Effects of Cone Adaptation on Variability in S-Cone Increment Thresholds

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PURPOSE. Short-wavelength automated perimetry (SWAP) has gained popularity as a clinical tool for the assessment of short-wavelength-sensitive (S)-cone visual function, but has also been shown to have higher threshold variability than conventional achromatic perimetry, possibly due to an imbalance between S-cone adaptation and long (L)- and medium (M)-wavelength-sensitive cone adaptation. To investigate potential causes for this relatively high variability, we studied the effects of luminance and S-cone adaptation on variability in S-cone increment thresholds.

METHODS. Foveal S-cone increment thresholds were measured on adapting backgrounds ranging from 1.17 to 4.17 log troland (Td) and from −0.16 to 3.66 log S-cone trolands (TdS). Within-session variability (slope of the psychometric function) was evaluated in 2 trained and 15 inexperienced normal observers. Test-retest variability was evaluated in the 2 trained observers, and interobserver variability in the group of 15 observers. Multiple linear regression was used to model the effects of log luminance, log S-cone adaptation, and second-site polarization (ratio of luminance and S-cone adaptation; log [Td/TdS]).

RESULTS. Test-retest variability was lower for conditions with higher levels of S-cone adaptation (F = 9.04, P = 0.013, for the trained observers). Adaptation conditions with lower levels of polarization were associated with lower within-session variability (F = 6.9, P = 0.011; trained observers) and interobserver variability (F = 35.7, P = 0.004; group of 15 observers).

CONCLUSIONS. Variability of S-cone increment thresholds can be reduced by using adaptation conditions with a higher level of S-cone adaptation and/or a more balanced ratio between luminance and S-cone adaptation than is used for SWAP. (Invest Ophthalmol Vis Sci. 2003;44:4140–4146) DOI:10.1167/iovs.02-1067

Assessment of the short-wavelength-sensitive cone (S-cone) pathways has been of interest to the clinical vision research community for decades. A recent application, short-wavelength automated perimetry (SWAP), maps S-cone increment thresholds across the central visual field. SWAP has gained popularity as a clinical tool for measuring functional abnormalities, most notably in patients with (or at risk for) glaucoma,1–6 but also in a variety of other conditions.7–10

An empiric observation has been that SWAP thresholds show higher variability than thresholds from conventional, achromatic, automated perimetry. Test-retest variability is higher in SWAP than in achromatic perimetry, both in normal subjects11 and in patients with glaucoma or ocular hypertension.12,13 Also, interobserver variability14,15 is higher in SWAP than in achromatic perimetry, even after correction for preretinal absorption of light from the short-wavelength target. These findings are unfortunate in situations in which statistical significance of quantitative SWAP results is crucial, such as early detection or measuring progression of visual field defects, both in ophthalmic practice and in clinical studies.

The cause of the increased variability in SWAP remains unclear, but may be due either to differences in intrinsic properties between the S-cone pathways and the luminance pathways, or to properties of the test conditions. Of note, review of published S-cone threshold data shows high variability in some studies,11–16 but not in all.17–19 This suggests that the increased variability in SWAP may be due to the test conditions used to measure S-cone sensitivity rather than to intrinsic properties of the S-cone pathway. In fact, the studies that did not show increased variability in S-cone-mediated detection thresholds were all performed under conditions of approximately equal adaptation of L-, M-, and S-cones. This is in contrast with SWAP, in which a middle-wavelength background desensitizes (i.e., adapts) the L- and M-cones while leaving the S-cones relatively unadapted.20 As a result, in SWAP, the visual system operates in a range in which S-cone sensitivity is near absolute threshold and, furthermore, in which there is a large imbalance between S-cone adaptation and L- and M-cone adaptation. This imbalance, in turn, could cause the color-opponent “second site” of S-cone visual processing to be considerably polarized21–24 which may increase the variability of detection thresholds.

Herein, we present S-cone increment threshold data obtained under a range of adaptation conditions. This study was undertaken in an attempt to determine whether threshold variability can be affected by the level of S-cone adaptation and/or the level of luminance adaptation (adaptation of the L- and M-cones). Based on the results, we suggest that threshold variability can be reduced by measuring S-cone increment thresholds in relatively neutral adaptation conditions (i.e., when there is less imbalance between luminance and S-cone adaptation).

METHODS

Apparatus

All stimuli were presented monocularly in Maxwellian view by a two-channel optical system with a 2-mm artificial pupil and a single 150-W tungsten light source for both channels. One channel was collimated and then attenuated by neutral density (ND) filters and a triple-cavity interference filter (10 nm bandwidth) to produce a 25°-diameter monochromatic background. The other channel was collimated and guided subsequently through a liquid crystal variable attenuator (Meadowlark Optics, Longmont, CO), an ND filter wheel, an interference filter, and a 2°-diameter aperture to provide the monochromatic target. The Gaussian waveform (2σ = 200 ms) of the target...
was produced by the variable attenuator (maximum attenuation 3 log units), and the ND filter wheel provided up to 4 log units of attenuation of the increments. The variable attenuator and the ND filter wheel were controlled by a microcomputer (Apple, Cupertino, CA) equipped with a 12-bit digital-to-analog converter, a timing board (DMA-8), and a digital input-output board (National Instruments, Austin, TX). The two channels were combined by a beam splitter. A chin rest and a rubber eyepiece enabled the observer to maintain a steady viewing position.

**Calibration and Units**

The retinal illuminance of all monochromatic target and background lights was measured with a photometer and a photometric probe (UDT Sensors, Hawthorne, CA) which together were accurate to within 5% for all visible wavelengths, and expressed in trolands (Td) according to the method of Nygaard and Frumkes. The level of adaptation of the S-cones was defined by using the model of Boynton and Kambe for cone-specific trolands. This model is based on the Judd standard observer and postulates that equal-energy white of 1 Td retinal illuminance results in S-cone excitation of 1 S-cone troland (1 Td S). S-cone trolands were calculated using equations provided by Miyahara et al.

Whereas all increment thresholds were measured in fractions (e.g., log(Td/TdS)), we converted the values for variability to a decilog scale (decibel units; 1 dB = 0.1 log), which is the scale commonly used in static perimetry.

**Stimuli, Threshold Measurements, and Adaptation Conditions**

Increment thresholds were determined for a foveal 460-nm target, presented on a series of monochromatic backgrounds of various wavelengths and intensities. A two-interval forced-choice staircase was used with 20 reversals and a two-down/one-up rule. Target luminance changed in 0.3-log steps until the second reversal and in 0.15-log steps thereafter. This resulted in staircases of typically 80 to 100 trials. Using maximum likelihood estimation, data from each staircase were fit with a three-parameter Weibull psychometric function:

\[ R(x) = 0.5 + (y - 0.5) \times (1 - 2^{(x-a)/b}) \]

where \( x \) denotes stimulus intensity, \( R(x) \) denotes the rate of correct responses, and 0.5 corresponds to chance level in the two-alternative forced-choice task. The three free parameters were threshold \( a \) (75% correct point), slope \( b \), and upper asymptote \( y \). This combination of staircase method and fitting technique has been shown to provide reliable estimates of threshold.

Observers adapted to the background for approximately 2 minutes before each staircase. Between staircases, the color and intensity of the background were changed to obtain data for six different adaptation conditions, chosen to span at least a 3-log unit range of luminance adaptation (retinal illuminance ranging from 1.17 to 4.17 log Td) as well as S-cone adaptation states (−0.16 to 3.66 log Td S; Table 1). This also provided a range of ratios of luminance and S-cone adaptation.

This ratio is termed (second-site) “polarization” herein, designates luminance adaptation relative to S-cone adaptation, and is expressed as log(Td/TdS). Polarization ranged from 0.51 to 3.29 log(Td/TdS). In the diagram of Figure 1, where log luminance adaptation is plotted against log S-cone adaptation for each condition, polarization varies along an oblique axis, as indicated. Note that conditions A and B have equal polarization, as do conditions E and F, and that condition D is similar to the conditions used for SWAP.

To determine whether thresholds in all adaptation conditions were mediated by S-cones and not by L- or M-cones or rods, additional thresholds were measured for 490-nm increments in adaptation conditions A and E in all observers, and the ratios of the 460-nm and 490-nm increment thresholds were compared to predicted values for detection by S-cone, luminance (L + M), red-green opponent (L − M), and rod mechanisms. These predicted values were based on data in the literature for the cone fundamentals, the photopic and scotopic luminosity functions, normal variability in density of lens and macular pigment, and adaptation effects on luminance and chromatic mechanisms. The 460/490-nm ratio varied by no more than 0.05 log unit when macular pigment density was changed from mean density to either the upper or lower 95% confidence limits for normal, whereas change in lens density from the standard 32-year-old lens to an average 77-year-old decreased the 460/490 ratio by 0.17 log unit. We generated ranges for 460/490 ratios for each of the different postreceptorial mechanisms, using the upper and lower 95% confidence limits for macular pigment density, and lens densities from 20 to 77

Table 1. Adaptation Conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Peak Wavelength (nm)</th>
<th>Luminance Adaptation (log Td)</th>
<th>S-Cone Adaptation (log TdS)</th>
<th>Polarization (log [Td/TdS])</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>510</td>
<td>1.17</td>
<td>0.66</td>
<td>0.51</td>
</tr>
<tr>
<td>B</td>
<td>510</td>
<td>4.17</td>
<td>3.66</td>
<td>0.51</td>
</tr>
<tr>
<td>C</td>
<td>540</td>
<td>3.18</td>
<td>1.50</td>
<td>1.68</td>
</tr>
<tr>
<td>D</td>
<td>570</td>
<td>3.16</td>
<td>−0.16</td>
<td>3.29</td>
</tr>
<tr>
<td>E</td>
<td>636</td>
<td>3.13</td>
<td>−0.16</td>
<td>3.29</td>
</tr>
<tr>
<td>F</td>
<td>636</td>
<td>4.13</td>
<td>0.84</td>
<td>3.29</td>
</tr>
<tr>
<td>SWAP*</td>
<td>585</td>
<td>2.85−5.29</td>
<td>0.29−0.73</td>
<td>2.56</td>
</tr>
</tbody>
</table>

* SWAP data are computed approximations based on a dominant wavelength of 585 nm, a mean luminance of 100 cd/m², and a pupil diameter range of 3.0 to 5.0 mm.

**Figure 1.** Log retinal illuminance (in trolands) versus log S-cone trolands (TdS) of conditions A through F ( ). Condition D falls in the approximate range of SWAP adaptation conditions ( ) and corresponds to a 3.0- to 5.0-mm pupil diameter range. The oblique axis (Polarization) denotes the ratio of luminance adaptation and S-cone adaptation in units of log [Td/TdS]. A polarization of 0 defines equal-energy white. Dashed lines show that conditions A and B have equal polarization, as do conditions E and F.
Finally, interobserver variability was defined as the SD of log threshold from retest variability (tvi) functions. 24 All analyses were performed on computer (MiniTab Statistical Software; Minitab Corp., State College, PA).

**RESULTS**

Figure 2 shows the means and standard errors of the increment thresholds of all observers for the six adaptation conditions. They are plotted as luminance increment thresholds (Fig. 2A) and in log S-cone trolands (B). **Solid curves** in (B) are S-cone threshold-versus-illuminance (tv) functions. 25
and S-cone increment thresholds (Fig. 2B). S-cone thresholds versus illumination (tvi) functions (see the Discussion section) were fit to data points of equal retinal illumination and are shown in Figure 2B. The equation for the tvi curves was log (threshold) = \( a_0 + \log(1 + a_1 \text{illumination}) \), where \( a_0 \) represents absolute threshold and \( a_1 \) represents the Weber fraction. The tvi functions for different retinal illumination levels differed only by a shift along a 45° angle. In the Discussion section we will argue that of the six conditions, only condition B brings the S-cones in a state of adaptation where Weber’s law holds. The finding that the remaining five conditions are not in the “Weber region” for the S-cones has implications for the variability of measurements in those conditions.

One-way ANOVA showed a significant effect of age on log threshold \((F_{(1,45)} = 24.3, P < 0.001)\). Post hoc linear regression analyses of log threshold on age for each condition separately, showed a significant age effect for conditions A, E, and F, a borderline significant age effect for condition D, and no age effect for conditions B and C (Table 2).

### Within-Session Variability

For the two trained observers, multiple regression of log β (five repetitions) on luminance adaptation, S-cone adaptation, and observer indicated that there was no significant effect of observer \((t = 1.59, P = 0.12)\) and that log β correlated with both luminance adaptation \((t = -2.07; P = 0.043)\) and S-cone adaptation \((t = 2.40; P = 0.020)\). Subsequent omission of the observer parameter from the analysis resulted in the linear regression equation, log β = 0.468 − 0.066 × (log luminance adaptation) + 0.065 × (log S-cone adaptation) \((F_{(2,57)} = 3.4, P = 0.040)\). Note that the coefficients for log luminance adaptation and log S-cone adaptation are almost equal in size but with opposite sign, indicating that within-session variability in the two trained observers was directly related to the amount of polarization (log[Td/Td_s]), or log[Td] − log[Td_s]) of the adaptation state. Regression of log β on polarization resulted in the equation, log β = 0.459 − 0.064 × polarization \((F_{(1,92)} = 6.9, P = 0.011)\), indicating that log β increases (variability decreases) for lower levels of polarization (i.e., for more neutral adaptation conditions). This relationship is illustrated in Figure 3, where log β is plotted against polarization for each of the five repetitions, for each condition, for each of the two observers.

A tendency to confirm these results was found in the group of 15 inexperienced observers, in which multiple linear regression of log β on luminance adaptation, S-cone adaptation, and age also yielded regression coefficients for luminance adaptation and S-cone adaptation that were comparable in size but with opposite sign, but the regression was not significant \((F_{(10,78)} = 1.45, P = 0.14)\). Fitting the model that was obtained in the trained observers to the data from the group of 15 inexperienced observers yielded an approximately uniform distribution of residuals, but the model explained only 11.5% of the variation in the data.) Within-session variability was not associated with age \((t = -0.16, P = 0.871)\) in this group of 15 observers.

### Table 2. Effects of Age on Log Threshold. Post Hoc Comparisons

<table>
<thead>
<tr>
<th>Condition</th>
<th>Regression Coefficient (dB/year)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.116</td>
<td>2.41</td>
<td>0.031</td>
</tr>
<tr>
<td>B</td>
<td>0.074</td>
<td>1.37</td>
<td>0.194</td>
</tr>
<tr>
<td>C</td>
<td>0.092</td>
<td>1.27</td>
<td>0.225</td>
</tr>
<tr>
<td>D</td>
<td>0.185</td>
<td>1.91</td>
<td>0.079</td>
</tr>
<tr>
<td>E</td>
<td>0.198</td>
<td>2.38</td>
<td>0.033</td>
</tr>
<tr>
<td>F</td>
<td>0.210</td>
<td>2.48</td>
<td>0.028</td>
</tr>
</tbody>
</table>

For each condition, the regression coefficients represent the result of linear regression of log threshold on age for the group of 15 inexperienced observers. The overall age effect was established by ANOVA \((F_{(14,78)} = 24.3, P < 0.001)\).
Test–Retest Variability

Test–retest variability, defined as the SD of log threshold across five repetitions (expressed in dB), was studied for the two trained observers. Multiple regression of test–retest variability on luminance adaptation, S-cone adaptation, and observer indicated a significant effect from S-cone adaptation (t = −2.67, P = 0.028), but not from luminance adaptation (t = 0.04, P = 0.97) or observer (t = 1.33, P = 0.22). Subsequent omission of luminance adaptation and observer from the analysis resulted in the regression equation: Test–retest variability (in dB) = 1.03 − 0.17 × (log S-cone adaptation) (F(1,10) = 9.04, P = 0.013), indicating that test–retest variability decreases for adaptation conditions with higher levels of S-cone adaptation. This relationship is illustrated in Figure 4, where the test–retest reliability is plotted against S-cone adaptation for each of the six conditions, for each of the two observers.

Interobserver Variability

Interobserver variability was defined as the SD of log threshold across the group of 15 observers (expressed in dB). Multiple linear regression of interobserver variability on luminance adaptation and S-cone adaptation indicated a significant effect from both log luminance adaptation (t = 3.74, P = 0.033) and log S-cone adaptation (t = −4.69, P = 0.018). This resulted in the regression equation: Interobserver variability (in dB) = 1.86 + 0.61 × (log luminance adaptation) − 0.63 × (log S-cone adaptation) (F(2,5) = 12.7, P = 0.034). Similar to what was found in the analysis of within-session variability, the coefficients for log luminance and log S-cone adaptation are almost equal in size but with opposite sign, suggesting that interobserver variability was directly related to the amount of polarization of the adaptation state. Regression of interobserver variability on polarization resulted in the equation, Interobserver variability (in dB) = 1.82 + 0.62 × polarization (F(1,4) = 35.7, P = 0.004), indicating that interobserver variability decreases for lower levels of polarization (i.e., for more neutral adaptation conditions). This relationship is illustrated in Figure 5, where the interobserver variability (based on the data from the group of 15 observers) is plotted against polarization for each of the six adaptation conditions.

DISCUSSION

We explored the effects of luminance adaptation and S-cone adaptation on within-subject and between-subject variability of S-cone increment thresholds. Within-subject variability was evaluated in terms of both within-session variability (slope of the psychometric function) and test-retest variability (variation in thresholds between sessions). A small set of adaptation conditions was sufficient to show that within-subject (within-session) and between-subject (interobserver) variability were lower on more neutral backgrounds, and test-retest variability was lower for higher levels of S-cone adaptation. Both of these effects are relevant for SWAP, in which the high polarization associated with the adapting background is expected to increase within-session variability, and the low level of S-cone adaptation is expected to increase test-retest variability. In patients with glaucoma, there may be additional effects of rapid changes in adaptation condition on S-cone-mediated detection.34

This study was not primarily designed to investigate the effects of aging. Nevertheless, a fairly wide range of ages was present among the observers. Log threshold was found to be associated with age in conditions A, E, and F and to some extent in condition D, with the average amount of threshold increase ranging from 0.116 to 0.210 dB/year (Table 2). This confirms published findings by Eisner et al.35 for foveal conditions quite similar to our condition D (S-cone increments on a 580-nm, 3.0-log Td background), by Johnson et al.36 for peripheral (central 30°) S-cone increments on low and high intensity broad-band yellow backgrounds, and by Remky et al.37 for perifoveal (central 10°) S-cone increments on a 594-nm, 3.7-log Td background. We must conclude therefore that part of the interobserver variability in the present study is probably explained by age. In addition to the observed increase of log S-cone threshold with age, Johnson et al.36 also reported an increase of interobserver variability in log S-cone threshold data for subjects aged more than 60 years. They attribute this to an increased interobserver variability in lens density.38 We will point out in the discussion to follow, as has been argued before (e.g., Refs. 14,15,20), that the magnitude of the effects of interobserver variability in lens density, pupil diameter, and the sensitivity of the cone mechanisms on perimeter thresholds is strongly dependent on the adaptation state of the visual system.

Published data on point-wise normal perimetric variability under conventional, achromatic, adaptation conditions vary...
among studies, and reported data are on the order of 1 dB for within-session variability,\textsuperscript{11,38} on the order of 2 dB for test-retest variability,\textsuperscript{11,59,40} and approximately 2 to 5 dB for interobserver variability.\textsuperscript{14} Variation in the results from different studies is presumably due in part to differences in methods of analysis. More pertinent to our findings, however, is the comparison of normal threshold variability between conventional perimetry and SWAP within single studies, which have shown a 1.6-fold lower within-session variability,\textsuperscript{11} a 2.1-fold lower test-retest variability,\textsuperscript{11} and a 1.9-fold lower interobserver variability,\textsuperscript{14} for conventional perimeter. These values correspond well with the current findings. The size of the effects observed (i.e., the change in variability across conditions) corresponded to a factor of 1.5 for within-session variability (from 4.0 to 5.8 dB, after converting the parameter \( \beta \) to the SD [in decibels] of a cumulative Gaussian function\textsuperscript{41}; Fig. 3, inset). Test–retest variability varied 2.6-fold (from 0.41–1.06 dB; Fig. 4), and interobserver variability varied 1.8-fold (from 2.14 to 3.86 dB; Fig. 5) across conditions.

Typically, psychophysical techniques show less variability when increment thresholds are measured (such as in conventional achromatic perimeter) than when absolute thresholds are measured (such as in dark-adapted perimeter\textsuperscript{22,45} or in measurements of dark adaptation). Part of the explanation is that, if the visual system operates at an adaptation level at which Weber’s law holds, increment threshold is proportional to the intensity of the background. As a result, thresholds are only minimally affected by fluctuations in the observer’s pupil size or cone sensitivity or by variations in lens density across observers. Absolute thresholds, in contrast, are directly affected by all three factors.

The mean threshold data show a pattern similar to that found by Yeh et al.\textsuperscript{24} When plotted as log S-cone increments as a function of log S-cone adaptation (as in Fig. 2B), thresholds obtained at a fixed luminance level follow Weber’s law for relatively high levels of S-cone adaptation (where the data points fall on a line of slope = 1), but not for lower S-cone adaptation levels (where the data level off to fall on a horizontal line). The curves in the Figure 2B are S-cone tvi functions that show the transition from constant threshold behavior at lower S-cone adaptation levels to Weber-like behavior for higher S-cone adaptation levels. For different luminance levels, these S-cone tvi functions are shifted along a 45\( ^\circ \) angle, which Yeh et al. interpreted in terms of second-site adaptation.

The Yeh et al.\textsuperscript{24} model was a modification of the chromatic discrimination model of Boynton and Kambe,\textsuperscript{26} which included gain control mechanisms that allowed fitting color discrimination data from a wider range of retinal illuminance. The Boynton and Kambe model, in turn, built on a long tradition of describing color discrimination thresholds in terms of opponent processing.\textsuperscript{21,22,44,45} That has been successful in capturing many aspects of classic color discrimination data. Our data are consistent with the data and the model of Yeh et al.\textsuperscript{24} and extend them to higher mean retinal illuminances.

SWAP test conditions are close to our condition D (assuming a pupil diameter between 3 and 5 mm; Table 1, Fig. 1) and must therefore be on the horizontal portion of an S-cone tvi function (Fig. 2B). For SWAP, this implies that the adaptation condition is such that the S-cones are near absolute threshold, and that Weber’s law does not hold for S-cone increment thresholds. This probably increases variability above the level that might be found in the region where Weber’s law holds.

The primary purpose of this study was to gain insight into the underlying causes of the relatively high variability in SWAP. The results indicate that lower S-cone threshold variability can be achieved in conditions with higher levels of S-cone adaptation. It is often implicitly assumed that minimizing the variability of a clinical measure is a desirable goal. Indeed, we believe that this is justified for many clinical uses of perimetry, such as early detection or longitudinal measurement of the progression of visual field defects. However, by using adaptation conditions that are in the Weber region, the test becomes less sensitive to any disease action that has equal effects on the stimulus and the adapting field. Only by testing near absolute threshold can such an effect of disease action be observed. On the one hand, for such tests that operate outside the Weber region, it is crucial to control properly for preretinal light absorption and pupil diameter. On the other hand, increasing S-cone adaptation to ensure Weber-like behavior results in a smaller amount of isolation of S-cone mechanisms from L- and M-cone mechanisms. In fact, requirements of isolation and of variability seem to counteract each other, and the choice of S-cone adaptation level should therefore reflect a tradeoff between the two. Currently, SWAP test conditions have been optimized to yield good isolation, thereby producing relatively high variability. This should be kept in mind and, wherever possible, compensated for by increasing the number of stimulus presentations or visual field measurements.

In conclusion, we showed systematic effects of S-cone adaptation and second-site polarization on different types of variability: S-cone increment thresholds. Further research is needed to explore the magnitude of these effects systematically and to extend these findings to perifoveal test conditions relevant for perimetry.

References

13. Hutchings N, Hosking SL, Wild JM, Flanagan JG. Long-term fluctuation in short-wavelength automated perimetry in glaucoma sus-