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LETTER

Near infrared spectroscopy reveals instability in retinal mitochondrial metabolism and haemodynamics with blue light exposure at environmental levels

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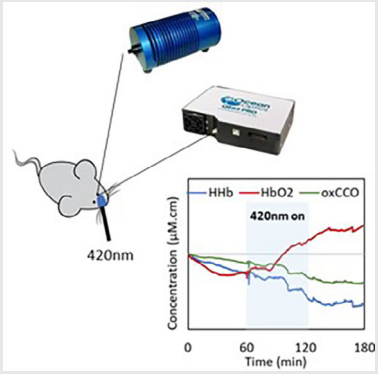
Abstract



Blue light (~400-470 nm) is considered potentially detrimental to the retina but is present in natural environmental light. Mitochondrial density is highest in the retina, and they exhibit a prominent optical absorption around 420 nm arising from the Soret band of their porphyrins, including in cytochrome-c-oxidase in their respiratory chain. We examine the impact of continuous 420 nm at environmental energy levels on retinal mitochondrial metabolism and haemodynamics in vivo in real time using broadband near-infrared spectroscopy. One hour environmental exposure to 420 nm induces significant metabolic instability in retinal mitochondria and blood signals, which continues for up to 1 h post blue exposure. Porphyrins are important in mitochondrial adenosine triphosphate (ATP) production and cytochrome-c-oxidase is a key part of the electron transport chain through which this is achieved. Hence, environmental 420 nm likely restricts respiration and ATP production that may impact on retinal function.

KEYWORDS

blue light, cytochrome-c-oxidase, environmental light, mitochondria, near-infrared spectroscopy

1 | INTRODUCTION

Blue light is regarded as harmful, but the reason for this is unclear other than the relatively high energy that it conveys. This is particularly true of the eye where light is focused on the outer retina, but we do not know if this potential damage extends to blue light in the natural environment. As the cornea and lens block UV [1–3],

short wavelength damage likely arises from wavelengths above 400 nm.

Mitochondria have a specific absorbance focused around 420 nm [4, 5], which is within the mammalian visual range, and is present in environmental light as well as many artificial light sources. This absorbance is likely due to Porphyrins that have highly phototoxic forms, notably protoporphyrin IX, which has been used in photodynamic cancer therapy where it undermines mitochondrial performance and increases reactive oxygen species (ROS) production inducing cell death [6–8]. Protoporphyrin IX is the immediate precursor in the synthesis of the haemoglobin responsible for oxygen transport in blood and hence an agent in both mitochondria and blood [9].

The potential damaging influence of 420 nm is highlighted in in vitro studies on the retinal pigment epithelium (RPE). Using different wavelengths, irradiances and exposure times, studies show that short wavelengths impact negatively on mitochondria and they agree that this is associated with elevated ROS [10–12]. However, Marie et al. scanned progressive 10 nm wavelength bands from 390 to 520 nm and showed those around 420 nm resulted in the greatest phototoxicity [13], consistent with known porphyrin absorption. Yet, 420 nm has rarely been used experimentally and not on the retina, which is both rich in mitochondria and where light is tightly focused.

Here, we examine retinal mitochondrial and haemodynamic responses to continuous 420 nm light at irradiances present in natural daylight in vivo in real time. This is achieved by analysing spectral signals embedded in reflected light from the retina using broadband near infrared spectroscopy (bNIRS), which is a non-invasive highly sensitive technique to investigate in vivo mitochondrial function and blood oxygenation [14]. bNIRS uses reflected light in the NIR range to provide metrics of changes in the concentration of tissue mitochondrial oxidised cytochrome-c-oxidase (Δ[oxCCO]) as well as oxygenated and deoxygenated haemoglobin (Δ[HbO_2] and Δ[HHb]) in real time.

2 | METHODS

Mice were used with University College London ethics approval and under a UK Home Office project licence (PPL 70/8379). All procedures conformed to the United Kingdom Animal Licence Act (1986) and local regulations. C57BL mice ($n = 7$, 6-7 months old) were anaesthetised with 0.3 mL of a mixture of 6% ketamine and 10% Dormitor (National Veterinary Services, UK) and 84% H_2O at 5 μ/g. The pupils were dilated with 1% Tropicamide (Bausch and Lomb, France) and the cornea lubricated with Viscotears (Novartis, Switzerland). Mice were unrestrained and placed on their sides for real-time measurement of retinal oxygenation and haemodynamics as well as metabolism embedded in light within 780 to 900 nm range using a miniature bNIRS system, miniCYRIL [15].

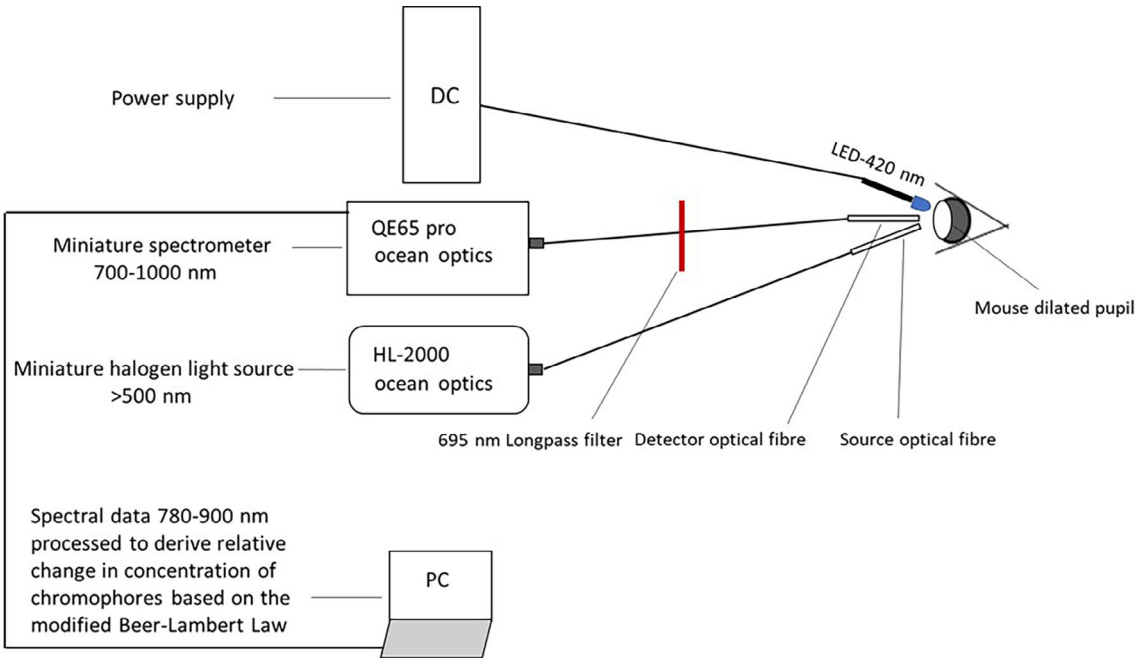


FIGURE 1 Schematic of the experimental setup. Light from the halogen white light source is filtered (>500 nm) and illuminates the dilated pupil of the mouse through an optical fibre. Reflected light is sent to the spectrometer via an identical optical fibre and is processed every second (780-900 nm). Real time changes in oxygenated and deoxygenated haemoglobin as well as oxidised cytochrome-c-oxidase are derived using the modified Beer-Lambert law and are displayed on the laptop during 1 h baseline, 1 h exposure to blue LED (420 nm) and up to 1 h after blue LED removal

Experiments were performed in the morning in a darkened room (total irradiance ~2 $\times 10^{-4}$ W/m^2) and the experimental set up is illustrated in Figure 1. A white light source, based on a halogen lamp, was used to generate the reflections with a yellow filter to remove the short wavelengths blocking light below 500 nm (HL2000, Ocean Optics, Inc., USA). Two custom-made optical fibres (LOPTEK, Germany) were placed in front of the eye, mounted on stereotaxic probe holders at ~40° to each other and perpendicular to the cornea to avoid specular reflection. These were not in contact with the cornea. The first fibre transmitted light from the source to the retina and the second collected the back reflected light carrying within it metrics of oxygenation and metabolism (HbO_2, HHb and oxCCO), which were quantified using the UCLn algorithm based on the modified Beer-Lambert Law [16]. The collected signal was filtered with a longpass colour filter (FGL695, Thorlabs, UK) removing wavelengths <695 nm to avoid second order 420 nm light interfering with the spectral measurements during exposure to blue LED. A single 420 nm LED with a full width half maximum (FWHM) of 18 nm (Roithner lasertechnik, Austria) was mounted above the two probes illuminating the eye with an irradiance of ~5 W/m^2 at the corneal surface. For this bandwidth, this is comparable to the average solar spectral irradiance at 420 nm on the ground which varies between 0.2 and 1.1 W/m^2/nm over a range of solar altitudes from 60° to 10° [17, 18].

Experiments were performed continuously over 3 h measuring real-time changes in HHb, HbO_2 and oxCCO recorded every second. The protocol consisted of 1 h baseline, 1 h exposure to 420 nm light, followed by 1 h recovery after 420 nm removal.

Data were analysed in MATLAB 2018b. We quantified the instability in the bNIRS signals through calculating the absolute mean change in the concentration of NIR chromophores (HHb, HbO_2 and oxCCO). In all animals, the absolute mean change in the concentration of each chromophore was calculated for every 1 h measurement period. One-tailed Mann-Whitney *U* test was used to compare the absolute mean changes in all the signals during exposure to 420 nm and recovery periods against their corresponding baseline values and statistical significance was considered as $P < .05$.

The experiment was also performed on a solid phantom with tissue-like properties to make sure the effect of 420 nm light was not caused by the instrumentation or optical artefact.

3 | RESULTS

No change, apart from small random noise, was observed in any of the NIRS signals measured on a solid phantom as a control during baseline, exposure to blue and after.

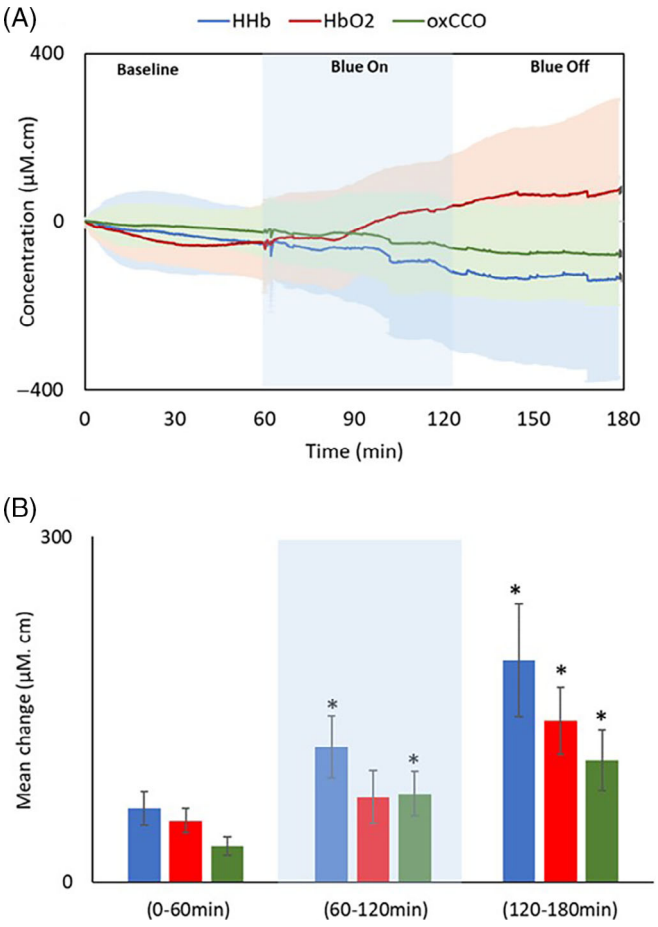


FIGURE 2 Significant instability in mitochondrial and blood signals in the mouse retina as a response to exposure to low power blue light (420 nm) measured in real time. The blue curve and blue bars show changes in deoxygenated haemoglobin (HHb). The red curve and red bars show changes in oxygenated haemoglobin (HbO_2). The green curve and green bars show changes in oxidised cytochrome-c-oxidase (oxCCO). The curves in (A) demonstrate mean data for $n = 7$ mice and the shaded area around each curve represent the standard deviations. The shaded area in blue between 60 and 120 min shows the period of exposure to a 420 nm LED (A) There was increased variation in oxygenated and deoxygenated haemoglobin (HbO_2 and HHb) as well as oxidised cytochrome-c-oxidase (oxCCO) during exposure to 420 nm LED (60-120 min) and the recovery period (120-180 min) compared to baseline (0-60 min). Baseline variations in HHb, HbO2 and oxCCO change their gradient after 420 nm light exposure which continues for 1 h post blue LED. (B) Data in (A) are re-represented in (B) in histogram form where changes are compiled over each of the three-time intervals: 0-60 min, 60-120 min, and 120 to 180 min. Error bars represent standard error and the absolute mean change for each chromophore during the 420 nm and recovery periods are compared against their corresponding baseline values using nonparametric one-way Mann-Whitney *U* test (*$P < .05$)

Progressive changes in retinal blood (HHb and HbO_2) and mitochondrial (oxCCO) signals in the retinae of mice is shown in Figure 2A during 1 h baseline, 1 h blue LED

(420 nm) exposure and 1 h recovery (after the blue LED was off). There were gradual changes in all three signals during baseline recordings in the first hour. The animals did not move but they were unrestrained, and no attempt was made to control for potential slow eye movements, although these were not obvious. These signals changed upon exposure to 420 nm both in terms of their direction and magnitude of change. This progressed over the 1 h 420 nm exposure and continued after. Divergence in the signals and their variability increased after the 420 nm LED was turned off. Hence, both mitochondrial and blood signals show significantly increased variation with respect to baseline (Figure 2A). The bar chart in Figure 2B represents the group data for the absolute mean change in mitochondrial and blood signals during baseline, blue LED exposure and recovery periods. Each signal shows significantly greater variability during exposure to 420 nm and the recovery period. Instability induced by 420 nm exposure in blood and mitochondrial signals is apparently not immediate but the result of cumulative exposure, which does not recover until 1 h after the LED is off. During the 1 h recovery, the absolute mean change in all the signals is still significantly greater than the baseline. Experiments were not extended beyond 3 h due to concerns over prolonged anaesthesia and associated changes in physiology. Further, cataract can develop in mice with extended anaesthesia and that undermines reflectance measurements.

4 | DISCUSSION

The retina has a very high metabolic rate and the greatest mitochondrial density in the body in photoreceptors where light is uniquely and tightly focused [19]. We demonstrated in real time that 1 h exposure to 420 nm light at environmental levels induces significant disturbance to normal retinal mitochondrial metabolism and oxygenation. This impact was gradual and did not subside when 420 nm was withdrawn. The instability or variations in all the signals were still significantly greater than the baseline even after the full 1 h recovery period.

The spectral irradiance of the extended blue light we used was comparable to that of the solar irradiance on the ground at 420 nm [18]. Even though we do not know exactly how much of its energy reached the retina, we can be certain that the majority of it did because of the large acceptance angel of the mouse's eye (~60°, almost three times greater than that for the human eye [20]).

Mitochondria are primary absorbers at 420 nm [4, 5] most likely due to porphyrin absorption [21]. We are unable to quantify porphyrin with our optical technology, however, the haem a3 at the binuclear metal centre of cytochrome-c-oxidase contains porphyrin, and measurement of the absorption spectra of oxidised cytochrome-c-oxidase has revealed a Soret peak at 420 nm [22, 23]. But our analysis also reveals changes in haemodynamics, and porphyrin is constituent of haemoglobin. It is possible that mitochondrial absorption of 420 nm initiated disturbance in mitochondrial oxidative metabolism that resulted in alterations in oxygen consumption with greater variations in oxy and deoxy haemoglobin signals. Alternatively and perhaps in addition, red blood cells do absorb 420 nm [24] that may result in blood signal disturbances. This is a key point as such changes may have a systemic impact. Evidence for a systemic effect comes from Stern et al. [25]. They exposed the human body to 450 nm for 30 minutes resulting in significantly elevated heart rate and reduced blood pressure during exposure, but this returned to a normal range relatively rapidly after blue light was removed. We do not know how the ocular exposures in our study impacted other tissues, which would have been difficult to monitor considering our experimental set up. However, the potential systemic effect of blue light remains an important issue.

With age mitochondrial membrane potential declines and this is associated with reduced production of adenosine triphosphate (ATP) and increased ROS that can drive inflammation [26]. Hence, an additional concern is the frailty of mitochondria in ageing and their behaviour that drives the ageing process and can trigger disease, and whether this may be associated with increased vulnerability to short wavelength exposure. In the retina, mitochondrial decline is associated with age related macular degeneration and diabetic retinopathy [27, 28]. In this study, we have explored mitochondrial response to 420 nm in 6-month-old mice, but we do not know how the metrics we have examined vary with age. Here there are unknown factors. While mitochondria are more vulnerable in ageing, the optics of the lens has reduced clarity particularly at short wavelengths and there are changes in its geometry that may act as a protective filter [29–32].

There are major differences between the eyes of mice and humans. We exposed our mice to daylight levels of 420 nm that, being nocturnal, they would strongly avoid. However, it is likely that humans regularly exposing their eyes to 420 nm in daily life are potentially disrupting mitochondrial function by interfering with the electron transport chain. But they rarely do it continuously. This has not been examined in the human retina partly because of the understandable current caution regarding blue light exposure. However, it is interesting that historic human psychophysical experiments on colour matching and adaptation show very long recovery times when short wavelength light is used, but not with longer

wavelengths [33]. It is possible that such effects result from the impact of short wavelengths directly upon retinal mitochondria that in turn modulate psychophysical adaptation.

There is evidence however that longer wavelengths around 670 nm have a positive impact on mitochondria, improving electron transport chain function. These wavelengths improve mitochondria membrane potential and ATP production and also significantly improve aged human visual function [34, 35]. While blue and red components of atmospheric light vary with time of day, season and changes in weather patterns, the blue variation is greater than the red [18]. Further, our developments in our artificial lighting in which we spend a large proportion of our time are increasingly biased towards short wavelengths [36]. Given the data presented here, it seems likely that retinal metabolism would respond to these features of light in a way that we do not yet appreciate but that may impact on our vision.

5 | CONCLUSION

Our results demonstrate that exposure to 420 nm light at approximately environmental levels disrupts retinal metabolism. This wavelength is specifically absorbed by mitochondria whose cellular density is greatest in the outer retina. The negative impact of this is probably largely corrected by longer wavelengths in natural light, but this may not be the case in some forms of artificial lighting that lack longer wavelengths.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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