Measuring Rod and Cone Dynamics in Age-Related Maculopathy

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PURPOSE. A cathode-ray-tube (CRT) monitor-based technique was used to isolate clinically significant components of dark adaptation. The utility of the technique in identifying adaptation abnormalities in eyes with age-related maculopathy (ARM) is described.

METHODS. A CRT dark-adaptometer was developed to assess cone and rod recovery after photopigment bleach. The following measures were obtained: cone recovery rate ($R_c$; in decades per minute) and absolute threshold ($T_f$; log candelas per square meter), rod recovery rate ($R_r$; decades per minute), and rod–cone transition (rod–cone break [$RCB$] in minutes). These components were isolated by appropriately selecting stimulus size, stimulus location, pigment bleach, and test duration and by coupling the CRT with judiciously selected neutral-density (ND) filters. The protocol was developed by using 5 young observers and was tested on 27 subjects with ARM in the study eye and 22 age-matched control subjects.

RESULTS. The parameters necessary for effective isolation of cone and early phase rod dark adaptation were a 2.6 ND filter (for a standard CRT monitor, 0.08–80 cd·m⁻² luminance output); a 4° foveated, 200-ms, achromatic spot; ~30% pigment bleaching; and a 30-minute test duration. These settings returned obvious rod and cone recovery curves in control and ARM eyes that were compatible with conventional test methods and identified 95% of participants with ARM as having delayed dynamics in at least one of the parameters. Cone recovery dynamics were significantly slower in the ARM group when compared with age-matched control subjects ($R_c$, 0.99 ± 0.35 vs. 2.63 ± 0.61 decades·min⁻¹, P < 0.0001). Three of the 27 eyes with ARM did not achieve $RCB$ during the allowed duration (30 minutes). The remaining eyes with ARM ($n = 24$) exhibited a significant delay in rod recovery ($R_r$, ARM, 0.16 ± 0.05 vs. controls, 0.22 ± 0.02 decades·min⁻¹, P < 0.0001) and the average time to $RCB$ (±SD) in the ARM group was significantly longer than in the control subjects (19.12 ± 5.17 minutes vs. 10.40 ± 2.49 minutes, P < 0.0001).

CONCLUSIONS. The CRT dark-adaptation technique described in this article is an effective test for identifying abnormalities in cone and rod recovery. Slowed cone and rod recovery and a delayed $RCB$ were evident in the eyes with ARM. The test method is potentially useful for clinical intervention trials in which ARM progression is monitored. (Invest Ophthalmol Vis Sci. 2008;49:55–65) DOI:10.1167/iovs.06-1048

Dark adaptation is abnormal in eyes with age-related maculopathy (ARM).1–3 The altered adaptation manifests as delayed rod or cone recovery time and/or an abnormal rod–cone transition (rod–cone break; $RCB$), as well as an elevated cone and rod absolute threshold. Documenting such changes may provide valuable diagnostic and prognostic information for identifying and monitoring patients with ARM. Currently, there is no simple technique that can be used to measure dark adaptation in routine clinical practice. The standard method for measuring dark adaptation can take longer than 30 minutes for complete recovery in normal individuals and more than 1 hour in patients with ARM,1 limiting its clinical usefulness. We developed a simple and reliable technique in which we used CRT technology that tracks dark adaptation for a limited time, yet still yields useful clinical outcomes.

CRT displays are powerful and flexible stimulus generators when calibrated and driven within their operating limits.4,5 and their use is widespread in basic and clinical science. However, application of the CRT to dark adaptation has certain restrictions. First, the lowest achromatic luminance of conventional CRT hardware (RGB) is approximately 0.08 cd·m⁻², which is some 3 to 4 log units above the normal rod absolute threshold. Neutral density (ND) filters can be used to reduce this luminance, however. Second, the entire range of cone and rod recovery spans some 5 to 6 log units,6 whereas a CRT’s output is constrained to ~3 log units.7 Given that the limited luminance range of the CRT will not fully define the entire recovery curve, we overcame this drawback by gradually attenuating the light output of the CRT by using various strength ND filters to expose the entire range of cone and rod recovery. We then investigated whether measuring only a section of the curve could return useful parameters of rod and cone recovery by judiciously selecting a single ND filter that exposes clinically important components, such as the late stages of cone recovery, the $RCB$, and the slope of the second phase of rod adaptation.3

Classic analytic methods, such as the Rushton template for photopigment bleach, have a limited capacity to describe the entire dark-adaptation process.8 However, we show that combining Rushton’s approach with modern theories of adaptation6 can isolate clinically valuable dark adaptation parameters in patients with ARM. As the time for dark adaptation depends on the level of photopigment bleaching,6,9 target size, and location,10,11 we optimized these parameters for the detection of ARM.
METHODS

The study was stratified as follows: First we determined the test parameters (density of the ND filter, stimulus size, and the level of photopigment bleaching). Then, we verified our method by comparing its outcome to the recovery obtained with a conventional Goldmann-Weekers adaptometer. Finally, we established the clinical utility of the method by applying our technique to a sample of participants with ARM and comparing their outcomes with those of age-matched controls.

Participants

The protocol was developed on a single healthy individual aged 40 years of age. Comparison with the established method to the Goldmann-Weekers test was undertaken on five normal observers, aged 26, 35, 36, 39, and 40 years. The clinical utility of the methodology was tested on 27 subjects with ARM (67.5 ± 5.0 [SD] years of age) and 22 age-matched control subjects (66.8 ± 5.9 [SD] years of age). Written informed consent was obtained from all individuals, and ethics approval for this project was provided by the Human Research and Ethics Committee of the Royal Victorian Eye and Ear Hospital, Melbourne (RVEEH). The study was conducted in accordance with the Declaration of Helsinki.

All participants had visual acuity assessment with a logMAR chart\(^1\) after autorefration (Humphrey Automatic Refractometer, model 597; Carl Zeiss Meditec, Inc., San Leandro, CA) with subjective refinement. Ophthalmoscopy, slit-lamp examination and assessment of ocular motility were performed, to exclude confounding ocular diseases in the participants with ARM and any ocular abnormalities in normal subjects. Lens opacities were graded with the Wilmer Cataract Grading System. Persons with significant lens opacity (nuclear, ≥2.0; cortical, ≥0.25; or posterior subcapsular, ≥1 mm\(^2\)) were excluded.

The fundus status was assessed via slit lamp examination with a 78-D lens and digital fundus photography (Canon CR6-45NM Non-paracentral scotoma (significant total deviation [International Pty. Ltd., Vermont, Victoria, Australia). Participants with neurologic diseases, such as Parkinson’s disease, Alzheimer’s disease, diabetes, significant cataract, glaucoma, visual field defects, narrow anterior chamber angles, fundus changes due to diseases other than ARM (e.g., vein occlusion or retinal scar), heterotropia, amblyopia, color vision abnormalities, and uncontrolled systemic hypertension; those taking medication that might compromise vision; and those with neurologic diseases, such as Parkinson’s disease, Alzheimer’s disease, or stroke. All normal participants (5 young subjects in the validation group and 22 aged control subjects) had no visible fundus disease, and visual acuity was better than 20/25 (<0.1 logMAR)\(^1\) in each eye. The study eye in the ARM group (n = 27) had at least one large high-risk druse (≥125 μm) within the inner macular area (3000 μm diameter, centered on the fovea), and visual acuity was better than 20/25 (<0.1 logMAR; Table 1). The nonstudy eye status in the ARM group varied from the presence of intermediate drusen to choroidal neovascularization (four cases) and geographic atrophy (one case), and the visual acuity of those eyes differed accordingly (Table 1).

Apparatus and Stimuli

Stimuli were presented on a calibrated\(^4\) high-resolution CRT monitor (Accuvue HM-7211-D; Hitachi, Tokyo, Japan) powered by an 8-bit video card (Nvidia, Santa Clara, CA, hosted by a Macintosh G4 computer; Apple Computer, Cupertino, CA). The CRT’s luminance output was γ-corrected\(^5\) and modified by using standardized ND filters (Schott AG, Mainz, Germany) mounted in light-tight goggles worn by the observer. In pilot trials, filters were chosen to expose the entire range of rod and cone recovery. A single ND filter was then chosen that could be used to sample a key 3.0 log units of recovery, and this filter was used in all subsequent testing.

We produced Ganzfeld photopigment bleaching ranging from 3% to 99%\(^6\) with a device constructed in our laboratory comprising a light tube (200 mm diameter and 1000 mm long) with a translucent dome positioned inside the tube at the observer’s end and a light source positioned at the other end with a 450-mm separation between the front surface of the light source and the back surface of the dome. The light source was either a photographic flash (Mecablitz 45 CL-I; Metz-Werke GmbH & Co., Zirndorf, Germany) or a halogen light source (12 V, 50 W; Philips Inc., Sydney, NSW, Australia). All light sources were calibrated with an integrating photometer (ILI700; International Light Inc., Newburyport, MA). The purpose for testing the bleaching level was to determine the minimum amount of bleaching needed to provide a fast and reliable clinical test result. In the clinical trial the photographic flash was used to produce a 30% rod bleach (11 ms duration; 6.48 log scotopic trolands/second) and a 10% cone bleach (5.66 log photopic trolands/second) with an 8-mm pupil. Throughout this article, we specify the level of pigment bleaching as the amount of rhodopsin bleached, unless stated otherwise.

Psychophysical Methods

Thresholds were measured for a 0.2-second, foveal, achromatic (1931CIE x = 0.267, y = 0.318) spot of various sizes (1°–6°). Stimuli were generated in the center of the monitor to avoid variations in luminance across the CRT.\(^7\) Subjects were asked to fixate the center of the screen indicated by four 0.5° (width) − 1° (length) markers (two vertical and horizontal) offset from the center of the monitor by 4°. The target appeared centered in the space between the markers. Fixation accuracy was monitored with an infrared (940 nm) gaze-tracking video system (ViewPoint EyeTracker; Arrington Research, Inc., Scottsdale, AZ). If inaccurate fixation was observed, the subject was verbally reminded to maintain proper fixation.

Post hoc analysis removed responses when fixation errors were ≥3°. Such deviations are not uncommon in clinical applications, but have little impact on psychophysical outcomes in the absence of a scotoma. Removing these data from our raw responses did not change the parameter estimates, but did reduce noise.

Recovery was monitored for a maximum of 30 minutes after bleaching, by using a modified Békésy tracking procedure.\(^19\) The stimulus timing is shown in Figure 1. Each trial was initiated by the appearance of fixation markers that remained ON for the duration of the trial (1.5 seconds; Fig. 1). After the onset of the fixation markers, a 0.3-second foreperiod was allowed to achieve fixation before stimulus presentation, which lasted 0.2 second. A 1.0-second response window was followed by a 1.0-second interstimulus delay. True trials were randomly interleaved with 50% false-positive (blank) trials. This procedure generated a spot on average every 3.75 seconds.
TABLE 1. Visual Acuity and Fundus Changes in the ARM Group

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<th>Fellow Eye</th>
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<tr>
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<td>27</td>
<td>R 0.14 5 CM 4</td>
<td>-</td>
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n = 27 participants with ARM.

* Drusen Size: 2, ≥63 μm to <125 μm; 3, ≥125 to <175 μm; 4, ≥175 to <250 μm; 5, ≥250 μm.
† Location: C, central subfield; M, middle subfield; O, outer subfield.
‡ Number of drusen: 2, 1–9; 3, 10–19; 4, ≥20.
§ Late Stage: CNV, choroidal neovascularization; GA, geographic atrophy.
|| Subjects who failed to reach RCB during the 30-minute testing time.
A. True - Trial

The observers signaled stimulus detection with a response button. Participants were told that auditory feedback would be provided on error responses (responses to blank trails). Observers were instructed, to minimize the occurrence of errors. Correct identifications resulted in a 3-dB decrease in stimulus luminance, whereas a NO response resulted in the true trial resulted in a 1-dB increase in luminance. Post hoc analysis of the average ARM group response profiles showed that recovery was sampled every 17.5 seconds on average. The luminance of the fixation markers tracked the threshold, being 1 log unit brighter than the stimulus level to which the subject last made a correct response, up to the maximum luminance of the monitor.

Procedure

Observers were tested monocularly after pupil dilation of more than 7 mm diameter. The pupil size was measured and monitored using the tracking system (EyeTracker; Arrington Research, Inc.). Average (±SD) pupil size on commencement was 7.80 ± 0.71 mm in the ARM group and 7.70 ± 0.58 mm in control subjects. Variation in pupil size during the test did not exceed 1 mm.

Testing was performed in a darkened room, with best refraction for the test distance being fixed in a lens frame in the goggles. Proper ventilation was provided in the room to avoid the effect of oxygen deprivation on photoreceptor recovery. Participants were exposed to the Ganzfeld bleach, and adaptation recovery was tracked immediately after light offset.

Modeling

The dynamics of the cone and rod recovery were modeled (Excel; Microsoft, Redmond, WA), by using a single exponential decay8,21 separately for the rod and cone components, according to equation 1 (see the Appendix for derivation):

\[ T(t) = \log(10^{Tf + Ti - R} + 10^{Tf}) \]  

where the threshold \( T \) (in log candelas per square meter) is a function of time (t) after offset of bleaching (in minutes), \( Ti \) is the initial threshold (log candelas per square meter) of the photoreceptor, \( Tf \) is the asymptotic threshold, and \( R \) is the rate of decay (in decades per minute). The variables are assigned to cone and rod mechanisms by subscripted alphanumeric c and r, respectively (e.g., \( Tfc \)). We found that for our psychophysical data, a single decaying function (equation 1) adequately defined each of the cone and rod components of dark adaptation. The rod rate of decay (\( R_r \)) value in equation 1 represents the second mechanism described by Lamb71 for rod dark adaptation.

Parameter optimization was achieved by minimizing the merit function (sum of squares; Solver module of an Excel spreadsheet; Microsoft, Redmond, WA).

We report the mean ± SD for the data and each of the model parameters, as well as the 95% confidence limit for the aged normal observers. Parametric (t-test) and nonparametric (Mann-Whitney) tests were used as appropriate, to compare the dark adaptation parameters between the ARM group and age-matched control subjects (\( R_c \) ARM versus \( R_c \) Controls; \( Tf \) ARM versus \( Tf \) Controls; \( RCB \) ARM versus \( RCB \) Controls; \( R_r \) ARM versus \( R_r \) Controls), with \( \alpha = 0.05 \). Receiver operating characteristic (ROC) curves were calculated with commercial statistical software (GraphPad, ver. 4; GraphPad, San Diego, CA). ROC parameters were compared by a twotailed z-statistic that allows for correlation between data sets derived from the same populations.\(^{22,23}\)

RESULTS

Test Parameters

Figure 2 shows threshold recovery (log10) in a normal individual measured with a foveated, 4° target as a function of time (in minutes) after a 96% pigment bleaching. The unfilled circles represent full recovery, exposed with progressively larger ND filters attenuating the brightness of the CRT stimulus. The progressively shaded gray horizontal bar along the abscissa indicates the time points of insertion of different filters. During the first 4.5 minutes, the initial phase of cone recovery was tracked by attenuating the CRT stimulus with a 1.0-ND filter (light gray bar). Interposing the 2.6 ND (4.5–23 minutes) exposes clinically important regions of adaptation: the cone plateau, the \( RCB \) and the second phase of rod recovery. At 23 minutes, a 3.3-ND filter was needed to expose the third phase of rod recovery. The best-fitting models to the cone branch (solid curve) and the rod branch (dashed curve) were estimated by using equation 1. The \( RCB \) in this normal participant was at 10.3 minutes (10 minutes, 18 seconds) and the decay rate for cones (\( R_c \)) was 1.04 decades · min⁻¹ and for rods (\( R_r \)) 0.22 decades · min⁻¹. The rate-limited recovery of the second phase of rod recovery\(^6\) found in our experiment is similar to the previously reported 0.25 to 0.24 decades · min⁻¹.\(^1,3,5\) Our results suggest that testing with the single 2.6 ND filter should expose the clinically useful domains of cone and rod recovery.

The filled diamonds on Figure 2 represent recovery of the same participant using a single 2.6 ND filter for the entire duration of dark adaptation (30 minutes). This procedure gave

B. False Positive (Blank) Trial

Parameter optimization was achieved by minimizing the merit function (sum of squares; Solver module of an Excel spreadsheet; Microsoft, Redmond, WA).

We report the mean ± SD for the data and each of the model parameters, as well as the 95% confidence limit for the aged normal observers. Parametric (t-test) and nonparametric (Mann-Whitney) tests were used as appropriate, to compare the dark adaptation parameters between the ARM group and age-matched control subjects (\( R_c \) ARM versus \( R_c \) Controls; \( Tf \) ARM versus \( Tf \) Controls; \( RCB \) ARM versus \( RCB \) Controls; \( R_r \) ARM versus \( R_r \) Controls), with \( \alpha = 0.05 \). Receiver operating characteristic (ROC) curves were calculated with commercial statistical software (GraphPad, ver. 4; GraphPad, San Diego, CA). ROC parameters were compared by a twotailed z-statistic that allows for correlation between data sets derived from the same populations.\(^{22,23}\)
-2 log units of rod recovery. There is reasonable exposure of the clinically important aspects of cone and rod recovery: the cone rate constant, the cone plateau, the RCB, and the second component of rod recovery. Recovery of these components was similar to that achieved with the serial ND filters (circles; Fig. 2).

Figure 3 shows the effect that a range of stimulus sizes had on the dynamics of dark adaptation for foveal thresholds after a 96% bleaching in a normal individual. Thresholds for the smallest (1°) target exposed limited rod activity. As target size increased, a greater proportion of rods were recruited, exposing an earlier and more prominent second phase of rod recovery. The 4° and 6° targets provided adequate definition of the key components of both rod and cone function in the macular area. Previous reports (histologic,24 psychophysical,25 and electrophysiological26) have shown that rods are most vulnerable in a ring-shaped macular area with the peak of loss being ~2° from the fovea. Therefore, we propose adopting a central 4° stimulus (filled symbols; Fig. 3) when testing eyes with ARM.

The effect that each of the six levels of photopigment bleach (3%-96%) had on the dynamics of dark adaptation are shown in Figure 4A for a normal individual. As the bleaching
level increased, the rod–cone transition occurred at a progressively later time. The time course of the second phase of rod recovery, as indicated by the straight solid lines in Figure 4A, was the same (0.23 decades - min⁻¹) in all cases, whereas recovery from the lowest level of bleach (3%) showed very fast recovery, being of a different shape from the other levels of bleaching, consistent with previous reports. When the effect of different levels of bleaching (Fig. 4B) was considered in terms of the time for rod second-phase recovery to reach a criterion threshold (horizontal dotted line, −3 log cd - m⁻², Fig. 4A), it showed a linear rate-limited relationship for recovery, except for the 3% bleaching level (Fig. 4B). Similar results were found in previous reports that show rate-limited recovery for bleaching levels above 20%. The rod–cone transition is considerably longer in ARM cases, therefore the smallest pigment bleaching that gives a rate-limited second phase of the rod recovery would keep test duration to a minimum. Our light source bleaches −50% of pigment in an eye with a 8-mm pupil (average pupil size in our ARM group was 7.80 ± 0.71 [SD] mm). This level of bleaching yielded a fast recovery (<30 minutes in normal eyes) and allowed exposure of the clinically significant cone components as well as the second, rate-limited component of rod recovery (Figs. 4A, 4B).

**Test Validation**

To test the comparability of our dark adaptometer with a conventional technique, we contrasted measurements obtained with a Goldmann-Weekers adaptometer to our CRT method in five normal observers using the following test parameters: 2° achromatic stimulus at 10° inferior field and >96% pigment bleach and the CRT luminance output attenuated with a single 2.6 ND filter. There were no statistically significant differences found when the average adaptation parameters (±SD) acquired with a Goldmann-Weekers adaptometer (GWA) were compared with our CRT method for cone recovery (R_c CRT, 1.19 ± 0.19 vs. R_c GWA, 1.18 ± 0.19; log cd - m⁻², P = 0.241; T_f CRT, 1.55 ± 0.21 vs. T_f GWA, −1.61 ± 0.18; log cd - m⁻², P = 0.140). Rod–cone break (RCB) was 8.49 ± 1.14 vs. RCB CRT 8.75 ± 1.50 minutes, P = 0.467) and the rod second-phase recovery (R_f GWA 0.26 ± 0.01 vs. R_f CRT 0.28 ± 0.09 decades - min⁻¹, P = 0.093). The rod third phase of the recovery was evident only with the GWA test, although this part of the dark adaptation curve is not clinically significant in ARM. The rod absolute threshold was also not evident with the CRT method.

**Dark Adaptation in People with ARM and Age-Matched Controls**

The previous experiments showed that a Ganzfeld bleaching of −30% coupled with a 4° stimulus and 2.6 ND filter exposed −3 log units of cone and rod recovery in normal participants. This procedure was applied to a group of 27 patients with ARM (67.5 ± 5.0 years of age) and 22 aged-matched control observers (66.8 ± 5.9 years of age). The age distributions in the ARM group and the control subjects were not significantly different (t = 0.42, P = 0.67). Visual acuities were also not significantly different between the groups (logMAR ± SD: 0.026 ± 0.078 ARM vs. −0.017 ± 0.083 control; L_v = 1.98, P = 0.067).

In the control group (n = 22), the average ± SD absolute threshold for cones (T_f) was −1.98 ± 0.30 log cd - m⁻² with a recovery rate (R_f) of 2.63 ± 0.61 decades - min⁻¹ (Table 2). The average time to RCB was 10.40 ± 2.49 minutes; and the average recovery rate for the second phase of rods (R_b) was 0.22 ± 0.02 decades - min⁻¹ (Table 2). The time course of dark adaptation for a representative age-matched control subject is shown in Figure 5A. Figures 5B-D show a sample of clinical cases with ARM reflecting a wide variation in recovery. Figure 5B shows an example of an ARM case with slower cone recovery, elevated cone threshold, and delayed RCB. Figure 5C is remarkable in that the participant had a very slow rod second phase. Figure 5D gives an example of a patient with no evidence of a rod-cone break during the 30 minute test.

In the ARM group (n = 27), the cone recovery rate on average ± SD was significantly slower than the control rate (R_f ARM, 0.99 ± 0.35 vs. R_f control, 2.63 ± 0.61 decades - min⁻¹, Mann-Whitney [MW] = 10.0, P < 0.0001; Fig. 6A, Table 2). The T_f was the least affected parameter (Fig. 6B); however,
the difference between ARM and control groups was still statistically significant ($T_f$, ARM, $-1.62 \pm 0.38$ log cd $\cdot$ m$^{-2}$ vs. $T_f$, control, $-1.98 \pm 0.30$ log cd $\cdot$ m$^{-2}$; $t_{27} = 3.64, P = 0.0007$, Table 2). The average cone absolute thresholds in the 7 ARM cases (26%) who had abnormal values was 0.8 log units removed from the average threshold in the control group ($T_f$, ARM, $-1.19 \pm 0.11$ log cd $\cdot$ m$^{-2}$ vs. $T_f$, control, $-1.98 \pm 0.3$ log cd $\cdot$ m$^{-2}$; $t_{27} = 7.40, P < 0.0001$; Table 2). It is important to note that an abnormal (higher) cone threshold yields a faster RCB, therefore reducing the diagnostic power of the RCB.

Comparison of the average RCB $\pm$ SD in the control ($n = 22$) and ARM ($n = 27$, including 3 cases with no RCB; >30 minutes) groups showed significant delays in ARM eyes (MW = 50.50, $P < 0.0001$). The average time of the RCB in the 24 participants with ARM who exhibited a rod–cone transition within the 30-minute test duration was significantly delayed ($19.12 \pm 5.17$ [SD] minutes) when compared with control subjects ($10.40 \pm 2.49$ minutes, $t_{44} = 7.18, P < 0.0001$; Table 2). $R_t$ rate of the second phase was significantly slower in the ARM ($n = 24$) group than in the control subjects ($R_t$, ARM 0.16 $\pm$ 0.03 vs. $R_t$, control 0.22 $\pm$ 0.02 decades $\cdot$ min$^{-1}$, $t_{44} = 6.77, P < 0.0001$; Fig. 5D).

Of all ARM cases ($n = 27$) 93% had at least one recovery parameter that was abnormal. Only two ARM cases had all parameters within the 95% confidence band of age-matched control observers. Cone parameters were abnormal in 22 patients (81%, Table 2) with all ($n = 22$) recording delayed recovery rates (Fig. 6A) and 7 (26% of all ARM) showing abnormal absolute thresholds (Fig. 6B). The rod–cone transition (RCB) was abnormal in 22 patients (81%; Fig. 6C). Three of the ARM patients failed to show an RCB within the 30 minutes of testing (e.g., Fig. 5D). Of the 24 remaining, 15 patients (63%; Fig. 6D) had abnormal rod dynamics in the second phase.

Figure 7 shows the receiver operating characteristic (ROC) curves for 22 control subjects and 27 patients in the ARM group. The plot shows test sensitivity for ARM against false alarm rate (1 – specificity) and demonstrates the diagnostic capacity, expressed as area under the curve (AUC) of the dark adaptation parameters. The recovery rate for cones yields a significantly better diagnostic capacity than does cone absolute threshold ($R_t$, AUC 0.983 $\pm$ 0.014 vs. $T_f$, AUC 0.761 $\pm$ 0.069; $z = 3.063, P < 0.001$). The recovery rate for rods and cones gave similar diagnostic capacity ($R_t$, AUC 0.924 $\pm$ 0.044; $z = 1.416, P = 0.078$), and although both were better than the RCB, the differences were not statistically significant ($R_t$, AUC 0.983 $\pm$ 0.014 vs. RCB AUC 0.904 $\pm$ 0.045, $z = 1.469, P = 0.071$; $R_t$ AUC 0.924 $\pm$ 0.044 vs. RCB AUC 0.904 $\pm$ 0.045, $z = 0.261, P = 0.397$). The lack of significant differences between the latter parameters most likely reflects

**Table 2. Dark Adaptation Parameters in Age-Matched Control and Age-Related Maculopathy Groups**

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<td></td>
<td></td>
<td></td>
<td>$n$ (%)</td>
<td>Average</td>
</tr>
<tr>
<td>$R_t$ (decades $\cdot$ min$^{-1}$)</td>
<td>2.63</td>
<td>0.61</td>
<td>0.99</td>
<td>0.35</td>
</tr>
<tr>
<td>$T_f$ (log cd $\cdot$ m$^{-2}$)</td>
<td>-1.98</td>
<td>0.30</td>
<td>-1.62</td>
<td>0.38</td>
</tr>
<tr>
<td>RCB (minutes)</td>
<td>10.40</td>
<td>2.49</td>
<td>19.12</td>
<td>5.17</td>
</tr>
<tr>
<td>$R_t$ (decades $\cdot$ min$^{-1}$)</td>
<td>0.22</td>
<td>0.02</td>
<td>0.16</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* The three participants with ARM who did not have RCB within the 30 minutes of the test duration are not included (ARM, $n = 24$).

![Figure 5](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/Journals/IOVS/932947/) Dark adaptation curves for four clinical participants. (A) Thresholds (•) and the fitted model (line) for an age-matched control; (B–D) thresholds (○) and fitted models (lines) for three representatives of the ARM group. Shaded zone: 95% confidence interval for the RCB for our 22 normal observers.
the low experimental power (0.23) for the sample sizes in our study.27

Given that cone and rod recovery, as well as the RCB, had the best diagnostic capacity, we applied these parameters to consider whether high-risk clinical profiles28,29 varied in their average ± SD recovery parameters in our ARM group (Table 1). Study eyes with drusen and no pigment change (D+P; n = 9) were no different from those that had both drusen and pigment change (D+P; n = 18; R+D+P 0.93 ± 0.21 vs. R−D+P 1.02 ± 0.40 decades · min⁻¹, P > 0.05; R+D−P 0.15 ± 0.06 vs. R−D−P 0.14 ± 0.06 decades · min⁻¹, P > 0.05; RCB D+P 18.37 ± 4.79 minutes versus RCB D−P 19.49 ± 5.46 minutes, P > 0.05). Study eyes with an AMD fellow eye (n = 5, Table 1), showed a nonsignificant trend for slower recovery compared with cases with bilateral ARM (R, AMD in the fellow eye, n = 5, 0.98 ± 0.36 vs. R, AMD in the fellow eye, n = 22, 1.05 ± 0.54 decades · min⁻¹, P > 0.05; R, AMD in the fellow eye, n = 5, 0.18 ± 0.03 vs. R, AMD in the fellow eye, n = 22, 0.16 ± 0.03 decades · min⁻¹, P > 0.05). The cases with unilateral AMD also showed a nonsignificant trend for slower RCB. Two cases (one geographic atrophy, one choroidal neovascularization [CNV]) did not show an RCB within the 30 minutes of testing (Fig. 5D) and the remaining three CNV cases had an average RCB of 22.86 ± 2.69 minutes compared with the 18.58 ± 5.26 minutes found in cases with bilateral ARM (n = 22, P > 0.05). Nevertheless, although there were trends for slower recovery in these clinical subgroups, the average trend was not statistically significant. This result is presumably due to the small sample size of our ARM cohort, and further investigation with larger sample sizes is warranted.

**DISCUSSION**

CRT technology provides a universally available and uniquely flexible presentation modality that can be used to display a variety of static or modulating stimuli, so that a dark-adaptation test can be combined easily with other tests of visual function.30 We have detailed how this technology can be used to measure dark adaptation in a clinical setting. Using this dark-adaptation method we identify four clinically important parameters: the rate of decay of cones, the cone absolute threshold, the RCB, and the rate of decay of rods.

Documenting visual recovery promises to be an essential adjunct to other clinical assessments when investigating ARM.1–3 However, current methods of measuring adaptation are not readily adaptable to clinical use. The CRT method described in this article is a simple and effective device for measuring the dynamics of dark adaptation. We show how a dynamic range that extends over the limit of the CRT luminance output can be exposed by using serial and progressively larger ND filters (Fig. 2). However, we also argue that the stepped approach of increasing ND filters is not needed to yield clinically useful outcomes. The single ND filter (2.6 ND; Figs. 2–5) will isolate ~4 log units of range, which provides useful information about receptoral dynamics, by way of cone recovery, the RCB, and the second phase of rod recovery.

Careful matching of the CRT luminance and ND filter ensures the greatest range of stimulus control for monitoring recovery. The 2.6 ND filter used in our configuration was adopted for our CRT monitor that had a minimum luminance of 0.08 cd · m⁻². However, different screen luminance will need different filters to achieve similar outcomes (e.g., a screen with a minimal background luminance of 0.2 cd · m⁻² will need a 3 ND to achieve the same output as our configuration). Although we developed our method by using a CRT monitor, the technique can be used with LCD technology. However, the number and density of ND filters should be varied because of the brighter output and limited operating range (~2 log units, data not shown) of LCD displays.

Stimulus location and size (foveated, 4°) have been chosen to span cones and macular rods, which have been reported as the most vulnerable to ARM damage.24,25 Coupled with the modified Békézy tracking procedure,19 our method provides the high temporal resolution needed for adequate sampling of both cone and rod recovery dynamics (Figs. 2–5) from the same retinal region under common test conditions. Overall, 93% of ARM cases had an abnormality in at least one rod or cone adaptation parameter, despite their good acuity (logMAR ± SD, 0.026 ± 0.078).
Although three phases define rod recovery, use of the 2.6 ND filter and ~30% bleaching exposed only the second rate-limiting component of rod adaptation \( ^{6,31} \) in most cases (Figs. 2–5). The third phase of rod recovery was found to be of limited diagnostic value, \(^3\) yet a slow second phase of rod adaptation and delayed RCB have been found to be sensitive indicators of ARM \(^{1,3,25}\) as well as good signs of practical disability. \(^{22}\) Although the central 4° retinal region has a modest rod population, selective rod losses in this location \(^{24,25}\) are predictive of the development of ARM. \(^{33}\) In our ARM cohort, of all 27 cases, 81% showed a delayed rod-cone break. Abnormal rate constants of the second rod phase were found in 63% of all cases with RCB within the 30 minutes of the test duration \((n = 24)\). Furthermore, the rod recovery rate and RCB measured with our method had a high diagnostic capacity (ROC, area under the curve, \( R_c \), 0.924 \( \pm \) 0.044; RCB, 0.904 \( \pm \) 0.045).

Although the RCB and the second phase of rod adaptation have been proposed as the best dark adaptation indicators of ARM, \(^{1,3,25}\) our technique shows that cone recovery also has high diagnostic value, with high sensitivity and specificity (ROC, area under the curve, \( R_c \), 0.983 \( \pm \) 0.014). Of the ARM group, 81% had delayed cone recovery, which supports earlier reports on abnormal cone dynamics. \(^{2,30}\) Coupled with our modest level of bleaching, the test time is restricted to a maximum of 30 minutes, making it suitable for clinical application. However, when time does not permit examination of the entire rod and cone recovery, clinically meaningful data can be obtained in less than 10 minutes (Figs. 2–5), given the high diagnostic capacity of the cone decay (ROC, area under the curve, 0.983 \( \pm \) 0.014). The three observers who failed to show an RCB all had an abnormally delayed cone recovery and were deemed abnormal on this attribute alone.

Five participants in our ARM group had end-stage lesions (four with CNV and one geographic atrophy [GA]) in their fellow eyes, indicating a higher risk status. \(^{29}\) All five cases showed either no RCB by 30 minutes \((n = 2)\), one case with CNV and one GA, or had considerable delays (20, 22, and 26 minutes) before rod recovery became evident. This finding may indicate that the degree of delay in rod recovery correlates with risk of progression to end-stage disease, an issue presently under investigation.

The cases with all abnormal adaptation parameters \((n = 5)\) had the worst clinical profiles \(^4\) in the study eye: large central drusen, pigment abnormalities, and an average \( \pm \) SD visual acuity of 0.068 \( \pm \) 0.054. The single case of GA was also in this group and had no RCB. The two remaining subjects who did not have RCB by 30 minutes also had all parameters abnormal except for cone absolute threshold in one of them. Participants with all normal parameters \((n = 2, \text{ visual acuity: } -0.06 \text{ and } -0.1)\) had only small drusen in the middle subfield \(^{14}\); however both cases had minor patches of hyperpigmentation.

Although we were able to measure dark adaptation in all our participants, our experience with a larger number of aged patients has shown that some 4% \((3/69)\) of participants were not able to perform this test reliably due to physical limitations and/or an inability to follow instructions. We
have considered the variability of the indices determined by our technique by retesting a subset of control subjects (n = 16) and patients (n = 24) after 16 ± 6.5 (mean ± SD) days. This returned a coefficient of variation for RCB on retest of 32% (controls) and 44% (ARM): ~1.5 dB which is similar to the variability found in the perimetry indicating that our method has potential as a clinical application for the early detection of persons with ARM. Also, comparison of our data with the conventional Goldmann-Weekers adaptometer for a similar bleaching level and target size showed similar profiles for recovery. As our logic is based on the linear relation between the bleaching source and recovery rate (Fig. 4B) we have calculated that the pupil diameter must exceed 6.5 mm and any cataract must have an opacity <0.2 on the Wilmer Cataract Grading System\(^{12,13}\) (pupil size and lens opacity together <0.2 log units) to achieve a level of bleaching (>20%) that yields the rate-limited response for our time criterion. Otherwise, a brighter bleaching source would have to be used.

The CRT dark-adaptation technique described in this article is a simple and effective device that can provide high temporal resolution assessment of both rod and cone dynamics under similar conditions. It has the potential to provide functional measures of ARM progression for monitoring the efficacy of interventional therapies in clinical trials.

### APPENDIX

The formula for threshold recovery has been specified as a decaying time-dependent exponential by Lamb.\(^{21}\) Pianta and Kalloniatis\(^{6}\) have shown that the exponential relationship, when expressed on a linear log scale, can be described by straight lines. This concept is formalized in Figure 1 and equations 9a and 9b of their paper. Although the authors implement the concept to describe three mechanisms of photoreceptor recovery (early recovery phase, late recovery phase, and absolute threshold or asymptotic constant), we adapted it for our clinical implementation as a two component recovery: one specifying the recovery phase after desensitization and the other specifying the asymptotic constant or absolute threshold (Fig. A1). This approach has been adapted as follows.

\[ Tr(t) = (T_f + T_i) - R_t \]  
(A1)

where \(Tr(t)\) represents threshold for the recovery phase in log units as a function of time. \(T_i\) is the initial threshold elevation in log units relative to absolute threshold (\(T_f\)), and \(R\) is the rate of recovery in decades per minute (this is related to the exponential decay constant \(\tau\), by \(R = \tau \ln(10)\)). Since the final threshold \(T_f\) is constant, thresholds measured during dark adaptation can be described by a combination of the recovery phase and the final asymptotic threshold as

\[ T(t) = \log(10^{T_f(t) - R} + 10^T) \]  
(A2)

This equation results in a smooth transition between these components, giving a threshold elevation of 0.3 log units (i.e., a factor of two), where the two components intersect. By substituting equations A1 and A2 we have the final equation

\[ T(t) = \log(10^{T_f(t) - R} + 10^T) \]  
(A3)

which is equation 1 in the text.

Figure A1 shows recovery curves for a range of parameters. Two families of three curves were plotted to demonstrate the effect that varying one of the parameters has on the curve.

### References


