Angioscotometry With the Scanning Laser Ophthalmoscope

Comparison of the Effect of Different Wavelengths

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Purpose. Angioscotomas are scotomata caused by vessel shadows. Their extent may be influenced by physiological and pharmacologic conditions and disease. In this study, the authors quantified angioscotomas in normal subjects using a fundus perimetry technique with a scanning laser ophthalmoscope. They further investigated the influence of two different wavelengths on scotoma depth.

Methods. For blue-on-yellow perimetry, the authors used two different lasers—an argon laser (\( \lambda = 458 \text{ nm} \)) for stimuli and a low background and a HeNe (\( \lambda = 594 \text{ nm} \)) for a superimposed yellow background. For red-on-red perimetry, the authors used another HeNe laser (\( \lambda = 633 \text{ nm} \)). Fundus illumination was provided by an infrared light. Five healthy subjects were examined. Twenty-one to 24 stimuli (200 msec duration, 0.4° × 0.4°) were presented at different intensities in randomized order in a 5° × 2.5° retinal test field, directly inferior and adjacent to the disk.

Results. The depth of scotomas caused by major vessels varied in all subjects and depended on perimetry condition. To quantify the influence of vessels on sensitivity, the authors analyzed psychometric functions for stimuli projected on the vessels and for those far from the vessels. The authors found a significant difference for targets on the vessel compared to those far, which was more pronounced for the blue-on-yellow condition.

Conclusions. Angioscotomas are detected better with blue targets on a yellow background than with red-on-red perimetry. The greater light absorption by hemoglobin and oxyhemoglobin at short wavelengths compared to longer wavelengths is not compensated for by visual mechanisms. Invest Ophthalmol Vis Sci. 1996;37:2350-2355.

Angioscotomata are visual field defects spatially correlated with retinal vessels that lie anterior to the photoreceptor layer. The mechanisms underlying these defects are not well understood. Interestingly, angioscotomas tend to be larger than the vessels themselves, indicating that the defects might be caused by factors in addition to the mere decrease of light reaching the photoreceptors. The severity of the defects seems to be affected by changes in physiological status. Widen-
techniques, not only white-on-white but also red-on-red, depend on luminance mechanisms and do not emphasize short wavelengths. These two types of stimuli differ as well in the amounts of absorption by the vessels. To compare the effects of different target and background conditions on angioscotomas, we tested two perimetry conditions, red-on-red and blue-on-yellow.

METHODS. Apparatus. A research SLO was used for all experiments. For retinal imaging, a diode pumped Cr:Li:SaF laser (Eye Scan SED, Concord, MA) (λ = 850 nm) provided illumination that appeared invisible to the subject but produced high-contrast images for the experimenter. Red-on-red perimetry was performed with a HeNe laser (λ = 633 nm) for background illumination (4200 td) and stimulus presentation. Blue-on-yellow perimetry was performed with an Argon blue laser (λ = 458 nm) and an additional HeNe laser, which produced a constant 594 nm yellow background (4100 td) superimposed on the blue background (93 td). The total background for each condition was relatively high, corresponding to approximately 600 cd/m² compared to conventional perimetry or even blue-on-yellow perimetry (200 cd/m²). But chosen to isolate short-wavelength-sensitive-cone responses.

The light level of each visible wavelength laser was modulated by an acousto-optical modulator, and the target laser acousto-optical modulator was controlled by a video graphics and digital image capture board (FG100-AT; Image Technology, Bedford, MA). Calibrations were performed with one laser source at a time with a photometer (EG&G, DR2550; Summa Scientific, San Diego, CA). The range of usable target intensity varied between 2 and 2.5 log units (maximal intensity—background intensity). To compare different conditions, data were normalized to a maximal range of 2 log units (20 dB).

Procedure. Five healthy subjects (one man, four women; age range, 28 to 45 years; visual acuity better than or equal to 20/20) were examined in both conditions. The tenets of the Declaration of Helsinki were followed in this study. Before participation in the study, the subjects received a detailed explanation of the procedures. The subjects gave their informed consent; they signed a consent form and followed a protocol approved by the Institutional Review Board of the Schepens Eye Research Institute.

Each experimental session consisted of a sequence of single sets of 21 to 24 stimuli, with the stimuli in each set presented in random order to prevent experimenter bias among conditions. The stimulus duration was 200 msec, long enough to provide stimulation of short-wavelength-sensitive-cone pathways and short enough to minimize artifacts caused by the onset of voluntary saccades in normal observers. A large cross of 2.3 × 2.3° at maximum intensity was presented for fixation.

The stimuli were presented in three closely spaced horizontal rows, on a 28.6° × 23° field. Stimuli that would fall too close to the optic nerve head were repositioned. The stimuli were 0.4° × 0.4°, sized approximately to Goldmann III, and a typical size for exploration around the blind spot. Thus, the targets were approximately 120 × 120 μm — smaller than the largest retinal veins, similar to the largest arteries, and larger than most retinal vessels after branching. The 5° × 2.5° retinal test field was directly inferior and adjacent to the disk (Fig. 1). The test field included the largest retinal vessels (150 to 200 μm) to ensure a high rate of stimuli intersecting vessel locations. Approximately 40% of the stimuli achieved this goal.

Targets were presented at 12 to 15 different intensities, with all stimuli in a single set presented in randomized location but at the same intensity (blocked design). This design permitted a rest period between each intensity to ensure better fixation stability. Each session included one condition and had a duration of 60 to 90 minutes. The spherical refractive error was corrected by the optics of the SLO.

The perimetry software to present targets, collect responses, and compute sensitivity with the SLO was version 3.0 of the Aachen software, plus a calculation program. The digitized image was displayed at a 60 Hz field rate on a large, high-resolution video monitor (650-A1; Tektronix, Beaverton, OR) during the entire stimulus presentation. The image quality was maintained so that small fundus landmarks, against which small eye motions were compared, were continuously visible. The SLO could be adjusted even during testing to improve image quality with respect to head position, light level, or video gain. At the same time, the stimulus was displayed on the fundus location tested, which was represented by a square.

The small microsaccades needed to maintain sensitivity were distinguished in real time by an experienced observer from larger eye movements that would displace the stimuli from the proper position. Furthermore, if an eye movement occurred during the target presentation, the image appeared blurred or contained noncontinuous features, such as segments of blood vessels. The examiner did not accept the response from this trial and retested this location at a later time.

To map the retinal location of the target, a fundus reference point, such as a vessel bifurcation, was chosen before the presentation of stimuli as a landmark. Directly after each target presentation, the retinal image was digitized by the same video board used for stimulus presentation. On this image, the examiner placed a cursor on the landmark to map the retinal
FIGURE 1. Responses after eye movement correction, one subject, without exclusion of data points. Three target intensities: (top) bright, (middle) medium, (bottom) dim stimuli. (A,C,E) Red-on-red. (B,D,F) Blue-on-yellow. (filled squares) Seen. (open squares) Not seen.

location of the target. A final image was digitized at the end of the test, for which the locations of stimuli were calculated. That large eye movements were eliminated successfully is shown by the regular appearance of the test grid after the recalculation of target location (Fig. 1). However, all eye movements cannot be eliminated during a target when using current SLOs; their timing is too slow. The total elimination of eye movements, which would result in sensitivity measurements under stabilized conditions, has not been examined with SLOs.

Data Analysis. Responses were excluded from analysis if the stimuli were projected too closely to the optic nerve head; this was seldom necessary. Using the final images (Fig. 1), the seen and unseen responses were counted in the image overlay for each target.
intensity to obtain psychometric functions. This was performed separately for the stimuli "on" a vessel (where "on" implies on or near) as opposed to far from a vessel. The criterion for stimuli "on" a vessel was an overlap of more than 30%, which was determined from the digitized image at the end of the stimulus presentation. If more than 30% overlap was required, fewer data points would be available for analysis, but 30% was enough to measure reliable effects (see Results). Some potential vessels are smaller than this target size. Stimuli “on” small vessels (less than 70 μm) were excluded from the analysis because much of the target falls off the vessel and the absorption effect might be small. The typical percentage of points used to calculate the psychometric function ranged from 87% to 100%, and subjects with the most excluded points had numerous small vessels. The data of the percentage seen versus target intensity (dB) were fitted by cumulative Gaussian distributions. Sensitivity was then defined as the interpolated 50% seen data point of the fitted curve.

RESULTS. The depth of the angioscotomas ranged from 0.1 to 5 dB, at a criterion of 50% seen. The average sensitivity for all subjects depended on the wavelength tested. For blue-on-yellow perimetry, the mean sensitivity was 12.53 dB for targets “on” a vessel and 15.28 dB for targets far from vessels (Table 1). For red-on-red perimetry, the mean sensitivity was 17.86 dB for targets “on” a vessel and 17.19 dB for targets far from vessels.

The change in response as a function of intensity was compared for the two perimetry conditions in Figure 1 (data from subject 2), with only three intensities shown per condition for brevity. For those stimuli “on” vessels, there was poor detection of the targets for all intensities shown for both conditions. The detection of stimuli “on” vessels versus far from vessels was relatively poorer in the blue-on-yellow (Figs. 1B, 1D, 1F) than in the red-on-red (Figs. 1A, 1C, 1E) perimetry data sets. That proximity to the optic nerve head has little effect on these data is evident from comparison among the panels. There was good detection near the optic nerve head (the second stimulus from the left in the top row of stimuli) as opposed to the poor detection of stimuli farther away (in the middle of the bottom row but “on” the vessels).

Figure 2 shows the psychometric functions for all subjects and both perimetric conditions. For all conditions, the curves typically were shifted to higher target intensity (lower dB) for the stimuli projected “on” the vessels compared to those far from the vessels. This was more pronounced for the blue-on-yellow condition. Furthermore, the psychometric functions appear broader for the stimuli “on” a vessel than for those far from vessels, primarily for the blue-on-yellow condition (4 of 5 subjects as determined by the psychometric function curve fits). From the psychometric functions, we obtained sensitivity as a 50% seen value. For both perimetric conditions, combined sensitivity was reduced significantly for the stimuli projected “on” a vessel versus far from vessels (analysis of variance for repeated measurements; P < 0.05). Blue-on-yellow perimetry resulted in a significantly lower sensitivity compared to the red-on-red condition (P < 0.01). Furthermore, the wavelength tested had a significant effect on the difference between “on” versus far vessel projected stimuli (P < 0.05).

DISCUSSION. In this study, angioscotomas were identified and quantified by a direct fundus perimetry technique. For stimuli “on” versus off vessels, there was a significant sensitivity loss and a significant effect of the wavelength used. These effects varied among subjects in a manner that may be attributed to different vessel sizes. The number of stimuli, however, was too small to permit subgroup analysis of vessel diameter. In contrast to studies with conventional perimetric techniques, the angioscotomas detected had a depth of only 0.1 to 5 dB in contrast to the reported 8 to 12 dB with conventional techniques. This might be the result of a difference in the perimetric conditions or calculation of the results. Newtonian view perimeters typically use dim background illuminances that fall within the lower part of the Weber region of a threshold versus intensity function for normal subjects. In

### TABLE 1. Average Sensitivity Values Derived From the Psychometric Function in Decibels for All Subjects and Perimetric Conditions

<table>
<thead>
<tr>
<th>Subject Number</th>
<th>Blue-on-Yellow</th>
<th>Red-on-Red</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Far Vessel</td>
<td>'On' Vessel</td>
</tr>
<tr>
<td>1</td>
<td>14.46</td>
<td>13.14</td>
</tr>
<tr>
<td>2</td>
<td>16.32</td>
<td>12.93</td>
</tr>
<tr>
<td>3</td>
<td>15.91</td>
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<tr>
<td>4</td>
<td>13.99</td>
<td>8.65</td>
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<tr>
<td>5</td>
<td>15.72</td>
<td>14.97</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>15.28 ± 1.00</td>
<td>12.53 ± 2.33</td>
</tr>
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within the Weber region. Therefore, reduction of losses has less effect as long as performance is determined by Weber’s law. The test wavelength had a significant effect on the depth of angioscotomas. The scotomata were more pronounced at 458 nm, where blood, hemoglobin, and oxyhemoglobin, have much greater absorption than at 633 nm. Theoretically, vessels act as filters in front of the retina, and transmission can be calculated based on the Lambert–Beer law. For example, for a 100 μm vessel with an assumed hematocrit of 45%, the transmission is 10% to 20% for 633 nm light and approximately 80% to 90% for 458 nm light.

The losses of sensitivity for targets "on" vessels found in this study may be attributed only partly to these transmission losses. Because sensitivity typically is determined by the relationship between target intensity and background intensity, the filter effect of the vessel may be counterbalanced by Weber’s law, averaged over a larger region than just a single blood vessel. Hypotheses for the sensitivity loss, therefore, are complex: Background illumination may be maintained by small eye movements or scattered light across the retina, despite a vessel in the path of incoming light. Thus, the long-term adaptation is set by the recent light history, averaged over space, in contrast to the relatively small, brief target. Transmission loss might cause light reduction to intensities at which the Weber ratio is not constant. Although this is not generally true for bright, achromatic targets in normal subjects, this could be the case for blue targets or in patients. In addition, the photoreceptors beneath the vessel may be less dense and may have different response ranges. This might have an effect particularly in blue-on-yellow perimetry because short wavelength-sensitive cone pathways have poorer spatial and temporal resolution, as well as markedly nonlinear response properties, and they respond differently from the shorter wavelength mechanisms.

Fundus perimetry with the SLO is useful in the quantification of small scotomatous areas, including angioscotomas. Results from fundus perimetry can be compared with those from conventional bowl perimetry, in which changes in eye position could be one source of the measured fluctuation in sensitivity. This source can be minimized, and the total number of stimuli needed can be reduced, when there is position correction of the stimuli. Nevertheless, when measuring changes in very small locations, such as angioscotomas, the experiments remain long in duration for both examiner and subjects. Furthermore, large eye movements during stimulus presentation can add noise and artifacts; thus, we used experienced subjects and excluded stimuli during detectable eye movements. Control of stimulus position can be improved by using fundus tracking procedures in combination with preplanned stimuli and advanced psychophysical test strategies. This study indicates that the quantification of angioscotomas is straightforward using SLO fundus perimetry in a sample of normally sighted adults.

These data indicate that angioscotomas may affect measures of visual sensitivity, even with modern perimetry techniques designed for clinical use. Angioscotomas as large as 5 dB were found with blue-on-yellow perimetry. Clearly, greater fluctuations in responses or decreased sensitivity in a region with large or many vessels could be caused by angioscotomas. The potential effects of angioscotomas should be considered in the interpretation of perimetry, particularly with modern, short-wavelength automated perimetry techniques (SWAP) because the meaningful differences
occur with eccentricity and aging. The current SWAP technique typically uses Goldmann size V stimuli, which are larger than those used in our study. Thus, the loss of sensitivity “on” or near vessels could depend not only on the wavelength content but also on the size of the target, depending on the integration area of the target and background adaptation state. Patients with poor fixation stability are difficult to test for the depth of the angioscotomas without retinal stabilization. The effects may be averaged over a larger spatial region and may influence perimetric results, but they remain difficult to quantify. Diseases known to affect an older population, such as age-related macular degeneration and glaucoma, also affect blue-on-yellow sensitivity. Thus, in the interpretation of scotomata, there is a need to distinguish between visual loss caused by various disease processes that may be progressing and loss that is caused by vessel shadowing.

Key Words

Angioscotomata, perimetry, retina, retinal vessels, scanning laser ophthalmoscopy, short wavelength automated perimetry.

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References