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CONTRAST SENSITIVE AND COLOR OPPONENT OPTIC TRACT FIBERS IN THE MEXICAN GROUND SQUIRREL: EVIDENCE FOR ROD (502 λ_{MAX}) INPUT

By
Lillian Tong

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Psychology)
in The University of Michigan
1977

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CHAPTER I

INTRODUCTION: ROD AND CONE CONTRIBUTION TO COLOR AND CONTRAST CODING

The neurophysiological basis for color vision has been a subject of great interest for many years. Many different investigators have studied color vision in a large number of animals, among which are goldfish, ground squirrels, cats, monkeys and man. For several reasons the Mexican ground squirrel (Citellus mexicanus) has been used as a model for the study of color mechanisms. Behavioral studies have shown that this diurnal animal has color vision since it can discriminate between colors independent of brightness differences (Jacobs & Yolton, 1971). Indirect evidence for color vision was obtained electrophysiologically by the discovery that some ganglion cells respond differentially to stimuli from different parts of the spectrum (Michael, 1968c). Furthermore, color vision was believed to be mediated by cones. It was thought that the ground squirrel had an all-cone retina making it an ideal preparation for study. Recently, however, evidence has been found to suggest that there are rod-like receptors in the ground squirrel retina. In view of this recent evidence, it seems worthwhile to re-examine the response of ganglion cells in the ground squirrel and to look for possible rod input.

Before describing the literature on the ground squirrel retina, I would first like to give some background information on responses of ganglion cells and the methods for determining rod and cone input which form the basis for these experiments.

Background - Ganglion cell studies

It has long been known that ganglion cells in many species respond with increased firing at the onset of a light ("on" response) or with increased firing at the offset of the light ("off" response), with inhibition of firing during the light-on period. In 1953 Kuffler used small spots of light to stimulate the cat retina and discovered that ganglion cell responses were more specific than this. Each cell was stimulated by a specific region of the visual field, the receptive field, and this receptive field was not homogeneous. Each receptive field consisted of a circular central region that produced an "on" or "off" response, and a surround region which produced the opposite sign response. Large stimuli covering both regions could produce an "on-off" response.

Various methods have been used to determine the receptor inputs to the "on" and "off" responses. Particularly relevant to this study are several methods used to determine if rods have input. One of the direct tests for rod input is to measure the spectral sensitivity curve for the response and to compare it with the absorption spectrum of the rod pigment, rhodopsin. Spectral sensitivity curves are measured by adjusting the intensity of the stimulus to produce a criterion response for wavelengths across the spectrum. Since rods and cones share neural pathways (Granit, 1947), the rod and cone responses must first be separated. One way is to choose lighting conditions which favor one mechanism over the other. Von Kries (1895) formulated the Duplicity Theory that summarized the evidence that rods function only in dim illumination and mediate colorless sensation while cones take over at higher levels of illumination and are responsible for color vision. By dark adapting

the animal and using low intensity stimuli, the rod response can be accentuated; and if rods contribute to the ganglion cell response, the spectral sensitivity curves should fit the rhodopsin absorption curve. If cones are present, increasing the background illumination will result in a Purkinje shift, which means that the shape of the spectral sensitivity curve will change.

Another way of differentiating rods and cones is by measuring the rate of recovery of sensitivity after a bleach. Cells with both rod and cone input have a characteristic dark adaptation curve with two phases. The first phase is due to recovery of cones and the second phase to recovery of rod sensitivity (Kohlrausch, 1922).

There are also methods to study the cone input to a cell. Spectral sensitivity curves can be measured on high intensity white backgrounds which favor cone responses. To determine if more than one cone mechanism has input, the spectral sensitivity curves can be measured on strong chromatic backgrounds whose wavelengths are chosen to suppress one mechanism more than another. If only one cone mechanism has input, the shape of the curve will be the same independent of the wavelength of the background. If there is more than one cone mechanism, however, chromatic adaptation should shift the curve. These spectral sensitivity curves can be compared with templates generated from Dartnall nomograms to identify the λ_{max} of the pigments involved. Details are described in Chapter 3.

The number of receptors with input to a cell and the organization of those inputs determines to some extent the type of information that could be coded in the response of the cell. Accordingly, researchers have classified cells in the visual system as contrast

sensitive or color opponent.

In contrast sensitive cells both "on" and "off" responses are mediated by the same receptor mechanism(s). Since the spectral sensitivity of the center and surround are the same, this cell would not respond differentially to different wavelengths. A cell's response to lights of different color in the center and in the surround would be indistinguishable from its response to lights of the same color with some difference in intensity. These cells would be sensitive to spatial aspects of the stimuli, however.

There are two classes of color opponent cells. Single opponent color cells are those with input from at least two receptor types, one mediating the "on" response and one mediating the "off" response. A stimulus at each wavelength would evoke a unique response determined by the interaction of the two opponent receptor mechanisms. Short wavelength stimuli may produce an "off" response and long wavelength stimuli may produce an "on" response, or vice versa. The "on" and "off" mechanisms may be spatially distinct in a center - surround organization, completely overlapping, or partially overlapping. Therefore, a change in the size of the stimulus might affect the response in the same way as a shift in the wavelength.

Double opponent cells have a center-surround organization. The center is color opponent, perhaps responding "on" to long wavelengths and "off" to short wavelengths, while the opponency is reversed in the surround; that is, "off" to long wavelengths and "on" to short wavelengths in this example. In other words, the center and surround are complementary single opponent systems. It has been hypothesized

that double opponent cells receive input from single opponent cells in a hierarchical manner (Daw, 1972). Double opponent cells are found in some species,such as the goldfish, at the ganglion cell level (Daw, 1968). However, in most mammals double opponency is found more centrally.

Mexican ground squirrel

In 1968 Michael, using the methods just described above for determining receptor inputs, studied ganglion cell responses in the Mexican ground squirrel and reported input from cones alone.

Earlier ERG and anatomical studies had already suggested that there were no rods in the ground squirrel retina. Arden and Tansley (1955) measured the spectral sensitivity of the European ground squirrel with the ERG and found a broad spectral sensitivity curve with a peak between 520 and 530 nm. Evidence from microspectrophotometry shows that mammalian rhodopsin should peak near 500 nm (Brown & Wald, 1963). Tansley et al. (1961) measured spectral sensitivity curves again with ERGs of four species of ground squirrels and found a more complex picture with two spectral peaks at 585 and 490-500 nm, and a hint of a hump at 450-460 nm. There was no shift in spectral sensitivity with light adaptation as would be expected if rods were present. In 1964, Dowling measured the dark adapted spectral sensitivity curve using the b-wave of the ERG and found only a single peak with maximum at 523 nm. Studying the visual pigment directly using microspectrophotometry on the same retina, P.K. Brown found a difference spectrum for one visual pigment which was identical to the ERG spectral

sensitivity. This in itself is surprising since the ERG measurements were made with the intact eye which has a highly pigmented yellow lens.

Early anatomical studies also reported that only cones were present. Walls (1942) claimed that diurnal squirrels had two types of receptors, which he classified as cones although he provided no evidence to support the claim. Vaidya (1964) studied aldehyde fixed, paraffin-imbedded 13-line ground squirrel retinas by light microscopy and described only cones. Dowling (1964) and Hollenberg and Bernstein (1966) used electron microscopy on 13-line ground squirrel retinas and agreed that the ground squirrel had an all-cone retina.

Therefore, at the time that Michael began single unit recording from ground squirrel ganglion cells, it was widely believed that no rods were present. Michael measured the spectral sensitivity of the "on" and "off" response for wavelengths in 20 nm steps across the spectrum. Under any lighting condition, dark adapted, light adapted, or chromatically adapted, the spectral sensitivity curves could be interpreted to show the presence of a pigment with peak sensitivity at 460 or 525 nm. No evidence was found for a 500 nm λ_{max} pigment.

In addition to reporting that two cones were present, Michael classified ganglion cells by the stimulus parameters on which the responses depended. He called them contrast sensitive, opponent color, or directional selective. This differentiation also depended on the types of receptor inputs to the cells and the spatio-temporal arrangement of the inputs. The contrast sensitive cells comprised about half the units recorded (Michael, 1968a). Both center and surround were reported to have the spectral sensitivity of the 525 nm pigment in dark adapted, light adapted, and chromatically adapted conditions. These

cells responded to long and short wavelength stimuli of the same spatial configuration with the same sign response.

The color opponent cells (24%) had an "on" response and an "off" response, mediated by two antagonistic receptor systems (Michael, 1968c). A wavelength could be found at which neither the "on" nor "off" response could be elicited, which was termed the neutral point. In the non-chromatically adapted conditions, therefore, the spectral sensitivity curves of the "on" and "off" responses were both narrow. When measured under chromatic adaptation with one receptor mechanism selectively suppressed, the curve of the other was broader and the peak sensitivity shifted toward the neutral point. The action spectra of the two receptor systems peaked at 460 and 525 nm when chromatically adapted. Michael further divided the class into three types on the basis of their spatial properties: complete spatial overlap of two receptor systems, partial overlap in which the blue system was more extensive than the green system, and no overlap in which the center was driven exclusively by the green receptor and the surround by the blue receptors. (See Fig. 5.3 in Discussion for illustration.)

Directional selective cells responded better to stimuli moving in one direction than in the opposite direction (Michael, 1968b). The directional selective cells (23%) had an "on-off" center with an antagonistic surround which inhibited both the "on" and "off" response of the center but did not evoke a response by itself. The spectral sensitivity of both center and surround (measured by its ability to reduce the center response) had a peak at 525-540 nm and showed the same shape. Michael reported that the receptor mechanism was the same as

in the contrast cells.

Ganglion cells of 13-line and Mexican ground squirrels have been more recently studied by Gur (1974) who concurred that only cones were present. Using a different anesthetic, Gur found the same three classes of cells as Michael. However, color opponent cells, which comprise 15-20% of his units had receptive fields different from those described by Michael. He speculated that a single type of cell, the BGG ("Blue-green-green"), responds with different properties under heavy anesthesia so as to produce the three types of color opponent cells of Michael. The spectral sensitivity curve of the BGG center was broader than a single pigment and chromatic adaptation revealed at least two pigments which together mediated the same sign response. Gur claimed that the B and G pigments were the 460 and 525 cones. The BGG was not color opponent to small stimuli. When the stimulus size was increased and response measured, green stimuli reached an optimal size beyond which the center response decreased, but blue stimuli did not. Large stimuli evoked a center sign response to blue and the opposite sign response to green. Gur used this as evidence that there is an antagonistic green, but no blue, surround mechanism. (See Fig. 5.3 in the Discussion for illustration.) In the presence of a very small white or green adaptation spot in the center, opponent responses in the center to blue and green stimuli were obtained. Gur suggested that this was evidence that the green surround mechanism extends through the center. Gur found that no opponent cell had a neutral point, perhaps because the opponency was between center and surround.

Behavioral studies by Jacobs and Yolton (1971) seemed to confirm

that ground squirrels had only cones. They trained the animals to choose from three panels the one panel that was different. The one panel differed from the others in that some intensity of monochromatic light was added to it. By measuring psychometric functions for each wavelength in 20 nm steps and noting the intensity for a 50% correct criterion, spectral sensitivity curves could be plotted. The curve was fit by a combination of 520 and 460 nm λ_{max} templates generated from the Dartnall nomogram. Because they noticed no Purkinje shift with light adapting backgrounds, Jacobs and Yolton concluded that only cones were present in the ground squirrel. In addition Jacobs and Yolton showed that there was a wavelength which animals could not discriminate from white, which they used as further evidence that the animal had only two types of cones.

In 1975, evidence for a rod-like receptor in the Mexican and 13-line ground squirrel retina was presented. West and Dowling (1975) examined ground squirrel retinas under light and electron microscopy and found anatomical evidence for rods. Only 4-5% of the receptors were rod-like. They differed from typical mammalian rods, having both rod and cone features. The rod-like receptors had inner segments that were often displaced proximally relative to the cone-like receptors. The rod-like receptors also appeared pale with glutaraldehyde fixation and had smaller synaptic terminals (West & Dowling, 1975). Recently retinas of two other species of ground squirrel have been studied anatomically and similar results were obtained (Jacobs, Fisher, Anderson, & Silverman, 1976; Fisher, Jacobs, Anderson, & Silverman, 1976).

The most recent ERG studies have also found physiological

evidence for a rod receptor in the Mexican, 13-line, California, and antelope ground squirrel retinas (Green & Dowling, 1975; Jacobs et al., 1971; Fisher et al., 1976). In one of these studies, Green and Dowling (1975) measured the spectral sensitivity curve for the dark adapted eye using a b-wave criterion amplitude. They claimed that the curve could be fit with the rhodopsin nomogram template. Upon light adaptation a small Purkinje shift was seen. Another way of showing the presence of rods was by measuring the incremental threshold function for a 500 and 600 nm light. As background luminance increased, sensitivity for the long wavelength stimulus increased indicating a change in the receptor types which mediate the response. Furthermore, Green and Dowling (1975) suggested that the Jacobs and Yolton (1971) behavioral results could be reinterpreted to support the presence of rods. They replotted the data and showed that the behavioral spectral sensitivity and ERG sensitivity matched, both showing a change between dark adapted and light adapted states.

The recent evidence that rods are present as well as cones raises the question, can rod response be seen in the ganglion cell, and if it can be found, how does it change the model of information coding as described by Michael? The model extends beyond the ganglion cell experiments to the lateral geniculate nucleus and the superior colliculus. At the LGN double opponent color cells were found as well as contrast sensitive cells similar to contrast sensitive ganglion cells (Michael, 1973). No directional selective cells were found in LGN, and apparently all directional ganglion cells project to the superior colliculus (Michael, 1972). Because so much of the processing of information in the ground squirrel takes place at

the peripheral levels of the visual system, all of which have been studied at the level of the single cell, it has become a very important example of neural coding of information. It is therefore important to show how the rod-like receptor affects this system. The major aim of the experiments to be presented in this thesis is to determine the contribution of rods to ganglion cell response.

In considering the role of the rod in processing of information, particularly color information, it may be helpful to look at the visual system of some other animals that have rods and color vision: the cat, monkey, goldfish, and human.

Cat

The cat has been shown to distinguish colors (Mello & Peterson, 1964; Sechzer & Brown, 1964) although it learns color discrimination tasks much slower than brightness discrimination tasks. In keeping with this finding, very few color opponent cells have been found in its visual system. At the ganglion cell level, no color opponent units have been found; in fact, in recent work only responses from rods and green cones have been reported (Daw & Pearlman, 1969; Hammond & James, 1971). A blue cone channel must exist, however, since double opponent cells with opponency between green and blue cones have been found in the lateral geniculate nucleus (Pearlman & Daw, 1970). If the hypothesis of hierarchical processing of color information is true in this animal, one would expect that eventually some single opponent ganglion cells will be found.

Ganglion cells found so far, however, could be called contrast sensitive. For most units, the scotopic spectral sensitivity curve for center and surround can be fit by the rhodopsin template. Upon

light adaptation the spectral sensitivity curve fits the 556 λ_{max} template. The sign of the center and surround response does not change with background intensity, indicating that rods and cones with input to the center or to the surround mediate the same sign response (Daw & Pearlman, 1969).

Two double opponent cells and one single opponent cell have been described at the level of the LGN (Pearlman & Daw, 1970). In the double opponent cells, spectral sensitivity curves of dark adapted center and surround response fit rhodopsin template suggesting that the rods mediate the response. Under photopic conditions the units were double opponent with opponency between the green (556) and blue (450) cones. The centers had both an "on" and "off" response mediate by the two cones, and the surrounds had "on" and "off" responses mediated by the reverse receptor mechanisms. In the change from dark adapted to light adapted conditions, the center rod-mediated response ("on" or "off") became opponent between the two cone systems with both "on" and "off" components, and likewise for the surround. There was not a consistent correlation between the sign of the rod and sign of either blue or green cone (Pearlman & Daw, 1970).

Macaque

Processing of color information has been studied in the macaque monkey, an animal which is assumed to have receptors similar to man (Marks, Dobelle, & MacNichol, 1964). Both color opponent and contrast sensitive ganglion cells have been recorded.

The nonopponent cells, called broad-band by DeMonasterio and Gouras (1975) had concentrically organized receptive fields. Spectral sensitivity curves of center and surround responses were identical,

both being broader than a single pigment template. DeMonasterio and Gouras suggest that the responses therefore are mediated by at least two cones. These cells were not studied in a completely dark adapted state. In previous work, nonopponent ganglion cells were shown to have input from both rods and cones, active at the same time under some lighting conditions (Gouras & Link, 1966).

Color opponent ganglion cells with input from only the green and red cones were reported in 1968 by Gouras. At the time it was thought that all color opponent ganglion cells were of this type. More recently, however, it was found that 24% of the color opponent cells have input from all three cone mechanisms (DeMonasterio, Gouras, & Tolhurst, 1975). The center mechanism generally received input from one type of cone and the surround from two types of cones in single opponent manner. All combinations of cones have been found: blue opponent to red + green, green opponent to red + blue, and red opponent to blue + green. It was also reported that color opponency was not evident against neutral backgrounds but was seen only in the presence of chromatic backgrounds in about one third of the color opponent cells. Therefore, the percentage of ganglion cells thought to be opponent must be larger than previously reported. Unfortunately the rod input was depressed by a strong white background and was not investigated in these studies.

Macaque LGN cells were studied earlier than the ganglion cells by several investigators. Wiesel and Hubel in 1966 reported that both contrast sensitive and color opponent cells could be recorded. Contrast sensitive cells, Type III, had spectral sensitivity curves broader than for a single pigment, like the contrast sensitive

ganglion cells just described. Upon dark adaptation, some spectral sensitivity curves fit the rhodopsin template indicating rod input, and others did not. Color opponent cells made up a large proportion of the lateral geniculate cells. These have been described by Wiesel and Hubel (1966), DeValois, Abramov, and Jacobs (1966), and more recently by Padmos and Norren (1975). Although the results differ, possibly due to differences in stimulation conditions, it appears that color opponent cells in LGN are similar to color opponent ganglion cells and little reorganization takes place in this nucleus. All or almost all are single opponent units. Many have input from all three types of cones and in some opponency is seen only with chromatic adaptation. Double opponent cells, like those found in the LGN of cat and ground squirrel, have been found in the cortex of the macaque (Hubel & Wiesel, 1968). This is in keeping with the general principle of greater encephalization of function in primates.

Goldfish

The goldfish has rods, red, green, and blue cones (Marks, 1965) and has been shown behaviorally to have color vision (McCleary & Bernstein, 1959). Like the mammals, it may process color through a hierarchy of cell types, with double opponent cells receiving input from single opponent cells; however, the processing takes place more distally.

Recording S-potentials of horizontal cells, Kaneko (1970) found some single opponent color cells in which there was hyperpolarizing to some wavelengths and depolarizing to others. There was no spatial organization in the receptive field (Kaneko, 1971). Horizontal cells received either rod or cone input but not both, and thus the color

opponent cells had no rod input (Daw, 1973). Stell (1975) proposed a way by which color opponent cells might get their input from the cone types based on his anatomical studies. He associated the anatomical classes with the physiological classes by inferring the probable soma size from the sampling distribution in the physiological studies, and then worked out the connections which would result in the appropriate physiological response. He concluded that each horizontal cell type received direct input from at least one class of cones. The other class of cone input could come by indirect pathways via other horizontal cells. He suggested that sites of contact may be in the cone pedicles.

Bipolar cells had a center-surround organization. The color opponent cells all had red cone input to the center and green to the surround; however, either cone could mediate hyperpolarizing or depolarizing responses. In nonopponent cells, the centers and surrounds were all mediated by the red cones (Kaneko,1973). Although these experiments were performed under mesopic conditions, Kaneko did not mention rod input.

At the ganglion cell level, Wagner, MacNichol, and Wolbarsht (1963) used small spot stimuli and found single opponent cells in which the green sensitive region extended farther than the red sensitive region. However, Daw (1968) found that a large majority of these units had an inhibitory surround seen only when annular stimuli were used. These units were termed double opponent, or O-type, with red and green opponency in center and surround. Another type of cell, Q-type, was essentially the same as the O-type but had stronger red cone input than green. The blue cone, when seen, had the same input to the

center and surround.

Almost all double opponent cells had rod input and all center-surround cells with rod input also had cone input (Daw, 1973). A particularly interesting finding about the rod input in goldfish is that rods and red cones are connected in the same way (Raynauld, 1972). For example, a cell which responded red-"on" and green-"off" in the center, and green-"on" and red-"off" in the surround, would be "on" center, "off" surround when dark adapted. The spectral sensitivity curve of the center and surround can be fit with the template of porphyropsin, the pigment in the goldfish rod but with a slight shift toward the long wavelengths, possibly due to red cone input. This connection between rod and one type of cone mechanism has not been found in cat (as stated earlier) or monkey (Daw, 1973).

Human

In a switch from neurophysiology to human psychophysics, I would now like to discuss some of the evidence that color vision is not mediated by cones alone; rods contribute to color sensation.

One line of evidence has used the failure of Grassmann's laws of trichromatic color matching to show that rods contribute to color matching. One of Grassmann's law is the Law of Additivity, which states that metamers, or nonidentical lights that match, can be added to metamers without upsetting the match. It has long been known that this law of additivity holds for a 2° stimulus but not for a 10° stimulus (Stiles, 1955). It has been suggested that rods which are present in higher density in the periphery are responsible for the nonadditivity by contributing to the sensation of color (e.g. Stabell & Stabell, 1976; Trezona, 1970).

Trezona tested this hypothesis in 1974 by making tetrachromatic rather than trichromatic matches to take into account possible rod intrusion. In one experiment she tested the matches for a special case of additivity called luminance metamerism in which the light added to the two half fields merely changes the luminance and should not upset the match. Tetrachromatic matches were made with red, yellow, blue, and cyan primaries at two luminance levels. Under high illumination a match was made adjusting only the red, yellow, and blue primaries. Changing to a dim luminance level below cone threshold, the match was upset. The cyan adjustment alone would return the match. By making repeated matches alternating between the two luminance levels, and thus between chromatic and achromatic fields, a tetrachromatic match could be found that was good at both levels. To test for additivity, Trezona looked at the amounts of each primary necessary for trichromatic and tetrachromatic matches to tests of 530, 546, and 610 nm at different luminance levels. The results were the same for all test stimuli. For the trichromatic match no change was necessary in the relative amounts of the red and yellow primaries. However, a large increase in blue primary was necessary as luminance decreased indicating a failure of additivity. In tetrachromatic matches the red, yellow, cyan, and blue primaries showed little or no change as luminance level decreased, thus showing additivity. Since trichromatic additivity errors were in the blue, Trezona suggested that rod color may be blue with rods and blue cones sharing a common pathway.

Other psychophysical evidence that rods contribute to color vision has come from a series of papers by Stabell and Stabell. In 1973 they suggested that after a prestimulus causing activity of cones,

a stimulus at an intensity which can only stimulate rod vision takes on a hue. The subjects reported that with a 650 nm prestimulus, a 510 nm stimulus superimposed on a 650 nm test first appeared bluish, then shifted toward the red. They concluded that rods can interact with cones to contribute to the sensation of color.

In order to quantify the effect, they studied the effect of rod activity on color matching (Stabell & Stabell, 1975). The subject was presented with a 650 nm or 510 nm test 6° extrafoveally that was 1.5 log units above its absolute threshold. The subject adjusted three primaries in a foveal comparison field to match the test. The test and comparison fields were presented in succession in .5 sec flashes. The 650 test was above cone threshold but the 510 nm test was below cone threshold. Next the two stimuli were superimposed and a match made. It was found that the mixture of the two tests did not obey additivity. The chromaticities of the achromatic scotopic stimulus, the photopic stimulus, and their mixture did not lie on a straight line in the WDW chromaticity diagram, but the mixture was displaced toward the yellow. Using scotopic tests of other wavelengths, the same shift toward yellow was produced suggesting that the rods alone mediated the scotopic stimulus and shifted the hue toward yellow.

In a second experiment they used the course of dark adaptation following light adaptation to separate the cone from the rod contribution (Stabell & Stabell, 1975; Stabell & Stabell, 1976). Matches were made between an extrafoveal test and a foveal comparison before the cone-rod break (6 min) and after 30 minutes to determine the chromaticity shift due to rod intrusion. On the WDW chromaticity diagram, the orange and green-yellow tests shifted toward yellow, and the blue-

green and violet tests shifted toward the blue. The saturation was reduced between 6 and 30 minutes. Stabell and Stabell suggested that this meant that the rod response is not a constant color quality. This differs from Trezona's suggestion that rod color is blue. There is agreement, however, that the rod contributes to color vision.

McKee, McCann, and Benton (1977) showed that rods contribute to color sensation using a simultaneous contrast effect. A multicolored scene is photographed first through a red filter, then through a green filter. When these two transparencies are projected (the long-wavelength transparency with long-wavelength light, and the middle-wavelength transparency with middle-wavelength light) and the images are superimposed, the viewer sees a scene with a variety of colors. It had been shown previously by McCann and Benton (1969) that colors can be seen if one stimulus is below cone threshold. In this experiment the transparency taken through the green filter was illuminated with a wavelength between 400 and 600 nm and the transparency taken through the red filter was illuminated with 656 nm light. At each wavelength the observer adjusted the intensity of the shorter wavelength light to give optimal color balance. The resulting plot of inverse optimum-color intensity as a function of wavelength fit the scotopic luminosity curve, suggesting that rods mediated one of the two channels contributing to the color vision. Furthermore, the angle of incidence of light on the retina did not affect the setting, which is consistent with the rod hypothesis. Because of the Stiles Crawford effect, cones would have been more sensitive to light passing through the center of the pupil than to light through the edge. Rods do not show this effect (Stiles, 1939).

These psychophysical reports of the role of rods in color

vision are particularly interesting in light of the findings to be presented here that show that rods play a role in color opponent as well as contrast sensitive units in the ground squirrel.

CHAPTER II

METHODS

The experiments in this study were designed to determine the role of rods in the Mexican ground squirrel (Citellus mexicanus) at the ganglion cell level. All animals were obtained from Otto Martin Locke (P.O. Drawer 76, New Braunfels, Texas, 78130). Over the three-year period during which these experiments were performed, changes were made in the procedures. The procedures used most recently will be described first. Later in the chapter mention will be made of procedures formerly used and the reasons for changing them.

Surgery and animal maintenance

The ground squirrel was dark adapted for at least 12 hours prior to surgery. All surgery was performed under dim red illumination or with a black occluding contact lens over the left eye to maintain maximal rod sensitivity. The animal was first lightly anesthetized with ether in its cage for easier handling. The cage was placed in an air-tight plastic bag with cotton balls soaked in ether. When the animals was awake enough to struggle and move, but not to run or bite, it was removed and an injection was given of sodium pentobarbital (60 mg/kg, IP).

When the animal was sufficiently areflexic, an incision was made in the skin over the trachea. The trachea was exposed, slit, and fit with a cannula of polyethylene tubing (PE 240) which was sewn in place for later artificial respiration. The right internal carotid artery was cannulated with polyethylene tubing (PE 50) filled with heparinized saline. The cannula was attached to a Statham P23 DC pressure transducer and blood pressure was monitored on a chart recorder. The femoral vein

was cannulated with polyethylene tubing (PE 50) filled with mammalian ringer.

When the animal began to recover from anesthesia, it was transferred to halothane (Fluothane) anesthetic. A controlled flow of oxygen and air from pressurized tanks was passed through a Fluotec 3 vaporizer. The vaporizer was set to regulate the percentage of Fluothane in the gas mixture: .5% during recording and as high as 2% during surgery. A tube from the vaporizer was loosely placed over the opening of the tracheal cannula and the animal initially breathed on his own from this open system of anesthesia. The transfer to fluothane anesthetic took place at least an hour before recording began.

The animal was then placed in a stereotaxic apparatus (Baltimore Instruments). This apparatus allowed a wide visual field, blocking only far temporal fields by the ear bars. The head was oriented to correspond with the sterotaxic atlas for the Mexican ground squirrel (Joseph, Knigge, Kalejs, Hoffman & Reid, 1966).

A rectal probe was used to monitor the animal's temperature and control a temperature regulating system which was connected to a heating pad under the animal. The DC power supply for the heating pad was automatically switched on and off so as to maintain temperature at 38° C.

Next, surgery was performed to allow access to the optic tract. A piece of skin was removed from the head and a hole drilled in the skull over the right optic tract with a dental drill. The dura was reflected and the brain surface kept moist with mammalian ringer. The position of the two millimeter square hole was determined from landmarks on the skull and stereotaxic coordinates found successful on

other animals. The majority of recordings were made approximately 6.0 mm AP, 3-4 mm lateral from midline. The atlas for the ground squirrel (Joseph et al., 1966) was not accurate for these animals. The histology showed that brain sections with electrode tracts made at 6.0 AP on our coordinate system correspond to sections labeled 7.7 AP in the atlas. Figure 2.1 shows one brain section in which the electrode tracts from several penetrations passed through the optic tract. This section corresponds with the structures seen in the atlas at 8.0 AP.

The following surgery was performed to stabilize the left eye. The upper and lower eyelids were removed and the conjunctiva cut around the globe. The cut edge close to the cornea was sutured with small stitches to a stainless steel ring 8 mm in diameter which fit around the eyeball. The ring was held rigidly by a bar to the stereotaxic frame limiting eye movement. This procedure has been used to stabilize the eye of the cat and rat (Rodieck, Pettigrew, Bishop, & Nikara, 1967; Cicerone & Green, 1977). Care was taken to keep the cornea moist at all times. Following surgery, atropine sulfate (1%) was applied to dilate the pupil. A contact lens wetted witha drop of commercially available contact lens solution was placed over the cornea.

The animal was paralyzed with gallamine triethiodide (Flaxedil^{R}, 12 mg/kg IV) once the optic tract had been located. Flaxedil was thereafter continuously infused IV using a Harvard infusion pump (Model 942)at a rate of 10 mg/kg/hr along with Rheomacrodex, a commercially available saline and dextrose solution,to equal an infusion rate of 2 cc/kg/hr of fluid. A Harvard small animal respirator

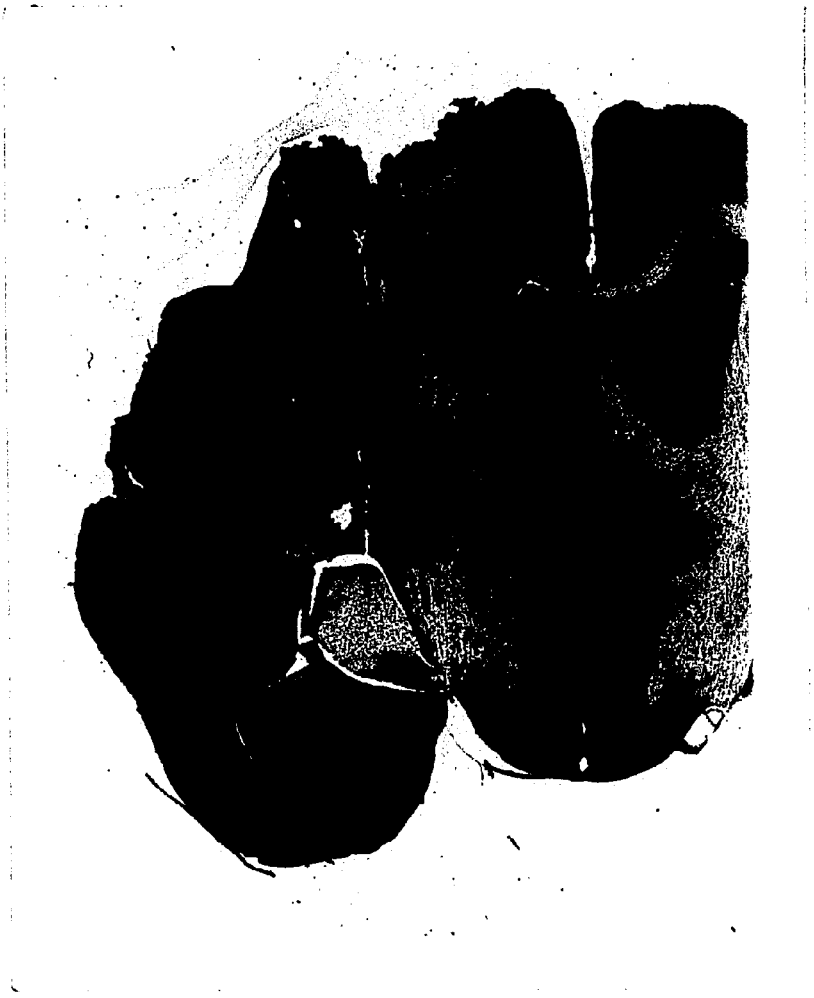


Figure 2.1. Histological section showing electrode tract. This histological section shows an electrode tract which passes into the optic tract. Visual fibers were recorded from this animal. The section corresponds to 8.0 AP in the stereotaxic atlas for the ground squirrel (Joseph et al., 1966).

(Model 680) was connected to the tracheal cannula, respirating the animal with the oxygen-air-fluothane mixture at 60 strokes/min, 2.5 cc/stroke. The respirator alternately forced the anesthetic into the ground squirrel through one leg of the Y-tube and allowed expired air to be released via the other leg of the Y-tube. The expired air was not recirculated. Depth of anesthesia depended not only on the percent fluothane in the oxygen and air mixture, but also on the stroke rate and volume of the respirator. Stroke rate and volume were adjusted to match the preparalysis breathing of the animal.

Blood pressure amplitude and expired CO_2 were monitored as indicators of physiological condition. Expired CO_2 samples were taken through a needle inserted into the tracheal cannula close to the animal and were analyzed with a Beckman CO_2 analyzer. Percent CO_2 was kept at 4.5% by adjusting the ratio of oxygen to air in the gas mixture. Respiration rate and volume were occasionally adjusted; however, these parameters also affected the amount of anesthetic received by the animal. It was found that if the preparalysis breathing rate and depth from the open system of anesthesia were matched, the percent expired CO_2 was normal requiring little or no adjustment. Usually the percent expired CO_2 fell only when the physiological condition was poor.

The blood pressure measurement was the best indicator of physiological condition. From the chart recording, mean pressure and heart rate could be monitored. The blood pressure amplitude was used as an index of the depth of anesthesia. Before paralysis, anesthetic was adjusted so that a noxious stimulus to the paw would produce withdrawal and no sudden change in blood pressure. After paralysis blood change in response to noxious stimuli was the only indicator of depth

of anesthesia. Since the animal was allowed to stabilize before paralysis, a matching of the breathing rate and depth was usually sufficient to match the preparalysis depth of anesthesia.

Blood pressure amplitude occasionally fell over the course of the experiment. If it could not be raised by adjusting anesthetic level or increasing oxygen, vasopressors were injected IV in some cases as an emergency measure. These drugs, such as phenylephrine hydrochloride (Neosynephrine^{R}) or metaraminol bitartrate (Aramine^{R}) stimulated an increase in blood pressure by constricting peripheral blood vessels, but these were rarely effective over a long period. In addition vasopressors are known to cause cardiac arrhythmia during halothane anesthesia which is sometimes irreversible (Katz, Matteo, & Papper, 1962).

Stimuli

Test stimuli, spots of light on a tangent screen, were generated by a Leitz xenon arc lamp. Light passed through a series of optical components as seen in Fig. 2.2. The lenses condensed the light and focused images of the aperture on the screen. A shutter (Uniblitz 225) was controlled by a pulse generator which regulated the timing of the stimulus presentation. The shutter was generally open for 500 msec every 2.5 sec. Fish Shurman NG-3 neutral density filters (.1, .2, .4, .8, 1.0_1, 1.0_2, 1.0_3, 2.0_1, 2.0_2) mounted in a filter box made by BB Instruments adjusted the intensity of the test stimulus. The wavelength of the stimulus could be changed by interposing different interference filters (Baird Atomic B1, 10 nm half band width). One of two discs, each with a series of holes of increasing size controlled the size of the stimulus. One disc had eight apertures producing stimuli from .75 to 10 degrees visual angle. The other pro-

Figure 2.2. Stimulus and recording apparatus. The test stimulus channel included a concave mirror (M_1), Leitz Xenon arc lamp (S_1), condensing lenses (L_1, L_2, L_3), Uniblitz 225 shutter (Sh), Baird Atomic B_1 chromatic filters (F_{ch}), Fish Shurman NG-3 neutral density filters (F_{ND}), an aperture disc (Ap), a flat mirror (M_2), and a tangent screen (TS). The background stimulus channel included a Kodak slide projector (S_2), condensing lens (C_4), Kodak Wratten or Melles Griot 03FIB broad band chromatic filters (F_B), Fish Shurman neutral density filters. The recording apparatus included a custom made FET preamplifier (Preamp.), a Tektronic RM 565 (CRT, Diff Ampl.), Tektronix R 564B (Storage CRT), an audio monitor and a Data General Nova 2 computer. See Text for Details.

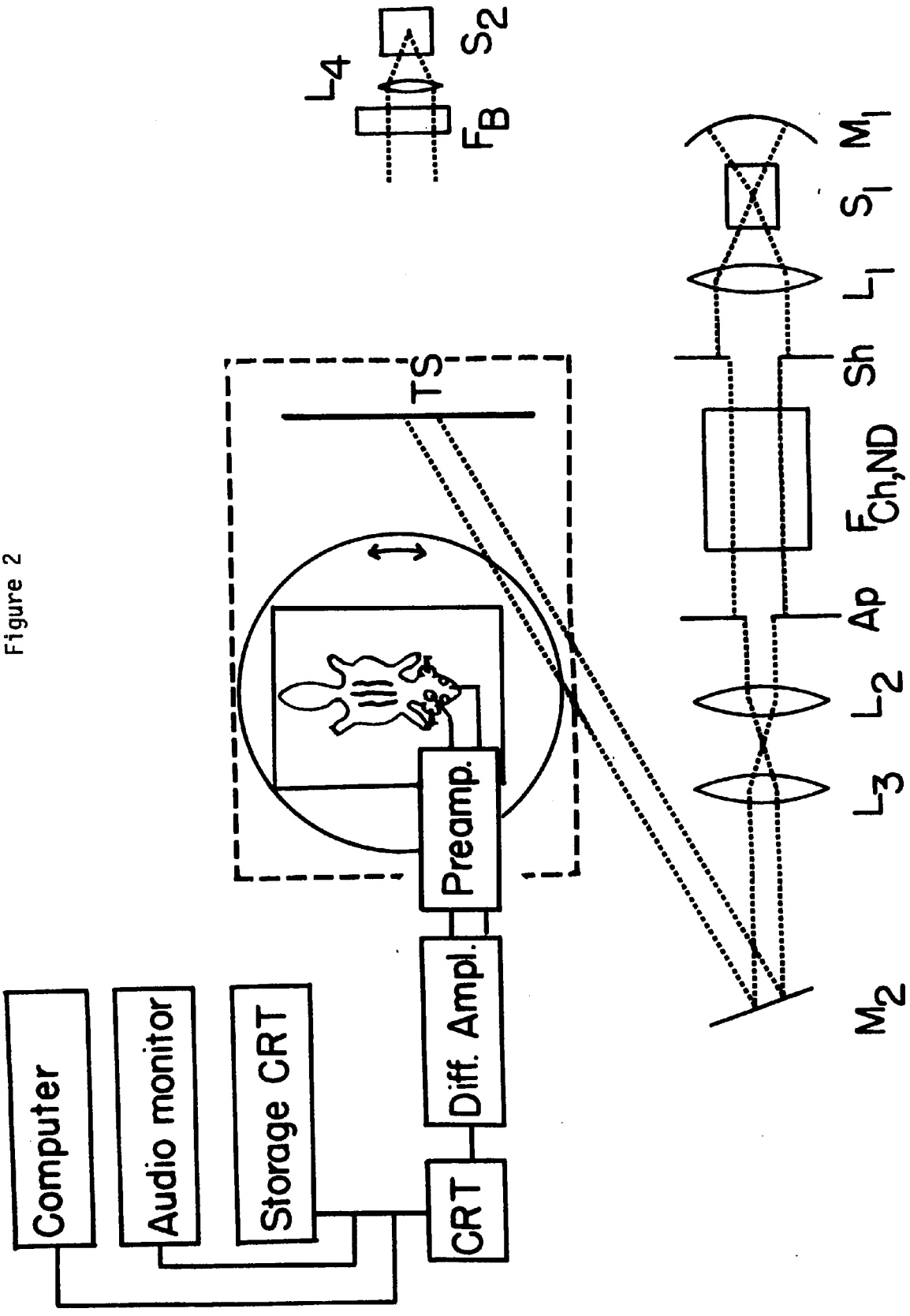

Computer
Audio monitor
Storage CRT
CRT
Diff. Ampl.
Preamp.
TS
L4
S2
FB
M2
L3
L2
Ap
FCh,ND
Sh
L1
S1
M1

Figure 2

duced six stimuli from 3 to 24 degrees visual angle. A glass cover slide with a centered black dot could be attached to the disc so that the dot was centered in a larger hole to produce an annular stimulus. Different size annuli could be produced by combining dots of different sizes with holes of different sizes. The light was reflected from an adjustable mirror onto a tangent screen, the back of a sheet of graph paper mounted in front of the ground squirrel eye.

The position of the stimulus in the ground squirrel's visual field could be adjusted by raising or lowering the tangent screen on a jack, and moving the mirror to reflect the stimulus to the appropriate height. Nasal-temporal placement of the stimulus was adjusted by rotating the stereotaxic apparatus on a turntable. Fine adjustment of the position of the stimulus was made by moving the aperture itself which was mounted on an adjustable x-y control.

Adaptation backgrounds were produced by one or two projectors from behind the tangent screen. Neutral density filters (Fish Shurman) varied the intensity and chromatic filters, broad band interference (Melles Griot 03FIB series) or Kodak Wratten, varied the wavelength. Care was taken that the projectors' lights did not shine directly into the eye of the animal.

Calibration

The luminance of the stimuli and background was measured using an SEI photometer. The maximal intensity of the white test beam was on the average 2.94 log candles/m^2, This was comparable to Michael's maximal white stimulus which was 3.0 log candles/m^2 measured with a MacBeth illuminometer. White background intensities could be as bright as 2.4 log candles/m^2. The maximal blue backgrounds were -0.27

log candles (Wratten 47) or 0.85 log candles/m^2 (Melles Griot, 450 nm λ_{max}). The maximal yellow backgrounds were 1.8 log x candles/m^2 (Wratten 21) and 2.0 log candles/m^2 (Melles Griot, 600 nm λ_{max}). These compared with Michael's 0-1.0 log candles/m^2 white backgrounds. In most cases the intensity of the background was adjusted to be as bright as possible, yet leaving the retina sensitive enough to permit measurement of a spectral sensitivity curve. This will be further described in the Procedure section.

The stimulating system was calibrated by measuring the relative energy transmitted with each interference filter placed in the stimulator. The stimulus was projected on the tangent screen and a photodiode (United detector UTC) was placed at the approximate position of the animal's eye. The measurements were corrected for the spectral response characteristics of the photodiode. The spectral transmission characteristics of each neutral density filter was determined with a Beckman Spectrophotometer. Using these measurements, the relative energy arriving at the cornea could be determined for any combination of interference and neutral density filters. These correction factors were applied to all data.

Focus

Many methods of refracting the animal were used over the course of the experiments, including streak retinoscopy and moving gratings of various frequencies across the screen to determine the best resolution. The most successful method was to plot the threshold in a series of traverses across the center of the field with various lenses in front of the eye. Another method of measuring the extent of the field was to mark the position of a moving spot where it first evoked

an "off" response in an "off"-center unit, approaching the field from many directions. This was repeated for various lenses to determine the best lens.

A contact lens with +10.25 D power, 5.0 base curvature, was used in almost all the experiments. A few units were studied with supplemental lenses in front of the eye in addition to the contact lens to make the smallest, sharpest receptive field. In many of the experiments the field center was adequately small with the contact lens alone that I assumed that focus was good.

Recording

The animal was grounded by a silver, silver-chlorided indifferent electrode inserted into the cheek of the animal.

Two types of recording electrodes were used: cerrelow in glass and etched tungsten in glass, both of which were made in the laboratory. The cerrelow electrode was a glass pipette with a tip of .5 - 1.0μ filled with cerrelow 136, a low melting point metal. The tip was plated with platinum and had a D.C. resistance of 4 megohms. The cerrelow electrode was used to locate the boundaries of the optic tract. The etched tungsten in glass electrode (Levick,1972) which isolated single units better was used for data recording.

Movement of electrodes was controlled by a Trent H. Wells Jr. hydraulic microdrive. The electrode was first lowered 5 mm from the surface of the brain using the course drive (2 revolutions/mm). Thereafter, it was lowered slowly by turning a fine drive (20 revolutions/mm).

Electrical signals recorded by the microelectrode were amplified ten times with a custom-made FET preamplifier. The signals were further amplified and displayed on a Tektronix RM 565 oscilloscope screen. An

internal trigger on the vertical output from the oscilloscope was adjusted to detect spikes exceeding a criterion. This separated the spikes from the background activity. A separately adjustable clipper circuit controlled what could be heard on an auditory monitor. Furthermore, every time the oscilloscope was internally triggered, a pulse was sent to the z-axis of a Tektronix R 564B storage oscilloscope. By this means, a dot was placed on the screen for every spike from a single fiber. The sweep of the storage oscilloscope was triggered by the onset of the stimulus light and successive presentations of the stimulus displaced the trace downward on the screen. The pattern formed by the dots showed the discharge rate of the unit, time locked to the presentation of a flash of light, for a large number of presentations. In later experiments a Nova 2 computer (Data General) generated post-stimulus time histograms from ten successive presentations. The dot patterns, histograms, and auditory response were used to determine a threshold response.

Procedure

All experiments were performed in a dark room with the stimulus lights as the only illumination. When searching for visual units the stimulus light was reflected off a mirror to uniformly light a half ping pong ball placed over the contralateral eye. The stimulus was presented for 500 msec every 2.5 sec. The optic tract was recognized by the synchrony of the spike discharge with the flashing light. As soon as it was determined that visual units could be recorded in the penetration, the electrode was retracted several millimeters and a drop of agar solution (4%) was applied around the electrode. This effectively kept the brain from pulsating which could move the neuron away

from the recording electrode. This allowed a longer recording time per unit. The electrode was lowered again to the level of the optic tract. Once the first visual response was evoked, all units whose neural activity could be isolated were studied, even if they did not appear to be visual since some fibers did not respond well to diffuse light. Of course, only those fibers with some spontaneous activity or some response to diffuse light could be studied. The electrode was not advanced more than 10-12 mm from the surface of the brain to protect the electrode from hitting the bone at the base of the brain. Upon reaching this depth the electrode was withdrawn, the brain surface cleaned, and a new penetration made in a different position.

Once a unit was encountered, the following procedure was used in studying the neural activity. First the internal trigger of the oscilloscope was adjusted to ensure that a single fiber was being studied. The standard criteria for distinguishing multiple from single fiber recording were applied. Only recordings with uniform spike amplitude and waveform were used. Interspike intervals of less than one millisecond were taken to indicate that more than one fiber was being sampled. When assured that a single fiber was being recorded, the ping pong ball was removed and the receptive field crudely located with a handheld ophthalmoscope projected on a piece of paper.

Receptive field centers were often tedious to find because of their small size or their poor response to large spots of light. However, the receptive field center, once found, usually produced a brisk response when stimulated. Some units were insensitive and the ophthalmoscope was too dim to elicit a response. For these units the xenon arc stimulus was reflected from a moveable mirror onto the paper to

search for the receptive field center. For some units the center was never found and the unit was abandoned.

When the center of the receptive field was located, the animal was rotated and the tangent screen height adjusted so that the receptive field lay approximately at the center. The location of the center, as determined with the ophthalmoscope stimulus, was marked on the back of the screen. The mirror was then moved until the xenon arc stimulus was centered on the marked position. At this time, the unit was tested for directional selectivity by moving the small bright spot in various directions across the receptive field center. This also served as a check to be certain that the center of the field had been properly identified.

A few quick tests were made to determine whether the unit's qualitative response characteristics matched those of Michael's contrast sensitive units, Michael's color opponent units, or Gur's BGG color opponent unit. To test for color opponency, blue (Wratten 47) and yellow (Wratten 21) filters were placed in the beam sequentially. These stimuli elicited approximately equal responses when mediated by a 502 λ_{max} mechanism. For each filter, small and large tests were flashed at an intensity to give a small response. The sequence was then repeated with stimulus intensity increased to give a large response. The relative response amplitude and sign of response were noted for all eight stimulus combinations. It is important to note that the receptor mechanisms could not be determined from these qualitative responses, only from spectral sensitivity curves. It could be determined, however, if a unit were color opponent or if the mechanism probably were not rhodopsin.

In general, color opponent units responded with an "on" response to one end of the spectrum and with "off" or "on-off" to the other end of the spectrum. Contrast sensitive units responded to lights at both ends of the spectrum with the same sign response. Some units responded with the same sign response to small tests of both yellow (Wratten 21) and blue (Wratten 47). However, for large tests, the blue test produced the same sign response as the small test, but the yellow test produced both "on" and "off" responses. These units qualitatively are like those color opponent units described by Gur as BGG.

To determine the optimal size stimulus for the center response and to determine the surround properties for the unit, a series of stimuli of increasing size were presented and responses of the unit compared. Starting with the smallest spot, as the diameter of a dim test increased over a range of size, the response increased due to spatial summation of the center mechanism. When the response to increasingly larger stimuli remained the same or showed evidence of a surround by becoming weaker or changing sign, I took this to indicate that the stimulus had exceeded the size of the center. The optimal size stimulus for the center response was noted.

Next, a dark adapted spectral sensitivity curve was measured using the optimal size stimulus as the test. Interference filters of wavelengths between 425 and 650 nm in 25 nm steps were placed in the test beam. For each filter, the intensity of the light was adjusted to elicit a small criterion response which was called threshold. Threshold was generally 2-3 additional spikes above spontaneous activity in response to the test, although there was some variability depending on the rate and stability of the spontaneous activity. The threshold response

was determined by ear and verified on the storage oscilloscope dot raster. Repeated determinations were usually within .1 log unit. In some cases, there was not enough light to evoke a response at wavelengths far from the peak sensitivity and a complete curve was not obtained. This occurred frequently when the units had low spontaneous activity.

A faster method of obtaining information about receptor input was often used to verify the spectral sensitivity curve. Two monochromatic interference filters, 460 and 600 nm, were placed sequentially in the test beam. For my apparatus, these filters would elicit equal responses if the receptor mechanism mediating the response were rhodopsin. This quick method could only determine if rhodopsin alone were mediating the response. If the responses were not equal, another mechanism or mechanisms had input. The identity of the mechanisms could not be determined without measuring a complete spectral sensitivity curve. This method had the advantage of determining two points on the curve rapidly, before sensitivity could change.

A light adapted spectral sensitivity curve was next measured to determine photopic receptor mechanisms. A background was produced by back illuminating the screen with a slide projector. The intensity of a white background was adjusted until it brought the full intensity 475 nm test to threshold. In this manner the background was made as bright as possible while still allowing a response to superimposed tests at enough wavelengths to determine the receptor inputs. In some very insensitive units, the background could not be very bright and had little effect in raising the threshold of the unit.

Chromatic adaptation with orange and yellow (Wratten 21 or Wratten 15) and blue (Wratten 47) was used to determine if more than

one mechanism mediated the response. In later experiments broad band interference filters of different wavelengths were used (Melles Griot 30FIB series). A short wavelength background should selectively adapt the shorter wavelength mechanism and reveal the spectral sensitivity curve of the longer wavelength mechanism. The long wavelength adaptation should have the opposite effect. The intensity of the chromatic background was adjusted in a similar manner to the white background. A monochromatic test of a wavelength on one end of the curve was flashed at full intensity while the background intensity was increased until the response to the test was at threshold. Then the rest of the spectral sensitivity curve was measured on this background. For long wavelength backgrounds, a short wavelength test (475) was chosen and for short wavelength backgrounds a long wavelength test (600) was chosen, although the exact wavelength depended on the properties of the unit. The brighter the background, the better the isolation, but if too intense, there was not sufficient test source intensity to measure the whole spectral sensitivity curve. (See Appendix A for the effective luminance of the backgrounds.)

For each lighting condition, dark adapted, light adapted, and chromatically adapted spectral sensitivity curves of the surround were measured if a surround response could be elicited. This determined the spatial distribution of the receptor mechanisms involved.

In some units a more quantitative method was used for measuring the optimal center and annulus sizes. Area sensitivity curves were measured in which threshold was determined for tests of increasing diameters. Because of spatial summation, the threshold decreases until the test exceeds the size of the center. The threshold then

remains the same, or the sign of the response changes depending on the presence and nature of the surround.

For a small number of units the dark adaptation curves following a three-minute full field bleach are measured. The bleaching light was an unattenuated xenon arc stimulus projected onto the white tangent screen, centered on the receptive field but covering a much larger area. This light exposure was roughly 7.3 log td-sec which would be sufficient to bleach over 90% of the rhodopsin in man (Alpern, 1971; see Appendix A).

The entire protocol might take four or five hours, depending on the ease of determining threshold. It was a rare unit which could be held this long.

Upon the completion of the study of one unit, the animal was allowed to dark adapt for at least a half hour before I looked for another unit. Since the next unit was not often found immediately, the period of dark adaptation was usually longer than a half hour. One unit was restudied after the half hour of dark adaptation. This unit had the same dark threshold as before light adaptation. This convinced me that the length of time between units was probably sufficient for complete dark adaptation.

History-Problems encountered

Over the three years of experiments changes have been made in the methods for various reasons. I initially used urethane (2.5g/kg, IP) as an anesthetic; however, after several months of experiments no color opponent units had been recorded. I therefore changed to sodium pentobarbital which is the anesthetic used by Michael. During the several months that I used sodium pentobarbital many color

opponent units were recorded. The anesthetic difference was not solely the cause of the change in unit sampling, however, since upon returning to urethane anesthesia, color opponent units were seen, although in lesser frequency. Differences in results due to anesthetic differences will be further discussed in Results II, Chapter 4.

I returned to urethane anesthesia because in personal communication with Moshe Gur I learned that sodium pentobarbital may cause physiological problems, such as low blood pressure. Furthermore, it was difficult to maintain a stable level of anesthesia under sodium pentobarbital using intraperitoneal administration of anesthetic. Urethane was more convenient since the initial injection was sufficient to produce anesthesia for 8 hours or more.

The next major change was initiated in an attempt to overcome the problem of cycling levels of spontaneous activity. This cycling was accompanied by a change in sensitivity of the unit which made consistent threshold measurements difficult to obtain. Personal communication with Gur informed me that he had solved similar problems by keeping the animal in good physiological condition with blood pressure in the normal range. I therefore began to monitor blood pressure and changed to halothane anesthesia, the anesthetic used by Gur. As an inhalent anesthetic the level of anesthesia could more quickly be adjusted.

The change in anesthetic necessitated other changes in procedure. Light halothane anesthesia did not prevent eye movements as adequately as urethane and the eye ring was not sufficient stabilization. The receptive field often moved during the experiment. Therefore the animal was paralyzed with Flaxedil and artificially respirated.

Because the animals were artificially respirated, we started monitoring the CO_2 expired since the oxygen content and blood pH was no longer under internal control.

In spite of the changes made, the units continued to cyclically change spontaneous activity. Because the experiments in this thesis were affected greatly by this cycling I will present here some of my observations on the phenomenon as well as the finding of others. Most of the observations were made in the course of the regular experiments. However some quantitative data were collected from cycling units which could not be used for the regular experiments. One must keep in mind that these were the units which cycled the most eratically.

The cycling of spontaneous activity did not seem to be due to a physiologically unsound preparation. It was found under all anesthetics tried. It was found under conditions of high, low, and normal (110-120 mm Hg) blood pressure. This is in agreement with Rodieck's finding that blood pressure is not correlated to the cycling (1973). The suturing of the eye ring was not causing the cycling: two units which cycled were driven by the ipsilateral, non-stabilized eye. Anoxia was most likely not a cause of the problem. From personal communication with Robert Lensenmeyer, who recorded oxygen percentage in the blood and in the vitreous near the retina, oxygen level is not correlated with the appearance of cycling. To further support the belief that cycling is not due to the surgery or an unsound preparation, Cavaggioni (1968), recording massed discharge in the optic nerve, found rhythmic activity in the unrestrained cat.

The properties of the cyclic activity differed from unit to unit. In some units the spontaneous activity cycled between two stable

levels of discharge, while in others the spontaneous activity cycled between different high and low levels. The transition between high and low spontaneous activity was very rapid; however, the length of time that the spontaneous activity remained stable differed between units. Rodieck and Smith (1966) recording cat ganglion fibers, described dark rhythms with cycles of 2/min to 2/hr. Rhythms in the same eye were found to be synchronized. In this study no simultaneous recordings were made from multiple units. Therefore it cannot be shown whether the rhythms are synchronous in the optic tract of the ground squirrel. Different cycling periods were found for successive units; however, the entire retina could have changed its cycling period during the time between units. The rhythms described by Rodieck and Smith (1966) disappeared with light adaptation unlike the ones seen in the ground squirrel.

The rhythmic spontaneous activity was accompanied by a rhythmic change in response to a flash of light which was not discussed by Rodieck and Smith (1966). Because of this change in response, data could only be taken for certain cells. Data from units with short cycles were frustratingly variable and could not be used. When the cycles had a long period, however, reliable threshold measurements during the same level of spontaneous activity could be made. Since cycling was generally between two stable levels of activity, the threshold values could be determined with fair repeatability in most units. The threshold intensity was found during one cycle and the response to that intensity confirmed at the same level of activity one cycle later. Redeterminations were made for some wavelengths and the averages of measurements were used in the spectral sensitivity curves

if the measurements differed.

One question is whether receptor mechanisms mediating the "on" response or the "off" response change during the different parts of the cycle. A test for this is to determine spectral sensitivity during each phase and compare the results. This was completed for only a few units. For this limited sample, spectral sensitivity curves measured at the peak and trough of the cycle were very similar in shape, suggesting that the mechanisms do not change.

Whenever possible, data were collected during the high spontaneous activity phase of the cycle. The major reason for this was that with zero spontaneous activity there was no measure of excitability of the cell and therefore response to a stimulus was not necessarily unique. In some units the response changed radically while the spontaneous activity remained at zero. One such unit is seen in Fig. 2.3. A stimulus was flashed repeatedly and a histogram made every minute of the response to 10 flashes. Plotted is the average number of spikes per 100 msec bin during the light-on period (500 msec). The spontaneous activity is plotted as the average of the number of spikes in the two bins preceding the light flash. Units such as these could not be used to determine spectral sensitivity curves. In other units, however, thresholds were as stable under low spontaneous levels as high activity levels. The threshold intensities were generally higher during low spontaneous activity than during high.

While the receptor mechanisms mediating the "on" or "off" response did not seem to change during the cycling, the "on" and "off" response mechanisms were not always affected equally during the various

Figure 2.3. Graph of spike discharge rate during cyclically varying response of fiber: "On" response and spontaneous activity. Post-stimulus time histograms for 10 presentations of a stimulus were made once per minute. The average number of spikes per 100 msec bin during the light-on period (5 bins) was found and plotted as a function of time. The spontaneous activity (SA) was the averaged number of spikes in two 100 msec bins preceding the stimulus onset. The spontaneous activity remained 0; however, the response to the flash varied cyclically.

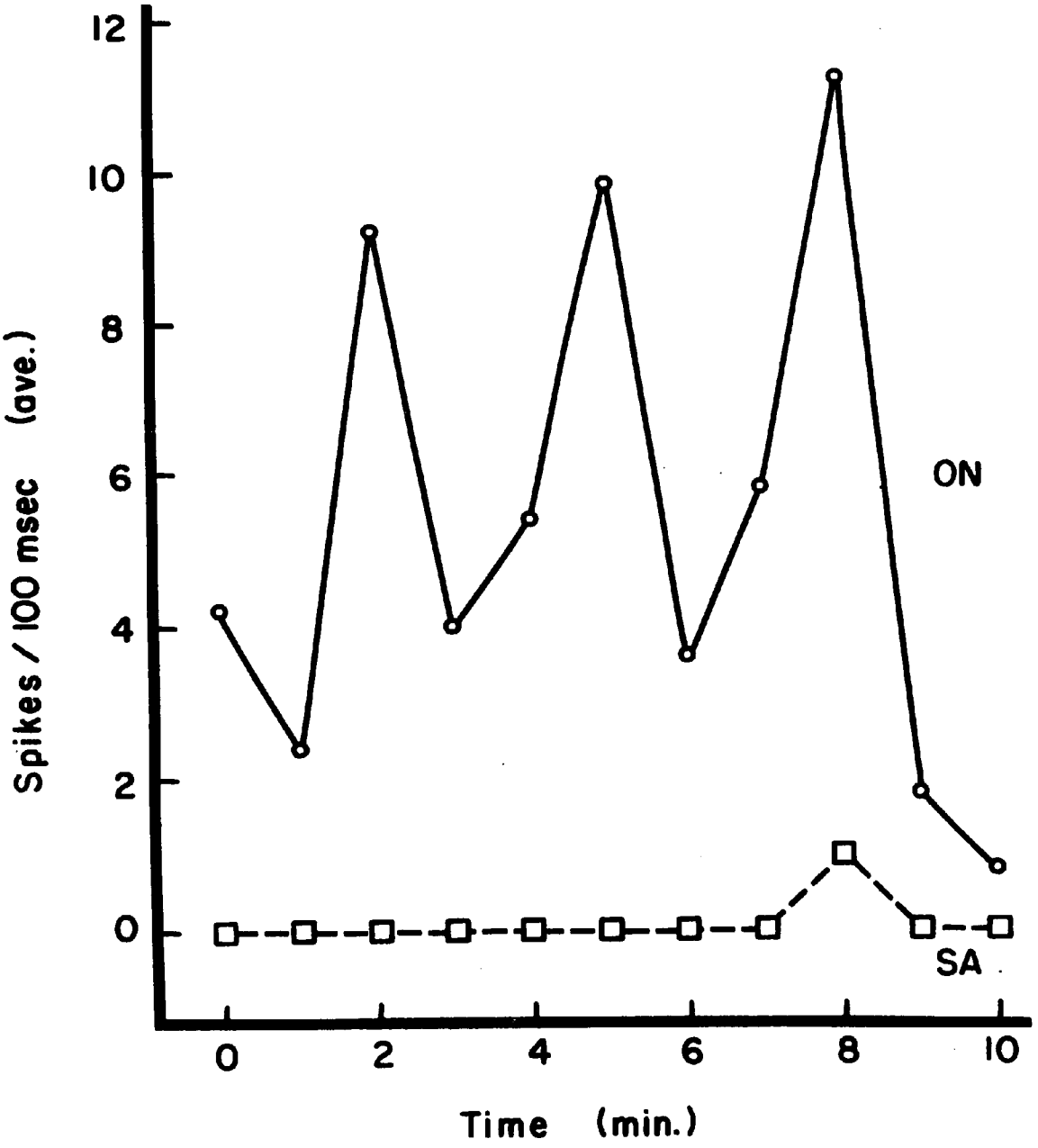


Figure 2.3

phases of the cycling. Thus, for several units an "on-off" response could become a pure "on" response during a different part of the cycle. Since the center and surround spectral sensitivity curves were measured at different times, the differential effect of cycling did not affect the data.

An example of a unit in which the "on" and "off" responses cycle differently is shown in Fig. 2.4. Data are from poststimulus time histograms made every minute for 10 presentations. The spontaneous activity plotted was the average number of spikes in two 100-msec bins prior to the stimulus. The "off" response was the average of the first two bins after light-off. The "on" response was to a large extent confined to one or two bins. As can be seen, the spontaneous activity and "off" response seem to cycle together. Spontaneous activity levels of zero show that the cell is below the firing threshold, but not how much below. It can't be determined, therefore, if sensitivity of the cell is changing or if the response is a constant number of spikes above excitability level. The "on" response is quite different. The cycle has the same period but the "on" response is greatest when the spontaneous activity is lowest. Therefore there is a different excitability cycle for the "on" response. This unit and others which showed the independent cycling of "on" and "off" responses suggest that the generator of the cyclic activity is prior to the ganglion cells.

The change in spontaneous activity levels was a great problem in this study which was not solved or explained. It limited the amount of data which could be obtained from each unit because data collection

Fig. 2.4. Graph of spike discharge rate during cyclically varying response of fiber: "On" response, "off" response, and spontaneous activity. Histograms for 10 flashes of a stimulus light were made once per minute. The number of spikes in two 100 msec bins preceding the presentation of the stimulus were averaged and plotted as a function of time (SA). The "off" response was the average of 2 100 msec bins at the offset of the light. The "on" response which was very brief was the number of spikes in a single 100 msec bin from 100 to 200 msecs after the flash. The SA and "off" response varied cyclically with the same period and in phase. The "on" response varied cyclically with the same period, but out of phase.

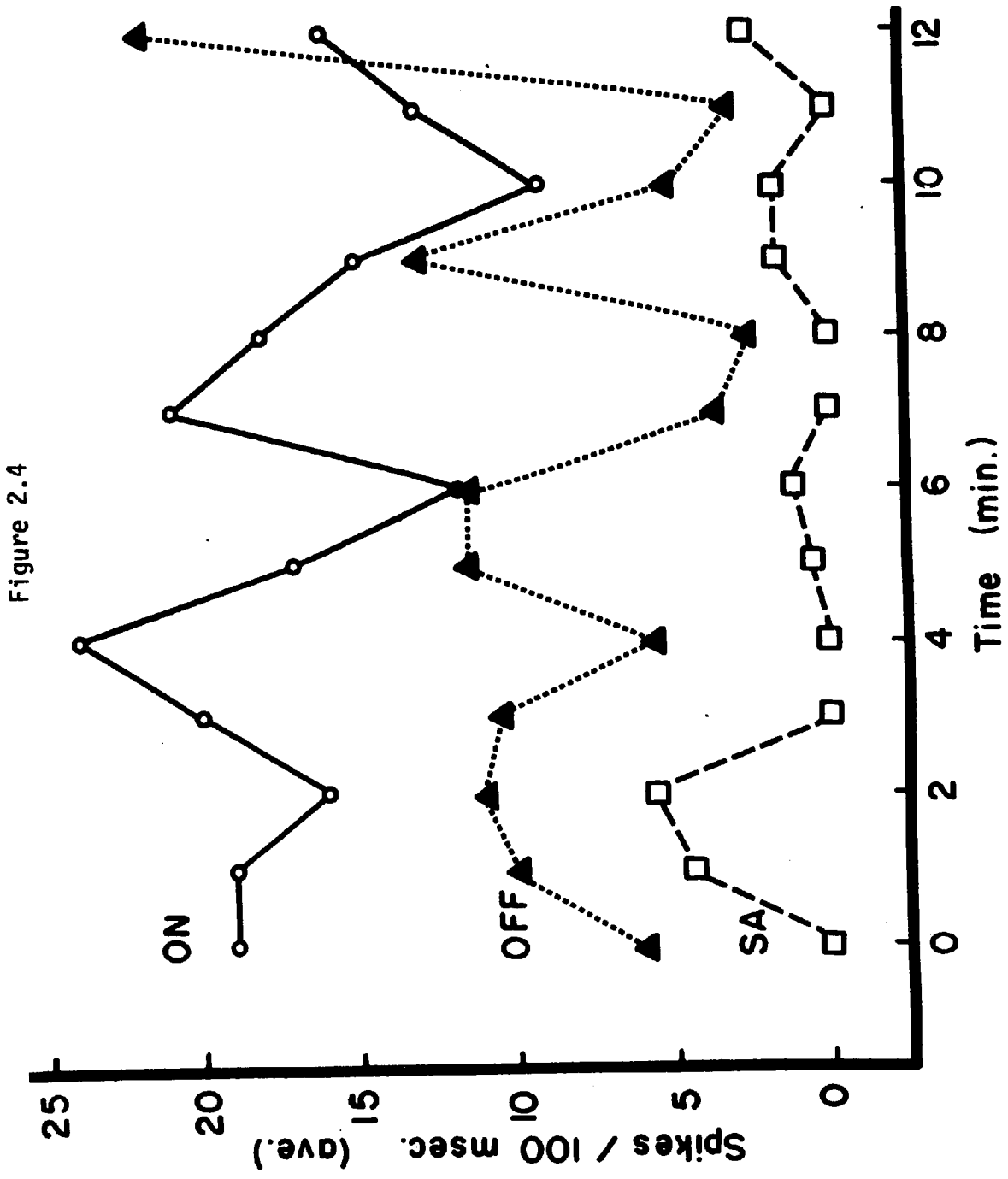


Figure 2.4

took more than twice the usual amount of time. In units with unstable cycling, repeatability was poor and the experiment was not continued.

CHAPTER III

RESULTS I

Spectral sensitivity curves for fibers were measured under dark adapted and various light adapted conditions. At each wavelength in 25 nm steps, the intensity of the light was adjusted so that a threshold response was evoked. When both "on" and "off" responses were present, spectral sensitivity curves were measured for each response. When the log reciprocal intensity for the "on" or "off" response is plotted as a function of wavelength, a spectral sensitivity curve such as in Fig. 3.1 is obtained.

The spectral sensitivity curves of ganglion cell responses measured would ideally be compared to the absorption spectra of known pigments so that the pigments present in the retina can possibly be identified. The rationale for this is that the response properties at all levels of the visual system are influenced by the absorption spectra of input pigments. As an example, a correspondence is found between the psychophysically determined scotopic spectral sensitivity curve of humans and the absorption spectrum of the pigment rhodopsin. The action spectrum is more complicated when more than one receptor mechanism has influence on the response. However, by varying the spectral composition of the background, one or more mechanisms could be selectively depressed leaving the other mechanism relatively more effective. The spectral sensitivity curve of this mechanism could then be compared with absorption spectra of pigments.

In these experiments the Dartnall nomogram templates will be used as an approximation to the real absorption spectra of pigments.

Figure 3.1. Dark adapted spectral sensitivity curve fit by a Dartnall nomogram template, and the template corrected for lens density. This dark adapted spectral sensitivity curve (circles) can be adequately fit by a 500 nm λ_{max} Dartnall nomogram template corrected for the highly pigmented lens. Lens correction measurements used are in Appendix B.

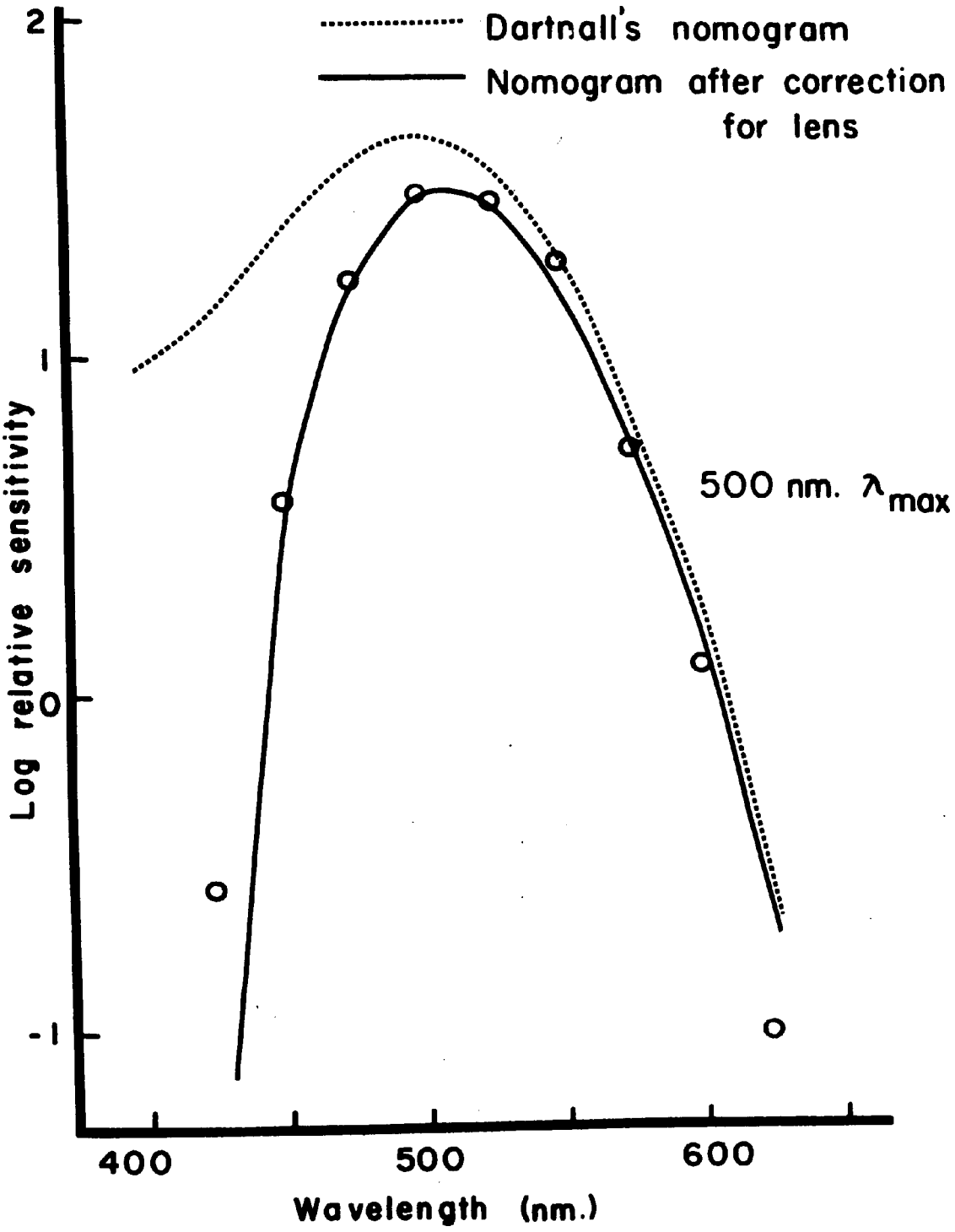

Dartnall's nomogram
Nomogram after correction for lens
500 nm. λmax
Log relative sensitivity
2
1
0
-1
400
500
600
Wavelength (nm.)

Figure 3.1

Dartnall (1953) reported that absorption curves of all isolated pigments plotted with frequency instead of wavelength as the abscissa, have a similar shape. He published a general vitamin A_1 pigment nomogram which could be used to generate pigment spectral absorption curves if the λ_{max} were known. Since mammals have vitamin A_1 based pigment, I felt that the Dartnall nomogram for A_1 pigments would generate hypothetical curves that would be a good approximation to the actual pigments.

Using the Dartnall nomogram for A_1 pigments, I plotted a series of template curves for pigments with λ_{max} in 5 nm steps. The nomogram templates generated were plotted as log sensitivity as a function of wavelength. The nomogram template for 500 nm λ_{max} is shown as the dotted line in Fig. 3.1. A 5 nm difference in λ_{max} between templates was close to the limit at which one template could be chosen as a best fit by eye to the data. Larger steps would have made the decision easier; however, the templates would have less accurately shown differences in the data. My hope was that the distribution of curves which best fit the templates would indicate which λ_{max} pigments were most likely present in the retina.

Before comparison can be made between the physiologically determined corneal spectral sensitivity curves and the nomogram templates for pigments, one must correct for losses of light in the ocular media. In the range of wavelengths 460 to 625 nm, most of the media loss is due to the ocular lens. Rather than correcting each log spectral sensitivity curve, the nomogram templates were transformed into equivalent corneal measurements by subtracting the log density of the animal's lens.

The correction factor for the ground squirrel lens used to transform the nomogram templates were an average of the Brown and Wooten

(unpublished) measurements and my own measurements. With the help of E. Boettner, I measured the spectral transmission of two lenses from Mexican ground squirrels as seen in Appendix B. The correction factor for the highly pigmented yellow lens significantly changed the shape of the nomogram templates. All templates referred to in this thesis will be corrected with these factors.

The Dartnall nomogram template for 500 nm λ_{max} following correction for the lens is also shown in Fig. 3.1. As can be seen, the fit between the template and the spectral sensitivity curve for this unit is very good. This type of comparison was done for all spectral sensitivity curves and the λ_{max} of the best fitting template was determined.

No correction was applied for density of pigment. The degree of broadening due to pigment density is only moderate for ordinary concentrations of pigment (Dartnall, 1953). Pigment density also has no effect on the location of the λ_{max}. Moreover, the templates without this correction fit the data quite well.

Experiments were performed on a total of 82 ground squirrels, 22 of which yielded no data. From the remaining 60 animals, 325 ganglion cell fibers were recorded, receptive fields found, and some response characteristics noted. More quantitative data were obtained from 191 units of the 325 total.

Dark adapted spectral sensitivity curves were obtained for 122 units. Figure 3.2 shows spectral sensitivity curves for two units. Some of the spectral sensitivity curves fit the template for a single pigment reasonably well as seen in Fig. 3.2a. This was unfortunately not always the case. The spectral sensitivity curve in 3.2b was chosen to illustrate a poorly fitting template. The curve was broader than

Figure 3.2. Two examples of dark adapted spectral sensitivity curves with best-fit nomogram templates. a) This spectral sensitivity curve is adequately fit by its best-fit template, 505 nm λ_{max}. b) This spectral sensitivity curve is poorly fit by its best-fit template, 490 nm λ_{max}.

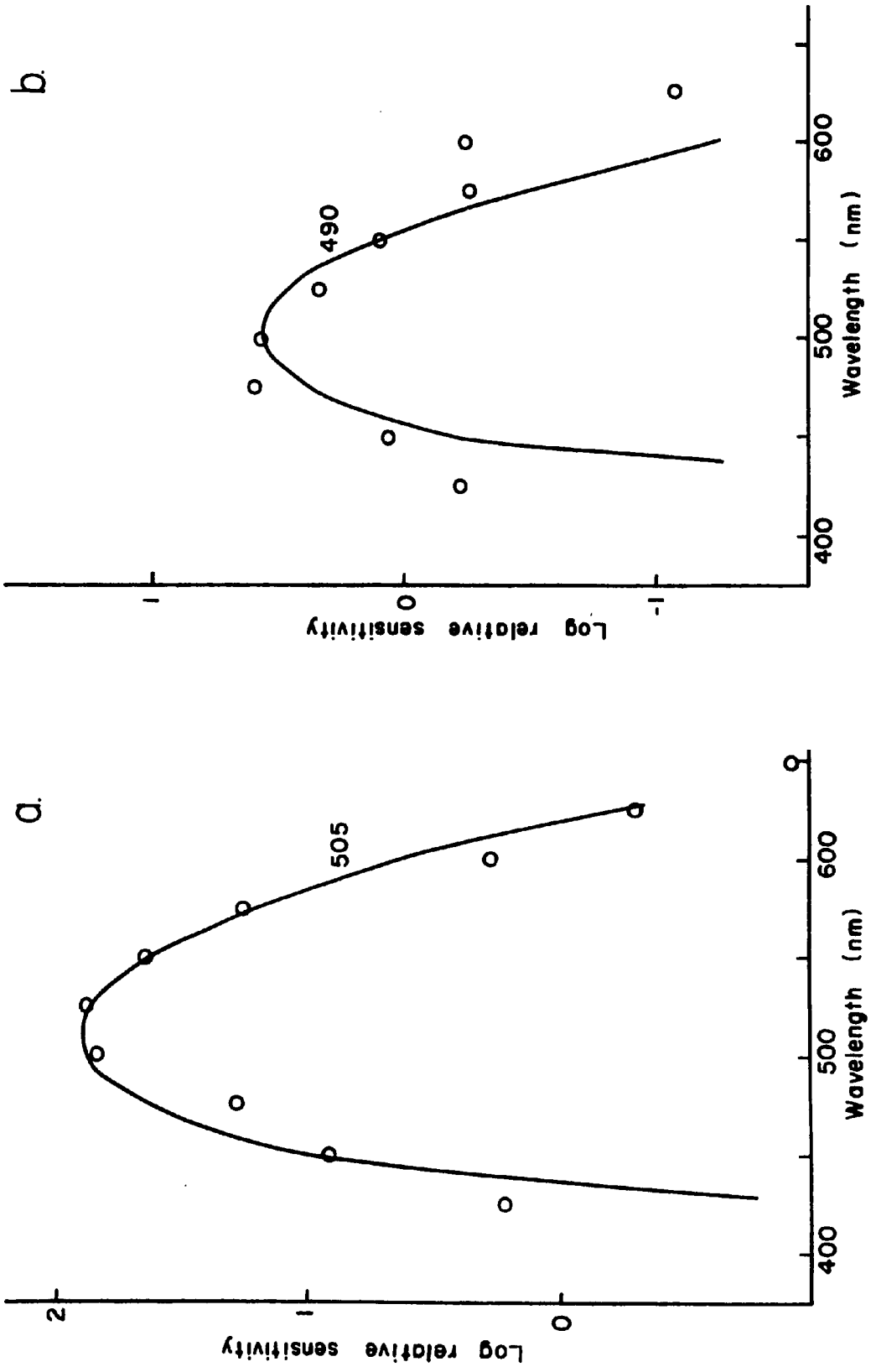

a.
Log relative sensitivity
2
1
0
505
400
500
600
Wavelength (nm)
b.
Log relative sensitivity
1
0
-1
490
400
500
600
Wavelength (nm)

Figure 3.2

a single pigment template, and even the best fitting template fit poorly. This could represent experimental error in measurement or inputs from more than one pigment. Rather than make a judgment on the relative likelihood of these two possibilities for each poorly fitting curve, all curves were fit with a single nomogram even if further evidence showed that two inputs were likely. Spectral sensitivity curves were also measured on a white background to test for a Purkinje shift. These curves were fit with templates in a similar manner.

A good fit between an experimentally determined spectral sensitivity curve and a template of a single pigment did not necessarily mean that one pigment alone had input. Chromatic adaptation provided a stronger test. In some units in which the dark adapted or light adapted spectral sensitivity curve fit the nomogram of a single pigment, chromatic adaptation merely shifted the spectral sensitivity curve on the vertical axis without changing its shape, indicating that indeed only one receptor mechanism had input. In other units, however, it was found that on a chromatic background this shape changed, indicating that a second receptor mechanism had input. The second input could have been dominated by the first, experimental error could have obscured the contribution from a second pigment, or a nonlinear combination of two pigments produced a resultant spectral sensitivity curve which was an approximation to a single template. The last possibility will be discussed further in Chapter 5. The results of the chromatic adaptation of a single fiber will be illustrated in the next chapter. Dark adapted and light adapted or chromatically adapted spectral sensitivity curves were completed for 52 units.

A histogram of the number of spectral sensitivity curves which

fit each nomogram template is found in Fig. 3.3. There are three peaks in the histogram suggestive of three pigments: 445, 500-505 (502), and 525. The 505 peak is the largest, most likely because the spectral sensitivity curve in the totally dark adapted condition was always measured first. In some units this was the only condition studied.

One question that might be asked is if there are three pigments, why doesn't the histogram show three unequivocable peaks? One way of addressing the question is to look at the difference in shape and spectral location of the nomogram templates most often fit by the data. In Fig. 3.4 are three spectral sensitivity curves of three different units: one which is fit by a 445 template (a) (measured under yellow chromatic adaptation), one fit by 502 (b) (dark adapted), and one fit by 525 (c) (dark adapted). The templates of all three λ_{max} pigments are included for comparison in a, b, and c. As can be seen the shape of the 445 and 502 λ_{max} templates are quite dissimilar. The variance in the short wavelength portion of the histogram is probably due to the small number of measurements in each action spectrum making it difficult to fit nomogram templates for most units. Not all curves had as many data points as the one shown in Fig. 3.4a. I do not have great confidence in the precise identification of the blue cone. However, it would not be difficult to distinguish a spectral sensitivity curve fitting 445 from one fitting 502. The 502 and 525 templates, however, are rather similar differing mainly in the long wavelength sensitivity. It is probable that experimental error would cause some spectral sensitivity curves mediated by either 502 or 525 to best fit a nomogram template with a λ_{max} between them.

There are several reasons other than variability in the

Figure 3.3. Histogram of the number of spectral sensitivity curves which best fit each nomogram template. The height of each bar represents the number of spectral sensitivity curves which are best fit by that λ_{max} template. Templates are drawn for λ_{max} in 5 nm steps. Shading indicates the light condition under which each curve was measured: dark adapted (DA), light adapted (LA), and chromatically adapted (blue or orange bkgd.). The histogram suggests three peaks: blue (445), 502-505, and 525 λ_{max}.

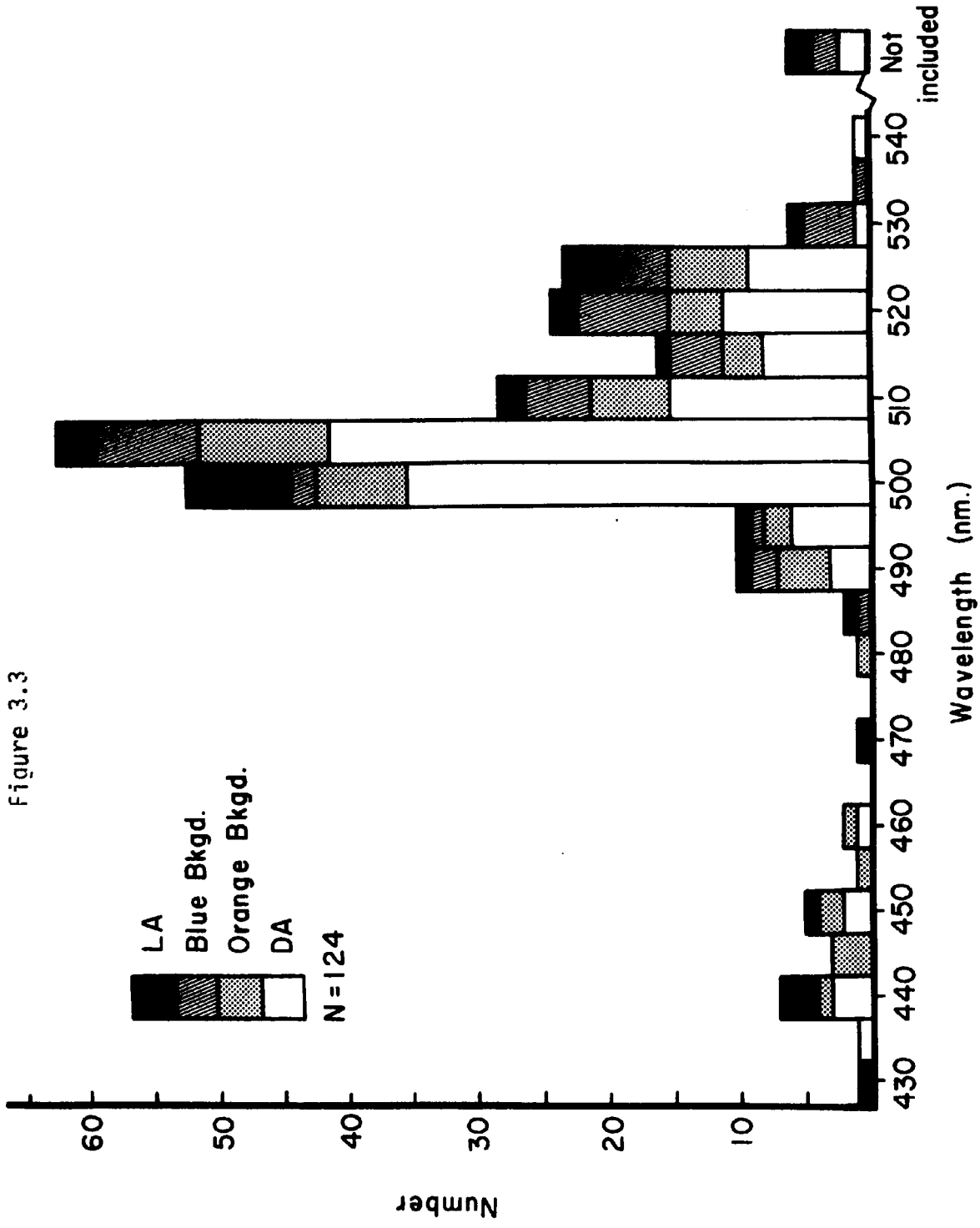

LA
Blue Bkgd.
Orange Bkgd.
DA
N=124
Number
60
50
40
30
20
10
430
440
450
460
470
480
490
500
510
520
530
540
Not included
Wavelength (nm.)

Figure 3.3

Figure 3.4. Three representative spectral sensitivity curves which fit nomogram templates for 445, 502, and 525 λ_{max}.

a) A spectral sensitivity curve measured on a yellow background is fit with a 445 λ_{max} template.

b) A dark adapted spectral sensitivity curve is fit with a 502 λ_{max} template and

c) a dark adapted spectral sensitivity curve is fit with a 525 λ_{max} template. 445, 502, and 525 λ_{max} templates have been included for comparison with all spectral sensitivity curves.

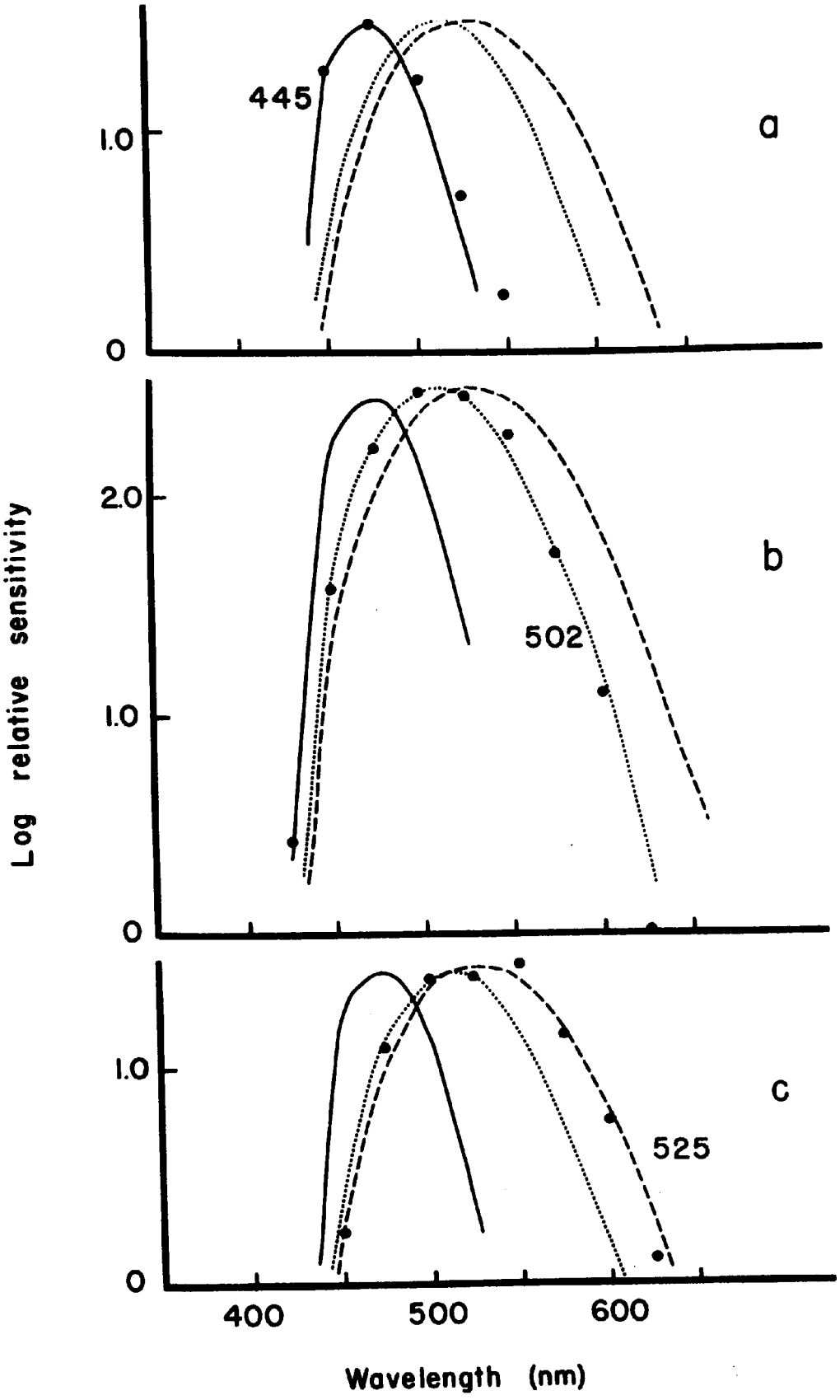

445
a
1.0
0
b
2.0
502
1.0
0
c
1.0
525
0
400
500
600
Wavelength (nm)
Log relative sensitivity

Figure 3.4

measurements why there would be curves which best fit templates between 505 and 525, even if there were actually two distributions. Units with spectral sensitivity curves broader than a single nomogram could sometimes upon chromatic adaptation be shown to have input from more than one receptor mechanism. However, the curve broader than a single template would still best fit a template with λ_{max} between the two, although poorly. Also spectral sensitivity curves of color opponent units were frequently distorted by opponency.

In spite of these explanations, it still can be argued that the results might be due to variance around a single pigment and not due to two separate pigments. To more convincingly show that the 505 and 525 peaks in the distribution are real, the data can be separated by lighting condition under which they were measured as seen in Fig. 3.5. In all four histograms, dark adapted, light adapted, orange adapted, and blue adapted, there are two peaks representing the middle and long wavelength part of the spectrum with a dip between occurring at the same place. I take this to be further indication that there are three, not two pigments in the ground squirrel retina. The shading of the bars represents the type of ganglion cell, color opponent and non-color opponent (contrast sensitive), from which the curve was measured. This aspect of the fibers will be discussed in the next chapter. Note also that the 445 peak was not seen on the blue background and was seen on the orange background. This is what would be expected if the background were completely effective in suppressing the blue mechanism. Under orange chromatic adaptation, the long wavelength mechanism was often still measurable, although depressed.

Due to small sample size and large variance the evidence is only

Figure 3.5. Histograms of the number of spectral sensitivity curves which best fit each nomogram template under each lighting condition. Notice that there is an indication of a blue peak, a 502 peak, and a 525 peak indicated by the dotted lines. Under all lighting conditions, there are both 502 and 525 peaks. Shading indicates the classification of the fiber from which the spectral sensitivity curve was measured.

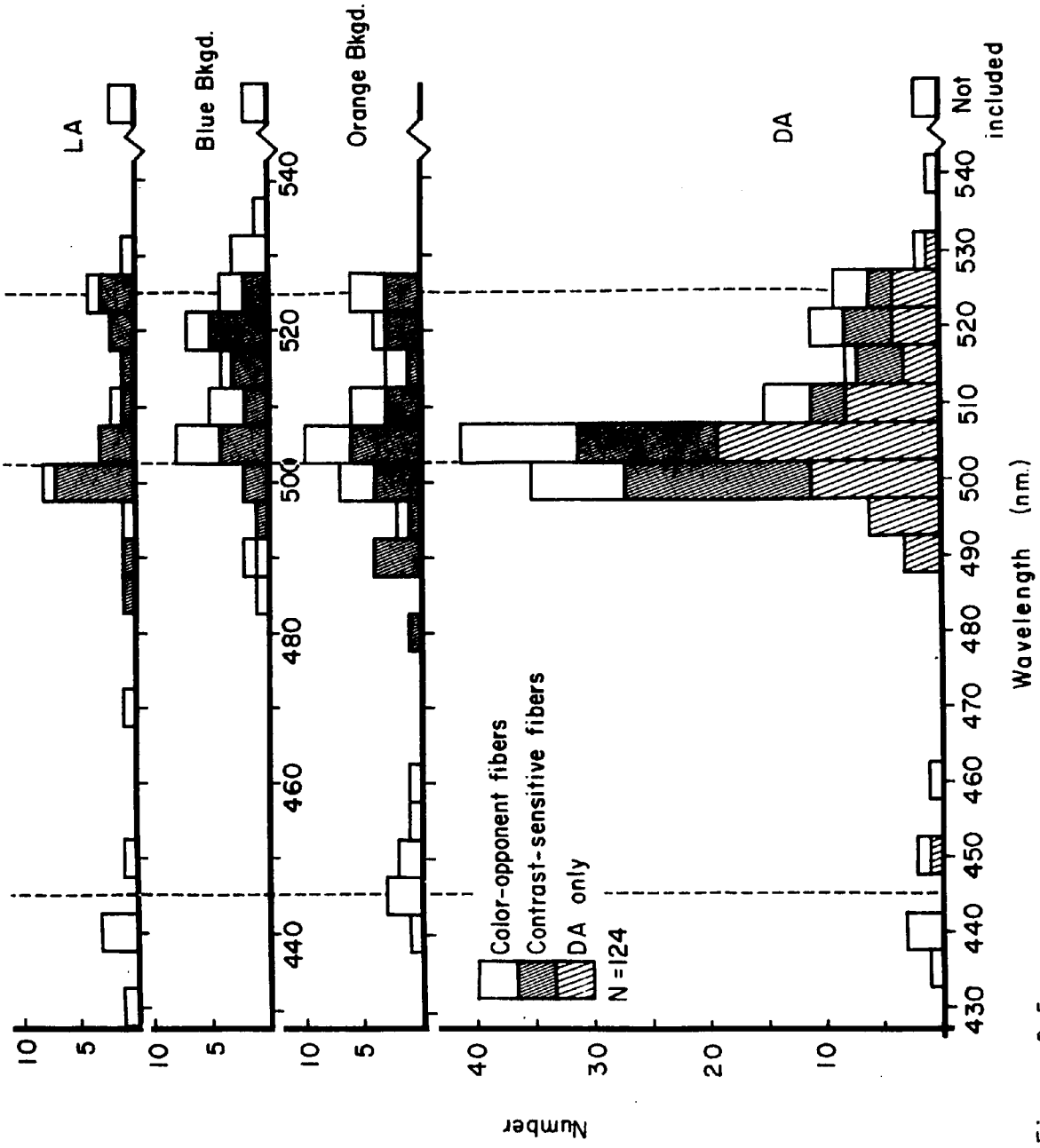

LA
Blue Bkgd.
Orange Bkgd.
DA
Not included
Color-opponent fibers
Contrast-sensitive fibers
DA only
N=124
Number
Wavelength (nm.)

Figure 3.5

suggestive of three peaks and therefore the existence of three pigment mechanisms. This method of looking at the distribution of all spectral sensitivity curves is a conservative way of showing three pigments however, since a response mediated by the additive combination of two receptor mechanisms would be represented as having a single input with an intermediate λ_{max}. Variance in the histogram might be due to several factors: variance due to error in measurement and variance due to differences in input to different units. The latter is what we would like to measure. An estimate of the former might be gained by knowing which of the curves under the different lighting conditions were measured from the same fiber. In the next chapter individual units and their spectral sensitivity curves measured under various lighting conditions will be described.

CHAPTER IV

RESULTS II

General comments

The fibers studied had a variety of response properties. For simplicity and because of the precedent set by Michael, the fibers have been separated into two categories, contrast sensitive and color opponent. An alternate categorization of units is presented in Appendix C.

Fibers were categorized as contrast sensitive (nonopponent) if they responded with the same sign response to all wavelengths of light. This does not mean that stimuli of all sizes elicited the same response. When the size of the spot was made large, some units changed their response, depending on the spatial organization of inputs. In any case, however, for contrast sensitive fibers orange and blue stimuli elicited the same type of response if the stimuli were the same size.

The spatial organization was determined for a fiber by increasing the size of the stimulus spot and noting the change in response. Three results were obtained: some fibers increased their response to a maximum which was maintained as the spot size was further increased; in others the response increased to a maximum, then decreased, indicating an inhibitory surround; in still others a response of the opposite sign appeared with larger stimuli. The optimal size for a center response for measuring spectral sensitivity was determined in this manner. The spatial extent of center and surround could be quantified by measuring Ricco's law summation area, the area over which intensity times area of the stimulus equals a constant at threshold.

Contrast sensitive units were found with "on" centers, and others were found with "off" centers.

Fibers were categorized as color opponent if they responded to long and short wavelength stimuli with different sign responses. Orange and blue test stimuli were presented as described in Chapter 2 to determine if the fibers were opponent. If the fiber responded in an opponent fashion to the large, bright stimuli, the unit was categorized as opponent. Verification came from measuring the spectral sensitivity for the "on" and "off" responses and finding that they were different.

As in the contrast sensitive units, a white stimulus of increasing size was used to determine the optimal size for the center response. Then units were tested with orange and blue stimuli of varying sizes to measure the spatial extent of the opponent mechanisms. Color opponent units were found with "on" centers and with "off" centers with the opponent mechanisms in the surround. Others were found with evidence for both "on" and "off" opponent mechanisms in the center.

The receptor mechanisms of each unit, contrast sensitive or color opponent, were determined by measuring spectral sensitivity curves and comparing them to nomogram templates as described in the previous chapter. In the previous chapter units were categorized by their best fit to one of a series of nomogram templates in 5 nm λ_{max} steps. The histogram of the distribution of spectral sensitivity curves suggested that there are three receptor mechanisms with λ_{max} approximately at 445, 502, and 525. In this chapter I will assume that this is true. All spectral sensitivity curves have therefore been recategorized as

representing one of the three.

The spectral sensitivity curves were measured under dark adapted conditions, then light adapted conditions to look for a Purkinje shift. Spectral sensitivity curves measured on chromatic backgrounds were used to determine if more than one receptor mechanism had input to a response. Chromatic adaptation also suppressed opponency in color opponent units so that a single pigment mechanism would better fit a nomogram template. Spectral sensitivity curves under the different lighting conditions were measured for both center and surround when a surround was present. The categories of units based on receptor inputs and evidence for them are described in this chapter.

Contrast sensitive fibers

a) 502 (rod) alone

Data from 16 fibers suggest that there is a class of fibers with input from the 502 receptor alone. Spectral sensitivity curves from one such fiber are seen in Fig. 4.1. The log relative sensitivity has been plotted as a function of wavelength as in all subsequent spectral sensitivity figures. The dark adapted spectral sensitivity curve shown as open circles was measured using a test of 2° visual angle. The spectral sensitivity curve has been fit with a 502 template for comparison (solid line) which fits quite well. The unit had a small receptive field and showed no evidence of a surround response to large tests. Like most fibers in this class, the dark adapted threshold was very low, approximately -2.0 log cds/m^2.

The light adapted spectral sensitivity curve was measured on a white background of 1 log cd/m^2 to determine if there were a Purkinje shift. The background intensities were chosen to be as bright as

Fig. 4.1. Spectral sensitivity curves of a contrast sensitive fiber fit with a 502 template: Dark adapted and light adapted. Spectral sensitivity curves, dark adapted (DA) and light adapted (LA) (white background, 1 log cd/m^2) have been superimposed with a 502 nomogram template. LA data have been shifted vertically upward 2.1 log units. Test stimuli were 2°. Templates in this and following figures are shown as solid lines and are labeled with their λ_{max}.

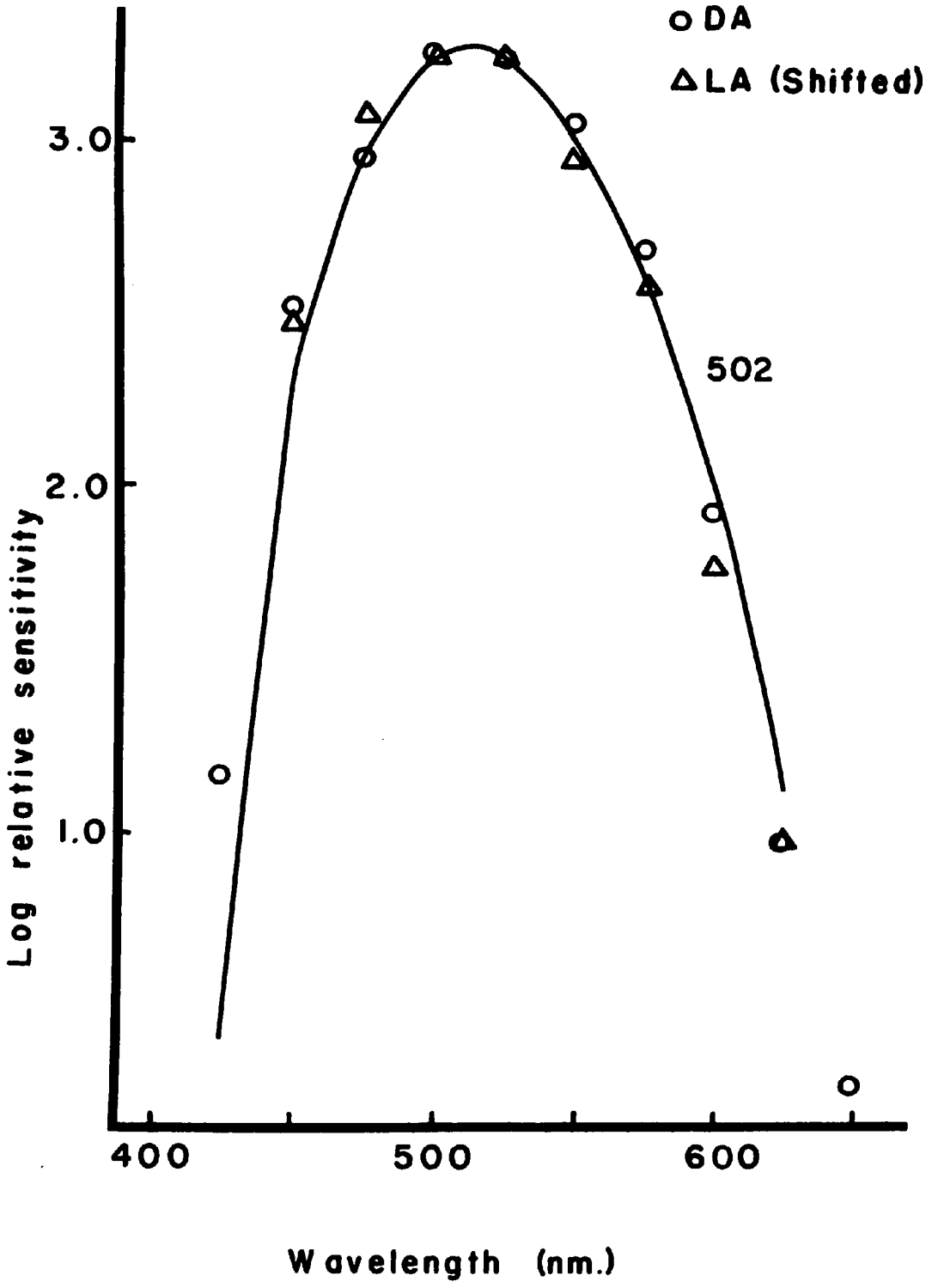

o DA
Δ LA (Shifted)
502
3.0
2.0
1.0
Log relative sensitivity
400
500
600
Wavelength (nm.)

Figure 4.1

possible, yet allowing enough sensitivity to measure an identifiable spectral curve, as described in Chapter 2. In this unit, the background raised the threshold 2.1 log units. The curve, shown as triangles, has been shifted vertically 2.1 log units so that the peak sensitivity matches that of the dark adapted curve for easier comparison. The shapes of the curves are very similar, both fitting the 502 template. This lack of a Purkinje shift suggests that only one receptor mechanism had input.

To be certain, however, spectral sensitivity curves under chromatic adaptation must be measured. The effects of chromatic adaptation on one fiber in this class can be seen in Fig. 4.2. As in the unit of Fig. 4.1, the dark adapted spectral sensitivity curve, shown as open circles, and the light adapted curve (unshifted), shown as squares, have been fit with 502 templates (solid lines), which fit adequately. As can be seen, the white background of 0.4 log candles/m^2 reduced sensitivity 2.0 log units from the dark adapted state. The spectral sensitivity curve measured on a blue (Wratten 47, -0.2 log cds/m^2) background is shown as inverted triangles. With the exception of the 575 nm point, the 502 template fits well. The upright triangles are the sensitivity measurements on an orange (Wratten 21, 0.3 log cds/m^2) background. A template has not been fitted to the curve; however, a 502 template would fit adequately. The chromatic backgrounds raised threshold approximately 2 log units. Because neither chromatic background systematically shifted the spectral sensitivity curve, it is likely that only one receptor mechanism mediated the response.

No surround response to longer tests and stimuli presented outside

Figure 4.2. Spectral sensitivity curves of a contrast sensitive fiber fit with 502 templates: Chromatic adaptation. Dark adapted (DA) and light adapted (LA) (white background, 0.4 log cds/m ^{2}) spectral sensitivity curves, and those measured on an orange background (Wratten 21, 0.3 log cds/m^2) and blue background (Wratten 47, -0.2 log cds/m^2 can be compared with 502 templates. The LA and blue adaptation curves are an average of two measurements each.

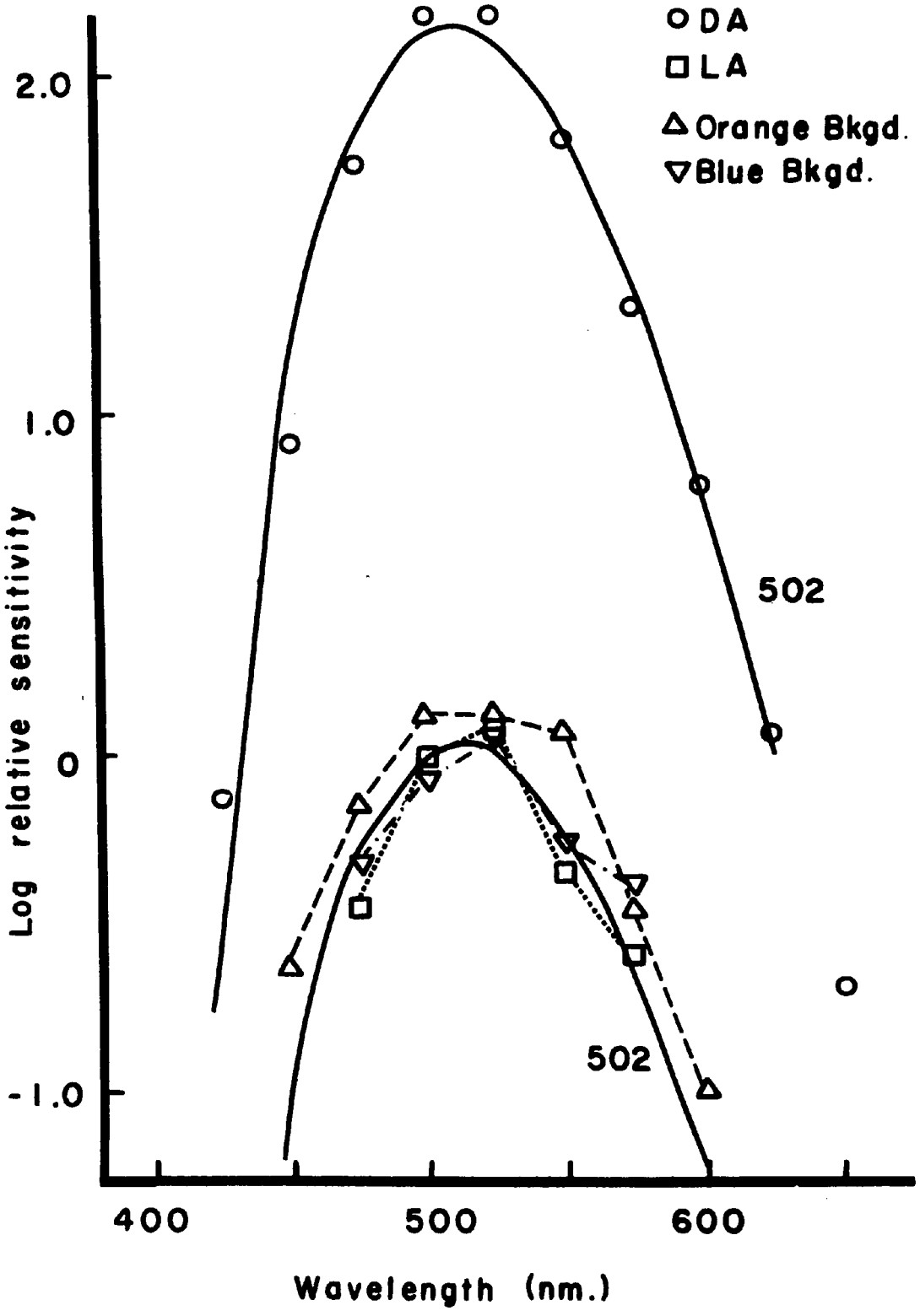

DA
LA
Orange Bkgd.
Blue Bkgd.
2.0
1.0
0
-1.0
Log relative sensitivity
502
502
400
500
600
Wavelength (nm.)

Figure 4.2

of the center was seen for the units in Figs. 4.1 and 4.2. Some units had surround responses, however, as illustrated in Fig. 4.3. This unit had an "off" center, "on" surround as determined by increasing the size of the test stimulus and noting the change in response from "off" to "on-off." Spectral sensitivity curves were measured separately for center and surround. The center test size was 1.5°, determined by increasing the size of the test until the "off" response was greatest. The annulus used to measure surround sensitivity had an outer diameter of 10° and an inner hole diameter of 1.5°. The spectral sensitivity curve for the center"off" response is seen as open circles. The fit of the data to the superimposed 502 template is fair. The surround was much less sensitive than the center. The spectral sensitivity (filled circles) for the "on" response at 500 nm is 2.6 log units down from the spectral sensitivity of the center. The spectral sensitivity curve for the surround has been fit with a 502 template. A light adapted spectral sensitivity curve (open squares) was measured only for the center response, and a 502 template has been superimposed. The background was approximately -1.0 log cds/m^2. The surround light adapted action spectrum was not measured, although qualitative observations of relative response to spectral lights suggested that it, too, was mediated by the 502 mechanism. This unit and others like it suggest that there are units with 502 input alone to both center and surround.

One might wonder if this class of units would show a dark adaptation curve typical of rods. The possibility exists that the 502 pigment could be in a cone. Figure 4.4 shows a unit for which the dark adaptation curve was measured. At the top of the figure (a) are the spectral sensitivity curves measured under dark adapted and

Figure 4.3. Spectral sensitivity curves of a contrast sensitive fiber fit with 502 templates: Center-surround organization. A 1.5° test was used to measure the spectral sensitivity curve for the center response ("off") in the dark adapted (DA) condition and the light adapted condition (LA) (white background, -1.0 log cds/m^2). An annulus (10° outer diameter, 1.5° center hold) was used to measure spectral sensitivity for the surround response ("on") in the dark adapted (DA) condition. All curves have been fit with 502 templates.

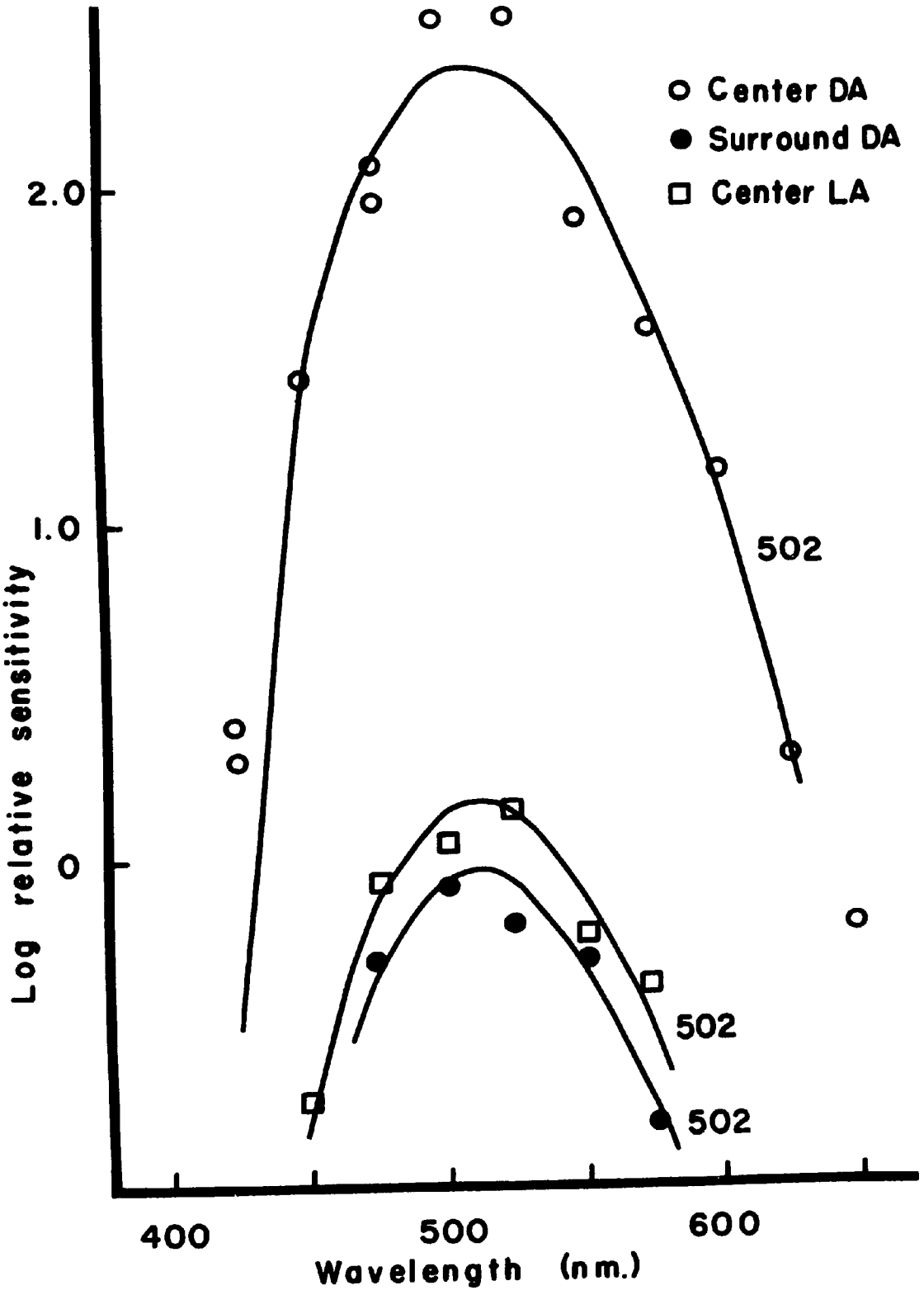

Center DA
Surround DA
Center LA
2.0
1.0
0
Log relative sensitivity
502
502
502
400
500
600
Wavelength (nm.)

Figure 4.3

Figure 4.4 Spectral sensitivity curves (a) and dark adaptation curve (b) of a contrast sensitive fiber with evidence for 502 input alone. a) Spectral sensitivity curves were measured with a 2° test under dark adapted (DA) conditions, on a red background (Melles Griot, 650 nm λ_{max}, 2.0 log cds/m^2) and on a blue background (Melles Griot, 450 nm λ_{max}, 0.85 log cds/m^2). All curves have been superimposed with 502 templates. b) A dark adaptation curve of threshold intensity for a 2° 500 nm test was plotted as a function of time following a 3-min. bleach. Dark adapted sensitivity (filled triangle) is recovered after 25-30 minutes.

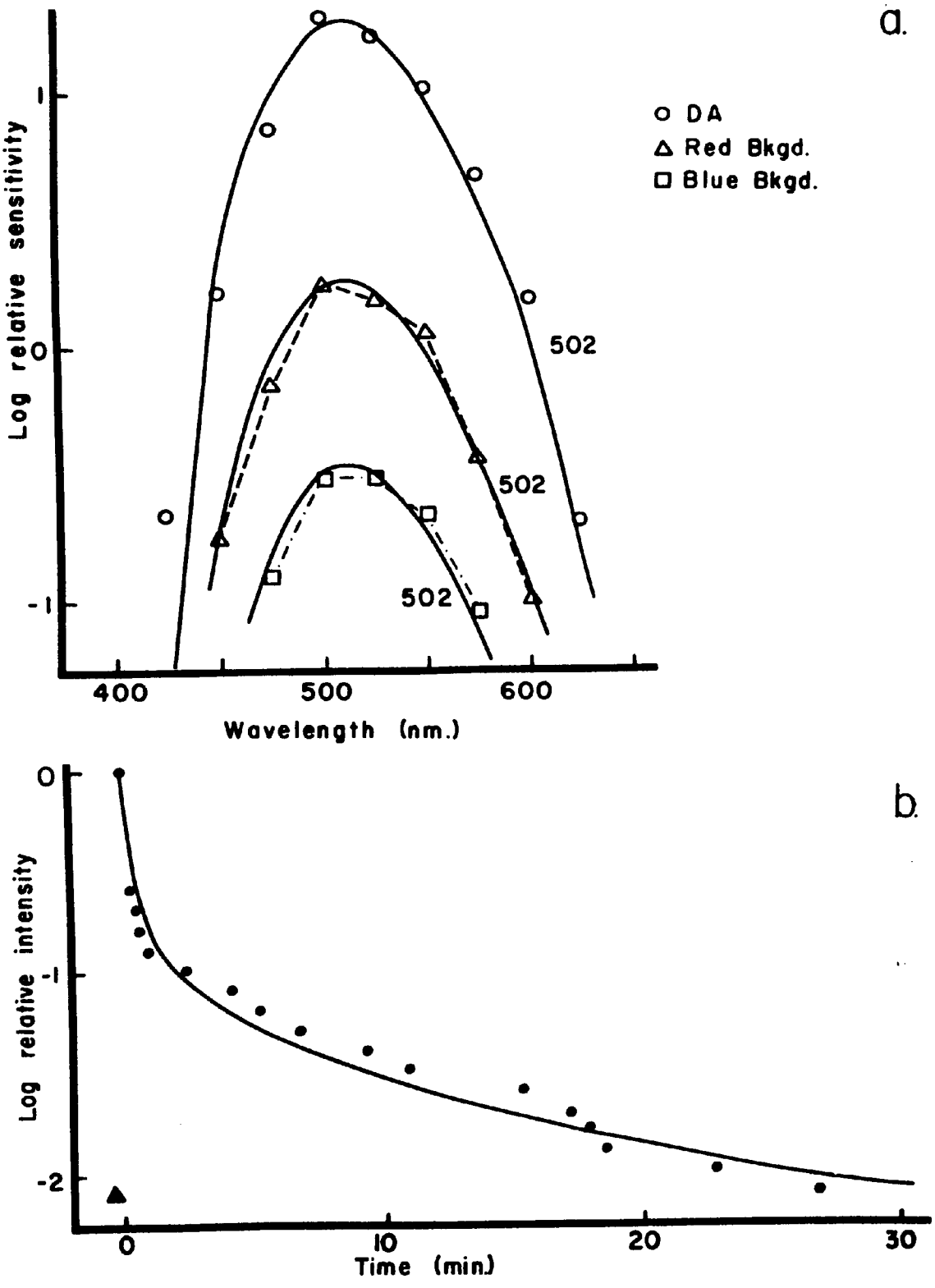

a.
o DA
Δ Red Bkgd.
□ Blue Bkgd.
502
502
502
Log relative sensitivity
1
0
-1
400
500
600
Wavelength (nm.)
b.
Log relative intensity
0
-1
-2
0
10
20
30
Time (min.)

Figure 4.4

chromatically adapted conditions with a 2° test. The backgrounds used were .85 log cds/m^2 for the blue (Melles Griot, 450 nm λ_{max}) and 2.0 log cds/m^2 for the red (Melles Griot, 650 nm λ_{max}). These are all fit by 502 templates. The dark adaptation curve seen at the bottom (b) was measured following a 3-minute full field bleach using the unattenuated Xenon arc source (2.94 log cds/m^2) directed onto the receptive field of the unit. This light would bleach over 90% of the pigment (See Appendix A). The log relative intensity of the test flash (2°, 500 nm) to give a threshold response is plotted as a function of time. The sensitivity returned to the dark adapted threshold for this stimulus (triangle) in 25to 30 minutes.

b) 502 (rod) + 525 (cone).

Thirteen cells showed some evidence that both 502 and 525 mechanisms mediated the same sign response. The fiber in Fig. 4.5 and 4.6 illustrates one variation of this receptor organization. To determine the optimal size stimulus and to determine if a surround were present, Ricco's law was tested for this unit by measuring the summation area as shown in Fig. 4.5. Threshold was found for stimuli of increasing diameter and the log intensity for threshold response plotted as a function of the log diameter. If there were complete summation, the data would fit a line with a slope of 2. At small diameters the data points approximate a slope of 2 as can be seen by comparison with the line drawn in. However, for diameters greater than 2.5° it is clear that the slope of the data decreases and is fit better by the second line with a slope of .75. For diameters greater than 9° the slope drops to zero. At no diameter was a surround response seen or an inhibition which could have caused the data to drop below the final zero summation

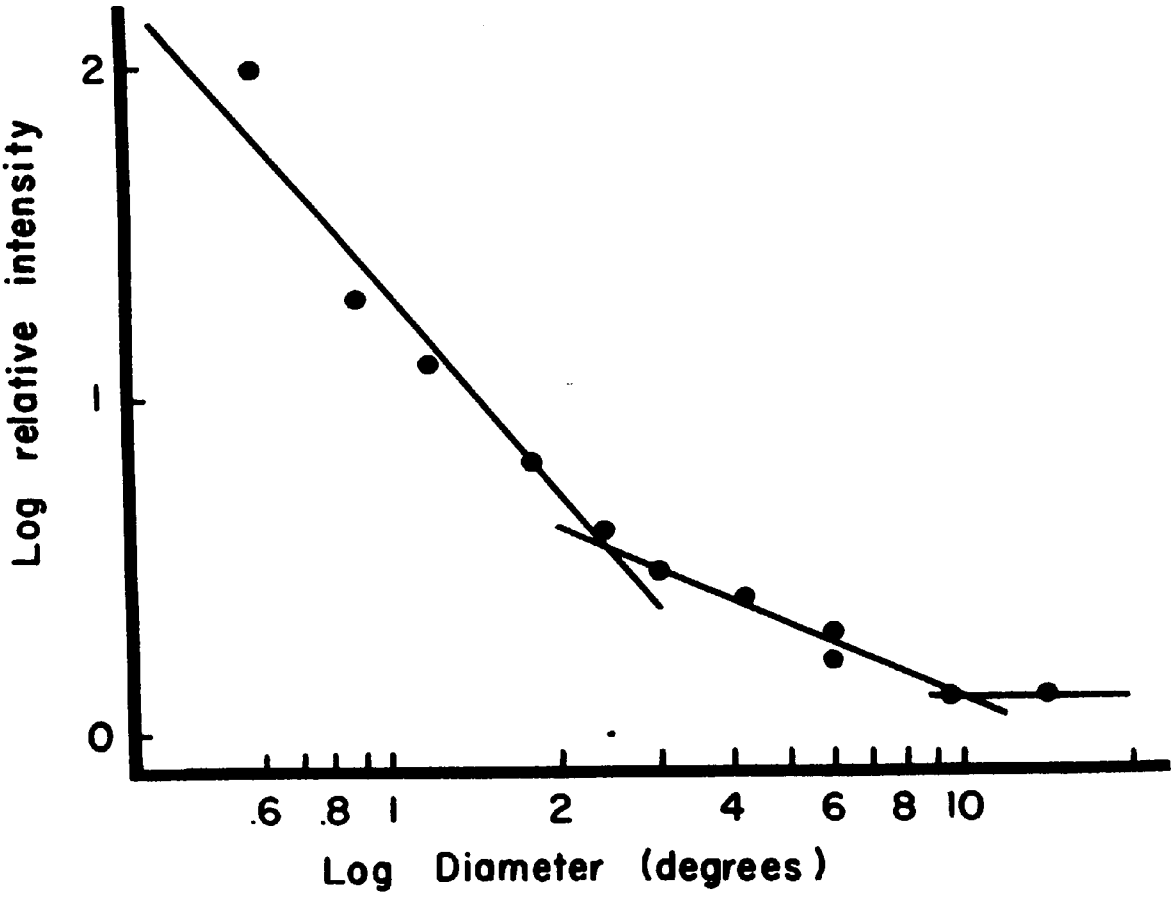


Figure 4.5. Ricco's law summation area measured for a contrast sensitive fiber. Log intensity for a threshold response is plotted as a function of log diameter of the stimulus for diameters .6 to 14.5°. A line of slope 2, then a line of slope .75, and finally a line of slope 0, have been drawn in for comparison.

Figure 4.6. Spectral sensitivity curves of the same contrast sensitive fiber as in Fig. 4.5 fit with 502 and 525 templates. Dark adapted (DA) spectral sensitivity curves measured with and without an opaque contact lens with a small pupil (2.5 mm) have been superimposed with a 502 template. The curve measured with the pupil has been shifted .7 log units. Spectral sensitivity curves measured on a white background (LA) (0.6 log cds/m^2) yellow background (Wratten, approximately 0.3 log cds/m^2) and blue background (Wratten 47, 0.1 log cds/m^2) have been compared to a 525 template. Test stimuli were 12°.

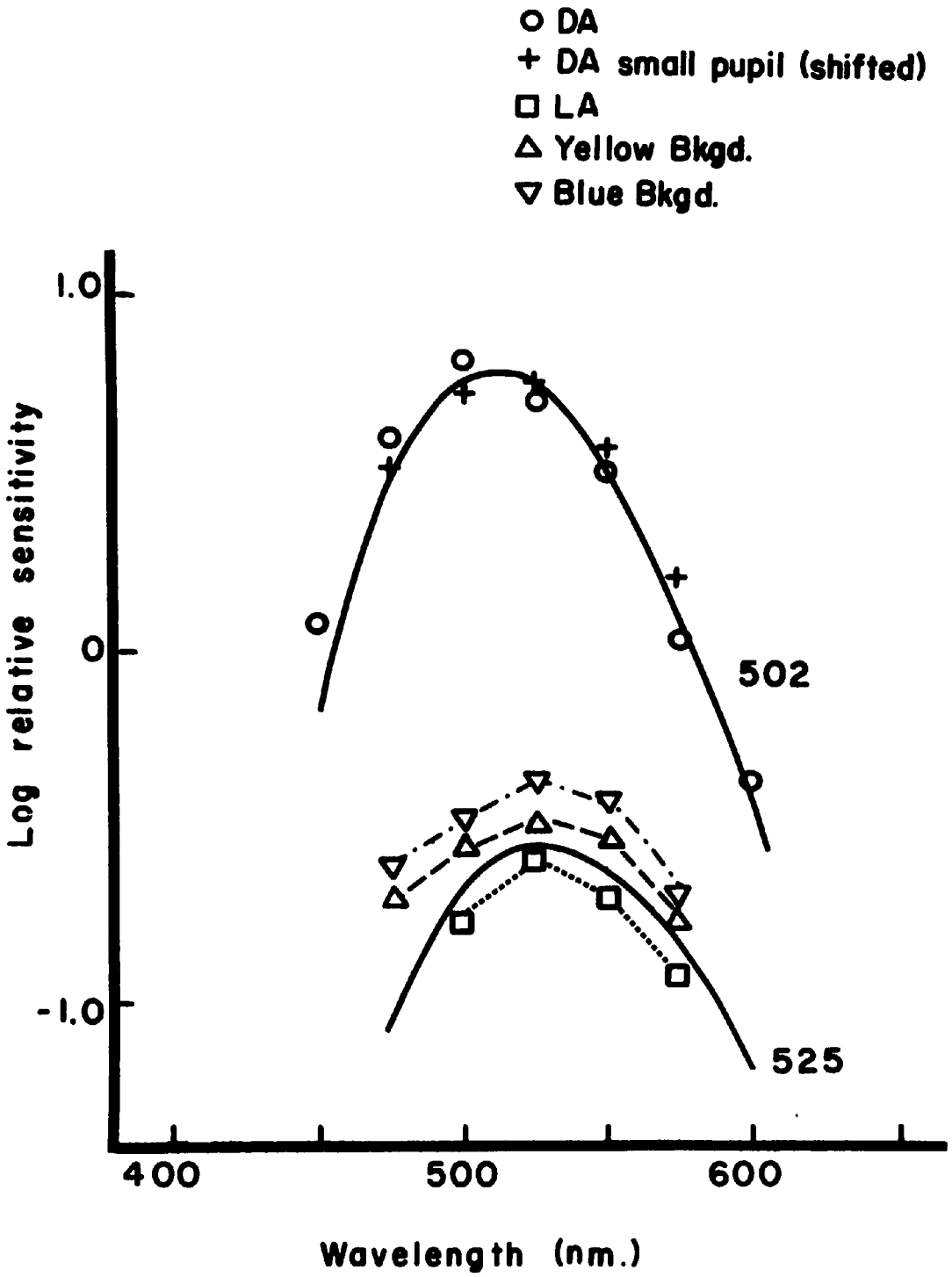

O DA
+ DA small pupil (shifted)
□ LA
△ Yellow Bkgd.
▽ Blue Bkgd.
1.0
0
-1.0
Log relative sensitivity
502
525
400
500
600
Wavelength (nm.)

Figure 4.6

line. Test stimuli of 12° were used to measure spectral sensitivity curves.

In this unit two dark adapted spectral sensitivity curves were measured: with and without a small opaque contact lens in which a small pupil had been drilled. The pupil, which was originally applied to increase depth of focus, was removed to allow more light to enter the eye. The curve measured with the pupil (plus signs) has been shifted up vertically 0.7 log units for comparison with the other dark adapted curves (circles) and the 502 template (solid line). Although the dark adapted curve deviates on the short wavelength limb somewhat, the template fits both curves adequately. A spectral sensitivity curve was next measured on a white background (0.6 log cds/m^2) which is shown in Fig. 4.6 as squares. Spectral sensitivity curves were also measured on blue (Wratten 47, 0.1 log cds/m^2) and yellow (Wratten 15, approximately 0.3 log cds/m^2) backgrounds, shown as inverted and upright triangles, respectively. A 525 template has been drawn for comparison with these three curves. As can be seen, the template fits well for all data points except 475nm. The sign of the response remained the same under all lighting conditions.

Spectral sensitivity curves from the unit just described and five others suggest that in some ganglion cells the 502 mechanism is more sensitive than the 525 mechanism: the 502 mediates the center response in the dark adapted condition and upon light adaptation the 525 mechanism dominates, mediating the same sign response.

In other units there is evidence that the 502 and 525 mechanisms have almost equal sensitivity in the dark adapted state. An example is shown in Fig. 4.7. In this unit the dark adapted spectral

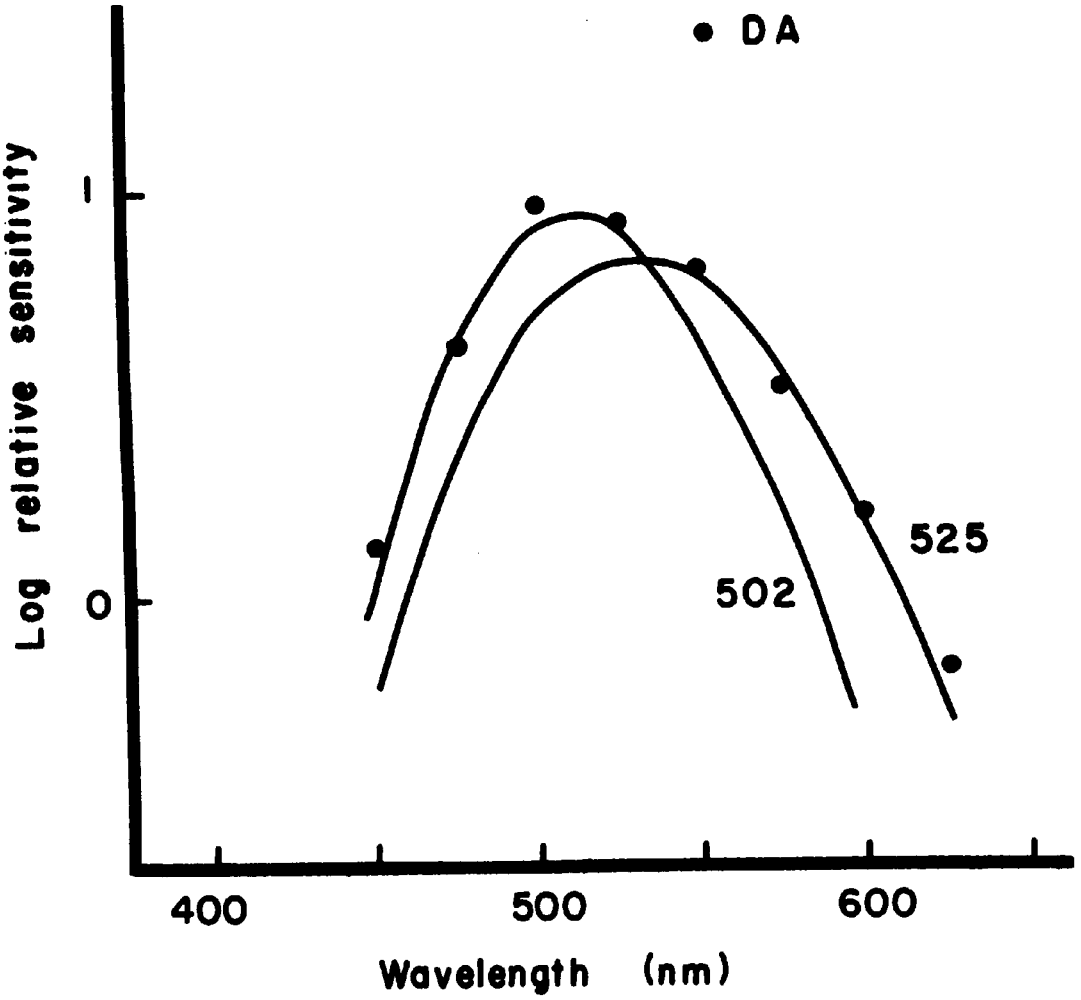


Figure 4.7. Spectral sensitivity curve of a contrast sensitive fiber fit with 502 and 525 templates: Dark adapted. The dark adapted (DA) spectral sensitivity curve measured with a 1.5° test has been superimposed with 502 and 525 templates.

sensitivity curve shown was measured with a 1.5° test and the threshold measurements were very replicable. The templates drawn through the points are 502 and 525 nm λ_{max}, with the 502 template fitting the short wavelength part of the curve and the 525 template fitting the long wavelength part of the curve. A total of 5 units showed similar results. These units should be viewed with caution, however. It is especially difficult to differentiate between curves with both 502 and 525 inputs, and curves with pure 525 input since the differences in the templates are primarily in the long wavelengths, and the 502 contribution would not be evident. Chromatic adaptation is needed to more positively identify inputs and to determine if the broader curve is actually due to a combination of 502 and 525 input.

One such fiber was tested with chromatic adaptation as seen in Fig. 4.8. A dark adapted spectral sensitivity curve (circles) was measured with a 5° test. The curve has been fit with a 525 template, although the input could possibly be from both 502 and 525 mechanisms. The fit is only fair. Under chromatic adaptation with orange (Wratten 21, 1.8 log cds/m^2) the spectral sensitivity curve shown as triangles, could be fit by the 502 template. Under adaptation with blue (Wratten 47, -0.27 log cds/m^2) the spectral sensitivity curve shown by squares could be fit by the 525 template. Thus chromatic adaptation conclusively reveals the two mechanisms. This unit showed no response of the opposite sign to large tests, suggesting there was no surround mechanism.

In two additional fibers, the 502 template fit the dark adapted spectral sensitivity curve but the light adapted curve was better fit by a combination of 502 and 525 templates. No units had a 525 dark adapted spectral sensitivity curve and a light adapted spectral sensitivity curve which fit 502 alone.

Figure 4.8. Spectral sensitivity curves of a contrast sensitive fiber fit with 502 and 525 templates: Chromatic adaptation. The dark adapted (DA) spectral sensitivity curve has been superimposed with a 525 template. Spectral sensitivity curves measured on a blue background (Wratten 47, -.27 log cds/m^2) and on an orange background (Wratten 21, 1.8 log cds/m^2) have been fit by 525 and 502 templates, respectively. Test stimuli were 5°.

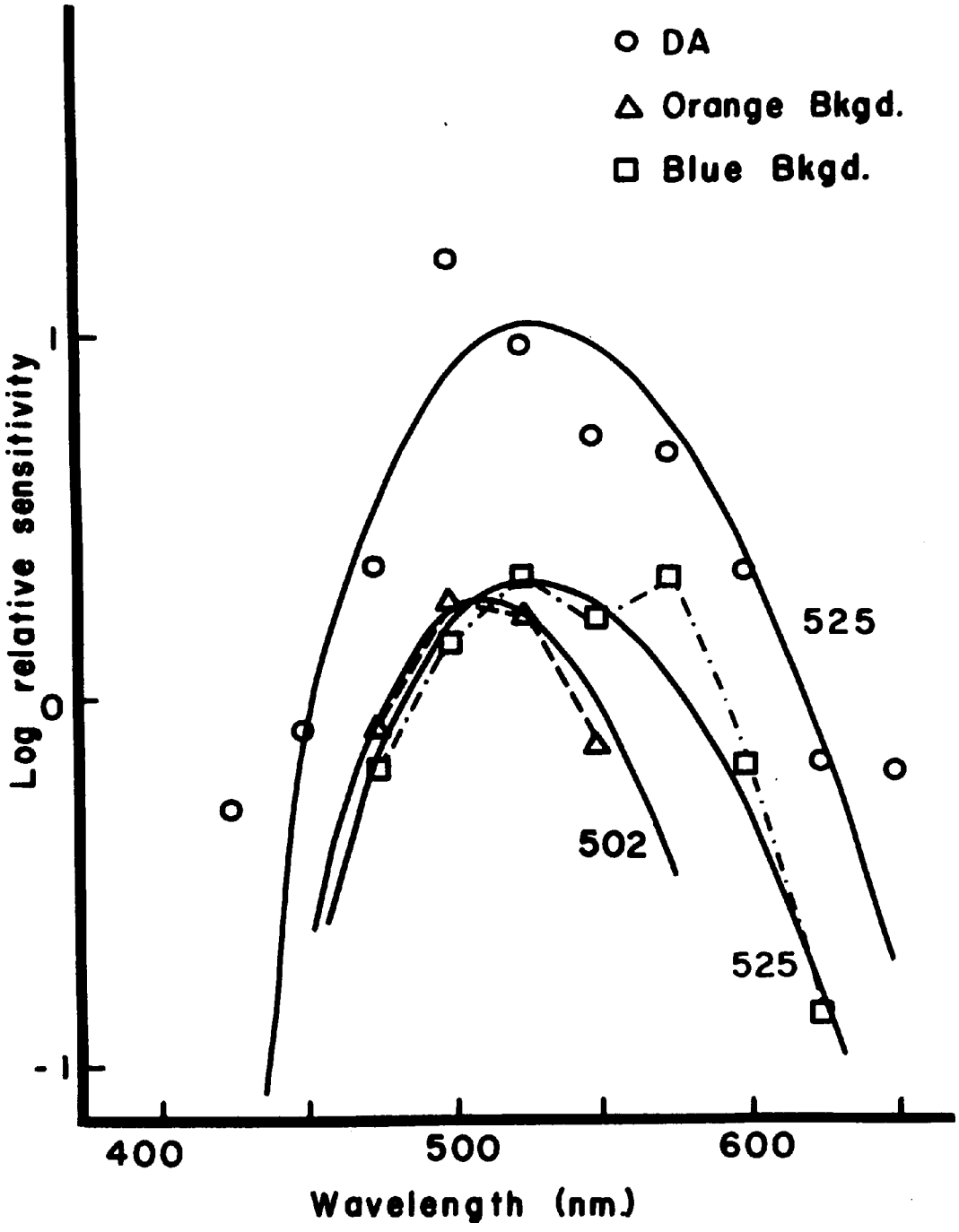

DA
Orange Bkgd.
Blue Bkgd.
Log relative sensitivity
1
0
-1
525
502
525
400
500
600
Wavelength (nm)

Figure 4.8

What seems to be the most important feature of these units is that there is evidence for input from two receptor mechanisms which both contribute to the same sign response. The 502 pigment is usually more sensitive in the dark adapted state and the 525 mechanism becomes relatively more active upon light adaptation. This is consistent with the properties of typical rods and cones. Three of the units included in this category showed inhibition to the center response when the test was made large. The surrounds were not studied, however. These units were tested for color opponency with large spots as were all units, and were not color opponent. The possibility remains, however, that they belong in a different category.

c) 525 (cone) alone

Some fibers showed evidence for only 525 cone input. Figure 4.9 shows an example of one such fiber. Figure 4.9a shows the spectral sensitivity curves measured with a large 7.5° test. The dark adapted curve (circles) has been superimposed with a 525 template for comparison and the fit is good except for the 600 nm data point. The threshold stimulus at 525 nm was 1.7 log cds/m^2. Note that the stimulus at threshold was much brighter than for units with 502 alone. The spectral sensitivity curves measured on blue (Melles Griot 450 nm λ_{max}, 0.85 log cds/m^2) and red (Melles Griot 600 nm λ_{max}, 650 nm λ_{max} approximately 2.0 log cds/m^2) backgrounds are shown as squares and triangles, respectively. The triangles are an average of the data for the two red backgrounds. The superimposed 525 template is an adequate fit for all curves suggesting that 525 input alone mediates the response.

Figure 4.9 Spectral sensitivity curves (a) and dark adaptation curve (b) of a contrast sensitive fiber with evidence for 525 input alone. a) The dark adapted spectral sensitivity curve (DA) and those measured on red backgrounds (Melles Griot 600 nm λ_{max} and 650 nm λ_{max}, approximately 2.0 log cds/m^2) and a blue background (Melles Griot 450 nm λ_{max}, 0.85 log cds/m^2) have been superimposed with 525 templates. The curve labeled "Red Bkgd" is an average of two measurements. Stimuli were 7.5^o. b) A dark adaptation curve of log threshold intensity for a 500 nm test as a function of time after a 3-min bleach shows that dark adapted sensitivity (triangle) returns in 8 minutes.

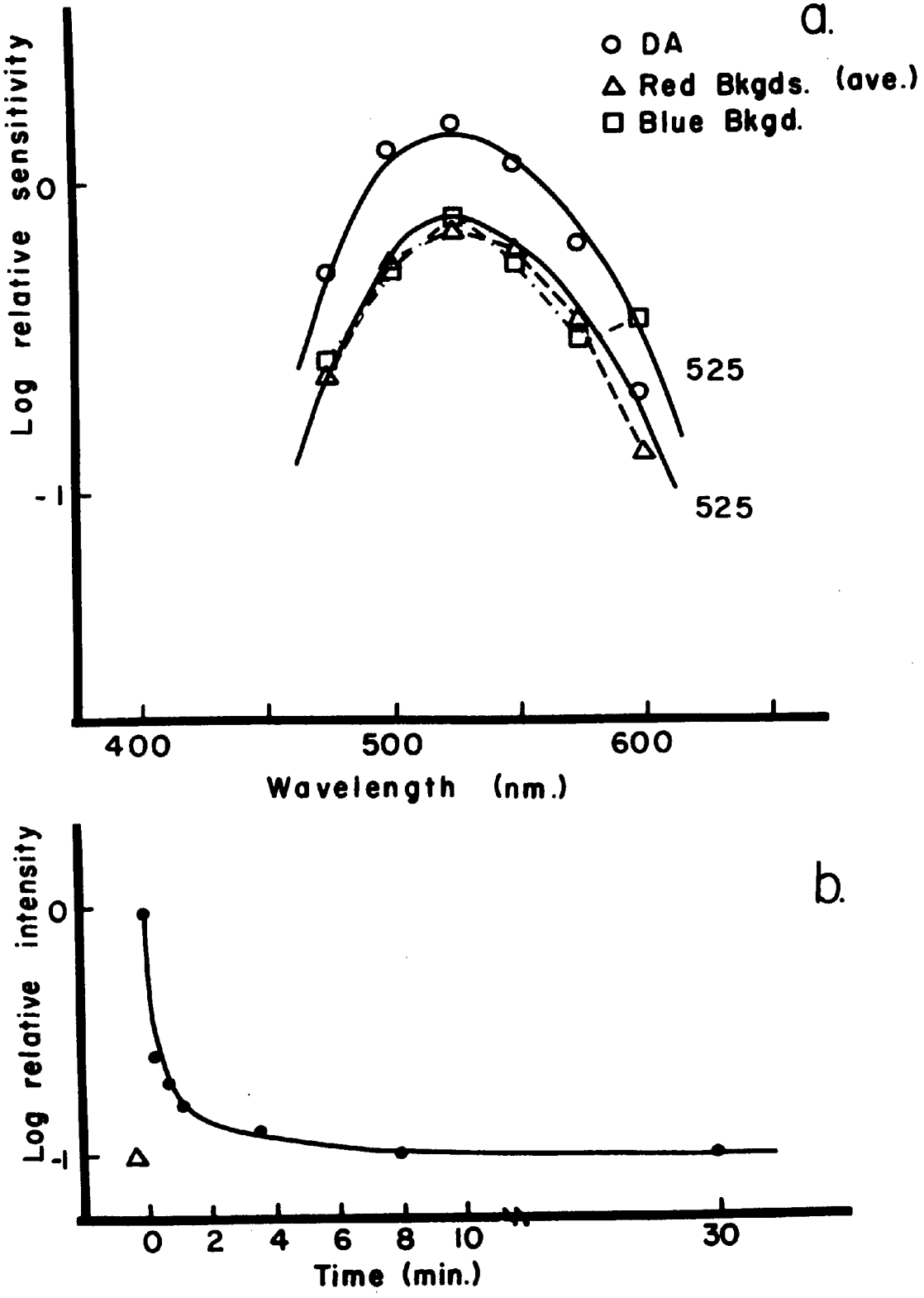

a.
DA
Red Bkgds. (ave.)
Blue Bkgd.
Log relative sensitivity
0
-1
525
525
400
500
600
Wavelength (nm.)
b.
Log relative intensity
0
-1
0
2
4
6
8
10
30
Time (min.)

Figure 4.9

The time course of dark adaptation was measured for this unit as seen in Fig. 4.9b. The rate of recovery of sensitivity following a three-minute exposure to a full field xenon arc illumination was tracked using a 500 nm test. Dark adapted sensitivity, shown as the triangle, was recovered in 8 minutes. This unit showed no surround response to annuli.

Some fibers showed evidence for both center and surround responses mediated by the 525 mechanism. The unit in Fig. 4.10 is an example. The center spectral sensitivity curve shown as filled circles was measured with a 3.7^{o} test. The surround spectral sensitivity curve, shown as open squares was tested with an annulus of 18° outer diamter with a 6° center hole. The surround spectral sensitivity curve has been shifted 0.35 log units for comparison. A 525 template has been drawn in, and the fit is only suggestive that 525 mediates the response. Spectral sensitivity curves measured on chromatic backgrounds did not shift the curve. Seven units showed evidence under all lighting conditions used for 525 input alone.

d) 445 (cone) + 502 (rod)

Data from four non-opponent fibers showed some evidence for 445 and 502 mechanisms contributing to the same sign response. An example is seen in Fig 4.11. The dark adapted spectral sensitivity curve (circles) measured with a 7.5^{o} test has been superimposed with a 502 template. The curve has been shifted vertically downward to allow it to be shown in the same figure. When spectral sensitivity was measured on a blue background (Wratten 47, -0.8 log cds/m^2), the peak sensitivity decreased 4.4 log units, but the shape of the curve did not change (squares). The orange background (Wratten 21, 1.8 log cds/m^2),

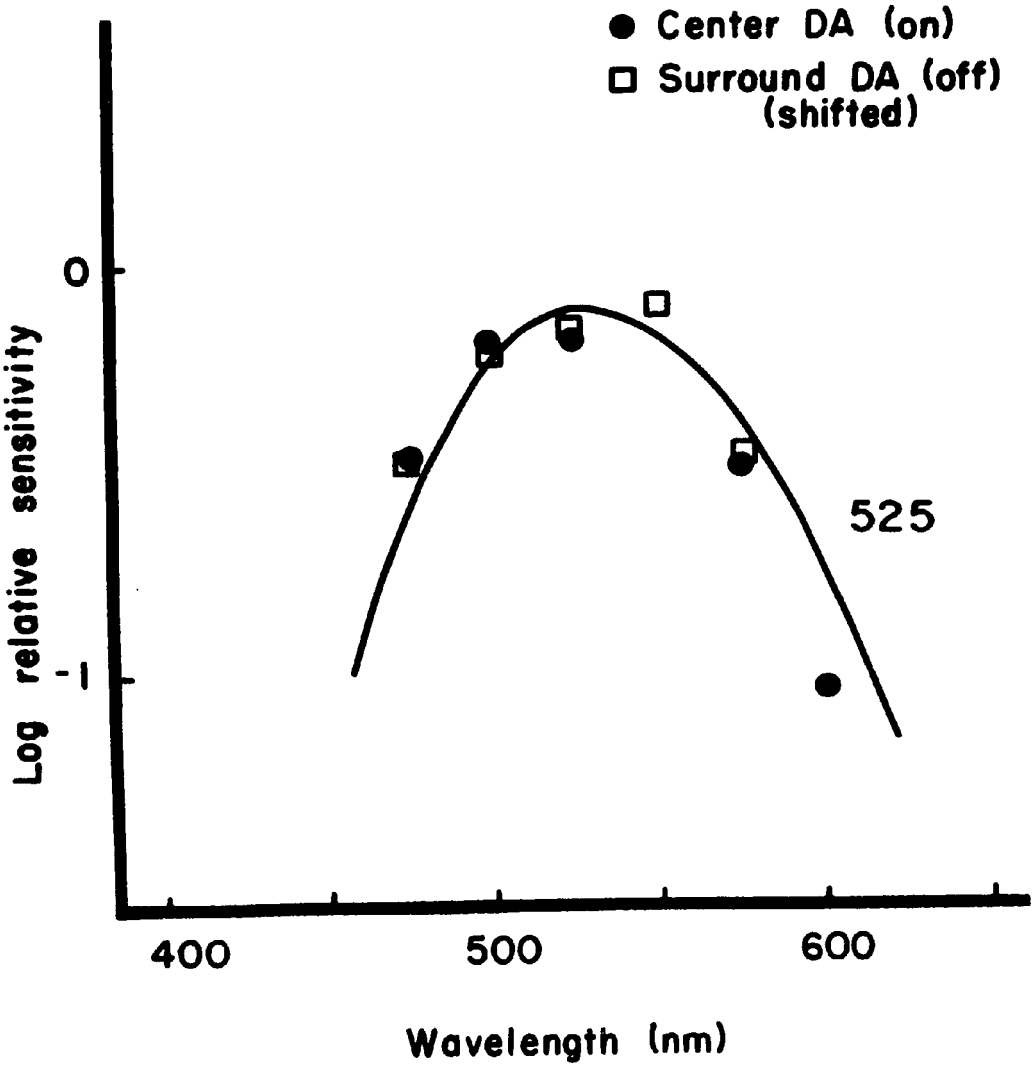


Figure 4.10. Spectral sensitivity curves for a contrast sensitive fiber fit with a 525 template: Center-surround organization. Spectral sensitivity curves measured for the center response "on" with a 3.7° test and for the surround response "off" with an annulus (18° outer diameter, 6° center hole) in the dark adapted (DA) state have been superimposed with a 525 template. The surround curve has been shifted .35 log units. Spectral sensitivity curves measured on orange (Wratten 21) and blue (Wratten 47) backgrounds are similar (not shown).

Figure 4.11 Spectral sensitivity curves for a contrast sensitive fiber fit with 445 and 502 templates. The dark adapted spectral sensitivity curve (DA) has been shifted downward vertically 3.7 log units, and superimposed with a 502 template. The curve measured on a blue background (Wratten 47, -0.8 log cds/m^2) is also fit with a 502 template. On an orange background (Wratten 21, 1.8 log cds/m^2) the curve is shifted toward the short wavelengths and has been fit with a combination of 445 and 502 templates. Test stimuli were 7.5^0.

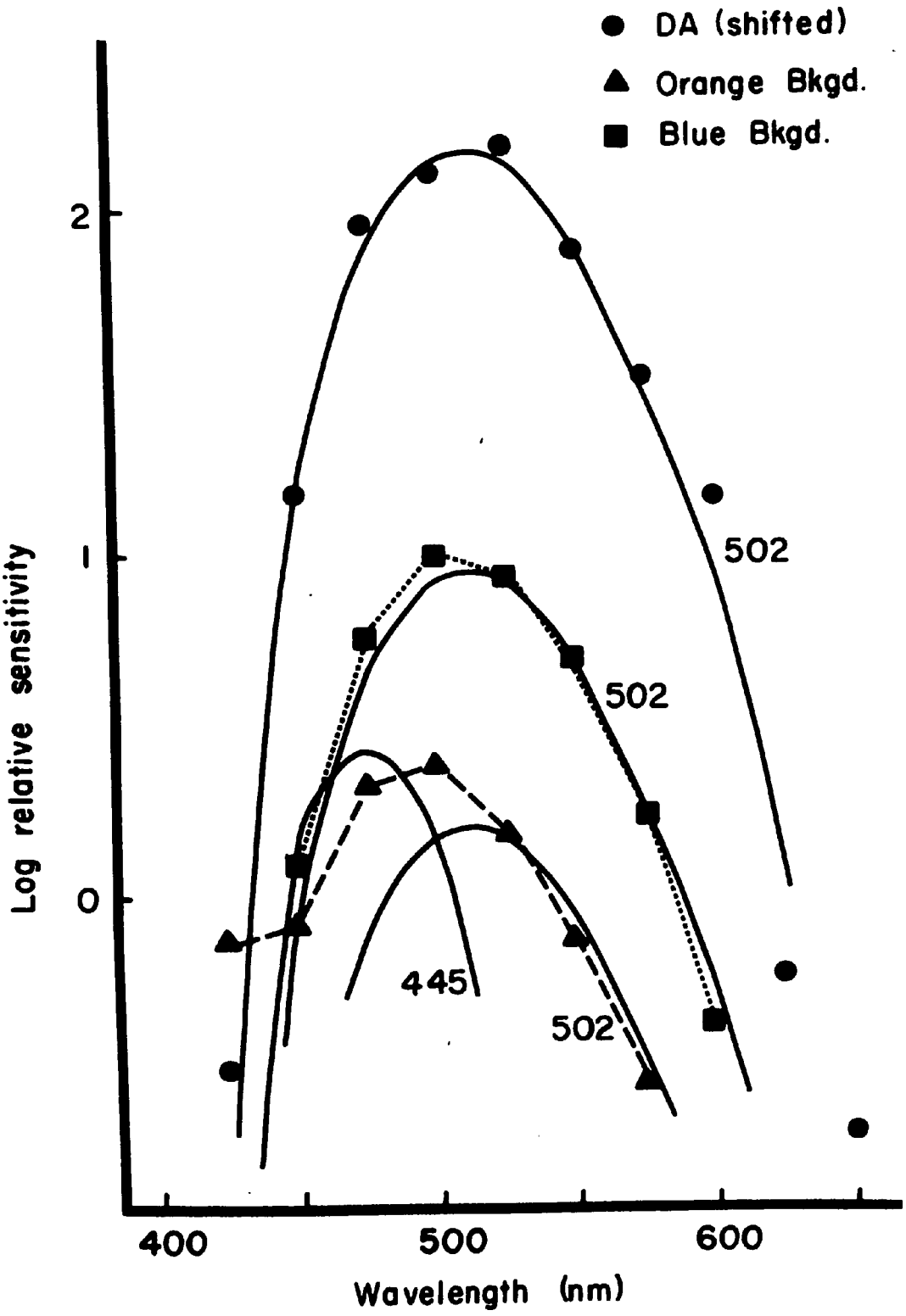

DA (shifted)
Orange Bkgd.
Blue Bkgd.
2
1
0
Log relative sensitivity
502
502
445
502
400
500
600
Wavelength (nm)

Figure 4.11

however, changed the shape of the spectral sensitivity curve, shown as triangles. The curve has been fit with a combination of 445 and 502 templates. These data are only suggestive of a 445 mechanism and the evidence is not strong. Four nonopponent fibers showed similar results with the dark adapted curve fitting a 502 template and the light adapted curve fitting a combination of 502 and 445 templates. This category of units must be further studied before a definitive statement can be made about the inputs.

Color opponent fibers

a) 445 (cone) vs 525 (cone)

To produce a color opponent response, the ganglion cell fiber must have input from at least two receptor mechanisms. Twelve units showed evidence for opponent input from the 445 and 525 cones. An example is the unit whose spectral sensitivity curves are seen in Fig. 4.12. In this unit blue stimuli evoked an "off" response and yellow stimuli evoked an "on" response. The white stimulus evoked a weak "on" response. The dark adapted spectral sensitivity curve was measured with a 10° test. The "off" response threshold could be measured for only one point at 450 nm (open circle). No response could be elicited at 475 nm which is termed the neutral point by Michael. The spectral sensitivity curve for the "on" response is seen as filled circles and peaks at a wavelength longer than 525 nm, around 550 nm. Chromatic adaptation with blue (Wratten 47,0.1 log cds/m^2) increased sensitivity of the long wavelength mechanism and shifted the peak sensitivity toward the short wavelengths (filled squares). The curve better fit the 525 nm template which has been drawn for comparison. Chromatic adaptation with orange (Wratten 21,2.1 log cds/m^2) similarly increased the sensitivity of the

Figure 4.12. Spectral sensitivity curves of a color opponent fiber fit with 445 and 525 templates. The dark adapted (DA) spectral sensitivity curve had two portions: "off" response to short wavelengths and "on" response to long wavelengths with a neutral point at 475 nm. Chromatic adaptation with orange (Wratten 21, 2.1 log cds/m^2) and blue (Wratten 47, 0.1 log cds/m^2) increased sensitivity of the mechanisms sensitive to the unadapted wavelengths. Spectral sensitivity curves have been fit with a 445 and 525 template under orange and blue adaptation, respectively. Test stimuli were 10°.

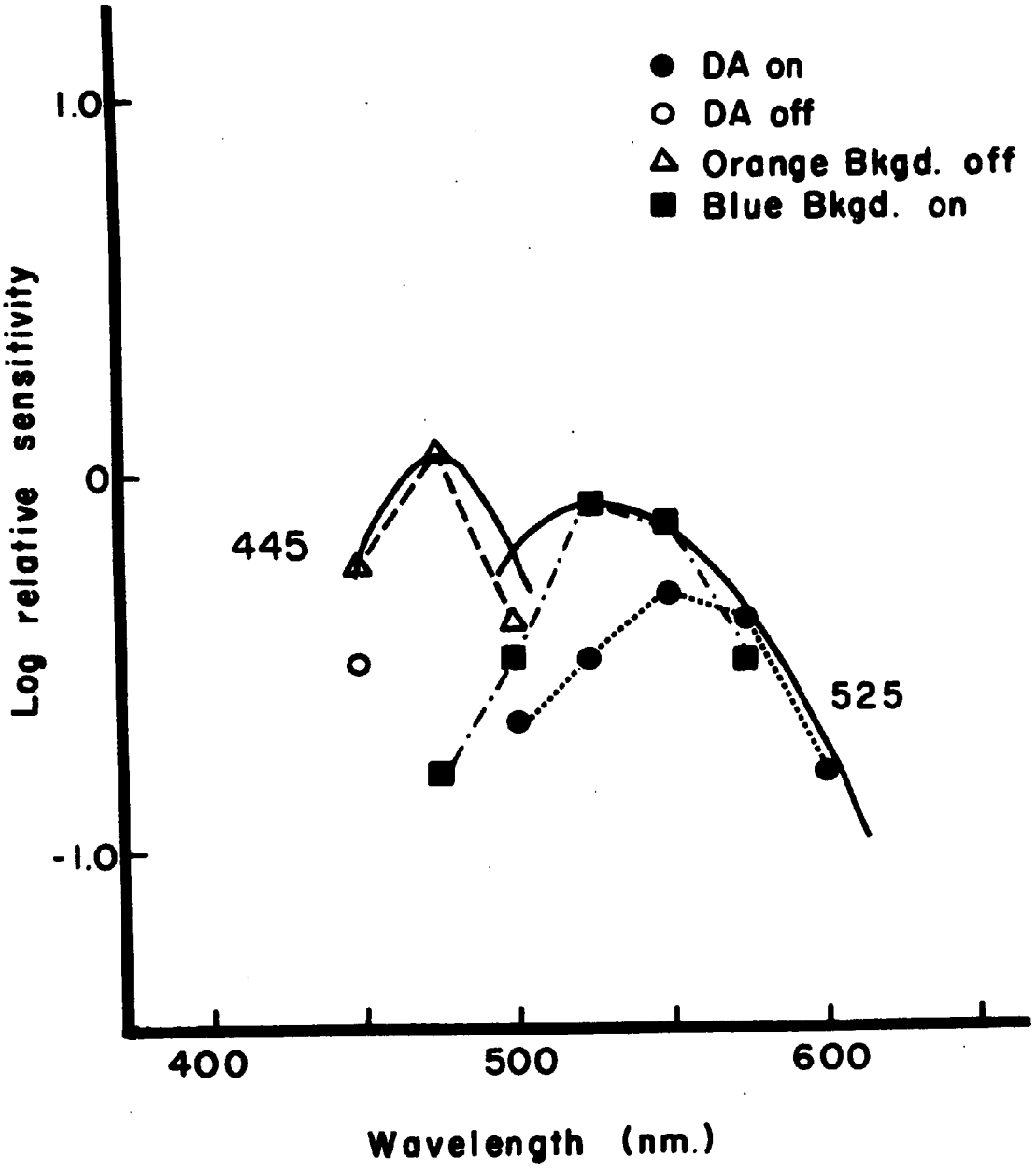

DA on
DA off
Orange Bkgd. off
Blue Bkgd. on
1.0
0
-1.0
Log relative sensitivity
445
525
400
500
600
Wavelength (nm.)

Figure 4.12

short wavelength mechanism and shifted the peak sensitivity toward the long wavelengths (open triangles). The curve has been fit with a 445 λ_{max} template. These curves suggest that the opponent response was mediated by the 445 and 525 cones.

Only two fibers showed evidence for only 445 and 525 cone input; the other fibers which showed evidence for 445-525 opponency also showed some evidence for 502 input. An example is shown in Fig 4.13. This unit was color opponent to the bright blue and yellow stimuli, both large and small (1.5^{o}). Chromatic stimuli at threshold intensity, however, did not give opponent responses. The dark adapted spectral sensitivity curve measured with a 1.5^{o} test is shown as filled circles in Fig. 4.13, and has been fit with a 502 template. At no wavelength did the sign of the response change from "on". The curve has been shifted vertically downward 1.5 log units. On a yellow background (Wratten 15, 2.0 log cds/m^2) the response had the same sign as the center response but the spectral sensitivity curve (triangles) peaked at a shorter wavelength. The 445 template drawn for comparison, fits adequately. On a blue background (Melles Griot 450 λ_{max} 0.85 log cds/m^2) the "off" response spectral sensitivity curve could be measured. For this unit a large test was used (10°). The curve peaks at 550 nm, but is most likely mediated by the 525 cone. The background may not have removed all the opponency of the short wavelength mechanism. A spectral sensitivity curve was not measured on a white background for this unit. In most units of this type, a neutral point was found between the long and short wavelength limbs of the curve when on a white background. Ten fibers gave this result of a dark adapted spectral sensitivity curve fit by a 502 template, and color opponency in the light adapted

Figure 4.13. Spectral sensitivity curves of a color opponent fiber fit with 502, 445, and 525 templates. The dark adapted (DA) spectral sensitivity curve which has been shifted down 1.5 log units, has been superimposed with a 502 template. No color opponency was seen. Upon light adaptation the fiber became color opponent. On a blue background (Melles Griot 450 nm λ_{max}, .85 log cds/m^2) the "off" response spectral sensitivity curve can be fit with the 525 template; on a yellow background (Wratten 15, approximately 2.0 log cds/m^2) the curve can be fit with a 445 template. Curves were measured with 1.5° tests (DA and yellow background) and a 10° test (blue background).

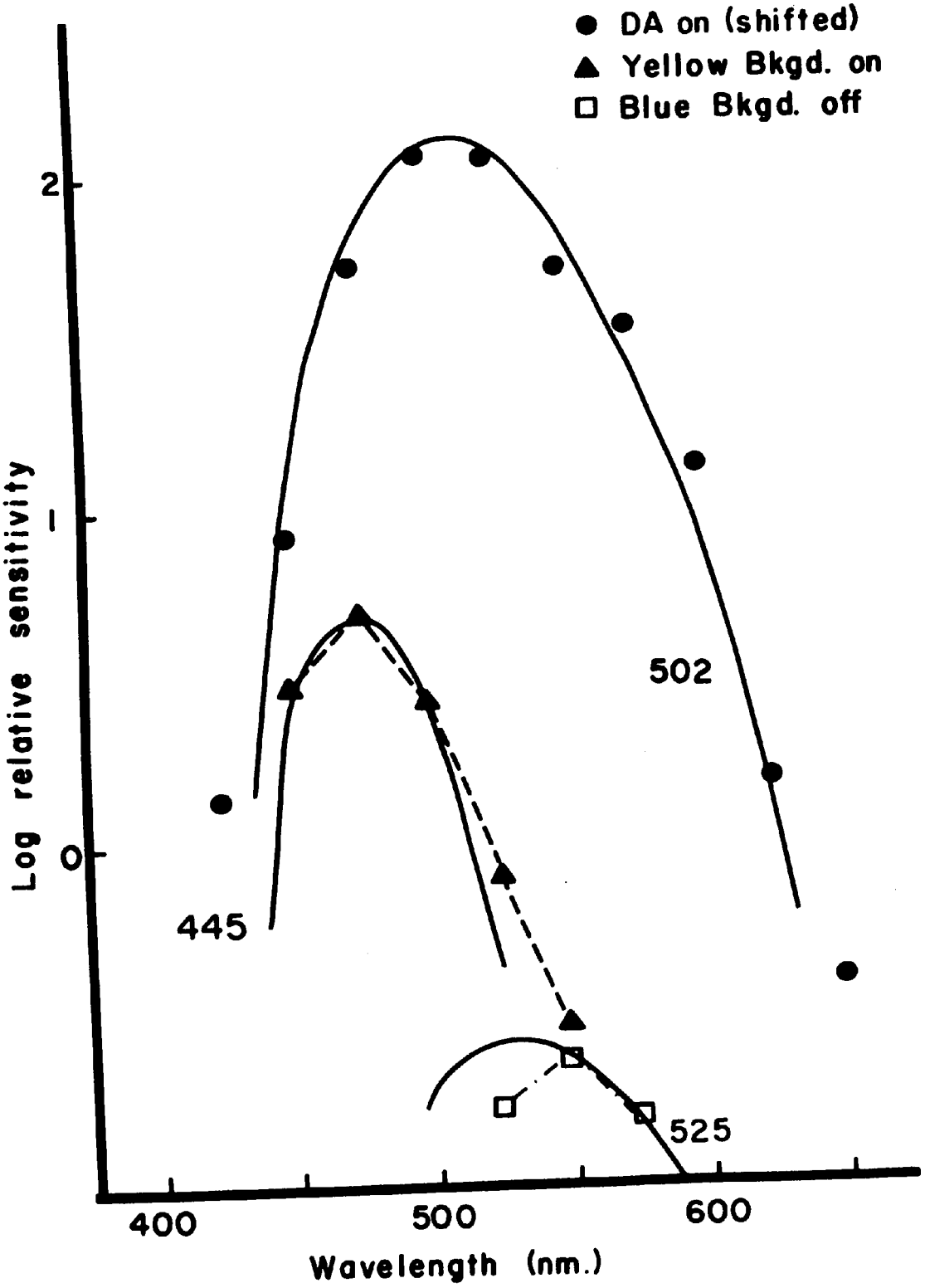

DA on (shifted)
Yellow Bkgd. on
Blue Bkgd. off
Log relative sensitivity
2
1
0
502
445
525
400
500
600
Wavelength (nm.)

Figure 4.13

condition with spectral sensitivity curves fit by 445 and 525 mechanisms. In nine of the ten units, the responses mediated by the 502 and 445 mechanisms had the same sign, as is the case in the unit in Fig. 4.13.

Spatial characteristics of the input mechanisms for this class cannot be described with certainty. In several units the "on" response and "off" response mechanisms had a center-surround organization. However, because small bright stimuli of different wavelengths could evoke the opponent responses, it is probable that the surround extends through the center. It is difficult to be sure because with bright stimuli, light most certainly is scattered to a possibly non-overlapping surround.

b) 502 (rod) vs 525 (cone)

In some color opponent fibers, spectral sensitivity curves showed no evidence for a blue cone, even upon chromatic adaptation. Instead, the curves for the opponent responses fit the 502 and 525 templates. There were several variations of the response characteristics.

One variation is illustrated by the unit shown in Fig. 4.14. In this fiber, broad-band yellow stimuli of any size gave the center response, "off." Blue stimuli when small gave a center response, but when large gave an "on-off" response. There was no neutral point. Gur reported that all color opponent units have surrounds which are more sensitive to longer wavelength light. This unit and others seen like it disprove this hypothesis. I will discuss this in greater detail in Chapter 5.

Spectral sensitivity curves were measured for center and surround responses. The center spectral sensitivity curve was measured using

Figure 4.14. Spectral sensitivity curves of a color opponent fiber fit with 502 and 525 templates. Center-surround organization. The dark adapted spectral sensitivity curve (DA) measured with a 1.5° test for the center "off" response has been superimposed with a 525 template. The surround "on" response spectral sensitivity curve was measured with an annulus (15° outside diameter, 4.5° center hole) and has been fit with a 502 template.

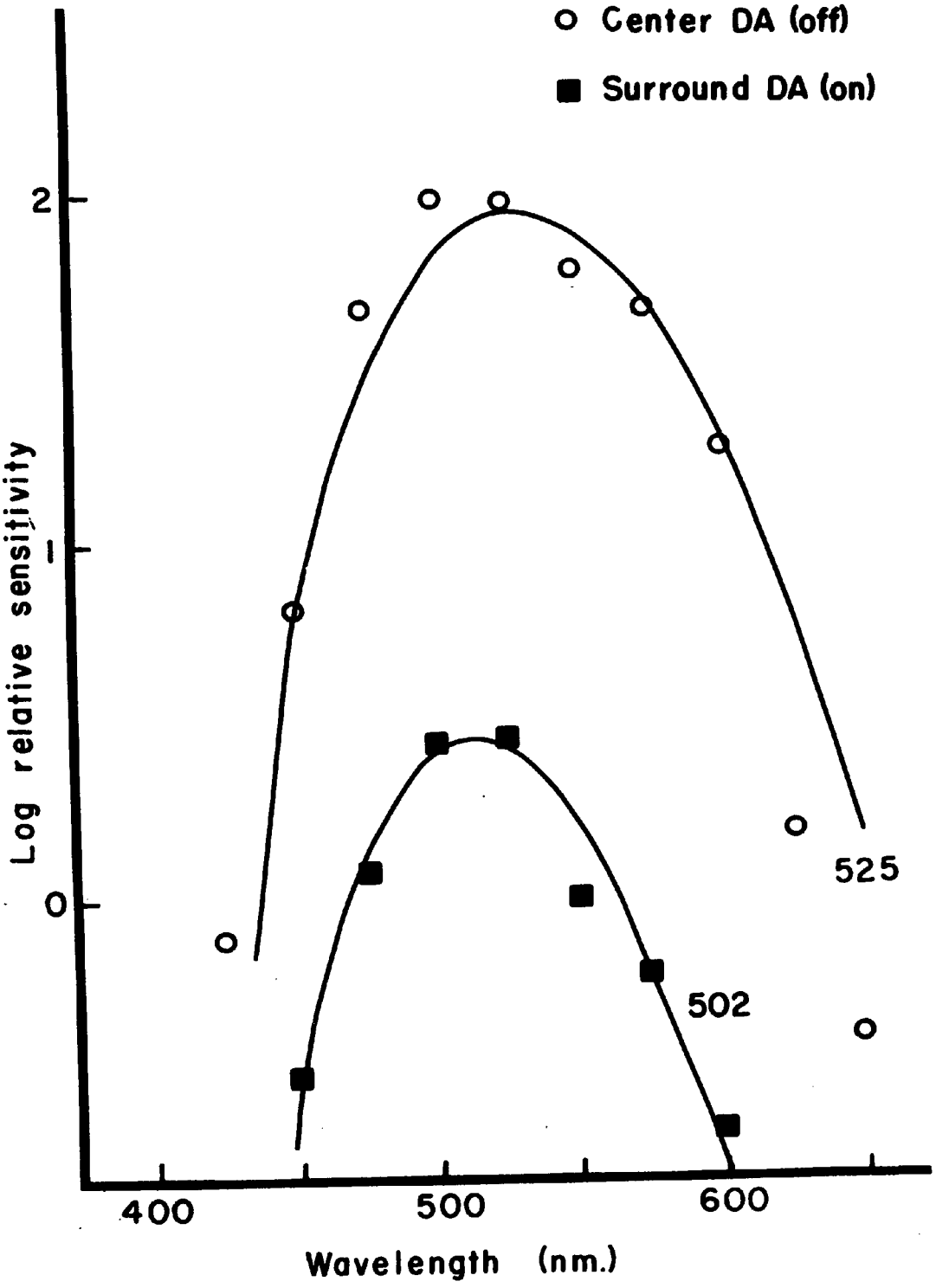

Center DA (off)
Surround DA (on)
2
0
Log relative sensitivity
525
502
400
500
600
Wavelength (nm.)

Figure 4.14

a 1.5° test (circles). The surround spectral sensitivity curve was measured using an annulus with 15° outside diameter and a 4.5° center hole (squares). As can be seen, the center was more sensitive to long wavelengths than was the surround, and the spectral sensitivity curves can be fit by a 525 and 502 template, respectively. This is in agreement with the qualitative responses of this unit mentioned previously.

Spectral sensitivity curves from three units showed this result: the center spectral sensitivity curve could be fit by 525, the surround by 502. In the unit in Fig. 4.14, threshold intensity for the surround was greater than for the center, as is usually the case in typical center-surround ganglion cells. For the other two units with this receptive field organization, the threshold intensities for an annulus and for a small central spot were almost identical. It seems reasonable that when the surround mechanism, usually less sensitive than the center, is mediated by the more sensitive pigment (502), the thresholds for center and surround may be similar.

Unlike the three fibers described above, one fiber had a dark-adapted spectral sensitivity curve which fit the 502 template, but the response had the sign of the surround. The light adapted spectral sensitivity curve for the center response fit the 525 template. The center of the receptive field was very small, however, and the test used was large. Perhaps if a smaller test had been used, the dark adapted spectral sensitivity curve would have fit a 525 template for the center response as in the previously described fibers.

Another variation in the organization of receptor inputs showed evidence for some 502 input to the center response. The unit shown in

Fig. 4.15 was extensively studied. This unit was opponent to large stimuli with an "off" response to blue and an "on-off" response to yellow. Small stimuli of all wavelengths gave the center "off" response. This qualitative response corresponds to the response described by Gur for the BGG unit in which the blue cone and green cone mediate the central response, and the green cone alone mediates the surround response which extends through the center. With more careful chromatic adaptation, however, it was shown that this was not the case for this unit. In no case was a 445 spectral sensitivity curve seen.

In the dark adapted condition, spectral sensitivity curves were measured both for the center and surround. The center was tested with a 4^{0} test (open circles, left) and the surround with an annulus with a 15^{0} outer diameter and a 4^{0} hole in the center (filled circles, right). The curves have both been superimposed with a 502 template. For the center response (left), chromatic adaptation in the long wavelength part of the spectrum (Wratten 21, 1.8 log cds/m^2) resulted in a spectral sensitivity curve (open triangles) which could be fit by the 502 nomogram template. Short wavelength adaptation (Wratten 47, -0.27 log cds/m^2) shifted the curve, revealing a curve which could be fit by a combination of 502 and 525 templates (open squares).

Measuring the surround response (right) under chromatic adaptation resulted in similar spectral sensitivity curves: under orange adaptation, the curve (filled triangles) could be fit with a 502 template and under blue adaptation the curve (filled squares) could be fit with a combination of 502 and 525 templates. Tests in the center under long wavelength adaptation responded "on-off", thus, the surround may extend through the center.

Figure 4.15. Spectral sensitivity curves for a color opponent fiber fit with 502 and 525 templates. Spectral sensitivity curves were measured for both center (4° test, left) and surround (annulus with 15° outer diameter, 4° center hole, right) under dark adapted conditions (DA) and on orange (Wratten 21, 1.8 log cds/m^2) and blue (Wratten 47, -0.27 log cds/m^2) backgrounds. For both center and surround, dark adapted curves and those measured on orange backgrounds have been fit with 502 templates. The curves measured on blue backgrounds have been fit with a combination of 502 and 525 templates. See text for details.

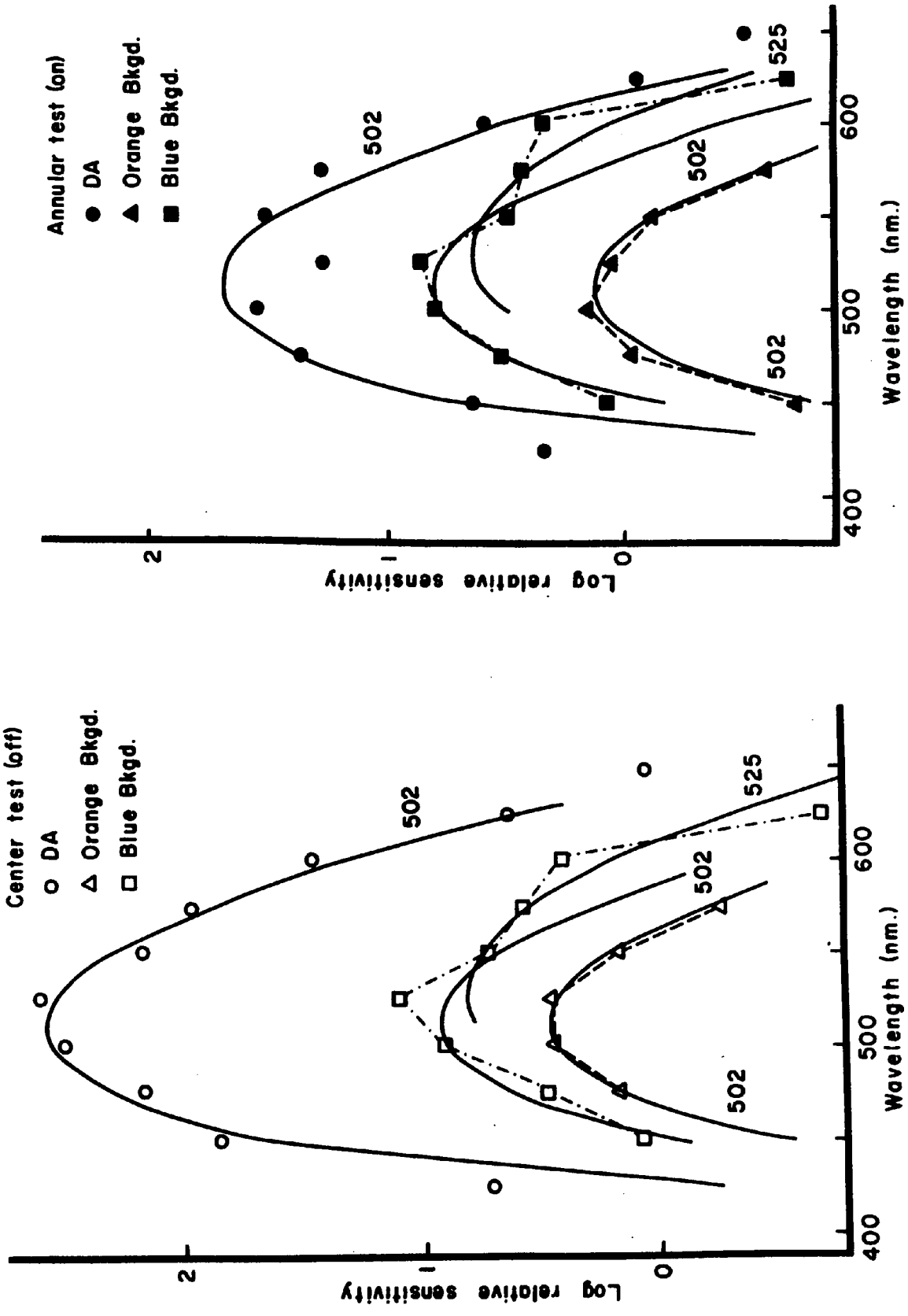

Annular test (on)
DA
Orange Bkgd.
Blue Bkgd.
502
502
502
525
Log relative sensitivity
2
1
0
400
500
600
Wavelength (nm.)
Center test (off)
DA
Orange Bkgd.
Blue Bkgd.
502
502
502
525
Log relative sensitivity
2
1
0
400
500
600
Wavelength (nm.)

Figure 4.15

Although the qualitative response characteristics show this fiber to be color opponent, the spectral sensitivity curves show both the center and surround responses to be mediated by a combination of 502 and 525 input. Thus one can conclude that the center mechanism with an "off" response has input from 502 and 525, but with relatively stronger 502 input; and the surround mechanism, "on" response, has input from 502 and 525 also, but with relatively more 525 input. This arrangement would predict a color opponent response as seen.

Three other units were smilar to the unit just described in that the spectral sensitivity curves suggested the same input mechanisms; however, the qualitative response characteristics were not identical, presumably because of a different balance between the 502 and 525 mechanisms in the center and surround. In all the units, 502 had some input to the center which dominated in the dark adapted state. This is in contrast to the variation formerly described in which 525 alone had input to the center.

c) 445 (cone) vs 502 (rod)

The final category of color opponent fibers, and that for which I have the weakest evidence is the one suggesting opponency between the 445 mechanism and the 502 mechanism. An example is seen in Fig. 4.16. This unit was color opponent to broad band stimuli and showed a neutral point. It was more sensitive to blue stimuli than to white. The spectral sensitivity curve measured in the dark adapted state with large stimuli had two limbs. The short wavelength limb was measured for the "on" response which has been fit with a 445 template. The long wavelength limb is threshold measurements for the "off" response and is fit with a 502 template. These data are hard to explain since the apparent

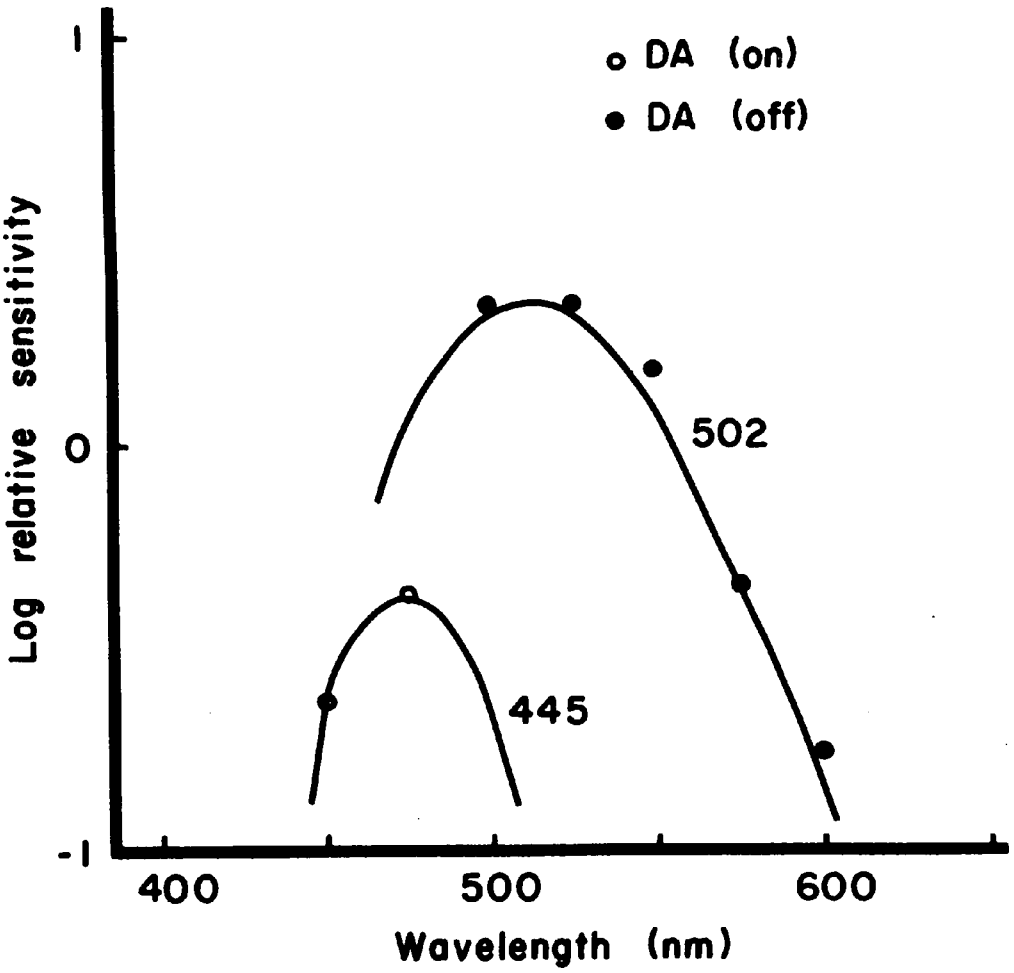


Figure 4.16. Spectral sensitivity curves for a color opponent fiber fit with 445 and 502 templates. The dark adapted spectral sensitivity curve for the "on" response has been fit with a 445 template; the dark adapted spectral sensitivity curve for the "off" response has been fit with a 502 template. Test stimuli were large.

opponency did not shift the shape of the spectral sensitivity curves: the nomogram templates seem to fit perfectly, even without chromatic adaptation. I am identifying the mechanisms as 445 and 502 only because they are the most likely mechanisms. Four units had similar spectral sensitivity curves but also were measured only in the dark adapted condition.

One unit had a slightly different receptive field organization. In the dark adapted state, the spectral sensitivity curve fit the 502 template and no opponency was seen. Upon light adaptation the unit became opponent, possibly between 445 and 502 although the data are not conclusive. Because none of the units were studied extensively, this category remains open for future study.

Directionally selective fibers

In spite of extensive searching for directional fibers, none were found definitively. Several units were first believed to be directional, but upon careful examination, it was found that the receptive fields had not been centered. Possible reasons for failure to find directional fibers will be proposed in Chapter 5. Units were found which responded "on-off" to diffuse white light, as did directional units described by Michael. However, further examination did not show directional selectivity.

Anesthetic effects

One might wonder if the anesthetic used during the experiment might have some affect on the results obtained. Three anesthetics were used during the course of this study. Of the 191 units from which quantitative data were obtained, 76 (40%) were recorded under urethane anesthesia, 52 (27%) under sodim pentobarbital and 63 (33%) under

fluothane.

It is difficult to assess the affects of different anesthetics on the results of this study. There is a correlation between the anesthetic used and the classes of units studied. Of the units in which spectral sensitivity curves were measured, 26% were color opponent using urethane,81% were color opponent using sodium pentobarbital,and 47% were color opponent using fluothane. The others were contrast sensitive. However, the anesthetics were not used in a random sequence, and this effect may not be purely an anesthetic effect. Furthermore, all of the large classes of units were found under all anesthetics suggesting that if there is an anesthetic effect, it is on the proportion of units found in each class, not on their existence.

The major difference between experiments in which different anesthetics were used was their position in the sequence in which experiments were performed. In the earliest experiments when urethane was the anesthetic, only contrast sensitive units were found. Many units had no surround mechanism. Upon changing to sodium pentobarbital, color opponent units were found and more units had surround opponency. At first, this appeared to be a large anesthetic effect, and I was concerned that urethane had suppressed color opponent units during the earlier experiments. However, urethane was used in subsequent experiments because sodium pentobarbital presented other problems. During the second set of urethane experiments, color opponent units were found and many units had center-surround organization.

In addition, to test whether urethane suppressed opponency, several experiments tried different anesthetics while recording from the same unit. In these animals, sodium pentobarbital injections (IP) were given

every hour. At one point in the experiment as the animal recovered from anesthesia, a color opponent unit was found and urethane was injected (IP) in an attempt to see a change in response characteristics to the blue (Wratten 47) and orange (Wratten 21) tests which were alternately presented. In one experiment the color opponency stopped after injection of urethane; however, the animal died shortly thereafter. In another attempt the unit remained opponent after injection of urethane. The experiment was difficult and rather impractical since only one unit could be tried per animal. Since the animal was waking, it was difficult to record the unit for a long enough time to see results. I take the one result, however, as some evidence that urethane does not inhibit color opponent units.

A possible source of the difference between the first and second set of experiments is that the animals were hibernating during the earliest experiments and were not in subsequent experiments. Perhaps the cone mechanism, or mechanisms, are suppressed during hibernation. Although the number of units studied from hibernating animals in the winter of 1974 and 1976 was small, there was a difference in the percentage with 502 inputs compared with the total sample. If an animal in hibernation is defined as one whose rectal temperature is less than 35°C, then four hibernating animals were studied. Of 10 units, nine (90%) were contrast sensitive fibers whose dark adapted spectral sensitivity curves fit Dartnall nomogram templates with 502 nm λ_{max}. For comparison, 72% of the total sample had dark adapted spectral sensitivity curves which fit the 502 template. The other unit was studied in 1976 and was color opponent but the receptor mechanisms were not determined.

One indication that hibernation may have an effect is that Remé and Young (1975) found that there is autophagy, or self destruction, in the receptors during hibernation. The receptor surrounds part of its own cytoplasmic organelles with its membrane and digests it. It has not been reported whether the cones are affected more than the rods, but an effect of this sort may be a possible explanation for my results. It would be important to know the time course of recovery from the hibernation-induced autophagy to correlate the physiology with the anatomy. The rectal temperature of the ground squirrel returned to normal (~37-38°C) around mid March, however no color opponents were found until May, 1974 when the anesthetic was changed from urethane to sodium pentobarbital. The abruptness of the change in proportion of color opponent units between the earliest urethane experiments and the sodium pentobarbital experiments, however, suggests that even if there is a hibernation effect, there may be some anesthetic effect in addition.

The large percentage of color opponent units studied under sodium pentobarbital also reflects the heightened interest by the experimenter in color opponent units after having seen only contrast sensitive units under urethane anesthesia. This biased the sample toward color opponent units. The Fluothane experiments, which were done last, represent a less biased sampling of ganglion cell types since all units isolated were studied. Under Fluothane anesthesia approximately half the units were color opponent, half were contrast sensitive.

CHAPTER V

SUMMARY AND DISCUSSION

Summary and comparison with previous studies

The results of this study suggest that the 502 receptor plays an active role in the response of ground squirrel ganglion cells. It can be found in both contrast sensitive and color opponent fibers. In fact, it can be one of the opponent mechanisms in a color opponent fiber. In Fig. 5.1 are histograms of the number of spectral sensitivity curves which are best fit by the nomogram templates with λ_{max} in 5 nm steps for contrast sensitive and color opponent units. These data are the same as shown in Figs. 2 and 3 in Chapter 3, except the spectral sensitivity curves are separated by the class of unit from which they were measured. Contrast sensitive units basically have input from 502 and 525 receptor mechanisms. Color opponent units have input from 445, 502, and 525 receptor mechanisms.

a) Contrast sensitive fibers

My view of the spatial organization of these inputs is summarized in Fig. 4.2. Basically there are three types of contrast sensitive units: those with 502 input alone, 502 + 525 input, and 525 input alone. The fibers with 502 input alone were the most sensitive of all the fibers seen. Cells of this type were found with and without a surround mechanism also mediated by 502. The second type of contrast sensitive unit had both 502 and 525 input to the same sign response. In some units the 502 dominated in the dark adapted condition while 525 was only evident upon light adaptation. In other units there was some

Figure 5.1. Histograms for contrast sensitive fibers and color opponent fibers of the number of spectral sensitivity curves which best fit each template. (λ_{max} in 5 nm steps). Each bar represents the number of curves which are best fit by the template at that λ_{max}. The shading indicates the lighting condition under which the spectral sensitivity curve was measured. The histogram suggests that there are three pigments with λ_{max} at 445, 502, and 525. Contrast sensitive cells do not show evidence for a 445 pigment.

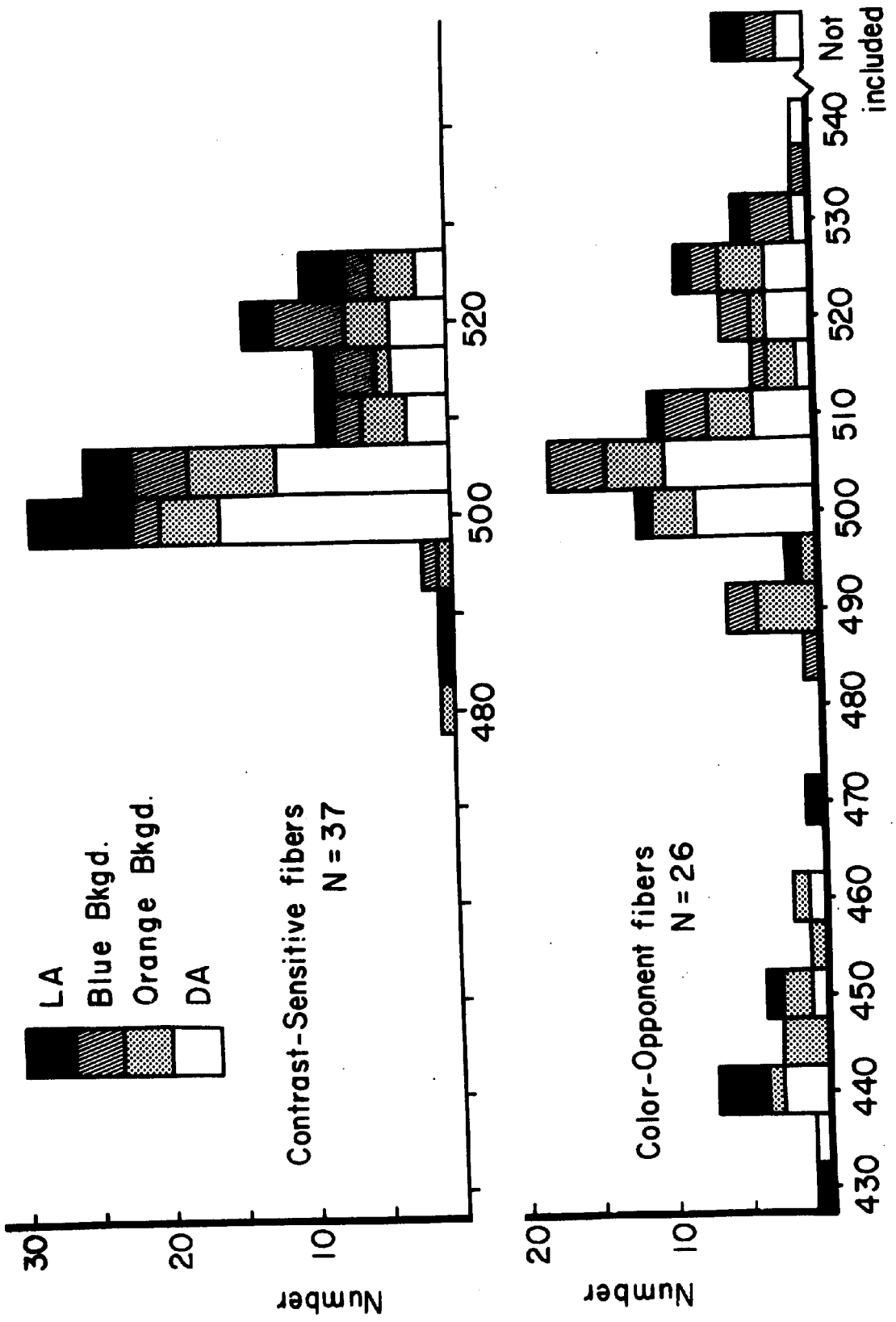
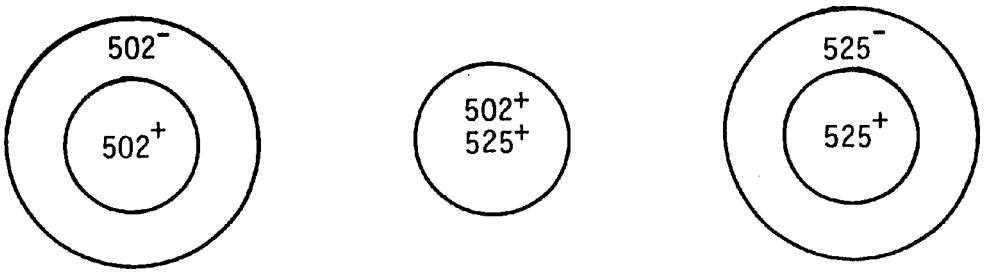

LA
Blue Bkgd.
Orange Bkgd.
DA
Contrast-Sensitive fibers
N = 37
Color-Opponent fibers
N = 26
Number
Wavelength (nm.)
Not included
430
440
450
460
470
480
490
500
510
520
530
540
10
20
30

Figure 5.1

Figure 5.2. Spatial organization of receptor inputs for contrast sensitive and color opponent fibers. The three major classes of contrast sensitive units and two classes of color opponent fibers are shown. Other spatial organizations were found, as described in the text.

CONTRAST SENSITIVE*

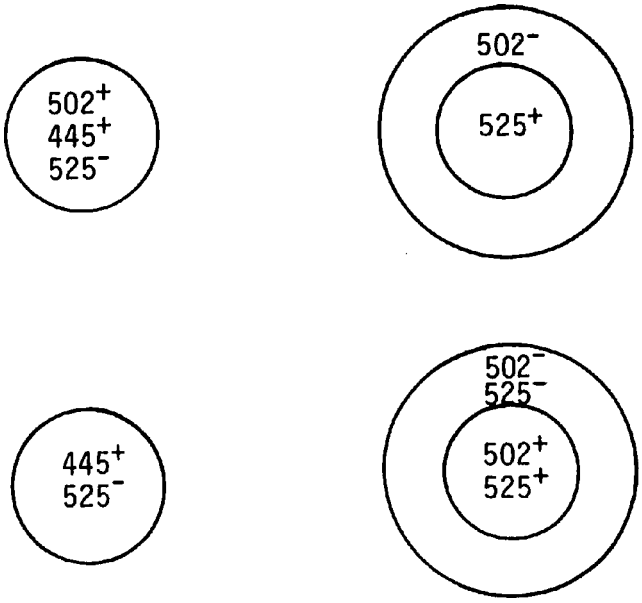


502 (ROD) ALONE

502 (ROD) + 525 (CONE)

525 (CONE) ALONE

COLOR OPPONENT*



445 (CONE) vs. 525 (CONE)

(502 ROD INPUT)

502 (ROD) vs. 525 (CONE)

* Off-response centers also seen.

Figure 5.2

evidence for 502 and 525 mechanisms being active in the same lighting condition. Not much can be said about the spatial organization of these fibers. Ricco's law summation area was measured for one unit in this class to determine the spatial organization (see Figs. 4.4 and 4.5). As log diameter increased, log intensity for threshold decreased with a slope of 2 initially, then fell to a slope of .75. This is consistent with the possibility that the 502 and 525 mechanisms may have unequal summation area. Some units in this class had surrounds but these were not investigated. The third class of contrast sensitive units had 525 input alone. Fibers in this class were found with and without surrounds. Other contrast sensitive units were found with some evidence for 445 and 502 input mediating the same sign response. This organization is not included in the summary figure (5.2) since none of these units were studied in detail.

The fibers with 502 alone and 525 alone were very different in response character as well as their spectral sensitivity. The units with 525 alone were on the average 1.6 log units less sensitive at their peak sensitivity than the units with 502 input alone. For units with 502 alone, test stimuli could be made bright enough to measure spectral sensitivity curves on backgrounds which raised threshold an average of 1.7 + .16 SE log units. However, because of the lower sensitivity of fibers with 525 alone, backgrounds had to be used which raised threshold only an average of .5 $\pm$ .08 SE log units. The course of dark adaptation was different for the two fibers on which it was measured. Sensitivity recovered in 8 minutes for the fiber with 525 input alone and in 25-30 minutes in the fiber with 502 fiber alone.

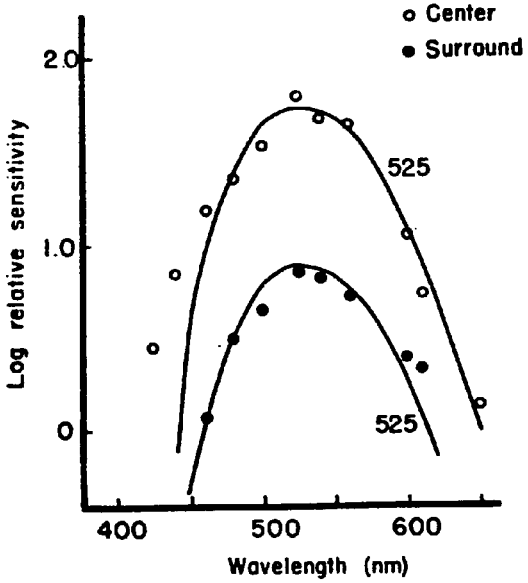
The results of this study create a different picture of the organization of the contrast sensitive fibers than that seen from the results of Michael and Gur. Neither saw any evidence of a 502 receptor mechanism. Of the three major classes of contrast sensitive fibers I found, only one type was described previously; the class with input from 525 alone, reported by both Michael (1965, 1968a) and Gur (1974). Figure 5.3 summarizes their results. The contrast sensitive unit shown on the left is from Michael's PhD thesis (1965, Fig. 8). The spectral sensitivity curves for center and surround responses are fit with the 525 λ_{max} template which I used in this study and the fit is good. The example of this class from Michael (1968a; Fig.5) is too narrow to fit the template of a single pigment and I suspect that the figure was drawn incorrectly from the data. Gur (1974) does not show a spectral sensitivity curve for this class of units; however, he reports that he also finds only this type of contrast sensitive cell.

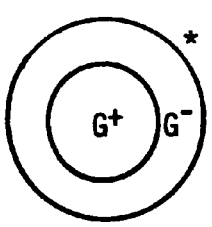
b) Color opponent fibers

An oversimplified summary of the spatial organization of color opponent units found in this study is shown at the bottom of Fig. 5.2. One category had opponency between the 445 and 525 mechanisms; the other had opponency between the 502 and 525 mechanisms. Most units with 445-525 opponency had some 502 input which was of the same sign as the 445 mediated response. These units were generally not opponent in the dark adapted condition and the response was mediated by 502. Upon light adaptation the unit became opponent between the 445 and 525 mechanisms. The spatial organization of this class shown in Fig. 5.2 is not the only organization possible since in some units the 525 mechanism extended larger than the 445 mechanism. I believe

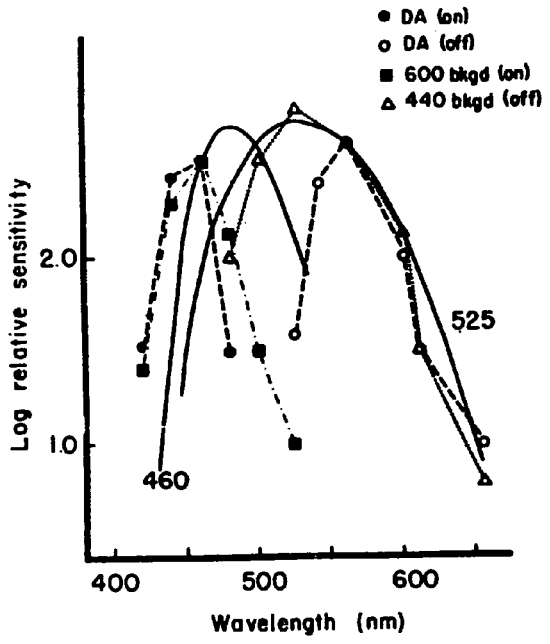
Figure 5.3. Summary of previous ganglion cell studied: Spectral sensitivity curves and spatial organization of contrast sensitive and color opponent fibers. (Left) Spectral sensitivity curves for center and surround of a contrast sensitive unit replotted from Michael (1965, Figure 8) and fitted with the 525 templates used in this thesis. (Middle) Dark adapted spectral sensitivity curves and those measured on chromatic backgrounds of a color opponent unit replotted from Michael (1968, Figure 1), and fit with the 460 and 525 templates used in this thesis. (Right) Spectral sensitivity curves from Gur (1974, Figs. 3.4 and 3.8). The open circles are an average from 10 BGG units fit by 460 and 525 templates. The remaining curves are from a single unit, measured dark adapted and on chromatic backgrounds. Below the spectral sensitivity curves are the spatial organizations for the same units reported by Michael and Gur.

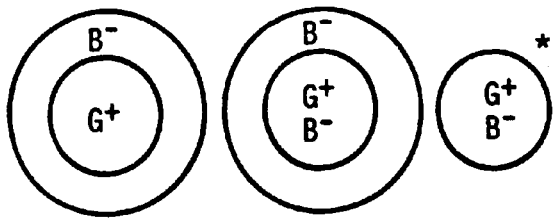
CONTRAST SENSITIVE
(MICHAEL, 1965)



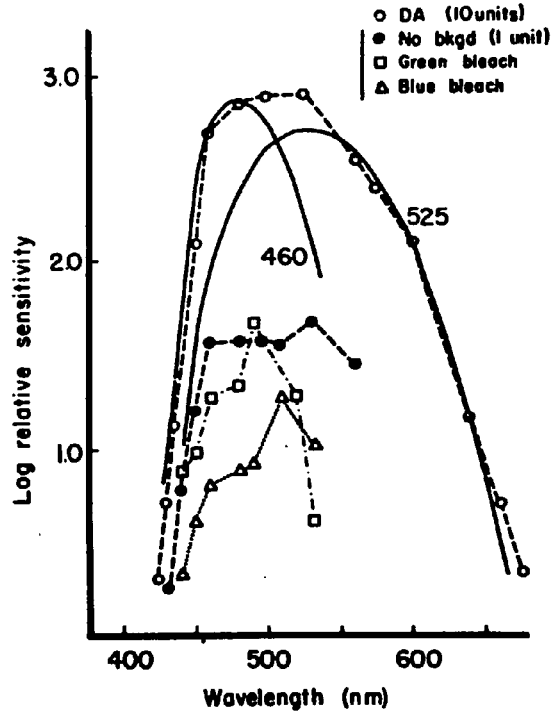


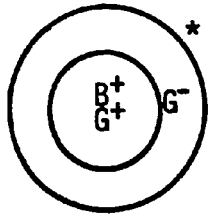
COLOR OPPONENT
(MICHAEL, 1968)





COLOR OPPONENT - BGG
(GUR, 1974)





*Off-response centers also seen

Figure 5.3

however, that the surround generally extends through the center.

Two units had 445 - 525 opponency with no 502 input. These units were opponent in both dark adapted and light adapted conditions.

Units with 502 - 525 opponency were generally of two types, shown in Fig. 5.2, although to repeat, this is an oversimplification of the classes seen. Some units had 525 mechanism mediating the center response, 502 mediating the surround. In others 502 and 525 had input to both center and surround. These units were most likely opponent because the relative influence of 502 and 525 on the response was different for center and surround although the input mechanisms were the same.

Some units showed evidence for 445 - 502 opponency. These units have not been studied thoroughly and are not included in the summary figure.

The color opponent units just described differ from those described previously by Michael and Gur primarily because they found no 502 input. The color opponent units described by Michael were opponent between a blue cone and a green cone, as seen in Fig. 5.3 in the example from Michael (1968, Fig. 1). The curves measured under chromatic adaptation can be used to identify receptor input. Michael found that chromatic adaptation with a blue background revealed a 525 mechanism and chromatic adaptation with red revealed a 460 mechanism. In the example shown, Michael's data have been fit with nomogram templates including lens correction used in my study. The 525 template fits adequately; however, the curve measured on the red background does not fit the 460 template. Apparently Michael called this mechanism 460 because it peaked at 460, not because the curves fit the template. I replotted all of the color

opponent units from Michael (1968c) and they all fit a 460 template poorly. The 445 template,which I found fits the blue peak in my data better than the 460 template, also fits these curves poorly. The pigment mediating the response most likely has a much shorter λ_{max}.

Gur's evidence for a 460 and 525 mechanism is less strong than Michael's. The spectral sensitivity curves on the right of Fig. 5.3 are from two figures in Gur (1974), Figures 3.4 and 3.8). The curve which is the average of 10 units is not extremely meaningful since individual differences are not shown. The curve has been fit with the 460 and 525 templates used in my study. Some units may have been better fit by a 502 nomogram while others may havefit the 525 template. The data points for the three longest wavelengths are from a single unit.

The remaining three spectral sensitivity curves are from a single unit measured under different lighting conditions. The spectral sensitivity curves measured under chromatic adaptation are not strong evidence for a 460 and 525 pigment input, particularly since the measurements were not made in the longer wavelengths where differences between the pigments are greatest. This makes it hard to suggest alternative receptor mechanisms.

While Michael and Gur agree on the receptor mechanisms involved, they do not agree on some characteristics of the units, and my results lie somewhere between their results. Michael found that all color opponent units have a neutral point in the spectrum between the two opponent mechanisms where no response can be evoked. The neutral point in the example shown in Fig. 5.3 from Michael (1968c, Fig. 1) is

at 500 nm. Gur found that no color opponent unit had a neutral point, and suggested that the classes reported by Michael were due to a physiologically unsound preparation. I found that there are some units with a neutral point as described by Michael and some without as described by Gur.

Michael and Gur also disagree on the spatial organizations of the receptor inputs. The spatial arrangements suggested are seen below the spectral sensitivity curves in Fig. 5.3. Michael found units with nonoverlapping, partially overlapping, or completely overlapping center and surround. Whenever there was a surround, it was most sensitive in the short wavelength part of the spectrum. Gur, however, found only one spatial arrangement, BGG. The center response was mediated by blue and green mechanisms, and there always was a surround sensitive to the longer wavelengths which extended through the center.

My results, once again, lie between those of Michael and Gur. I find that some units have qualitative characteristics which resemble the BGG. The BGG unit described by Gur was not opponent for small centered spots of light, but was opponent for large spots; short wavelength stimuli retained the sign of the center response, but long wavelength stimuli elicited an "on-off" response.When spectral sensitivity curves are measured on some units I found that two mechanisms can mediate one response, as in the BGG. However, I do not find that the two mechanisms are the blue and green cone suggested by Gur. Furthermore, not all color opponent cells in this study are BGG by qualitative description. It is difficult to prove that the animal was in "good physiological condition" when these units were recorded, but for many the animals met Gur's criterion of normal blood pressure. The most

compelling argument that color opponent units exist which are not BGG were the few units I saw that were qualitatively opposite in response characteristics: long wavelength stimuli evoked the same sign response to large and small stimuli while the response to short wavelength stimuli changed sign when the stimulus was made large. I have not succeeded in measuring spectral sensitivity curves on this type of unit. They resemble those of Michael in which the surround was always more sensitive to blue.

There are other color opponent units which more resemble those described by Michael. Two units had the same 445 - 425 opponency and had a neutral point. Others were opponent but had no surround mechanism.

General discussion

a) Possible explanations for differences with former electrophysiological studies.

One question which my results raise is why did I find 502 input to ganglion cells and Michael and Gur did not? The answer is not simple. If I had found only a few units with 502 input, one could say that the rod-like receptors which anatomically comprise only 4 - 5% of the receptors have input to so few ganglion cells that Gur and Michael could have missed them. But with my experimental procedures a large percentage of cells have rod input. The other simple explanation, that the dark adaptation procedures used previously were not adequate for rod activity, is also not supported by the results. The 502 receptor is active over a broad range of background intensities including levels where cones are active. Insufficient dark adaptation is not likely to be the explanation. Another possible explanation for the

discrepancies is that Gur and Michael only infrequently measured spectral sensitivity curves after having established classes of units. In most instances they used qualitative response characteristics to quickly identify units. Michael was primarily interested in classifying units by their response properties. Since the literature at that time suggested that the ground squirrel had only cones, it seems likely that units with rod input were seen, but inappropriately identified.

I suggest this possibility because the qualitative response characteristics of units seen by Michael and Gur are similar to those seen in this study, although a more detailed analysis of the spectral sensitivity curves revealed that the receptor mechanisms differed.

Another difference between the results of this study and the results of Michael and Gur is that I found no directional units. Michael found that directional units respond "on-off" to diffuse light. I found this response characteristic for many units; however, it was generally due to response of both center and surround of non-directional units. All units were tested for directionality while receptive field centers were being located.

There are several possible reasons why I did not find directional fibers. Directional cells have been shown to belong to the W-cell category in the cat which have the smallest fibers (Stone & Hoffmann, 1972). A possible explanation for the difference may be a sampling bias of my electrodes toward the larger fibers. However, this explanation seems unlikely because ganglion cell somata diameter, and presumably the axon diameters as well, are much more uniform than in the cat (West,1976) and electrode bias should not be an important factor. Also, Michael recorded in optic nerve with etched tungsten electrodes

similar to the ones used in this study. Since Gur recorded from cell bodies, his sampling would not be affected by fiber diameter.

Another possible explanation of the difference is that directional fibers, which terminate in superior colliculus (Michael, 1972) may form a bundle in one part of the optic tract as the fibers leave the chiasm. It is possible that the population of tectal-bound fibers was not sampled in this study. Since Gur and Michael both recorded distal to the chiasm, this fiber organization would not affect their sampling.

b) Possible explanations for behavioral dichromacy

An explanation is also necessary for discrepancies between this study and the behavioral results of Jacobs and Yolton (1971). It is not difficult to explain their conclusion that the ground squirrel retina had only cones. This conclusion is based on spectral luminosity functions. They trained ground squirrels to detect a monochromatic light added to one of three achromatic stimulus windows. Then psychometric functions were obtained for wavelengths in 20 nm steps. Spectral luminosity functions at four luminance levels were obtained from the psychometric functions using a criterion of 50% correct. Jacobs and Yolton concluded that the curves did not change from dark adapted to light adapted conditions, suggesting that cones alone were present. However, the functions have been reinterpreted (Green & Dowling, 1975) to actually support the newer finding of rod input. The behavioral spectral luminosity curves do show a change between dark and light adapted states similar to that found in the ERG.

It is more difficult to explain their evidence for two receptor channels. They found a behavioral neutral point, a wavelength which could not be discriminated from white. This in itself is not

inconsistent with a finding of three receptor mechanisms. However it is inconsistent if those receptors contain Dartnall nomogram pigments, as my data indicate, and comprise three separate channels. In trying to reconcile the evidence for three receptors in my study with the behavioral data, three hypotheses will be considered.

One possible explanation is that at the light intensities used in the behavioral study, only two of the three receptor mechanisms were active. In my study the 502 mechanism was most sensitive and the 525 and 445 cones mechanisms were less sensitive. It is difficult to compare the relative sensitivities of the 445 and 525 mechanisms in my study because the 445 mechanism was never found in a nonopponent unit. Because the lens absorbs strongly in the blue, however, it seems possible that relatively more light is required to stimulate the blue mechanism. If this is true only the 502 and 525 mechanisms may be active at low light levels. To evaluate this possibility, it is necessary to determine the light levels used in the behavioral neutral point experiments. The light levels used in my experiment cannot be compared easily with those used in the behavioral experiment because the pupil was dilated during my experiment. However, we can compare the light levels used in the various behavioral experiments. The white light to be matched in the neutral point study was .08 log cs/m^2, about the same as the brightest background used to measure the luminosity functions (.15 log cd/m^2). However the test stimulus used in the luminance function experiments was brighter than the background. Therefore, in the range of stimulus intensities used by Jacobs and Yolton, the neutral point study used relatively dim lights. It is

possible that the intensity was too dim to stimulate the blue cones. More information is necessary to make any firm conclusions, however. The position of the neutral point, between 500 and 510, is consistent with color vision mediated by 502 and 525 pigment mechanisms.

Another hypothesis is that two of the three receptors share a common color channel at some level of the visual system. The sharing of a channel has previously been found in the goldfish in which rods and red cones signal with the same sign response in ganglion cells (Raynauld,1969). However, Raynauld found that the rod and cone input in goldfish ganglion cells were not active under the same lighting conditions as they are in the ground squirrel. Rod stimulation seemed to inhibit the response of the cones in color opponent cells so that stimuli intense enough to evoke cone response in a light adapted retina, produced only a rod response in a dark adapted retina. Raynauld (1969) used the finding that rods and red cones share a pathway to explain the Q-type cell described by Daw (1968). Daw described two types of color opponent cells: the O-type cell with opponency between red and green cones, and the Q-type cell with the same opponency but with the green mechanism depressed. Raynauld speculated that if the retina were not sufficiently light adapted to suppress rods, the rods which share the red cone pathway may inhibit the green cones which are maximally sensitive to the same part of the spectrum. Q-type cells may actually be an O-type cell with some active rods. The idea of rod-cone opponency has been proposed for the goldfish to explain behavioral color discrimination, also; however, in that case rods and red cones have been implicated (Powers, 1977). This is difficult to explain, if rods and red cones share a common pathway. Color discrimination using

rods and red cones also requires that under some lighting conditions rods cannot completely inhibit cones in the goldfish.

In the ground squirrel there is some evidence for the 502 mechanism and blue cone sharing a common channel. In this study, cells which had a 502-mediated response in the dark adapted state and became color opponent (445 vs 525) in the light adapted state had responses mediated by the 502 receptor and the 445 cone of the same sign. If the blue cone and 502 receptor share a common pathway, there would be only two color channels: the "rod"-blue cone channel and the green cone channel. Thus the ground squirrel would behave like a dichromat.

There are two teleological reasons to suppose why the blue cone and "rod" would share a pathway. The blue cone is most sensitive at wavelengths which are strongly absorbed by the yellow lens. Therefore color vision would only be possible with extremely bright lights if the animal depended on cone systems for color vision. If the "rod" mechanism shared the blue cone pathway, however, "rods" could mediate color vision at lower intensities.

Problems with this hypothesis arise because of the five units which exhibit color opponency between the 445 and 502 mechanisms (See Fig. 4.16). However, since spectral sensitivity curves were measured only in the dark adapted state for 4 of the 5 units, they may be improperly classified. The possibility that blue cones and 502 receptors share a pathway is an unresolved question.

The third hypothesis is that the animal has a behavioral neutral point because two, not three, receptors are present in the retina. Perhaps the two receptor types are the 502 "rod" and the 525 cone. The 445 peak in the spectral sensitivity curves might be due to

opponency between the 502 and 525 mechanisms in which the 445 peak is the linear or nonlinear difference between the 502 and 525 mechanisms. In Fig. 5.4athe 525 nomogram template was linearly subtracted from a 502 template resulting in a curve with a peak around 475 nm. In Fig. 5.4b the 502 - 525 curve was replotted on a log scale for comparison with the nomogram template with 445 nm λ_{max}, which is my best estimate of the shape of the blue peak. The curves are quite similar. It is likely that a better fit could be obtained using a nonlinear subtraction as described by Sirovich and Abramov (1977); however, it is not very meaningful to fit a template of a pigment. Ideally one would fit the data. The variability in my data is too great and the number of points per curve too few to make this worthwhile.

The hypothesis that a rod and green cone mechanism can be opponent to yield a blue peak has recently been proposed to explain discrepancies between gray squirrel behavior data, ERG data, and microspectrophotometric data. The gray squirrel scotopic spectral sensitivity curve measured using the ERG fits a 543 λ_{max} template except for a small hump around 460 nm which some have interpreted as a blue cone (Silver, 1966). However, the microspectrophotometry by Loew (1975) of individual receptors, found evidence for only two pigments with 500 λ_{max} and 543 λ_{max} with no evidence for a blue cone. Recently Silver (1976) has measured a spectral sensitivity curve using a behavioral heterochromatic flicker technique and concurred with the ERG evidence that the blue peak was real. She suggested that the blue peak may result from opponency between the 502 rod and the 543 cone. This hypothesis may also be valid for the ground squirrel, an animal with similar rod-like and cone-like receptors (West & Dowling, 1975).

Figure 5.4. Illustration of the hypothesis that the peak sensitivity at 445 nm may be due to a differencing between the 502 and 525 pigment mechanisms. a) Linear Dartnall nomogram templates (λ_{max} 502 and 525) without lens correction are plotted as well as the linear difference between 502 and 525. b) The difference (502 - 525) in a) is replotted as log sensitivity as a function of wavelength. The curve is also plotted with lens correction and compared with the 445 template, which is an approximation for actual data.

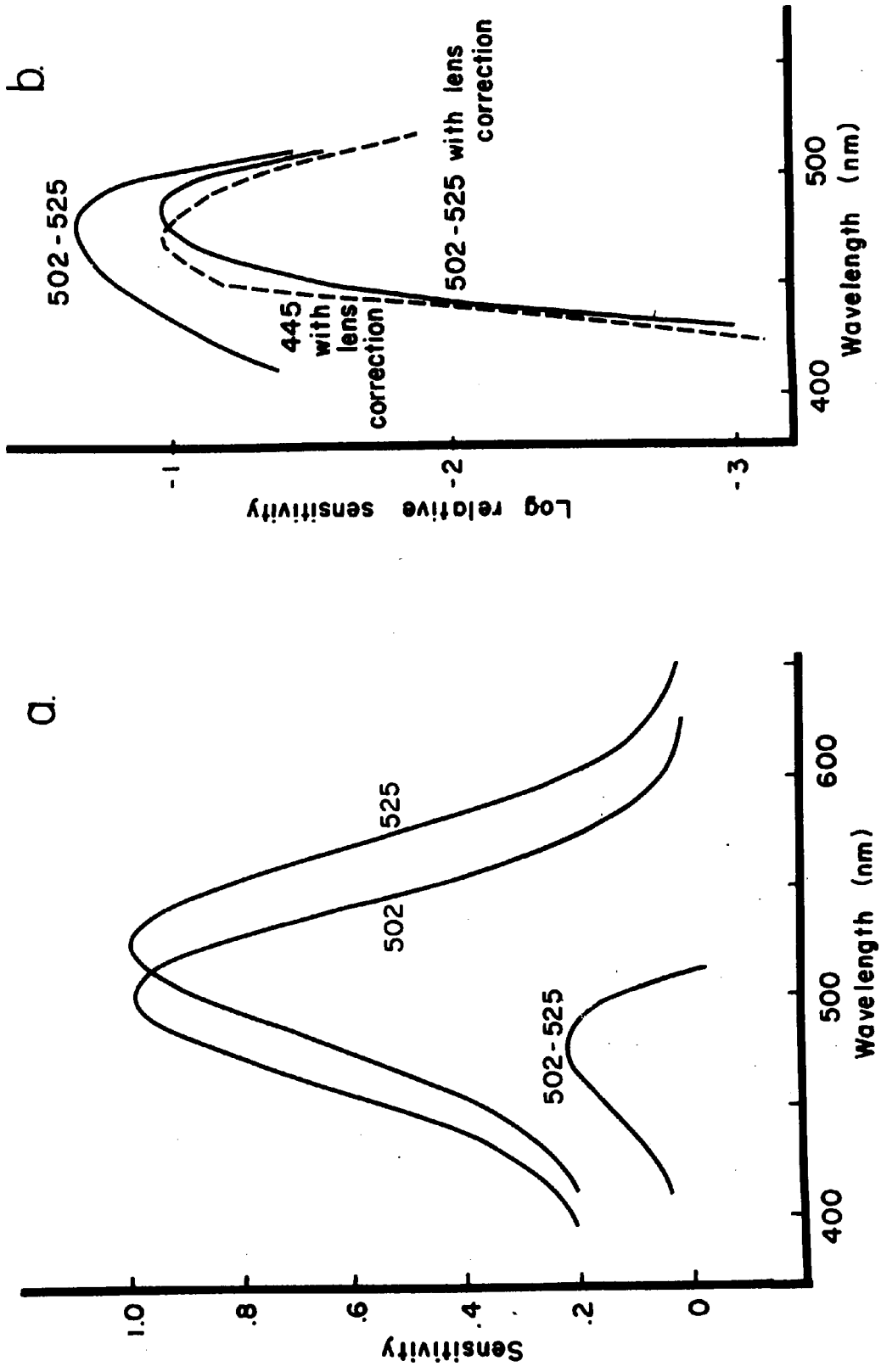

a.
Sensitivity
1.0
.8
.6
.4
.2
0
400
500
600
Wavelength (nm)
525
502
502-525
b.
Log relative sensitivity
-1
-2
-3
400
500
Wavelength (nm)
502-525
445 with lens correction
502-525 with lens correction

Figure 5.4

There is also precedence for this type of color opponency in the monkey LGN in which a narrow long wavelength peak has been explained as the result of antagonistic input from the medium and long wavelength cones (Padmos & Norren, 1975). DeMonasterio, Gouras, and Tolhurst (1975) found similar antagonism in ganglion cells of the monkey. Their major finding was that units appearing to be nonopponent were actually the result of silent opponency which could be seen only upon chromatic adaptation.

The theory that the "blue" cone is a result of rod green cone opponency is interesting because it could explain some of the observations made in this study. It can explain the finding that the mechanism mediating the 502 response and the 445 response have the same sign. If the response mediating the blue peak is actually the 502 mechanism with the spectral sensitivity curve depressed on the long wavelength side by the 525 cone, it would of course have the same sign as the dark adapted 502 response. In addition, this hypothesis could explain the observation that the color opponent cells which show 445 input all had spatially overlapping color opponent mechanisms such that small chromatic stimuli as well as large stimuli could evoke the opponent responses. The color opponent units with 502 vs 525 opponency all had center-surround organization. Perhaps in order for the subtractive opponency to occur, producing a 445 peak, the 502 and 525 mechanisms must be spatially overlapping. When they are not spatially overlapping, as in the center-surround organization, the spectral sensitivity curves reveal 502 and 525 mechanisms and no peak. The sample size is too small to use this as a strong argument, however.

The hypothesis that there is no 445 pigment is pure speculation

with little evidence besides the utility in explaining some observations. The data in fact tend to weigh against it,although not unequivocably. If there were no 445 mechanism, the spectral sensitivity curve with the short wavelength peak of color opponent units should shift toward 502 with long wavelength chromatic adaptation since the antagonistic 525 mechanism would be suppressed. What actually happens, however, is that in most units long wavelength chromatic adaptation causes the 445 peak to become more prominent, just as would be expected with a 445 pigment. Another problem is explaining the units which are opponent 445 vs 525, yet have no 502 input. Also the five units which are opponent 445 vs 502 cannot be explained by this hypothesis any better than by the hypothesis that rods and blue cones share a pathway. It is possible that the units are misclassified,however, as mentioned before.

Of the three hypotheses presented, I consider this hypothesis most unlikely. What needs to be done, however, is to measure spectral sensitivity curves on long wavelength backgrounds of increasing intensity. The possibility remains that the chromatic adaptation I used was not intense enough to shift the 445 peak toward the 502 λ_{max}. Light adaptation may first serve to make the 525 cone more active relative to the 502 rod mechanism and thus increase the effect of the 525 opponency. If made brighter, the chromatic adaptation should decrease the oppnency. Such experiments were in fact attempted; however, the test intensity was not sufficient to evoke a response on the brighter backgrounds. Also the units were rarely held long enough to carry out this long sequence of experiments. Another attempt to solve this question was to use backgrounds of longer wavelength

composition to affect only the 525 mechanisms and leave the 502 relatively unaffected. The 502 mechanisms may be more adaptable than the 525 pigment and the bright background affects it more, even if the background is closer to the peak wavelength of the 525 mechanism.

c) Use of the Dartnall nomogram

Another question that could be asked is if there are three pigments, how sure can we be that we have identified them correctly. The identification of the receptor inputs is based on the assumption that the Dartnall nomogram can accurately predict the absorption spectrum of the pigments in the ground squirrel. The 502 and 525 nomogram templates fit the data well and the nomogram is probably a good approximation. There is too much variability in the short wavelength peak, however, to determine if the approximation is good. Furthermore, it has recently been found that the Dartnall nomogram is not valid over the entire range of $\lambda_{max's}$ of visual pigment but only holds for the middle wavelength region. Pigments with λ_{max} less than 460 would be broader than the Dartnall nomogram template and a modification in the nomogram has been suggested by Ebrey and Honig (1977). I drew the modified nomogram templatefor 445 nm λ_{max} and compared it to the unmodified Dartnall nomogram templates, both including lens correction. The 445 modified template is almost the same as the 450 nm Dartnall nomogram at the wavelengths at which spectral sensitivity measurements were made. Therefore I feel confident that the results would not have changed much had I used the Ebrey and Honig modified nomogram for the template.

d) 502 λ_{max} mechanism: rod?

The new finding in this study is that rods may have input to

ganglion cells in an opponency to a cone such that color information can be coded. In the past, coding of color information has been considered the cone domain. This finding raises the question, is the 502 pigment actually from a rod receptor or from a cone with 502 pigment? One way of addressing the question is to see how properties of the ground squirrel 502 receptor compare with properties of typical rods. Some of the properties which separate typical rods from cones have been discussed in the introduction.

The most convincing property of the 502 mechanism which supports its location in rods is its greater sensitivity. Cells with input from the 502 mechanism are more sensitive than units with all cone input. Furthermore, no cells were found in which the dark adapted spectral sensitivity curve fit 525 and a light adapted spectral sensitivity curve fit 502 alone. The fiber in Fig. 4.10 is the only unit in which a 525 template fit the dark adapted spectral sensitivity curve and chromatic adaptation in the light adapted condition showed both 502 and 525 input. It is likely in this case that the 525 input hid the presence of the 502 when not chromatically adapted. While the sensitivity is greater than the cones, like a typical rod, the 502 pigment mechanism is atypical in that it does not saturate at higher light levels.

The time course of dark adaptation also suggests that the 502 pigment is in a rod. Dark adaptation curves were only completed for four non-opponent units. Unfortunately, none of the units had both 502 and 525 input. However, the unit with all rod input required 25 - 30 minutes to return to dark adapted sensitivity while the unit with only 525 input required only 8 minutes. There was, however, no difference in the early time course of adaptation for these two units. Several

determinations of the time course of dark adaptation on each unit would have been more convincing. The behavioral dark adaptation curves of Jacobs and Yolton (1971) show return of maximum sensitivity within 10 minutes, although one curve shows a hint of a second branch.

Other tests to distinguish between rods and cones such as the test for a Stiles Crawford effect were not attempted for technical reasons. Flicker fusion frequency which has been used to separate rods from cones has recently been shown not to be a definitive test (Green & Siegel, 1975; Conner and MacLeod, 1977).

The most that can be said at this time is that the rod is not typical although it contains the 502 pigment and has greater sensitivity than the cone mechanisms. The fact that it is atypical makes it easier to explain the unusual finding that some ganglion cells receive all-rod input. Other interpretations are possible, however. There may be a 502 rod and a 502 cone which operates at higher light intensities.

e) Correlation with morphology of the retina

Since the morphology of the ground squirrel retina has been so carefully studied using Golgi stain with conventional light microscope and electronmicroscopy methods (West & Dowling, 1975; West, 1976), it is tempting to try to correlate physiological with morphological data. Basically there are fifteen distinguishable types of ganglion cells with synapses in various different bands of the inner plexiform layer and with different percentages of amacrine and bipolar synapses. Some of the ganglion cells have bistratified dendrites, others terminate in one layer, and others have synapses in many different layers. The extent of the dendritic tree also varies between classes of morphological classes. My finding that there are many functionally different types

of ganglion cells complements West's finding of the many morphological types. It is impossible, however, to say which ganglion cell classes from my study correlate with West's morphological types. While the sizes of the receptive fields of the ganglion cells vary greatly, no particular class of cells consistently had small fields. Furthermore, it would be purely speculation to attribute specific complex response properties to specific anatomical complexities. Furthermore, West does not state which bipolars receive rod input. Perhaps with this information one could at least correlate the anatomical and physiological classes which receive rod input.

f) Fluctuating spontaneous activity

As an unexpected and unwanted bonus of this study of ground squirrel ganglion cells some data were collected on cyclic fluctuation of spontaneous activity. Although it is not pertinent to the questions addressed in this study, I would like to discuss one possible explanation for the phenomenon. As mentioned in Chapter 2, cycling of spontaneous activity in ganglion cells has been seen by other investigators and the source is still unknown.

I recorded responses to light during the cycling in many units, and one observation seemed particularly interesting. In most units the response amplitude cycled with the spontaneous activity; usually high spontaneous activity accompanied a large response amplitude and vice versa. However, in some units the "on" and "off" responses cycled 180° out of phase, one response type cycling with the spontaneous activity and the other out of phase. This suggests that the source of the cycling is distal to the ganglion cell. One could speculate on possible mechanisms.

One hypothetical system for mediating this result might be based on the stratification of the inner plexiform layer. West (1976) described some bistratified ganglion cells with terminals in two distinct layers. Possibly the "on" response and "off" responses are mediated through different strata in some cells by different amacrines and bipolars. If amacrine cells in only one layer cyclically facilitate or inhibit signals from reaching the ganglion cells, then the "on" and "off" responses may cycle independently, with the same period but in different phases. There are, of course, many other possible explanations.

Most investigators, myself included, have considered the cycling to be a nonphysiological phenomenon to be eliminated, particularly since it is so disruptive to the measurement of other aspects of the response. I have unsuccessfully tried to eliminate it by improving the procedures of monitoring and maintaining the physiological condition. Even in animals with normal blood pressure the cycling occurred. The possibility exists that there is a physiological mechanism controlling the cycling, since it is so widely found, even in freely moving animals (Cavaggioni, 1968). Perhaps the phenomenon should be studied in a more detailed manner.

Appendix A. THE EFFECTIVE LUMINANCE OF STIMULI AND BACKGROUNDS

One might wonder how effective the test and background lights were. To address this question calculations were made to estimate the illuminance on the retina of the lights in units of ground squirrel trolands. The numbers are only approximations, however, because of assumptions that must be made.

In humans the conversion to trolands involves multiplying the luminance in candles/m^2 by the area of the pupil in mm^2. Because the ground squirrel eye is smaller than the human eye and light falls off as the square of distance, an additional factor must be included comparing the posterior nodal distances. The posterior nodal distance of the man is 17 mm; the posterior nodal distance for the ground squirrel has not been measured, but was estimated to be half the diameter of the globe, or 4 mm. The conversion factor is therefore $(17/4)^2$ = 18.06. The pupil diameter for the ground squirrel was estimated to be 3mm, and therefore the area of the pupil was 7.1 mm^2. These estimates were used for all the conversions to troland units.

To determine the effectiveness of the test light, the luminance of an unattenuated 525 nm test was measured with an SEI photometer. The luminance was 2.1 log cds/m^2 (125.9 cds/m^2). The test was on for .5 second. Therefore the retinal illuminance was 125.9 cds/m^2 x 18.06 x 7.1 m^2 x 1/2 sec = 8071.83 photopic td-sec (3.91 log photopic td-secs). This was converted to scotopic troland-secs using conversion factors for humans of +.45 log units for 525 nm (Wyszecki & Stiles, 1967), which results in 4.35 log scotopic td-secs for the brightest 525 stimulus. Since the conversion factors are based on human scotopic and photopic luminosity functions, the calculation is only an approximation.

However, in the middle wavelengths the conversion factors are probably most accurate.

To determine if the brightest stimuli could be bleaching a significant amount of pigment, this illuminance was compared to the human data on the bleaching of rhodopsin from Alpern (1971). Assuming that ground squirrel rhodopsin is similar to that of man, 4.35 log scotopic td-secs would only bleach .16% of the pigment.

In the dark adaptation experiments it is important that the bleaching light be effective. This stimulus was an unattenuated xenon arc light projected for three minutes onto the tangent screen, over an area larger than the receptive field. The luminance was 2.94 log cds/m^2 (870.96 cds/m^2) measured with an SEI photometer. Using the same assumptions as in the conversion for the 525 test, the bleaching source was found to be 7.31 log photopic td-secs. This can be converted to 7.7 log scotopic td-secs by adding the conversion factor for a xenon arc with a color temperature of 6000°K. This would bleach more than 90% of the pigment if ground squirrel rhodopsin is like human rhodopsin. The conversion factor from photopic to scotopic trloands again is from human data.

The background luminance can also be converted to troland units to determine if backgrounds should saturate typical rods. The unattenuated long wavelength background was approximately 1.8 log cds/m^2 (Wratten 21) which can be converted to 3.94 log photopic trolands. The unattenuated short wavelength backgrounds were -.27 log cds/m^2 (Wratten 47) or .85 log cds/m^2 (Melles Griot 450 nm λ_{max}) which convert to 1.84 or 2.96 log photopic tds, respectively. One cannot validly compare these numbers with human data, since the ground squirrel and human are

quite different for those wavelengths. The ground squirrel has no red cone as in humans, and therefore the long wavelength backgrounds would not be as effective as for humans. In the short wavelengths the ground squirrel lens absorbs strongly, which would reduce the effectiveness of the blue backgrounds. It is, therefore, of some question whether these backgrounds would saturate rods. In the human, rods saturated at 3.3 scotopic trolands (Aguilar & Stiles, 1954). It is difficult to determine how much the chromatic backgrounds would bleach since the measurements are in photopic trolands and cannot be compared to the human data.

Appendix B: LENS TRANSMISSION IN THE MEXICAN GROUND SQUIRREL

With the help of Edward A. Boettner,* I measured the spectral transmission of the lenses of two Mexican ground squirrels using a spectrophotometer (American Instruments DW-2). This double beam instrument compares the transmission through a sample with the reference beam. For a baseline measurement, the transmission of a control solution of mammalian ringer was first measured in the same mount in which the lens sample was later measured. This was necessary because the size of the beam of radition of the instrument (about 6 x 6 mm) was larger than the opening in the mount (4 mm diameter).

The lens sample was prepared immediately after sacrificing the animal. The eyeball was removed and the lens dissected out. The lens was placed in a hole (4 mm diameter, 2 mm depth) in a black opaque disk, taking care that the lens was oriented so that the rays of light would pass parallel to its axis. A drop of mammalian ringer was applied to displace any air bubbles. The lens and disc were sandwiched between two glass plates. This mount was designed so that the glass would press the lens down to fill the hole in the disc.

Measurement of the transmission of the lens was plotted by the machine continuously from 400 nm to 675 nm. The measurements were completed within an hour of the animal's death. The baseline transmission of the ringers was subtracted. Correction was applied for the thickness of the lens in the intact eye. This value was determined by averaging the thicknesses of four lenses and was 3.33 mm $\pm$ .055.

The transmissions of the two lenses measured agreed well and the results were averaged. As can be seen in Fig. B1, my measurements

were very similar to the results of Brown and Wooten (unpublished) which were used in the ERG study of Green and Dowling (1975). Measurements made by Michael showed less density in the short wavelengths; however, the only correction factor radically affected was for 425 nm. The correction factors used in this thesis were an average of my measurements and those of Brown and Wooten. The lens corrections were applied to the templates generated by the Dartnall nomogram at the wavelengths in 25 nm steps.

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Figure B1. Mexican ground squirrel lens absorption spectra. The absorption spectra of 2 ground squirrel lenses were measured and averaged (circles). The squares are the measurements of Brown and Wooten (unpublished). The dotted line is a by eye fit to the combined data.

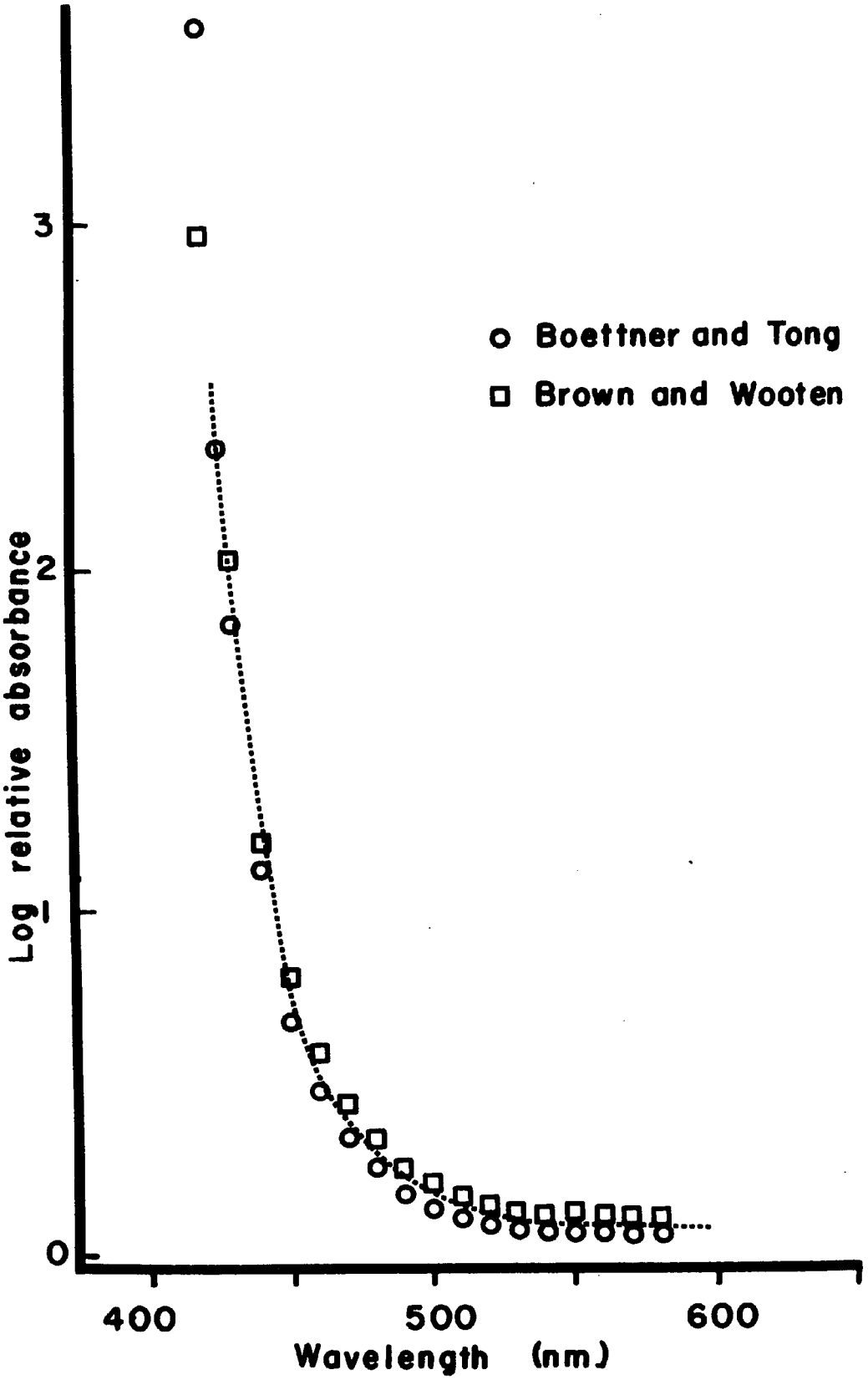
Boettner and Tong
Brown and Wooten
Log relative absorbance
3
2
1
0
400
500
600
Wavelength (nm)

Figure B1

APPENDIX C: FIBERS CATEGORIZED BY THEIR INPUTS DURING DARK AND LIGHT ADAPTED CONDITIONS.

Fibers can be classified by the input mechanisms active during dark adapted and light adapted conditions. The results are summarized in Table C1. Because of the arrangement in the table of the input mechanisms, the contrast sensitive units are found in the upper left corner of the table: above and to the left of the double lines. Contrast and color opponent fibers have not been differentiated to the left of the double line in the row labeled "LA not done." In the same row to the right of the double line, all fibers are color opponent. The units that were color opponent in in the dark adapted state were found to be still color opponent in the light adapted state. In the units in this row, however, spectral sensitivity curves were not measured on bright backgrounds, many because the test source was not bright enough. The sample is biased toward dark adapted spectral sensitivity measurements since these were always measured first.

Table C1

Ganglion Cells Categorized by The Likely Receptor Mechanisms with Input under Dark Adapted and Light Adapted Conditions

Light Adapted \ Dark Adapted	445	502	525	445 + 502	502 + 525	445 vs 502	445 vs 525	502 vs 525	Total
445									
502		14							14
525		6	7						13
445 + 502		4							4
502 + 525		2	1		1				4
445 vs 502		1							1
445 vs 525		10					1		11
502 vs 525		4	1						5
LA not done		46	11		3	4	1	2	67
Total		87	20		4	4	2	2	119

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