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EDITED BY
Weihua Yang,
Jinan University, China

REVIEWED BY
Qiaobing Xu,
Tufts University, United States
Bin He,
Swiss Federal Institute of Technology Lausanne, Switzerland
Min Sun,
Army Medical University, China

*CORRESPONDENCE
Jun Yu
✉ yujun4@aierhhr.com
Liang Jia
✉ ccvd328@163.com

†These authors have contributed equally to this work

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Photobiomodulation therapy for congenital color vision deficiency: results of a preliminary randomized clinical trial

Yidi Liu[1†], Qinghua Yang[2†], Jun Yu[3*] and Liang Jia[2*]

[1]Department of Laser Medicine, the First Medical Center, Chinese PLA General Hospital, Beijing, China, [2]Senior Department of Ophthalmology, the Third Medical Center, Chinese PLA General Hospital, Beijing, China, [3]Department of Ophthalmology, Yiwu Aier Eye Hospital, Yiwu, China

Purpose: This study presents a novel randomized controlled trial investigating photobiomodulation (PBM) therapy as an intervention method for color vision deficiency (CVD).

Methods: A total of 74 participants with CVD were assigned to either the PBM group or the control group. In the PBM group, participants wore virtual reality (VR) goggles twice daily, with a 12-h interval, over a four-week period. The VR video consisted of alternating red and green images, each presented for 5 s, totaling 6 min and 20 s. No treatment was administered to the control group. Color vision improvement was assessed using Yu's, Ishihara's pseudoachromatic plates, Color Blindness Check (CBC), and FM-100 Hue total error score (TES).

Results: After 4 weeks, in terms of Yu's and Ishihara's Plates, the patients in PBM group could identify increasing pieces (before: 1.6 ± 1.6, 2.3 ± 2.2; 4 weeks: 6.5 ± 4.4, 5.4 ± 2.9), while in control group, the number was before: 2.6 ± 3.4, 2.6 ± 2.5; 4 weeks: 3.3 ± 3.6, 2.9 ± 2.2. As for CBC scores, the patients in PBM also showed improved high scores (before: 2353.3 ± 700.0; 4 weeks: 2693.6 ± 642.5). Moreover, PBM treatment resulted in a significant reduction of FM-100 scores (before: 298.0 ± 211.3; 4 weeks: 202.1 ± 114.4).

Conclusion: These findings suggest that PBM therapy holds promise as a potential treatment option for individuals with CVD.

Clinical trial registration: The study received approval from the Ethics Committee of PLA General Hospital, China (KY2021-017). Additionally, it was registered as a Chinese domestic clinical trial (ChiCTR2200056761) at "http://Chictr.org.cn/index.aspx".

KEYWORDS

color vision deficiency, deutan, protan, photobiomodulation, phototherapy

1 Introduction

Color vision deficiency (CVD) is a congenital or acquired functional disorder that results in confusion and difficulty in recognizing colors (1). It is inherited in an X-linked recessive manner, leading to a higher prevalence of CVD in males compared to females (2). The reported prevalence of CVD varies across different geographical locations, ranging from 2 to 8% in boys (3–5) and 0.4–1.7% in girls (6). In China, the incidence rate was approximately 4.7% in boys and 0.7% in girls (7), indicating that more than 35 million people are affected by this disorder.

Individuals often remain unaware of their CVD until they undergo entrance examinations. According to the "Guiding Opinions on Physical Examination for Enrollment in Ordinary Colleges and Universities," more than 50 professions are restricted to individuals with CVD. Consequently, people with CVD are excluded from certain professions such as the military, aviation, and specific medical fields (8, 9), due to their distinct disadvantage in performing visual tasks (10). As a result, CVD significantly impacts people's daily lives, hindering them from achieving their goals and ambitions (11, 12).

In recent years, there has been increasing interest in the treatment of CVD, and extensive research has yielded significant progress. Gene therapy has shown promising results in non-human primates but remains untested in humans (13, 14). Additionally, individuals with CVD often rely on wearable devices to cope with everyday challenges. Tinted glasses or lenses are a common choice, with companies like Chromagen developing red contact lenses; however, their effectiveness varies among subjects (15, 16). Furthermore, smart goggles incorporating image processing algorithms, such as those developed by Google, have been used in CVD research, but their bulky size renders them impractical for everyday use. Therefore, it is crucial to discover a convenient, effective, and safe method to assist individuals with CVD in addressing their vision problems.

Low-Level Light Therapy (LLLT) (17) a form of photobiomodulation (PBM) therapy, involves the therapeutic application of low-power visible and infrared light sources, such as lasers, LEDs, and broadband light. PBM is a non-thermal process that utilizes endogenous chromophores capable of absorbing light and inducing photochemical events. In recent years, PBM has been investigated for its potential to improve visual function, particularly in age-related macular degeneration (AMD). For instance, Grewal et al. utilized a handheld 670 nm light source with a power of 40 mW/cm^2 for 2 min daily to treat AMD; although the results indicated insensitivity to the light source in AMD, the control group with aged eyes exhibited improved scotopic thresholds (18). Similarly, Shinhmar et al. demonstrated that illuminating the dominant eye with 670 nm LEDs at 40 mW/cm^2 for 3 min every morning over 2 weeks enhanced photoreceptor performance (19). In 2021, Shinhmar et al. further conducted a study revealing that single 3-min exposures of 670 nm light (8 mW/cm^2) in the morning significantly improved cone-mediated color contrast thresholds in aging populations, effectively restoring levels comparable to those observed in younger subjects for up to 1 week (20). These findings suggest that PBM can enhance rod and cone function. Motivated by these studies, we hypothesized that PBM could improve cone function in individuals with protan-deutan color blindness, which accounts for 95% of all CVD cases. Thus, we designed a novel alternating red-green light PBM schedule to treat individuals with this form of CVD (see Figure 1). To the best of our knowledge, this study is the first to utilize PBM for improving color discrimination.

2 Methods

2.1 Study design

From January to June 2022, participants were randomly assigned to either the PBM group or the control group. All subjects had confirmed cases of CVD but were unaware of the specific research objectives. The control group was recruited for the study without receiving any interventions.

Sample Size: No relevant studies have reported on the use of PBM for treating CVD. Considering the results of the preliminary experiment and the necessity to recruit as many subjects as possible, the improvement rate was set at 40% for the PBM group and 5% for

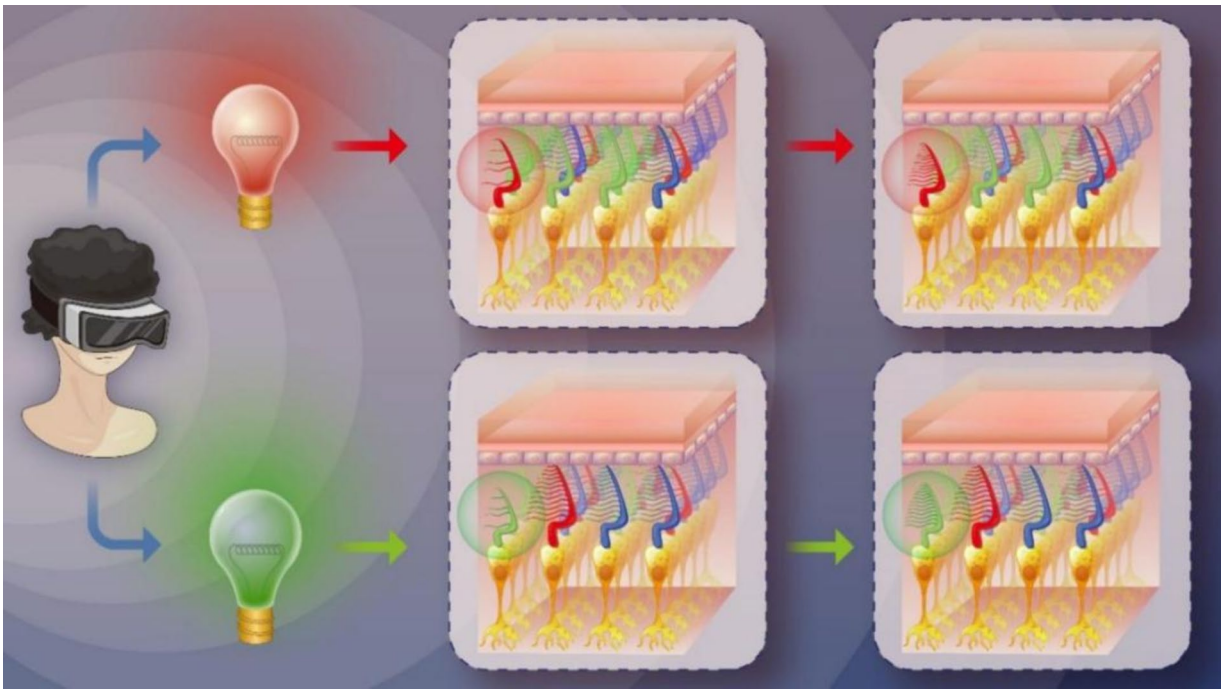


FIGURE 1
The assumption of PBM treating CVD.

the control group. Employing 1:1 matching and accounting for a 30% dropout rate, we calculated that each group would require 72 subjects to achieve a power of 0.90 and a significance level of 0.05 when evaluating the difference between the PBM and control groups using Pass 15 statistical software (NCSS, Kaysville, UT).

The study adhered to the principles of the Declaration of Helsinki and received approval from the Ethics Committee of PLA General Hospital, China (KY2021-017). Additionally, it was registered as a Chinese domestic clinical trial (ChiCTR2200056761) at Chictr.org.cn/index.aspx, and written informed consent was obtained from all participants.

2.2 Participants and color vision assessment

Subjects were recruited for this study via an advertisement seeking individuals with CVD to participate in an experiment. Accurate diagnosis of CVD involved a combination of medical history, comprehensive clinical examination, and multiple color vision tests. Specifically, clinical manifestations typical of this condition were taken into consideration during the diagnosis.

Pseudo-achromatic tests known as the Yu's and Ishihara's plates, developed in China and Japan, are utilized to assess an individual's CVD status (21, 22). Nonetheless, individuals with CVD frequently struggle to differentiate between the colors on the Yu's and Ishihara's plates, thus experiencing difficulty in identifying the numbers.

The Color Blindness Check (CBC) is a test assessing an individual's color vision using a mobile application downloadable from the Google Play Store.[1]

The FM-100 Hue Total Error Score (TEM) serves as an indicator of color vision, with higher scores indicating more severe color blindness.

Inclusion criteria required: (1) Visual acuity corrected to normal 20/25 or better; (2) Recognition score of less than 20 on the Yu's or Ishihara's plates (specifically 2nd to 16th, 37th to 40th, and 42nd for Yu's plates, and 2nd to 17th, 22nd to 25th for Ishihara's plates); (3) FM-100 TES score exceeding 50 (23).

Exclusion criteria: (1) Ability to correctly identify at least 20 Yu's or Ishihara's Plates; (2) FM-100 TES score of 50 or less (23); (3) CBC Severity equals 0.

The study profile for the enrolled subjects is depicted in Figure 2.

2.3 Procedures

Subjects in the control group received no treatment, while those in the PBM group underwent color vision training by watching a specially designed video (refer to Supplementary material S1) using virtual reality (VR) goggles twice a day, with a 12-h interval, over a duration of c During the VR goggle usage, all participants remained awake and in a resting state, with their eyes open and able to blink normally. Color vision assessments (using Yu's plates, Ishihara's plates, CBC, FM-100 TES) were conducted at the end of the first, second, and fourth week of PBM treatment. We specifically designed this video for subjects with protan and deutan defects.

The video comprises two parts: red light irradiation and green light irradiation (refer to Figure 3). Each part lasted for 5 s and the entire video had a duration of 6 min and 20 s. The red light had a central wavelength of 621 nm with full-field illumination, and an energy density of approximately $8.69*10^{-3}$ W/m^2 at the cornea. The green light had a central wavelength of 524 nm with full-field illumination, and an energy density of approximately $2.21*10^{-2}$ W/m^2 at the cornea (24).

2.4 Data analysis

Baseline values were presented as Mean ± SD. Differences in baseline characteristics between groups were analyzed using Wilcoxon and Fisher exact tests. A repeated measures analysis of covariance (ANCOVA) was conducted using a mixed model, with the baseline change serving as the dependent variable and PBM treatment, time, and the interaction of treatment times as the independent variables. All statistical analyses were performed using SPSS software (version 26.0, SPSS, Inc.).

3 Results

3.1 Baseline of the recruited participants

Baseline assessment was initiated with 134 participants according to Figure 1, of whom 114 were subsequently randomized. Fifty percent ($n = 57$) were assigned to the PBM treatment group, while the remaining 50% ($n = 57$) were allocated to the follow-up-only control group. Among them, 64.9% ($n = 37$) in each group completed the study (Figure 1). Table 1 presents the baseline characteristics of the 74 enrolled participants. In both the control and PBM groups, 89.2% ($n = 33$) were male. The mean age was 27.2 ± 10.9 years in the control group and 27.8 ± 11.3 years in the PBM group. Regarding color vision defects, the control group had 35.1% ($n = 13$) with protan defects and 64.9% ($n = 21$) with deutan defects, while the PBM group had 27.0% ($n = 10$) with protan defects and 73.0% ($n = 23$) with deutan defects.

3.2 Effectiveness of PBM therapy in treating CVD

To evaluate the improvement of color vision in CVD subjects, we utilized five different methods: Yu's plate recognition, Ishihara's plates recognition, CBC scores, CBC severity scores, and FM-100 TES. Table 2 summarizes the effects of PBM treatment on CVD subjects at various time points (1 week, 2 weeks, and 4 weeks).

3.2.1 Yu's Pseudoisochromatic plates

The effectiveness of PBM in enhancing subjects' ability to recognize Yu's plates is demonstrated in Figure 4A. The dependent variable, representing the effect of PBM, was calculated by subtracting the pre-treatment value from the post-treatment value. As depicted in Figure 4A and Table 2, PBM exhibited significant improvement in the

1 https://play.google.com/store/apps/details?id=ch.colblindor.colorblindcheck

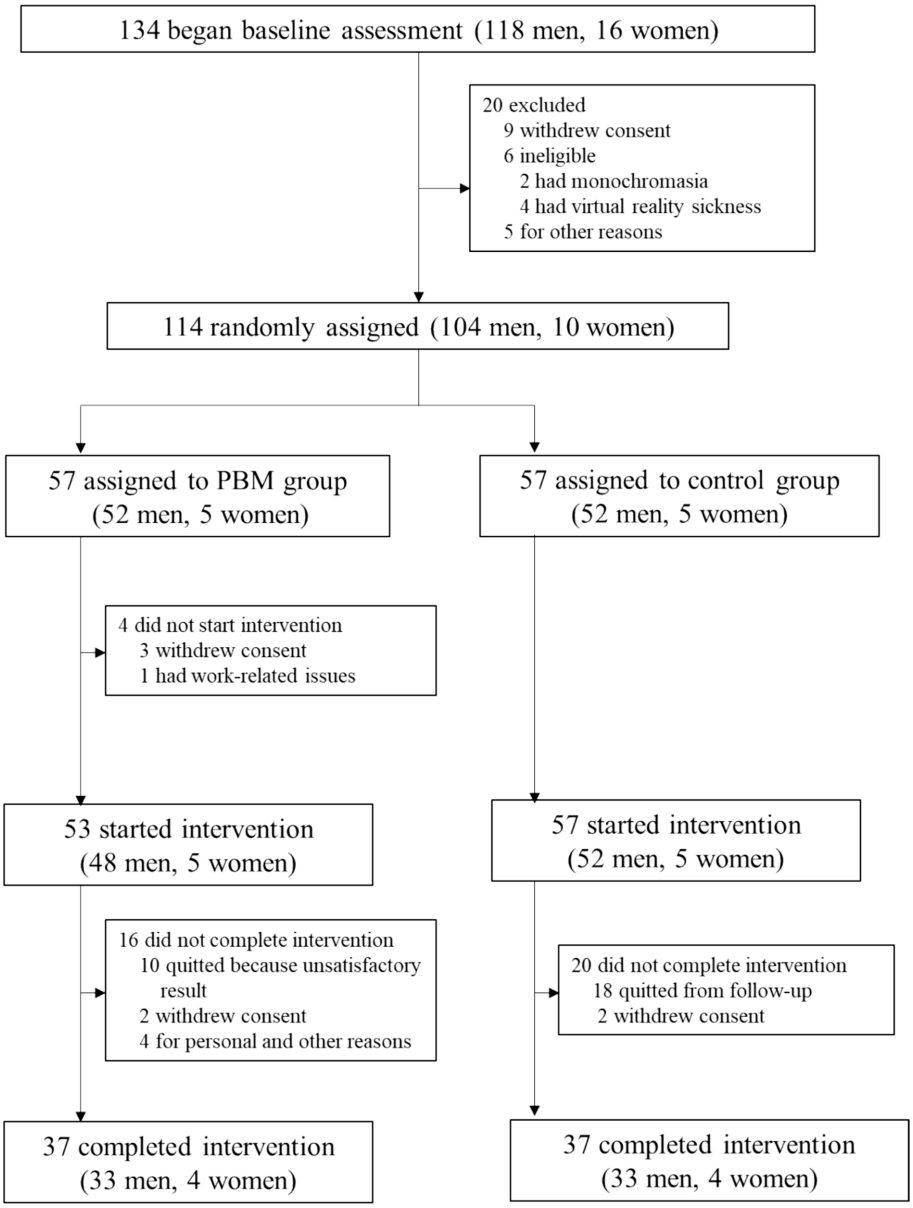


FIGURE 2
Trial profile.

recognition rate as early as 1 week after treatment, with a progressively increasing effect. After 4 weeks, the PBM group demonstrated the highest number of correct identifications (before: 1.6 ± 1.6; 1 week: 3.7 ± 2.9; 2 weeks: 4.9 ± 3.7; 4 weeks: 6.5 ± 4.4). Additionally, repeated ANOVA analysis revealed a positive correlation between treatment duration and the effectiveness of PBM ($p < 0.05$).

In the control group, it is evident that color vision did not improve over time without any treatment (before: 2.6 ± 3.4; 1 week: 2.6 ± 3.4; 2 weeks: 3.1 ± 3.9; 4 weeks: 3.3 ± 3.6). Furthermore, a statistically significant difference was observed between the two groups at each time point ($p < 0.0001$).

3.2.2 Ishihara's pseudoisochromatic plates

The effects of PBM on Ishihara's pseudoisochromatic plates were clearly observed, as depicted in Figure 4B and Table 2. Following 1 week of PBM treatment, subjects demonstrated an improvement in identifying color fragments, with a mean of 3.8 ± 2.1 pieces compared to 2.3 ± 2.2 pieces before treatment. Notably, the effect of PBM increased over time (2 weeks: 4.0 ± 2.4; 4 weeks: 5.4 ± 2.9). Moreover, the PBM group achieved the highest recognition rate after 4 weeks, and repeated ANOVA analysis indicated a significant positive relationship between treatment duration and effectiveness ($p < 0.05$).

In contrast, the control group did not demonstrate any significant improvement in recognizing Yu's plates (before treatment: 2.6 ± 2.5; 1 week: 2.8 ± 2.6; 2 weeks: 3.0 ± 2.2; 4 weeks: 2.9 ± 2.2). Moreover, a statistically significant difference was observed between the experimental and control groups at each time point except the baseline ($p < 0.0001$).

3.2.3 CBC scores and CBC severity

The CBC scores serve as a reliable measure of the subjects' color vision ability, where higher scores indicate superior performance.

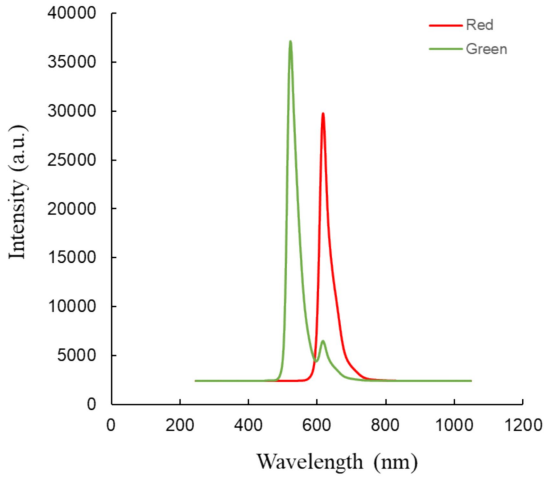


FIGURE 3
The wavelength of therapeutic light.

TABLE 1 Baseline of the recruited subjects.

	Control group	PBM group
Male (n, %)	33, 89.2%	33, 89.2%
Age	27.2 ± 10.9	27.8 ± 11.3
Protan defects (n, %)	13, 35.1%	10, 27.0%
Deutan defects (n, %)	21, 64.9%	23, 73.0%
Total	37	37

Following PBM treatment, the CBC scores exhibited a progressive increase from 2353.3 ± 700.0 to 2693.6 ± 642.5, as illustrated in Figure 4C and Table 2.

The CBC values of the control group exhibited no significant alterations throughout the study period (Before: 2569.1 ± 762.8; 1 week: 2480.3 ± 793.2; 2 weeks: 2554.5 ± 774.7; 4 weeks: 2585.8 ± 764.9, as presented in Table 2).

The severity of color blindness confusion (CBC) is determined based on the CBC scores, with higher severity indicating a reduced ability to perceive colors. Figure 4D and Table 2 illustrate a decreasing trend in CBC severity within the PBM group (Before: 64.6 ± 25.7; 1 week: 63.9 ± 26.3; 2 weeks: 59.8 ± 25.1; 4 weeks: 56.1 ± 26.0), while the control group exhibited no change (Before: 57.0 ± 32.0; 1 week: 60.1 ± 31.7; 2 weeks: 58.2 ± 32.7; 4 weeks: 57.6 ± 32.5).

The findings from repeated ANOVA analysis ($p < 0.05$) demonstrated the time-dependent impact on both CBC values and severity. Furthermore, PBM treatment exhibited a remarkable superiority over the control group in enhancing color vision at all time points ($p < 0.0001$).

3.2.4 FM-100 TES

The FM-100 is an internationally recognized color vision test, with higher scores indicative of poorer color discrimination. Our study revealed that PBM treatment resulted in a significant reduction of FM-100 scores (Before: 298.0 ± 211.3; 1 week: 236.6 ± 146.2; 2 weeks: 225.7 ± 155.1; 4 weeks: 202.1 ± 114.4), whereas the control group remained unchanged throughout the study period (Before: 234.6 ± 148.0; 1 week: 248.9 ± 154.0; 2 weeks: 244.9 ± 156.9; 4 weeks: 242.4 ± 147.9), as depicted in Figure 4E and Table 2.

Repeated ANOVA analysis indicated that the duration of PBM treatment had a positive correlation with its effectiveness ($p < 0.05$). Furthermore, our findings demonstrated a significant difference in outcomes between the PBM and control groups at every time point ($p < 0.0001$).

3.2.5 The effect of PBM on protan and deutan subjects

In our investigation, we sought to determine whether PBM had a differential effect on protan and deutan subjects. Table 3 presents a summary of the changes in five color vision assessments before and after PBM treatment. However, there was no statistically significant difference between protan and deutan subjects, indicating that PBM had the same effect on different types of CVD.

Our study aimed to investigate whether PBM had a differential effect on protan and deutan subjects. Table 3 summarizes the changes in five color vision assessments before and after PBM treatment. Our findings indicate that there was no statistically significant difference between the two groups, suggesting that PBM had a similar effect on both types of CVD.

4 Discussion

Normal color vision is mediated by cones containing blue-, green-, and red-sensitive opsin pigments, often referred to as short (S, 426 nm, blue), medium (M, 530 nm, green), and long (L, 557 nm, red) wavelength-sensitive pigments (25).

Deuteranomalous and protanomalous deficiencies are characterized by a greater degree of overlap in spectral sensitivity than normal, with peak sensitivity shifts occurring in green-sensitive and red-sensitive pigments, respectively (25). In deuteranomaly, the normal M cone is replaced by an abnormal cone, denoted as L', with spectral sensitivity close to that of the normal L cone. Similarly, in protanomaly, the L cone is replaced by M', with spectral sensitivity close to that of the normal M cone. Deuteranomalous and protanomalous deficiencies are characterized by a significant spectral overlap between the sensitivities of the middle and long wavelength cone types, leading to a reduction in chromatic contrast within the corresponding region of the visible spectrum. Consequently, color discrimination is impaired (26, 27).

Our study shows the efficacy of photobiological modulation (PBM) in treating color vision deficiencies (CVD). Following 1 week of PBM therapy, deuteranomaly and protanomaly patients demonstrated significant improvement in color vision, with increased response rates to pseudoisochromatic plates and CBC, and decreased FM100 TES and severity scores, compared to the control group. Furthermore, PBM treatment demonstrated sustained efficacy with longer treatment durations of up to 4 weeks.

Photobiological modulation (PBM) is a novel approach that utilizes photon energy to regulate cellular and tissue function and correct chromatic aberrations. This preliminary study investigated the efficacy of PBM in improving color vision deficiencies in subjects with weak red or green color perception. Red light (621 nm) with an energy density of $8.69*10^{-3}$ W/m^2 and green light (524 nm) with an energy density of $2.21*10^{-2}$ W/m^2 were

TABLE 2 PBM effect on CVD at different time points.

	Control group (n = 37)	PBM group (n = 37)	Between-group p value
Yu's pseudoisochromatic plates			
Baseline	2.6 ± 3.4	1.6 ± 1.6	0.105
1 week	2.6 ± 3.4	3.7 ± 2.9	/
2 weeks	3.1 ± 3.9	4.9 ± 3.7	/
4 weeks	3.3 ± 3.6	6.5 ± 4.4	/
Change at one-week	0.6 ± 1.4	2.1 ± 2.0†	<0.0001
Change at two-week	0.5 ± 1.5	3.3 ± 2.8†	<0.0001
Change at four-week	0.7 ± 1.4	4.9 ± 3.6†	<0.0001
Ishihara's pseudoisochromatic plates			
Baseline	2.6 ± 2.5	2.3 ± 2.2	0.625
1 week	2.8 ± 2.6	3.8 ± 2.1	/
2 weeks	3.0 ± 2.2	4.0 ± 2.4	/
4 weeks	2.9 ± 2.2	5.4 ± 2.9	/
Change at one-week	0.3 ± 1.5	1.5 ± 2.1§	<0.0001
Change at two-week	0.5 ± 1.6	1.7 ± 2.1§	<0.0001
Change at four-week	0.4 ± 1.2	3.1 ± 2.5†	<0.0001
CBC scores			
Baseline	2569.1 ± 762.8	2353.3.6 ± 3.4	0.215
1 week	2480.3 ± 793.2	2493.2 ± 672.5	/
2 weeks	2554.5 ± 774.7	2609.3 ± 641.5	/
4 weeks	2585.8 ± 764.9	2693.6 ± 642.5	/
Change at one-week	−88.8 ± 235.0	139.9 ± 256.5	<0.0001
Change at two-week	−14.6 ± 240.2	256.0 ± 262.3	<0.0001
Change at four-week	16.7 ± 203.4	340.3 ± 307.5*	<0.0001
CBC severity			
Baseline	57.0 ± 32.0	64.6 ± 25.7	0.271
1 week	60.1 ± 31.7	63.9 ± 26.3	/
2 weeks	58.2 ± 32.7	59.8 ± 25.1	/
4 weeks	57.6 ± 32.5	56.1 ± 26.0	/
Change at one-week	3.2 ± 8.8	−0.65 ± 9.7	<0.0001
Change at two-week	1.2 ± 8.0	−4.8 ± 10.7	<0.0001
Change at four-week	0.6 ± 7.3	−8.4 ± 11.9	<0.0001
FM-100 TES			
Baseline	234.6 ± 148.0	298.0 ± 211.3	0.145
1 week	248.9 ± 154.0	236.6 ± 146.2	/
2 weeks	244.9 ± 156.9	225.7 ± 155.1	/
4 weeks	242.4 ± 147.9	202.1 ± 114.4	/
Change at one-week	14.3 ± 54.4	−61.4 ± 127.3	<0.0001
Change at two-week	10.2 ± 52.5	−72.3 ± 150.7	<0.0001
Change at four-week	7.8 ± 34.7	−95.9 ± 153.4*	<0.0001

All p values reflect Bofferroni correction. *$p < 0.05$, §$p < 0.01$, ¶$p < 0.001$, †$p < 0.0001$ vs. baseline.

applied twice daily for 6 min and 20 s. The study demonstrated significant improvement in color vision ability, as evidenced by an increased number of plates recognized on the Yu's and Ishihara's tests after 2 and 4 weeks of treatment. Upon literature review and inference of the possible mechanism, there is evidence indicating a significant increase in the gain of the S-(L + M) mechanism between the retina and cortex (28, 29). This suggests that the function or number of S, L, and M cones undergoes dynamic

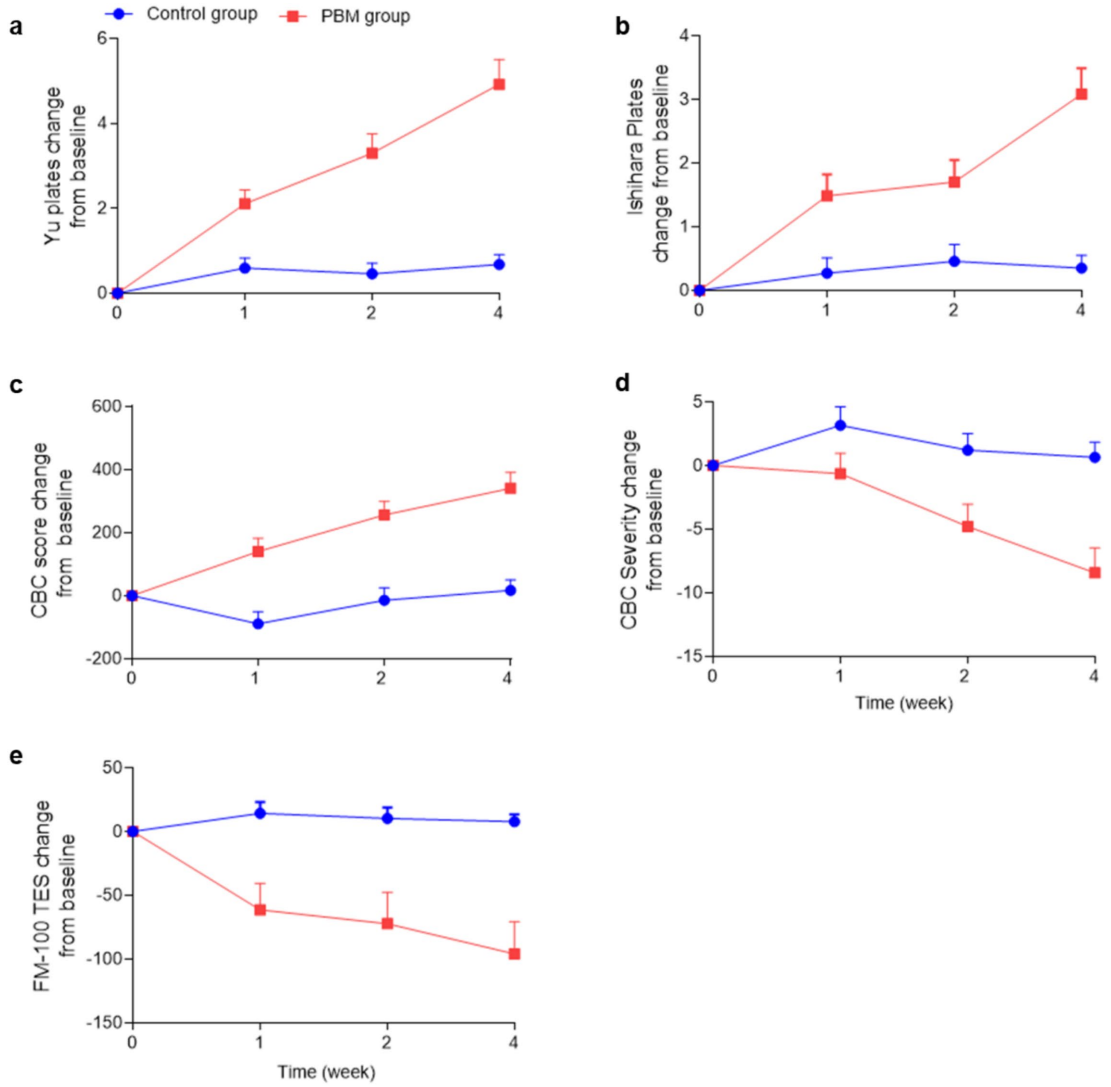


FIGURE 4
Color vision changes in the PBM and control groups. ("CBC" is an abbreviation for "Color Blindness Check", "FM-100 TES" is an abbreviation for "FM-100 Hue Total Error Score").

changes. These adjustments likely take place during natural adaptation processes that calibrate sensitivity and neural gain based on environmental stimuli (30–32), optimizing the utilization of neurons' limited response range (33). Following exposure to red (621 nm) and green (524 nm) light, the L' cones converted into normal M cones, and the M' cones transformed into normal L cones, suggesting adaptive adjustments.

There are still some limitations existed in our study. Our study is relatively preliminary and the core purpose is to find whether PBM is effective in treating CVD. In control group, the patients included receive no treatment, however, the more proper way is letting patients watch white light video which lasts the same time as treatment group. Moving forward, the tests of the optimum wavelength, light intensity, duration of PBM should be further designed to be sure. The underlying mechanism of PBM is also crucial, which need to be delved deeper.

PBM represents a safe, convenient, and cost-effective therapeutic approach for managing CVD. Our study has effectively showcased the untapped potential of PBM, instilling confidence that it holds tremendous benefits for a wider population afflicted by CVD.

5 Conclusion

PBM applies light of low density on the lesion tissue or monolayer cells to cause non-destructive and non-thermal biological reactions for therapeutic purposes. In this study, red light (621 nm) with an energy density of $8.69*10^{-3}$ W/m^2 and green light (524 nm) with an energy density of $2.21*10^{-2}$ W/m^2 were applied twice daily for 6 min and 20 s. Following 1 week of PBM therapy, deuteranomaly and protanomaly patients demonstrated significant improvement in color vision. PBM treatment presented sustained efficacy of up to 4 weeks. However, our study represents an initial investigation into the comparative effectiveness of PBM. Moving forward, we aim to ascertain the optimal wavelength and duration of PBM, while also expanding our participant pool. Furthermore, it is crucial to figure out the underlying mechanism of PBM.

TABLE 3 Effects of PBM on individuals with protan and deutan color vision deficiencies.

	Protan group (n = 10)	Deutan group (n = 27)	Between-group p value
Yu's pseudoisochromatic plates			
Baseline	1.2 ± 1.2	1.7 ± 1.7	0.371
Change at one-week	1.8 ± 2.2	2.2 ± 1.9	0.575
Change at two-week	3.3 ± 3.1	3.3 ± 2.6	0.997
Change at four-week	5.3 ± 4.1	4.8 ± 3.3	0.702
Ishihara's pseudoisochromatic plates			
Baseline	1.3 ± 1.2	2.7 ± 2.3	0.095
Change at one-week	1.5 ± 1.8	1.5 ± 2.1	0.981
Change at two-week	1.6 ± 2.1	1.7 ± 2.1	0.861
Change at four-week	3.2 ± 3.3	3.0 ± 2.1	0.864
CBC scores			
Baseline	2116.0 ± 771.8	2441.1 ± 649.9	0.221
Change at one-week	134.1 ± 242.2	142.1 ± 261.5	0.935
Change at two-week	212.8 ± 275.4	272.0 ± 255.4	0.555
Change at four-week	289.3 ± 230.5	359.2 ± 329.5	0.552
CBC severity			
Baseline	72.8 ± 27.3	61.5 ± 24.5	0.248
Change at one-week	−3.6 ± 6.1	−0.4 ± 10.6	0.275
Change at two-week	−7.5 ± 8.5	−3.8 ± 11.2	0.361
Change at four-week	−7.5 ± 9.8	−8.8 ± 12.6	0.780
FM-100 TES			
Baseline	354.4 ± 249.6	277.1 ± 191.1	0.337
Change at one-week	−90.9 ± 74.8	−50.4 ± 140.4	0.405
Change at two-week	−78 ± 151.3	−70.2 ± 150.5	0.893
Change at four-week	−143.6 ± 169.8	−78.3 ± 142.9	0.262

All p values reflect Bofferroni correction. *$p < 0.05$, §$p < 0.01$, ¶$p < 0.001$, †$p < 0.0001$ vs. baseline.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by Ethics Committee of PLA General Hospital, China (KY2021-017). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

YL: Formal analysis, Writing – original draft. QY: Data curation, Investigation, Resources, Writing – review & editing. JY: Conceptualization, Investigation, Writing – review & editing. LJ: Conceptualization, Data curation, Investigation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1497501/full#supplementary-material

SUPPLEMENTARY MATERIAL S1
The treatment video.

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Weeklong improved colour contrasts sensitivity after single 670 nm exposures associated with enhanced mitochondrial function

Harpreet Shinhmar, Chris Hogg, Magella Neveu & Glen Jeffery✉

Mitochondrial decline in ageing robs cells of ATP. However, animal studies show that long wavelength exposure (650–900 nm) over weeks partially restores ATP and improves function. The likely mechanism is via long wavelengths reducing nanoscopic interfacial water viscosity around ATP rota pumps, improving their efficiency. Recently, repeated 670 nm exposures have been used on the aged human retina, which has high-energy demands and significant mitochondrial and functional decline, to improve vision. We show here that single 3 min 670 nm exposures, at much lower energies than previously used, are sufficient to significantly improve for 1 week cone mediated colour contrast thresholds (detection) in ageing populations (37–70 years) to levels associated with younger subjects. But light needs to be delivered at specific times. In environments with artificial lighting humans are rarely dark-adapted, hence cone function becomes critical. This intervention, demonstrated to improve aged mitochondrial function can be applied to enhance colour vision in old age.

Metabolic rate and ageing are both regulated by mitochondria. Mitochondrial membrane potential, however, declines with age resulting in reduced adenosine triphosphate (ATP) production, which is a major source of cellular energy. Cellular decline is further accelerated with ageing by increased production of pro-inflammatory reactive oxygen species (ROS)[1]. Retinal photoreceptors have the greatest mitochondrial density and metabolic demand in the body and age rapidly[2]. A pivotal point in their functional ageing in the human retina appears to be around 40 years[3]. Subsequently 30% of central rods progressively die, and while cones remain, they have reduced functionality[4–7]. Hence, regenerating aged cone function is vital because in an environment where artificial lighting results in humans rarely being fully dark-adapted and needing their rods, cone function becomes critical.

Exposure to long wavelength light (650–900 nm) in animals improves mitochondrial function, increasing ATP production and reducing ROS[8,9]. It also reduces the pace of age related cell death in the retina[10]. These mitochondrial changes translate into improved electrophysiological responses from the ageing retina in both insects and mice[11,12]. The mechanism may be due to reduced nanoscopic interfacial water layer viscosity around ATP rota pumps, increasing their efficiency[13]. As neuronal membrane pumps consume large amounts of ATP, mitochondrial decline impacts widely on the energy demands of the nervous system as age erodes pump efficiency[14]. Hence, improving ATP availability optically has potentially widespread impact including improved patterns of ageing in mammals and extended lifespan in insects[15]. While few human experiments have been undertaken with long wavelength light in ageing, daily exposures to relatively bright 670 nm light over weeks in aged humans has been demonstrated to improve rod and cone function[3,16]. But we have no knowledge of how much exposure is needed and how long its influence persists.

Results

A single 3 min 670 nm morning exposure to the eye at energy levels approximately a log unit greater than found in environmental light[17] significantly improved subject colour contrast thresholds for both tritan and protan axes when tested 3 h later. Tritan threshold measures are improved by an average of 17% ($P < 0.0001$) and protan by an average of 12% ($P < 0.0001$. Figure 1). When the population is subdivided by progressive age, percentage

Institute of Ophthalmology, University College London, 11-43 Bath Street, London EC1V9EL, UK. ✉email: g.jeffery@ucl.ac.uk

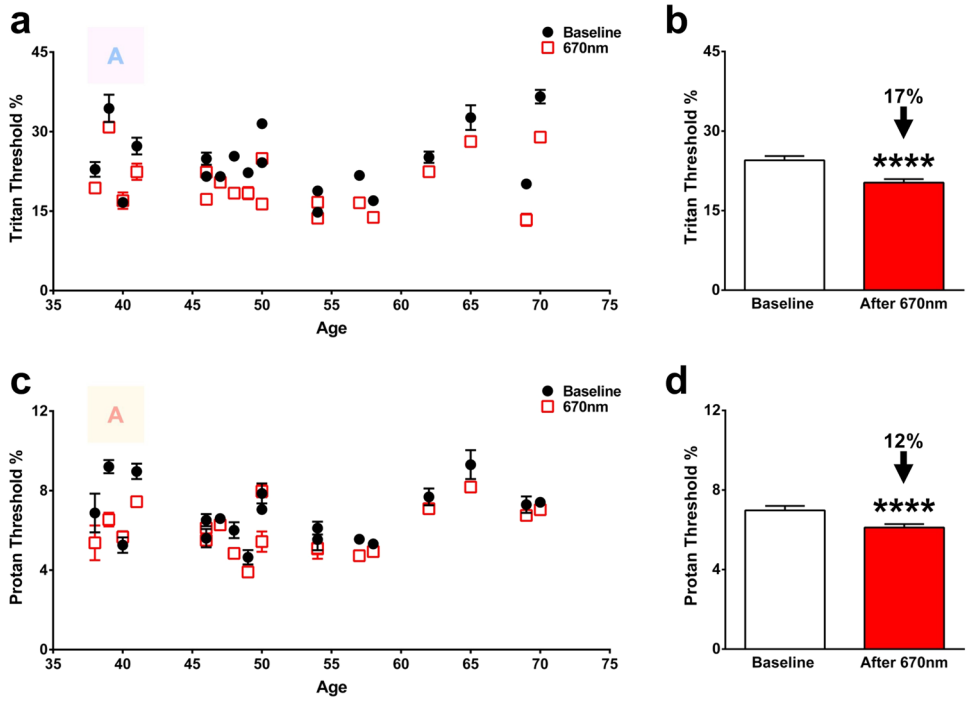


Figure 1. Colour contrast sensitivities (CCS) measured 3 h following a morning exposure (8-9AM) of 670 nm. CCS of tritan (**a**) and protan (**c**) axes measured in 20 healthy subjects and their response to 670 nm exposure. Black closed circles represent baseline measurements and red open boxes are those in the same individuals measured 3 h following a single 3 min 670 nm exposure delivered in the morning. The colour letter A in both graphs is an example of the target to be identified as it appeared on the screen. (**b**) Thresholds for tritan function in individuals 38–70 years. Most subjects displayed a significant decrease to their tritan thresholds following light exposure. Overall, there was a 17% reduction to thresholds across the population. (**d**) Thresholds for protan function in individuals 38–70 years, with half the population displaying a significant decrease to their protan thresholds after 670 nm exposure. Total protan thresholds were reduced by 12% across all subjects. Wilcoxon matched-pairs signed rank test was used for statistical analysis. Data are presented as means ± SEM. ****$p < 0.0001$. Age related differences were not apparent in the baseline measurements as was apparent in Shinhmar et al. (2020). This was because the age range covered here was shorter than in the previous study with a tendency for clustering in the middle age range.

threshold reductions for the tritan axis in those 38–49 years is 14% ($p < 0.0001$), in those 50–59 is 20% ($P < 0.0001$) and those over 60 is 19% ($P < 0.0001$). The same figures for the protan axis according to subdivided age are 13% ($P < 0.001$), 11% ($P < 0.001$) and 12% ($P < 0.01$) respectively.

Overall threshold reductions along the tritan axis were 17% for both males and females ($P < 0.0001$). Threshold reductions along the protan axis were 10% ($P < 0.001$) in males and 14% ($P < 0.0001$) in females. Subdividing by age group, visual axis and sex was not realistic because of the limited sample size. These changes are for measurements before and after 670 nm exposure within individuals. However, an additional control was undertaken where the subjects had their colour contrast thresholds measured at the same time in the morning as above and were then re-tested 3 h later without exposure to 670 nm light (Fig. 2). These showed no difference between the two measurements confirming that differences in the main group were due to 670 nm light exposure.

Similar methods were applied to a subgroup ($N = 6$, Fig. 3) of subjects across a comparable age range with exposure to 670 nm light in the afternoon and tested 3 h later. The results showed no impact across either colour axis following afternoon light exposure. Hence only the morning light dose effective. However, it is possible that there are fundamental differences in the patterns of colour contrast sensitivities over the day that could explain this finding independent of 670 nm light exposure. Consequently, a subgroup of individuals covering the full age range, had their colour contrast thresholds measured repeatedly across the day at time 0, + 3, + 6 and + 9 hs. Data for protan measurements were more variable within subjects than tritan, but neither showed any consistent pattern between across time in the subjects (Fig. 4).

A subgroup ($N = 10$, Fig. 5) of subjects covering a comparable age range that had light exposure in the morning were re-tested 1 week later, without any further 670 nm exposure in the intermediary period. Significant threshold improvements in this population remained across both colour axes, although reduced compared to the 3 h measurements. For the tritan axis, threshold reductions were 10% ($P < 0.001$) and for the protan axis, they were 8% ($P < 0.0001$). Subdividing this population further was not feasible because subject numbers were not large enough.

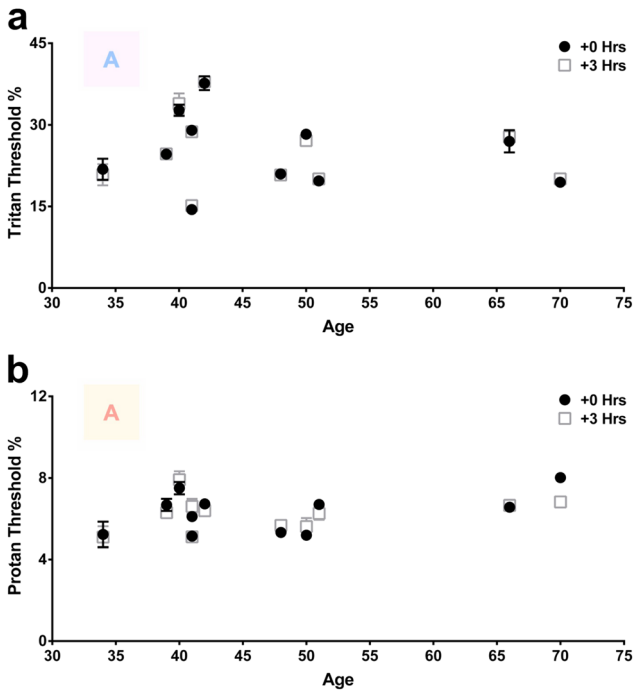


Figure 2. Colour contrast sensitivities (CCS) from a control cohort measured at the same time of day (8-9AM) with no 670 nm light exposure. CCS of tritan (**a**) and protan (**b**) axes measured in 10 subjects (34–70 years) at 8-9AM and 3 h later in the same manner as morning 670 nm light exposure and re-testing. Black closed circles represent measurements taken at 8-9AM to mimic baseline measures and time of 670 nm light exposure. Grey open boxes are those in the same individuals measured 3 h later between 11AM-12PM to mimic the time interval a perceived effect to 670 nm light exposure was achieved. The colour letter A in both graphs is an example of the target to be identified as it appeared on the screen. Thresholds for tritan function revealed no significant difference ($p = 0.4648$) when measured 3 h apart, simulating the same time of day for morning 670 nm light exposures. Similarly, thresholds for protan function revealed no significant difference ($p = 0.9658$) across all 10 subjects. Wilcoxon matched-pairs signed rank test was used for statistical analysis. Data are presented as means ± SEM.

Discussion

Single exposures to 670 nm light delivered in the morning, at only 8 mW/cm^2 have the ability to improve cone photoreceptor function in aged subjects to levels commonly found in much younger individuals and can be sustained for up to a week[3]. This result is substantiated in both a within subject repeated measure design and also compared to a control group which received no light. Our subject population was ≥ 35 years because we have previously shown that the impact of 670 nm light on retinal function is reduced in younger subjects, presumably due to the relatively healthy state of their retinal mitochondria[3]. This is consistent with the mitochondrial theory of ageing[1].

Time of exposure is critical, as 670 nm light is only effective in the morning. This time dependent effect is likely due to the demonstrated shift in mitochondrial function across the day, and light exposure is likely only effective when synchronised to an aspect of this process. The mitochondrial biology behind shift over the day includes variation in complex activity and ATP production. Here the differential impact of 670 nm has been mapped in fly[18]. Within the mornings when mitochondrial function can be improved, there are times when mitochondria appear to be particularly sensitive. However, it would be surprising if their function could be improved uniformly over this period. Hence, we are currently mapping sensitivities over this time.

Despite the clarity of our results, some of the data are noisy. While positive effects are clear for individuals following 670 nm exposure, the magnitude of improvements can vary markedly between those of similar ages. Hence, caution is needed in interpretation of our data. It is possible that there are other variables between individuals that influence the degree of improvement that we have not identified so far and would require a larger sample size.

Previous studies in animals and on the human retina have used much higher energies than those applied here, which are approximately a log unit higher than found in environmental light[17]. A common target is approximately 40 mW/cm^2 [3,19]. But the energies used here are significantly lower at only 8 mW/cm^2. Hence, both the number of exposures and the energy applied is much lower than previously reported.

Tritan function was more responsive to 670 nm light exposure than protan, and this difference likely relates to the respective mitochondrial populations of different cone types. Short wavelength sensitive cones uniquely have relatively few mitochondria and appear to derive their ATP via glycolysis[20]. They are also selectively vulnerable

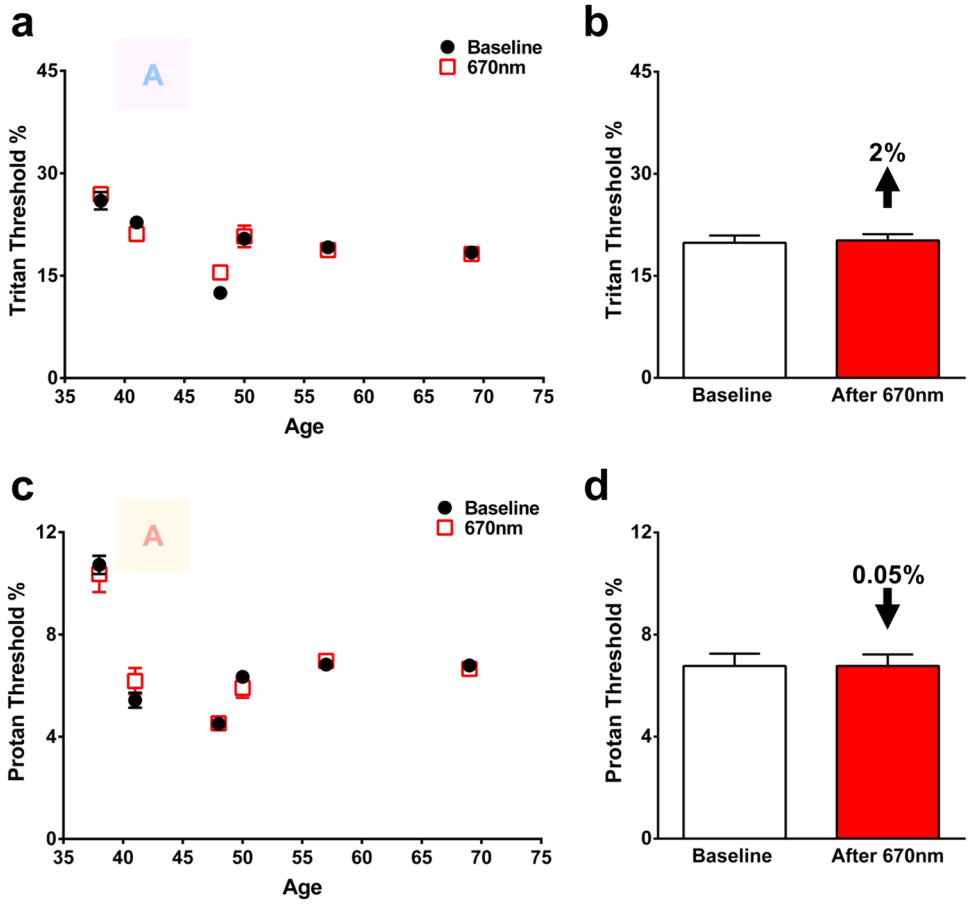


Figure 3. Colour contrast sensitivities measured 3 h following an afternoon exposure (12-1PM) of 670 nm. CCS of tritan (**a**) and protan (**c**) axes measured in 6 healthy subjects and their response to 670 nm exposure. Black closed circles represent baseline measurements and red open boxes are those in the same individuals measured 3 h following a single 3 min 670 nm exposure delivered in the afternoon. The colour letter A in both graphs is an example of the target to be identified as it appeared on the screen. (**b**) Thresholds for tritan function in individuals 38–69 years. No significant change to tritan thresholds ($p = 0.3047$) were found when subjects were exposed to 670 nm light in the afternoon. (**d**) Thresholds for protan function in individuals 38–69 years. 670 nm exposure showed no effect on protan thresholds ($p = 0.2577$) when it was delivered in the afternoon. Wilcoxon matched-pairs signed rank test was used for statistical analysis. Data are presented as means ± SEM.

in ageing and metabolic diseases[21]. Mitochondria absorb strongly at 420 nm[22] and their presence in the light path of S cones would reduce photons reaching the outer segments of these. This may be the reason why S cones have switched to glycolysis to produce ATP[20]. It is possible that 670 nm exposure results in the recruitment of what may be otherwise relatively few dormant mitochondria in S cones in terms of ATP production. If correct, then it is possible that the signatory characteristics of S cones including early saturation may change due to increased ATP availability[23].

Our choice of measuring cone function at 3 h was based on our finding that this was the time needed to increase ATP in experiments on aged flies exposed to 670 nm[24], but improved whole body respiration occurs in less than 0.5 h post 670 nm exposure in the fly[24]. The difference in timing may be due to shifts in patterns of ATP production versus its consumption. However, there is a need to determine the temporal course of increasing improvements in human cone function following 670 nm exposure. It is likely that this may start much earlier than shown in this study. Despite this, it is clear that improved colour contrast remains for both colour domains for up to a week. Here there is more consistency between fly and human data, as improved respiration in aged flies following 670 nm exposure can be found to be sustained for over 4 days[24].

We have employed a stringent colour contrast test, which is used in many clinical environments for a range of colour defects[25–29] but is not otherwise available widely. We are currently exploring if the improvements we find with 670 nm can also be detected using the more labour intensive, but more accessible Farnsworth Munsell 100 Hue test. However, as a measure of sensitivity, subjects were asked after 670 nm exposure if they thought their colour perception had changed in their normal visual environment and around 10% reported a subjective difference.

This study cannot rule out the possibility of adaptive mechanisms to the 670 nm exposure, although this is unlikely. But in data not reported in our previous study where 670 nm exposure was delivered daily for 2 weeks[3],

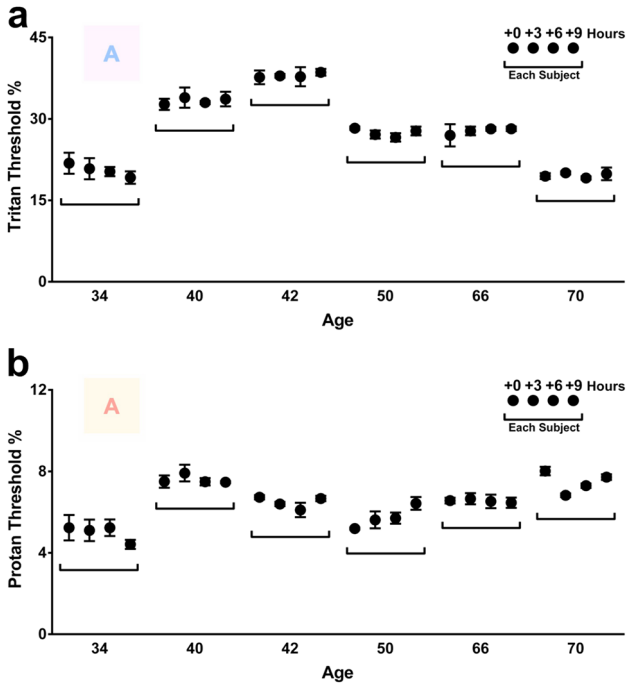


Figure 4. Variation in colour contrast sensitivities (CCS) across the day in 6 subjects. CCS of tritan (**a**) and protan (**b**) axes measured in 6 subjects (34–70 years) in 3 h intervals across the day. The four black closed circles within the bar represent each subject, measurements were taken in 3 h intervals at 8AM, 11AM, 2PM and 5PM. The colour letter A in both graphs is an example of the target to be identified as it appeared on the screen. Thresholds for tritan function revealed no significant variation across the day when measured in a 34 ($p = 0.6898$), 40 ($p = 0.8902$), 42 ($p = 0.9388$), 50 ($p = 0.3890$), 66 ($p = 0.8592$), and 70 ($p = 0.7613$) year-old. Similarly, thresholds for protan function revealed no significant variation across the day when measured in a 34 ($p = 0.5802$), 40 ($p = 0.6149$), 42 ($p = 0.1937$), 50 ($p = 0.0943$), 66 (0.9588), and 70 ($p = 0.2222$) year-old. An ordinary 1-way ANOVA and a multiple comparison test was used for inter-subject comparison and statistical analysis. Data are presented as means ± SEM.

we did examine interocular transfer. There was no difference found in the unexposed eye over the treatment period. Hence, the repeat measures design for testing which here shows the mechanism found with a single exposure is almost certainly retinal.

The energies at 670 nm that we have used are within a log unit of those that can be found in sunlight. This naturally raises the question of whether daylight may impact positively on mitochondrial function in the retina. While this may be the case, we are only recently coming to appreciate the optical absorbance characteristics of mitochondria and their functional consequences. Mitochondria strongly absorb at shorter wavelengths[22] that are present in environmental light and these are known to reduce their function[30–32]. Consequently, long, and short wavelengths in sunlight may have opposing and perhaps balancing influences on mitochondrial function in the retina.

Humans live in a world where we now rarely employ rod function exclusively because we control our light environment. This in combination with the fact that unlike rods, ageing cones do not die but have reduced function highlights the critical need to improve cone ability in progressively ageing populations. We demonstrate that we can significantly improve cone mediated colour contrast thresholds for a week using a single 3 min 670 nm light exposure by an average of 17% and in some older subjects by > 20%. This simple and highly economic intervention applied at the population level will significantly impact on the quality of life in the elderly and likely result in reduced social costs that arise from problems associated with reduced vision.

Methods

The study was conducted in accordance with the Declaration of Helsinki and approved by the UCL research ethics committee (16547/001). Each participant provided written informed consent prior to testing.

Subjects were healthy, of both sexes with an age range of 34–70 years. Each had normal colour vision and were given a general questionnaire regarding eye health prior to testing. Different numbers of subjects were used in different experiments but all experiments on each were tested for thresholds of colour contrast sensitivity along both tritan and protan axes. Anonymised subject identity numbers, their ages and cohort of the experiment in which they were used are given in Table 1. In the case of multiple testing per subject, they were given a wash out period to the effects of 670 nm light for a few months. All exposures to 670 nm were for 3 min to the subjects' dominant eye either in the morning between 8 and 9 AM or the afternoon between 12 and 1 PM and were

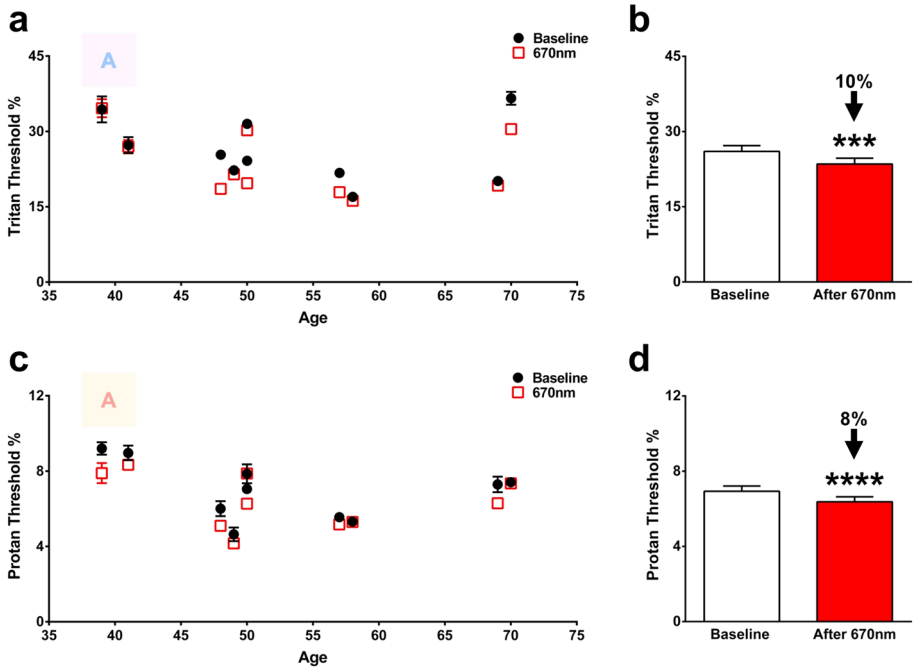


Figure 5. Colour contrast sensitivities measured 1 week later from a morning exposure of 670 nm. CCS of tritan (**a**) and protan (**c**) axes from 10 healthy subjects with their response to a single dose of 670 nm measured 1 week later, without any further 670 nm exposure in the intermediary period Black closed circles represent baseline measurements and red open boxes are those in the same individuals measured 1 week later from a single 3 min 670 nm light exposure delivered in the morning. Some subjects displayed a return of their thresholds to baseline, whereas some still had a reduction. The colour letter A in both graphs is an example of the target to be identified as it appeared on the screen. (**b**) Thresholds for tritan function in individuals 39–70 years. There was a 10% sustained reduction to tritan thresholds ($p = 0.0001$) across the population from baseline measures. (**d**) Thresholds for protan function in individuals 39–70 years. There was an 8% sustained reduction to protan thresholds ($p < 0.0001$) across the population from baseline measures a week following 670 nm exposure. Wilcoxon matched-pairs signed rank test was used for statistical analysis. Data are presented as means ± SEM. ***$p \leq 0.0001$, ****$p < 0.0001$.

assessed at either 3 h post exposure or 1 week later without further 670 nm exposure in the intervening period. Morning exposures measured 3 h later comprised 20 subjects (13 Females and 7 Males). Afternoon exposures measured 3 h later comprised of six subjects (three Females and three Males). For morning exposures measured 1 week later there were ten subjects (seven Females and three Males). The above were a repeat measures design within subjects. However, an additional control was undertaken where colour contrast thresholds were measured in the morning and then re-measured 3 h later without exposure to 670 nm. This comprised of ten subjects (six Females and four males). To determine if there were significant shifts in colour contrast sensitivities across the day that were independent of 670 nm and might undermine outcome measures for their exposure, six subjects were repeatedly tested at 0, + 3, + 6 and + 9 h (four Females and two Males).

The 670 nm light devices were supplied by CH electronics (UK) and based on commercial DC torches with nine 670 nm LEDs mounted behind a light diffuser so that energies at the cornea were approximately 8 mW/cm^2. 670 nm light was delivered down a white internally reflecting tube that fitted over the eye with an internal diameter of 3.2 cm. Based on subject's perception the region of the retina illuminated was centred on the macular and extended into the equator but did not include the far periphery. Estimates of the exact retinal region of illumination are hard to derive because the pupil will variably close in response to the light. However, 670 nm will penetrate the iris[33] and this will most likely be associated with scatter. The energy delivered at this wavelength is less than a log unit greater than that found in environmental light[17].

Colour contrast sensitivity (CCS) was measured based on the ChromaTest and a computer graphics system called Cavity, which assesses colour contrast thresholds across the protan axis (red-green visual axis,) and tritan axis (blue-yellow visual axis) axes[25–29]. Table 2 below defines the coordinates of these axes in the CIE colour space. These coordinates are the endpoints and are not adjusted for any subject according to the flicker balance.

Subjects were seated at a fixed distance from the stimulus monitor so that that opto-type letter subtended a 1.3-degree angle on the retina. Target letters were displayed against a background of the opposing colour with the colour contrast between the 2 varying and appeared either in red or blue for the protan and tritan axes respectively. The subject discrimination task was to identify the presented letter at varied contrasts against the background. Contrast was defined as 0% when the letter had the same hue as the background and 100% when the letter and the background difference was at its maximum achievable by the monitor. Hence, for example, a

	Age	Gender	Cohort	Subject also in Shinhmar et al. 2020
1	34	F	d, e	Yes
2	38	F	a, d	
3	39	F	a, b, c, d, e	
4	40	F	a, d	Yes
5	41	M	a, b, c, d	
6	42	F	d, e	Yes
7	46	F	a	
8	46	M	a	
9	47	M	a, d	
10	48	F	a, c	
11	49	F	a, b, c, d, e	Yes
12	50	F	a, c	Yes
13	50	F	a, b, c, d	
14	54	M	a	
15	54	F	a	
16	57	F	a, c	
17	57	M	b	
18	58	F	a, c	
19	62	F	a	
20	62	F	a	
21	65	M	a	
22	66	M	d, e	Yes
23	69	M	a, b, c, d, e	Yes
24	70	M	a, c	

Table 1. Subject demographics: a breakdown of the subjects used in different parts of the study. *F* Female, *M* Male. a = AM light exposure. b = PM light exposure. c = AM light exposure tested a week later. d = AM control, no light exposure. e = variation across the day.

	x	y	x	y
	Red		Green	
Protan	0.494	0.386	0.336	0.475
	Blue		Yellow	
Tritan	0.299	0.23	0.433	0.439

Table 2. End-point x, y coordinates defining the protan and tritan axis according to the CIE colour space.

low contrast letter T presented in red on a blue background where target and background were isoluminant. Isoluminace was set for each subject prior to threshold testing, by making a heterochromatic flicker balance match between red and green and blue and green monitor guns until there is minimum flicker i.e. a balanced output for each subject taking into account the subject's optical pathway. The output is used to adjust the values in the look-up table to produce equiluminous colours for the subject being tested. Thresholds were then determined by a modified binary search algorithm where if a response is correct, on the next presentation the colour difference between letter and background is halved. If the response is incorrect, the colour contrast is increased by half. This method of determining thresholds leads to finite steps which reach a plateau at the subject's threshold, the minimum colour contrast required to identify a letter correctly.

We used a within subjects repeat measures design because this reduces effects due to individual differences independent of the metric examined. Further, separate controls using different wavelengths of light could not be used because a wide range of wavelengths impact on mitochondrial function both positively and negatively and these are present in different proportions in white light[31,32]. All results were taken in triplicate and analysed from initial baseline recordings and final recordings taken after 670 nm exposure at the indicated time points. Test–retest variability was 1.6% for protan thresholds and 6% for tritan thresholds (N = 20 subjects) calculated from separate normative data with identical equipment and methods. The test–retest variability was calculated via a repeatability coefficient as $1.96\sqrt{2}\sigma$. Data were graphed and analysed using GraphPad Prism 6 (GraphPad, San Diego, CA) with a Wilcoxon matched-pairs signed rank test for significance, and an ordinary 1-way ANOVA for inter-subject comparison in variation across the day.

Data availability

The data that support the findings of this study are available on request from the corresponding author GJ. The data are not publicly available due to them containing information that could compromise research participant privacy.

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H.S. undertook all of the experiments and the analysis. C.H. designed and built the light devices. M.N. and G.J. designed the experiments and assisted with the manuscript.

Competing interests
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Additional information
Correspondence and requests for materials should be addressed to G.J.

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