Special Issue

Slowed Dark Adaptation in Early AMD: Dual Stimulus Reveals Scotopic and Photopic Abnormalities

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Purpose. The recovery of visual sensitivity after a photobleach in early AMD is slowed in rods but cones also may be abnormal. The purpose of this article was to test different stimulus locations to investigate cone function and its relation to rod abnormalities.

Methods. Stimuli were presented at two locations, 3.0° and 5.5°, in the inferior visual field. Post photobleach dark adaptation (DA) curves from 50 early-AMD patients were compared with those from 15 healthy controls of similar age. Curves were characterized in terms of four parameters: ct, cone threshold; α, the transition point from cone to rod function; S2, the slope of the second rod-mediated component; and β, the transition from the second to the third rod-mediated component.

Results. There were strong location effects for the healthy group and the AMD group. Cone threshold was higher for the outer compared with the inner stimulus ($P = 0.001$), S2 was steeper for outer compared with inner ($P < 0.001$), α was shorter for outer ($P = 0.004$), and β was shorter for outer than inner ($P = 0.002$). The high variance in the patient data, particularly for α and β, explained the absence of a group*location interaction in the statistics.

Conclusions. The data provide a novel perspective on abnormal cone- and rod-sensitivity recovery in early dry AMD. The comparison of pairs of DA curves from different locations highlights the involvement of cones in the underlying pathology of AMD. Dynamic measures of visual function are particularly sensitive to early AMD.

Keywords: dark adaptation, rods, cones, dual location

AMD is a major clinical problem. It has been the leading cause of severe visual impairment in older people for many years.[1] The early/dry form of the disease is classified from fundus photographs in terms of fundus abnormalities such as drusen and pigmentary changes.[2–5] This grading system allows an estimate for the risk of progression to more severe disease.[6] Other methods of assessing morphological changes in AMD that rely on more sophisticated imaging technology, such as optical coherence topography (OCT)[7] and fundus autofluorescence (FAF),[8,9] have been described.

None of these approaches, however, provide information on the functional deficit associated with early AMD. There is now a substantive body of evidence to show that the rod photoreceptors are particularly susceptible to the disease. For example, Steinmetz et al.[10] showed scotopic sensitivity abnormalities and prolonged sensitivity recovery after a bleach in patients with compromised Bruch's membrane. Subsequently, in a study using donor eyes, Curcio et al.[11] reported histological evidence that cones survive longer than rods in AMD patients. This observation is amply supported by data obtained using psychophysical methods for measuring sensitivity recovery after a bleach. These show that rod function appears to be selectively impaired in AMD.[12–15]

The recovery of sensitivity after a photobleach is typically presented in terms of log sensitivity recovery against time and is referred to as a dark adaptation (DA) curve. The function has three distinct components, the first mediated by cones and the other two by rods as described below. The photobleach induces profound loss of vision due to the degradation of receptor photopigment. The recovery of sensitivity in total darkness is controlled by a cascade of biochemical reactions that culminate in regenerated pigment being gradually deposited in the photoreceptor outer segments.[16] The DA curve is an accurate bioassay of the time course of this process. Cones improve sensitivity quickly and mediate the initial 5 to 10 minutes of the recovery function.[16] The transition from cone to rod function is called the α point. Following the α point there are two components, referred to as S2 and S3, which have markedly different time constants. The transition from S2 to S3 is described in Lamb and Pugh[16] and has been referred to as the β point.[17]

Techniques for measuring DA in AMD provide information about dynamic as opposed to static measures of function. However, in a series of articles, Dimitrov et al.[14,15,18] showed that both dynamic and static aspects of vision may be abnormal in early/dry AMD. These observations indicate that character-

izing the functional deficit in early AMD in terms of only a rod-based scotopic deficit is an oversimplification. Although rod recovery is affected in the very early stages of the disease, cone-based flicker sensitivity can be an effective index of disease severity.[14] This observation is also supported by the recent work of Cocce et al. (2018)[19] who reveal a deficit in cone contrast sensitivity and in other functional measures.

A further complication when linking the structural deficit with scotopic sensitivity abnormalities in early AMD is the fact that there is a well-established, age-related, rod-specific deficit in the older normal eye.[20] Others (e.g., Jackson et al.[21] and Patryas et al.[17]) have confirmed that, on average, the slope of the second rod component is reduced by approximately 0.2 log units/decade. This means that, when using rod function to grade AMD severity, age should ideally be taken into account.

It is thought that impaired sensitivity recovery is due to reduced hydraulic conductivity and compromised diffusion characteristics of Bruch's membrane.[22] Rods are dependent on the RPE for regeneration of photo-activated, or bleached, visual pigment via a complex chain of biochemical reactions called the visual cycle. As is well known, in this pathway, all-*trans* retinol is recycled to 11-*cis* retinal by the RPE.[16] However, recent studies[23] have demonstrated the existence of a second mechanism, the retinal visual cycle, which is much faster and specific to cones. In the retinal visual cycle, all-*trans* retinol is isomerized in glial Müller cells and then oxidized in cones to 11-*cis* retinal, allowing them to adapt to ambient illumination much more rapidly than rods.

Both photoreceptor types are dependent on the choroid for delivery of essential nutrients and removal of toxic waste products. Bruch's membrane is the conduit for this complex active transport system between the choroid and the RPE. The impaired transport of vitamin A, a precursor of rod pigment rhodopsin, almost certainly accounts for slowed DA in some older healthy adults. According to in vitro studies of Bruch's membrane from donor eyes, hydraulic conductivity and diffusional transport decline exponentially with age, resulting in a 10-fold reduction in diffusion in older eyes.[24] The build-up of debris from partially digested outer segment material increases with age, leading to the formation of a lipid barrier impeding transport.[25] In some cases, this metabolic insult results in inflammation and the subsequent death of RPE cells and photoreceptors leading to sight-threatening AMD.

Previously, most measures of dark adaptation have been obtained from one location in a single session so that only a small region of the retina is tested. In diseases such as AMD, specific regions of the retina appear to be particularly susceptible to impaired rod recovery. Indeed, Owsley et al.[12] showed regional deficiencies that were most severe at 2° to 4° from the fovea and decreased with increasing eccentricity. More recently, Fraser et al.[26] tested AMD patients at 4°, 6°, and 12°, on the inferior and superior retina, finding the area within the central 6° to be particularly affected.

The location of the test field is important when investigating the extent of rod and cone defects because of the marked spatial inhomogeneities in the distribution of the two photoreceptor types. Owsley et al.[27] used a 1.7°-diameter target located at 12° eccentricity. They reported virtually no cone involvement according to cone time constant and cone threshold. This may be because there are few cones (approximately 1500) compared with rods in such a small retinal region at 12°.

On the other hand, Dimitrov et al.[14] reported marked impairment in both rod and cone function in a population of 293 patients with a wide spectrum of severity of AMD. They used foveal targets to test both dynamic and static aspects of cone sensitivity. A main finding was that steady-state cone threshold matched the severity of early AMD according to fundus photograph-based drusen classification, suggesting that such tests may be an effective tool for monitoring disease progression. It is clear from their data that patients with early-stage AMD have a conspicuous cone deficit, but that identifying this defect is critically dependent on the location of the stimulus.

The aims of the present study were 2-fold. First, we wanted to establish the extent to which abnormal sensitivity recovery in early dry AMD varies with retinal region by simultaneously testing two retinal locations. A second aim was to understand the underlying pathophysiology in more detail, concentrating in particular on abnormalities in α, mediated mainly by cones, and S2, mediated exclusively by rods. To achieve these aims, we have used a technique, described in Tahir et al.,[28] in which two DA curves are obtained concurrently from a pair of stimuli using a single bleach.

Methods

The apparatus and procedure are described in detail in Tahir et al.[28] The following is a brief outline.

Setup

The setup is illustrated in Figure 1. Arc-shaped white (1931 CIE $x = 0.31$, $y = 0.316$) stimuli were presented on a black background on a calibrated high-resolution cathode ray tube (CRT) monitor (GDM-F500R; Sony, Tokyo, Japan). The stimuli were segments of annuli located at 3.0° and 5.5° eccentricity, as described in Tahir et al.[28] A ViSaGe stimulus generator (Cambridge Research Systems, Rochester, UK) and the Visual Psychophysics Engine software, written by one of the authors (NRAP), was used. The dynamic range of the CRT was expanded using a technique previously described.[17,18] The left half of the screen was covered with a 1.2 log unit neutral density (ND) filter, and the right half with 3.6 log ND filter. The fixation/stimulus ensemble initially appeared in the left

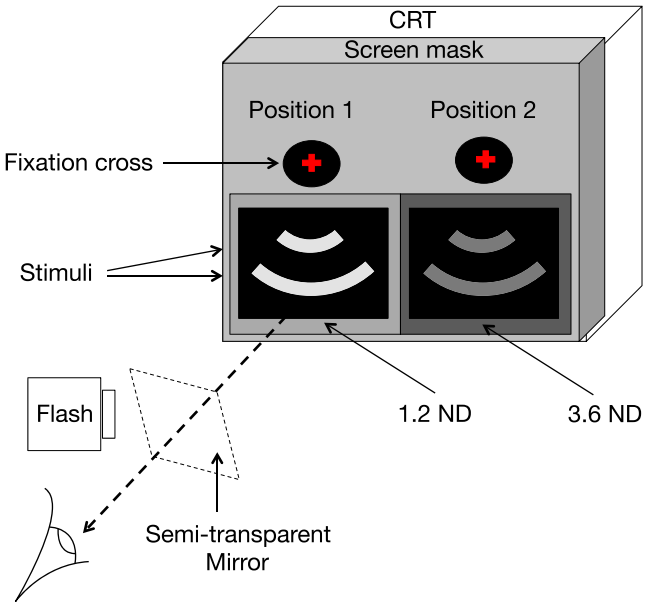

Figure 1. Experimental setup. Observers initially fixated the *red cross* at position 1. When sensitivity increased so that the intensity was below −2.5 log Scotopic cd.m^{-2}, the red fixation light and the stimuli moved to position 2. Before each experimental session the flash unit delivered a selective bleach to the area of the retina corresponding to the stimulus. The foveal region was not bleached.

Table 1. Distribution of the Study Population ($n = 65$) in Groups Corresponding to AREDS Grade

AREDS Grade	*n*	Age (SD)	Sex	Pupil Size, mm (SD)	Bleach %, Minimum–Maximum
Healthy					
0	8	67.2 (9.13)	7M:1F	5.47 (0.70)	~84%–99%
1	7		0M:7F		
AMD					
2	22	73.8 (7.29)	19M:3F	5.32 (0.53)	~70%–99%
3	28		0M:28F		
P		0.32		0.9	

P values for comparisons between healthy individuals and AMD patients.

window (position 1 in Fig. 1) and for the first part of the experiment the observer responded to the stimuli presented in this position. When sensitivity increased such that stimulus intensity was below −2.5 log scotopic cd.m^{-2}, the fixation and stimuli shifted rightward and the observer responded to stimuli in position 2. This technique allows a total filtered luminance range of approximately 6.5 log units (0.8 to −5.7 log scotopic cd.m^{-2}). Note that approximately 2.5 times more rods are stimulated by the outer (5.5°) arc than the inner (3.0°) arc. See Figure 4 in Tahir et al.[28] for receptor count corresponding to the two stimuli.

A localized retinal bleach was delivered through a pellicle (see Fig. 1) using a calibrated photographic flash (Speedlight SB800; Nikon, Tokyo, Japan) of 6 ms duration. The integrated intensity of the flashgun was set at either 5.91 or 6.08 $\log_{10}$ cd.s.m^{-2}, which, depending on the pupil size, bleached the photopigment by 70% to 99%.[29] The retinal region of the bleach was 9° × 9° and thereby included that part of the retina corresponding to the stimuli. The bleach was delivered through the pellicle, so as to expose the correct area of the retina to the bleach without including the fovea.

Observers

Fifteen healthy observers (mean age 67.2 ± 9.13) and 50 individuals with early AMD (mean age 73.8 ± 7.29) were recruited for the study. Their fundus status was determined using digital fundus photography (TRC50DX Non-Mydriatic Retinal Camera; Topcon, Tokyo, Japan). The images were assessed by two of the authors (TA and ER-D). The level of early-stage disease was scored according to the Age-Related Eye Disease Study Research Group (AREDS)[5,30] and observers who were AREDS grade 2 to 3 were assigned to the patient group. See Table 1 for details.

All observers had undergone an eye examination in the 12 months before being included in the study and all healthy subjects were free from any ocular disease (e.g., glaucoma, AMD, cataract). Pupils were measured under the experimental (scotopic) light levels; a UV source with a surrounding magnifier was used to illuminate the pupil so that it could be measured with a conventional millimeter ruler. In some control participants ($n = 5$), pupils were larger than 5 mm in total darkness (just before the bleach) so that in these cases pupil dilation was regarded as unnecessary. All AMD subjects were dilated using 1% Tropicamide. The older healthy group mean pupil size was 5.47 (±0.7) mm. The AMD group had a mean pupil size of 5.32 (±0.53) mm and this was not significantly different from the older healthy group ($t = -0.128$, $P = 0.9$).

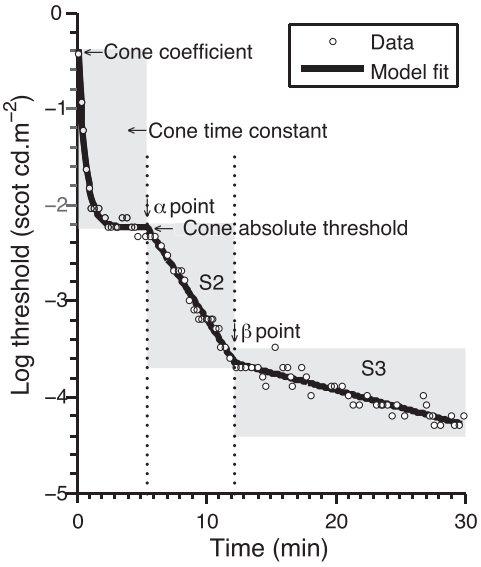

Figure 2. Typical DA curve for a young, healthy observer measured inferiorly at 5.5° eccentricity, following a bleach of 97%. *Grayed areas* show the cone-dominated recovery phase and the S2 and S3 phases. S2 = −0.26 log10 scot cd.m^{-2}.min^{-1}, α = 5.39 minutes and β = 12.24 minutes.

Procedure

Recovery of sensitivity to the stimuli was measured by presenting them alternately, beginning with the inner, detected typically within the first 20 to 40 seconds after the bleach. Thresholds were obtained using a modified method of adjustment (start point 0.8 log scotopic cd.m^{-2}, step size 0.05 log units) described in detail elsewhere.[28] The observers pressed the up and down keys on a computer keyboard to adjust stimulus luminance. When the threshold for the inner target was indicated (by space bar press) the outer target was immediately presented at a below-threshold luminance and threshold for this was determined by the observer by slowly increasing luminance until the target was detected. Testing continued in this nonseeing to seeing strategy, alternately presenting the outer and inner stimuli, for 40 minutes.

If no β point was reached, the test was continued for a further 20 minutes. A defined β point required at least 4 to 5 measurements of the S3 phase. This meant we could assign patients to either a standard or slow recovery group. As we did not test to absolute threshold sensitivity, the true value of S3 was not determined and S3 is not used in the analysis. Note that, in nine of the AMD subjects, testing continued until 60 minutes with no β point being achieved.

Data Analysis

DA curves were obtained for all observers. Example data from a young healthy observer are presented in Figure 2. For the overall analysis, a seven-parameter model was used.[17] The parameters were optimized with nonlinear regression[31] using a MATLAB (MATLAB 2013a; The MathWorks, Inc., Natick, MA, USA) script. The script uses a multistart algorithm and the Nelder Mead[32] simplex method to calculate minima for the nonlinear response function. In nine cases, curves were best described with a five-parameter model.

The seven-parameter model used in the description of the data is as follows.

$$Thres(t) = \theta_1 + \theta_2.\exp\left(-\frac{t}{\theta_3}\right) + \theta_4.h(t,\theta_5) + \theta_6.h(t,\theta_7) \quad (1)$$

$$h(t,\theta) = \begin{cases} 0, t-\theta \le 0 \\ t-\theta, t-\theta > 0 \end{cases},$$

where

Thres (t) = threshold sensitivity as a function of time (t)
θ_1 = cone threshold ($\log_{10}$ cd.m^{-2})
θ_2 = constant derived from the initial cone sensitivity ($\log_{10}$ cd.m^{-2})
θ_3 = cone time constant (minutes)
θ_4 = S2 slope ($\log_{10}$ cd.m^{-2}.min^{-1})
θ_5 = α-point (minutes)
θ_6 = S3 slope ($\log_{10}$ cd.m^{-2}.min^{-1})
θ_7 = β-point (minutes)
h(t, θ) is a step function

As stated above, the standard duration of the test for the patient group was 40 minutes. If a β point was not reached after this time, the test continued until approximately 60 minutes had expired. Hence, 27 patients composed the standard group and the remaining 23 were assigned to the slower group.

Data for the four parameters included in the analysis were checked for normality using the Kolmogorov-Smirnov test. All were normally distributed apart from α and these were normalized where appropriate using a square root transformation. Parametric (repeated measures and multivariate ANOVA) tests were used to model the effects of Location and Group. A correlation analysis was used, controlling for the effect of age, to investigate the association between α and S2 (IBM SPSS Statistics for Mac, version 20.0; IBM Corp, Armonk, NY, USA).

Consent and Ethics

Informed consent was obtained from all participants after the nature of the investigation was explained. The tenets of the Declaration of Helsinki were followed. This study was approved by the South Manchester Research Ethics Committee (ref: 12/NW/0546).

Results

Overview of Main Effects

A multivariate ANOVA, with the four DA parameters as dependent variables and Group and Location as factors, was conducted. The results of this analysis are presented in Table 2. There are strong effects for Location and Group with a small interactive effect that does not reach statistical significance. Note that Pillai's trace is regarded as relatively robust to departures from assumptions and appropriate where there are different cell sizes. Its range is from 0 to 1 with larger values indicating bigger contribution to the model (see Ref. 32).

Analysis of main effects, showing how the effect of Location is divided between the different dependent variables, is presented in Table 3. The results show strong effects for all four variables.

Note that, as the variances for the patients are substantially higher than for the healthy observers, particularly in the case of alpha and beta, there is no statistical interaction between Group and Location factors. Essentially this means that the difference between parameters obtained from the inner and outer stimuli are similar for the two groups. This point is further amplified in the Discussion.

Table 2. Multivariate ANOVA Based on $n = 56$, 15 Healthy Individuals, 41 AMD Patients

Effect	Pillai's Trace	*F*	df	*P*
Location	0.62	20.67	4,51	<0.001*
Group	0.46	10.88	4,51	<0.001*
Location*Group	0.08	1.14	4,51	0.35

* Statistical significance.

In Figure 3, we present box plots for the two groups comparing performance obtained from the inner and outer stimulus for the four main DA parameters. It is apparent that there is no one characteristic feature to represent abnormal DA in early AMD. Where differences between parameters are statistically significant, there is a horizontal bracket between the measurements accompanied by an asterisk and *P* values for the Group effect. Apart from cone threshold, all parameters are grossly abnormal. When comparing data from the two groups, it is apparent that the most notable difference between them is the higher variance in the AMD group.

The sensitivity recovery functions obtained from the two stimuli for the 50 AMD patients are illustrated in Figure 4. For

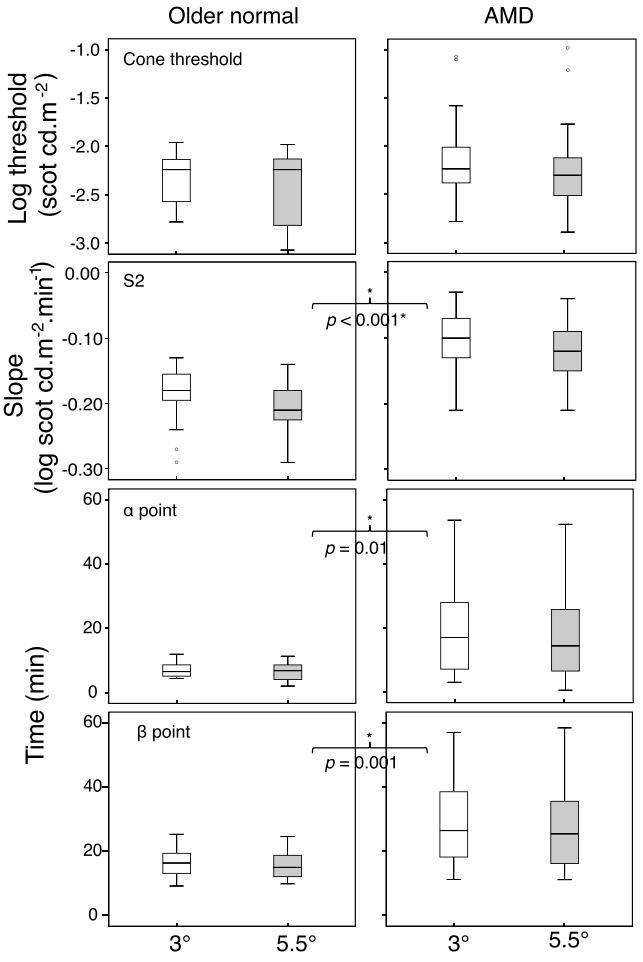

Figure 3. *Box plots* of the four DA parameters for controls (*left*) and AMD (*right*) groups. Locations indicated at the bottom of the plots. In each box, the *central line* is the median and the *edges* of the box are the interquartile range. Comparisons between groups (indicated by a *thin horizontal bracket*) are marked with an *asterisk* if statistically significant.

Table 3. Location Effect Comparisons (Bonferroni Corrected)

			3.0°		5.5°		
Parameter	**Group**	***n***	**Mean**	**SD**	**Mean**	**SD**	***P***
Cone threshold, $\log_{10}$ cd.m^{-2}	Older healthy	15	−2.34	0.28	−2.48	0.38	0.001*
	AMD	50	−2.17	0.35	−2.28	0.38	
S2, $\log_{10}$ scotopic cd.m^{-2}.min^{-1}	Older healthy	15	−0.18	0.05	−0.21	0.05	<0.001*
	AMD	50	−0.10	0.05	−0.12	0.04	
α point, min	Older healthy	15	6.93	2.27	6.47	2.8	0.004*
	AMD	50	18.79	13.95	17.09	12.93	
β point, min	Older healthy	15	16.53	4.48	15.58	4.48	0.002*
	AMD	41	29.5	13.7	27.55	12.78	

Note that $n = 41$, as β was absent in nine patient cases.
* Statistically significant effect of location for both groups.

illustration purposes, the data derived from each stimulus are divided into standard ($n = 27$) and slower ($n = 23$) duration according to the criteria set out in the Methods section. The left-hand panel indicates recovery functions obtained from the inner stimulus and the right-hand panel from the outer stimulus. It is interesting to note that delayed α did not necessarily give rise to extremely shallow S2, suggesting that these two parameters are controlled by different mechanisms. This issue is further explored below.

Note that, of the 27 patients for whom the test was completed in 40 minutes, 17 were AREDS 2 and 10 were AREDS 3. For those who did the 60-minute test, 5 were AREDS 2 and 18 were AREDS 3. Although not a clear-cut distinction, this suggests that the patients needing the longer-duration test had more advanced disease.

It is quite clear from Figure 4 that those patients completing the slower test had substantially poorer overall sensitivity for both inner and outer stimuli. There is also evidence for much longer α times and higher cone thresholds for these patients. Again, this is confirmed for both locations. Note that the slope of S2, the second rod-mediated recovery function, is not very different for the long and standard duration group. The main difference between the standard and slower groups, apart from overall sensitivity, is the α point.

In Figure 5, we provide example data to illustrate the many ways in which abnormalities in sensitivity recovery are manifest in AMD. The DA curves for patient PH (upper left plot) have slightly shallower S2 slopes than normal (dashed lines). However, this patient has a nearly 3-minute difference, Δα (α_{inner}-α_{outer}), in α point time between the two locations. The combination of this and the shallow S2 values confirms the patient to have impaired DA.

The upper right plot (DM) illustrates abnormal S2 and much delayed α and β points. Note that in this case, there is only 1 minute between α measured at the two locations. The bottom left plot (JN) is similarly abnormal in showing very shallow S2 values but again with a substantial Δα of 6.4 minutes. In this case, there is an obvious difference between S2 (inner) and S2 (outer). The bottom right plot (DP) is an example of a patient with prominently delayed α points at both locations (particularly for the inner location), shallow S2 values, and an absent β point 60 minutes after the photobleach. Note that in this case the data were fitted with the constrained five-parameter version of the model.

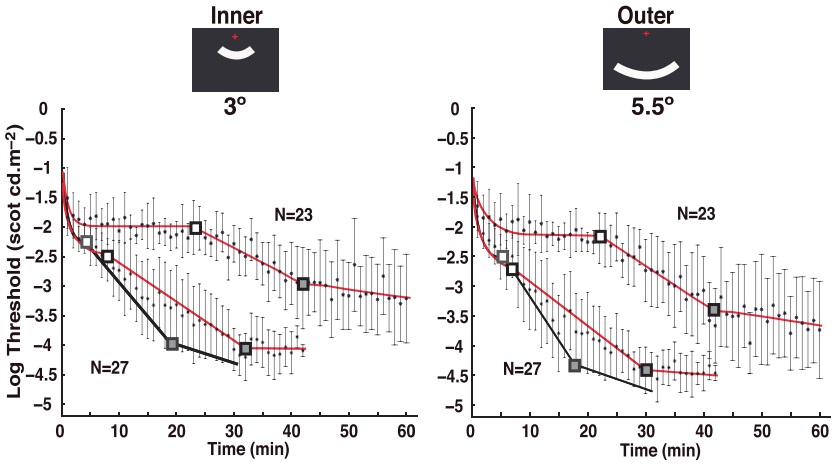

Figure 4. DA curves for the 50 patients. *Left*, DA curves for the inner stimulus. *Right*, DA curves for the outer stimulus. *Data points* are means and *error bars* are SDs. *Open squares* indicate α and *gray squares* indicate β. One-minute bins have been used for the *x*-axis for clarity. In each panel, the curves are divided into standard test time ($n = 27$; lower curve) and slower test time ($n = 23$; upper curve). *Black lines* represent the data of older healthy individuals without error bars for clarity.

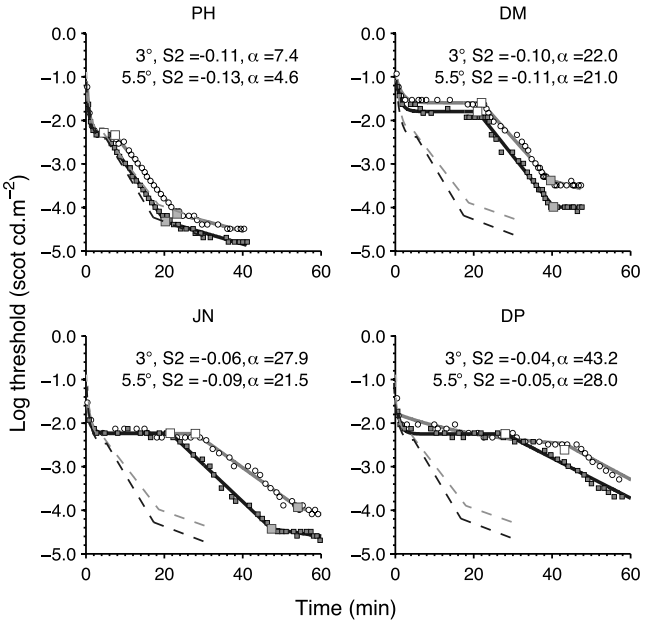

FIGURE 5. Example data for four AMD patients. *Black lines* represent curves obtained from the outer stimulus and *gray lines* are from the inner stimulus. α points are indicated by *white squares* and β points by *gray squares*. Model estimates for the S2 and α time for each curve fit are given for the individual patients. The average population-based model fits for the healthy group for outer and inner stimuli are shown in the *gray* and *black dashed lines*, respectively.

The Effect of Location

In Figure 6, we present the box plots for Δα and Δβ (defined above) for the two locations to show their distribution across the population. For the healthy population, values of α are longer for the inner compared with outer stimulus, as might be expected, so Δα is >0. This holds for the patient group. This effect also applies to β. Again, β is longer for the inner stimulus but there is much more variance in the patient data. In fact, the means and medians are not very different between groups, which explains why the statistics did not detect an interaction effect between Group and Location.

As described in the next section, this observation highlights one of the major benefits of testing two locations in the same session. The difference in alpha values, Δα, for the two locations appears to be of particular significance. Note that mean Δα for the healthy observers was 28 seconds, whereas for the patients it was 1.8 minutes. This is likely to be representative of the population of dry AMD patients. It will be important for accurately identifying abnormalities in an individual patient.

Association Between S2 and α

Although the multivariate ANOVA indicates which parameters reach statistical significance, it cannot tell us the extent to which individual variables account for the overall effect. As illustrated in Figures 4 and 5, α may be grossly abnormal when S2 is close to normal and vice versa, thus giving the impression that S2, the slope of the second rod component and α, the time to rod-cone break, are mediated by different mechanisms.

Of the four parameters investigated, S2 and α are most likely to reveal differences between photopic and scotopic mechanisms. We therefore conducted a correlation analysis between these parameters, correcting for age because of the well-documented age effect in healthy individuals, particularly in S2.

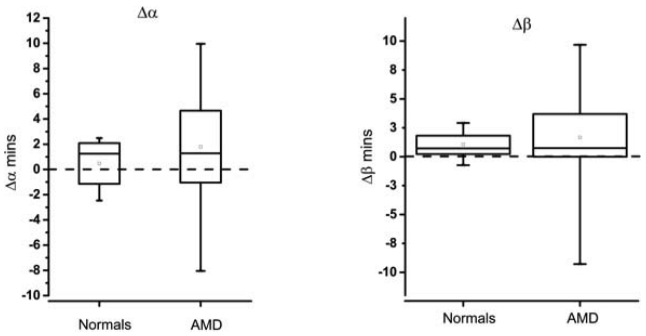

FIGURE 6. *Box plots* illustrating Δα (*left*) and Δβ (*right*) for the two groups and inner and outer stimuli.

There was a weak nonsignificant association between α and S2 for the inner stimulus (Pearson $r = 0.25$, $P = 0.078$) and a stronger association for the outer stimulus (Pearson $r = 0.44$, $P = 0.005$). As discussed more fully below, the implication of these findings is that recording sensitivity functions from an arc centered on 3° from the fovea appears to bias the DA curve in favor of cones, as might be expected from the configuration of the stimuli and the starkly heterogeneous distribution of photoreceptors in this region.

DISCUSSION

We investigated sensitivity recovery following a bleach in healthy older observers and patients with early AMD. DA curves, recorded within a single measurement session from two retinal locations, are described. We find that, although rod-mediated parameters were profoundly abnormal in the AMD group, many patients also exhibited substantively longer values of α, the inflexion point between cone- and rod-mediated portions of the DA curve. For the inner stimulus, there was a weak nonsignificant association between α, representing mainly cone dynamics, and S2, the second rod-mediated parameter. This confirms that the underlying deficit in early AMD is not confined to sensitivity recovery of rods.

Dual Stimuli

As described in Tahir et al.,[28] the stimuli were designed to excite approximately equal numbers of cones but, due to their location, they activate a different mix of rods and cones, with the outer stimulus stimulating approximately 2.5 times more rods than the inner. In that article, the sensitivity recovery functions and statistical analysis obtained with young and older healthy observers showed that the two stimuli were matched photopically but not scotopically. This is exactly what would be expected given the location and configuration of the stimuli. Importantly, the recovery curves are obtained using the same bleach.

As indicated above, almost half of our patient group (23/50), exhibited particularly long α times from both inner and outer stimuli. This meant that they did not reach the β point before 40 minutes after the bleach. Using this criterion (described in Methods), the patient group readily divided into two distinct groups, as illustrated in Figure 4. From the AREDs grading, those with extended α appear to have more advanced disease. This is supported by the fact that, in addition to delayed α, they have reduced sensitivity. They do not, however, have conspicuously shallower values of S2 than the short-duration group, as might be expected if the severity of the underlying pathology were reflected entirely in slowed rod recovery. From inspection of Figure 4, the slope of S2 is not

radically different between the long-duration and short-duration groups, and this is particularly evident for the inner stimulus.

Diversity of Sensitivity Recovery Functions

The statistics and even the figures hide the true structure of the data because of the diversity of DA curves obtained from the patient group. We have included Figure 5 to emphasize this point. Overall, it is clear that slowed sensitivity recovery is attributable to extended α and shallow S2. But what of the linkage between the two? Such is the heterogeneity in impaired dynamics that it could be argued that each of the examples in Figure 5 could represent a different category of defect. For example, S2 may be shallow, indicating abnormal rod recovery, but α may be close to normal for the inner stimulus and abnormal for the outer, suggesting localized cone abnormalities (see patient PH). On the other hand, both S2 and α may be abnormal but show little difference for the two stimulus locations (patient DM). Invariably, however, there are substantial differences in α between the two locations, as shown for patients JN and DP.

We can conclude that cone and rod mechanisms are frequently abnormal but that abnormalities in cone recovery are best revealed by comparing α for the two locations. As discussed further below, this approach has the added advantage that it takes account of any age effect because the comparison is being made between two different regions of the same retina.

The Link Between S2 and α

A major finding in the present article is the poor correspondence between α and S2 derived from the inner stimulus. As described in Tahir et al.,[28] slightly fewer than one-third of the photoreceptors stimulated by the inner stimulus are cones, but cones constitute only one-seventh of the photoreceptors excited by the outer stimulus. This would mean that the value of α derived from the inner stimulus will have a much stronger input from cones than that from the outer stimulus. Our analysis of the relative association between α and S2 for the two stimuli strongly supports this as outlined below.

When corrected for age, the Pearson correlation coefficient (r) between α and S2 for the inner stimulus was 0.25, giving an r^2 value of 0.06, indicating that 6% of the variability in S2 can be attributable to the variability in α; however, there is a stronger association between the two variables derived from the outer stimulus. Here $r = 0.44$, giving r^2 of 0.19, indicating that 19% of the variability in S2 is due to variability in α.

Clearly, the two parameters α and S2 are controlled by different mechanisms and the extent to which either rods or cones mediate α will depend on the stimulus conditions, as mentioned in Jackson et al.[21] Where there is substantial input from cone photoreceptors, α can be expected to be dominated by the regeneration rate of cone opsin. Rapid pigment regeneration is crucial to enable cones to maintain sufficient pigment levels in steady bright ambient illumination and to recover sensitivity after being exposed to intense light levels.

Physiological studies have shown that rods and cones use quite different mechanisms to regenerate their photopigment and therefore recover sensitivity after a bleach. Cones recover circulating current within 20 ms, whereas rods take approximately 20 minutes. This much faster response time is due to a rapid, cone-specific pathway that does not involve the RPE.[23] Note that, in the well-known rod-based visual cycle, the all-*trans* retinol released from rod outer segments following a bleach is converted back to 11-*cis* retinal by the RPE.[16,33]

However, cone pigment is regenerated in a separate process called the retinal visual cycle. This much faster process is mediated by Müller cells from where cones can access recycled retinoids.[34] An important feature of the retinal cycle is that it maintains an efficient supply of chromophore without competition from rods, particularly important in retinae that are numerically rod-dominated. In humans, cones constitute only 5% of the total number of photoreceptors (see Lamb[35] for an interesting commentary), so most of the chromophore produced by the RPE is consumed by rods.

Benefits of the Dual-Stimulus Setup

One benefit of the dual-stimulus method is that, under the assumption that, as argued by Tahir et al.,[28] the age effect in healthy observers is probably pan retinal, one part of the retina can act as control for detecting localized abnormalities. This makes the technique robust to the well-known slowing of sensitivity recovery in the normal older eye,[17,28,36] because we find individuals exhibit substantial differences in parameters derived from the two locations. For example, mean $\Delta\alpha$ ($\alpha_{inner} - \alpha_{outer}$) for patients is 1.8 minutes as opposed to 28 seconds for the controls. We might speculate that, apart from age, systemic diseases, such as liver disorders, vitamin A deficiency, and Chron's disease, are likely to induce DA abnormalities that are homogeneous across the retina. Obtaining data from two locations would provide a means of differentiating slow recovery due to these conditions from that due to early dry AMD.

The dual stimulus effectively caters for an obvious gap in the literature; the fact that the stimuli are designed to preferentially activate either photopic-biased or scotopic-biased regions of the retina means the technique is likely to be sensitive to both cone and rod photoreceptor dysfunction, as described by Dimitrov et al.,[14] and to localized abnormalities, as reported by Owsley et al.[12] The arc-shaped configuration allows for the characteristic distribution of photoreceptors. For an arc of a given eccentricity, the ratio of rods to cones is constant, whereas at the same time a large region of retina can be activated. For example, in our inner stimulus, more than 5000 cones are excited in an area of 0.27 mm^2. Note this would not be the case for a circular stimulus of equivalent size because the rod-cone ratio would be different at different regions of the circle due to the marked photoreceptor heterogeneity of the human parafoveal retina.

Although the parameters from the two stimuli cannot be expected to be statistically independent, we wanted to test whether a receiver operator characteristic (ROC) analysis, based on S2 and α, might confer a diagnostic advantage when results from a single stimulus are compared with those from two stimuli. The ROCs were obtained assuming the fundus photograph grading represented the definitive diagnosis. By this criterion, we determined the extent to which α or S2 obtained from either a single or double stimulus predicted the presence of disease. The outcome of these computations was that the area under the curve (AUC) in the ROC for α was 0.76 $\pm$ 0.06, regardless of whether the data were from single stimuli or from a combination of both stimuli. However, for S2 there was a more substantial benefit of combining the stimuli, with the AUC changing from 0.88 $\pm$ 0.04 for individual stimuli to 0.94 $\pm$ 0.03 for the combined stimuli. As part of this analysis, we also measured sensitivity and specificity for measurements made after 10 minutes post-bleach. We found that relying only on this parameter and comparing with the fundus photographic data, the technique yielded sensitivity of 88.0% and specificity of 66.7%.

The location effect observed here, in which the outer stimulus, which activates a relatively higher proportion of rods, gives slightly steeper values for S2, has been reported by

Dimitrov et al.[15] They described steeper S2 in stimuli at 10.0° eccentricity than at 2.5°. We found S2 to be significantly higher between the two testing locations for the healthy subjects and the AMD patients. The AMD subjects were highly abnormal at both locations for all parameters. The kinetics of rod recovery, represented by the mean S2 slopes, at both retinal locations in the normal older eyes (–0.18 ± 0.05, inner and –0.21 ± 0.05 outer) match well with the values measured in previous studies (–0.23 ± 0.04[37]; –0.24[16]; –0.23 ± 0.03[17]).

As illustrated in Figure 5, some patients have a normal α point but shallow S2; others an extended α point and shallow S2. In some cases, there are marked differences in S2 from the inner and outer stimulus; in others, there are minimal differences. In more advanced disease, there are substantial differences in α and S2 for the two locations. The common feature in most early-AMD patients is slowed S2. But equally there is a marked difference between alpha points obtained from the two locations, as mentioned above, with Δα for patients of 1.8 minutes as opposed to 28 seconds for the controls.

Concluding Comments

Photoreceptor dysfunction can act as a marker, warning of pathological changes in the retina,[15,38] and it has strong relevance to the management of early AMD. Although the association between rod function and macular disease is not fully understood, it has been shown that those with AMD have an increased scotopic threshold and a prolonged time course for photopigment regeneration.[13,37,39,40] In many AMD patients, this loss in scotopic sensitivity occurs before any photopic loss can be detected.[14] According to some reports, this change does not have a clear link to changes in photopic sensitivity, either in time course or magnitude.[41] In the present article, we can support this observation and add detailed analysis of the link between photopic and scotopic dynamics by obtaining sensitivity recovery functions from different locations that sample from stimuli activating different relative numbers of rods and cones.

In conclusion, by recording pairs of DA curves from different retinal locations, we provide a novel perspective on the characteristics of photoreceptor dysfunction in early AMD. The technique has many benefits without being burdensome on patients. It provides additional insight into the extent to which cone and rod-sensitivity recovery abnormalities occur, an issue on which there are stark differences in the literature. The data confirm the importance of measuring DA in early AMD and illustrate the value of the technique when assessing future strategies for impeding the progress of the condition.

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References

1. Leibowitz HM, Krueger DE, Maunder LR, et al. The Framingham Eye Study monograph: an ophthalmological and epidemiological study of cataract, glaucoma, diabetic retinopathy, macular degeneration, and visual acuity in a general population of 2631 adults, 1973–1975. *Surv Ophthalmol*. 1980;24:335–610.
2. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Surv Ophthalmol*. 1995;39:367–374.
3. Ferris FL, Davis MD, Clemons TE, et al. A simplified severity scale for age-related macular degeneration: AREDS Report No. 18. *Arch Ophthalmol*. 2005;123:1570–1574.
4. Klein R, Davis MD, Magli YL, Segal P, Klein BE, Hubbard L. The Wisconsin age-related maculopathy grading system. *Ophthalmology*. 1991;98:1128–1134.
5. Age-Related Eye Disease Study Research Group. The Age-Related Eye Disease Study system for classifying age-related macular degeneration from stereoscopic color fundus photographs: the Age-Related Eye Disease Study Report Number 6. *Am J Ophthalmol*. 2001;132:668–681.
6. Davis MD, Gangnon RE, Lee LY, et al. The Age-Related Eye Disease Study severity scale for age-related macular degeneration: AREDS Report No. 17. *Arch Ophthalmol*. 2005;123:1484–1498.
7. Farsiu S, Chiu SJ, O'Connell RV, et al. Quantitative classification of eyes with and without intermediate age-related macular degeneration using optical coherence tomography. *Ophthalmology*. 2014;121:162–172.
8. Bindewald A, Bird AC, Dandekar SS, et al. Classification of fundus autofluorescence patterns in early age-related macular disease. *Invest Ophthalmol Vis Sci*. 2005;46:3309–3314.
9. Delori FC, Fleckner MR, Goger DG, Weiter JJ, Dorey CK. Autofluorescence distribution associated with drusen in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2000;41:496–504.
10. Steinmetz RL, Haimovici R, Jubb C, Fitzke FW, Bird AC. Symptomatic abnormalities of dark adaptation in patients with age-related Bruch's membrane change. *Br J Ophthalmol*. 1993;77:549–554.
11. Curcio CA, Medeiros NE, Millican CL. Photoreceptor loss in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 1996;37:1236–1249.
12. Owsley C, Jackson GR, Cideciyan AV, et al. Psychophysical evidence for rod vulnerability in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2000;41:267–273.
13. Curcio CA, Owsley C, Jackson GR. Spare the rods, save the cones in aging and age-related maculopathy. *Invest Ophthalmol Vis Sci*. 2000;41:2015–2018.
14. Dimitrov PN, Robman LD, Varsamidis M, et al. Relationship between clinical macular changes and retinal function in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2012:5213–5220.
15. Dimitrov PN, Robman LD, Varsamidis M, et al. Visual function tests as potential biomarkers in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2011;52:9457–9469.
16. Lamb TD, Pugh EN Jr. Dark adaptation and the retinoid cycle of vision. *Prog Retin Eye Res*. 2004;23:307–380.
17. Patryas L, Parry NR, Carden D, et al. Assessment of age changes and repeatability for computer-based rod dark adaptation. *Graefes Arch Clin Exp Ophthalmol*. 2013;251:1821–1827.
18. Dimitrov PN, Guymer RH, Zele AJ, Anderson AJ, Vingrys AJ. Measuring rod and cone dynamics in age-related maculopathy. *Invest Ophthalmol Vis Sci*. 2008;49:55–65.
19. Cocce KJ, Stinnett SS, Luhmann UFO, et al. Visual function metrics in early and intermediate dry age-related macular degeneration for use as clinical trial endpoints. *Am J Ophthalmol*. 2018;189:127–138.

20. McFarland RA, Fisher MB. Alterations in dark adaptation as a function of age. *J Gerontol*. 1955;10:424–428.
21. Jackson GR, Owsley C, McGwin G Jr. Aging and dark adaptation. *Vision Res*. 1999:3975–3982.
22. Bird AC. Bruch's membrane change with age. *Br J Ophthalmol*. 1992;76:166–168.
23. Wang JS, Kefalov VJ. The cone-specific visual cycle. *Prog Retin Eye Res*. 2011;30:115–128.
24. Lee Y, Hussain AA, Seok JH, Kim SH, Marshall J. Modulating the transport characteristics of Bruch's membrane with steroidal glycosides and its relevance to age-related macular degeneration (AMD). *Invest Ophthalmol Vis Sci*. 2015;56: 8403–8418.
25. Holz FG, Sheraidah G, Pauleikhoff D, Bird AC. Analysis of lipid deposits extracted from human macular and peripheral Bruch's membrane. *Arch Ophthalmol*. 1994;112:402–406.
26. Fraser RG, Tan R, Ayton LN, Caruso E, Guymer RH, Luu CD. Assessment of retinotopic rod photoreceptor function using a dark-adapted chromatic perimeter in intermediate age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2016;57: 5436–5442.
27. Owsley C, McGwin G Jr, Jackson GR, Kallies K, Clark M. Cone- and rod-mediated dark adaptation impairment in age-related maculopathy. *Ophthalmology*. 2007;114:1728–1735.
28. Tahir HJ, Rodrigo-Diaz E, Parry NR, Kelly JM, Carden D, Murray IJ. Slowed dark adaptation in older eyes; effect of location. *Exp Eye Res*. 2017;155:47–53.
29. Rushton WA, Powell DS. The rhodopsin content and the visual threshold of human rods. *Vision Res*. 1972;12:1073–1081.
30. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch Ophthalmol*. 2001;119:1417–1436.
31. McGwin G Jr, Jackson GR, Owsley C. Using nonlinear regression to estimate parameters of dark adaptation. *Behav Res Methods Instrum Comput*. 1999;31:712–717.
32. Nelder JA, Mead R. A simplex method for function minimization. *The Computer Journal*. 1965;7:308–313.
33. Saari JC. Vitamin A metabolism in rod and cone visual cycles. *Annu Rev Nutr*. 2012;32:125–145.
34. Kaylor JJ, Yuan Q, Cook J, et al. Identification of DES1 as a vitamin A isomerase in Muller glial cells of the retina. *Nat Chem Biol*. 2013;9:30–36.
35. Lamb TD. Why rods and cones? *Eye (Lond)*. 2016;30:179–185.
36. Jackson GR, Owsley C, Cordle EP, Finley CD. Aging and scotopic sensitivity. *Vision Res*. 1998;38:3655–3662.
37. Owsley C, Jackson GR, White M, Feist R, Edwards D. Delays in rod-mediated dark adaptation in early age-related maculopathy. *Ophthalmology*. 2001;108:1196–1202.
38. Owsley C, McGwin G Jr, Clark ME, et al. Delayed rod-mediated dark adaptation is a functional biomarker for incident early age-related macular degeneration. *Ophthalmology*. 2015;123:344–351.
39. Haimovici R, Owens SL, Fitzke FW, Bird AC. Dark adaptation in age-related macular degeneration: relationship to the fellow eye. *Graefes Arch Clin Exp Ophthalmol*. 2002;240:90–95.
40. Scholl HP, Bellmann C, Dandekar SS, Bird AC, Fitzke FW. Photopic and scotopic fine matrix mapping of retinal areas of increased fundus autofluorescence in patients with age-related maculopathy. *Invest Ophthalmol Vis Sci*. 2004;45: 574–583.
41. Jackson GR, Owsley C, Curcio CA. Photoreceptor degeneration and dysfunction in aging and age-related maculopathy. *Ageing Res Rev*. 2002;1:381–396.

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