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The effect of pre-adapting light intensity on dark adaptation in early age-related macular degeneration

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Abstract

Background: This study aimed to identify the pre-adapting light intensity that generated the maximum separation in the parameters of dark adaptation between participants with early age-related macular degeneration (AMD) and healthy control participants in the minimum recording time.

Methods: Cone dark adaptation was monitored in 10 participants with early AMD and 10 age-matched controls after exposure to three pre-adapting light intensities, using an achromatic annulus (12° radius) centred on the fovea. Threshold recovery data were modelled and the time constant of cone recovery (τ), final cone threshold, and time to rod-cone-break (RCB) were determined. The diagnostic potential of these parameters at all pre-adapting intensities was evaluated by constructing receiver operating characteristic (ROC) curves.

Results: There were significant differences between those with early AMD and healthy controls in cone τ and time to RCB (p < 0.05) at all pre-adapting ‘bleaching’ intensities. ROC curves showed that the diagnostic potential of dark adaptometry was high following exposure to all three pre-adapting intensities; generating an area under the curve (AUC) in excess of 0.87 +/- 0.08 for cone τ and time to RCB for all conditions.

Conclusions: Dark adaptation was shown to be highly diagnostic for early AMD across a range of pre-adapting light intensities and therefore the lower pre-adapting intensities evaluated in this study may be used to expedite dark adaptation measurement in the clinic without compromising the integrity of the data obtained. This study reinforces the suggestion that cone and rod dark adaptation are good candidate biomarkers for early AMD.

Key words
early age-related macular degeneration, dark adaptation, diagnostic potential, pre-adapting light intensity, psychophysics
Introduction

Age-related macular degeneration (AMD) is a degenerative disease of the central retina that typically presents in patients over 50 years of age. Characterised by dysfunction of the photoreceptors, retinal pigment epithelium (RPE), Bruch’s membrane and choriocapillaris, it is the leading cause of irreversible vision loss in the developed world [1-4]. Current treatments, such as anti-vascular endothelial growth factor (anti-VEGF) therapy [5, 6], are effective only for the neovascular (wet) form of the disease. Given that the incidence and prevalence of AMD is likely to increase over the coming decades due to increases in life expectancy [7], there is a strong research drive to develop new treatment strategies to target the early stage of the disease, which occurs prior to the development of noticeable vision loss, [8]. In order to expedite the development of new interventions and to monitor treatment outcomes, there is a need for ‘functional biomarkers’ that are sensitive to the very earliest changes in visual function.

Clinically, high contrast visual acuity is commonly used to monitor visual function in AMD. However, abnormalities affecting a range of other aspects of visual function have been shown to precede the loss of visual acuity in patients developing the disease. These include changes to contrast sensitivity [9-11], colour vision [12-13], flicker sensitivity [13-14], temporal thresholds [15-17], microperimetry [11, 18-19], photostress or glare recovery [11, 20-22] and dark adaptation [12-14, 23-26]. When measured alongside these other visual functions, dark adaptation abnormalities have emerged as the most sensitive markers for early AMD [12-14, 23].

Given the high sensitivity of dark adaptation assessment to early AMD, it clearly has potential to be used as a functional biomarker for the condition. In order to optimise this potential, it is useful to identify the characteristics of the stimulus and pre-adapting ‘bleaching’ light which provide maximal discrimination between the healthy retina and a retina with early AMD. In a recent publication, it was determined that an annular stimulus of 12º radius, centred on the fovea, was optimal for dark adaptation assessment in early AMD [26]. At this retinal location the time constant of cone recovery (τ) and the time to the rod-cone break (RCB) were both highly diagnostic for early AMD. In addition to stimulus parameters, the intensity and duration of the pre-adapting ‘bleaching’ light are also known to affect the time course of subsequent dark adaptation [25, 27-32]. The full biphasic dark adaptation function is only evident following exposure to high pre-adapting light intensities.
A reduction in the intensity of the adapting light causes a lateral shift of the dark adaptation function to the left [27-29], i.e. any given threshold is attained more rapidly at lower adapting intensities. Thus the implementation of a low pre-adapting intensity for the measurement of dark adaptation in early AMD is attractive clinically because it reduces the time over which data needs to be recorded. However, it is vital that any reduction in the intensity of the adapting light does not compromise the diagnostic capacity of the threshold recovery data and that sufficient cone threshold points are obtained to allow data to be fitted reliably with an exponential recovery model. Dimitrov et al. (2008) assessed dark adaptation following exposure to a range of pre-adapting intensities in one healthy participant, in order to develop a recording protocol for measuring dark adaptation in participants with early AMD [25]. However, there are currently no published reports examining the effect of pre-adapting intensity on dark adaptation in participants with early AMD.

The current study aimed to identify the intensity of the pre-adapting light that generated the maximum separation in the parameters of dark adaptation between participants with early AMD and healthy controls in the minimum recording time.

**Methods**

Twenty participants took part in the study. Ten participants had a diagnosis of early AMD and were recruited from the Eye Unit at the University Hospital of Wales, Cardiff, and the Eye Clinic at Cardiff University. That is, these participants had one or more soft drusen (>63µm) within the macula and hyperpigmentation and/or hypopigmentation of the retinal pigment epithelium (RPE) in at least one eye, in the absence of any co-existing ocular or fundus abnormality [33]. The diagnosis was confirmed using 37º fundus photographs (Canon CR-DGi Camera) obtained at the baseline examination. Ten age-matched control participants, with a normal retinal appearance in both eyes, were recruited from the Eye Clinic at Cardiff University. Participants recruited to both groups were aged at least 55 years, with a corrected visual acuity of 6/9 or better in the test eye and no history of systemic disease or medication known to affect visual function, or significant media opacity according to the LOCS III scale [34]. Based on data presented in a recent publication [26] which recorded large differences in cone dark adaptation 12° from the fovea between 10 participants with early AMD and 10 control participants, a sample size of 20 participants will allow detection of a difference in mean cone τ of 1.44 minutes and mean time to rod-cone-break of 4.49 minutes with 80% power at the 5% significance level.
All participants provided informed written consent prior to participation. The study was approved by the South East Wales Research Ethics Committee and all procedures adhered to the tenets of the Declaration of Helsinki.

**Apparatus**

Thresholds were recorded in response to a 12° radius amber annulus ($\lambda = 595$nm; $x$, $y$ chromaticity co-ordinates = 0.480, 0.480), 0.5° wide, 200 msec duration, centred on the fovea. The stimulus was presented on a calibrated, high resolution CRT monitor (Iiyama LS 902UT) driven by an 8-bit (nVIDIA Geforce 9) graphics board under software control (Matlab). The luminance output of the monitor was $\gamma$-corrected [35-36] and modified by neutral density filters mounted on the screen to expose the full range of retinal recovery. A 1.2 ND filter was positioned in front of the screen throughout all recordings. As the lower end of the luminance range approached, additional filters could be added to keep the monitor working within its linear range.

A Maxwellian View optical system was used to deliver a series of ‘long duration’ (120 seconds) photopigment bleaches to the central 43.6° of the test eye. An amber filter (LEE filters HT 015 ‘deep straw’) was used to modify the spectral output of the ‘white light’ LED that was used as the source in the Maxwellian viewing system. This modification reduced the scotopic retinal illuminance to ensure that approximately equal bleaches of cone photopigment and rhodopsin were attained at the highest intensity. Table 1 describes the fraction of rod and cone photopigment bleached by the three pre-adapting intensities (where Low Bleach 1 denotes the lowest pre-adapting intensity, Mod Bleach the middle, and High Bleach 3 the highest pre-adapting intensity) [37-38]. The system was calibrated using a photometer (LS-110; Konica Minolta, Osaka, Japan) at the highest bleaching intensity (High Bleach) and additional neutral density filters were positioned in front of the adapting light to attenuate the luminance in order to attain the two lower bleaching intensities: 0.3 ND for Mod Bleach and 0.6 ND for Low Bleach.

Table 1. about here.

**Experimental procedure**
Participants attended the laboratory on two days. Baseline examinations were completed at the start of the first visit. These included patient history, logMAR visual acuity (ETDRS), central visual field screening (C-40, Humphrey Field Analyser), stereoscopic fundus examination, fundus photography (Canon CR-DGi Camera) and LOCS III media opacity grading [34]. Any participants with a central visual field defect or a LOCS III grading of four or more were excluded from the study. Participants were dilated with one drop of 1.0% Tropicamide in each eye prior to dark adaptation (mean pupil diameter after dilation = 7.5mm). The eye selected for testing was the one with early AMD, or the eye with better visual acuity in bilateral early AMD or control participants. The contralateral eye was occluded and refractive correction was worn during the dark adaptation program if required.

All participants were instructed how to use the dark adaptation program and then underwent a 5 minute familiarisation trial. This was extended at the examiner’s discretion, until the participant produced consistent thresholds and was considered competent with the procedure.

The computerised dark adaptation program was based on a psychophysical method that was previously implemented by Jackson et al. (1999) using a modified Humphrey perimeter [39]. Each stimulus was presented for 200 msec, followed by a 600 msec response window and then a randomly determined interstimulus delay of 0.9-2.4 seconds. If the participant responded to the stimulus within the response window, the luminance was reduced by 0.3 log units for the next presentation. Conversely, if the participant responded to the stimulus outside of the response window, or failed to respond at all, the intensity was increased by 0.1 log units on the following presentation. Threshold was recorded when the stimulus first became visible on an ascending staircase.

Threshold was monitored for 30 minutes, in the dark, after exposure to one of the three pre-adapting light intensities, selected at random. Upon termination of the bleach, participants replaced any spectacles, placed their chin on a rest in front of the computer and the dark adaptation program started immediately. Participants were instructed to fixate the centre of the screen, marked by a fixation cross and to indicate perception of the stimulus via the computer keyboard. The investigator verbally encouraged the participant to maintain accurate fixation at regular intervals throughout the test, but fixation was not monitored. At the second session, this procedure was repeated for the remaining two bleaching intensities, separated by a washout period of an hour. The long duration pre-adapting light used was sufficient to
produce an equilibrium bleach [37], which ensured that all individuals reached the same level of photopigment bleach regardless of any small differences in pre-bleach adaptational status.

**Statistical analysis**

The dynamics of visual recovery were determined by fitting an exponential model of dark adaptation to the cone threshold recovery data and a two linear model to any rod threshold recovery data, after McGwin et al. (1999) (Equation 1), on a least squares basis, using Microsoft Excel [40]. An exponential model has previously been shown to provide a suitable approximation of cone photopigment regeneration after near total photopigment bleaches [41].

**Equation 1.**

\[
T(t) = (a + (b \times \exp(-t/\tau))) + (c \times (\max(t - rcb, 0))) + (d \times (\max(t - rrb, 0)))
\]

where \( T \) is the threshold (log cd/m²) at time \( t \) after cessation of the bleach, \( a \) is the final cone threshold, \( b \) is the change in cone threshold from \( t = 0 \), \( \tau \) is the time constant of cone recovery, \( c \) is the slope of the second component of rod recovery, \( \max \) is a logic statement, \( rcb \) denotes the time from bleach offset to the RCB, \( d \) is the slope of the final component of rod recovery and \( rrb \) denotes the time from bleach offset to the transition between the second and final components of rod recovery. Although the RCB was the only aspect of rod recovery assessed during the analysis, rod recovery was modelled in order to identify the time to RCB. For those participants that failed to reach a RCB within 30 minutes, the RCB was given a nominal value of 30 minutes.

The median and interquartile (IQ range) cone \( \tau \), final cone threshold and time to RCB were calculated, before Mann-Whitney U tests were used to make comparisons between early AMD and control groups. The diagnostic potential of the parameters that showed a statistically significant difference between groups was assessed using receiver operating characteristic (ROC) curves, constructed using statistical software (SPSS, Version 16.0).

**Results**

Dark adaptation data were obtained from 10 participants with early AMD and 10 control participants. There were no significant differences in age between early AMD (median age = 73.5 years, IQ range: 66.5-76 years) and control (median age = 74.5 years, IQ range: 72.3-
Similarly, there were no significant differences in logMAR acuity between the test eyes of early AMD and control groups median acuity = 0.1 logMAR (IQ range: 0.05-0.12) 0.01 for early AMD participants and logMAR (IQ range: -0.06-0.13) for control participants (p = 0.481).

Dark adaptation data for a typical control participant recorded following exposure to each of the pre-adapting ‘bleaching’ intensities are shown in Figure 1a. As expected, the RCB occurred progressively later as the intensity of the adapting light increased. Equivalent dark adaptation curves for a typical participant with early AMD are shown in Figure 1b. In comparison to the control data, this participant with early AMD had prolonged cone adaptation, and only displayed a clear RCB within the 30 minute recording period after the lowest intensity pre-adapting light (Low Bleach 4).

Table 2 summarises the mean dark adaptation parameters for the early AMD and control groups. There were significant differences between groups in cone τ and the time to RCB (p < 0.05) at all pre-adapting ‘bleaching’ intensities. This distinct separation in median cone τ and time to RCB between participants with early AMD and control participants is illustrated in Figure 2. In contrast, there were no significant differences in cone final threshold between the two groups at any of the adapting intensities (Table 2 and Figure 2b).

Receiver operating characteristic curves were constructed for all of the dark adaptation parameters that differed significantly on univariate analysis and the area under the curve (AUC) is given in Table 4 to illustrate the diagnostic ability of each parameter. For cone τ, the higher two pre-adapting intensities (Mod and High Bleach) were equally capable of discriminating participants with early AMD from healthy controls, both yielding an AUC of 0.92 +/- 0.07. This was marginally superior to the AUC of 0.87 +/- 0.08 obtained for cone τ at the lowest pre-adapting intensity (Low Bleach), however there were no statistically significant differences in the AUC obtained for cone τ at any of the pre-adapting intensities (z
< 1.96) [42-43]. Similarly, the ROC analysis showed that the time to RCB had a high diagnostic capacity for early AMD at all of the pre-adapting intensities and that there were no significant differences between the AUCs generated for time to RCB at any of the pre-adapting intensities (z < 1.96) [42-43]. Sensitivity and specificity values of between 80 and 100% were obtained for optimal cut-off values of cone τ and time to RCB after all of the photopigment bleaches, further illustrating their diagnostic potential (Table 3).

Table 3. about here.

**Discussion**

These results show that cone τ and time to RCB are highly diagnostic for early AMD over a range of pre-adapting ‘bleaching’ intensities. The pre-adapting conditions used here yielded an AUC in excess of 0.87, and participants with early AMD were discriminated from healthy control participants with sensitivity and specificity of between 80 and 100%. These results provide support for a previous study in which prolonged cone dark adaptation was demonstrated 12° from fixation. This study used a separate cohort of people with early AMD, and reported an area under the ROC curve of 0.99 +/- 0.02 for cone τ and 0.96 +/- 0.04 for time to RCB [26].

These results suggest that, compared to the highest pre-adapting intensity, the lower two pre-adapting intensities (Low and Mod Bleach) may be used to expedite the measurement of cone and rod dark adaptation in the clinic, without compromising the diagnostic value of the data obtained. For example, the optimal cut-off value given by the ROC analysis for time to RCB after High Bleach was 18.67 minutes, compared to just 8.11 minutes after Low Bleach. Despite the potential value of dark adaptation testing, it has not previously been widely used by clinicians. This is likely to be the result of barriers such as the lack of a standardised recording protocol, the significant time recording time required, as well as the availability of a dark environment and the patient co-operation required. The standardised protocol described in this study may be used to measure dark adaptation in a clinically viable timeframe, therefore minimising the demands placed on the patient during testing.

Visual difficulties in low illumination have been identified as a cause of trips and falls in elderly individuals [44] and a recent study reported that cone dark adaptation kinetics become progressively slower throughout adulthood [45]. The findings of current study suggest that
cone dark adaptation kinetics are slower still in participants with early AMD. This indicates that the performance of individuals with early AMD is likely to be impaired during routine visual tasks in which light levels change rapidly, for example the recovery of vision following exposure to oncoming headlights when driving at night.

Cone $\tau$ was shortest for the lowest pre-adapting light intensity (Low Bleach) and became progressively longer as the intensity of the pre-adapting light increased, indicating a slowing of cone photopigment regeneration at higher light intensities in both participants with early AMD and healthy controls. This is consistent with previous literature in which the exponential time constant of cone recovery ($\tau$) has been shown to vary with bleaching intensity and duration in the healthy retina [41, 46-47]. This behaviour is inconsistent with a first-order process, in which recovery of threshold is proportional to the concentration of a particular photochemical. Consequently it has been proposed that recovery of threshold during dark adaptation is a rate-limited process, potentially due to the delivery of 11-cis retinal to the photoreceptor outer segment [46] or the depletion of the pool of 11-cis retinal [47] after exposure to an adapting light source.

The primary interest of this study was to determine the pre-adapting intensity that best distinguished those with early AMD from healthy controls, that is, detecting clinically significant differences, rather than identifying small differences in mean values. Even with a relatively modest sample size ($n = 20$), there was a marked separation of participants with early AMD and controls in the cone recovery and RCB data. Furthermore, cone adaptation, which can be assessed rapidly in the clinic, showed similar diagnostic sensitivity as the time to RCB, a measure influenced by the rate of rod adaptation.

This study has confirmed that cone dark adaptation is a sensitive functional biomarker for early AMD. However, as cross-sectional studies are unable to determine the true diagnostic potential of a biomarker, longitudinal investigations are needed to explore the long-term potential of cone dark adaptation as a biomarker for early AMD in patients that are at risk of developing the disease, but without observable signs of AMD at the time of enrolment.

In conclusion, this study reinforces the suggestion that cone and rod dark adaptation are good candidate biomarkers for early AMD. Dark adaptation was shown to be highly diagnostic for early AMD across a range of pre-adapting light intensities and therefore the lower pre-
adapting intensities evaluated in this study may be used to expedite dark adaptation measurement in the clinic without compromising the integrity of the data obtained.

Acknowledgements
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References


30. Haig C (1941) The course of rod dark adaptation as influenced by the intensity and duration of pre-adaptation to light. J Gen Physiol 24: 735-751.


**Figure legends**

**Figure 1.** Dark adaptation curves recorded after exposure to three different photopigment ‘bleaches’ for a typical control participant (a) and a participant with early AMD (b). For each pre-adapting light intensity the raw data is shown with the best fitting model of dark adaptation given by equation 1 (circles = Low Bleach 1, triangles = Mod Bleach 2, and crosses = High Bleach 3). The Bleach Low 1 data is correctly placed with respect to the y-axis. All other data are displaced upwards by an additional 0.5 log units from the previous (lower intensity bleach) data to aid visualisation.

**Figure 2.** Summary of mean cone τ (a), cone final threshold (b) and time to RCB (c) at each pre-adapting intensity, shown with 95% confidence intervals. Filled symbols represent the early AMD group and open symbols the control group. * indicates those parameters that demonstrate a significant difference between groups.
Table 1. Percentages of cone photopigment [33] and rhodopsin [34] bleached at the three adapting intensities.

<table>
<thead>
<tr>
<th>Bleach</th>
<th>log photopic trolands (duration: 120 seconds)</th>
<th>% cone photopigment bleach</th>
<th>% rhodopsin bleach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleach Low</td>
<td>4.90</td>
<td>71</td>
<td>51</td>
</tr>
<tr>
<td>Bleach Mod</td>
<td>5.20</td>
<td>84</td>
<td>74</td>
</tr>
<tr>
<td>Bleach High</td>
<td>5.50</td>
<td>91</td>
<td>90</td>
</tr>
</tbody>
</table>
**Table 3.** Comparison of mean (standard error) median (IQ range) dark adaptation parameters in control and early AMD groups. **Bleach Low** denotes the lowest pre-adapting light intensity and **Bleach High** denotes the highest pre-adapting light intensity.

(* where there was no RCB within the recording time for an individual, 30 minutes was attributed as the time to RCB)

<table>
<thead>
<tr>
<th>Pre-adapting intensity</th>
<th>Control</th>
<th>Early AMD</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cone τ (mins)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleach Low</td>
<td>0.46 (0.24-0.87)</td>
<td>1.85 (1.05-4.72)</td>
<td>p = 0.004</td>
</tr>
<tr>
<td>Bleach Mod</td>
<td>1.45 (0.96-1.80)</td>
<td>4.78 (3.09-7.24)</td>
<td>p = 0.001</td>
</tr>
<tr>
<td>Bleach High</td>
<td>1.47 (0.96-2.10)</td>
<td>5.33 (3.58-9.56)</td>
<td>p = 0.001</td>
</tr>
<tr>
<td><strong>Final cone threshold (log cd/m²)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleach Low</td>
<td>-1.58 (-1.77- -1.44)</td>
<td>-1.76 (-1.86- -1.64)</td>
<td>p = 0.436</td>
</tr>
<tr>
<td>Bleach Mod</td>
<td>-1.77 (-1.87- -1.74)</td>
<td>-1.76 (-1.87- -1.49)</td>
<td>p = 0.631</td>
</tr>
<tr>
<td>Bleach High</td>
<td>-1.70 (-1.90- -1.57)</td>
<td>-2.03 (-2.08- -1.80)</td>
<td>p = 0.105</td>
</tr>
<tr>
<td>**Time to RCB (mins) ***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleach Low</td>
<td>5.12 (1.79-7.56)</td>
<td>17.08 (10.92-29.02)</td>
<td>p = 0.001</td>
</tr>
<tr>
<td>Bleach Mod</td>
<td>9.68 (7.76-11.43)</td>
<td>28.92 (17.70-30.00)</td>
<td>p = 0.001</td>
</tr>
<tr>
<td>Bleach High</td>
<td>13.36 (10.60-14.60)</td>
<td>30.00 (26.87-30.00)</td>
<td>p = 0.001</td>
</tr>
</tbody>
</table>
Table 4. Sensitivity and specificity of the dark adaptation parameters that differed significantly on univariate analysis, calculated according to the optimal cut-off value given by the ROC curve. **Bleach Low** denotes the lowest pre-adapting light intensity and **Bleach High** denotes the highest pre-adapting light intensity.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC</th>
<th>Optimal cut-off value (mins)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cone τ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleach Low</td>
<td>0.87 +/- 0.08</td>
<td>0.91</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td>Bleach Mod</td>
<td>0.92 +/- 0.07</td>
<td>2.99</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>Bleach High</td>
<td>0.92 +/- 0.07</td>
<td>2.74</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Time to RCB</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Bleach Low</td>
<td>0.94 +/- 0.05</td>
<td>10.39</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>Bleach Mod</td>
<td>0.93 +/- 0.07</td>
<td>13.94</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>Bleach High</td>
<td>0.92 +/- 0.08</td>
<td>18.67</td>
<td>90</td>
<td>100</td>
</tr>
</tbody>
</table>