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## MERIDIAN VARIATIONS IN SPECTRAL DARK ADAPTATION

O. Joseph Calabrese, MA Harry Zwick, PhD

Division of Ocular Hazards



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#### ABSTRACT

Spectral dark adaptation as a function of retinal meridian and of eccentricity was investigated by testing the absolute threshold dark adaptation curves for eight human volunteers at two retinal meridians. The horizontal and vertical meridians separately and together at eccentricities of 2 degrees and 16 degrees from a fixation point were studied. A microprocessoroperated dark adaptometer was used, and analysis of variance was performed on the data. The study indicated that meridian differences in the dark-adapted retina exist and that the horizontal meridian is more sensitive than the vertical meridian when the retina is tested at 16 degrees with medium wavelength light. This may indicate meridian differences in photoreceptor distribution.

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#### Meridian Variations in Spectral Dark Adaptation

#### Introduction:

On today's modern battlefield soldiers are required to fight twenty-four hours a day. As long as soldiers are called upon to carry out their missions at night and in reduced light research to increase our understanding of the complicated process of dark adaptation is needed.

Dark adaptation is dependent on several factors including increased pupil diameter, increased retinal photosensitivity, the shift from primarily cone to primarily rod vision, and a shift in the neural pathways within the visual system. As in earlier studies (1-4), subjects indicated their perception of a test light which was presented for a fixed duration. The intensity of the test light was then reduced in measured The reduction in intensity proceeds until increments. the test light is barely visible. The absolute thresholds will continue to indicate greater sensitivity until the curve reaches an asymptote and a minimum threshold of light perception is indicated. The darkadaptation functions, dependent upon light adaptation, test light wavelength and retinal placement and can reflect the photopic, mesopic, and finally the scotopic range of vision.

Although control of pupil diameter through the use of an artificial pupil had been considered in earlier investigations (5,6), a natural pupil was found to be completely effective in dark adaptation investigations (1,7-9) and was used in this study.

When light adaptation period and test stimulus are held constant and subjects are permitted to use any part of their retinas to detect a test light, greater variability from subject to subject has been found (10). This may be due in part to variable rates of adaptation of different areas of the retina, or may indicate uneven distribution of rod and cone cells through the vertical and horizontal meridians. A fixation light was used in all tests in this study to control placement of the stimuli on the retina.

The previous work describing the sensitivity of the dark-adapted retina has focused primarily on overall retinal reception of broadband light sources and has assumed that dark adaptation is the same regardless of

meridian (1,2,4,11). A possible difference in sensitivity between horizontal and vertical meridians has been suggested (12). The superior portion of the vertical meridian was found to be the most sensitive area of the retina. However, only limited areas (15 degrees eccentricity and only one subfoveal meridian) were investigated, and only broad spectrum (white) light was used. That the superior portion is the most sensitive is not supported by a recent histological study of the retinas of the eyes of four human donors, which found the greatest number of cone cells [color vision and sharp focus] to lie in an elliptical pattern aligned with the horizontal meridian (13). Thus, one would expect to find greater sensitivity to long wavelengths within this portion of the retina. The suggested lack of concentricity leads to the possibility interactions between meridian and degree of of eccentricity and between color and degree of eccentricity. An interaction between color and meridian has been observed at 8 degrees eccentricity (14), suggesting that retinal photoreceptors are not concentrically distributed around the central retina but differentially with respect to meridian.

In the present study two wavelengths were used, a medium wavelength (green) and a long wavelength (red) light, to test three hypotheses concerning spectral dark adaptation at two different eccentricities at both the horizontal and vertical meridians. The present research explores how meridian differences may reflect the actual photoreceptor distribution of the human eye.

#### Methods

Statistical Methods and Design: The experimental design was a 3A (retinal meridian) x 2B (eccentricity) x 2C (hue) factorial design with repeated measures on all factors. Each subject was tested under each of the twelve experimental conditions. The unit of the dependent variable (absolute threshold) was the log of the average pulse width which corresponds directly to the average luminance in lumens/cm<sup>2</sup> steradian (lm/cm<sup>2</sup>sr). Average luminance is the product of the peak luminance value (12.00 x 10<sup>-6</sup> lm/cm<sup>2</sup> sr) and the duty cycle. The duty cycle is the ratio of pulse width to total time. The duty cycle ratio ranges from 1/10,000 to 1. The range of the pulse width varies from 1-10,000

microseconds and is presented at a frequency of 100 Hz, which is much faster than the human flicker-fusion threshold. The apparently continuous levels of luminance produced by the LEDs in the instrument are generated by a pulse width modulation circuit. Peak illumination is continuous at a pulse width of 10,000 microseconds at 100 Hz. Minimum threshold values were obtained by averaging the responses over the last five minutes of each fifteen minute dark adaptation test. The analysis of variance of the absolute threshold data was analyzed using BMDP. Post hoc comparisons were made using Tukey's Honestly Significant Difference test (HSD) to determine any significant difference between particular cell means. In house programs and hand held calculators were used for these additional tests.

**Hypotheses:** 1. Greater sensitivity will be found when the vertical and horizontal meridians are presented together because the chance of detecting one of four simultaneously presented test lights is greater than the chance of detecting one of only two test lights and the horizontal meridian will be found to be more sensitive than the vertical meridian; these results will support the recent histological findings of Curcio (13).

2. In the horizontal condition, greater sensitivity will be found with the red light-emitting diode (LED) at 2 degrees than with the green LED at 2 degrees, once again corresponding with the horizontal elliptical pattern of cones found by Curcio (13).

3. Greater sensitivity will be found at 16 degrees with the green LEDs than at 16 degrees with the red LEDs, corresponding to the study done at 8 degrees with green and red LEDs by Zwick which suggested an uneven distribution of photoreceptors (14).

	Algebraic Expression of Hypotheses										
1.	H (1): A3 > A2 > A1										
2.	H (2): A2 x B2 x C2 > A2 x B2 C1										
3.	H (3): B1 x C1 > B1 x C2										

Figure 1. Hypotheses expressed algebraically:A, retinal meridian (A1, vertical; A2, horizontal; A3, both vertical and horizontal together); B, degree of eccentricity from fovea (B1, 16 degrees; B2, 2 degrees); and C, hue (C1, green; C2, red).

**Euran Volunteers:** Eight normal healthy male and female human subjects whose ages ranged from 22 to 33 years (X=26.2) were tested. Complete ophthalmological examinations were given prior to testing, to determine normal ocular media, retina, and visual functions. This examination included tests to ensure normal vision (20/20 Snellen correctable, stereopsis, phoria, and color sense).

**Apparatus:** Spectral dark adaptation thresholds were measured using a microprocessor controlled dark adaptometer. The target test stimuli employed in this equipment are produced by LEDs which produce both red (610-660 nm) and green (555-575 nm) light (9). A select segment of the visible spectrum can be emitted by LEDs, unlike the incandescent light source used in conventional dark adaptometers, which emit broadband light.

The LED light sources were arranged in a cross pattern (Upper left Figure 1) and covered a 20-degree retinal area. The red and green LED displays were equated in peak luminance at approximately (12.00 x  $10^{-6} \text{ lm}/(\text{cm}^2 \text{ sr})$  by using radiometric measurement from an EG&G 580 Radiometer. All LEDs in both horizontal and vertical meridians were adjusted to meet this luminance output within a tolerance range of plus or minus 5 percent. The LED panels were mounted at the rear of a Ganzfeld hemisphere 30 cm from the chin and headrest. There are two crosses of LED light sources (one green and one red). Each display has an LED light source of its respective color at its center to function as an optional fixation light.



Figure 2. Schematic illustration of LED dark adaptation (14). Upper right: sample dark-adaptation function showing how the threshold pulse width decreases as dark adaptation increases. Lower right: pulse width modulation. Lower left: relative spectral transmission curves of the LED sources; green (C) and red (E) were used in this study. Top left: the cross pattern of the LEDs with the "x" marking the fixation points.

Procedure: Following the ophthalmological examinations the test procedure and the proper method of controlling the hand-held springloaded control button were explained to each subject. The subject was seated in an adjustable chair in the low-light dark adaptation The head and chin rests, which position the room. subject's line of sight in the Ganzfeld hemisphere, were adjusted. The experimenter exited the room and announced the start of the 2-minute light-adaptation period. The-25 watt incandescent lamp was extinguished, and the green LED display was activated.

The subject was instructed to fixate on a central LED of variable luminance at the same wavelength as the target stimuli presented in the parafoveal fields of vision. At first, the lights from the stimulus LEDs (2 or 4) were indiscernible, but as the luminance increased the subject was able to detect them. At this point the subject depressed a response button and kept it pressed until the target lights faded to a dimness below threshold. Thresholds were indicated both ascendingly and descendingly when the subject pressed and held down a button to indicate the perception of the stimuli. Once the stimuli faded just beyond threshold, the subject released the button and went on to another trial that tested the other wavelength. The red LEDs came on immediately and continued to alternate with the green LEDs during the 15-minute test period. Both green and red dark adaptation-functions were thus obtained.

The tests consisted of a pretest of global dark adaptation by using a variable luminance fixation light and both red and green LEDs, which were presented in all four quadrants simultaneously. These tests were followed by four additional tests: two testing the vertical meridian at both 2 degrees and 16 degrees eccentric to the fovea and two testing the horizontal meridian at the same two degrees of eccentricity. Each subject received an initial practice period of trial run and, as necessary, rest periods between runs.

### Results

Meridian variations were found. The horizontal was the more sensitive of the two meridians tested. The green led at 16 degrees was the most sensitive horizontal meridian condition  $(\bar{X} \approx .89)$ . The least sensitive condition with green light at 16 degrees was the vertical meridian condition (X=1.21). In Figures 2a and 2b, mean dark adaptation functions measured for red and green light for vertical, horizontal, and both vertical and horizontal meridians together are presented. The mean dark-adaptation functions were measured at both 16 and 2 degrees from fixation. The greatest sensitivity was found when the retina was tested at 16 degrees with green light for both meridians together  $(\bar{X}=.72)$ .



Figure 3a. Mean Dark Adaptation Functions at 16 Degrees Eccentricity for both Red and Green LEDs across all levels of Meridian. Note: Sensitivity increases as pulse width decreases.



Figure 3b. Mean Dark Adaptation Functions at 2 Degrees Eccentricity. [The curves for all three meridian conditions within hue group were almost identical-for the sake of clarity they are each represented by one curve.]



Figure 4. Main effect for factor A (Meridian)

Anova results indicated a significant main effect for factor A (meridian)  $\underline{F}(2,14) = 5.98$ ,  $\underline{p} < 0.0133$ (Figure 4.). These results support hypothesis 1. Main effects were also found for both factor B (eccentricity)  $\underline{F}(1,7) = 40.76$ ,  $\underline{p} < 0.0004$  (Figure 5.), and for factor C (color)  $\underline{F}(1,7) = 173.46$ ,  $\underline{p} < 0.0000$  (Figure 6.).



Figure 5. Main effect for factor B (Eccentricity).



Figure 6. Main effect for factor C (Color).

A Tukey's Honestly Significantly Different test (HSD) was performed to make specific post hoc comparisons of cell means (Table 1). The test indicated that both meridians together at 16 degrees with green

light A3 x B1 x C1,  $(\bar{X}=.71)$ , produced significantly lower thresholds than the , vertical meridian at 16 degrees with green light A1 x B1 x C1,  $(\bar{X}=1.21)$  (p < 0.05), this can be seen graphically (Figure 3a).

Comparisons of means of the meridians at 16 degrees with green light ( $\bar{X}$ = 0.94) with those at 16 degrees with red light ( $\bar{X}$ =2.37) (p < 0.05)(Figure 3a.)indicated greater sensitivity in the green light condition. Tukey's HSD test showed that there were no significant differences among means when subjects were tested at 2 degrees of eccentricity. These functions can be observed (Figure 3b).



Figure 7. Two - Way Interaction between factor A (Meridian) and factor B (Eccentricity).

A two-way interaction was found between meridian and eccentricity (Figure 7), F(2,14) = 10.60, p < 0.0016with significantly lower thresholds at 16 degrees with green light but not with the red.



Figure 8. Two - Way Interaction between factor A (Meridian) and factor C (color).



Figure 9. Two - Way Interaction between factor B (eccentricity) and factor C (color).

Significant two - way interactions were also found between meridian and color (Figure 8) F.(2,14) = 12.94, p < 0.0007, and eccentricity and color (Figure 9) F(1,7) = 511.88, p < 0.0000.

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## TABLE 1

TABLE	OF	MEANS
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<u></u>							
MERIDIAN	EC( TR	CEN- ICITY	LED COLOR	LEVELS A	of B	FACTORS C	CELL MEAN
Vertical	16	Deg.	Green	1	1	1	1.21
Vertical	16	Deg.	Red	1	1	2	2.70
Vertical	2	Deg.	Green	1	2	1	2.25
Vertical	2	Deg.	Red	1	2	2	2.19
Horizontal	16	Deg.	Green	2	1	1	.89
Horizontal	16	Deg.	Red	2	1	2	2.18
Horizontal	2	Deg.	Green	2	2	1	2.34
Horizontal	2	Deg.	Red	2	2	2	2.14
Both	16	Deg.	Green	3	1	1	.71
Both	16	Deg.	Red	3	1	2	2.22
Both	2	Deg.	Green	3	2	1	2.11
Both	2	Deg.	Red	3	2	2	1.71

A = retinal meridian; B = degree of eccentricity from fovea; C = hue; LED = light-emitting diode; Deg. = degrees.

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## TABLE 2

		DEGREES			
	SUM OF	OF	MEAN	-	TAIL
SOURCE	SQUARES	FREEDOM	SQUARE	F	PROBABILITY
MERIDIAN	3.44745	2	1.72373	5.98	.0133 *
ERROR	4.03814	14	.28844		
DEGREES	5.26098	1	5.26098	40.76	.0004 *
ERROR	.90346	7	.12907		
COLOR	8.86525	1	8.86525	173.46	.0000 *
ERROR	.35775	7	.05111		
MERIDIAN					
X DEGREES	1.78168	2	.89084	10.60	.0016 *
ERROR	1.17645	14	.08403		
MERIDIAN					
X COLOR	.64836	2	.32418	12.94	.0007 *
ERROR	.35076	14	.02505		
DEGREES					
X COLOR	16.48533	1	16.48533	511.88	.0000 *
ERROR	.22544	7	.03221		
MERIDIAN					
X DEGREES					
X COLOR	.44621	2	.22310	3.63	not
ERROR	.86027	14	.06145		significant

#### ANOVA SUMMARY TABLE

\* Significant at the 0.01 level or greater as stated.

#### Discussion

These data suggest that the photoreceptor mosaic is not concentric around the fovea in function or in agreeing with a recent histological distribution, investigation (13) which found that cone density contours were elliptical and aligned with the horizontal meridian. Conversely, the less sensitive vertical meridian may have less dense aggregations of photoreceptors and their distribution may be something other than concentric. The assumptions of concentricity drawn from Osterberg's histological investigation of one section of the retina of one human donor eye (16) may not be indicative of photoreceptor distributions in other areas of the retina. The distribution and density of photoreceptors may depend upon the area investigated. These data may indicate meridian effects which support a retinal model consisting of a differential dispersion of photoreceptors throughout the macula. The horizontal meridian has been shown to be more sensitive at 16 degrees than the vertical meridian at 16 degrees. Different densities of photoreceptors along the horizontal and vertical meridians or the different alignment of photoreceptors in the horizontal and vertical meridians may account for the difference in sensitivity. The retinal area of greatest sensitivity may actually be best described as an arc ranging from the horizontal temporal meridian up through the superior vertical meridian in the paramacular region and around and down to the horizontal meridian. However, further investigations with additional wavelengths at various degrees of eccentricity will be necessary to confirm this suggestion.

One possible explanation for the difference in meridian sensitivity is that the ratio of rods to cones in the horizontal meridian may be different from that in the vertical meridian; their grouping may also be different. Different forms of aggregation may mediate rod-cone inhibitory interactions. There may be fewer competing cone photoreceptor cells in the horizontal meridian at 16 degrees than in the vertical meridian at 16 degrees, leading to the possibility of an energy conservation model, which is described below.

A cone inhibitory effect may occur as the rods increase in sensitivity. The energy conserved by the rods, if there are fewer cone cells to inhibit, may be translated into rod sensitivity by intensifying the neural signal transmitted to the visual cortex. The amount and nature of the processing, which occurs at higher visual centers, may be determined by the origin of the signal and the particular group of photoreceptors from which the signal emanated.

Another possible reason for the difference in meridian sensitivity may be that in the horizontal periphery the orientation of the rods is more precisely toward the pupil than in the corresponding area of the vertical meridian (17,18).

More basic than this ontological possibility is the physiological explanation for the slower differentiation of the subfoveal vertical meridian, which may make the horizontal meridian seem more sensitive overall (19). Since the superior and inferior portions of the vertical meridian and the temporal and nasal portions of the horizontal meridian were tested together, it is not possible to know from these data which portion of each meridian is primarily responsible for the greater portion of sensitivity. Further study of these radians with the present device would be necessary. However, an investigation of the vertical meridian at 15 degrees of eccentricity using broadband light (12) found the superior portion to be significantly more sensitive. The lower sensitivity of the vertical meridian may be part to the lack of sensitivity of its due in subfoveal component, which may be affected by the embryonic fissure and subsequent slower differentiation of this area of the human retina (6).

A fourth reason for the differential findings of meridian sensitivity may be an effect of learning. The human visual system may or may not be without bias at birth, but since so much of human vision involves horizontal scanning, adaptation by the affected retinal area may produce differences. Thus, the higher sensitivity of the horizontal meridian may be the result of active horizontal scanning, which provides a higher rate of stimulation for the horizontal paramacular meridian, and which may, through frequent use, translate into greater visual sensitivity.

Our study indicates that meridian differences in the dark adapted retina exist. Dark-adaptation functions measured in the horizontal meridian at 16 degrees are steeper in slope and achieve lower final thresholds than those measured in the vertical meridian at 16 degrees.

The horizontal meridian shows consistently greater sensitivity than the vertical meridian in the parafoveal measurement made at 16 degrees. These new findings suggest that the distribution of retinal photoreceptors may not be organized concentrically in the macular region of the retina and that a differential dispersion of rod and cone photoreceptor clusters may exist with regard to retinal meridian.

We have postulated that extra-retinal functions such as cortical processing, learning and embryonic development are involved in the dark-adapted sensitivity of the human visual system.

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