Minireview

OCT imaging of rod mitochondrial respiration *in vivo*

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Impact statement

New clinical imaging biomarkers of photoreceptor mitochondrial function are needed to improve early diagnosis and interventions in order to prevent vision loss in a range of common retinopathies. The focus of this review is on the development and application of an imaging index of mitochondria-evoked changes in subretinal space (SRS) water content during dark and light conditions, an under-appreciated contributor of outer retina health and disease involving pro-survival factors in the interphotoreceptor matrix. Translating the promising OCT results from animal studies to humans will result in improved diagnosis of mitochondria-based threats to sight in aging and disease, and improve the success rate when translating treatments from bench-to-bedside.

Abstract

There remains a need for high spatial resolution imaging indices of mitochondrial respiration in the outer retina that probe normal physiology and measure pathogenic and reversible conditions underlying loss of vision. Mitochondria are involved in a critical, but somewhat underappreciated, support system that maintains the health of the outer retina involving stimulus-evoked changes in subretinal space hydration. The subretinal space hydration light–dark response is important because it controls the distribution of vision-critical interphotoreceptor matrix components, including anti-oxidants, pro-survival factors, ions, and metabolites. The underlying signaling pathway controlling subretinal space water management has been worked out over the past 30 years and involves cGMP/mitochondria respiration/pH/RPE water efflux. This signaling pathway has also been shown to be modified by disease-generating conditions, such as hypoxia or oxidative stress. Here, we review recent advances in MRI and commercially available OCT technologies that can measure stimulus-evoked changes in subretinal space water content based on changes in the external limiting membrane-retinal pigment epithelium region. Each step within the above signaling pathway can also be interrogated with FDA-approved pharmaceuticals. A highlight of these studies is the demonstration of first-in-kind *in vivo* imaging of mitochondria respiration of any cell in the body. Future examinations of subretinal space hydration are expected to be useful for diagnosing threats to sight in aging and disease, and improving the success rate when translating treatments from bench-to-bedside.

Keywords: Diffusion MRI, optical coherence tomography, mitochondria, photoreceptors

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Introduction

Detection and treatment of sight-threatening retinal disease typically involve imaging structural damage of, for example, the photoreceptors and retinal pigment epithelium (RPE). However, clinical identification of histopathology is often at a stage with irreversible damage and thus at a time when interventions would not restore lost vision. Before morphological manifestation of ocular diseases, there are usually functional/mitochondrial defects in photoreceptors and/or their support system; however, such dysfunction is not usually measured. New functional biomarkers are needed to evaluate photoreceptor function and their mitochondrial-based support system that maintain a healthy environment and evaluate treatment efficacy against vision loss. Preferably this will be achieved using instruments already available in clinic and laboratories. The focus of this review is on the development and application of imaging indices of a key aspect of this support system: measuring mitochondria-evoked changes in subretinal space (SRS) water content during dark and light conditions, an under-appreciated contributor of outer retina health and disease.

Current functional indices of cone and rod photoreceptors

Cone and rod function are often evaluated using the electroretinogram (ERG) (both Ganzfeld and multi-focal ERG [mfERG]).[1–7] These methods measure an integrated signal from the entire retina indicating phototransduction and outer retina synaptic transmission. However, their no-to-limited spatial resolution makes lesion detection sensitivity and specificity low.[1–7] For example, ERG has not been able to predict loss of visual performance in models of retinal degeneration nor in aging in the absence of overt pathology.[8,9] In addition, changes in retinal laminae electrical resistance will alter ERG readout confounding any interpretation of the role of mitochondria. To address these shortcomings, new optoretinographic (ORG) methods are being developed that encode light-based changes (thickness or reflectance) in cone and rod photoreceptors with very high spatial and temporal resolution, and are likely insensitive to changes in electrical resistance.[10–12] While much progress has been made in identifying the mechanisms underlying ORG signals, including a report of a mitochondrial contribution, the earliest change appears to reflect electromechanical deformation in individual human cone photoreceptors.[10–15] It is unclear at present whether the fast ORG response is able to evaluate modifiable pro-survival factors within the photoreceptor-retinal pigment epithelium (RPE) support network.

The photoreceptor support system depends on light–dark changes in SRS hydration

The SRS is the extracellular fluid space between RPE cells and photoreceptors, spanning from the posterior border of the Müller glia (i.e. the external limiting membrane [ELM]) to the apical RPE.[16] This space is relatively isolated by tight junctions at these the two borders. In most mammalian (rod-dominate) retina, the inner and outer segments of rod photoreceptors largely occupy the SRS which contains the interphotoreceptor matrix (IPM) and extracellular fluid.[17–22] The IPM is thought to contribute to the essential maintenance and healthy performance of photoreceptor cells by regulating nutrient and retinoid transport, pro-survival/anti-oxidant factors, and ion composition.[17,18,23,24] Notably, the IPM has been implicated in neurodegenerative disease.[18,25]

Somewhat surprisingly, many components of the SRS normally undergo a major shift in their distribution within the IPM between light and dark conditions; abnormal distribution appears to be of pathogenic importance.[18,20,24,26] This shift in IPM content dispersal is linked to two other stimulus-induced changes. First, as shown by microelectrode studies, pH outside of rod photoreceptors in the dark decreases (i.e. acidify) in cat retina *in vivo*.[27,28] Light-induced extracellular alkalinization near rod photoreceptors was also reported for *ex vivo* toad and rabbit retinas.[29,30] These findings support the large and well established increase in mitochondria respiration in the dark over that in the light which is linked to keeping cGMP channels in the open position.[31–33] Second, in the dark substantial shrinkage of SRS, volume has been measured in frog and chick retinal preparations *ex vivo*, and in cat retina *in vivo* using microelectrodes and an extracellular marker.[27,34–38] Intriguingly, blocking RPE ion channels with 4,4′-diisothiocyanostilbene-2,2′-disulfonate (DIDS) inhibited the stimulus-dependent IPM volume change.[36] Also, lowering IPM pH alone with carbonic anhydrase inhibitors (i.e. acetazolamide or benzolamide) is sufficient to decrease SRS volume in perfused light-adapted chick retina–RPE-choroid preparation; in-line with this response, acetazolamide is useful for increasing subretinal fluid resorption in patients.[39] Further, hypoxia, a disease-generating condition that can lower the pH outside of rods, also suppresses light–dark SRS volume changes.[40,41] Mechanistically, Adijanto *et al.* showed how lower pH can trigger a reduction in SRS volume.[33] In RPE cells, they found that dark-like increases in CO_2 and waste water can acidify the SRS, an event that turned on water removal co-transporters in RPE that caused rapid removal of the acidified SRS water in order to protect rods and cones.[33,37,42–48]

In summary, the above findings from 1990 to 2009 provide strong support for the following signaling pathway (summarized in Figure 1). In the dark, cGMP levels in the outer segments are relatively higher and ion channels stay open which requires greater mitochondrial respiration resulting in acidification of SRS due to increased CO_2 production. In turn, this acidified IPM upregulates water efflux co-transporters in the RPE. As a result, higher RPE fluid transporter activity occurs that overcomes enhanced water production of increased mitochondrial respiration and reduces the SRS water content causing ELM-RPE thickness to shrink (Figure 1(a)). In the light (Figure 1(b)), when cGMP levels in the outer segments are low, ion channels are closed, requiring less energy to maintain circulating current and, therefore, supporting mitochondrial respiration is reduced. Reduced mitochondrial respiration also generates less CO_2 byproduct, and pH in the SRS is thus relatively more basic. This pH keeps water efflux co-transporter activity in the RPE low. Although reduced mitochondrial respiration generates less water as another byproduct, down-regulated fluid co-transporters on the RPE lead to accumulation of water in the SRS producing a relatively thicker ELM-RPE compared to that in the dark.

So, is this light-evoked SRS expansion or dark-evoked SRS shrinkage?

The literature largely refers to the above events as a light-evoked expansion of the ELM-RPE.[38] This description does not, in our opinion, adequately capture the active mechanism (see Figure 1).[38] Our preference is to refer to the above signaling pathway as a dark-evoked thinning of the ELM-RPE in order to focus on the fact that dark actively triggers water removal from the SRS via upregulated pH-sensitive RPE co-transporters; herein, we refer to this as the cGMP →

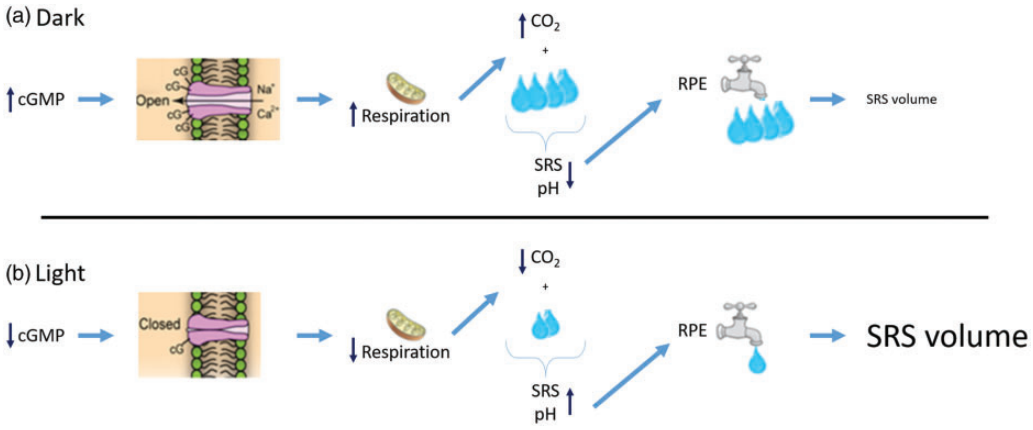

Figure 1. Working model outlining how the SRS volume changes hydration with dark and light conditions. (a) In the dark, cyclic nucleotide–gated ion channels (left most cartoon) are maintained in an open position via availability of cGMP (up arrow), causing increased ion pumping and associated increase (up arrow) in mitochondrial (second cartoon) respiration. In turn, rods produce more waste water (# of droplets) and CO_2 that together lower (down arrow) the pH of the SRS. Water + CO_2 make an acidified environment that upregulates water efflux co-transporters (faucet cartoon) on RPE causing a reduction in SRS volume (indicated by smaller font). A smaller SRS volume is measured by a thinner ELM-RPE thickness. (b) In the light, cGMP is hydrolyzed reducing its concentration and causing cyclic nucleotide-gated channels to close, reversing the events in (a) and producing a larger SRS volume than in the dark with a thicker ELM-RPE region. Each step in this signaling pathway has been experimentally demonstrated (see text for details). (A color version of this figure is available in the online journal.)

mitochondria → pH → RPE water removal signaling pathway.

Measuring light–dark SRS hydration changes *in vivo*: Diffusion MRI

In 2012, Bissig and Berkowitz non-invasively showed a dark-evoked decrease in the apparent diffusion coefficient (ADC) in SRS region in rats as measured by MRI.[49] Diffusion MRI has a spatial detection sensitivity of ~5 μm and is exquisitely sensitive to changes in water mobility due to changes in cellular barriers and shapes, as shown for a mouse eye (Figure 2(a)).[50,51] Thus, the light–dark diffusion MRI changes likely reflected the expected dark-evoked shrinkage of the SRS volume. Another finding from this study was that water mobility in the SRS region is much higher than in the rest of the retina suggesting a distinct and localized water management system.[49] Also, light–dark changes in water mobility occurred in the axial direction (in the direction parallel to the rods) and not in the transverse direction.[49] This was also the first study to indicate light–dark changes in SRS hydration in rats, and subsequently shown in mice.[50–52]

In 2015, diffusion MRI also showed light–dark changes in the SRS region in mice (Figures 2(b) and 3).[52] Further, the diffusion SRS photoresponse was first shown to be phototransduction-dependent because it was absent in *GNAT1* knockout mice (Figure 3).[52] Intriguingly, stimulus-evoked SRS changes were also not seen in diabetic mice but could be recovered 30 min after giving an antioxidant, demonstrating an unexpected sensitivity to oxidative stress (Figure 3).[52,53] One likely mechanism for this surprising result is that oxidative stress is associated with acidification, a condition expected to inhibit light–dark SRS hydration changes (see above).[54] In support of this notion, in 2016, we presented data showing *in vivo* that lowering SRS pH alone with acetazolamide given to non-diabetic mice also prevented light–dark SRS changes measured by diffusion MRI (Figure 3).[55]

In summary, these studies from 2012 to 2016 demonstrated that light–dark SRS changes are sizable enough *in vivo* to generate endogenous contrast that is detectable with imaging. These studies opened the way to considering similar imaging studies in patients using optical coherence tomography, a more clinically accessible technology than MRI.[17–22]

OCT studies of light–dark SRS hydration changes *in vivo*

OCT is commonly used both in clinic and research for providing a non-invasive optical section of the retina. In 2016, Li *et al.* reported shrinkage of outer retina thickness after dark-adaptation in the same mouse.[56] To quantitate outer retinal layer thickness changes, outer retina thickness was measured from two clearly distinguishable OCT markers, external limiting membrane (ELM) and basal side of RPE layer. In commonly used C56B/6J mice, 4–6 μm reductions in ELM-RPE thickness were observed on OCT images after over-night dark-adaptation (Figure 4(a)); more recent work finds that the ELM-RPE phenotype in the light can be converted to a thinner dark-like phenotype with a phosphodiesterase 6 inhibitor sildenafil (Figure 4(b)).[56,57] Good agreement was noted with light–dark changes in staining of the actin in the IPM as measured histologically.[56] In addition, the authors noted a novel OCT photoresponse with a strong hyporeflective band between the RPE and photoreceptor-tip layers in the light that was suppressed in the dark; work is on-going to understand how this hyporeflective band and ELM-RPE changes are related.[56,58]

OCT studies demonstrating mitochondria control of light–dark SRS hydration changes

We then asked if differences in mitochondria respiration could be interrogated based on the ELM-RPE thickness.

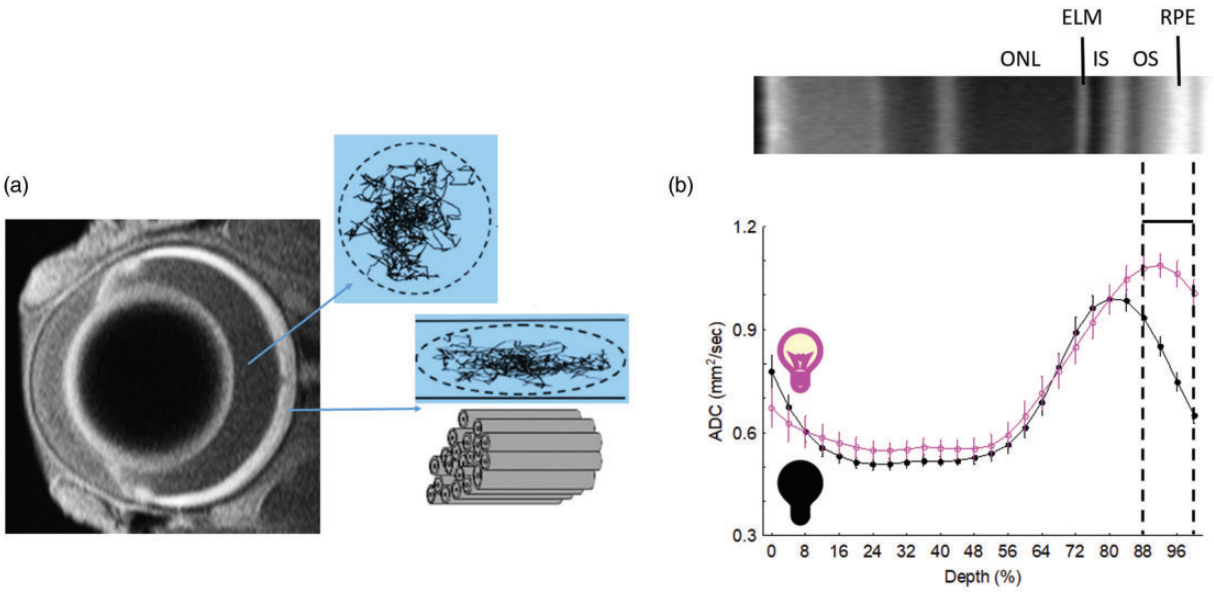

Figure 2. Diffusion MRI measures dark–light differences in the SRS volume *in vivo*. (a) Typical MRI image of a mouse eye (left-most image) indicating that the shape of isotropic water movement (as measured by diffusion MRI) changes from circular/spherical in the absent of barriers (e.g. in the vitreous) to more constrained/oblong in the extracellular fluid surrounding rod photoreceptors. Thus, a decrease in SRS volume is predicted to decrease isotropic water movement as water encounters more barriers. (b) Summary of retinal water mobility (i.e. apparent diffusion coefficient [ADC]) profiles as a function of retinal depth during dark (black, dark lightbulb) and 20 min of ~500 lux light (pink, yellow light bulb) for wildtype C57BL/6J mice. A representative OCT image is presented at the top of the graph (after aligning the vitreous-retina and retina-choroid boundaries) to provide a guide for assigning a particular ADC value to a particular retinal laminae; indicated are the outer nuclear layer (ONL), external limiting membrane (ELM), inner segments (IS), outer segments (OS), and retinal pigmented epithelium (RPE). Profiles were spatially normalized to whole retinal thickness for each mouse (0% = vitreous/retina border; 100% = retina/choroid border). Data are means ± SEM. Black horizontal line = region with significant differences ($P < 0.05$) between profiles. This graph highlights (i) that in the light water mobility is greatest in the SRS relative to other parts of the retina, and (ii) that a dark-evoked decrease in ADC is localized to the SRS region (e.g. 92–100% retinal depth), as predicted by the signaling pathway in Figure 1 and microelectrode studies in the literature.

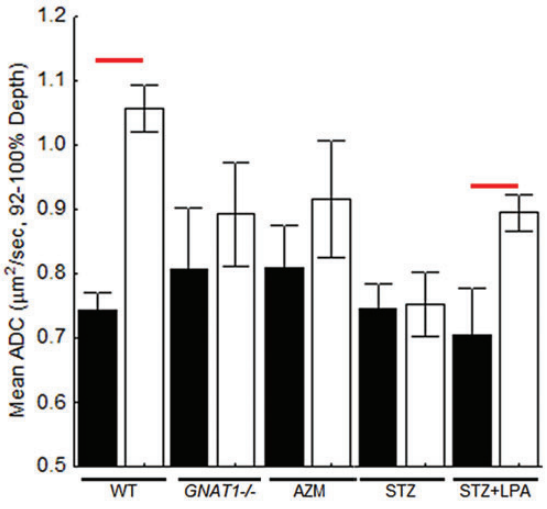

Figure 3. Diffusion MRI data supporting signaling pathway factors in Figure 1 that contribute to the SRS volume changes in light and dark. Summary of paired data (filled = dark, open = light) of wildtype (WT) mice) showing a nice reduction in ADC in the dark, mice without the phototransduction protein transducing ($GNAT1^{-/-}$ mice) do not show a light–dark difference, acidified SRS by acetazolamide (AZM) do not show a light–dark difference, and vehicle or an antioxidant (α-lipoic acid, LPA) treated two-month diabetic mice (STZ, STZ + LPA, respectively) show the presence of oxidative stress (which can acidify neurons). Red horizontal line, $P < 0.05$.

In 2018, we compared two mouse strains with different mitochondrial respiration efficacies (lower C57BL/6J vs. higher 129S6/SvEvTac).[58] Mitochondrial respiration efficacy refers to the amount of oxygen needed to produce ATP; mice with less efficient mitochondria (e.g. C57BL/6J) are vulnerable to retinal degeneration involving, for example, oxidative stress.[59,60] Dark-stimulated outer retina layer water content was determined by proton density MRI, structure and thickness by ultrahigh-resolution OCT, and water mobility by diffusion MRI.[58] In C57BL/6J mice, dark adaptation triggered a decrease in water content of outer retina *in vivo* together with a decrease in the ELM-RPE thickness, and in water mobility (Figure 5(a)). In contrast, dark did not change SRS hydration or decrease water mobility in the 129S6/SvEvTac mice; a significant but relatively smaller decrease in ELM-RPE thickness was noted (Figure 5(a)).[58] In other words, a large light–dark ELM-RPE thickness change was linked to less efficient mitochondrial respiration. These studies raise the possibility of the size of the light–dark ELM-RPE thickness change may be a useful mitochondrial injury biomarker.

Next, we took advantage of mitochondrial uncouplers, such as 2,4-dinitrophenol (DNP), which shuttle protons across the mitochondrial inner membrane thus disrupting the mitochondrial proton gradient that is used to generate ATP, to stimulate metabolism if sufficient reserve capacity is available.[61] In the light, DNP produced dark-like ELM-RPE thickness in both C57BL/6J and 129S6/SvEvTac mice, with a larger thinning in C57BL/6J consistent with its relatively less efficient mitochondria respiration (Figure 5 (b)).[58,59,61] In the dark, when photoreceptor mitochondria activity is high, DNP had no significant effect on ELM-RPE thickness in dark-adapted C57BL/6J mice (implying little mitochondrial reserves) but did cause thinning in

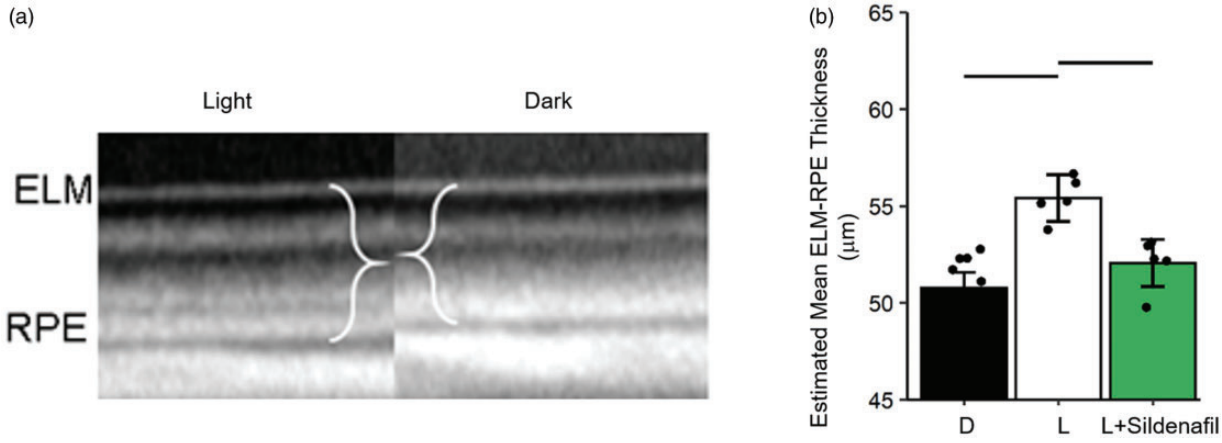

Figure 4. Modifiers of the ELM-RPE thickness. (a) Light vs. dark: Zoomed-in region of the ELM-RPE in a representative mouse that was exposed to either 5 h of lab light or was overnight dark adapted; brackets provide a visual guide to highlight dark-evoked shrinkage of the ELM-RPE thickness. Quantitation of this light–dark change is provided in the first two bar graphs in (b) (also in Figure 5). (b) Pharmacology: Bar graph summary comparing dark (D, black bar) and light (L, white bar) adapted ELM-RPE thicknesses to a light-adapted mouse given the phosphodiesterase 6 inhibitor sildenafil (green bar) 1 h prior to examination. Data are means ± 95% confidence intervals; data for each mouse also shown as individual points. Black horizontal lines = significant differences between groups ($P < 0.05$). (A color version of this figure is available in the online journal.)

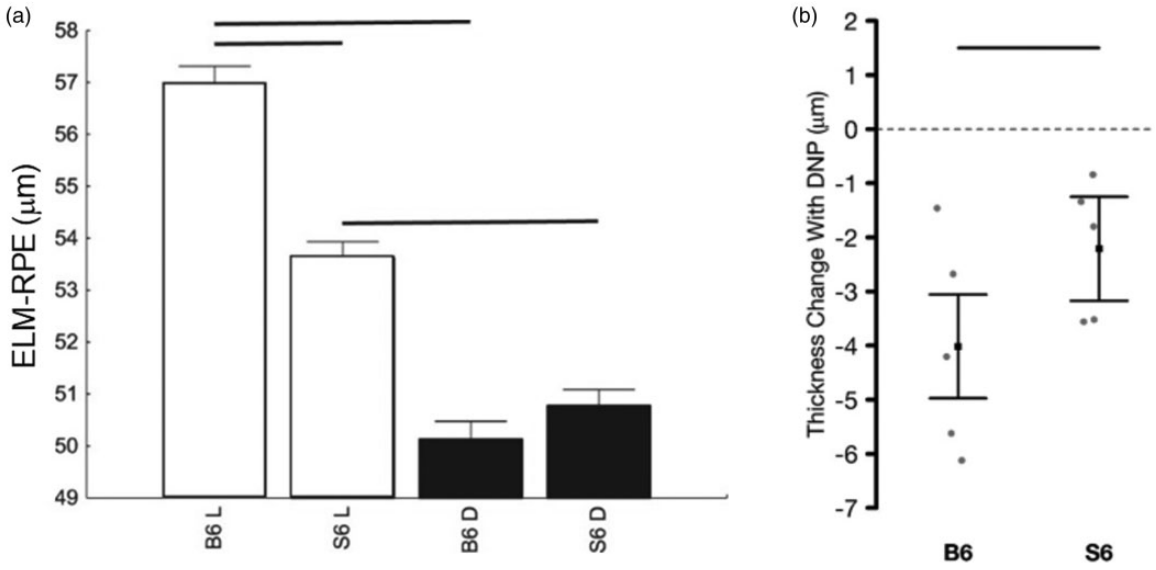

Figure 5. Evidence that mitochondria drives changes in ELM-RPE thickness. (a) Light vs. dark: Summary of outer retina thickness, measured from OCT images in light (L) and dark (D) for mice with relatively inefficient mitochondria respiration (C57BL/6J [B6]) and with relatively more efficient mitochondrial respiration (129S6/SvEvTac [S6]). This means that in the light, S6 mice have a lower basal level of mitochondria activity than B6 mice resulting in lower waste water production and thus shorter ELM-RPE thickness and smaller light–dark difference. (b) Pharmacology: Paired differences (before-after DNP) are presented to account for changes within mice. Individual data points (= number of eyes examined; one eye per mouse) represent the replicate average for each mouse to illustrate animal-to-animal variation. Specific stimulation of mitochondria with a protonophore again resulted smaller ELM-RPE in light-adapted S6 mice compared to that in B6 mice, in agreement with the light-differences in (a). In all graphs, horizontal range bar indicates the region with significant differences ($P < 0.05$); error bars represent 95% confidence intervals.

129S6/SvEvTac mice (consistent with residual mitochondrial reserves) (data not shown).[61] Work is on-going to test the prediction that acidifying the SRS with systemic acetazolamide will produce a dark-like ELM-RPE phenotype in light-adapted mice.

Can OCT measure oxidative stress in subretinal space?

Subretinal oxidative stress is thought to be a pathogenic factor for many blinding diseases, such as age-related macular degeneration, diabetic retinopathy, and retinitis pigmentosa, but until recently was not measured *in vivo* using OCT.[62–66] As noted above, MRI measured an impact of oxidative stress on SRS water mobility suggesting that excessive free radical production prevented the expansion of the ELM-RPE thickness in light-adapted mice. This effect is consistent with the expected acidification by oxidative stress that would be expected to inhibit light–dark SRS hydration changes (see above).[54,67,68] Thus, we asked if OCT could also detect oxidative stress in the SRS based on anti-oxidant recovery of absent light/dark ELM-RPE changes, as described in the previous section. Using a pharmacologically induced oxidative stress model,[68] we demonstrated that light–dark differences in outer retina on OCT images are eliminated in the presence of oxidative stress.[67]

After acute administration of antioxidants (i.e. a "quench" of oxidative stress), ELM-RPE thickness is restored to control levels. Therefore, comparing light/dark OCT responses in outer retina with and without antioxidants "quench" demonstrates a new functionality for OCT: measuring oxidative stress. We call this approach QUEch-assiSTed (QUEST) OCT. As OCT systems are commonly available in both clinic and laboratories, QUEST-OCT technique is expected to provide a useful non-invasive and convenient index of oxidative stress in the retina for diagnosis and intervention.

Future opportunities and challenges

As discussed above, to study mitochondria in photoreceptors, extended periods of dark vs. light conditions are usually used to produce SRS hydration changes as measured by OCT. However, it is unclear if these prolonged light/dark conditions are suited for routine clinical use. One potential solution to this problem is to take advantage of FDA-approved pharmaceuticals to more quickly interrogate mitochondrial respiration. In this case, two OCT images can be collected, before (i.e. light adapted) and 1 h after inducing a dark-like condition, either with a low dose of mitochondrial uncoupler or phosphodiesterase 6 inhibitor (e.g. sildenafil, Figure 4(b)).

Shorter light exposure protocols (i.e. ORG) might also be useful to speed up probing of the signaling pathway in Figure 1 but more work is needed to unravel the mechanism underlying the ORG responses. In 2017, Zhang *et al.* reported changes on OCT images of C57BL/6J mice elicited by a brief flashes instead of hours long dark vs. light conditions.[69] With this faster light exposure protocol, they saw a change in IS to RPE (IS-RPE) length with the thickness in light being about 2 μm greater than in the dark and was observed using a non-commercial OCT system. Much smaller light–dark changes in outer retina thickness have been made in human eyes using phase-detection of small optical length changes, submicron light–induced elongation of both rod and cone photoreceptors has been reported.[10,70,71] However, whether these changes directly interrogate photoreceptor function or the photoreceptor support system is currently an open question because the time scales are so different. In addition, a fast (millisecond) but smaller magnitude of photoreceptor shrinking was also reported.[12] Based on optical changes of cultured neurons in response to electrical firing,[72] it has been postulated that these light-stimulus-induced changes reflect optical responses to both electrical and osmotic changes with induced phototransduction.[73] It is hoped that next generation commercial OCT's will be able to address the smaller light–dark difference associated with short light stimuli in order to improve the detection sensitivity/dynamic range to disease and treatment efficacy. In any event, more work is needed to determine if the underlying mechanism (i.e. cGMP → mitochondria → pH → RPE water removal signaling pathway) also explains the IS-RPE changes to brief flashes.

As OCT continues to be considered for studying photoreceptor cell biology in addition to structure, careful attention is needed to variables that had not been previously considered. For example, as shown above, genetic differences in mitochondrial respiration can influence the light–dark ELM-RPE outcomes.[58,67] This problem can be addressed by using each subject as their own control in a paired design. It has also been noted that light–dark ELM-RPE thickness showed diurnal variations.[74] Differences in photoresponses as a function of sex is also possible.

Another key parameter is the duration of dark and light exposure times. Microelectrode studies in excised retina and *in vivo* investigated changes from dark adaptation after 1–5 min of light.[34,37] The first diffusion MRI study examined SRS changes following 2–4 min light dark cycles.[49] In the next study, the diffusion profiles across the retina were compared at 5 and 20 min of light exposure in mice.[52] The light dark difference was noted at both time points but was substantially larger at 20 min.[52] The kinetics of light/dark changes in ELM-RPE thickness was further investigated with OCT in a study by Li *et al.*[56] Compared with ~6 μm changes in ELM-RPE thickness for fully light-adapted (>5 h exposure to room light) and overnight dark-adapted mice, 15 min and 2-h light exposure induced elongation of about 2 and 4 μm in ELM-RPE thickness.[56] Conversely, 15 min and 2-h dark-adaptation produced shortening to nearly the same extent in ELM-RPE thickness.[56] In humans, using a 30-min dark adaptation time and bleaching rhodopsin to different extents over several minutes showed a maximum change in ELM-RPE of about 1.5 μm that depended on the bleaching extent.[11] Overall, it is clear that the size of the light–dark difference in SRS volume is a function of the light exposure period and dark adaptation period. Together these considerations highlight the need to standardize these periods to facilitate clinical acceptance of the light–dark SRS index and to avoid confounding interpretation of the results between groups.

Conclusions

In this review, we described how OCT can measure mitochondria respiration *in vivo* for use in diagnosis and intervention based on measuring the photoreceptor-retinal pigment epithelium (RPE) support network signaling pathway that controls modifiable pro-survival factors within the SRS. Our results raise the possibility of measuring mitochondrial respiration in patients using FDA-approved pharmaceuticals.[75] Overcoming the challenges of performing similar experiments in the clinic will provide a powerful new way to diagnose threats to sight in aging and disease, and improve the success rate when translating treatments from bench-to-bedside.

AUTHORS' CONTRIBUTIONS

All authors participated in the design, interpretation of the studies and review of the manuscript. Both BAB and HQ contributed to the writing of the manuscript.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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REFERENCES

1. Barile GR, Pachydaki SI, Tari SR, Lee SE, Donmoyer CM, Ma W, Rong LL, Buciarelli LG, Wendt T, Horig H, Hudson BI, Qu W, Weinberg AD, Yan SF, Schmidt AM. The RAGE axis in early diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2005;**46**:2916–24
2. Horio N, Clermont AC, Abiko A, Abiko T, Shoelson BD, Bursell SE, Feener EP. Angiotensin at(1) receptor antagonism normalizes retinal blood flow and acetylcholine-induced vasodilatation in normotensive diabetic rats. *Diabetologia* 2004;**47**:113–23
3. Johnsen-Soriano S, Garcia-Pous M, Arnal E, Sancho-Tello M, Garcia-Delpech S, Miranda M, Bosch-Morell F, Az-Llopis M, Navea A, Romero FJ. Early lipoic acid intake protects retina of diabetic mice. *Free Radic Res* 2008;**42**:613–7
4. Midena E, Segato T, Radin S, di Giorgio G, Meneghini F, Piermarocchi S, Belloni AS. Studies on the retina of the diabetic db/db mouse. I. Endothelial cell-pericyte ratio. *Ophthalmic Res* 1989;**21**:106–11
5. Samuels IS, Lee CA, Petrash JM, Peachey NS, Kern TS. Exclusion of aldose reductase as a mediator of ERG deficits in a mouse model of diabetic eye disease. *Vis Neurosci* 2012;**29**:267–74
6. Ball SL, Petry HM. Noninvasive assessment of retinal function in rats using multifocal electroretinography. *Invest Ophthalmol Vis Sci* 2000;**41**:610–7
7. Nusinowitz S, Ridder WH, Heckenlively JR. Rod multifocal electroretinograms in mice. *Invest Ophthalmol Vis Sci* 1999;**40**:2848–58
8. Bissig D, Goebel D, Berkowitz BA. Diminished vision in healthy aging is associated with increased retinal L-Type voltage gated calcium channel ion influx. *PLoS One* 2013;**8**:e56340
9. McGill TJ, Prusky GT, Douglas RM, Yasumura D, Matthes MT, Lowe RJ, Duncan JL, Yang H, Ahern K, Daniello KM, Silver B, LaVail MM. Discordant anatomical, electrophysiological, and visual behavioral profiles of retinal degeneration in rat models of retinal degenerative disease. *Invest Ophthalmol Vis Sci* 2012;**53**:6232–44
10. Azimipour M, Valente D, Vienola KV, Werner JS, Zawadzki RJ, Jonnal RS. Optoretinogram: optical measurement of human cone and rod photoreceptor responses to light. *Opt Lett* 2020;**45**:4658–61
11. Lu CD, Lee B, Schottenhamml J, Maier A, Pugh EN, Fujimoto JG. Photoreceptor layer thickness changes during dark adaptation observed with ultrahigh-resolution optical coherence tomography. *Invest Ophthalmol Vis Sci* 2017;**58**:4632–43
12. Pandiyan VP, Maloney-Bertelli A, Kuchenbecker JA, Boyle KC, Ling T, Chen ZC, Park BH, Roorda A, Palanker D, Sabesan R. The optoretinogram reveals the primary steps of phototransduction in the living human eye. *Sci Adv* 2020;**6**:eabc1124
13. Yao X, Kim TH. Fast intrinsic optical signal correlates with activation phase of phototransduction in retinal photoreceptors. *Exp Biol Med* 2020;**245**:1087–95
14. Ma G, Son T, Kim TH, Yao X. In vivo optoretinography of phototransduction activation and energy metabolism in retinal photoreceptors. *J Biophotonics* 2021;**14**:e202000462
15. Kim TH, Wang B, Lu Y, Son T, Yao X. Functional optical coherence tomography enables in vivo optoretinography of photoreceptor dysfunction due to retinal degeneration. *Biomed Opt Express* 2020;**11**:5306–20
16. Peng Y, Tang L, Zhou Y. Subretinal injection: a review on the novel route of therapeutic delivery for vitreoretinal diseases. *Ophthalmic Res* 2017;**58**:217–26
17. Mieziewska K. The interphotoreceptor matrix, a space in sight. *Microsc Res Tech* 1996;**35**:463–71
18. Ishikawa M, Sawada Y, Yoshitomi T. Structure and function of the interphotoreceptor matrix surrounding retinal photoreceptor cells. *Exp Eye Res* 2015;**133**:3–18
19. Adler AJ, Southwick RE. Distribution of glucose and lactate in the interphotoreceptor matrix. *Ophthalmic Res* 1992;**24**:243–52
20. Uehara F, Yasumura D, LaVail MM. Development of light-evoked changes of the interphotoreceptor matrix in normal and RCS rats with inherited retinal dystrophy. *Exp Eye Res* 1991;**53**:55–60
21. Hewitt AT, Lindsey JD, Carbott D, Adler R. Photoreceptor survival-promoting activity in interphotoreceptor matrix preparations: characterization and partial purification. *Exp Eye Res* 1990;**50**:79–88
22. Porrello K, Yasumura D, La Vail MM. The interphotoreceptor matrix in RCS rats: histochemical analysis and correlation with the rate of retinal degeneration. *Exp Eye Res* 1986;**43**:413–29
23. Gonzalez-Fernandez F. Interphotoreceptor retinoid-binding protein – an old gene for new eyes. *Vision Res* 2003;**43**:3021–36
24. Hodson S, Armstrong I, Wigham C. Regulation of the retinal interphotoreceptor matrix Na by the retinal pigment epithelium during the light response. *Experientia* 1994;**50**:438–41
25. Yokomizo H, Maeda Y, Park K, Clermont AC, Hernandez SL, Fickweiler W, Li Q, Wang CH, Paniagua SM, Simao F, Ishikado A, Sun B, Wu IH, Katagiri S, Pober DM, Tinsley LJ, Avery RL, Feener EP, Kern TS, Keenan HA, Aiello LP, Sun JK, King GL. Retinol binding protein 3 is increased in the retina of patients with diabetes resistant to diabetic retinopathy. *Sci Transl Med* 2019;**11**:eaau6627
26. Uehara F, Matthes MT, Yasumura D, LaVail MM. Light-evoked changes in the interphotoreceptor matrix. *Science* 1990;**248**:1633–6
27. Yamamoto F, Borgula GA, Steinberg RH. Effects of light and darkness on pH outside rod photoreceptors in the cat retina. *Exp Eye Res* 1992;**54**:685–97
28. Padnick-Silver L, Linsenmeier RA. Quantification of in vivo anaerobic metabolism in the normal cat retina through intraretinal pH measurements. *Vis Neurosci* 2002;**19**:793–806
29. Oakley B, Wen R. Extracellular pH in the isolated retina of the toad in darkness and during illumination. *J Physiol* 1989;**419**:353–78
30. Dmitriev AV, Mangel SC. Circadian clock regulation of pH in the rabbit retina. *J Neurosci* 2001;**21**:2897–902
31. Linton JD, Holzhausen LC, Babai N, Song H, Miyagishima KJ, Stearns GW, Lindsay K, Wei J, Chertov AO, Peters TA, Caffe R, Pluk H, Seeliger MW, Tanimoto N, Fong K, Bolton L, Kuok DLT, Sweet IR, Bartoletti TM, Radu RA, Travis GH, Zagotta WN, Townes-Anderson E, Parker E, Van der Zee CEEM, Sampath AP, Sokolov M, Thoreson WB, Hurley JB. Flow of energy in the outer retina in darkness and in light. *Proc Natl Acad Sci U S A* 2010;**107**:8599–604
32. Okawa H, Sampath AP, Laughlin SB, Fain GL. ATP consumption by mammalian rod photoreceptors in darkness and in light. *Curr Biol* 2008;**18**:1917–21
33. Adijanto J, Banzon T, Jalickee S, Wang NS, Miller SS. CO2-induced ion and fluid transport in human retinal pigment epithelium. *J General Physiol* 2009;**133**:603–22
34. Li JD, Govardovskii VI, Steinberg RH. Light-dependent hydration of the space surrounding photoreceptors in the cat retina. *Vis Neurosci* 1994;**11**:743–52
35. Gallemore RP, Li JD, Govardovskii VI, Steinberg RH. Calcium gradients and light-evoked calcium changes outside rods in the intact cat retina. *Vis Neurosci* 1994;**11**:753–61
36. Govardovskii VI, Li JD, Dmitriev AV, Steinberg RH. Mathematical model of TMA+ diffusion and prediction of light-dependent subretinal hydration in chick retina. *Invest Ophthalmol Vis Sci* 1994;**35**:2712–24
37. Li JD, Gallemore RP, Dmitriev A, Steinberg RH. Light-dependent hydration of the space surrounding photoreceptors in chick retina. *Invest Ophthalmol Vis Sci* 1994;**35**:2700–11
38. Huang B, Karwoski CJ. Light-evoked expansion of subretinal space volume in the retina of the frog. *J Neurosci* 1992;**12**:4243–52

39. Wolfensberger TJ, Dmitriev AV, Govardovskii VI. Inhibition of membrane-bound carbonic anhydrase decreases subretinal pH and volume. *Doc Ophthalmol* 1999;**97**:261–71
40. Cao W, Govardovskii V, Li JD, Steinberg RH. Systemic hypoxia dehydrates the space surrounding photoreceptors in the cat retina. *Invest Ophthalmol Vis Sci* 1996;**37**:586–96
41. Yamamoto F, Steinberg RH. Effects of systemic hypoxia on pH outside rod photoreceptors in the cat retina. *Exp Eye Res* 1992;**54**:699–709
42. Nakashima Y, Uehara F, Ohba N. Interphotoreceptor matrix in the colored-light-adapted rat. *Ophthalmic Res* 1992;**24**:308–15
43. Asteriti S, Gargini C, Cangiano L. Mouse rods signal through gap junctions with cones. *Elife* 2014;**3**:e01386
44. Aung MH, Park Hn Han MK, Obertone TS, Abey J, Aseem F, Thule PM, Iuvone PM, Pardue MT. Dopamine deficiency contributes to early visual dysfunction in a rodent model of type 1 diabetes. *J Neurosci* 2014;**34**:726–36
45. Aït-Ali N, Fridlich R, Millet-Puel G, Clérin E, Delalande F, Jaillard C, Blond F, Perrocheau L, Reichman S, Byrne LC, Olivier-Bandini A, Bellalou J, Moyse E, Bouillaud F, Nicol X, Dalkara D, van Dorsselaer A, Sahel JA, Léveillard T. Rod-derived cone viability factor promotes cone survival by stimulating aerobic glycolysis. *Cell* 2015;**161**:817–32
46. Jeon CJ, Strettoi E, Masland RH. The major cell populations of the mouse retina. *J Neurosci* 1998;**18**:8936–46
47. Jin N, Zhang Z, Keung J, Youn SB, Ishibashi M, Tian LM, Marshak DW, Solessio E, Umino Y, Fahrenfort I, Kiyama T, Mao CA, You Y, Wei H, Wu J, Postma F, Paul DL, Massey SC, Ribelayga CP. Molecular and functional architecture of the mouse photoreceptor network. *Sci Adv* 2020;**6**: eaba7232
48. Azimipour M, Migacz JV, Zawadzki RJ, Werner JS, Jonnal RS. Functional retinal imaging using adaptive optics swept-source OCT at 1.6 MHz. *Optica* 2019;**6**:300–3
49. Bissig D, Berkowitz BA. Light-dependent changes in outer retinal water diffusion in rats in vivo. *Mol Vis* 2012;**18**:2561–2 xxx
50. Wang Q, Song SK, Zhang H, Berkowitz BA, Chen S, Wickline SA, Chen J. Photoreceptor degeneration changes magnetic resonance imaging features in a mouse model of retinitis pigmentosa. *Magn Reson Med* 2011;**65**:1793–8
51. Chen J, Wang Q, Zhang H, Yang X, Wang J, Berkowitz BA, Wickline SA, Song SK. In vivo quantification of T1, T2, and apparent diffusion coefficient in the mouse retina at 11.74T. *Magn Reson Med* 2008;**59**:731–8
52. Berkowitz BA, Grady EM, Khetarpal N, Patel A, Roberts R. Oxidative stress and light-evoked responses of the posterior segment in a mouse model of diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2015;**56**:606–15
53. Berkowitz BA, Kern TS, Bissig D, Patel P, Bhatia A, Kefalov VJ, Roberts R. Systemic retinaldehyde treatment corrects retinal oxidative stress, rod dysfunction, and impaired visual performance in diabetic MiceSystemic retinaldehyde treatment in diabetic mice. *Invest Ophthalmol Vis Sci* 2015;**56**:6294–303
54. Mulkey DK, Henderson RA 3rd, Ritucci NA, Putnam RW, Dean JB. Oxidative stress decreases pHi and Na(+)/H(+) exchange and increases excitability of solitary complex neurons from rat brain slices. *Am J Physiol Cell Physiol* 2004;**286**:C940–51
55. Berkowitz BA, Bissig D, Roberts R. MRI of rod cell compartment-specific function in disease and treatment in vivo. *Prog Retin Eye Res* 2016;**51**:90–106
56. Li Y, Fariss RN, Qian JW, Cohen ED, Qian H. Light-Induced thickening of photoreceptor outer segment layer detected by ultra-high resolution OCT imaging. *Invest Ophthalmol Vis Sci* 2016;**57**: 105–11
57. Berkowitz BA, Podolsky RH, Lins Childers K, Saadane A, Kern TS, Roberts R, Olds H, Joy J, Richards C, Rosales T, Schneider M, Schilling B, Orchanian A, Graffice E, Sinan K, Qian H, Harp L. Sildenafil-evoked photoreceptor oxidative stress in vivo is unrelated to impaired visual performance in mice. *PLoS One* 2021;**16**:e0245161
58. Berkowitz BA, Podolsky RH, Qian H, Li Y, Jiang K, Nellissery J, Swaroop A, Roberts R. Mitochondrial respiration in outer retina contributes to Light-Evoked increase in hydration in vivo. *Invest Ophthalmol Vis Sci* 2018;**59**:5957–64
59. Berkowitz BA, Podolsky RH, Lenning J, Khetarpal N, Tran C, Wu JY, Berri AM, Dernay K, Shafie-Khorassani F, Roberts R. Sodium iodate produces a strain-dependent retinal oxidative stress response measured in vivo using QUEST MRI. *Invest Ophthalmol Vis Sci* 2017;**58**:3286–93
60. Kooragayala K, Gotoh N, Cogliati T, Nellissery J, Kaden TR, French S, Balaban R, Li W, Covian R, Swaroop A. Quantification of oxygen consumption in retina ex vivo demonstrates limited reserve capacity of photoreceptor mitochondria. *Invest Ophthalmol Vis Sci* 2015;**56**:8428–36
61. Berkowitz BA, Olds HK, Richards C, Joy J, Rosales T, Podolsky RH, Childers KL, Hubbard WB, Sullivan PG, Gao S, Li Y, Qian H, Roberts R. Novel imaging biomarkers for mapping the impact of mild mitochondrial uncoupling in the outer retina in vivo. *PLos One* 2020;**15**:e0226840
62. Biswal MR, Justis BD, Han P, Li H, Gierhart D, Dorey CK, Lewin AS. Daily zeaxanthin supplementation prevents atrophy of the retinal pigment epithelium (RPE) in a mouse model of mitochondrial oxidative stress. *PLoS One* 2018;**13**:e0203816
63. Bonilha VL, Bell BA, Rayborn ME, Samuels IS, King A, Hollyfield JG, Xie C, Cai H. Absence of DJ-1 causes age-related retinal abnormalities in association with increased oxidative stress. *Free Radic Biol Med* 2017;**104**:226–37
64. Hollyfield JG, Bonilha VL, Rayborn ME, Yang X, Shadrach KG, Lu L, Ufret RL, Salomon RG, Perez VL. Oxidative damage-induced inflammation initiates age-related macular degeneration. *Nat Med* 2008;**14**:194–8
65. Handa JT. How does the macula protect itself from oxidative stress? *Mol Aspects Med* 2012;**33**:418–35
66. Beatty S, Koh HH, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of Age-Related macular degeneration. *Surv Ophthalmol* 2000;**45**:115–34
67. Berkowitz BA, Podolsky RH, Lins-Childers KM, Li Y, Qian H. Outer retinal oxidative stress measured in vivo using QUEnch-assiSTed (QUEST) OCT. *Invest Ophthalmol Vis Sci* 2019;**60**:1566–70
68. Berkowitz BA, Podolsky RH, Farrell B, Lee H, Trepanier C, Berri AM, Dernay K, Graffice E, Shafie-Khorassani F, Kern TS, Roberts R. D-cis-Diltiazem can produce oxidative stress in healthy depolarized rods in vivo. *Invest Ophthalmol Vis Sci* 2018;**59**:2999–3010
69. Zhang P, Zawadzki RJ, Goswami M, Nguyen PT, Yarov-Yarovoy V, Burns ME, Pugh EN. In vivo optophysiology reveals that G-protein activation triggers osmotic swelling and increased light scattering of rod photoreceptors. *Proc Natl Acad Sci U S A* 2017;**114**:E2937–E46
70. Hillmann D, Spahr H, Pfäffle C, Sudkamp H, Franke G, Hüttmann G. In vivo optical imaging of physiological responses to photostimulation in human photoreceptors. *Proc Natl Acad Sci U S A* 2016;**113**:13138–43
71. Zhang F, Kurokawa K, Lassoued A, Crowell JA, Miller DT. Cone photoreceptor classification in the living human eye from photostimulation-induced phase dynamics. *Proc Natl Acad Sci U S A* 2019;**116**:7951–6
72. Ling T, Boyle KC, Zuckerman V, Flores T, Ramakrishnan C, Deisseroth K, Palanker D. High-speed interferometric imaging reveals dynamics of neuronal deformation during the action potential. *Proc Natl Acad Sci U S A* 2020;**117**:10278–85
73. Boyle KC, Chen ZC, Ling T, Pandiyan VP, Kuchenbecker J, Sabesan R, Palanker D. Mechanisms of light-induced deformations in photoreceptors. *Biophys J* 2020;**119**:1481–8
74. Zhang P, Shibata B, Peinado G, Zawadzki RJ, FitzGerald P, Pugh EN. Jr., Measurement of diurnal variation in rod outer segment length in vivo in mice with the OCT optoretinogram. *Invest Ophthalmol Vis Sci* 2020;**61**:9
75. Geisler JG. 2,4 Dinitrophenol as medicine. *Cells* 2019;**8**:280