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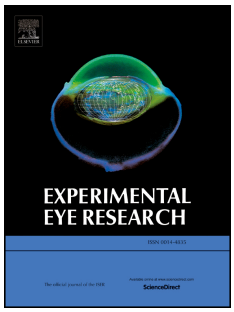

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Improving mitochondrial function significantly reduces the rate of age related photoreceptor loss

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Abstract

Declining mitochondrial function drives ageing. With age, mitochondrial membrane potential declines, reducing ATP production and elevating pro-inflammatory reactive oxygen species production leading to cell death. In the retina mitochondrial density is high and there is a 30% photoreceptor loss with normal ageing. But aged mitochondrial membrane potential and ATP can be improved by long wavelengths absorbed in mitochondrial respiration. Hence, we ask if exposure to such wavelengths for 8 months in 12 month old mice can reduce aged photoreceptor loss. We expose aged mice daily for 10mins to 670nm light then counted their photoreceptors. Exposure significantly retarded aged photoreceptor loss. Control mice suffered an approximate 30% decrease in photoreceptor outer segments and in the thickness of the retinal layer containing their nuclei compared to 2 month old mice. But in aged mice exposed to 670nm over 8 month, reductions in outer segments were only <15% and reductions in their nuclear layer were only <10%. Hence, improving mitochndrial function reduces the impact of aged cell loss.

Mitochondria provide much of the energy that drives cellular function in the form of adenosine triphosphate (ATP). The mitochondrial theory of ageing argues the progressive mutations in their DNA (mtDNA) that reduce mitochondrial function and increase production of reactive oxygen species (ROS) are key drivers of the ageing process (Harman, 1956, Lopez-Otin et al., 2013).Hence, aging is associated with reduced cellular energy, progressive inflammation and cell loss. Initiation of aged changes are associated with reduced mitochondrial membrane potential and result in release of cytochrome c that induces the formation of the apoptosome required for caspase activation and the cascade of events that lead to cell death (Gottlieb et al., 2003). However, mitochondrial membrane potential can be manipulated optically. Cytochrome c oxide (COX), the rate limiting enzyme in mitochondrial respiration absorbs specific wavelengths in the deep red (Mason et al., 2014, Karu, 2008, Gibson and Greenwood, 1965).This shifts COX redox (Kaynezhad et al., 2016), improves mitochondrial membrane potential (Kokkinopoulos et al., 2013) and ATP production (Gkotsi et al., 2014). This is subsequently associated with reductions in inflammation and reduces cell loss in experimental pathology (Fitzgerald et al., 2013, Shaw et al., 2010).

Given the association between declining mitochondrial membrane potential and the initiation of the cascade of signals leading to cell death, exposure to longer wavelengths that improve membrane potential may reduce the probability of age driven apoptosis. The retina is the perfect model to test this hypothesis, not only because it is open to the visual environment, but also because it has the highest concentration of mitochondria in the body in photoreceptor inner segments and the greatest energy demand of any tissue (Country, 2017, Futterman and Kinoshita, 1959, Winkler, 1981, Stone et al., 2008). High energy demands are associated with faster rates of ageing (Pearl, 1928, Speakman, 2005, Wang et al., 2010), and in both rodents and humans aged photoreceptor loss is a significant event. In the rod dominated rodent eye (Carter-Dawson and LaVail, 1979) there is a 30% pan retinal photoreceptor loss with age (Cunea and Jeffery, 2007), while in humans a similar number of central rods are lost by 70 years

of age (Curcio et al., 1993). In rodents, rod loss is a feature of retinal ageing from approximately 12 months onward (Cunea and Jeffery, 2007).Hence, here we age C57BL/6 mice until this point and then expose a cohort to 670nm daily for a further 8 months. We ask if such light exposure has the ability to reduce the pace of normal age related photoreceptor loss in these animals.

All experimental procedures were undertaken with local UCL ethical approval and under UK national Home Office legislation. C57BL/6 mice were used throughout. Three groups of mice were employed with n=5 in each. The first group was 2 month old animals. This age was selected because at this stage the size of the mouse eye is adult like, but it is prior to any significant age related photoreceptor loss. Hence, these young animals provided baseline data for photoreceptor numbers. The 670nm light experimental exposure was similar to Begum et al. (Begum et al., 2013). Mice were exposed once a day at 10am for 10 mins. Age related rod photoreceptor loss is initiated around the start of the second year of life in mice although cone photoreceptor cell death is initiated earlier, however cones form only around 3% of the total photoreceptor population in these animals and hence are a minimal component of the photoreceptor population (Carter-Dawson and LaVail, 1979).

Mice were killed by cervical dislocation and the eyes removed and placed in 2% paraformaldehyde and 2% glutaraldehyde in phosphate buffer (PB). After approximately 24h fixation the cornea and anterior chamber were removed and the eye cups placed in 1% osmium tetroxide for 1h. Eye cups were then washed in PB and dehydrated through a graded series of alcohols before being embedded in plastic (Technovit 7100 historesin solution, Taab Laboratories equipment, UK). Eye cups were sectioned at 5µm, mounted on glass slides and Nissl stained with cresyl violet before being mounted in DPX and cover slipped.

Counts were made of photoreceptor outer segments at X1000 in central sections in 7 adjacent regions in the central retina in sections close to that containing the optic nerve head. These counts were within the

central third of the retina. Independently, separate measurements were made by a separate person of the width of the outer nuclear layer (ONL). These were made from retinal images undertaken at a microscope magnification of X400 on a computer screen where the magnification was increased by X4. Results of the separate measurements were not shared between observers until complete and after statistical analysis, which employed Mann-Whitney non-parametric methods.

There was a significant decline in both the number of photoreceptor outer segments counted and the thickness of the ONL measured between 2 and 20 months of approximately 30% in both metrics in control mice. There was also a decline in these metrics in mice exposed to 670nm light. However, the age related decline found in mice exposed to 670nm was significantly less than found in aged matched controls. While both the thickness of the ONL and the number of outer segments declined in the 670nm treated group compared to young mice, the reduction was only of the order of 10% and 15% respectively. In the case of both metrics this was significantly different from levels found in old untreated controls but not significant compared to young mice. Hence, exposure to 670nm significantly retards age related photoreceptor cell loss over a critical period of ageing in this animal (Fig. 1).

Cell death is key feature of CNS ageing and is often linked to changes in metabolism (Dorszewska, 2013). This is marked in the outer retina (Cunea and Jeffery, 2007, Curcio et al., 1993), which has such a high metabolic demand (Country, 2017, Futterman and Kinoshita, 1959, Stone et al., 2008, Winkler, 1981). Our demonstration of reduced age related photoreceptor loss is consistent with our understanding of the mechanism of action of long wavelength light. This shows that 670nm significantly improves aged mitochondrial membrane potential and ATP production that are key elements in ageing and cell death (Kokkinopoulos et al., 2013, Gkotsi et al., 2014). Many studies have shown that exposure to long wavelengths improves experimental pathology where necrosis is often more likely the underlying mechanism driving cell loss (Fitzgerald et al., 2013). However, in natural ageing, the

mechanisms are likely based on apoptosis and fundamentally different, which highlights the novelty of our data and also potentially its importance.

Many factors influence cell survival in ageing, hence, it is not surprising that 670nm exposure did not completely block age related rod photoreceptor loss. With age there is thickening of Bruch's membrane (Ramrattan et al., 1994) and reductions in its permeability (Moore and Clover, 2001) that likely result in progressive outer retinal hypoxia, impacting on the high oxygen demand of photoreceptor mitochondria. There are also a range of other key drivers of ageing independent of mitochondria, including genomic instability, cellular senescence and telomere erosion that all play a role and likely interact (Lopez-Otin et al., 2013). There is no evidence that 670nm light impacts directly on these mechanisms.

The influence of 670nm light is not restricted to aged cell survival, but also impacts on cell function. Relatively short exposure to 670nm has been shown to improved aged mouse retinal function over 1 month (Sivapathasuntharam et al., 2017). Also in aged Drosophila, single exposures that improve whole body respiration also improve exploratory behaviour (Weinrich et al., 2018). In these cases, changes are likely the result of improved ATP and subsequent signaling mechanisms that impact on metabolism.

There is now a degree of clarity over our understanding of the interactions between light and mitochondrial function and the mechanism resulting in neuroprotection (Karu, 2008). Given the imperatives that arise from ageing populations and their cost, the use of this economic and relatively non-invasive route may have advantages.

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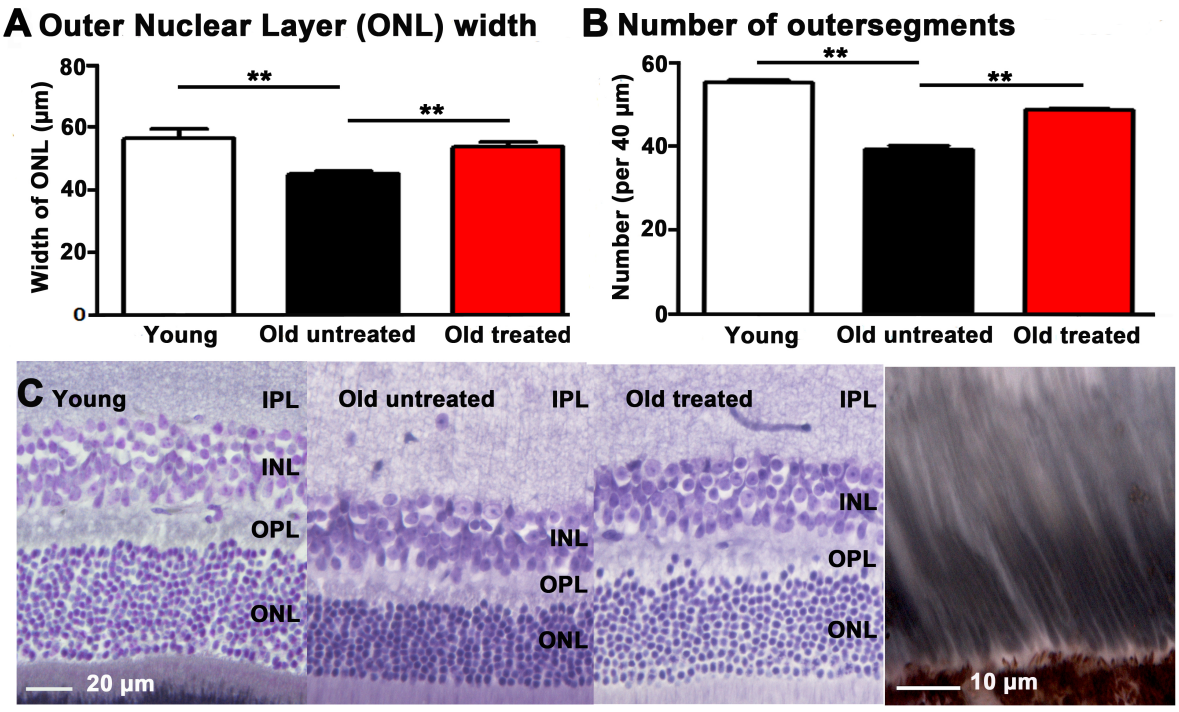
Figure Legend

Fig. 1. Daily exposure to 670nm light reduces the pace of age relate photoreceptor loss. Three groups of mice were used: young control at 2 months, old untreated control at 20 months and old treated who were exposed daily to 670nm for 10 mins from 12 months onward until 20 months old. The width of the outer nuclear layer (A and C) and the number of photoreceptor outer segments (B and C right hand panel) were assessed as independent metrics. Both showed significant aged reductions of approximately 30% between young and old untreated. Reductions were also present in old treated mice, but these were not significant against young animals. However, 670nm treatment resulted in significant differences between old treated and untreated mice with both a wider ONL and more outer segments in treated mice. Hence, extended 670nm exposure in aged mice reduces the pace of retinal apoptosis. Abbreviations: IPL, inner plexiform layer. INL, inner nuclear layer. OPL, outer plexiform layer. ONL, outer nuclear layer. Statistical significance, ** $P \leq 0.01$.



1] We chart murine age related photoreceptor loss between 2-20 months showing a ~30% decline

2] 670nm light exposure improves mitochondrial function that regulates apoptosis

3] 670nm exposure from 12-20 months significantly reduced normal aged cell loss

4] 670nm has been used protectively in experimental pathology. We extend this to normal ageing