Mean values, standard deviations, and p-values of Student's t test are reported in Table II. No statistically significant differences in Cp were found between blue and green ($p > 0.05$). However, a statistically significant difference in PSRT was found between blue and green PSRTs. The difference is evident by observing Fig. 2b, PSRT being clearly lower for blue than for green for a fixed irradiance. In addition, blue and green PSRT was also calculated for each subject at Log(irradiance(quanta s-1 cm-2))=14, similarly as performed for C_p. Mean values and standard deviations are reported in Table II, together with the p-values, as determined from the Student's t-test, which is far below the level of significance of 0.05 and indicates a clear statistically-significant difference between the PSRT for the two colors.

Second phase

PSRT was measured in each subject in four experimental conditions (Table I). PSRT distributions were normal only for the two highest level of irradiance of white LED.

A significant correlation was found between average PSRT and level of irradiance for white LED with Spearman coefficient r = 0.99 (P<0.001).

With the white, PSRT differences between the four levels of irradiance were statistically significant (Friedman's ANOVA, $p<0.001$).

In order to compare results of the two phases of the experiment, the measures of irradiance of LEDs (green, blue and white) in the two experiments were corrected, taking into account the optical absorption of the MP. PSRT values are presented in Fig. 3 against the new values of irradiance on a logarithmic scale. In the inset of Fig. 3, the transmittance spectrum of human MP is shown as deduced by calculating 10^{-OD}, where OD is the optical density spectrum reported in ref.24. Irradiance values after correction for MP absorption are here indicated as $I_{corrected}$. The difference between mean PSRTs corresponding to different colors is much less obvious when compared to Fig. 2b. PSRT values for a specific irradiance value ((i.e. Log(irradiance(quanta s-1 cm-2))=14) were determined from linear regression of data for each subject. Mean values, standard deviations, and p-values of Student's t test at $Log(I_{corrected}(quanta\ s^{-1}\ cm^{-2}))=14$ are reported in Table III. No statistically significant differences were found between blue, green, and white ($p > 0.05$). All mean data from Fig. 3 were included to obtain a single equation by linear regression of PSRT as a function of $I_{corrected}$ (dashed line in Fig. 3).

Discussion

Discussion begins with the comparison between C_p and data in the literature. As mentioned in some classical literature pieces early 20th century, a certain correspondence is expected between pupil response to light and retina sensitivity.[25-28] Hecht and Pirenne[29] described the relative pupil sensitivity curve, defined as the reciprocal of the energy causing the same pupil contraction at different wavelengths. They found the maximum size for the human pupil to be at 515 nm, similarly as other authors reporting it to be at 510 nm.[30] However, these curves are not directly comparable with previous curves in the literature, as in many old

papers, pupil size is reported at different wavelengths for equal incident energy. On the contrary, the sensitivity curve, as described by Hecht, Pirenne[29] and later authors, is the reciprocal of the relative energy required, at different wavelengths, to produce the same pupil contraction. More recently, intrinsically photosensitive retinal ganglion cells were found to influence non-image-forming functions, including pupillary light reflex.[19,31-39] Gamlin et al.[19] discussed in detail the melanopsin role, and they reported data that can be directly compared with the results in Fig. 2a of this work. Pupil size was measured during illumination at ten different wavelengths as a function of irradiance in (quanta cm^{-2} s^{-1}) in logarithmic scale from 9 to 15.[19] Pupil response for stimuli between 452 nm and 552 nm was comparable in size. At Log(irradiance(quanta s^{-1} cm^{-2}))=14 quanta cm^{-2} s^{-1}, Gamilin et al. reported pupil size to be of about 3 mm for both green and blue,[19] which corresponds to C_p of about 50% (the typical mean value for D_{max} in eq. (2) is about 6 mm[40]). The values in Fig. 2a and Table II in this work are in accordance with this data. As in Gamlin et al.,[19] no substantial difference is observed here between blue and green (450 and 523 nm) in the irradiance range under investigation (confirmed by Student's t statistics, Table II). Concerning C_p, a last comment concerns the conversion between the number of photons and irradiance measured in lumen/m^2, after correction for human spectral sensitivity.[41] By correcting irradiance data in Fig. 2a, blue LED irradiance values are much lower than green ones, and the overlap between the two ranges in lumen/m^2 is poor (data were converted in lumen/m^2, but they are not shown here). In the restricted overlap region (55$\pm$15) lumen/m^2), C_p is higher for blue compared to green. This behavior is compatible with data in the literature showing a stronger constriction for blue incident light compared to higher wavelengths of similar irradiance in lumen/m^2.[42-44]

The scenario differs for PSRT. A non-negligible difference between blue and green is clearly observed in Fig. 2b, and is confirmed by Student's t statistics (Table II). Notwithstanding a similar pupil response, the same number of incident photons is less critical for blue PSRT,

compared to green. In a first attempt to understand the origin of this difference, for each PSRT from 3 s to 7 s, the equations of the two lines in Fig. 2b were compared to obtain the factor with which to multiply the irradiance of the blue so that blue and green PSRTs would coincide. This multiplicative factor is found to be (0.31 ± 0.05), in agreement with the ratio (~0.33) between the optical transmission of the human MP at 450 nm (~30%) and at 523 nm (~ 90%) (inset of Fig. 3,[24]). This motivated us to correct the measured irradiance by taking into consideration the MP optical absorption (Fig. 3). With this correction, the difference between blue and green at fixed $I_{corrected}$ is no longer present (6.6 ± 0.8 s to be compared with 6.7 ± 1.7 s, p = 0.571). Therefore, the difference of PSRT between blue and green light, with the same number of incident photons is almost entirely attributable to the three-times greater transmittance of the MP in the green compared to the blue. An additional contribution is expected to be the wavelength-dependence of the light transmission by the crystalline lens. In adults and elderly people, its transparency is partially compromised, mainly at the lower wavelengths.[45] Below the age of 30 years, the difference of transmittance between blue and green is expected to be of few percentage points. Transmittance can be reasonably assumed to be approximately 95% and 90% at 523 and 450 nm, respectively.[45] Therefore, the product of the transmittances of crystalline lens and MP is estimated to be about 27% at 450 nm and about 86% at 523 nm, their ratio (0.31) complying with the measured ratio (0.31 ± 0.05) between green and blue irradiances to produce the same PSRT. The previous considerations confirm the major and relevant role of MP in attenuating photostress. Stringham et al. widely discussed the benefits of MP for photostress recovery, and demonstarted that PSRT is significantly shorter for subjects with higher MP density.[13-15] In the present work, the experimental evidence of the role of MP as an optical filter is the wavelength-dependence of PSRT, which follows the wavelength-dependence of MP absorption. For this reason, data of PSRT was also reported in Fig. 3 as a function of irradiance corrected for the MP absorption ($I_{corrected}$), together with additional data of recovery

time taken with a white LED. No significant differences were observed between blue, green, and white as a function of $I_{corrected}$, as expected. In conclusion, MP plays the role of an optical filter which attenuates photostress. When comparing blue and green, after correcting the incident irradiance by the MP absorption, PSRT is substantially proportional to the number of photons regardless of their wavelength. These conclusions support the hypothesis that the effect of MP on photostress is attributable to its role of optical filter rather than to other physiological effects of visual processing, such as photopigment regeneration. The physiological role of the MP on other visual functions is out of the scope of this work, and deserves to be further investigated. The last comment concerns the conversion between the number of photons and irradiance measured in lumen/m^2 after correction for human spectral sensitivity (40). The difference between blue and green with equal irradiance would not be negligible if irradiance was expressed in lumen/m^2 both before and after correction for the MP absorption.

Funding: This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Declaration of Interests: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Table I. The eight conditions for which PSRT was measured for each of the 39 subjects in the first phase of the study, and the four conditions for which PSRT was measured for each of the 27 subjects in the second phase of the study.

LED colour	blue (450 nm) PHASE 1				green (523 nm) PHASE 1				white PHASE 2			
Condition (different filter)	1	2	3	4	1	2	3	4	1	2	3	4
Irradiance (W/m^2)	0.32	0.62	0.90	1.56	0.14	0.29	0.46	0.77	0.12	0.24	0.36	0.61
Log(Irradiance(quanta s^{-1} cm^{-2}))	13.86	14.15	14.31	14.55	13.57	13.88	14.08	14.31	13.53	13.82	13.99	14.22

Table II. Mean and standard deviation (SD) of pupil constriction C_p and recovery time PSRT on 39 subjects, at Log(irradiance(quanta s^{-1} cm^{-2}))=14 deduced from the linear equations obtained by regression of the experimental data measured on each subject at variable irradiance. P-values of Student's t paired test are also reported between blue and green.

	C_p (%)		PSRT (s)	
	green	blue	green	blue
mean	50.6	51.0	6.7	3.8
SD	7.3	6.5	1.7	0.8
p-value	0.385		< 0.001	

Table III: Mean and standard deviation (SD) of recovery time at Log ($I_{corrected}$(quanta s^{-1} cm^{-2}))=14 deduced by the linear equations obtained by regression of the experimental data at variable $I_{corrected}$ measured on each subject. Paired(P)/unpaired(U) p-values of Student's t test are also reported.

	Recovery time (s)		
	green	blue	white
mean	6.7	6.6	7.5
SD	1.7	1.5	2.2
p-value	0.571 (P)		
			0.074 (U)
		0.129 (U)	

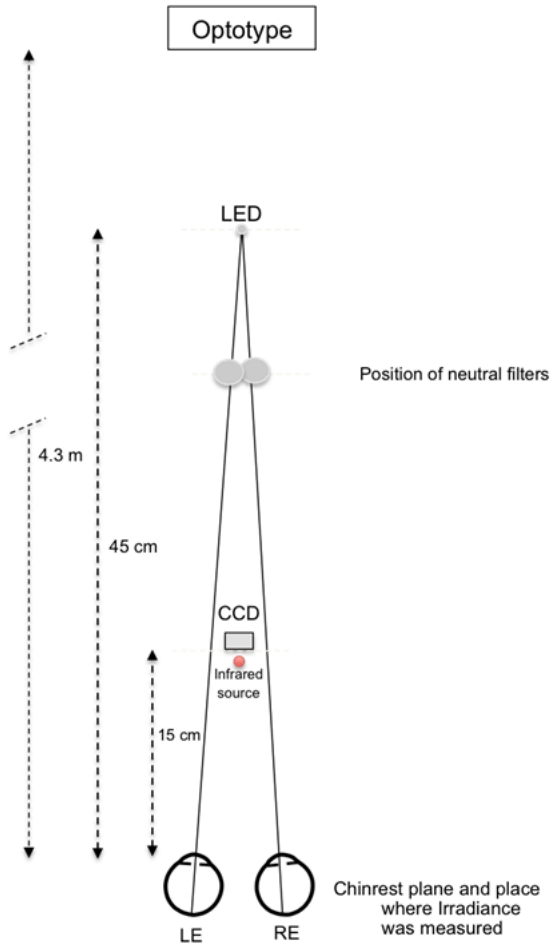

Figure 1. **Setting**. Sketch of the experimental setting.

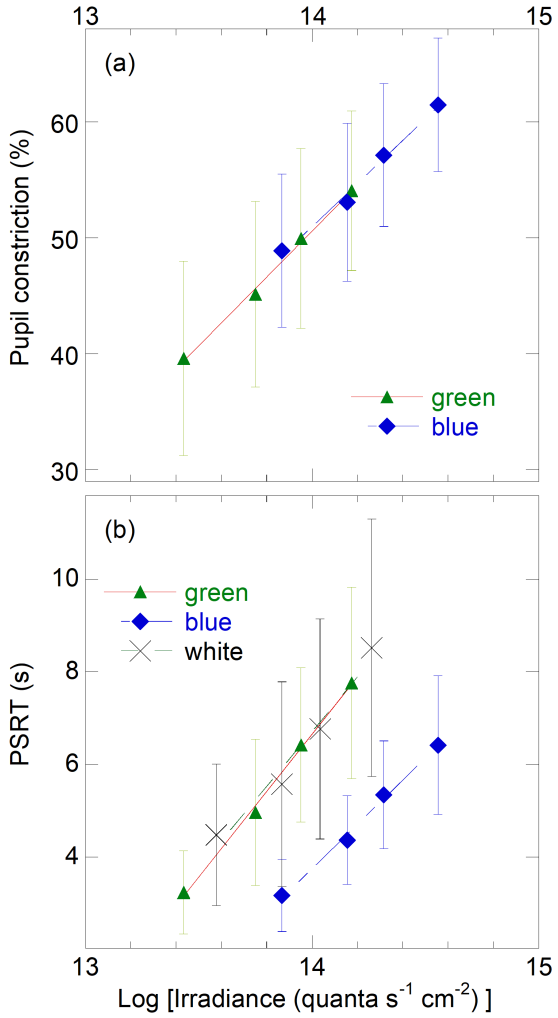

Figure 2. **Pupil constriction (C_p) and recovery time (PSRT) for blue and green light.** (a) Mean pupil constriction (39 subjects) under blue (♦) and green (▲) photostress and (b) mean PSRT (39 subjects) after blue (♦), green (▲), and white (×) photostress, as a function of irradiance on the pupil plane. Error bars: standard deviations of measured data. Lines: results of linear regression of data (blue pupil constriction: y = -212.90 + 18.86x, R = 0.995; green pupil constriction: y = -227.72 + 19.88x, R = 0.998; blue recovery time: y = -63.24 + 4.79x, R = 0.998; green recovery time: y = -79.73 + 6.17x, R = 0.998; white recovery time: y = -75.83 + 5.90x, R = 0.984).

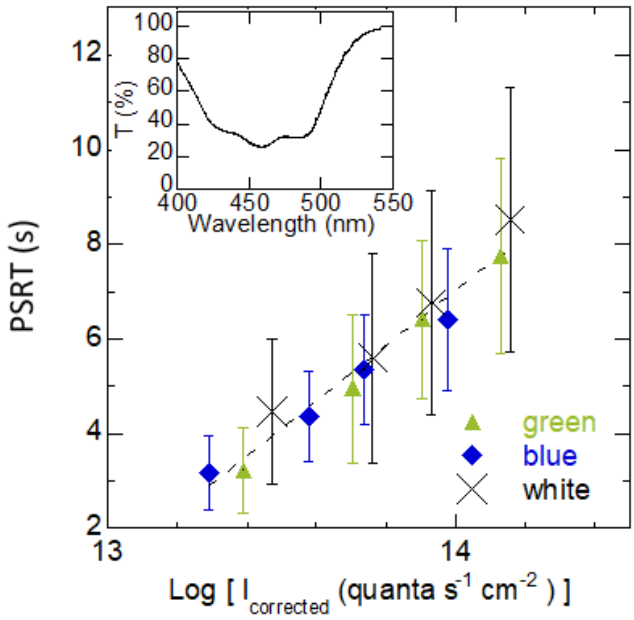

Figure 3. **Recovery time (PSRT) as a function of irradiance corrected for the absorption of macular pigment.** Mean recovery time after blue (♦), green (▲), and white (×) photostress as a function of irradiance on the pupil plane corrected for the optical absorption of the macular pigment. Error bars: standard deviations of measured data. Dashed line: result of linear regression of all mean data (blue, green, white): y = -74.40 + 5.82x, R = 0.981). Inset: transmittance spectrum of the macular pigment calculated as 10^{-OD}, where OD is the optical absorption reported in ref. 24.