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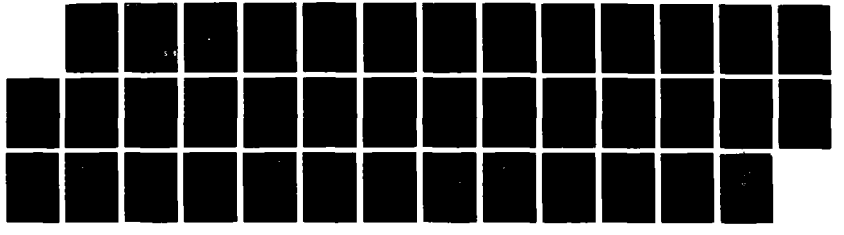

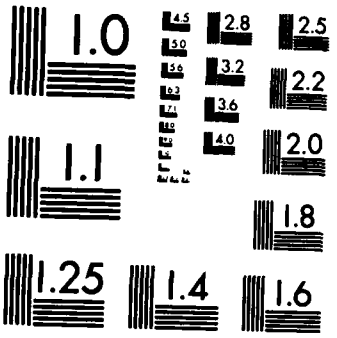

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PSYCHOPHYSICAL, BIOCHEMICAL AND HISTOLOGICAL STUDIES OF SPECTRALLY SELECTIVE PHOTIC DAMAGE TO PRIMATE RETINA

ANNUAL/FINAL REPORT

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February 25, 1987 ~~1986~~

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD 17-83-C-3003

University of Texas Health Science Center
Houston, Texas 77225

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SECURITY CLASSIFICATION OF THIS PAGE AD-A188113

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION University of Texas 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) Health Science Center at Houston P.O. Box 20036 Houston, Texas 77225	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-83-C-3003
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 61102A; PROJECT NO. 3M1-61102BS10; TASK NO. CF; WORK UNIT ACCESSION NO. 273

11. TITLE (Include Security Classification)
PSYCHOPHYSICAL, BIOCHEMICAL AND HISTOLOGICAL STUDIES OF SPECTRALLY SELECTIVE PHOTIC DAMAGE TO PRIMATE RETINA

12. PERSONAL AUTHOR(S)
Sperling, H.G., Wright, A.A.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual/Final	FROM 82/11/1 TO 87/02/01	87/02/25	34

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
20	06		spectral sensitivity; hue discrimination; argon-ion laser exposure; blue-blindness; brief exposures; blue laser light, under anaesthesia
20	05		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Spectral sensitivity and hue discrimination data are presented which show an irrecoverable loss of blue sensitive cone (B-cone) response following as little as 64. milliwatts per cm^2 of 458.5nm argon-ion laser exposure to rhesus monkey central retina over 4 hours of intermittent exposure. If reciprocity of intensity and exposure is linear, blue-blindness and attendant loss of discrimination of colored signals could result from fewer than 240 brief exposures to blue laser light over a several hour period. Evidence is also shown for green lights. During the current contract period, success was obtained in destroying the B-cone response in single sessions under anaesthesia and in obtaining spectral sensitivity measures from the focal ERG which are sufficiently stable and detailed to measure the response of R-, G- and B-cones, rods and the interactions between them. Evidence is presented, from the focal ERG, that the color-opponent type neural inhibition exists in rhesus, as in fish and amphibia, in the outer-plexiform layer of the retina, since it is detectable in the late receptor potential (a-wave).

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	UNCLASSIFIED

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Virginia M. Miller	301/663-7325	SGRD-RMI-S

DD Form 1473, JUN 86 Previous editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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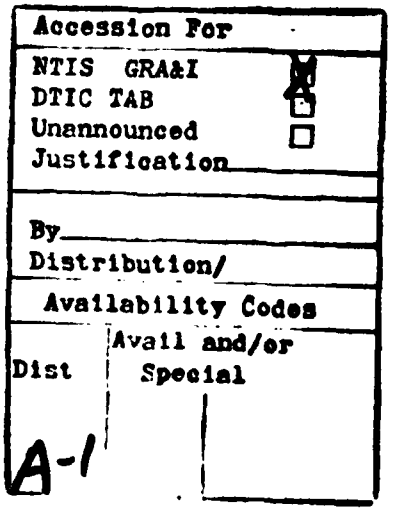

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A. Introduction

The goals of this program are to study the color blinding lesion which we reported in previous years (Harwerth and Sperling, 1971; 1975; Sperling, Johnson, and Harwerth, 1980) using rhesus monkeys. In those experiments, we found that short wavelength lights of intensities well below those which had previously been reported to produce retinal pathology, when applied intermittently on a 50 percent light-dark schedule for as little as 40 minutes per day for six to ten days produced irreversible loss of blue sensitivity in the case of blue light exposures and upwards of one week loss of green sensitivity with green light exposures. Histological study of the blue light exposed retinas revealed a pattern of degenerating receptors which matched that of primate blue-sensitive cones labeled by a cytochemical technique (Marc and Sperling, 1977; Sperling et al., 1980)

We also learned that steady light exposures of roughly equivalent energies and durations to the intermittent ones which caused the cone lesions produced an entirely different pattern of retinal damage, with pronounced changes in the pigment epithelium but only secondary damage to cones (Sperling et al., 1980). An early study had been undertaken to see if we could produce the color-blinding effect without the very costly and time-consuming behavioral training and testing. A trained rhesus was used, so comparative psychophysical measurements could be made. It was

deeply anaesthetized with nembutal, to the point of eliminating eye-movements and maintained that way for 80 minutes of exposure to 8×10^{-4} watts/cm^2 of 463 nm $\pm$ 6 nm blue light, on a 4 secs on, 4 secs off duty cycle, over a 30^{o} region, centered on the fovea. This was repeated on six successive days. Within one week the animal's spectral sensitivity was nearly identical in that eye with pre-exposure data. There was no "blue-blinding". Three months later the same animal was exposed to the same light (over the central 18^{o}) under our usual behavioral control procedure, resulting in complete foveal blue-blindness by our criteria. Thus, it appears that barbiturate anaesthesia prevents the color-blinding effect but not the RPE "photochemical" lesion, further distinguishing these two lesions.

We further investigated the anaesthesia question by incubating freshly excised rhesus retina in a NBT-succinate-Ringers medium with several anaesthetic agents. It is clear that nembutal interferes with NBT reduction to diformazan in the cones by more than 50 percent. Another anaesthetic agent, ketamine, was found to not produce such interference. Nembutal may prevent the effect by reducing the metabolism of the cell; if true, these results support our previously stated hypothesis that the light induced primary degeneration of cones--our color-blinding lesion--involves overdriving the metabolism of the cell.

In order to further investigate the nature of the color-blinding lesion, we planned to obtain multiple laser exposures on

the same monkey retina and attempt to make independent spectral sensitivity measurements on each exposed area. This would enable us to obtain action spectra of the lesion and to study the time course of recovery on an affordable number of animals and would save us the enormous training and testing times required by our earlier behavioral technique. To achieve these goals, we proposed to expose the eyes under ketamine anaesthesia and to test the exposed and control areas using the focal ERG technique, also under ketamine.

B. Progress During Present Contract Period

During the current contract period, we have made considerable progress on color-blinding and testing spectral sensitivity in rhesus monkeys under anaesthesia, with the use of ketamine and using the focal ERG to discriminate the three classes of cone activity and derive spectral sensitivity functions (or action spectra). We have compared photoreceptor activity from the a-wave with bipolar level activity from the b-wave, and both with behavior from our increment-threshold psychophysical measurements and found good correspondence. We accomplish this under ketamine anaesthesia, plus a retrobulbar block using either Duranest (etidocaine HCl) or marcaine plus xylocaine. We maintain the animals eye movement free during the entire period of a full spectral sensitivity measurement or a prolonged intense light exposure by repeated small doses of the

ketamine and of the retrobulbar block.

1. Anaesthetized Exposure Experiments

a. Apparatus and Procedure

Our successful intense light exposures under ketamine plus retrobulbar block utilize a three watt argon ion laser set to the 458 nm line. The optical system converges the beam to the shutter, then allows it to spread to fill a final lens which is very close to the pupil of the eye. The image subtends 22^{o} in Maxwellian view. The image is placed relative to retinal landmarks, which are illuminated by a tungsten light beam, superimposed on the laser beam by means of a beam splitter, as seen in figure 1. The two are placed using a small, moveable mirror, which also allows a simultaneous view of the fundus.

The apparatus and procedure for behavioral testing of spectral sensitivity has been described elsewhere (Harwerth and Sperling, 1975).

b. Results

Figure 2 shows a series of spectral sensitivity functions obtained on the right eye of a behaviorally trained rhesus, following exposures. The December 1982 and May and July 1983 functions were obtained following a single four hour exposure made in November of 1982, at a retinal irradiance of 6.4

milliwatts/cm^2 over the 22^o centrally placed exposure image. The June 1984 and December 1984 functions were obtained following a 64.0 milliwatts/cm^2 exposure for a total on-time of four hours, made in May, 1984. Note that the peak in the blue was lost following the higher irradiance exposure and had not recovered in seven months. This agrees wtih the data previously obtained from blue light-exposures under behavioral control (Harwerth and Sperling, 1971; 1975). We have now tested this animal behaviorally and with focal ERG's, and there has been no recovery of the blue peak in 18 months since the higher exposure. Figure 2b shows spectral sensitivity functions on the unexposed eye at coordinate times. The solid curve in 2a is the mean of the unexposed eye data. Figure 3 shows the mean of a number of determinations on the right eye before and after exposure. Clearly, the threshold for irreversible damage to the blue sensitive cones lies between 6.4 and 64.0 milliwatts/cm^2 for a total exposure time of four hours.

It is interesting to speculate on the minimum duration that we will eventually find experimentally for irreversible blue-sensitive cone damage. It is generally accepted that the threshold for gross thermal damage to the retina lies in the neighborhood of 1 watt/cm^2 (Sliney and Wohlbarsht, 1980). If this is the case, we can increase the energy of exposure as high as one watt/cm^2 over our retinal image, and if linear reciprocity holds between intensity and duration for our blue-cone lesion, we

can then reduce the exposure time by a factor of 15 without a gross burn. Of course, we must find out whether the one watt value is the threshold for gross damage, and we also must test linear reciprocity in this system. These steps are early on the agenda for the next grant period, but it is interesting to consider what minimum duration for blue cone lesions will place military personnel at hazard. One might argue that steady fixation of a large image for an extended period is unlikely in a military situation. However, that is not how our exposures are made. The animal is exposed in a 4 sec on, 4 sec off duty cycle, and the four hour figure quoted for this experiment was a cumulation of the on-time. Thus, if our assumptions are correct, 240 exposures of 4 sec. each within some period, probably as long as several hours, would cause the irreversible blue cone lesion. This is unlikely, but not impossible, for operators performing a repetitive task with a faulty system. And, of course, if a linear relationship does not exist between sensitivity and exposure time as we go to the higher energy levels, a fraction of that number of exposures might produce the lesion. Thus, laser hazards in the field become a very real possibility. Clearly, from a military standpoint, it is worth pursuing the threshold exposure regime in our future studies.

2. Focal ERG Spectral Sensitivity Studies

a. Apparatus and Procedure

Subjects

Two male macaca mulatta adults 4 to 6 years of age and about 8 kg in weight were used as subjects.

Apparatus

Optics

Light for both repetitive stimulation of the ERG and for the adapting fields is produced by a 1600 watt xenon compact arc lamp. A series of mirrors, lenses, and beam splitters produces a Maxwellian view on the retina of the subject (Figure 4). The test flash is adjustable in size from 0.5 to 16 degrees of angular subtense. The background can vary up to 21 degrees. For these studies, the background was always 21 degrees and the test flash 12 degrees. The wavelength composition of the test field was obtained with Oriel interference filters (half-bandwidth = 10 n.m.). A 30,000 troland white background was always present; an additional chromatic adapting background was sometimes added. This was produced with interference or broad-band Wratten gelatin filters and was coincident with the 21^{o} white background. Test intensity was controlled with neutral density filters; background intensity by means of a neutral density wedge. Calibration was done with an EG&G model 580 radiometer.

Electronics

The ERG was recorded from an ERG-jet corneal contact lens electrode manufactured by Universo Sa-La Chaux-de-Fonds of Switzerland. A wire threaded superficially through the skin just below the gumline served as the reference electrode; ground was at the ipsilateral ear.

The signal was amplified by a Princeton Applied Research (PAR 113) pre-amplifier with the bandpass set from .1 to 30 Hz and the gain at 10K. This A.C. coupled signal was fed through a Mentor F-60 notch filter to remove 60 Hz noise, then into a Nicolet 4094 digital oscilloscope where waveforms were cumulated, averaged, and stored on diskettes.

Procedure

The stimulation frequency of 2.5 Hz was chosen to produce 150 repetitions of the presentation in one minute. The light duty cycle was varied according to the portion of the ERG waveform on which we wished to focus our attention. In general, to favor the on responses (a- and b-waves), the on-portion of the light cycle was 70 ms, the off-portion 330 ms.

The animal was anesthetized with 5 mg/kg of ketamine HCl mixed with .05 mg/kg acepromazine maleate. Drops of 1% atropine sulfate (for pupil dilation), 1% cyclopentolate HCl (to relax accommodation), and .5% proparacaine HCl (for corneal anesthesia) were placed in the eye. A retrobulbar block was administered by

threading a Critikon Cathlon IV catheter placement unit into the orbit beneath the eyeball and injecting .75% marcaine HCl into this cavity to temporarily paralyze the muscles governing eye movements.

The animal was next placed on a table with several degrees of translation. Electrodes were attached and the test beam was visually guided through the center of the pupil to the fovea by adjustment of the table. Stimulation began after a few minutes of adaptation to the background.

In general, five or more intensities for each of several wavelengths were chosen. These intensity series were regularly attempted at 10 nm intervals for all wavelenghs between 420 and 660 nm, excepting 510, 630, and 650 nm. Wavelengths at the spectral extremes were frequently unobtainable, particularly in the presence of an added chromatic field, due to a poor signal-to-noise ratio where ERG signals were weak. For each of these intensity-wavelength combinations, 150 stimulus presentations were averaged into a single waveform. Figure 5a shows a sample waveform obtained this fashion, with the a-, b-, and d-waves marked. Figure 5b shows sample voltage-intensity curves and how the spectral sensitivity plot in Figure 5c is derived from them by estimating what radiant intensity is required to produce a criterion voltage. Dropping a line to the radiant intensity axis (vertical dashed line) from the intersection of the voltage-intensity curve with the criterion response (horizontal dashed

line), the threshold intensity can be read directly on the abscissa. The height of the a- and b-waves were measured by computer. The b-wave was measured "peak-to-peak", that is, the height of the displacement from the negative peak of the a-wave to the peak of the b-wave was calculated. A-waves are usually measured from the baseline at stimulus onset to the maximal negative displacement just prior to onset of the b-wave. We did not use this procedure because we felt that since the temporal onset of the b-wave varies as a function of both intensity and wavelength, a-waves measured in this fashion might be contaminated by this variable b-wave onset. We therefore measured all waveforms to find the earliest b-wave onset and measured all a-waves before this point, which occurred very close to 28 milliseconds after stimulus onset. In support of this logic, when waveforms obtained in the presence of the b-wave blocker APB were compared with those obtained before the drug took effect, the a-waves were coincident up to at least 28 ms after light onset. Figure 6 demonstrates this for a pair of waveforms. The bold curve was taken shortly after APB injection. The b-wave has already begun to disappear somewhat, but still is larger than the a-wave in amplitude. The lighter curve was taken an hour later and shows much less b-wave contamination. The leading slope of the a-wave, however, is identical in both cases. If the b-wave were intruding upon our measurement of the a-wave, it would presumably cause a deflection that would be detectable in this figure. Instead, the a-wave slope remains identical for

the two curves up to the very instant that the slope of the curve changes to a positive-going state. This supports the conclusion that our a-wave measurements are uncontaminated by b-wave intrusion.

To generate intensity-voltage curves, the height of the b-wave was plotted for each wavelength versus the intensity that produced it. For a given criterion response, e.g., 10 microvolts, the intensity that would produce that response was then read interpolated on these curves for each wavelength. Plotting these intensities versus wavelength then yields a spectral sensitivity function. The criterion response amplitude was chosen so as to fall within the range of sampled voltages. The b-wave criterion was taken at 10 microvolts, the a-wave at 5 microvolts. For the purpose of comparing absolute sensitivities of b- with a-waves, b-waves can be plotted displaced by the average amount of light intensity required to move from 10 to 5 microvolts on their voltage-intensity curves.

The size of the ERG measurements tended to vary somewhat over the course of a session. To remove this variance from the measurements, a normalization procedure was employed. Before each set of wavelength determinations, a standard wavelength-intensity measurement was made. An average of the standard taken before and after a set of wavelength-intensity determinations was used to normalize those measurements to an average level for the entire session.

b. ERG Results

Figure 7 shows mean spectral sensitivty functions obtained from the a-wave and the b-wave of an animal's eye, and compares them with behavioral threshold spectral sensitivity obtained on the same eye. These results show several very interesting findings relevant both to the applied goals of this program and to some very basic issues in the physiology of retinal function. We obtained consistent functions from the focal ERG which show some of the features of our behavioral threshold functions. The solid lines drawn through the three functions are from our linear interaction model of threshold spectral sensitivity (Sperling and Harwerth, 1971). Both the a- and the b-wave show a mid-spectral channel which is best modeled by +G-R interaction. Both show a long wave channel which is best modeled by +R-G interaction. The dashed line fitted to the a-wave data is the envelope of the red sensitive and green sensitive cones acting without subtractive interaction (putting the -R and -G terms to zero). Clearly the mean data, whose one standard deviation variability is shown, are better fitted using the inhibitory interactions. It is also clear that those interactions, involving the middle and long wave channels, increased considerably by the time the mean behavioral threshold sensitivity is achieved (bottom curve).

The short-wave channel contributed by blue sensitive cones, is not visible in spectral sensitivity curves obtained with the ERG measured against a neutral background. In Figure 8, however,

we show results from the blue-blinded animal of Figure 2, obtained from the b-wave against an intense orange background. The normal eye, represented by the open circles, shows a clear blue peak. The blue-blinded eye, represented by the crosses is missing this peak. Figures 7 and 8 taken together demonstrate that we can adequately and reliably quantify the response of the three classes of cones using our focal ERG technique.

As to the basic significance of the results, since no one has succeeded in recording from the photoreceptors or the bipolar cells of mammals using intracellular microelectrode techniques, these data represent a significant new finding. If the data shown here continue to hold up, they establish that opponent-color interactions in primates, as in fish and amphibia, are found in the cones themselves, because the a-wave is produced in the photoreceptors (see Brown, 1968). They undoubtedly result from cone to horizontal cell to cone feedback, as in those lower vertebrates. The behavioral data show a considerable increase in the depth of the 580 nm notch, making it clear that at or above the ganglion cell level there is considerably enhanced inhibitory interaction between red and green cones.

3. Psychophysical Hue Discrimination Results

The combination of behavioral and neurophysiological results make this research program particularly strong. Neither can really be complete in itself. The neurophysiological experiments

lead us to identify the mechanisms and site of the effects of intense light exposure. The behavioral experiments document the degree to which these exposures and the resulting retinal changes, affect vision. Our hue discrimination experiments are particularly important in this regard as they amplify and support our spectral sensitivity and ERG results.

a. Apparatus and Procedure

A complete description of the apparatus and procedure are in press (Wright, Sperling, and Mills, 1987). As shown in figure 9, the apparatus utilizes four Maxwellian view pathways, for the background and three spectral targets. Each spectral target is supplied by a separate double monochromator. These paths combine to provide an 18°, 3000 td, equal energy white surround, through which are focused three spectral beams, apertured to .75 deg. diameter and arranged in an apex-down triangle whose maximum subtense is 2 deg. The monkey sees a large white circle with three homogeneous spectral circles in its center. These are adjusted to equal luminance based on the monkey's increment threshold spectral sensitivity to equivalent spectral bands against the same background.

The spectral stimulus may be set to any wavelength of the visible spectrum. The center spot is considered the standard. The monkey must judge whether the right or left stimulus is different from the center one. The location of the differing stimulus is randomized from trial to trial. The monkey hears a

tone, holds down a lever, and the three spectral targets appear. He must then move the lever right or left, signifying the stimulus which is different. Hue discrimination thresholds are measured by the method of limits beginning with large wavelength differences and reducing them in steps. An incorrect judgement following a series of correct ones is taken as a threshold value.

b. Hue Discrimination Results

Figure 10 shows that, for our unilaterally blue-blind monkey, differences in hue discrimination in the blue region are much larger than by other measures. Figure 11 compares the blue-blinded eye with human tritanopes from Wright's (1952) classical study on congenital tritanopes. It will be noted that our monkey has the same shape function as the tritanopes, and that the quantitative loss is very close. We have determined that this monkey is relying solely upon color cues to perform this task. In addition to correcting the intensity of the stimuli daily based upon that day's spectral sensitivity measures, we have adjusted them based upon the human's standard photopic luminosity function. This large change in brightness values did not change the hue-discrimination curve. Thus, we establish that the animal uses color, not brightness cues to make hue discriminations. These data allow us to conclude from a second, more sensitive psychophysical technique that we cannot reject the hypothesis that the intense blue light exposure regime renders primates tritanopic. Thus, we can venture that the perceptual losses of

humans exposed to such light regimes may be very similar to those perceptual losses shown to occur in congenital tritanopes. Our future program will further test these predictions but they appear justified on the basis of the hue discrimination results to date.

Figure 12 shows the results obtained during the recovery from a green-blinding exposure. The individual curves are shown sampling a period of nineteen days following the last day of exposure, during which period there was complete recovery from the green-blinding based on our threshold spectral sensitivity measurements. Here we see that on the first day there is enormous elevation of the wavelength discrimination thresholds over the region from 490 to 590 nm, the green part of the spectrum. Of considerable significance is the fact that there is no elevation of threshold discrimination at the two minima, 490 nm and 590 nm.

We have obtained corroborating evidence from two subsequent experiments which further validate that the minima do not move and that considerable discrimination loss occurs only between them. Two manuscripts are to be submitted to Vision Research. The first, now in press, deals with hue discrimination and spectral sensitivity of our monkey's blue blinded and normal eye. Comparisons are made to human tritanopia. The second deals with green blinding and recovery from green blindness. We have several such green exposures on each eye. They make an

impressive series of data that exemplify the power of our approach.

One important finding from this research is that none of the existing models of color discrimination are capable of explaining these hue discrimination results following green-blinding. All such models are based on either a weighted difference or a weighted ratio of red to green and blue to green receptor response, or on a composite of rate of change of response in all three classes of cones for different spectral regions. In no case can the various models predict a change in discrimination over the green region with no elevation at the 590 minimum. Our model will have to account for this important finding as well as to take into account that (1) our green-blinding cannot be complete, that is we are not rendering the animals completely dichromatic by the hue discrimination test (as we are in the blue-light case), (2) some feedback or second stage gain control must exist which holds the minimum down despite a considerable loss of green sensitive cone response.

C. Conclusions and Future Indications

It is clear that this research program is making progress on basic color vision questions and practical ones that bear upon hazards that may occur in future high-technology military settings. We have succeeded in color blinding under anaesthesia

in single sessions (as well as in behavioral settings over multiple days) and are now poised to determine the minimum duration over which the color-blinding lesion can be obtained by increasing the retinal irradiance using an ion laser. The anaesthetized technique will be useful in much more easily producing the green-blindness as well as the blue-blindness, thus facilitating further study of the interestings finding just discussed. Also, we are now ready to perform action spectrum studies and to study the time course of both recovery and of degeneration, because we can make multiple exposures to the two retinas and test each area independently using the focal ERG with sufficient accuracy to gauge partial loss of each class of cones. In working to achieve these interim goals, we have turned up important basic evidence in both the area of retinal color interactions and of modeling color discrimination data.

D. Dosimetry

Intensities of all light sources are measured with an EG&G radiometer (Model 580) and spectroradiometer (Model 585), equipped additionally with a laser probe and narrow beam adapter. Measurement of beam intensities for both chromatic and white beams is done by converting the radiometric intensity as read from the machine to watts by the equation:

$$\text{watts} = A \times I / S$$

where A is the area of the radiometer window, I is the intensity

read by the radiometer, in amps, and S is the radiometric calibration constant that converts the result to watts. Further conversion to watts/cm^2 requires an estimate of the diameter of the eye in order to calculate the area illuminated. Since the beam is focused on the pupil, the lens of the eye can be ignored, as it will not further refract the focused beam. Using a standard equation from optical systems, the size of the retinal image (S_R) can be calculated from the diameter of the collimated beam (S_B), the focal length of the final lens (F_L), and the diameter of the eye (F_E). This equation,

$$S_R = S_B (F_E / F_L) .$$

allows specification of the illuminated area in cm^2. The solid angle in steradians is calculated as $4*pi \sin^2 (A/4)$, where A is the angle of the convergent beam in radians. The power of the beam in watts/steradian can then be calculated.

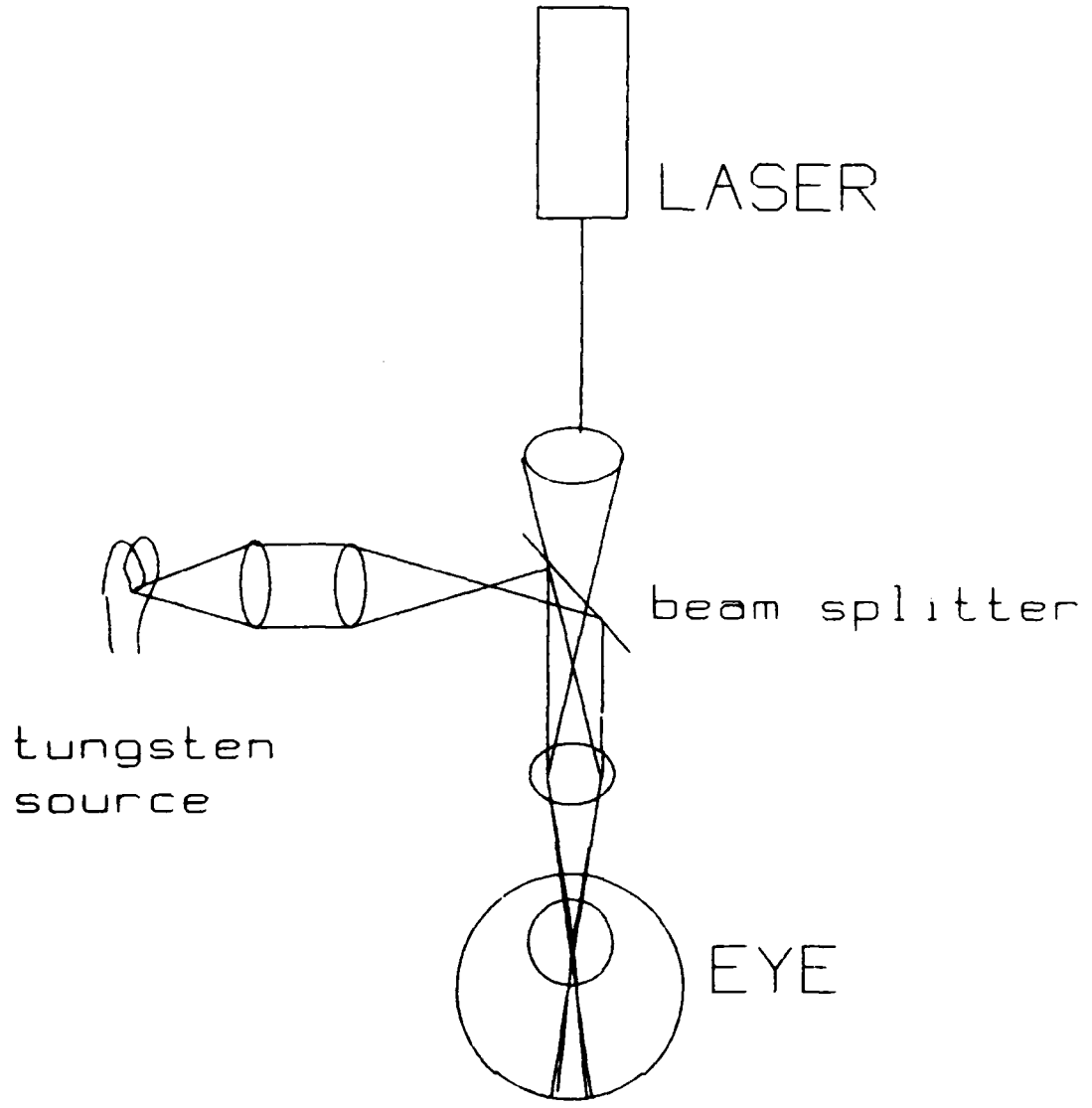

Figure 1

Apparatus for exposing monkey eyes to laser radiation.

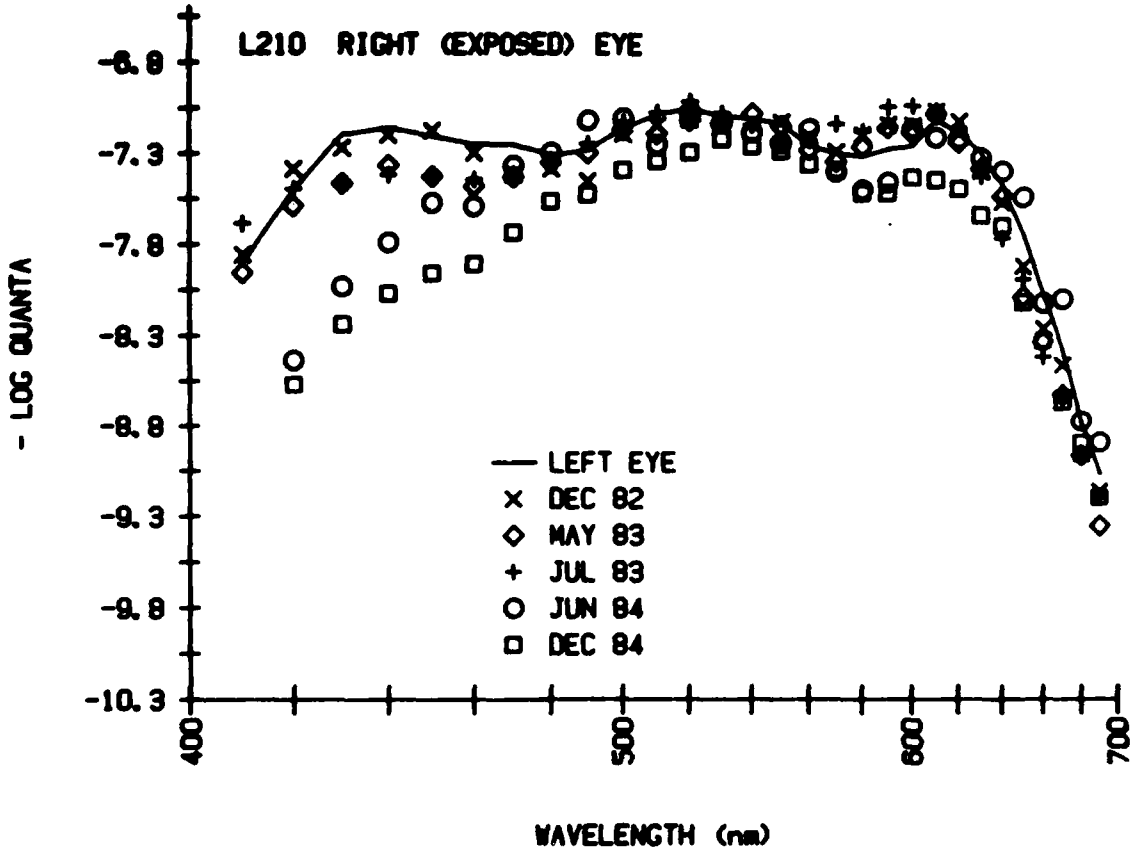

Figure 2a

Spectral sensitivity of a monkey's right eye measured 1 month (x), 6 months (◊), 8 months (+) after a four hour anaesthetized exposure to alternating 458. mm, 6.4 mw/cm^2 irradiance centered on the fovea, and 1 (o) and seven (□) months after exposure for 4 hours to alternating 458.5 nm light of 64 mw/cm^2 on the fovea. Solid line is mean sensitivity of the unexposed eye in December, 1984.

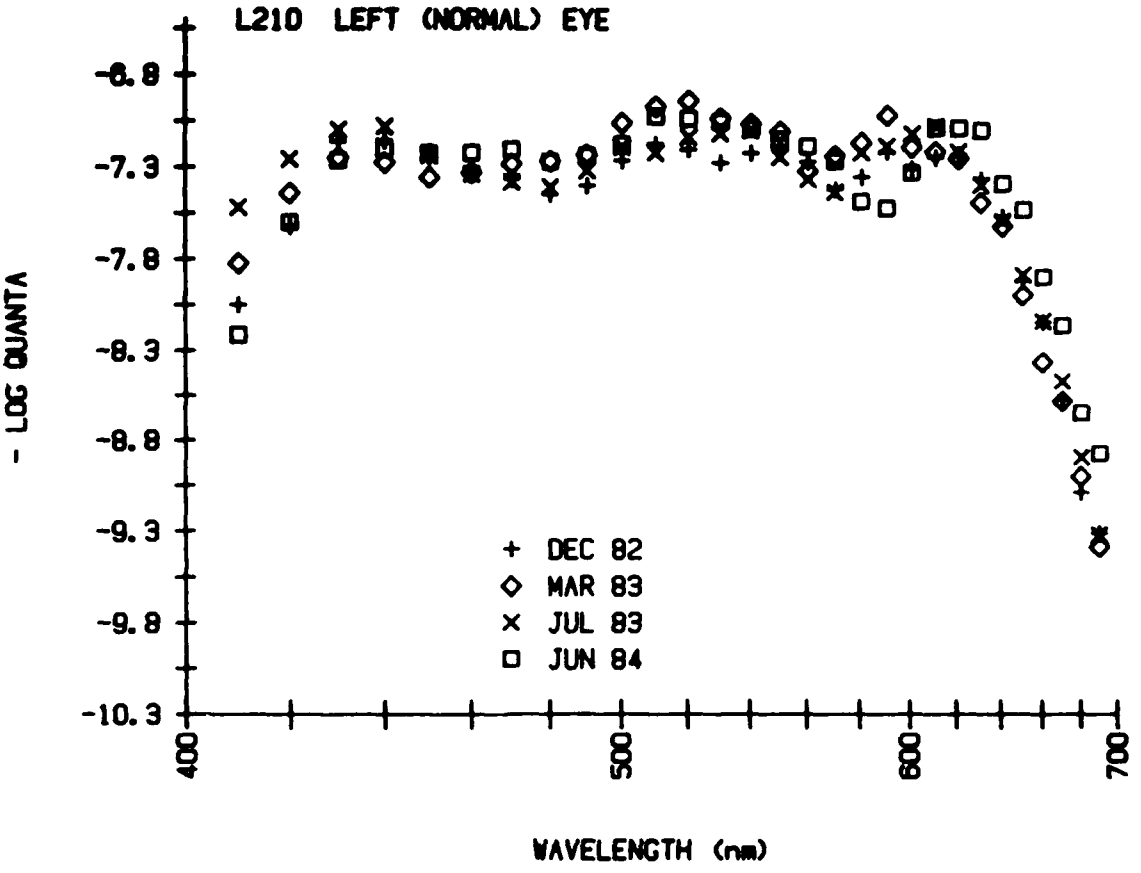

Figure 2b

Spectral sensitivity of unexposed (left eye) at coordinate times after right eye exposure.

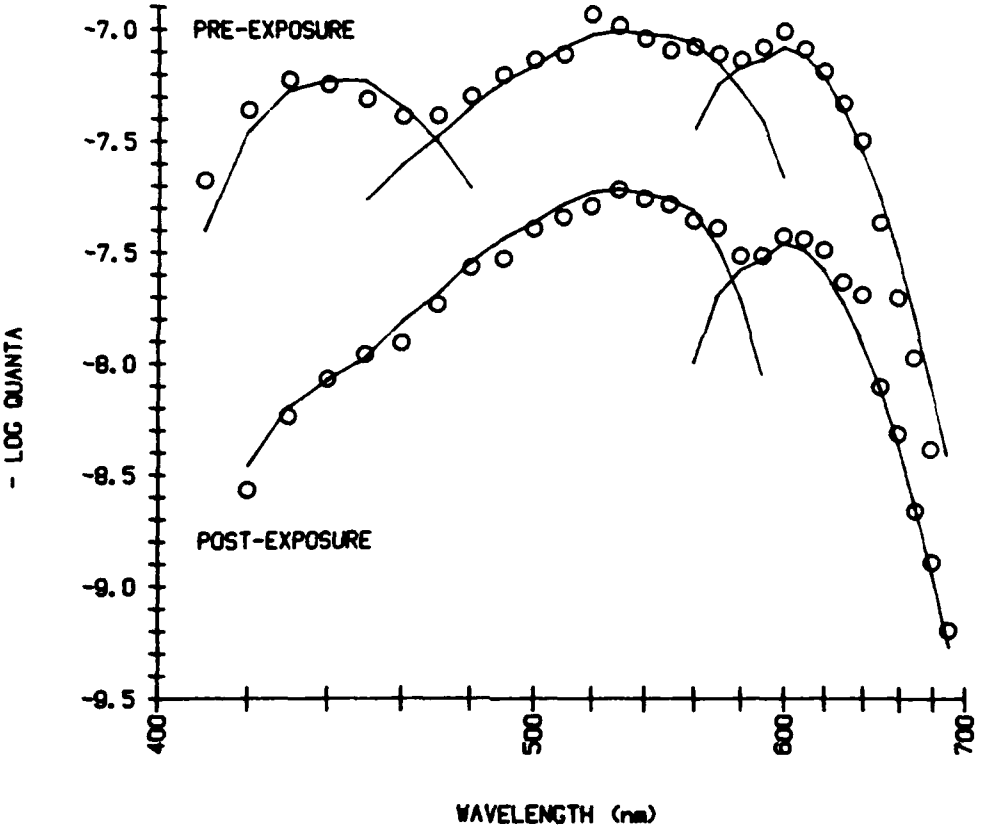

Figure 3

Mean spectral sensitivity of exposed eye before and after exposure. Curves are from Sperling and Harwerth (1971 model. Pre-exposure data requires a blue cone channel. Post-exposure data are fit by red and green cones alone.

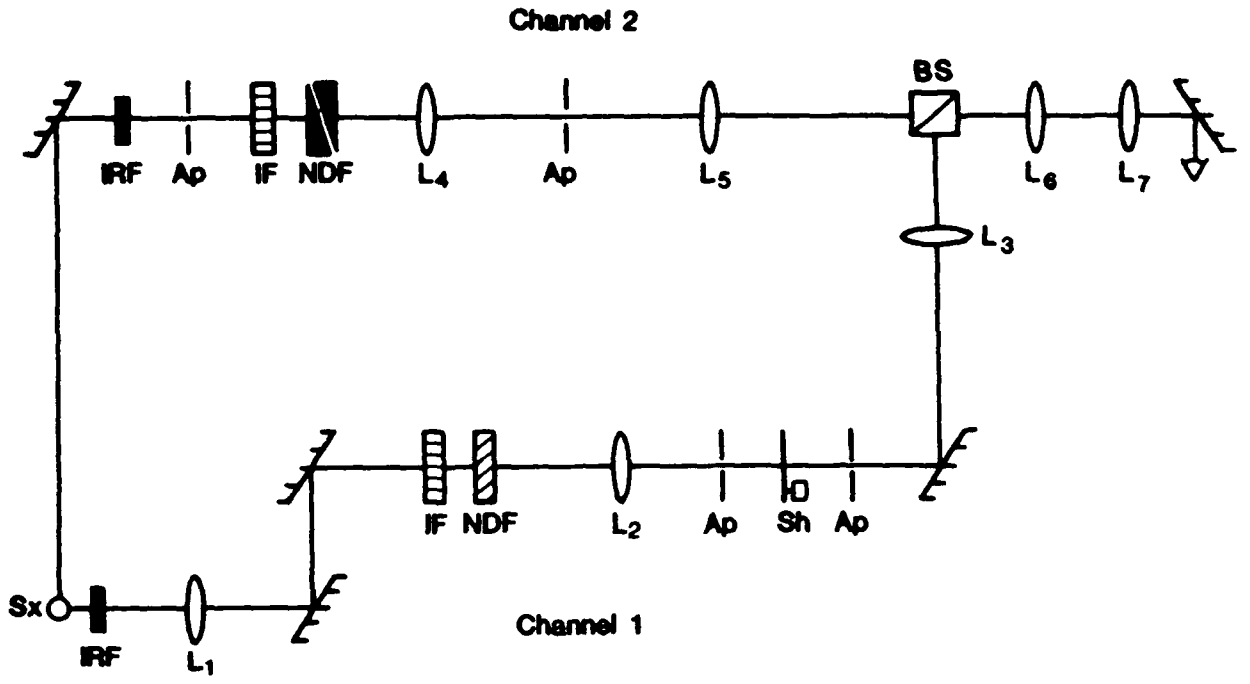

Figure 4

ERG apparatus

Components: Ap, aperture; BS, beam splitter; IRF, interference filter; L, lens; NDF, neutral density filter; Sh, shutter; Sx, xenon source lamp

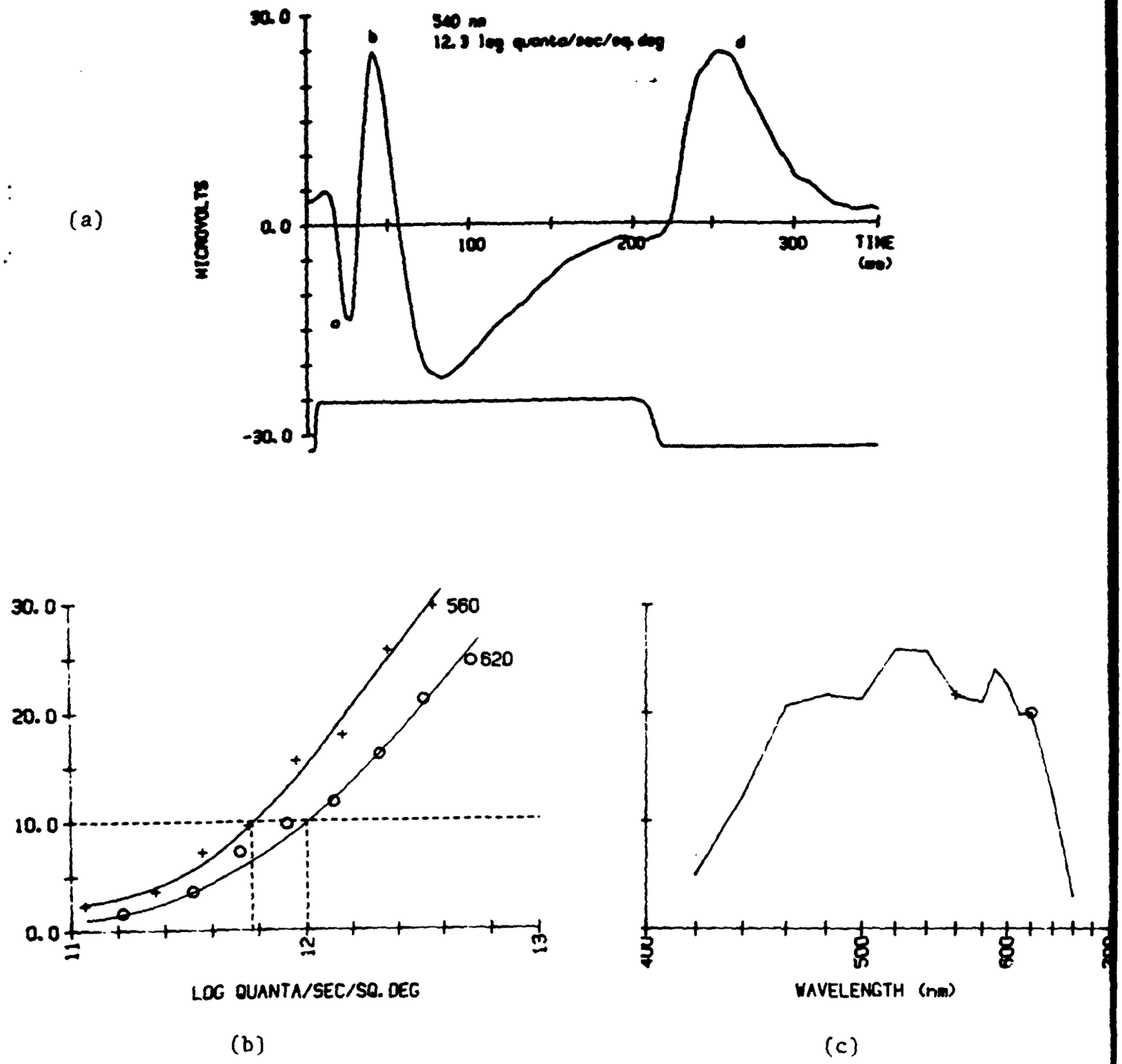

Figure 5

Calculation of spectral sensitivity curves from the ERG (a) A typical averaged ERG, with the a-, b-, and d-waves marked. (b) Two voltage-intensity curves with a 10 microvolt criterion response shown. (c) A spectral sensitivity curve, with the two threshold log quanta measurements derived from (b) shown.

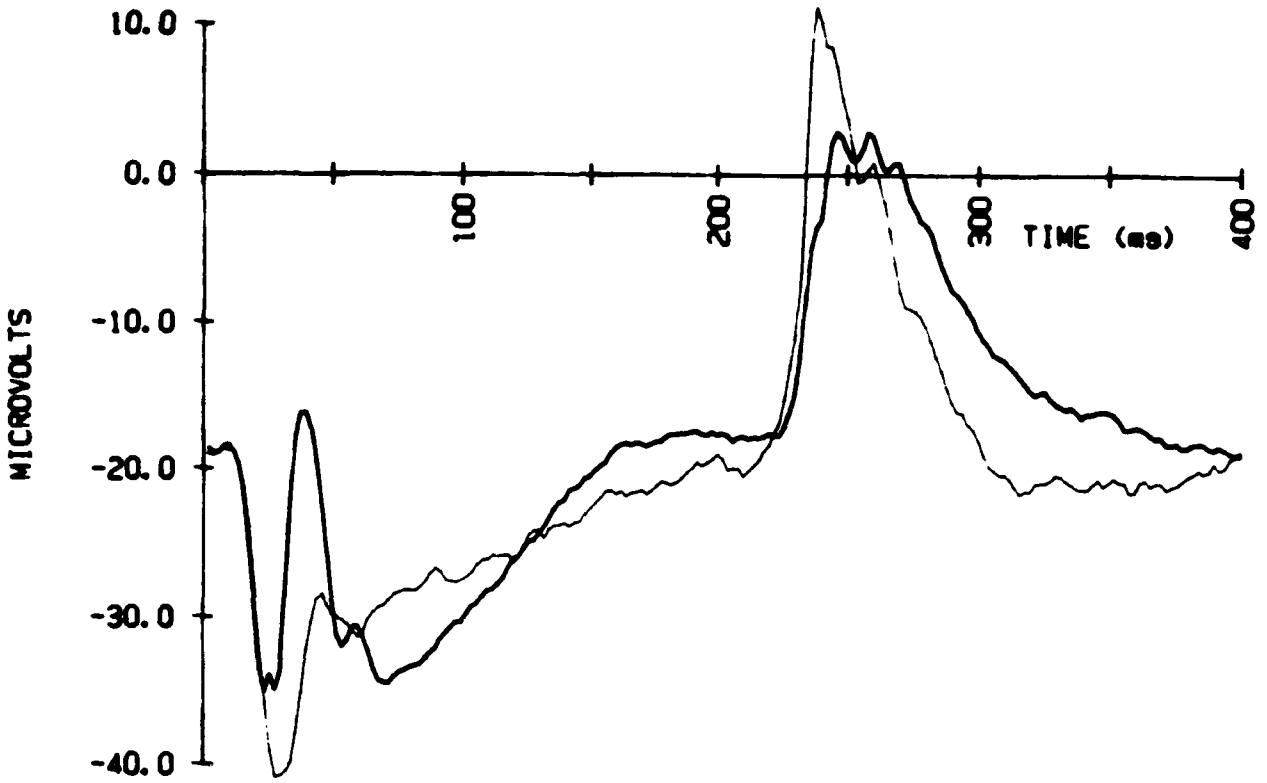

Figure 6

The ERG measurements taken after injection of APB. The bold line represents measurements 15 minutes after injection, when the b-wave is still prominent. The lighter line was taken 1 hour after injection; the b-wave is largely blocked.

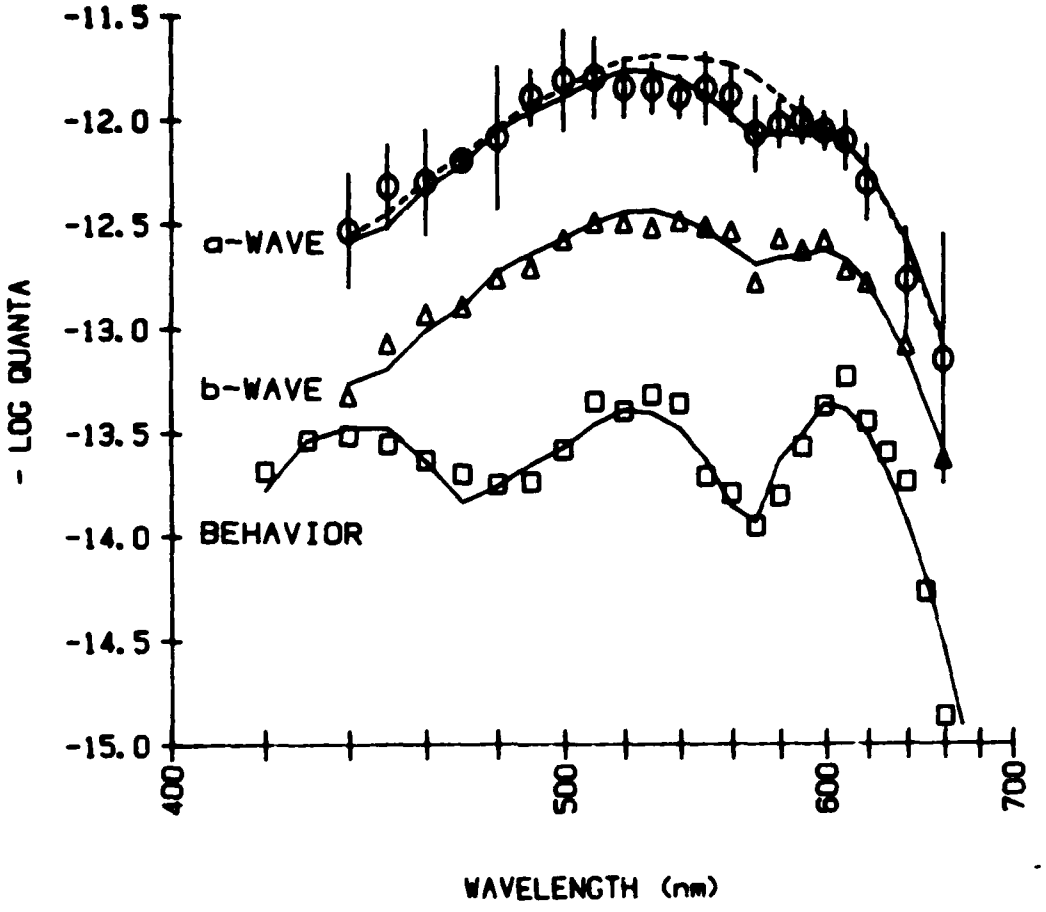

Figure 7

Spectral sensitivity functions for rhesus eye derived from focal ERG a-wave, b-wave, and behavioral increment thresholds.

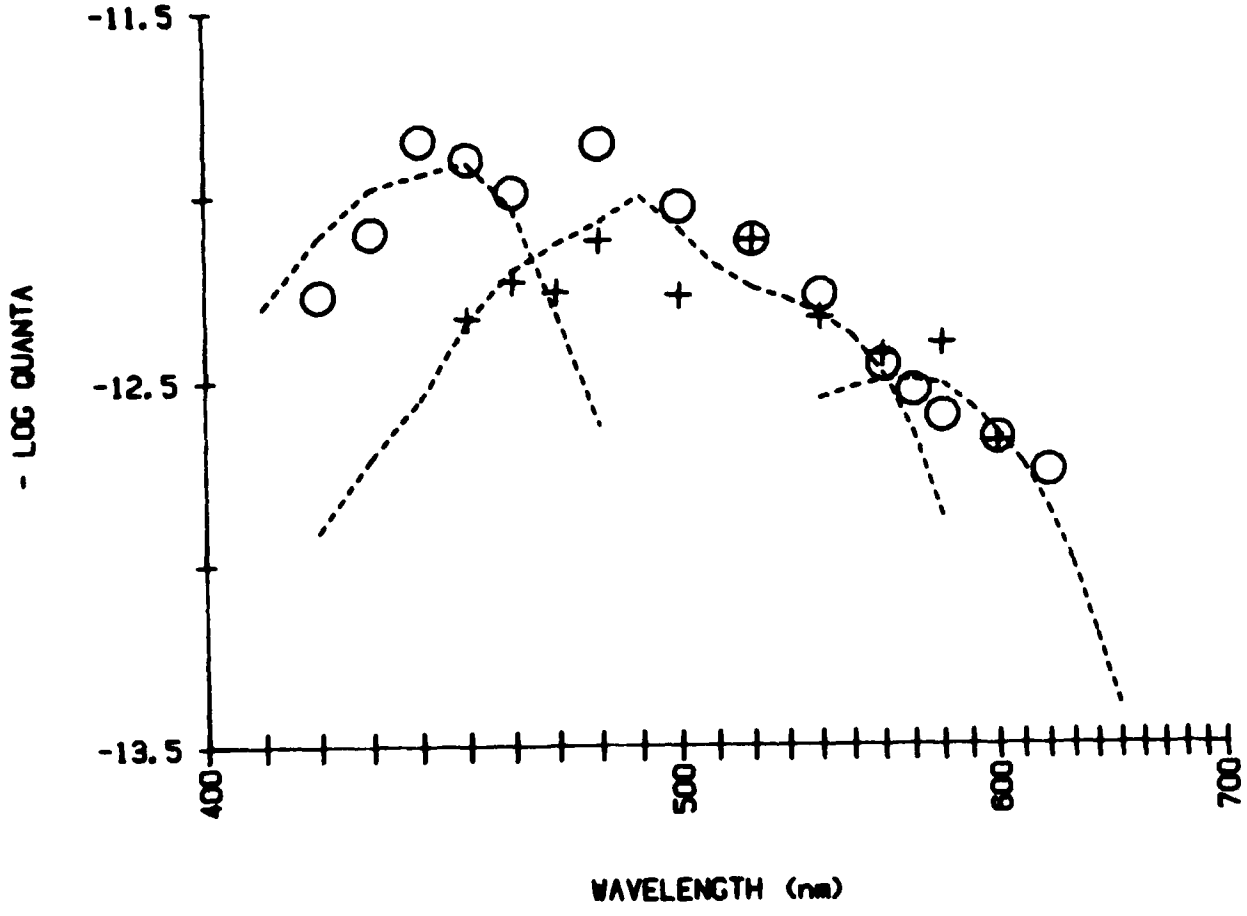

Figure 8

Spectral sensitivity functions derived from focal ERG b-wave against 10^6 troland 640nm background taken on normal (open circles) and blue-blind (crosses) eye of monkey of figures 1 and 2.

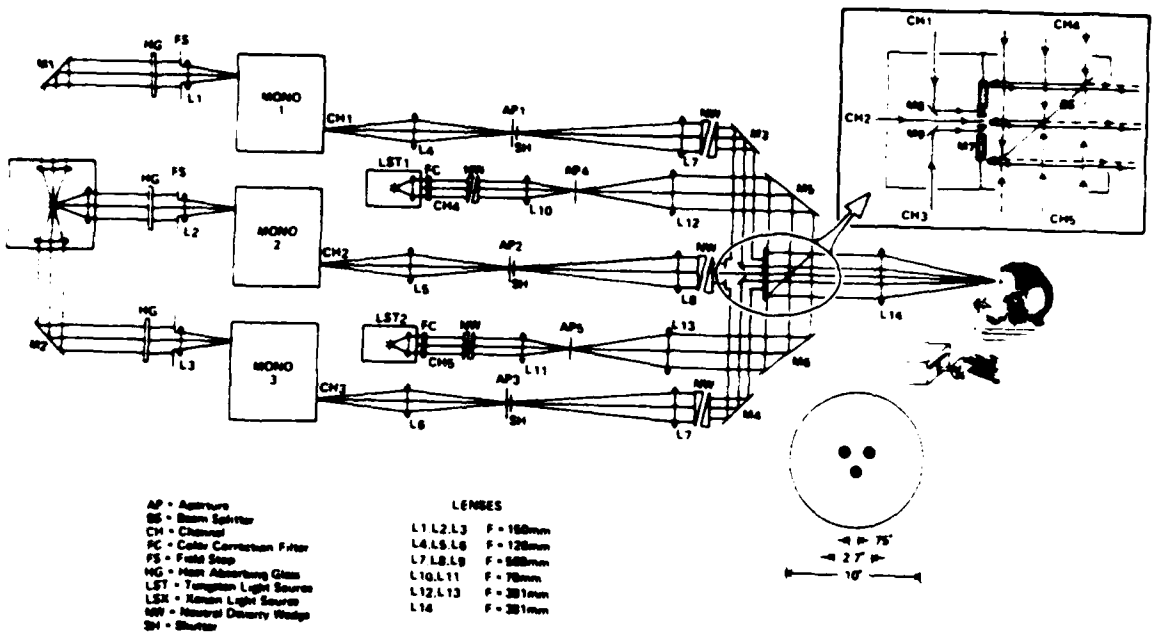

Figure 9

Hue discrimination apparatus

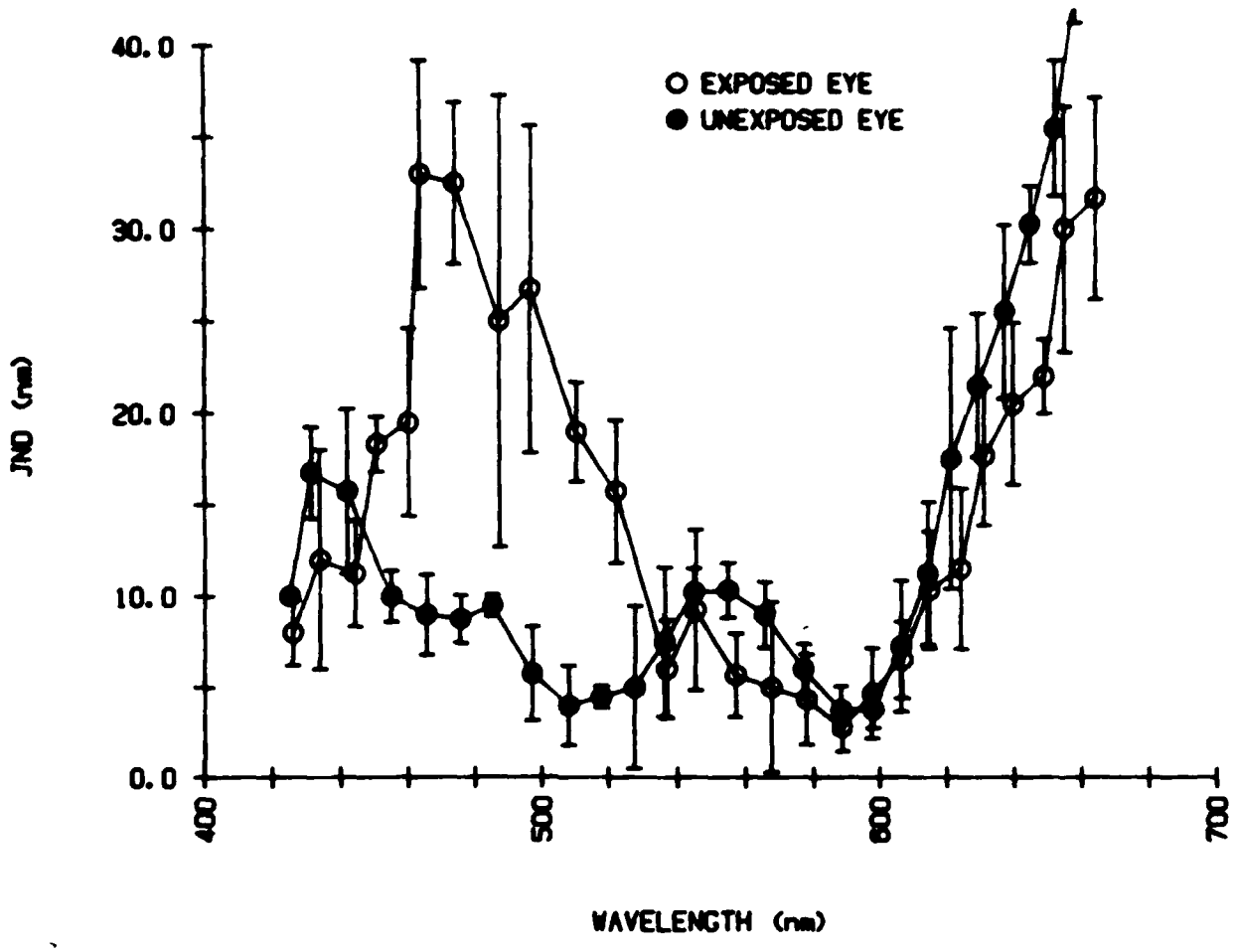

Figure 10

Mean hue discrimination function of rhesus' normal (filled circles) and blue-blinded (open circles) eyes. Vertical bars are ±1 standard deviation.

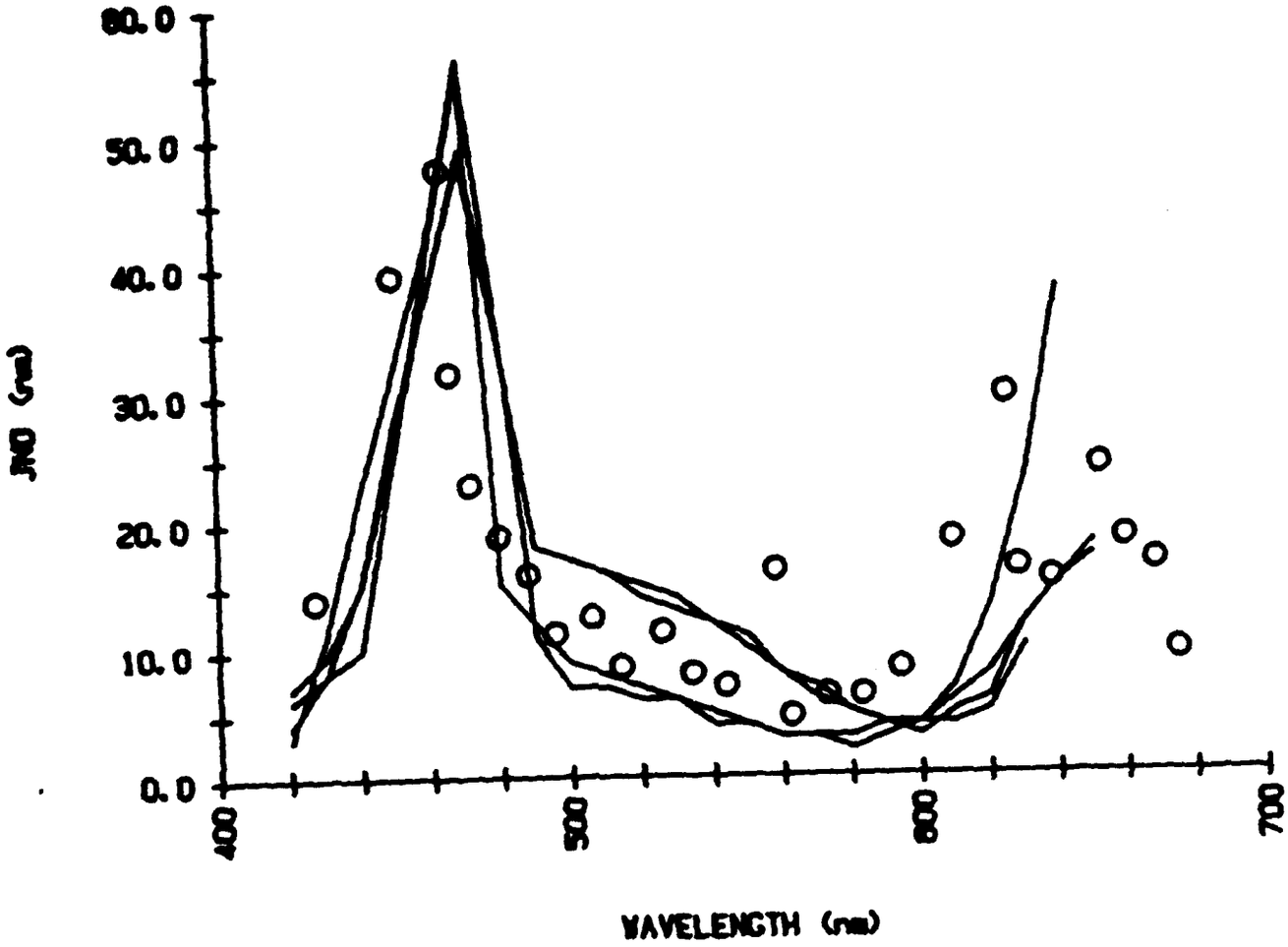

Figure 11

Mean hue discrimination of monkey's blue-blinded eye (circles) compared with hue discrimination functions of W.D. Wright's four congenital human tritanopes (solid curves).

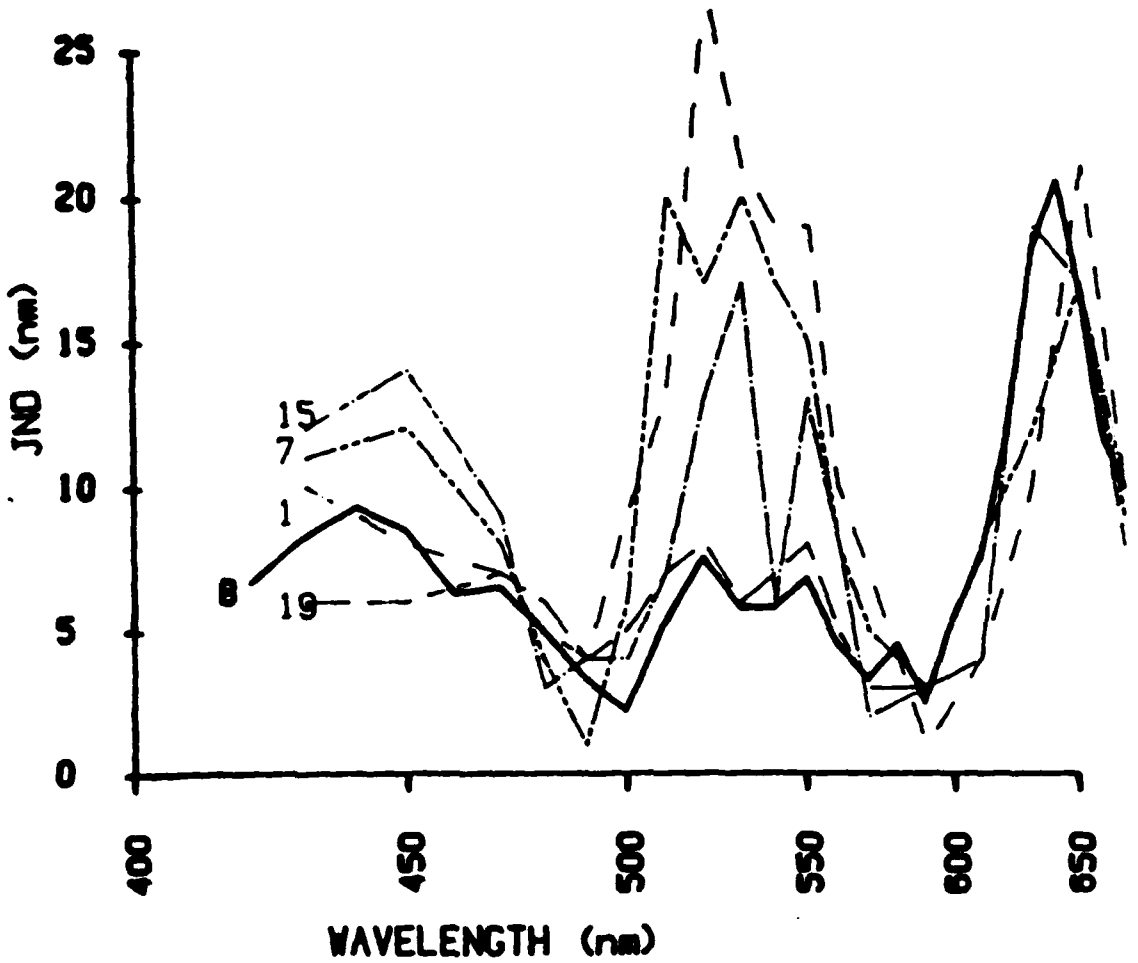

Figure 12

Hue discrimination before (heavy solid) and at different number of days after (as labeled on left) green-blinding of rhesus normal eye. Note that by 19th day recovery is complete.

LITERATURE CITED

Brown, K.T., The electroretinogram: Its components and their origins. Vision Research, 8, 633 (1968).

Harwerth, R.S. and H.G. Sperling. Prolonged color blindness induced by intense spectral lights in rhesus monkeys. Science, 174, 520-523 (1971).

Harwerth, R. S. and H. G. Sperling. Effects of intense visible radiation on the increment-threshold spectral sensitivity of the rhesus monkey eye. Vision Research, 15, 1193-1204, (1975).

Sperling, H.G. and R.S. Harwerth. Red-green cone interactions in the increment threshold spectral sensitivity of primates, Science, 172, 180-184 (1971).

Sliney, D. and Wolbarsht, M., Safety with lasers and other optical sources, Plenum Press, New York, 1034 pgs., (1980).

Sperling, H.G., ed., Intense light hazards in ophthalmic diagnosis and treatment. Proceedings of a Symposium, Vision Research, 20(12), (1980).

Wright, A.A., Sperling, H.G., and Mills, S.L. Researches on a unilaterally blue-blinded rhesus monkey. Vision Research, 27(9), 1551-1564 (1987).

Wright, W.D. The characteristics of tritanopia. Journal of the Optical Society of America, 42, 509-521, (1952).

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