COLOR VISION IN *DROSOPHILA*

Thesis by

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In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1976

(Submitted May 3, 1976)

This thesis is dedicated to Jenijoy La Belle

who taught me about Thomas Hardy who in
turn taught me about color vision and memory.

NEUTRAL TONES

by Thomas Hardy, 1867

We stood by a pond that winter day,
And the sun was white, as though chidden of God,
And a few leaves lay on the starving sod
    -They had fallen from an ash, and were gray.

Your eyes on me were as eyes that rove
Over tedious riddles of years ago;
And some words played between us to and fro
    On which lost the more by our love.

The smile on your mouth was the deadest thing,
Alive enough to have strength to die;
And a grin of bitterness swept thereby
    Like an ominous bird a-wing...

Since then, keen lessons that love deceives,
And wrings with wrong, have shaped to me
Your face, and the God-curst sun, and a tree,
    And a pond edged with grayish leaves.

ACKNOWLEDGEMENTS

I would like to thank Seymour Benzer for being great. Besides being an inspiration as a scientist, he has been an inspiration as a person. The support, good humor, and friendship both he and Dotty have given me through the proverbial "thick and thin", which in this case was not just proverbial, I deeply appreciate.

It was a honor to be the first recipient of the Gordon Ross Medical Foundation Fellowship. I thank Dr. Gordon Ross for this privilege and for the personal interest he has taken in my graduate career. The research reported in the thesis was also supported in part by an Earle C. Anthony Fellowship (to W.A.H.), NSF Grant GB27228 (to Seymour Benzer), NSF Grant GB-M574-12817 (to William S. Stark). Johns Hopkins Biomedical Sciences Research Award from NIH 3-S05-RR07041-OHS1 (to William S. Stark), NIMH Training Grant 5-T01-MH06742-16 (to John A. Walker), and an NIH Fellowship (to William G. Quinn).

The members of my thesis committee merit commendation both for the assistance they provided in the final construction of this thesis and for the cruellest April Fool's joke it has ever been my pleasure to be the brunt of, and so richly deserved.

The experiments and results presented in this thesis are

not entirely mine. They are the result of two collaborative efforts. I thank Bill Stark for the years and energy he has devoted to helping in the visual mutant project, and Chip Quinn for allowing me to join him at the beginning of his adventures into fly learning, and both for their friendship. Ed Lipson has been a virtual collaborator on the spectrophotometric experiments, and more than a virtual friend.

Many people have assisted in this research technically: Eveline Eichenberger, Joyce Gorn, Barbara Butler, Howard Seliger, William Green, William Hamilton, Glenn Pransky, and William Zitzmann. I thank them all. Jane Koenig, John Merriam, Ed Lewis, and George Lefevre have generously provided several mutant stocks.

Many of my ideas were not my own; they originally came from those people it has been my pleasure to associate with during my graduate student career. I thank Martin Heisenberg, and Bill Pak for their interesting correspondence; Tom Hanson, Alain Ghysen, Yuh-Nung and Lily Jan, Jim Hudspeth, Duncan Byers, and Yadin Dudai for criticisms, ideas, and discussion of work in progress. Jeff Hall and Doug Kankel made significant contributions to the direction of my research and have permanently warped me as a person. It is very difficult for me to thank Don Ready, not because he doesn't deserve it.

Finally there is Jenijoy La Belle. My respect for her

is as great as my love for her. Because of her alone, my years as a graduate student have been the best years of my life.

ABSTRACT

In Chapter I an historical introduction to the study of invertebrate color vision is given, with emphasis on insects, especially Drosophila.

In Chapter II three mutations which eliminate specific types of photoreceptors in Drosophila are described. Of the 8 photoreceptors in each facet, two mutations delete the outer 6 (R1-6). The third eliminates R7, one of the two central photoreceptors. Double mutants were constructed in which only photoreceptor R8 is present. The spectral sensitivities, photopigments, and behavioral properties of these mutants were investigated. R1-6 have two sensitivity peaks, near 350 and 470 nm. These receptors contain a rhodopsin with these absorption peaks. It interconverts with a metarhodopsin that absorbs around 570 nm. R7 is a UV-receptor, containing rhodopsin that absorbs around 370 nm and interconverts with a metarhodopsin which absorbs around 470 nm. R8 is a non-adapting blue-receptor with a third type of rhodopsin. The properties of these photopigments account for the different sensitivities and spectral adaptation phenomena of the various photoreceptors. All the photoreceptors have input into phototaxis. Spectral analysis of this behavior provides evidence for integration

of the input from the different receptors.

In Chapter III experiments are described showing learning and color vision in Drosophila. Populations of Drosophila were trained by alternately exposing them to two odorants, one coupled with electric shock. On testing, the flies avoided the shock associated odor. Pseudoconditioning, excitatory states, odor preference, sensitization, habituation, and subjective bias have been eliminated as explanations. The selective avoidance can be extinguished by retraining. All flies in the population have equal probability of expressing this behavior. Memory persists for 24 hr. Another paradigm has been developed in which flies learn to discriminate between light sources of different color.

In Chapter IV the results of these experiments are discussed, relating them to previous work and future directions.

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Chapter I

GENERAL INTRODUCTION

Since von Frisch (1914) refuted the then-popular idea that invertebrates are color blind, a great deal of effort has been expended in attempting to understand the biological basis of invertebrate color vision. Ideally one might integrate photochemistry, photoreceptor physiology, and neural circuitry into a scheme that will explain this behavioral phenomenon. This thesis is such an attempt.

The introduction presents an historical review of invertebrate and insect vision, with emphasis on Drosophila. In the body of the thesis, experiments are described showing that Drosophila has three spectrally different photoreceptor classes, each with a different photopigment, and that all these photoreceptors have inputs into visually guided behavior. Thus, the machinery for color vision is present. Experiments are then described which demonstrate the use of this capacity for learning in Drosophila.

A number of reviews on invertebrate vision have recently appeared including the comprehensive and well organized review of insect vision by Goldsmith and Bernard (1974). Other useful reviews are Wolken (1971), Eakin (1972), Goldsmith (1972), Abrahamson and Fager (1973), Ebrey and Honig

(1975), Wolken (1975), and Pak (1976). This introduction incorporates material from these reviews as well as material from original papers.

A. INVERTEBRATE AND INSECT VISION

1. The Structure of the Retina

All insect eyes are remarkably alike. The principal photoreceptive organs of adult insects are compound eyes, each composed of structural subunits called ommatidia. The primitive bristle-tail, Lepisma, has only about a dozen ommatidia per eye while the dragonfly may have as many as 10,000 (Goldsmith and Bernard, 1974). Drosophila has approximately 800 ommatidia per eye.

On the distal end of each ommatidium is a dioptric apparatus consisting of a corneal lens and a crystalline cone. Proximal to the cone are a group of photoreceptors (the retinula), and surrounding the retinula is a sheath of pigment cells. The ommatidia are limited proximally by a basement membrane. Exner (1891) found that the ommatidia of nocturnal insects have short, fat rhabdomeres that are separated from the crystalline cone by a relatively large distance. In these insects the screening pigments in the pigment cells migrate distally upon light adaptation. Since this arrangement allows light entering the cone of one ommatidium to stimulate receptor cells in neighboring ommatidia, he called these "superposition eyes". In diurnal insects,

the rhabdom is generally long and thin, extending from the basement membrane to the cone, and light entering a given ommatidium stimulates only the receptors in that ommatidium. In these insects the screening pigments in the pigment cells do not migrate, while those in the retinular cells can migrate radially toward the rhabdom upon light adaptation. Exner called these "apposition eyes". Although Exner's terminology has recently been questioned (Horridge, 1971; Goldsmith and Bernard, 1974), his classification of eyes into these two groups is still useful. Drosophila eyes are of the apposition type (Waddington & Perry, 1960).

The corneal lens, which in most species is colorless and transparent in the near UV (Carricaburu, Chardenot, 1967; Kolb, Autrum & Eguchi, 1969) is secreted by cone cells or primary pigment cells (Goldsmith & Bernard, 1974). In diptera, the lens consists of alternating layers of cuticle of high and low refractive index (Bernard & Miller, 1968), sometimes giving a characteristic coloration pattern to the eye which may be important in enhancing the contrast of colored objects (Bernard, 1971). In Drosophila, there are about 60 layers of corneal cuticle, bent into a doubly convex lens of high refractive index (Waddington & Perry, 1960). Refraction at the corneal outer surface leads to light convergence at the rhabdomere tips (Franceschini & Kirschfeld, 1971a; 1971b).

Below the lens is the cone, which is produced by four cone cells (Semper's cells). There are four types of cone

in insects (Grenchier, 1879; Goldsmith and Bernard, 1974): 1) acone, in which the cone cells are transparent but largely undifferentiated, 2) eucone, in which the cone cells contain a clear, hard, intracellular core, 3) pseudocone, in which the cone cells secrete a soft clear gelatinous or liquid material which is held in place laterally by the primary pigment cells, distally by the cornea, and proximally by the cone cells, and 4) exocone, in which the cone cells secrete an extension of the corneal lens and sometimes a crystalline thread down the center of the ommatidium (Horridge, 1969a). Drosophila has a fluid filled pseudocone (Waddington & Perry, 1960) as do other closely related diptera (Trujillo-Cenoz & Melamed, 1966; Boschek, 1971).

Photoreceptors come in two fundamental categories, ciliary and rhabdomeric (Eakin, 1963). The ciliary category includes vertebrate rods, cones, and other cells in which the photoreceptoral organelle arises from a cilium-like growth of the receptor cell. The rhabdomeric category includes all photoreceptors which are invaginated to form villi or lamellae, as found in many invertebrates (Eakin, 1972). All insects have rhabdomeric photoreceptors in their compound eyes. Insect ommatidia may each have from seven to twelve retinular cells that are not necessarily of identical type. In some insects, the retinula is stratified into proximal and distal photoreceptor layers. In others, photoreceptors run the entire length of the ommatidium.

Still others have photoreceptors in which the rhabdomeres interdigitate with each other proximo-distally (Goldsmith & Bernard, 1974). In Drosophila, the receptor cells are arranged in an asymmetric trapezoid. The outer six rhabdomeres are roughly the same size and run the entire length of the ommatidium. The central two are stacked one above the other, each running half the ommatidial length (Waddington & Perry, 1960).

The rhabdomeres of an ommatidium in various insect species can be arranged in one of two ways: 1) the closed (or fused) rhabdom, in which the rhabdomeres of all the receptor cells combine to form a single bundle in the center of the eye, as in the honeybee, 2) the open rhabdom, in which the rhabdomeres do not contact each other (Eakin, 1972). In Drosophila the outer six rhabdomeres are separate, but the central two are fused, one lying directly on top of the other (Dietrich, 1909; Waddington & Perry, 1960).

An insect rhabdomere is a collection of closely packed microvilli arising from outfoldings of the sensory cell membrane (Perry, 1968a). These are usually packed in a hexagonal array. These microvilli, varying in length from 5,000 to 50,000 Å and in diameter from 200 to 1,000 Å, contain the visual pigments (Langer and Thorell, 1966; see also discussion by Eakin, 1972).

The soma of the invertebrate photoreceptor is distinguished by a high concentration of mitochondria in the

immediate vicinity of the photoreceptor apparatus (Eakin, 1972). Pigment granules are also present in many insect photoreceptors. In Drosophila these ommochrome pigment granules (Nolte, 1961; Fuge, 1967) migrate towards the rhabdomere in bright light (Franceschini, 1975). In the crab and mosquito one also finds near the rhabdomere pinocytotic vesicles, multivesicular bodies (MVB), lamellar bodies (LB), and mixed bodies all of which increase in number with light adaptation (Eguchi and Waterman, 1967; White, 1967). The pinocytotic vesicles are coated with material that appears similar in the electron microscope to the inner surface of the microvilli. The MVBs are membrane-limited vesicles, about 1μ in diameter, filled with hundreds of smaller vesicles about 0.05μ in diameter. The LBs, also about 1μ in diameter, appear to be multilayered coils of unit membrane. In the mixed bodies, both coils and small vesicles are seen. Also near the rhabdomere are the so called clear perirhabdomal vesicles, which decrease in total volume with light adaptation (Eguchi and Waterman, 1967). Such morphological changes due to light adaptation have been used to identify the spectral sensitivities of different receptor cells in the same ommatidium (Gribakin, 1969; Eguchi, Waterman & Akiyama, 1973).

All these structures can be found in Drosophila photoreceptors (Waddington & Perry, 1960; Eakin, 1972), although the effect of adaptation on all but the pigment granules has

not been studied. In the mosquito it has been suggested that the substantial decrease in rhabdomere size during light adaptation (White & Lord, 1975) occurs by pinching off the ends of the microvilli nearest the cell body, creating coated pinocytotic vesicles which lose their coats, becoming incorporated into MVBs, which in turn degrade this membrane forming LBs. In darkness, protein synthesis may be responsible for the re-enlargement of the rhabdomeres (White & Lord, 1975).

The axons of the photoreceptor cells of the compound eye pass through the basement membrane towards the optic ganglia. In most insects there are four optic ganglia. From periphery inward, they are: 1) the lamina ganglionaris, 2) the medulla, 3) the lobula, 4) the lobular plate (Goldsmith & Bernard, 1974). In the fly, the lamina is arranged in "optic cartidges" (Cajal & Sánchez, 1915), each of which contains large second order interneurons which receive input from six photoreceptors. The six axons come from each of six neighboring ommatidia that receive input from the same point in visual space (Braitenberg, 1967; Kirschfeld, 1967). This has been called a "neural superposition" eye (Kirschfeld, 1967), as distinct from the optical superposition eye of Exner. In the fly, the two central photoreceptors do not synapse in the lamina, but do so first in the medulla (Cajal & Sánchez, 1915; Trujillo-Cenóz, 1965). Although these results were obtained using larger flies, they are also true

for Drosophila (Hanson, unpublished).

As in other insects, each Drosophila ommatidium contains two primary pigment cells which surround the cone (Waddington & Perry, 1960). There are also six secondary pigment cells, each shared between two neighboring ommatidia, and three tertiary pigment cells at alternate vertices of the hexagonal ommatidium, each shared with three neighboring ommatidia. At each other vertex there is a mechanoreceptive bristle complex of four cells (Perry, 1968b). The primary pigment cells contain brown ommochrome pigment (ommatin) granules, while the secondary pigment cells contain ommochrome and red pteridine (drosopterin) granules (Shoup, 1966). These pigments prevent the penetration of light to ommatidia which are not axial to the light beam. Red light, however, is not well absorbed and will pass from one ommatidium to another. The absorption peaks of these pigments are close to the wavelengths to which the eye is most sensitive (Stark, 1973a).

2. The Physiology of the Retina

The Electroretinogram (ERG). The insect ERG records the electrical potential change between an electrode placed on the cornea and another electrode placed in some electrically unresponsive part of the animal. It arises from the current flow in the extracellular medium of the eye, due to the summed activities of the photoreceptors and higher order

neurons (Pak, 1976). It samples many different types of cells and can be recorded with ease. The dipteran electroretinogram consists of a corneal-positive spike-like "on-transient" followed by a sustained negative wave that lasts throughout the illumination period, followed by a transient, corneal-negative off-effect. The sustained negative component of the ERG originates from the receptor potential while the transients originate from higher order neurons. The evidence for this, summarized by Goldsmith and Bernard (1974), is as follows: 1) If the receptor layer is surgically removed from the optic ganglia, the ERG from the ommatidial layer contains only the sustained component (Bernard, 1942; Jahn & Wulff, 1942; Autrum & Gallwitz, 1951; Naka & Kuwabara, 1959, Autrum, Autrum & Hoffman, 1961); 2) The sustained component can be isolated by the use of nicotine, CO_2, and anoxic conditions, which are presumed to interfere with synaptic transmission but have no effect on isolated photoreceptors (Autrum & Hoffman, 1957; Autrum & Hoffman, 1960; Goldsmith, 1960; Wolbarsht, Wagner & Bodenstein, 1966); 3) Eye imaginal discs, transplanted into the abdomen of the host and differentiated into retinas with normal receptors but no second-order connections give only the sustained response (Eichenbaum & Goldsmith, 1968); 4) The sustained component can be recorded, free of on and off transients, if the ERG is measured between one electrode on the corneal surface and the other at the basement membrane (Ruck, 1961; Pak, Grossfield & White, 1969;

Heisenberg, 1971); 5) Intracellular recordings from receptor cells show a maintained potential with a time course comparable to that of the maintained component of the ERG. This is true even when recording from the axons of retinular cells (Burkhardt & Autrum, 1960; Naka, 1961; Naka & Eguchi, 1962a & 1962b; Washizu, 1964; Kirshfeld, 1966; Järvelehto & Zettler, 1970; Alawi & Pak, 1971).

The Receptor Potential. Although much information has come from ERGs of Drosophila, such as spectral sensitivity and localization of defects in visual mutants, intracellular recording has furnished the most important advances in understanding the receptor potential mechanism. Small, discrete, voltage fluctuations commonly called "quantum bumps", can be recorded intracellularly from the photoreceptor cells of several species of arthropods both in the dark (spontaneous bumps) and in dim light (light-induced bumps) (Limulus lateral eyes: Yeandle, 1957; Fuortes & Yeandle, 1964; Adolph, 1964; Limulus ventral eyes: Millechia & Mauro, 1969; Locusta: Scholes, 1965; Musca: Kirschfeld, 1966; and Drosophila: Wu & Pak, 1975). These bumps appear to be the building blocks of the receptor potential (Dodge, Knight & Toyoda, 1968). Each bump appears to be generated by absorption of a single photon or spontaneous conversion of a rhodopsin molecule. The probability of bump production parallels the probability of photon absorption under various conditions in Drosophila. This means that the spectral

sensitivity of a Drosophila photoreceptor is due to the probability of a rhodopsin absorbing a photon, rather than the size of the quantum bump varying as a function of wavelength (Wu & Pak, 1975). In Calliphora, however, there are claimed to be two bump sizes, a larger one produced by UV light and a smaller one produced by yellow light (Horridge, 1975), indicating two photopigments and two transduction processes in the same receptor cell.

Drosophila retinular cells have a resting potential of about 20 mV inside negative, a specific membrane resistance of about 8×10^3 Ω cm^2, and respond to light, as most rhabdomeric photoreceptors do, by depolarizing in a graded fashion with intensity (Alawi, 1972). Unlike in some other insects spikes are not seen (Naka, 1961; Alawi, 1972) but the response does have a dynamic and a static phase as in other dipterans (Burkhardt & Autrum, 1960; Washizu, 1964; Kirschfeld, 1966; Scholes, 1969). The decline from dynamic to static is presumably due to an adaptation mechanism similar to that in Limulus. In Limulus calcium ions entering the cells in response to a light flash are somehow responsible for decreasing sodium conductance (Lisman & Brown, 1972, 1975a, 1975b). The receptor potential itself which is similar to that in other rhabdomeric photoreceptors (McReynolds & Gorman, 1970), is caused by an increase in membrane conductance principally to sodium ions (Fuortes & O'Bryan, 1972). It propagates electrotonically down the axons (Järvëlehto and

Zettler, 1971).

Another photoreceptor mechanism occurs in Drosophila photoreceptors after an intense flash of blue or ultraviolet light (Cosens & Briscoe, 1972; Minke, Wu & Pak, 1975a; Stark, 1975). This mechanism has been most extensively studied in the barnacle lateral ocelli (Hillman, Hochstein & Minke, 1972; Hochstein, Minke & Hillman, 1973; Minke, Hochstein & Hillman, 1973a) and in the Limulus median eye (Nolte, Brown & Smith, 1968; Nolte & Brown, 1972; Minke, Hochstein & Hillman, 1973b). In these photoreceptors, an intense colored stimulus, which shifts a substantial amount of visual pigment (rhodopsin) to its thermally stable product (metarhodopsin) induces a prolonged depolarizing afterpotential (PDA) which saturates the voltage response of the cell for some time after the stimulus has been turned off. Once a maximal PDA has been induced, no further depolarizing response can be obtained until (a) the PDA decays or (b) metarhodopsin regenerates to rhodopsin either thermally or photically (Minke, Wu & Pak, 1975a). Recent investigations of the transient receptor potential mutant (trp) of Drosophila show that the PDA is actually caused by the summation of quantum bumps which continue to occur after the light has been turned off (Minke, Wu & Pak, 1975b). In the mutant the PDA declines rapidly. Constituting on the tail of this response, normal quantum bumps are seen, suggesting that the coupling between the photoexcitation of the visual

pigment and the production of the bump is not direct (Minke, Wu & Pak, 1975b).

The M-Potential. The early receptor potential, ERP, of vertebrates and invertebrates has been shown to be correlated with the photoconversion of the visual pigment (Pak, 1965; Arden, Ikeda & Siegal, 1966; Cone, 1967; Hagins & McGaughy, 1967; Pak & Boes, 1967; Minke, Hochstein & Hillman, 1973a). In Drosophila a rapid diphasic (corneal negative followed by corneal positive) potential can be elicited by a high energy flash when most of the visual pigment is in the metarhodopsin state (Pak & Liddington, 1974). This has been termed the M-potential. The M-potential of Drosophila has the spectral sensitivity of Drosophila R1-6 metarhodopsin, it is proportional to the amount of pigment excited by the stimulus flash, and arises as a result of the photoreversal of metarhodopsin back to rhodopsin (Pak & Liddington, 1974). In most vertebrates and invertebrates studied, the largest component of the ERP originates from rhodopsin bleaching (Brown & Murakami, 1964; Cone, 1964; Pak, 1965; Smith & Brown, 1966; Hagins & McGaughy, 1967; Hillman, Dodge, Hochstein, Knight & Minke, 1973), but in Drosophila only the metarhodopsin reaction is evident. This M-potential is especially prominent in the $norpA^{P12}$ mutant where it does not have to compete with the on-transient or receptor potential (Pak & Liddington, 1974). These results show that the M-potential is produced by Drosophila metarhodopsin converting

to rhodopsin but suggest that it may be generated by a mechanism different from that responsible for the classical ERP (Pak & Liddington, 1974).

The Different Photoreceptor Cell Types. Spectral adaption combined with ultrastructural changes were used to tell which were the violet-sensitive and yellow-sensitive cells in the ommatidia of the crayfish retina (Eguchi, Waterman & Akiyama, 1973), and which the green-yellow, blue, and violet photoreceptors in the honeybee ommatidium (Gribakin, 1969, 1972). Behavioral studies and microspectrophotometry in which the stimulation is restricted to selected photoreceptors are also useful for this purpose and will be discussed later.

ERG colorimetry has been used to study insect spectral sensitivities (Mazokhin-Porshnyakov, 1959; 1960a; 1960b; 1966). A test light composed of two (or in general n) monochromatic lights in variable proportions is matched to achieve color equality with each of the spectral colors. The comparison here is made by shifting alternately from test to reference light, using the absence of response as the criterion for a match. This method is suspect for several reasons (Goldsmith & Bernard, 1974) the chief of which is that the spectral characteristics of the screening pigment are not taken into account (Goldsmith, 1965).

Intracellular recordings have been used to investigate the spectral sensitivity of photoreceptors in several species of insects. In many dragonflies the ommatidia are large in

the dorsal half of the eye and small in the ventral half. Single unit analysis in the dorsal half (Horridge, 1969b; Eguchi, 1971) reveals UV receptors with maxima from about 350 to 380 nm and green receptors with peaks from 475 to 520 nm. It is suggested by a combination of morphological and physiological studies (Eguchi, 1971) that the UV-sensitive cells are the proximal two receptors in each ommatidium, while the green sensitive cells are the distal five. In the ventral part of the eye (Autrum & Kolb, 1968; Horridge, 1969b) single unit recording also demonstrated two spectrally different receptors, one green type (λ_{max} near 500 nm) and one blue type (λ_{max} near 420 nm). Both had secondary maxima in the UV at about 350 nm. Whether or not the green receptors are the same in the dorsal and ventral parts of the eye is not known, nor is it known in the ventral part of the eye how the two receptor types are situated with respect to each other within the ommatidium.

In the locust, all units recorded had a peak sensitivity at about 430 nm, with variable sensitivity at about 515 nm (Bennet, Tunstall & Horridge, 1967). Because the coupling ratio between receptors in the same ommatidia is low (Shaw, 1967), this suggests that there may be variable amounts of a second rhodopsin present in one photoreceptor.

The dorsal part of the cockroach compound eye contains two receptor types, a UV receptor (λ_{max} near 365 nm) and a green receptor (λ_{max} near 507 nm), present in approximately

equal numbers (Mote & Goldsmith, 1970). Earlier ERG experiments on the cockroach had shown the green light response could be selectively adapted by long wavelength light (Walther, 1968), which is consistent with the intracellular results.

Spectral sensitivity analysis of single retinula cells in the backswimmer Notonecta revealed three cell types, UV (λ_{max} near 350 nm), violet (λ_{max} near 420 nm), yellow-green (λ_{max} near 470 nm), and possibly a fourth, blue (λ_{max} near 460 nm) (Bruckmoser, 1968).

In bees, as in cockroaches, there is good agreement between single-unit and selective adaptation studies (Goldsmith & Bernard, 1974). There are three receptor types with maxima at about 340, 450, and 530 nm (Autrum & von Zwehl, 1964). Earlier ERG analysis (Goldsmith, 1960) had shown that the eye of the honeybee was dominated by a green receptor with a sensitivity maximum at about 535 nm which could be selectively adapted with yellow light. When in the yellow-adapted state, the ultraviolet receptor system of the eye was revealed, having a maximum sensitivity at about 345 nm.

In dipterans the evidence is not quite so clear "despite the large amount of work done on flies" (Goldsmith & Bernard 1974). In Calliphora, Burkhardt (1962) distinguished three classes of receptor with maximum sensitivities at about 470 nm, 490 nm and 520 nm using intracellular

recording. All three classes had rather large secondary maxima in the UV at about 350 nm. Because 490 nm sensitive photoreceptors were most frequently recorded from, it was assumed that these represent the six peripheral cells in each ommatidium while the 470 and 520 nm peaks represent the central photoreceptors. Later intracellular studies (McCann & Arnett, 1972; Rosner, 1975; Horridge & Mimura, 1975) showed one class of receptors in Musca and Calliphora, these having sensitivity peaks at 350 and 480 nm, although the relative heights of these peaks varied with the polarization angle. In Drosophila, only one spectral sensitivity class of photoreceptors has been found by intracellular recording (Wu & Pak, 1975; Minke, Wu & Pak, 1975a), with sensitivity maxima in the UV and at about 480 nm. However, studies with selective adaptation using the ERG have demonstrated two other classes of photoreceptors in Drosophila. These are presumably the central ones, one with a peak in the UV (λ_{max} near 350 nm), another with a peak in the blue (λ_{max} near 470 nm (Minke, Wu & Pak, 1975a; Stark, 1975; Cosens & Wright, 1975). In Musca and Calliphora, ERG studies show a sensitivity curve with peaks in the UV and blue-green regions (Goldsmith & Fernández, 1968; Walther & Dodt, 1959; Hoffman & Langer, 1961). Although there is some disagreement on the relative heights of these two peaks, there has been no evidence for selective adaptation in these flies (see Hamdorf & Rosner, 1973; Muijsler, Leutscher-Hazelhoff, Stavenga &

Kuiper, 1975; Stark & Zitzmann, 1976). Furthermore, 620 nm sensitivity peaks which are seen in some ERG studies of diptera seem to arise from an artifact, the transparency of the screening pigments to long wavelengths (Burkhardt, 1962; Goldsmith, 1965).

Purely ultraviolet photoreceptors (λ_{max} near 350 nm) have recently been found in the compound eyes of two diptera, Eristalis and Calliphora, by intracellular techniques (Bishop, 1974; Smola & Meffert, 1975; Horridge, Mimura & Tsukahara, 1975) but were not identified by staining. In Calliphora intracellular recordings of receptor potentials from the first optic chiasm (Meffert & Smola, 1976) which are presumably from the axons of the central photoreceptor cells (since the outer six end in the lamina) show two types of spectral sensitivity, one with a λ_{max} near 345 nm, and one with a double peak near 350 and 450 nm.

3. The Photochemistry

Our understanding of invertebrate visual pigments is largely based on the photochemical principles that were first analysed in detail in the squid (Hubbard & St. George, 1958). Invertebrate visual photochemistry differs from that of vertebrates. Light bleaches vertebrate (11-cis) rhodopsin through a series of intermediates (Prelumi., Lumi., Meta I., Meta II., Meta III.) to a mixture of all-trans retinal and opsin. To regenerate vertebrate rhodopsin all-trans retinal has to be

enzymatically stereoisomerized to 11-cis which can recombine with opsin (reviewed by Abrahamson & Fager, 1973). Invertebrate rhodopsin, on the other hand, does not convert to retinal plus opsin in the light. Instead the majority of the retinal remains tightly bound to opsin and can be extracted only with denaturing organic solvents (Wald, 1941). Although squid rhodopsin does not bleach, it is photosensitive, with a λ_{max} at 493 nm which causes it to convert to bathorhodopsin → lumirhodopsin → acid metarhodopsin (St. George & Wald, 1949; Hubbard & St. George, 1958; Kropf, Brown & Hubbard, 1959; Yoshizawa & Wald, 1964). Unlike vertebrate metarhodopsin, which is quickly hydrolyzed to retinal and opsin, above 0°C, squid metarhodopsin is stable at 20°C (Hubbard & St. George, 1958). Squid metarhodopsin exists in two forms, depending on pH. It is red (λ_{max} 500 nm) in neutral and mildly acid (pH 5 - 7) conditions (acid metarhodopsin), and yellow (λ_{max} 380 nm) in alkaline (pH 9.5 - 10) solution (alkaline metarhodopsin). Light, in addition to converting squid rhodopsin to metarhodopsin, also brings about the reverse reaction, the regeneration of rhodopsin from metarhodopsin. Continuous irradiation of the squid retina therefore produces a steady state, independent of light intensity, but dependent on wavelength (Hubbard & St. George, 1958). Alkaline metarhodopsin, although it has spectroscopic properties similar to retinal, is not retinal. When squid metarhodopsin is denatured chemically or thermally, it

releases retinal. This is demonstrated by treating it with hydroxylamine to form the retinal oxime, or with alcohol dehydrogenase or reduced DPN to produce vitamin A. None of these agents reacts with undenatured alkaline metarhodopsin (Hubbard & St. George, 1958). Furthermore, the molar ratio of squid visual pigment to protein bound retinal is almost exactly 1 (Hubbard & St. George, 1958), indicating that squid rhodopsin does use the retinal chromophore.

Another important question about squid visual pigment concerns the stereoisomeric configurations of retinal in the rhodopsin and metarhodopsin states. Thermal denaturation releases retinal from cattle opsin without altering its stereochemical configuration (Hubbard, 1958). If this is done with squid visual pigment, the rhodopsin releases 11-cis retinal while both the metarhodopsins release all-trans retinal (Hubbard & Kropf, 1958). This, along with *in situ* changes in extinction coefficient (Hubbard & St. George, 1958) give very strong support to the idea that squid rhodopsin uses 11-cis retinal as the visual chromophore, and that 11-cis changes to all-trans during or closely after the initiation of the photochemical reaction. It has already occurred by the bathorhodopsin (prelumi.) stage (reviewed in Abrahamson & Fager, 1973).

Other invertebrate visual pigments have not been characterized in quite such detail. The rhodopsin ↔ metarhodopsin (acid and alkaline) scheme has been shown in several

species of decapods and octapods (Brown & Brown, 1958; Hara & Hara, 1967; Hamdorf, Schwemer & Täuber, 1968). All have rhodopsins with λ_{max} near 480 nm for rhodopsin, 500 nm for acid metarhodopsin, and 380 nm for alkaline metarhodopsin.

In many crustacea, vitamin A in the 11-cis form has been found in high concentration in the basal ends of ommatidia, where the rhabdomeres are (Goldsmith, 1972). As with cephalopod photopigment, most crustacean photopigments that have been studied in the extracted state convert to intermediates (metarhodopsins) which absorb at about the same wavelength as the parent pigments (λ_{max} near 500 nm) and which are relatively stable (reviewed by Goldsmith, 1972). All crustacean photopigments tested use retinal as the chromophoric group, as judged by the antimony trichloride (Carr-Price) reaction or the spectral location of the product of thermal bleaching, or both (Goldsmith, 1972).

Insect photopigments are only recently beginning to be understood as similar to other better characterized invertebrate photopigments. The reason for the lag in our understanding of insect visual pigment chemistry, claims Wolken (1975), is that "... to extract and isolate the visual pigments from insect eyes is extremely difficult; it requires that thousands of insects be collected, dark adapted, decapitated, and their eyes dissected, all in the cold and in red light." Six to fourteen grams of Drosophila, grasshopper, and dragonfly tissues failed to yield a clear test for the

presence of vitamin A (Wald and Burg, 1957). Extracts of dark-adapted honeybees (Goldsmith, 1958), however, did show a small amount of retinal localized in the head. This was done by using the Carr-Price reaction, and reducing the retinal to vitamin A by potassium borohydride. A difference spectrum of the aqueous extract of dark-adapted bee heads before and after 7 minutes of yellow light, indicated a photosensitive pigment with a λ_{max} near 440 nm (close to the λ_{max} of one of the physiologically identified photoreceptors). Subsequently, small amounts of retinal were found in Musca rhabdomeres (Wolken, Bowness & Scheer, 1960). Dark-adapted bees were found to have about a 4:1 ratio of retinal to vitamin A, while the reverse ratio was found in light-adapted bees (Goldsmith & Warner, 1964). This is the only instance among invertebrates where there is evidence for the participation of vitamin A in the visual cycle (Goldsmith, 1972).

Another line of attack on the question of whether retinal is part of the insect visual pigment has been to deprive the developing insect of carotenoids. When this was done with Musca (Goldsmith, Barker & Cohen, 1964; Goldsmith & Fernández, 1966), Drosophila (Zimmerman & Goldsmith, 1971; Stark & Zitzmann, 1976), and mosquitos (Brammer & White, 1969), there was a large, 2 - 4 log unit decrease in the sensitivity of the retina as judged by the ERG, and this reduction was true for the UV peak as well as the blue peak (Goldsmith & Fernández, 1966). In addition, morphological analysis of

vitamin A-deprived mosquitoes (Brammer & White, 1969) showed ultrastructural changes in the photoreceptor cells, notably the absence of MVBs even after light adaptation. All these effects are prevented or reversed by feeding the insects food enriched with β-carotene (Goldsmith & Bernard, 1974).

The compound eyes of the neuropteran Ascalaphus macaronius have predominantly ultraviolet-sensitive photoreceptors with a peak near 350 nm, as judged by ERG and intracellular recording (Gogala, 1967). A UV photopigment (λ_{max} 345 nm) was extracted from these eyes by 2% aqueous digitonin which, upon UV irradiation, was converted to a stable product with a λ_{max} near 480 nm. This product reconverts to the UV pigment when illuminated with light of longer wavelengths. Raising the pH to 9.3 converts the 480 nm photoproduct to one with a λ_{max} near 375 nm. Bleaching in the presence of hydroxylamine produces a product with the spectral characteristics of retinal oxime (Gogala, Hamdorf, & Schwemer, 1970). This scheme is completely analogous to the rhodopsin, acid metarhodopsin, alkaline metarhodopsin scheme in the squid (Hubbard & St. George, 1958), except that the λ_{max}'s are different. These conversions were also seen using microspectrophotometric methods on Ascalaphus rhabdomeres (Hamdorf, Paulsen, Schwemer & Täuber, 1972). Thin layer chromatography of extracts from Ascalaphus retinae revealed the presence of compounds which migrated with the characteristics of 1) all-trans retinal, 2) 11-cis retinal,

and 3) 13-cis retinal (Paulsen & Schwemer, 1972). The most critical test of the presence of 11-cis retinal was to add native cattle opsin to thermally denatured Ascalaphus pigment. Normal cattle rhodopsin was formed, as judged from action spectrum and reaction with hydroxylamine. If the UV pigment was irradiated with 353 nm, in order to achieve a photo-equilibrium between it and its stable intermediate, the amount of cattle rhodopsin formed was less and in good agreement with the amount of UV pigment expected to be present in the equilibrium state (Paulsen & Schwemer, 1972). These studies indicate that the UV photopigment of Ascalaphus is indeed a rhodopsin containing 11-cis retinal as the chromophoric group. The acid metarhodopsin (λ_{max} 480 nm) contains all-trans retinal and has an increased (by a factor of 1.7) extinction coefficient, which is characteristic of the change from 11-cis to all-trans retinal. This rhodopsin is the first shown to absorb maximally at a wavelength shorter than the 380 nm, the λ_{max} of the free chromophore. This hypsochromic shift, in contrast to the bathochromic shift usually observed, implies that the ordinary Schiff base linkage of retinal to the ε-amino group of lysine, characteristic of vertebrate rhodopsin, is not used for this pigment. It is suggested (Paulsen & Schwemer, 1972) that since unprotonated retinylidene imines show maximal absorbance about 20 nm shorter than free retinal, such a linkage of the 11-cis retinal may contribute to the hypsochromic shift found in

Ascalaphus rhodopsin. Other possible linkages of retinal to the protein moiety could also produce such a shift (Goldsmith, 1972).

A study of the photochemistry of the sphingid moth, Deilephila elpenor, which is shown by selective adaptation ERG experimnts to have three photoreceptor classes λ_{max}'s 350, 440 and 525 nm (Höglund, Hamdorf, Langer, Paulsen & Schwemer, 1973), revealed three different photopigments. Spectrophotometry of digitonin extracts and microspectrophotometry (not on single rhabdomeres) showed visual pigments, rhodopsins with λ_{max}'s near 350, 440, and 525 nm. The metarhodopsins of all three pigments have a higher absorbance than their rhodopsins and λ_{max}'s all near 480 nm. Adding hydroxylamine to the irradiated extract caused a shift to about 360 nm and an increase in absorbance, strongly suggesting that retinal is the chromophore of these pigments (Höglund, Hamdorf, Langer, Paulsen & Schwemer, 1973).

Fly photochemistry since the early vitamin A deprivation studies (Goldsmith, Barker & Cohen, 1964), has been studied by microspectrophotometric methods. Examination of the visual pigment of the larval mosquito by this technique (Brown & White, 1972) showed that it has a λ_{max} near 515 nm which upon irradiation converts to a stable intermediate which has λ_{max} near 480 nm. Long exposures in the presence of 0.1 M neutralized hydroxylamine produce a product with the spectral characteristics of retinal-oxime (λ_{max} 365 nm) at the expense of the visual pigment. When the ocelli were

irradiated for a long time, in the presence of potassium borohydride, vitamin A was formed, as judged by spectral characteristics. This is strong evidence that the chromophore of mosquito rhodopsin and metarhodopsin is retinal (Brown & White, 1972).

Results with the white-eyed mutant "chalky" of Calliphora (Langer & Thorell, 1966) showed that the six peripheral rhabdomeres all have absorption maxima at about 510 nm, with a secondary peak at about 360 nm. The central rhabdomeres absorbed maximally near 470 nm with a secondary peak near 380 nm. These results were complicated by the fact that the measuring lights were intense enough to establish a photo-equilibrium between rhodopsin and metarhodopsin (Langer, 1972; and Langer's comments on Stavenga, Zantema & Kuiper, 1973). In accordance with this earlier mistaken result, however, a digitonin extract from Musca heads (Marak, Gallik & Cornesky, 1970) was found to have a light-sensitive λ_{max} near 510 nm.

Better controlled attempts at microspectrophotometry with Calliphora (Stavenga, Zantema & Kuiper, 1973) revealed a rhodopsin in the outer rhabdomeres with a λ_{max} near 470 nm which converts with a stable metarhodopsin (λ_{max} near 580 nm), and a rhodopsin in the central rhabdomere with a λ_{max} near 350 nm. Later work, in the same lab (Stavenga, 1974), claimed that the former suggestion of a UV pigment in the central rhabdomeres was not correct, and that there was only one visual pigment.

This pigment was also found by other investigators in Calliphora (Hamdorf, Paulsen & Schwemer, 1973) and Drosophila (Ostroy, Wilson & Pak, 1974). Drosophila rhodopsin (λ_{max} near 480 nm) and metarhodopsin (λ_{max} near 580 nm) was shown to be based on retinal by bleaching in the presence of 1% Triton-X and noting the characteristic retinal peak appear at 387 nm (Ostroy, Wilson & Pak, 1974). The spectral characteristics of this pigment fitted well with the spectral sensitivity, the M potential, and the PDA seen in the outer six photoreceptors, but did nothing to explain the spectral properties of the central two.

4. The Behavior

von Frisch (1914) fed bees in a dish placed on blue paper squares interspersed in a checkerboard arrangement with papers of various shades of gray. After adequate training, the bees always returned to the blue paper, even when it was free of food, rearranged in the checkerboard, and covered with a glass plate. The conclusion reached was that bees must perceive blue as a color distinct from gray. The same type of experiment (von Frisch, 1914) showed bees to be red-blind. Later, it was shown, using mercury spectrum lines (Kühn & Pohl, 1921; Kühn, 1927) that bees could also distinguish blue-green and ultraviolet. Extensions of these results (Bertholf, 1931; Hertz, 1939; Daumer, 1956) showed that behaviorally, bee color vision could be understood in

terms of three spectrally different photoreceptors, one in the ultraviolet, one in the blue, and one in the yellow. Any color could be matched by a colorimetric combination of these three, but no two would suffice. Three spectrally different photoreceptors were later identified electrophysiologically (Autrum & von Zwehl, 1964). Only one photopigment (with a λ_{max} near 440 nm) has been found in bee eyes (Goldsmith, 1958).

Color vision in ants has also been studied behaviorally. Ants were trained to respond to monochromatic lights (Tsuneki, 1953; Wehner & Toggweiler, 1972) and then tested in a discrimination task between this wavelength and some other light. Although colorimetric matching was not attempted, discrimination between similar wavelengths was tested. It was found that this discrimination function had two maxima near 380 and 550 nm, which corresponded to the two minima of the three-peaked spectral sensitivity distribution (Wehner & Toggweiler, 1972). This suggests trichromatic vision in ants.

In the above examples of invertebrate color vision, conditioning was used as the basis for color discrimination. In an animal that does not learn, color vision would be much more difficult to demonstrate. Until recently, flies had the reputation of being incapable of learning, therefore, earlier examinations of fly spectral vision did not attempt to use learned discriminations as a basis for color vision (though see Isle, 1949).

Drosophila was found to be positively phototactic

(Carpenter, 1905), and this phototaxis was shown to be an hereditary trait, since flies bred for sixty-nine generations in the dark were just as phototactic as those bred in normal light (Payne, 1911). It was found (McEwen, 1918) that the spectral effectiveness for phototactic stimulation is a function of the Drosophila eye color. For white eyed flies the order was: violet most effective, then green, then red. Ultraviolet light (less than 390 nm) was also able to stimulate Drosophila phototaxis (Lutz & Richtmeyer, 1922).

The first attempt at demonstrating color vision in Drosophila (Hamilton, 1922) was done by stimulating the animals to go towards one end of a tube by light of one wavelength. After enough time for a marked decrease in the positive reaction of the flies to this "fatiguing light", a second "stimulating light" was turned on at the other end of the tube. This light had been balanced previously against the first for physiological attractiveness. It was found that the "fatiguing light", if of sufficiently different wavelength from the "stimulating light", upset the previously established balance. This experiment, interpreted as color vision, could equally well be explained by differential absorption of the screening pigments, two visual pigments in the same photoreceptor, two states of the same photopigment, or habituation.

The extent and spectral efficiency of the spectrum for phototaxis was measured in a Y-maze by finding a white light of equal physiological intensity to a series of monochromatic lights. This spectrum extends from about 250 nm to about

600 nm and has two peaks, a large one near 360 nm and a smaller one near 470 nm (Bertholf, 1932). When this was measured, using a blackened arm instead of white-light (Brown & Hall, 1936), a stronger response is found to the green than to the ultraviolet. The same experimental setup with varying intensity of monochromatic lights (Fingerman, 1952) showed that intensity response curves were not parallel for all wavelengths causing a shift in the shape of the action spectrum at different intensities. In red-eyed flies, this shift, from a peak near 470 nm at low intensity to one near 510 nm at high intensity, has been called a "Purkinje shift" (Fingerman & Brown, 1952). This shift is strongly affected by the screening pigments, and may be accounted for entirely by them (Fingerman, 1952). Another experiment that claimed color vision for Drosophila (Fingerman & Brown, 1953) consisted of repeating Hamilton's (1922) experiment in a Y-maze. This experiment is subject to the same criticisms as Hamilton's. Similar experiments (Schümperli, 1973) involved matching, in a Y-maze, a constant intensity white light with a variable intensity monochromatic light. When the intensity of the monochromatic light was changed, the shape of the phototactic action spectrum changed. At higher intensities the UV content of the constant light was also important. For low intensity constant light, there were approximately equal peaks near 350 and 500 nm, while at high intensity, the 500 nm peak shifted to near 480 nm and

the UV peak increased in relative height to 45 times the green peak height. This UV peak could be selectively adapted. These results were obtained with red eyed flies, so the effect of screening pigments is not known. Nevertheless, the results suggest the presence of three different spectral mechanisms in the fly, one with maxima in the green and UV, one purely in the UV, and one in the blue. They do not show color vision, however, because the input of these three systems may not be kept separate in the central nervous system.

Optical studies in Musca (Kirschfeld & Franceschini, 1968) demonstrated that the acceptance angle for light of the outer six photoreceptors is about 3°, while that of the 7, 8 system is only about 1½°, since the outer rhabdomeres are larger than the central. From pigment migration analysis at different light levels (Kirschfeld & Franceschini, 1968) it was shown that retinular cells R1-6 are more sensitive than R7 and R8. These pieces of information were used (Eckert, 1971) to show that the spectral sensitivity of the peripheral system was different from that of the central one. Monochromatic lights, dim enough to stimulate only the peripheral system, were used to find its action spectrum for driving the optomotor response of flies (see Hecht & Wald, 1934). On the other hand, stripes wide enough to stimulate the central system (but too narrow to stimulate the peripheral system) were used to find its optomotor action spectrum.

The peripheral system showed two maxima near 350 and 490 nm, while the central system showed only one near 470 nm. The peripheral photoreceptors, known as the high sensitivity system (HSS), and the central photoreceptors, known as the high acuity system (HAS), exist in Drosophila, and mutants have been found which selectively affect the input from one or the other system (Heisenberg, 1972).

In this connection, it should also be noted that theoretical studies of how light propagates down the rhabdomere (Snyder & Miller, 1972; Snyder & Pask, 1973) show that changes in rhabdomere diameter profoundly affect the wavelength of optimal absorption. This waveguide theory predicts the spectral sensitivity for the outer six rhabdomeres of Calliphora to peak near 490 nm with a slightly smaller peak near 350 nm, the spectral sensitivity for the seventh rhabdomere should peak near 340 nm with a much smaller secondary maximum near 470 nm. The eighth rhabdomere, since it has the same diameter as the seventh, should have identical spectral characteristics, except that the seventh being directly above the eighth, acts as a UV filter. Thus the primary peak for the eighth photoreceptor should be at 470 nm.

5. The Genetic Approach

Two recent reviews cover this material thoroughly (Heisenberg & Götz, 1975; Pak, 1976). The study of the genetics of Drosophila vision has gone through three phases

since its conception. The earliest work dealt with the question: "Is it possible that certain single gene mutations can influence behavior, for example, the phototactic and optomotor behavior of the fly?" The answer to this question was "Yes," (McEwen, 1918, Brown & Hall, 1936; Kalmus, 1943; Scott, 1947). This was not surprising, especially since most of the mutants tested affected the external morphology of the fly eye.

The second phase in the study of the genetics of Drosophila vision originally asked the question: "Are there aspects of behavior, such as phototaxis in a fly, which can be genetically modified by artifical selection techniques in the laboratory?" The answer to this question was also, "Yes", (Hirsch & Boudreau, 1958; Hadler, 1964; Dobzhansky & Spassky, 1967). This kind of research is still active, but the question now seems to be: "What is the number and the nature of the genes that influence this behavior?" (Hirsch, 1962; Woolf, 1972; Dobzhansky, Judson & Pavlovsky, 1974).

The third phase has the following question in mind: "How much can we find out about behavior and the nervous system, for example, vision in Drosophila, using genetics as a tool?" The techniques of isolating single sex-linked mutations which affect phototaxis (Benzer, 1967) and producing mosaics (Hotta & Benzer, 1970; 1972), offer a powerful way to localize the primary focus of any genetic defect. Several mutations that affect the

electroretinogram have been thus shown to act autonomously in the eye (Hotta & Benzer, 1970).

Phototransduction is being investigated, using phototactic mutants of Drosophila (Cosens & Manning, 1969; Alawi, Jennings, Grossfield, & Pak, 1972). Some of the important results of this type of work have already been reported in this introduction (see Physiology and Photochemistry).

The optomotor response of flies has also been investigated by the use of mutations that interfere with it (Götz, 1970; Heisenberg, 1972).

Collectively these laboratories, and several others working on related problems, have amassed a large number of single gene mutations which affect Drosophila vision. Some of these mutations were put to use in the experiments to be described in this thesis.

B. THE DIVISION OF LABOR

The following describes work done in collaboration with various people.

In the chapter "Genetic Dissection of the Photoreceptor System in the Compound Eye of Drosophila melanogaster", the basic idea, the microscopy, the genetics, and the photochemistry were my work, while the physiology was done by William Stark and John Walker at the Johns Hopkins University in Baltimore. In the chapter, "Conditioned Behavior in

Drosophila melanogaster", the work on the visual paradigm was mine, while the idea of conditioning *Drosophila* in order to investigate the learning process and most of the work on the olfactory paradigm were done by William Quinn and Seymour Benzer.

Chapter II

GENETIC DISSECTION OF THE PHOTORECEPTOR SYSTEM IN THE COMPOUND EYE OF *DROSOPHILA MELANOGASTER*

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This chapter has been published in
the *Journal of Physiology*
Volume 256, page 415-439, 1976

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INTRODUCTION

The compound eye of Drosophila consists of about 800 ommatidia each containing eight photoreceptor cells. The question arises: are these photoreceptors different from each other, and how do they combine to give normal visual function?

Optical and behavioral studies in Musca (Kirschfeld & Franceschini, 1968; Kirschfeld, 1969) indicated that the six peripheral photoreceptors, R1-6, in each ommatidium form a high-sensitivity low-acuity system, while the two central photoreceptors, R7 and R8, form a separate high-acuity, low-sensitivity system. Eckert (1971) obtained low-acuity sensitivity maxima at 360 and 486 nm and a high-acuity function with a single maximum at 464 nm. Burkhardt (1962) using intracellular recordings from Calliphora found three classes of photoreceptors: (1) with sensitivity maxima at 350 and 490 nm; (2) with maxima at 350 and 450 nm; (3) with maxima at 350 and 520 nm. Burkhardt did not histologically confirm cell type. More recently, McCann & Arnett (1972) were only able to record from cells like Burkhardt's first and most numerous class. In Drosophila, no recordings from R7 or R8 have been established (Alawi & Pak, 1971; Alawi, 1972; Minke, Wu & Pak, 1975a). Bishop (1974) has reported finding a new dipteran ultraviolet receptor by intracellular recording from the drone fly Eristalis but did not determine cell type.

Extracellular mass electroretinograms (ERGs) present an

electrophysiological alternative to intracellular recording. They do not yield single cell data but have the advantages of technical ease and reliability. Stark (1975) and Minke et al. (1975a) have shown that differential adaptation can be used to isolate three receptor components in the ERG: (1) a highly sensitive component with ultraviolet and visible sensitivity maxima; (2) a less sensitive component in the visible region; and (3) a component in the pure ultraviolet region. Both reports present evidence linking the latter two components to R7 and R8. Pak and Liddington (1974) and Stark (1975) have used ERG techniques to determine the spectral properties of the major rhodopsin component and its thermostable photo-interconvertible state, metarhodopsin. The rhodopsin has maxima around 350 and 470 nm while the metarhodopsin has a maximum around 570 nm.

Langer and Thorell (1966) used microspectrophotometry of thick retinal slices of Calliphora eyes to show that R1-6 had absorption maxima around 500 and 350 - 380 nm while the central rhabdomeres had a 470 nm maximum. The original interpretation has been questioned by the author (Langer, 1973).

Hamdorf and Rosner (1973), Ostroy, Wilson and Pak (1974), and Stavenga, Zantema and Kuiper (1973) demonstrated the interconversion of a 470 nm absorbing rhodopsin with a 580 nm absorbing metarhodopsin in the dipteran retina. None of these techniques resolves the receptor function of R7 and R8. The

present study uses the techniques of 'genetic dissection' to clarify dipteran retinal function using mutations which selectively eliminate classes of photoreceptors. Two mutants lacking R1-6, one mutant lacking R7, and double mutants in which R1-6 and R7 are missing were characterized genetically and anatomically. By comparing these 'simplified' mutant retinas with normal eyes, the function of all three receptor classes (R1-6, R7 and R8) can be deduced. The existence of a mutant selectively eliminating R7 allows clear tests of R7 versus R8 function. The results clearly establish that R1-6, R7 and R8 all differ from each other in spectral sensitivity, resident photopigments, and function.

MATERIALS AND METHODS

Mutants. Two visually defective, X-linked recessive mutant stocks, rdgB and sev were isolated at the California Institute of Technology in Seymour Benzer's laboratory by chemical mutagenesis and screening for deficits in phototaxis (Benzer, 1967). The third chromosome mutant ora was isolated by Koenig and Merriam (1975) by chemical mutagenesis and appropriate inbreeding crosses, followed by screening for ERG deficits. Each mutation is in a different gene (cistron) and the mutant is named by giving the cistron name followed by the individual mutant in superscript (the allele designation): receptor degeneration B (first characterized by Hotta & Benzer, 1970), $rdgB^{KS222}$; sevenless, sev^{LY3}; and

outer rhabdomeres absent, ora^{JK84}. In this study the allele designation will be omitted for convenience. The two X-linked mutants rdgB and sev were made white-eyed by recombination with the X-linked mutation white (w), which eliminates eye color screening pigments (Lindsley & Grell, 1968). Independent assortment of chromosomes allowed construction of white-eyed ora flies.

Double mutants rdgB sev and ora;sev were constructed, either by recombination or by assortment of chromosomes. Ommatidial screening pigments were eliminated from these two double mutants by crossing in mutations which eliminate them (w; white, or cn bw; cinnabar brown, see Lindsley & Grell, 1968). Establishment of these multiple mutants was confirmed with pseudopupil inspection and electron microscopy.

Chromosome mapping. The mutants sev and rdgB were mapped on the X-chromosome using standard mapping crosses to multiply-marked X-chromosomes and counting the resulting recombinant progeny. The multiply-marked X-chromosomal stocks used were y cho cv sn^3 f (provided by S. Benzer, California Institute of Technology), v t^2 f (provided by E. B. Lewis, California Institute of Technology) and g^{53d} sd (provided by P. T. Ives, Amherst College). Deficiencies and duplications were also used to localize the mutations more precisely. Dfv^{64}, Dfv^{62-II}, Dfv^{73-IV}, Dfras v^{17Cc18}, and $Dpv^{+}Yy^{+}$ (provided by G. LeFevre, California State University at Northridge) were used to map sev. $Df(1)g^{1}$ (used to map rdgB) and Df(3R)P14,

Df(3R)Dl, Df(3R)bxd^{110}, and T(1,3)05 (used to map ora) were provided by E. B. Lewis (California Institute of Technology).

Mosaic production. The focus of gene action was determined in the case of the X-linked mutants rdgB and sev by making haplo-X diplo-X mosaics (a technique previously used with X-linked visual mutants by Hotta & Benzer, 1970). A marked chromosome containing sev was obtained by crossing sev with a y w stock (yellow body color and white eyes) and collecting recombinant triple mutant males y w sev. The y and cho (chocolate eye color) mutations were crossed onto the chromosome containing rdgB. Males with such marked X-chromosomes were mated with virgin females heterozygous for the unstable ring-X chromosome In(1)w^{vC} (see Lindsley & Grell, 1968), which contains the wild-type alleles for vision, body color, and eye color genes. Approximately 7% of all the progeny of these crosses were gynandromorphs in which mutations for both body and eye color markers were expressed in the hemizygous male tissue but not in the heterozygous female tissue (see Hotta & Benzer, 1970).

Optical neutralization of the cornea. The individual rhabdomeres could be examined directly without sectioning the eye by the technique of optical neutralization of the cornea. The procedure was that of Franceschini and Kirschfeld (1971), except that the head of the fly to be examined was cut off at the neck with a razor blade, then mounted in clear nail polish on a clean glass slide before inspection.

Histology. For light and electron microscopy, heads of flies were cut off, sliced midsagittally, and fixed immediately, following the techniques of Poodry and Schneiderman (1970). This tissue was embedded in epon-raldite mixture. Sections of 1.5 μ for light microscopy, were collected on a glass slide and stained with toluidine blue. Thin sections of about 1200 Å were cut for electron microscopy, picked up on copper grids, and stained with lead citrate (Reynolds, 1963).

Electroretinograms. The ERG techniques used are described by Stark (1975). Spectral sensitivities shown are inverse intensities (in log quantum flux) required to elicit small ERG receptor potentials for 11 wavelengths from 350 to 600 nm. Three criteria were used depending on genetic strain and adaptation conditioning. These were chosen to lie on steep portions of their respective intensity-response curves to minimize errors of measurement. Normal ERG waveforms from dark-adapted cn bw and w sev were sufficiently large so that a 3.0 mV criterion was used. In all other mutants and adaptation conditions a 1.25 or a 0.5 mV criterion was used. Separate experiments on 4 subjects for each abnormal ERG waveform and on 8 dark-adapted cn bw subjects showed that the spectral sensitivity shapes derived using 0.5, 1.25, and 3.0 mV were the same within experimental error. These same experiments were used to normalize all sensitivity curves to a 0.5 mV criterion, (470 nm sensitivity data were used for this

recalibration).

Spectrophotometry. For each experiment, exactly 100 flies were placed in a small glass bottle which was then dipped in liquid nitrogen for 1 min. Vigorous shaking of the bottle decapitated the frozen flies. Nylon mesh filters were used to separate the heads from the bodies. The heads were then homogenized in ~0.6 ml of 0.1 M phosphate buffer pH 7.2, and the homogenate was then placed in a cuvette for spectrophotometry at room temperature.

A dual-wavelength spectrophotometer was used to measure light-induced absorption changes at two adjustable wavelengths in these experiments. This machine was constructed by Dr. Edward Lipson at the California Institute of Technology to study photopigments in Phycomyces. The light source for the measuring wavelengths was a 200 W tungsten halogen bulb (General Electric Model Q6.6A/T4/CL200W) which fed through two Jarrel Ash Model 82-410 monochromators. A beam chopper was used to alternate the two wavelengths passing through the sample. The resulting light signal was detected by a United Detector Technology PIN-25 silicon photodiode. The photodiode current was preamplified and fed into a Princeton Applied Research HR-8 lock-in amplifier. Records were kept on a Bausch & Lomb VOM-5 chart recorder. This assembly was sensitive enough to give accurate readings to about 10^{-4} O.D.

Intense monochromatic light used to convert photopigments was supplied by a 500W Xenon arc lamp (PEK Labs Inc.

Model X-500-A), the beam of which was focused to a diameter of 1.5 cm at the sample location after passing through a 5 mm Schott KG1 heat filter. Monochromatic wavelengths were obtained by placing interference filters, spanning 350 to 650 nm at 20 nm intervals (Balzers Filtraflex B-40), of bandwidths 9-12 nm in the light path. The intensity was adjusted using Wratten neutral density gelatin filters. The intensity of the pigment converting light was measured by a Hewlett-Packard Model 8330A Radiant Flux Meter and a Model 8334A Radiant Flux Detector.

Data were obtained subsequent to converting a maximal amount of photopigment into the rhodopsin or metarhodopsin state. Increasing and decreasing wavelength scans were taken and found not to differ significantly. To establish spectral efficiency functions for interconverting rhodopsin and metarhodopsin of the R1-6 system, responses produced by fixed intensities of various wavelengths were located onto the intensity-response curve produced by either 470 or 590 nm to determine the intensity of 470 or 590 nm which is equally effective as the fixed intensities of the other wavelengths. Action spectrum sensitivity was taken as the ratio of 470 or 590 nm intensity which produced an absorption change equal to that of the fixed intensity variable wavelength to the actual intensity at that wavelength. All these data were taken on the steep part of the intensity-response curve in order to avoid ceiling effects. For the R7 system 370 nm was used to

generate the intensity response curve for rhodopsin to metarhodopsin conversion and 470 nm was used for the reverse conversion.

Phototaxis. The apparatus was a black lucite Y-maze described in Quinn, Harris and Benzer (1974). Entry was from a polystyrene test tube (17 x 100 mm, No. 2017, Falcon Plastics) covered with black masking tape; the arms of the maze were uncovered polystyrene tubes. Illumination was from a 500W Xenon arc lamp placed 1 meter in front of the maze. Balzers interference filters and Wratten neutral density filters were used to adjust the wavelength and intensity of the light. Energy calibrations near the decision point in the maze were made with a Hewlett-Packard Flux Meter and Detector. Flies in a Y-maze were given the choice between a white light (the attenuated output of a Xenon arc) of a fixed intensity or a monochromatic light at an adjustable intensity. The flies were put under intense white light prior to testing to achieve a controlled level of light adaptation. About 40 flies were placed in a start tube at the entrance of the horizontal Y-maze. They were given 30 sec to choose between the two arms after which a sliding door was closed making their decisions irrevocable. The number of flies in each arm was counted. The intensity of the colored light was then adjusted and the experiment repeated. Such adjustments were made until the flies went 50:50 to the two lights. Thus it was possible to find the intensity at several wavelengths

between 350 and 590 nm necessary to produce the same phototactic response. From these data spectral sensitivities were calculated. Spectral sensitivities which were run taking data in both directions (increasing and decreasing λ) gave the same results.

RESULTS

The main results of this paper are outlined in Table 1 and described in detail below.

The genes. Of the three visual mutations used in the present study, two, sev and rdgB, were located on the X-chromosome, and the other, ora, was located on the third chromosome. All three mutations were completely recessive; in heterozygotes for each mutant and the corresponding wild-type allele, no receptors were found missing. Preliminary mapping of sev and rdgB was done against the multiply-marked X-chromosome y cho cv sn^3 f. To determine more precisely the location of sev it was then mapped against the closely flanking markers of the t^2v t chromosome, and checked with various duplications and deficiencies (see Methods). The map position of sev was calculated to be 1-33.2 ± 0.2; the gene is contained in salivary band region 10A2-10A3. rdgB was also mapped more precisely using the markers g^{53d} sd and the deletion Df(1)g^1. rdgB has a map position 1-42.7 ± 0.6 and is contained in salivary band region 12A-12E. The third chromosomal map position for ora was calculated to be

3-65.3 ± 0.4 by Koenig and Merriam (1975). Duplications and deficiencies (see Methods) reveal that ora is in salivary band region 88C-92C but neither in 90C2-91A2·3 nor in 91C7-92A2·3. Scoring for the presence or absence of rhabdomere defects was done by direct examination of the retina using the deep pseudopupil technique described by Franceschini (1972).

Light and Electron Microscopy

Wild-type (normal). In normal Drosophila each ommatidium of the compound eye contains eight photoreceptive structures or rhabdomeres, R1-R8, in which the visual pigments are localized (Langer & Thorell, 1966). The rhabdomeres are arranged in a rigorously determined trapezoidal array. Rhabdomeres R1-6 surround R7 and R8, which are stacked in one column, R7 being distal, R8 proximal (Dietrich, 1909). The trapezoidal pattern is easily seen in a microscope by the technique of optical neutralization of the cornea (see Methods). The rhabdomeres, which act as optic fibers, can be seen as seven bright spots in a trapezoidal array within each ommatidium (Pl. 1). R8 is not visible since it lies under R7. Each rhabdomere is a process of a separate cell body which occupies a wedge-shaped area around the ommatidium center. The cell body of R7 is between the cell bodies of R1 and R6, while the cell body of R8 is between the cell bodies of R1 and R2 (Dietrich, 1909)(Fig. 1).

Electron microscopy reveals that the rhabdomeres are composed of rows of parallel microvilli (Pl. 1). The microvilli of a rhabdomere are always oriented in the same direction as the stalk of the cell body to which the rhabdomere is attached. R7 and R8 thus have orthogonally oriented microvilli. The diameter of the central two rhabdomeres (~0.9 μ), is considerably smaller than the diameter of the outer six rhabdomeres (~1.5 μ).

The photoreceptor cell axons pass through the basement membranes of the eye toward the optic ganglia in a highly ordered array (Braitenberg, 1967). The axons of the R1-6 cells terminate in the first optic ganglion, the lamina. The axons of the R7 and R8 cells, however, pass through the lamina and make their first synapses in the second optic ganglion, the medulla (Trujillo-Cenóz & Melamed, 1966).

sev. This mutant is characterized by the complete absence of R7 rhabdomere in every ommatidium (Pl. 2). Serial sections through the eye, however, reveal that R8 is present (Pl. 6). The cell body of R7 is present although much smaller than normal. The absence of the R7 rhabdomere in newly emerged sev flies and the lack of any degeneration debris suggest that the defect is one of nonformation rather than degeneration.

The trapezoidal arrays in the ommatidia of sev are distorted, probably due to collapsing of the remaining rhabdomeres. It is still possible to identify R1 through R6

unambiguously in most ommatidia. The lamina, the medulla, and the brain in these mutants appear normal at the light microscopic level.

rdgB. On eclosion, these flies have apparently normal photoreceptors, both by light and electron microscopy (Hotta & Benzer, 1970). By the time these flies are about one week old, however (if raised in a normal light-dark cycle at 25°C), R1-6 have completely degenerated. Both rhabdomeres and the cell bodies (Pl. 3) of R1-6 decompose. The mechanism of this degeneration has been investigated (Harris & Stark, in preparation). The rhabdomeres and cell bodies of R7 and R8, however, remain intact in every ommatidium. Even 30 days post-emergence there is no sign of degeneration in R7 or R8.

Light microscopy reveals small holes in the lamina of these flies after degeneration has occurred, but it is unknown whether this is the result of degeneration of the photoreceptor synaptic terminals or transsynaptic degeneration of the second order neurons. The medulla and the brain of rdgB flies look completely normal at the light microscopic level.

ora. This mutant lacks R1-6 rhabdomeres in every ommatidium. The absence of the outer rhabdomeres is due to non-formation rather than degeneration (Koenig & Merriam, 1975); there is no degeneration debris in the eye (Pl. 4). Although the outer rhabdomeres are absent, the cell bodies of these photoreceptors are present, appear normal, and possess axons.

At the very distal end of each of these photoreceptor cells there exists a tiny vestigial tip of a rhabdomere. These tips are ~0.5 μ in diameter and about 4 μ long. As in rdgB, both R7 and R8 seem perfectly normal in ora at the level of both light and electron microscopy (Pl. 4). The lamina, medulla, and brain also appear normal.

sev rdgB. The phenotype of this double mutant is the sum of the two individual mutant defects. R7 is not formed as in sev; and R1-6 degenerate as in rdgB. R8, unaffected by either mutation, is present and apparently normal (Pl. 5).

sev;ora. This double mutant shows nonformation of R7 and R1-6 as though the two mutations acted independently. Only R8 remains in each ommatidium (Pl. 5).

Mosaic Studies

sev. To determine what tissue causes the defect in the sev mutant, gynandromorphs were made by the somatic loss of the unstable ring-X chromosome In(1)w^{vC} in developing females heterozygous for y w sev (see Methods). In the 40 mosaics examined, male eyes always showed the mutant phenotype no matter what the genotype of the rest of the fly, and female eyes never showed the mutant phenotype. In several cases the mosaic dividing line passed through the eye. Two such eyes were serially sectioned for light microscopy. In both of these mosaic eyes the red pigmented ommatidia had R7 and the unpigmented ommatidia lacked R7. On the borderline

between wild-type and mutant regions, several ommatidia were of mixed genotype in that some of the surrounding pigment cells had pigment and others did not. These mixed ommatidia sometimes had R7 and sometimes lacked it. The presence or absence of R7 in these ommatidia was uncorrelated with the genotype of the nearest neighboring pigment cells (Pl. 6), but R7 was always pigmented when present. These studies show that sev is a completely autonomous defect localized in the receptor cells themselves.

rdgB. Hotta and Benzer (1970) showed that rdgB (then called rdgII), was an autonomous defect localized in the eye. Mosaicism within the eye shows that the pigment cells are not involved in the degeneration, since, on a mosaic borderline, some of the receptor cells of an ommatidium may degenerate while others remain intact (Pl. 6). Thus in this mutant also, the defect is one of the receptor cells themselves.

Electroretinograms

All electroretinograms (ERGs) were measured with white-eyed flies. This phenotype has frequently been used to eliminate the absorbing effects of ommatidial screening pigments (see Alawi, Jennings, Grossfield & Pak, 1972; Burkhardt, 1962; Goldsmith, 1965; Stark & Wasserman, 1974). Genetic backgrounds used to produce white eyes are given in Table 1.

ERG waveforms. ERGs of wild-type flies, dark-adapted,

470 nm-adapted and 370 nm-adapted are shown in Fig. 2. In the case of the dark-adapted eye, transient on and off potentials flank a steady corneal-negative potential which lasts for the duration of the stimulus. Adaptation with 370 nm or 470 nm light produces a monophasic ERG without transients. The waveform of sev is similar to wild-type; in all other mutants the dark-adapted ERGs are monphasic, similar to light-adapted wild-type.

The lamina generates the on and off transients whereas the receptors generate the steady potential (see Pak, 1976). The lack of transients in the 470 or 370 nm-adapted wild-type, and sev, and in other mutant flies under all adaptation conditions, implies that under these conditions lamina activity is abolished. Since only R1-6 form synapses in the lamina, these results are consistent with the idea that short wavelength adaptation eliminates R1-6 activity and that the two mutants, rdgB and ora, but not sev delete R1-6.

Spectral Sensitivity Profiles

Wild-type. Fig. 3 (from Stark, 1975) shows the spectral sensitivity of wild-type using the steady component of the ERG. The dark-adapted spectral sensitivity has nearly equal peaks near 350 and 470 nm; this will be called a Type 1 sensitivity profile. Intense 470 nm-adaptation decreased sensitivity at all wavelengths especially near 470 nm resulting in a function with a UV peak (Type 2 profile). Intense 370

nm-adaptation of either the dark-adapted or 470 nm-adapted eye selectively depressed the UV sensitivity peak resulting in a function with a primary peak near 470 nm (Type 3 profile); sensitivity at wavelengths greater than about 450 nm is the same after 370 or 470 nm adaptation. These three spectral sensitivity profiles illustrate the only functionally separable components of the wild-type retina.

sev. Spectral sensitivities for sev in which R7 is missing are shown in Fig. 4. The shape, (Type 1), and absolute location of the dark-adapted sensitivity closely resembles that of dark-adapted wild-type. The sensitivities after 470 or 370 nm adaptation are nearly identical with one another, both in location and shape (Type 3). Intense short wavelength adapted sev sensitivity at 470 nm (similar at all wavelengths) is about 0.8 log units greater than that of 370 nm-adapted wild-type.

rdgB. Although upon eclosion the R1-6 system looks anatomically normal in this mutant (see Microscopy Results), it is already functionally defective if the flies have been raised in normal room light conditions. Fig. 5 shows the dark-adapted and 370 nm-adapted spectral sensitivities of such rdgB flies. The dark-adapted rdgB function closely resembles the 470 nm-adapted wild-type profile (Type 2). Adaptation by 370 nm selectively lowers the UV sensitivity, resulting in a Type 3 function.

ora. ora (like rdgB) is missing R1-6. Dark-adapted

ora has a Type 2 sensitivity profile (Fig. 6). Only 370 nm-adaptation affects the profile significantly resulting in a Type 3 profile (Fig. 6).

sev;ora. The dark-adapted, 470 nm-adapted, and 370 nm-adapted spectral sensitivities of sev;ora in which R1-6 and R7 are missing are shown in Fig. 7. The dark-adapted state has a Type 3 profile. No intense adapting stimuli significantly alters the shape or location of the curve.

sev rdgB. In this double mutant, as in sev;ora above, R1-6 and R7 are functionally eliminated. Dark-adapted sev rdgB also has a Type 3 sensitivity profile (Fig. 8) which cannot be altered by adaptation (data not shown).

These ERG results are consistent with the following model: (1) R1-6 generate the Type 1 profile, and genetic elimination of R1-6 or intense 470 nm-adaptation selectively eliminates the Type 1 profile; (2) R7 generates the UV peak in the Type 2 profile, and genetic elimination of R7 or intense 370 nm-adaptation eliminates this peak; (3) R8 alone generates the Type 3 profile, and no light adaptation can alter this profile.

Spectrophotometry

Recent reports (Hamdorf, Schwemer & Gogala, 1971; Minke, Hochstein & Hillman, 1973a; Goldsmith, 1974), suggest that invertebrate photopigments can occur in two thermostable forms: rhodopsin (R) and metarhodopsin (M). One wavelength, the

rhodopsin absorption maximum ($R_{\lambda max}$) is most effective for converting R to M and for eliciting the receptor generator potential; another wavelength ($M_{\lambda max}$) is most effective for reconverting M to R without generation of a receptor potential.

In this study a dual-wavelength spectrophotometer was used to measure action spectra as well as to interconvert photopigments (see Methods). Eyes were made white with w or cn bw so that eye color pigment absorption would not complicate the photopigment data.

Wild-type. Previous literature (Ostroy et al., 1974) indicated that the predominant photopigment in Drosophila has photointerconvertible 470 nm $R_{\lambda max}$ and 580 nm $M_{\lambda max}$ forms. In the present study the maximum absorbance change of the sample following R to M or M to R photoconversion was about 1.2%. Figure 9 shows an intensity-response function for the conversion of R to M by 470 nm, and for the reverse conversion (M to R) by 590 nm; intensities of these two stimuli were varied using neutral density filters. Figure 10 shows action spectra for R and M photoconversions for wild-type extracts. It is evident that the predominant photopigment system in white-eyed wild-type eyes has an $R_{\lambda max}$ near 470 nm and an $M_{\lambda max}$ near 570 nm; these results are very similar to previous spectrophotometric data from Drosophila (Ostroy et al., 1974) and Calliphora (Stavenga et al., 1973; Hamdorf & Rosner, 1973). The R has a secondary maximum in the ultraviolet. From the intensity-response curve it is possible to calculate

the partial molar extinction coefficients of the rhodopsin and metarhodopsin at 470 and 590 nm, respectively, using the relationship $p = \exp(-\sigma I \Delta t)$ where p is the proportion of photopigment unconverted, σ is the cross section for photoconversion, I is the intensity of the light pulse used, and Δt is the duration of the light pulse. The partial extinction coefficient, E, for photointerconversion can then be determined from σ. In this case: E_{470} (R) is 3.3×10^4 cm^2/mole, E_{590} (M) is 4.3×10^4 cm^2/mole. These values are very similar to that found for the total extinction coefficient of bovine rhodopsin which is 4.06×10^4 cm^2/mole (Wald & Brown, 1953).

sev. The photopigment system of sev samples (Fig. 11) was indistinguishable from that of wild-type. The size of the maximum light-induced absorbance change indicated that sev had approximately the same amount of 470 nm R-570 nm M as wild-type.

ora. Eliminating R1-6 with ora eliminates the 470 nm R-570 nm M photopigment system. Actinic light of either of these two wavelengths did not change the absorbance of the sample significantly at any wavelength. UV light, however, was found to increase absorbance at 470 nm. Thus, by eliminating the 470 nm R-570 nm M system, the R1-6 deficiency reveals a minor photopigment with a 370 nm R and 470 nm M which must have been masked in wild-type eyes. From the size of the maximum induced absorption change (~0.04%), using 370

nm and 470 nm as measuring lights, it was estimated that this new photopigment is 30 times less abundant than the R1-6 photopigment in normal eyes. Since this estimate is based on small relative absorption changes, it is tentative. The action spectrum of the photointerconversion of this new pigment is shown in Fig. 12. The most efficient wavelengths are 370 nm for the R to M reaction, and 470 nm for the reverse reaction. The partial extinction coefficients for photoconversion were calculated from the intensity-response curve for this photopigment (Fig. 13). E_{370} (R) is 1.0×10^4 cm^2/mole; E_{470} (M) is 1.8×10^4 cm^2/mole.

sev;ora. Elimination of R7 by sev (in addition to the removal of R1-6 by ora) eliminates the 370 nm R-470 nm M photopigment. In fact, no matter what wavelengths between 370 and 600 nm were used as the measuring light, there was no detectable change in absorption in these samples. If R8 had the same photopigment as R7, eliminating R7 should only halve the amount since R8 is approximately the same size as R7; this much or even 10 times less 370 nm R would have been detected. Since our spectrophotometric method depends on light-induced absorption change, no estimate of the R8 photopigment absorption spectra could be derived.

The presence of considerable debris and degenerating material in the eyes of rdgB mutants made them less desirable for photopigment analysis than ora which produces a clearer lesion. Therefore only data from ora and sev;ora (not rdgB

and sev rdgB) are presented.

The picture which emerges from these spectrophotometric studies is that R1-6 contain a 470 nm R-570 nm M photopigment system, R7 has a second 370 nm R-470 nm M system, and R8 a third photopigment system in which multiple forms are not resolvable by light-induced absorption changes.

Phototaxis

To investigate the roles which the different photoreceptors play in the phototactic behavior of Drosophila, phototactic spectral sensitivities for white-eyed wild-type and white-eyed visual mutants were obtained. These are shown in Fig. 14. The mutant ora does not show phototaxis, a paradoxical finding since rdgB which lacks the same photoreceptor system is phototactic. One hypothesis which accounts for this is that the ora mutation causes secondary defects in the optic ganglia whereas rdgB does not. Other explanations are possible but the truth is simply not known. For this reason ora and sev;ora could not be used in the behavioral study.

To find the proper relative placements of phototaxis spectra, the absolute threshold intensity of phototaxis using 470 nm light was determined. This was done using the visual Y-maze described in Methods, with one of the arms black and the other with 470 nm light of variable intensity. Below threshold the flies ran 50:50 to the two arms; above

threshold they mostly ran to the 470 nm arm. The error in our threshold measurements is high, roughly 0.6 log units. The 470 nm threshold values measured for wild-type and sev were the same; rdgB and sev rdgB were about one log unit less sensitive. The intensity of white light chosen as a reference in the spectral sensitivity measurements was 1.6 log units above the threshold for phototaxis.

As Fig. 14 shows, the wild-type phototaxis curve peaks in the UV and the blue; the phototaxis of sev is much reduced in the UV, and the spectra of rdgB and sev rdgB are very similar to each other and resemble the ERG spectrum of R8.

DISCUSSION

Three single-gene mutations were found which eliminate specific photoreceptors in the compound eye of Drosophila. The mutation ora prevents formation of R1-6 rhabdomeres while rdgB causes their degeneration. R7 rhabdomeres are absent in sev flies. Mosaic analysis shows that the defects in sev and rdgB are autonomous to the receptor cells themselves. Mouse hereditary retinal degeneration (in the rd mutant), appears also to be autonomous to the receptor cells (LaVail & Mullen, 1974).

Electroretinogram measurements show that wild-type flies have three spectral sensitivity profiles: (1) dark-adapted with sensitivity peaks near 350 and 470 nm (Type 1); (2) 470 nm-adapted with a primary UV sensitivity (Type 2); and (3)

370 nm-adapted, similar to 470 nm-adapted at long wavelengths, lower at UV wavelengths (Type 3). Wild-type and sev flies in which R1-6 are present, have a Type 1 profile, while all mutants in which R1-6 are absent do not. Therefore, R1-6 must mediate the dark-adapted Type 1 sensitivity of wild-type flies. Furthermore, since 470 nm adaptation produces sensitivity profiles similar to mutants which lack R1-6, this wavelength must inactivate R1-6 phototransduction. In addition, the 470 nm rhodopsin 570 nm metarhodopsin system found by dual-wavelength spectrophotometry in flies which have R1-6 but not in mutants which lack R1-6 indicates that this photopigment system is localized in R1-6. Thus R1-6 inactivation results from changing the 470 nm rhodopsin to the 570 nm metarhodopsin. The finding that 570 nm is the most effective wavelength for reactivating R1-6 (Pak & Liddington, 1974; Stark, 1975) confirms this interpretation. The ERG results also confirm the laminar origin of the on and off transients. Only R1-6 have synapses in the lamina, and the transients are lacking when R1-6 are missing or inactivated.

The UV-sensitivity peak (Type 2 profile) in all flies where R7 and R8 are present and functioning (470 nm-adapted wild-type, dark-adapted rdgB and ora) and its absence in all flies where R7 is missing (even if R8 is present) (470 nm-adapted-sev, dark-adapted sev;ora and sev rdgB) indicate that R7 is the UV-photoreceptor. A UV-sensitive rhodopsin was present in flies with R7 and R8 but not in flies with only

R8, a finding which proves that this photopigment, which photointerconverts with a 470 nm metarhodopsin, resides in R7. The finding that this UV sensitivity peak can be suppressed by 370 nm adaptation indicates that 370 nm inactivates R7, while 470 nm reactivates it (Stark, unpublished).

The spectral sensitivities of 470 nm-adapted wild-type and dark-adapted rdgB and ora are all similar and represent three different measurements of the combined R7 and R8 spectral sensitivity. These results agree with those of Minke et al. (1975a).

The spectral sensitivities of flies with only R8 intact (sev rdgB and sev;ora) indicate clearly that the Type 3 sensitivity curve they possess is mediated by R8. (Type 3 profiles also result from 470 nm-adapted sev and 370 nm-adapted wild-type, rdgB, and ora.) The compound mutants sev rdgB and sev;ora showed no sensitivity alterations by intense 370, 470, or 570 nm adaptation. Furthermore, spectrophotometric measurements showed no light-induced absorption changes in sev;ora samples. Therefore, if R8 rhodopsin has a stable metarhodopsin it is likely that the R and M absorption spectra are indistinguishable. An alternative explanation is that R8 metarhodopsin is extremely unstable and very quickly reverted to rhodopsin.

To summarize the ERG and spectrophotometric results: (1) R1-6 receptors have 350 and 470 nm sensitivity peaks and a 470 nm rhodopsin-570 nm metarhodopsin; (2) R7 is an

ultraviolet receptor with a 370 nm rhodopsin-470 nm meta-rhodopsin; (3) R8 is a nonadapting blue receptor with a third photopigment. The only inconsistency in these results lies in the higher ERG sensitivities of mutants relative to the corresponding spectral components in white-eyed wild-type. We do not feel that this is important since: (1) there is considerable error in estimating absolute sensitivity; (2) genetically eliminating receptors may change electrical resistance in the retina; and (3) the mutations may alter the optics in the compound eye.

The suppression of UV-phototaxis in sev relative to wild-type indicates that R7 plays an important role in phototaxis. The high threshold for phototaxis in rdgB and sev rdgB relative to wild-type and sev is consistent with the results arrived at by optomotor experiments: that R1-6 are a high-sensitivity system while R7 and R8 form a low-sensitivity system (Kirschfeld, 1969; Eckert, 1971). The phototactic spectral sensitivities of rdgB and sev rdgB are similar to each other and to the ERG spectrum of R8. Thus it may be that for R7 to operate in phototaxis R1-6 must be functioning. An alternative is that rdgB disrupts the input of R7 without affecting its ability to produce a receptor potential. These results also show that R8 alone is sufficient for phototactic behavior.

In this study we have separated the contributions of anatomically different classes of receptors in the compound

eye of Drosophila using mutants (and combinations of mutants) in which one or more classes were missing. Each of the three classes has a different sensitivity spectrum, a different photopigment, and probably plays a distinct role in behavior.

TABLE 1

SUMMARY OF RESULTS

Genetic Strain	Rhabdomes present	Allele used	Genes used to eliminate screening pigments	Map position	Primary focus	Dark-Adapted spectral sensitivity	470 nm-adapted spectral sensitivity	370 nm-photo-spectral sensitivity	Major photo-pigment absorption changes	Phototaxis spectrum peaks
wild-type	1-6,7,8	a	w or cn bw	a	a	Type 1	Type 2	Type 3	470R-570M	High sensitivity, UV & blue
sev	1-6,8	LY3	w	1-33.2, 10A2-10A3	Photo-receptor cells	Type 1	Type 3	Type 3	470R-570M	High sensitivity, blue
rdgB	7,8	KS222	w	1-42.7 12A-12E	Photo-receptor	Type 2	Type 2	Type 3	c	Low sensitivity, blue
ora	7,8	JK84	w	3-65.3, 88C-92C	b	Type 2	Type 2	Type 3	370R-470M	c
sev rdgB	8	a	cn bw	a	a	Type 3	Type 3	Type 3	c	Low sensitivity, blue
sev;ora	8	a	w	a	a	Type 3	Type 3	Type 3	None	c

a = not relevant; b = not done; c = see discussion in text.

FIGURE LEGENDS

Fig. 1. Schematic of ommatidia of normal and mutant Drosophila eyes. CL: corneal lens; CC: cone cells; 1°,2° and 3° PC: primary, secondary and tertiary pigment cells; R: rhabdomere; RC: receptor cell; BC: bristle cell. At right, diagrammatic representation of mutants; filled circles indicate photoreceptors present, dotted lines show missing photoreceptors. These diagrams are used in subsequent figures to identify the mutant type.

Fig. 2. Typical ERGs of dark-adapted (top), 470 nm-adapted (middle), and 370 nm-adapted (bottom) wild-type. Bottom trace of each pair is 470 nm stimulus, top trace is response. Corneal negative is shown downward.

Fig. 3. Spectral sensitivity of wild-type dark-adapted (circles), 470 nm-adapted (triangles), 370 nm-adapted (squares) (n=8). Each curve plots the sensitivity inversely in log (quanta/cm^2-sec) needed to elicit a 0.5 mV criterion ERG receptor potential (see Methods). Standard errors are shown only when larger than data points after each curve for each fly had been normalized to the mean value to eliminate absolute differences between subjects.

Fig. 4. Spectral sensitivity of sev. Dark-adapted (circles), 470 nm-adapted (triangles), and 370 nm-adapted (squares) sensitivities of sev (n=4). These and all following ERG spectral sensitivity functions have been plotted in the same manner as Fig. 3.

Fig. 5. Spectral sensitivity of rdgB. Dark-adapted (circles) and 370 nm-adapted (squares), (N=4).

Fig. 6. Spectral sensitivity of ora. Dark-adapted (circles), 470 nm-adapted (triangles), and 370 nm-adapted (squares), (n=2). Six other subjects run dark-adapted and 4 run 570 nm adapted, all gave Type 2 spectral sensitivity profiles.

Fig. 7. Spectral sensitivity of sev;ora. Dark-adapted (circles), 470 nm-adapted (triangles), and 370 nm-adapted (squares), (n=2). Six other subjects run dark-adapted and 4 run 570 nm adapted, all gave Type 3 profiles.

Fig. 8. Spectral sensitivity of sev rdgB. Dark-adapted, (n=4).

Fig. 9. Intensity-response curve for R1-6 rhodopsin and metarhodopsin in wild-type. A response of 1.0 indicates maximum obtainable photointerconversion. Circles indicate 470 nm R → 570 nm M produced by a 470 nm actinic light. Triangles indicate M ⟶ R produced by 590 nm light.

Duration of the pulse was 5 sec.

Fig. 10. Action spectra of predominant rhodopsin and metarhodopsin in wild-type. Maximal sensitivities have been normalized to 100. Circles indicate spectral sensitivity of R → M interconversion; triangles indicate spectral sensitivity of M → R interconversion.

Fig. 11. Action spectra of predominant photopigment in sev. Plotted as in Fig. 10, circles indicate R → M, triangles M → R.

Fig. 12. Action spectra of photopigment found in ora. Plotted as in Fig. 10, triangles indicate R → M interconversion, squares M → R.

Fig. 13. Intensity-response curve for R7 rhodopsin and metarhodopsin. Plotted as in Fig. 9, triangles indicate 370 nm R → 470 nm M produced by a 370 nm bleaching light, and squares indicate 470 nm M → 370 nm R produced by a 470 nm bleaching light. Duration of the light pulse was 10 sec.

Fig. 14. Spectral sensitivity of phototaxis for wild-type (circles), sev (triangles), rdgB (squares), and sev rdgB (hexagons).

Fig. 1

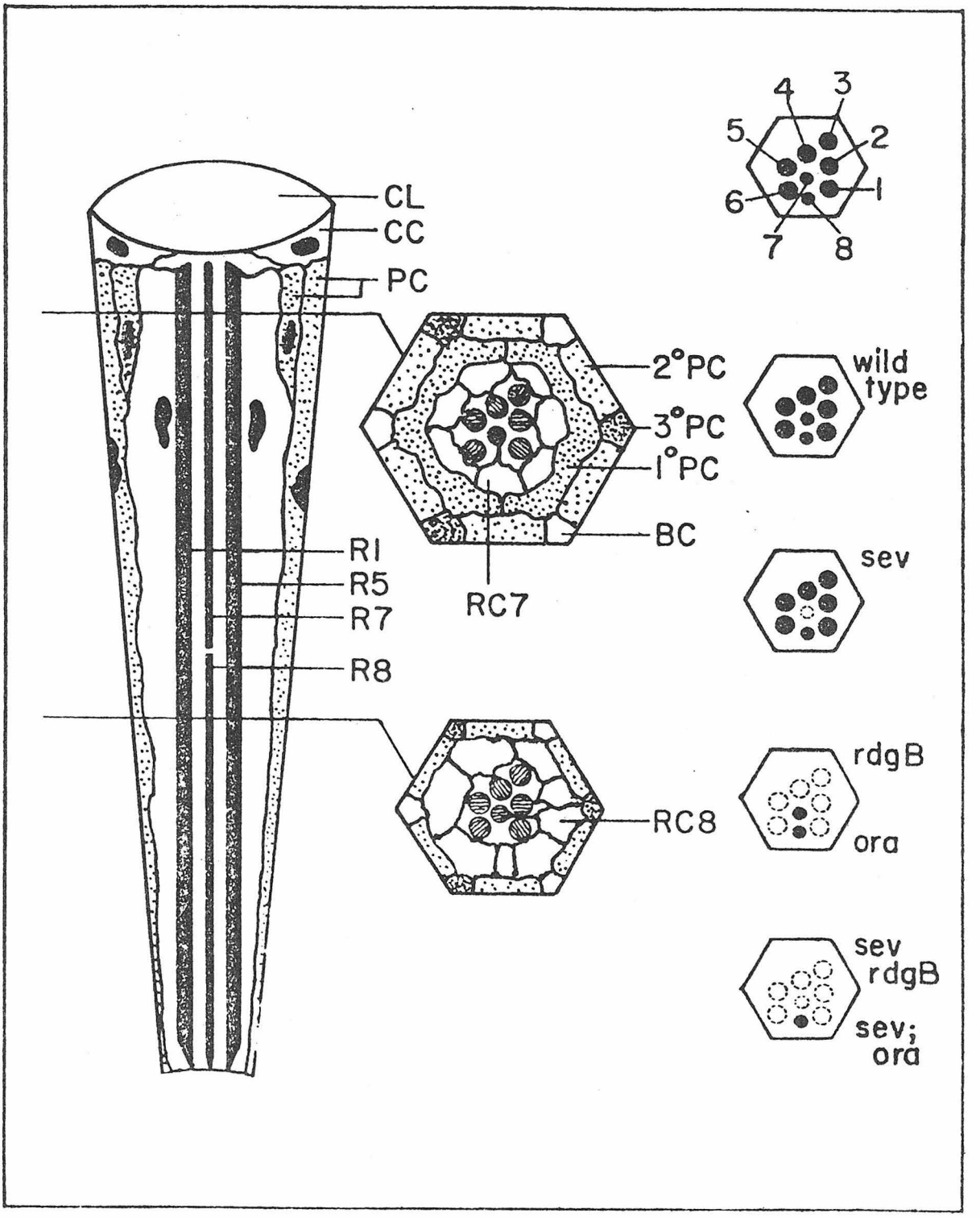

4
3
5
2
6
1
7
8
CL
CC
PC
2°PC
3°PC
1°PC
BC
RC7
R1
R5
R7
R8
RC8
wild type
sev
rdgB
ora
sev
rdgB
sev;
ora

FIG. 2

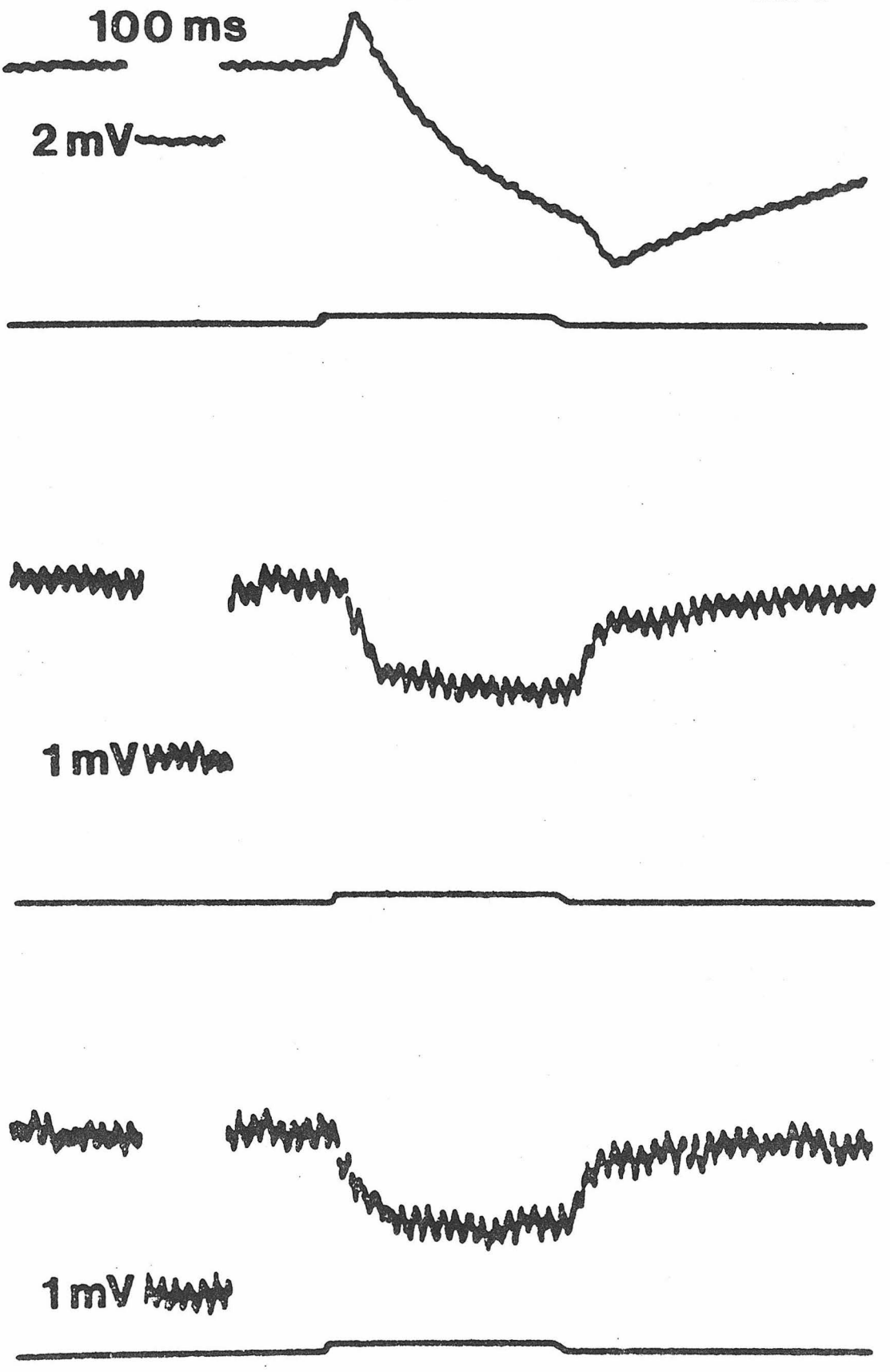


Fig. 3

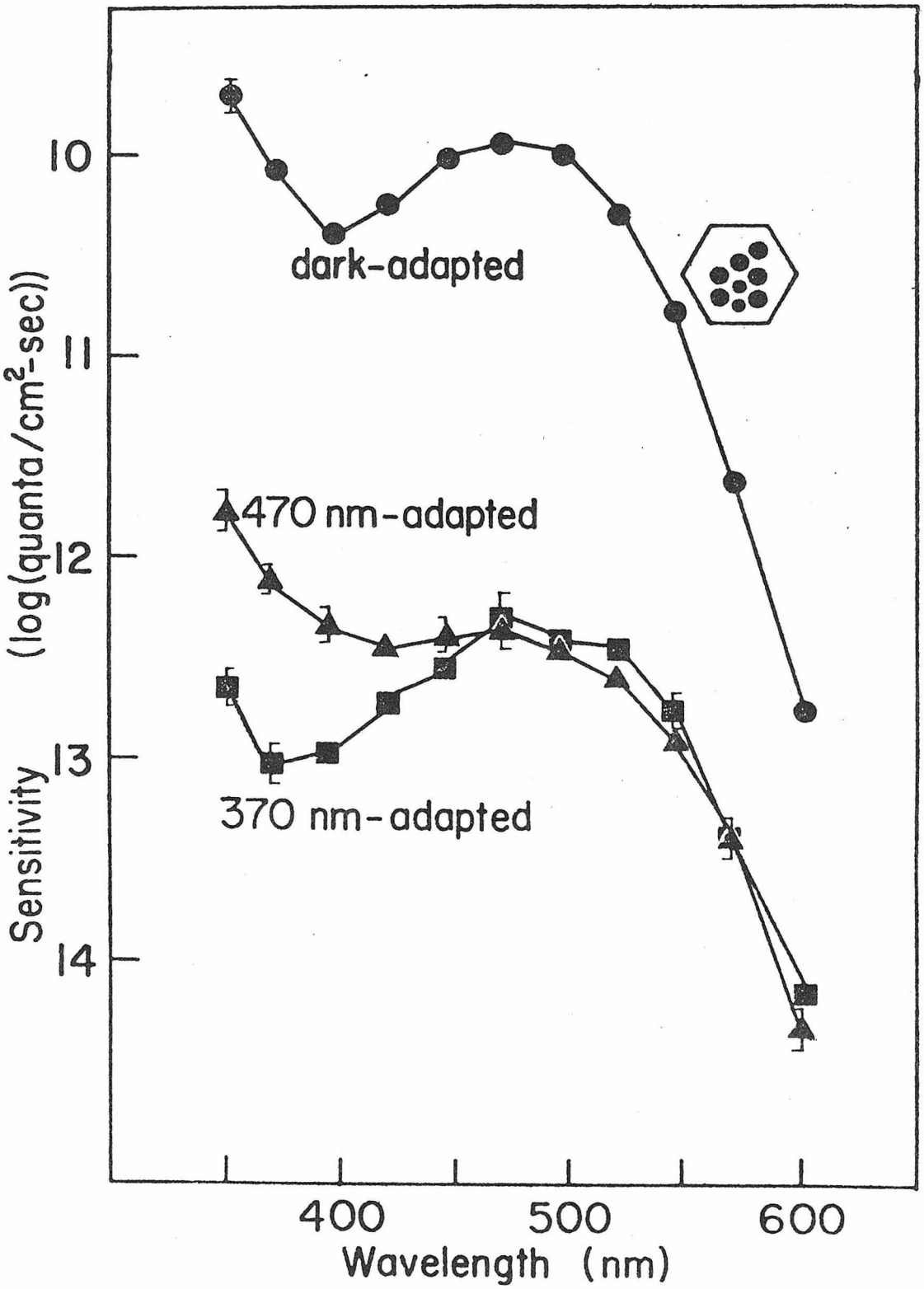
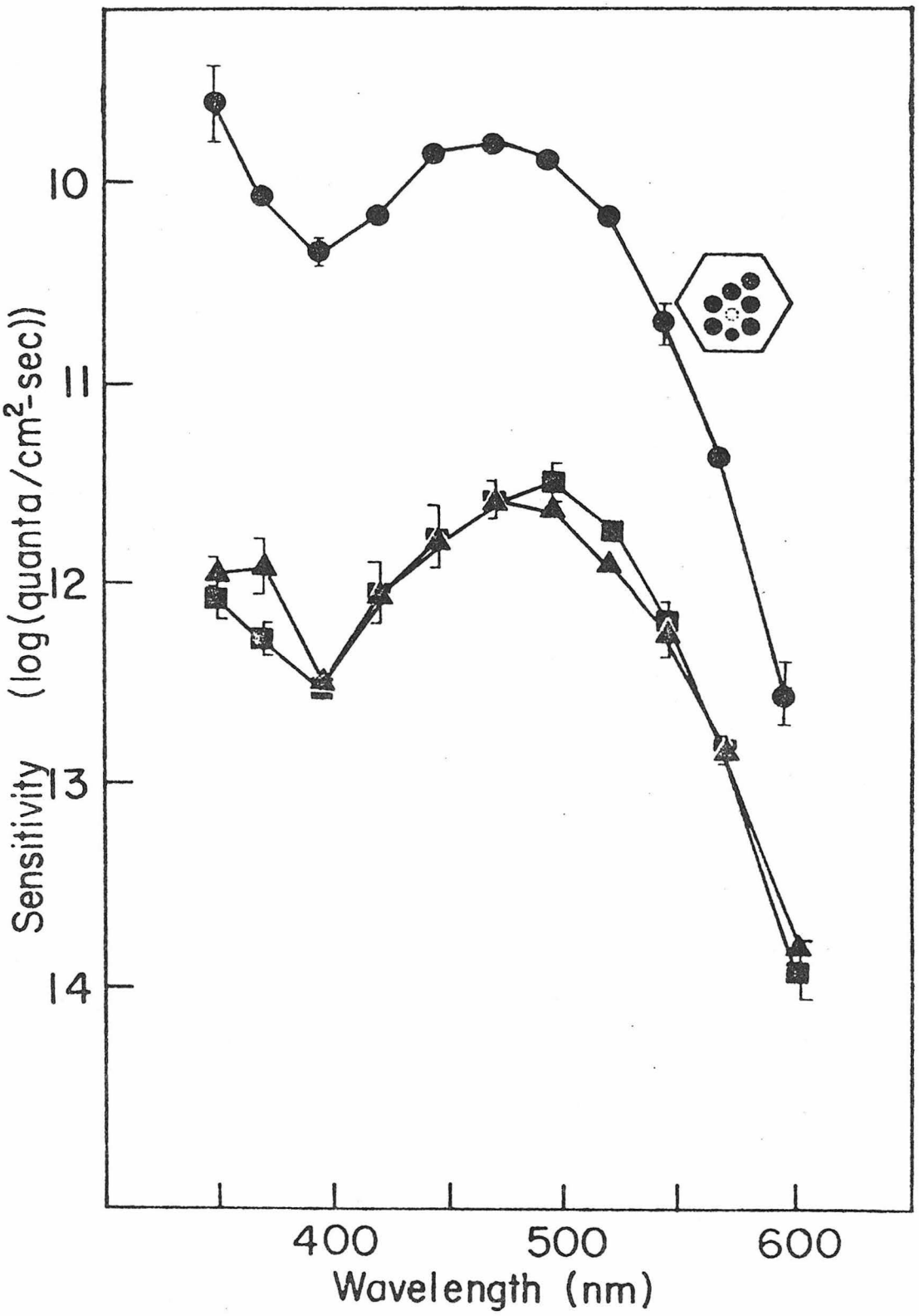

10
11
12
13
14
Sensitivity (log(quanta/cm²-sec))
dark-adapted
470 nm-adapted
370 nm-adapted
400
500
600
Wavelength (nm)

Fig. 4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11
12
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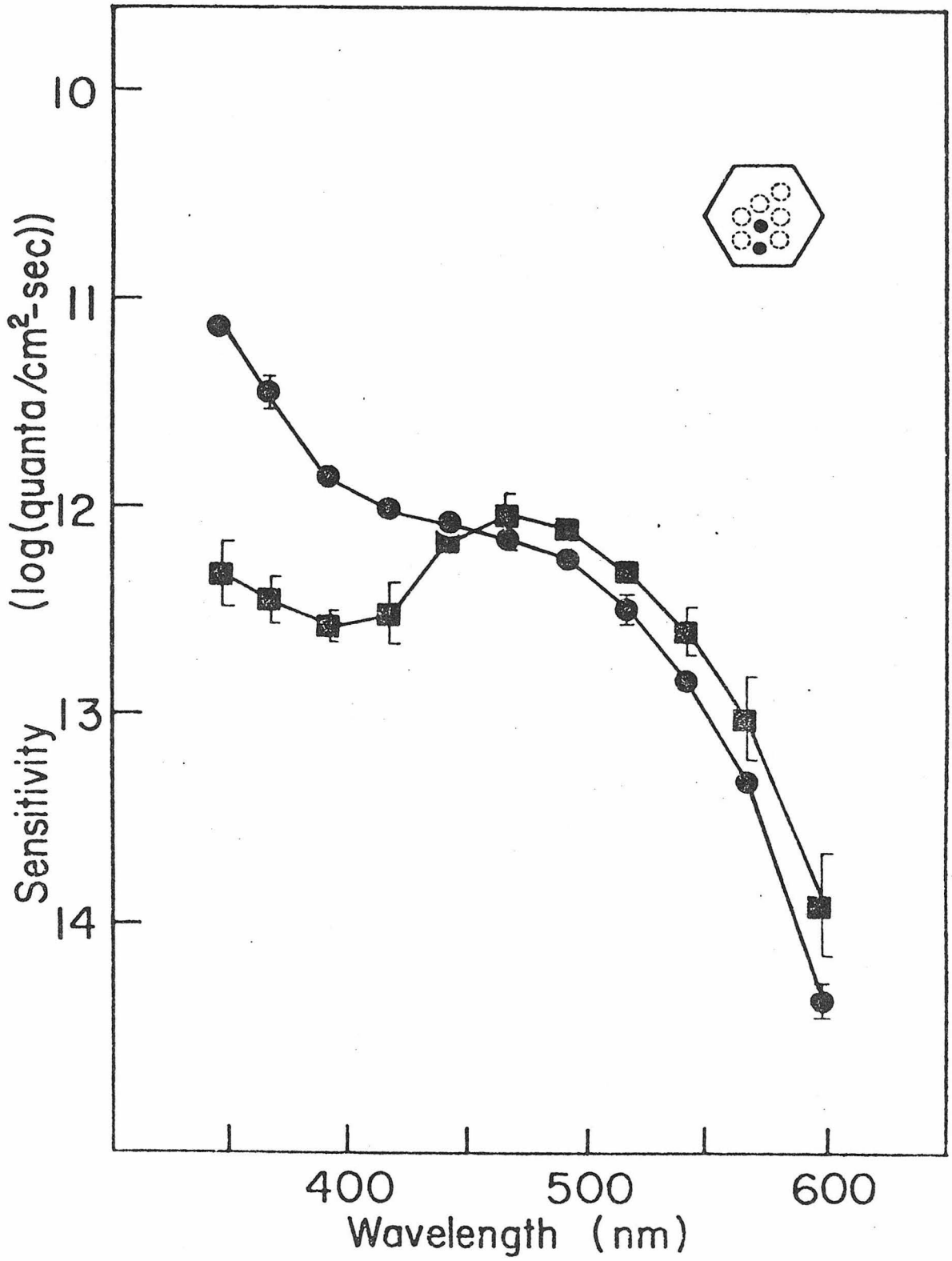
14
Sensitivity (log(quanta/cm²-sec))
400
500
600
Wavelength (nm)

Fig. 5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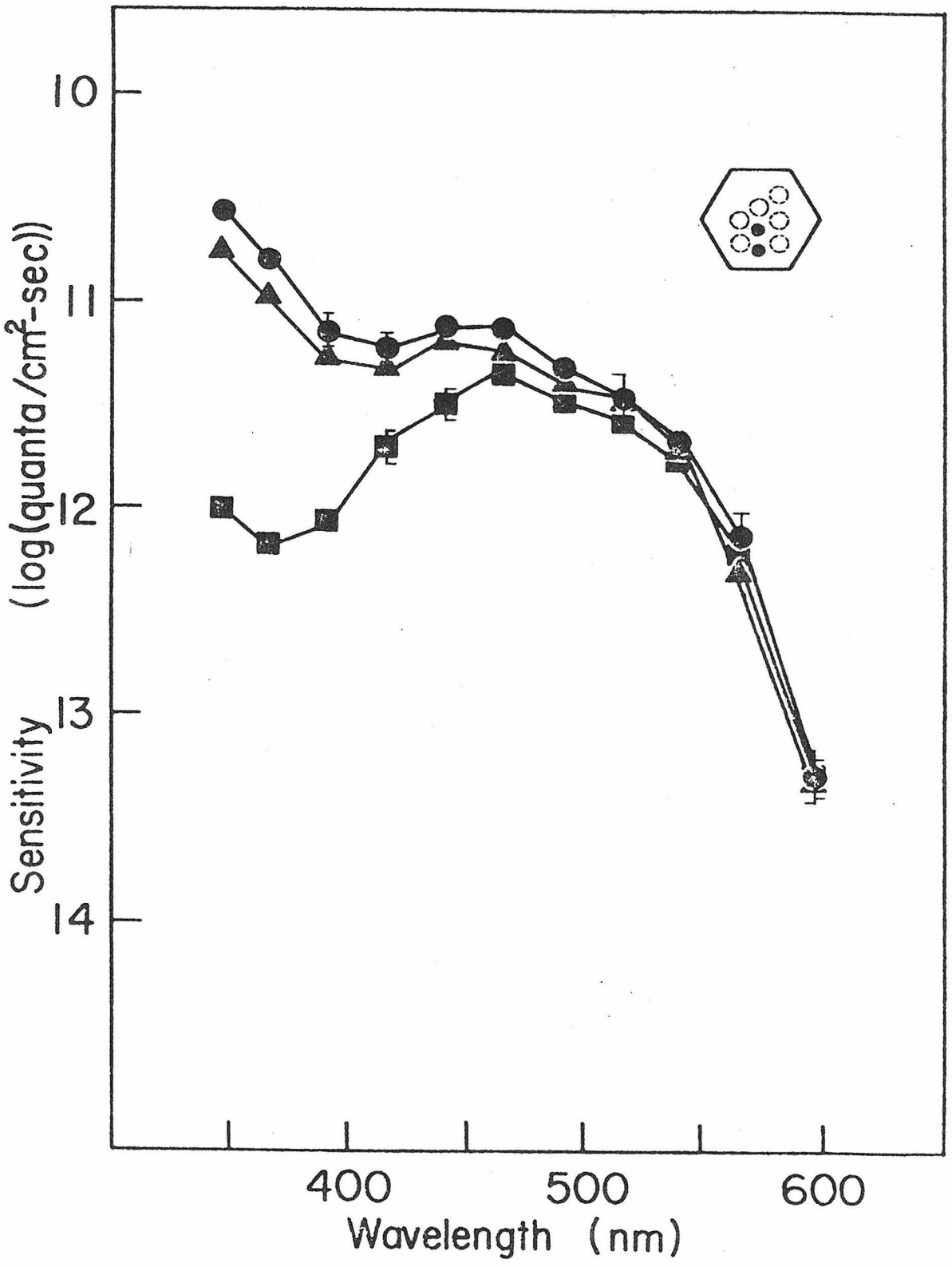
11
12
13
14
Sensitivity (log(quanta/cm²-sec))
400
500
600
Wavelength (nm)

Fig. 6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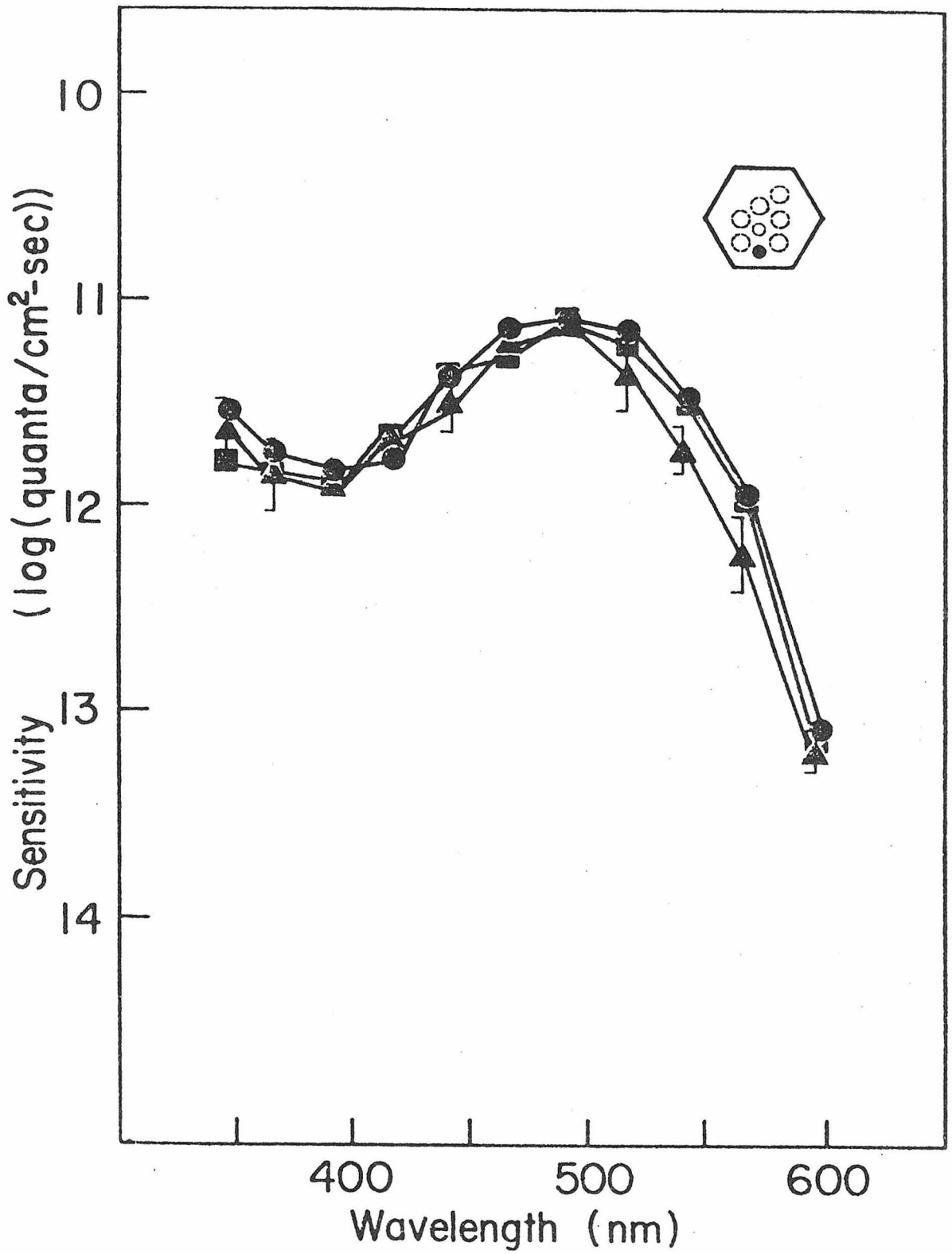
14
Sensitivity (log(quanta/cm²-sec))
400
500
600
Wavelength (nm)

Fig. 7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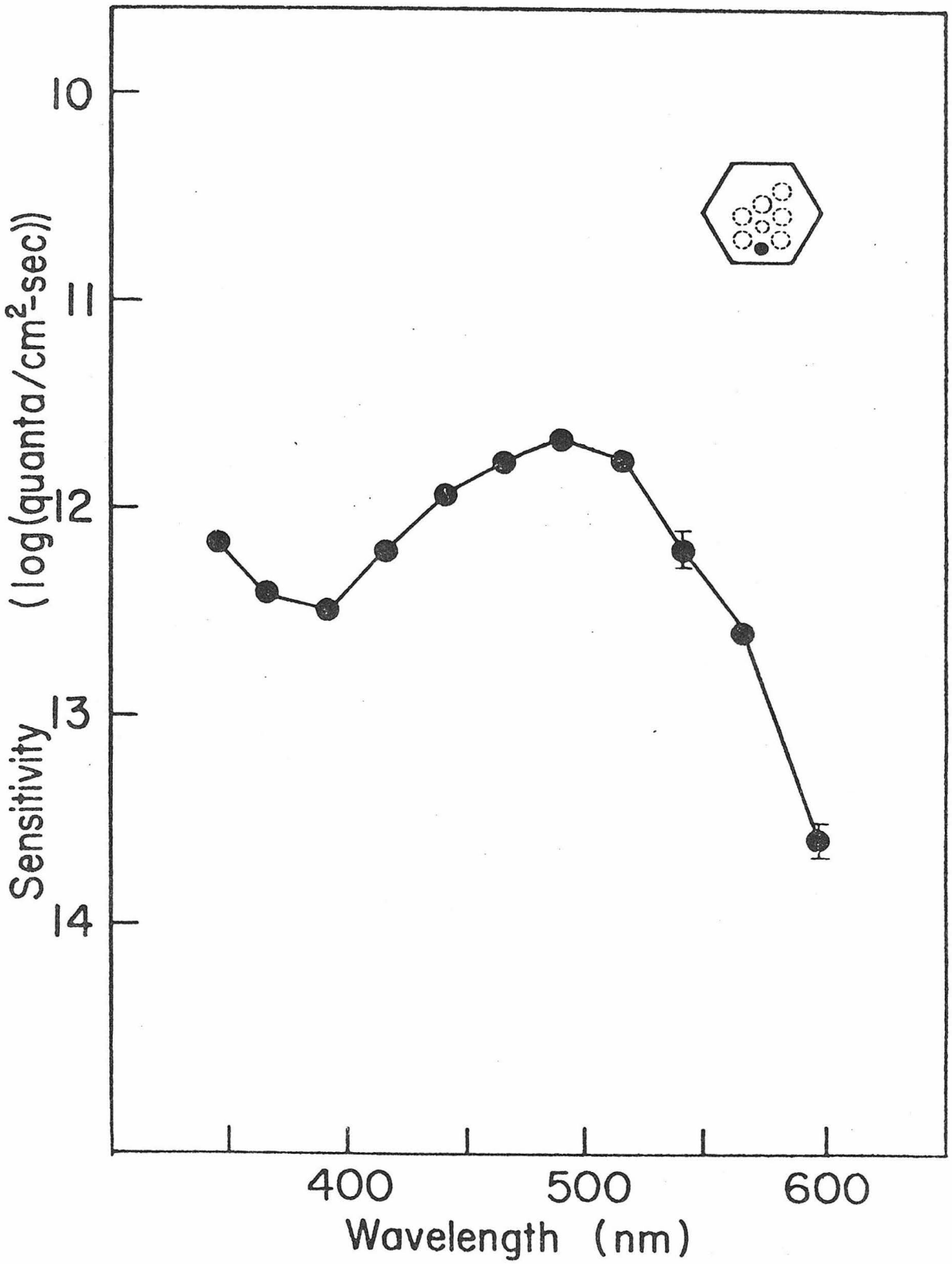
11
12
13
14
Sensitivity (log(quanta/cm²-sec))
400
500
600
Wavelength (nm)

Fig. 8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11
12
13
14
Sensitivity (log(quanta/cm²-sec))
400
500
600
Wavelength (nm)

Fig. 9

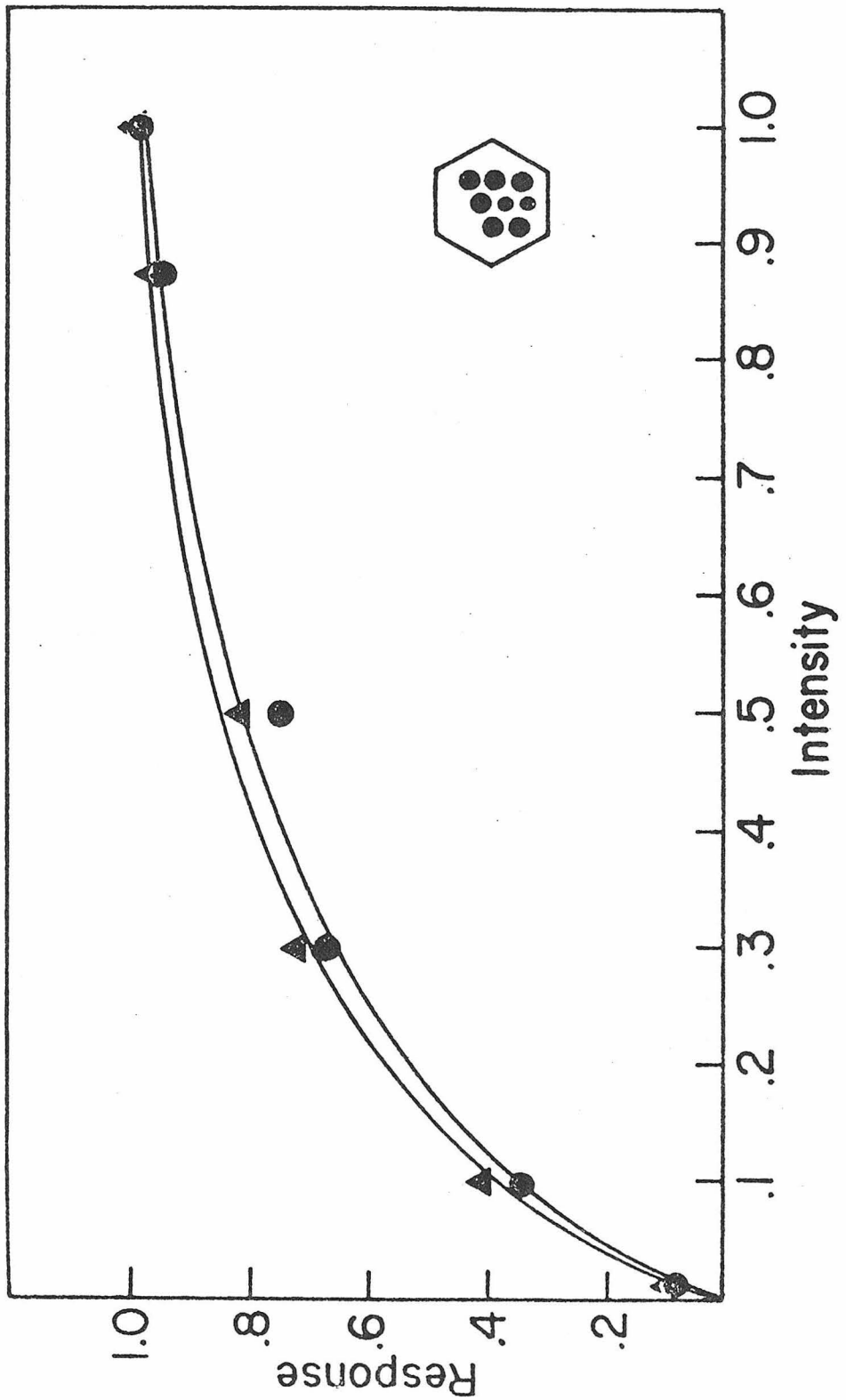


Fig. 10

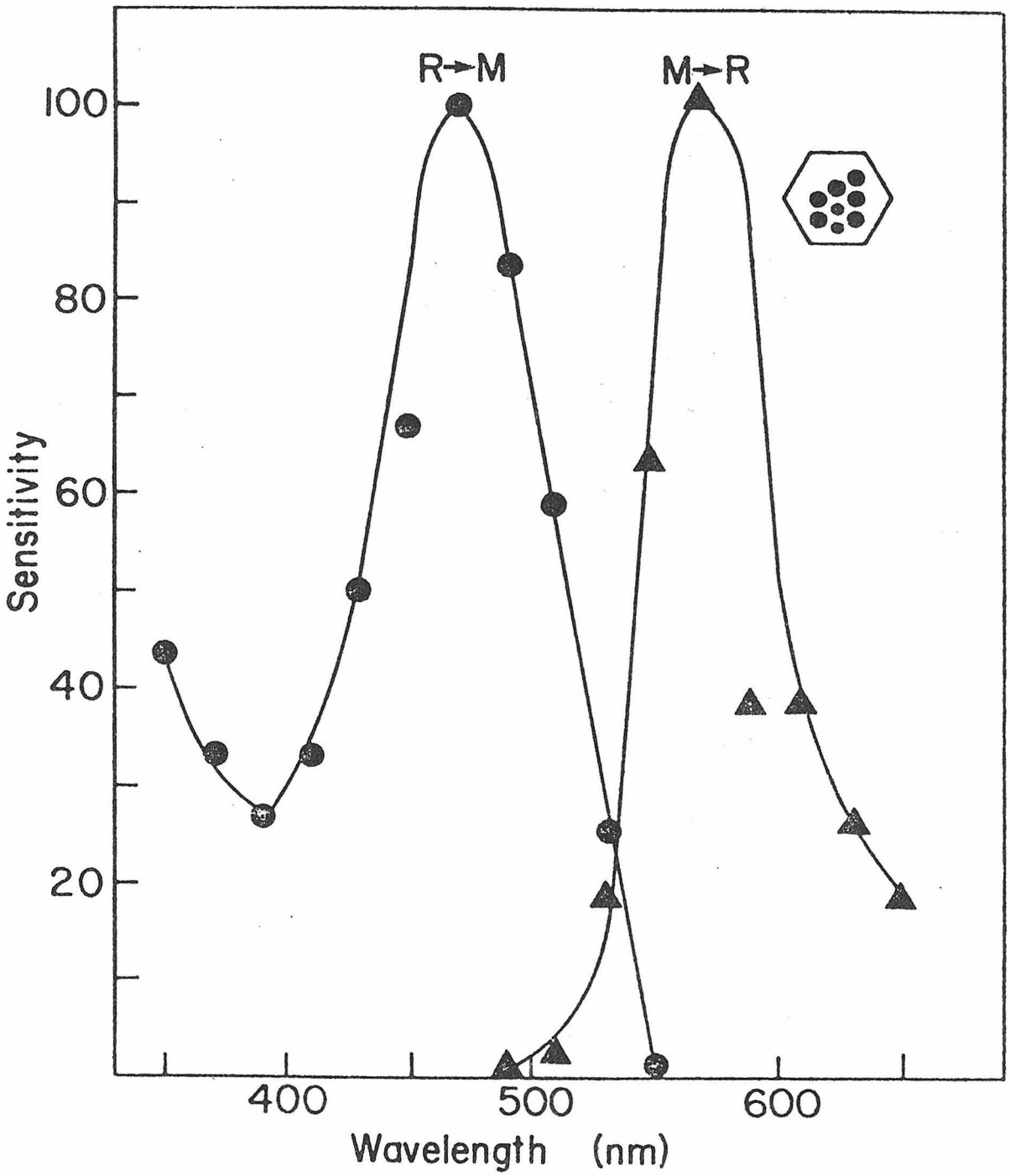


Fig. 11

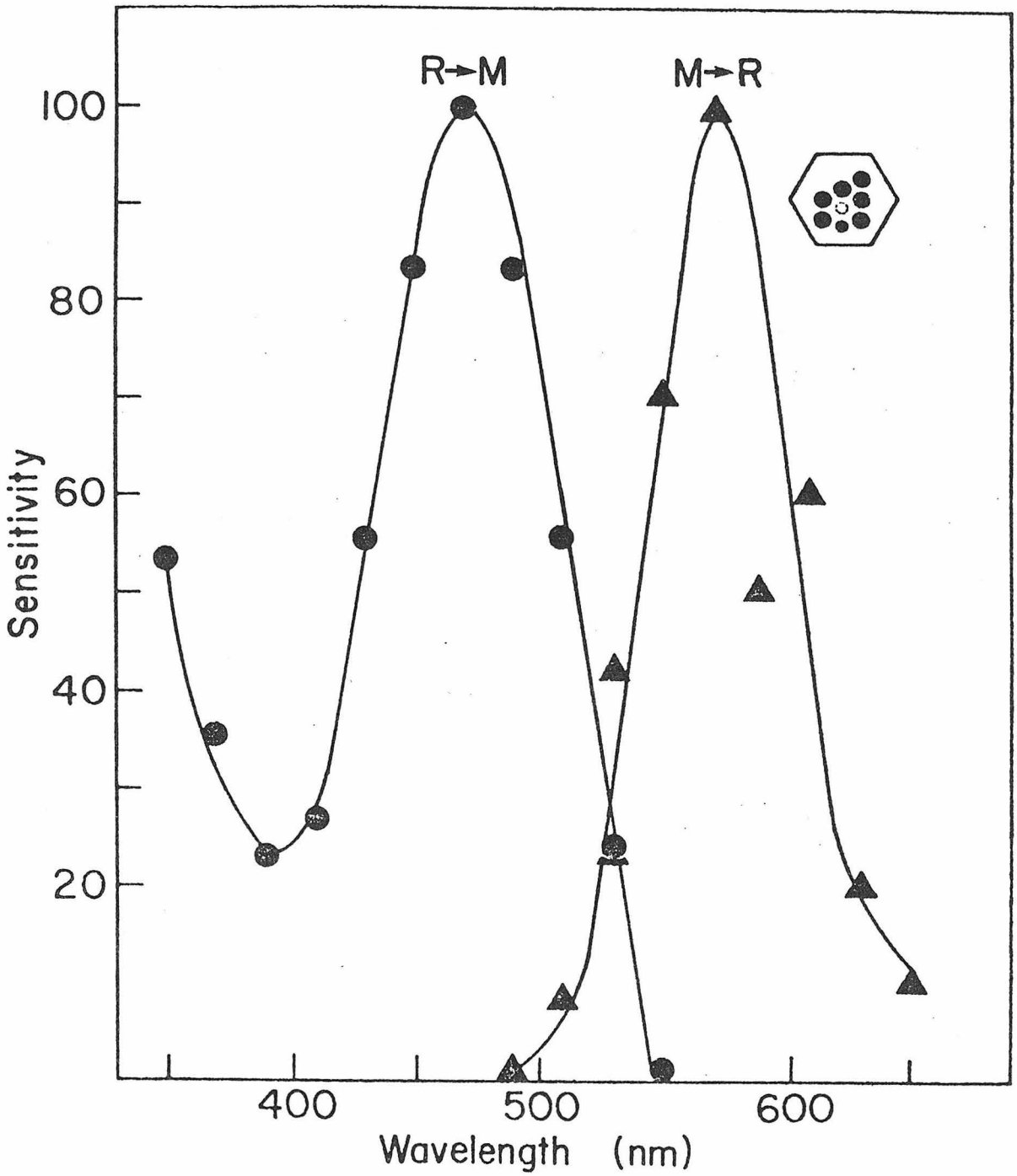


Fig. 12

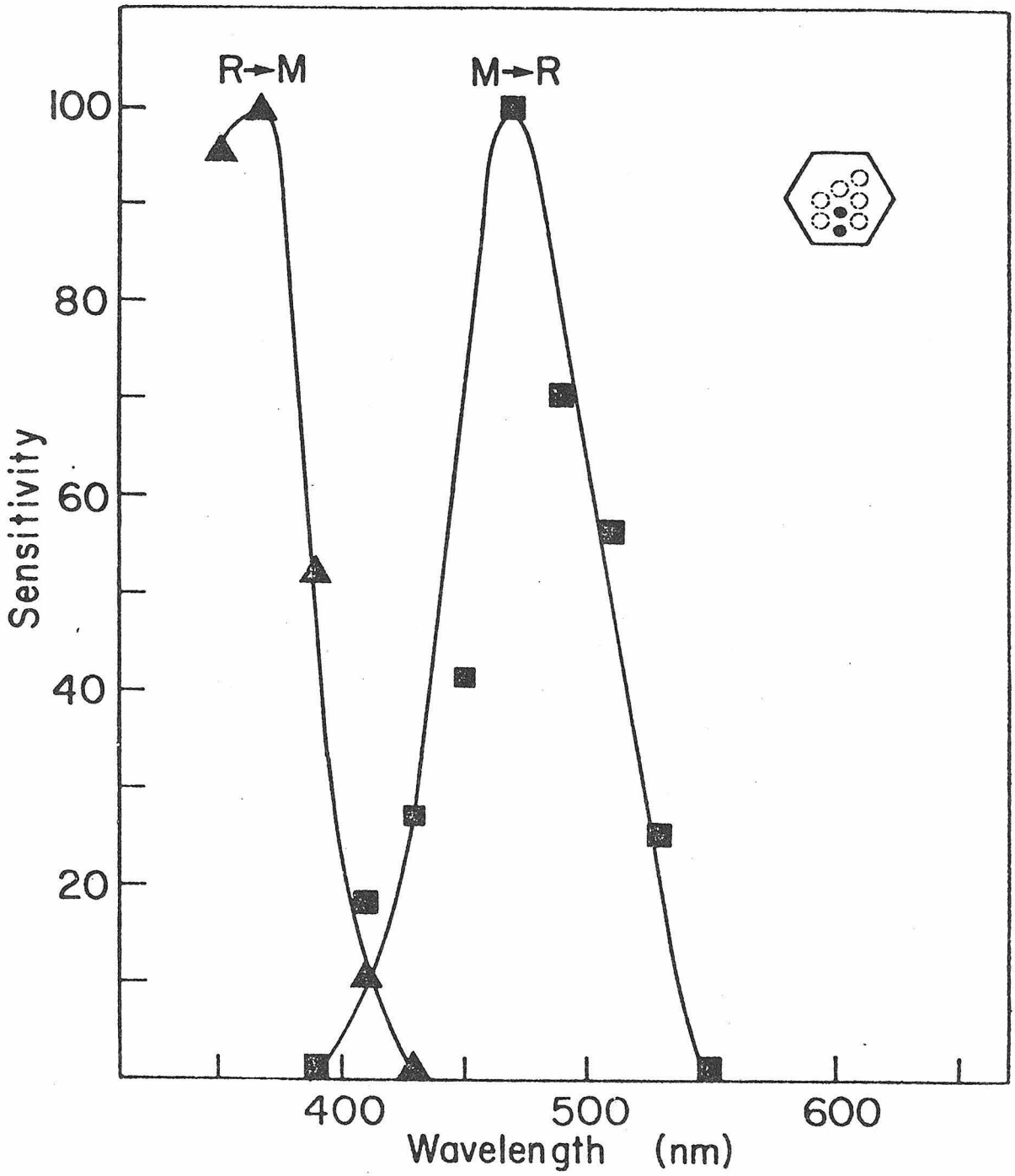


Fig. 13

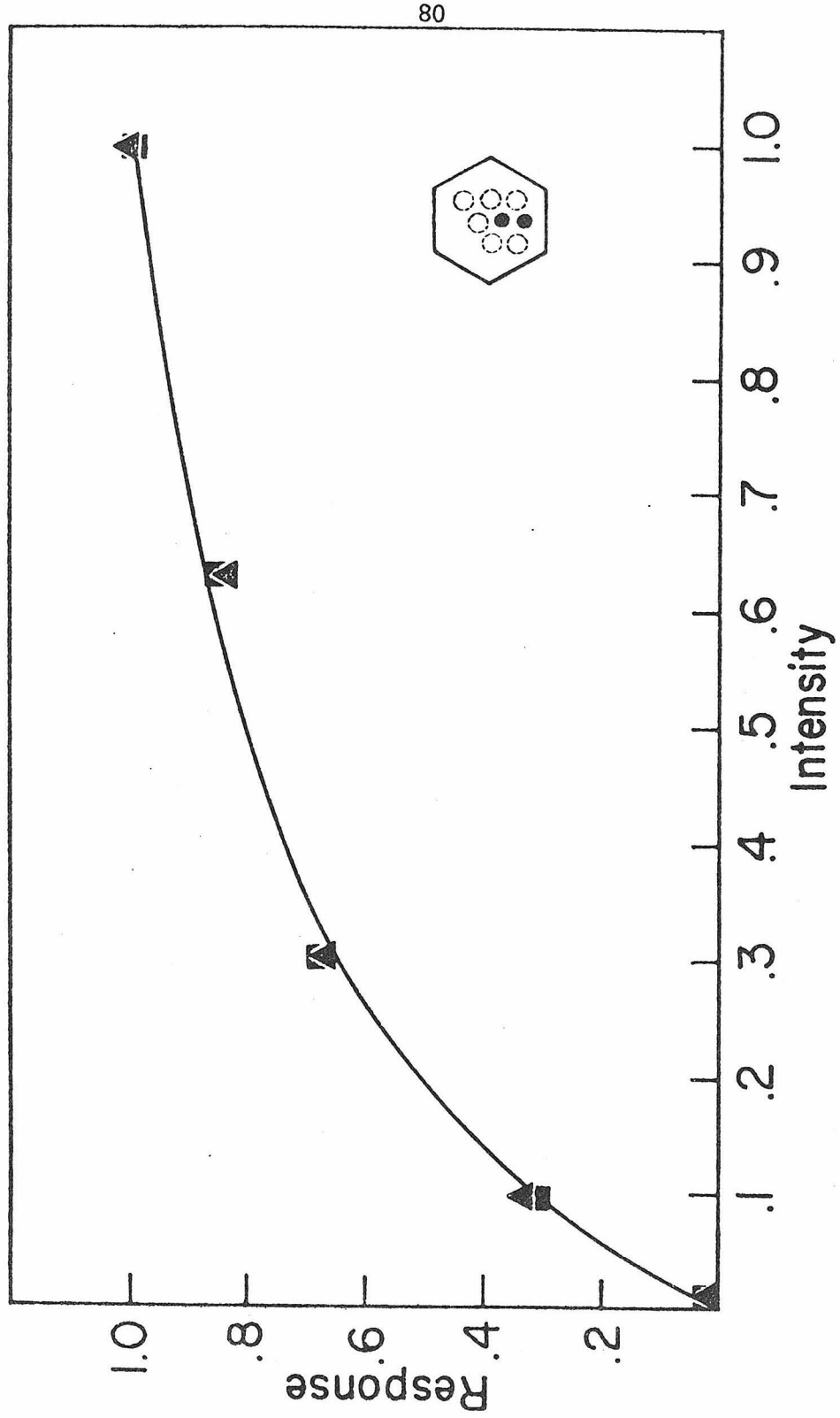


Fig. 14

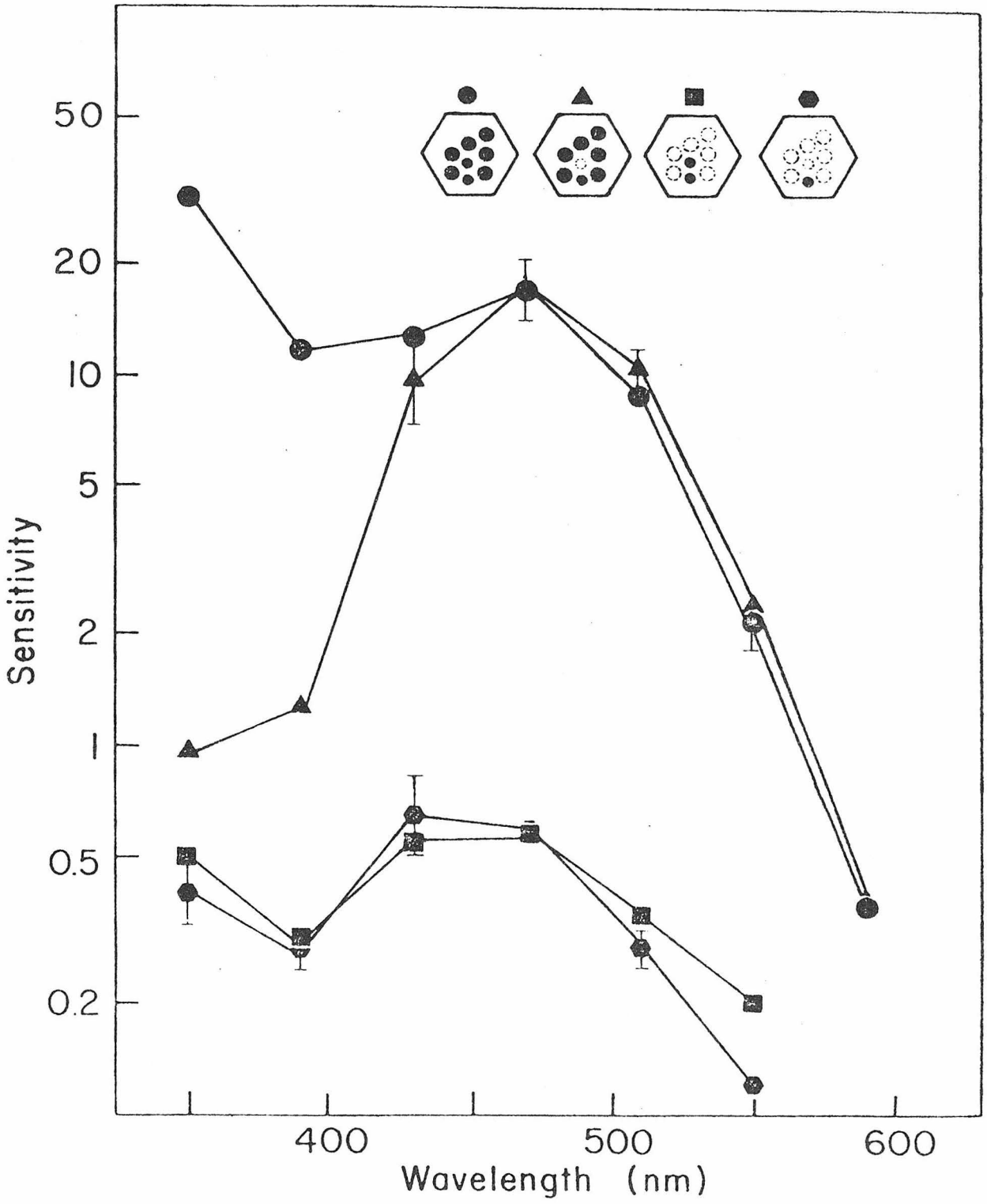


PLATE LEGENDS

Pl. 1. Wild type retina. (a) deep pseudopupil (bar = 100 μ), (b) optical meutralization (bar = 10 μ), (c) light microscopy (bar = 10 μ), (d) electron microscopy (bar = 2 μ).

Pl. 2. sev retina. (a), (b), (d), (d) as Pl. 1.

Pl. 3. rdgB retina. (a), (b), (c), (d) as Pl. 1.

Pl. 4. ora retina. (a), (b), (c), (d) as Pl. 1.

Pl. 5. Retinas of sev;ora (a) and sev rdgB (b). Light micrographs. Arrows in upper left point to ommatidia in which the section passes through at a relatively peripheral level, no central receptors are present; arrows in lower right point to ommatidia sectioned more deeply. Central rhabdomeres, R8, are present (bars = 10 μ).

Pl. 6. Mosaic dividing lines. Light micrographs. (a) and (b) of w sev/+. Arrows with short shafts point to completely normal ommatidia, top right. Arrows with long shafts point to R7 next to mutant pigment cell in (a), and in (b) R7 next to mutant pigment cell, and absence of R7 next to normal pigment cell. Arrow heads point to R8 in normal and mutant ommatidia at the bottom of (a). (c) y cho rdgB/+. Arrows point to ommatidia on dividing line with

some receptors degenerate, others not (bars = 10 μ).

Plate 1

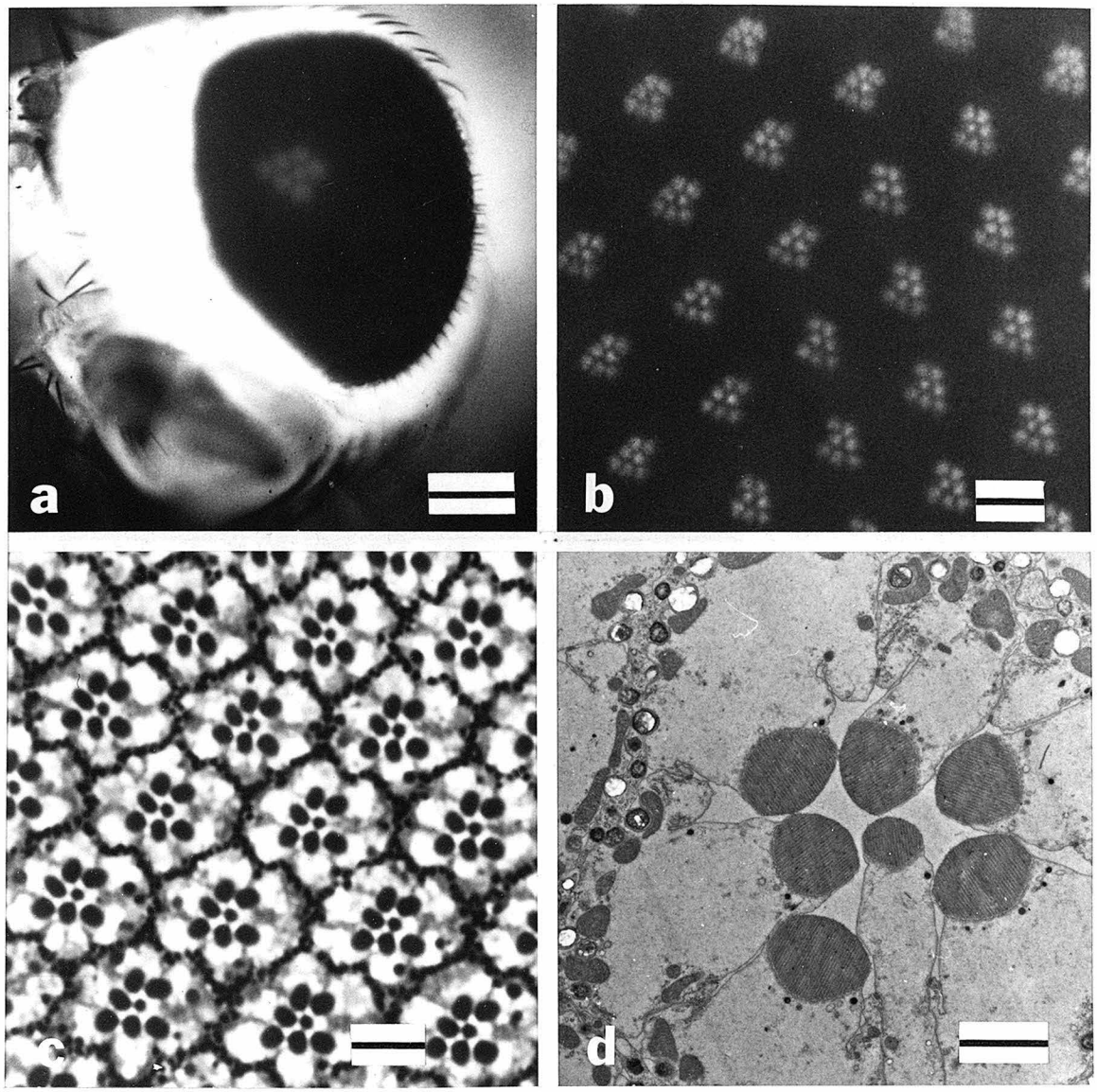
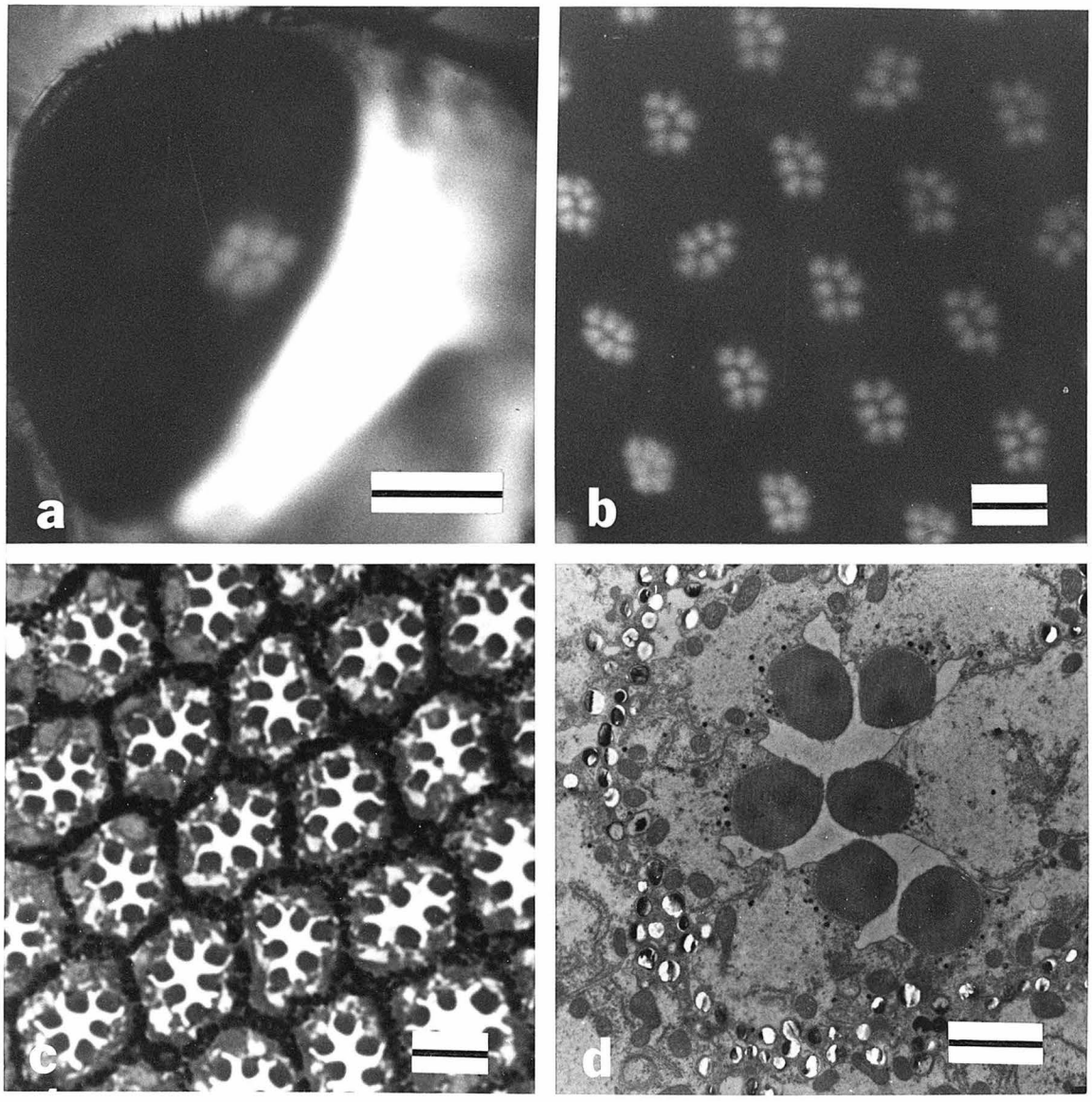


Plate 2



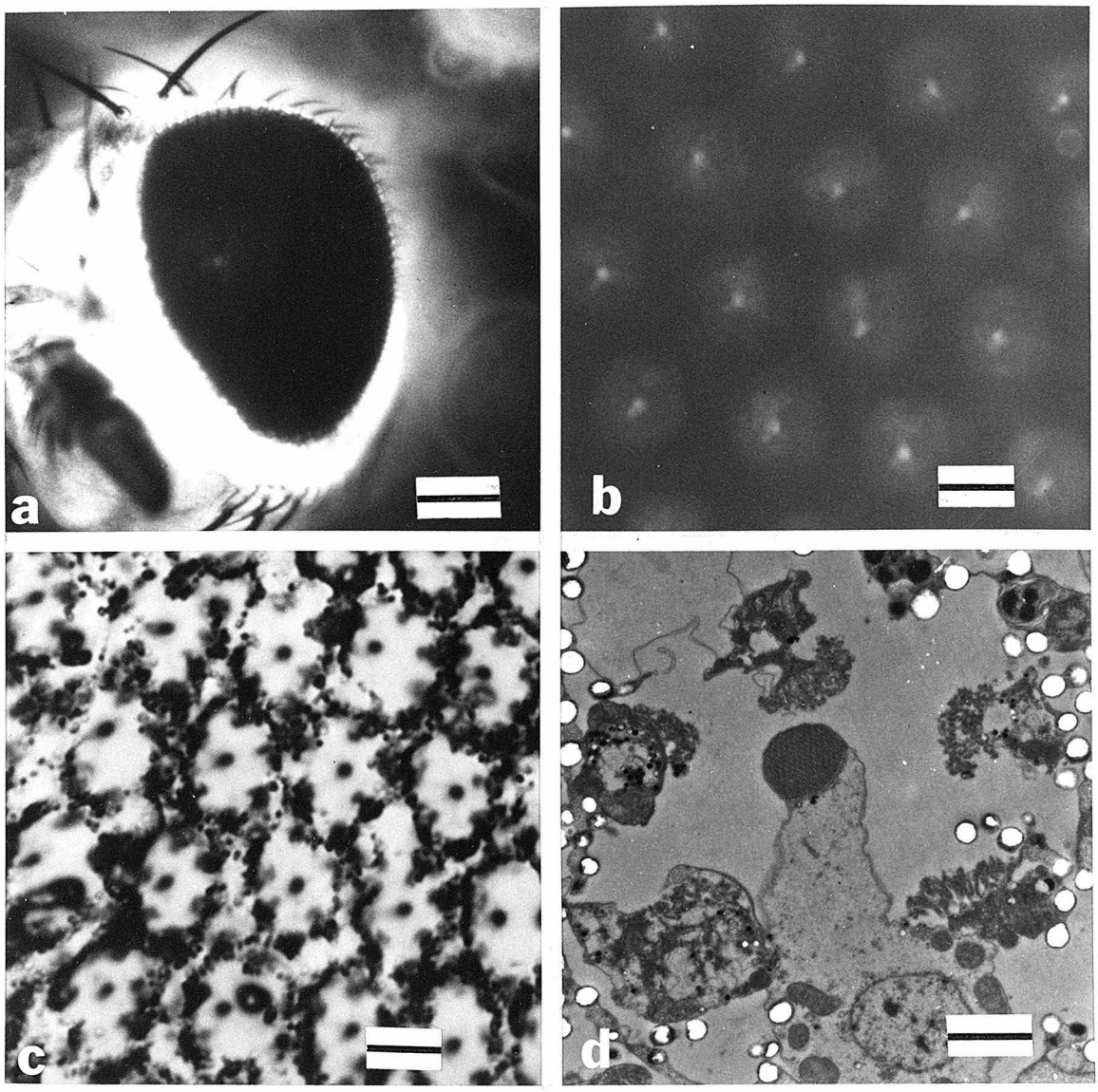
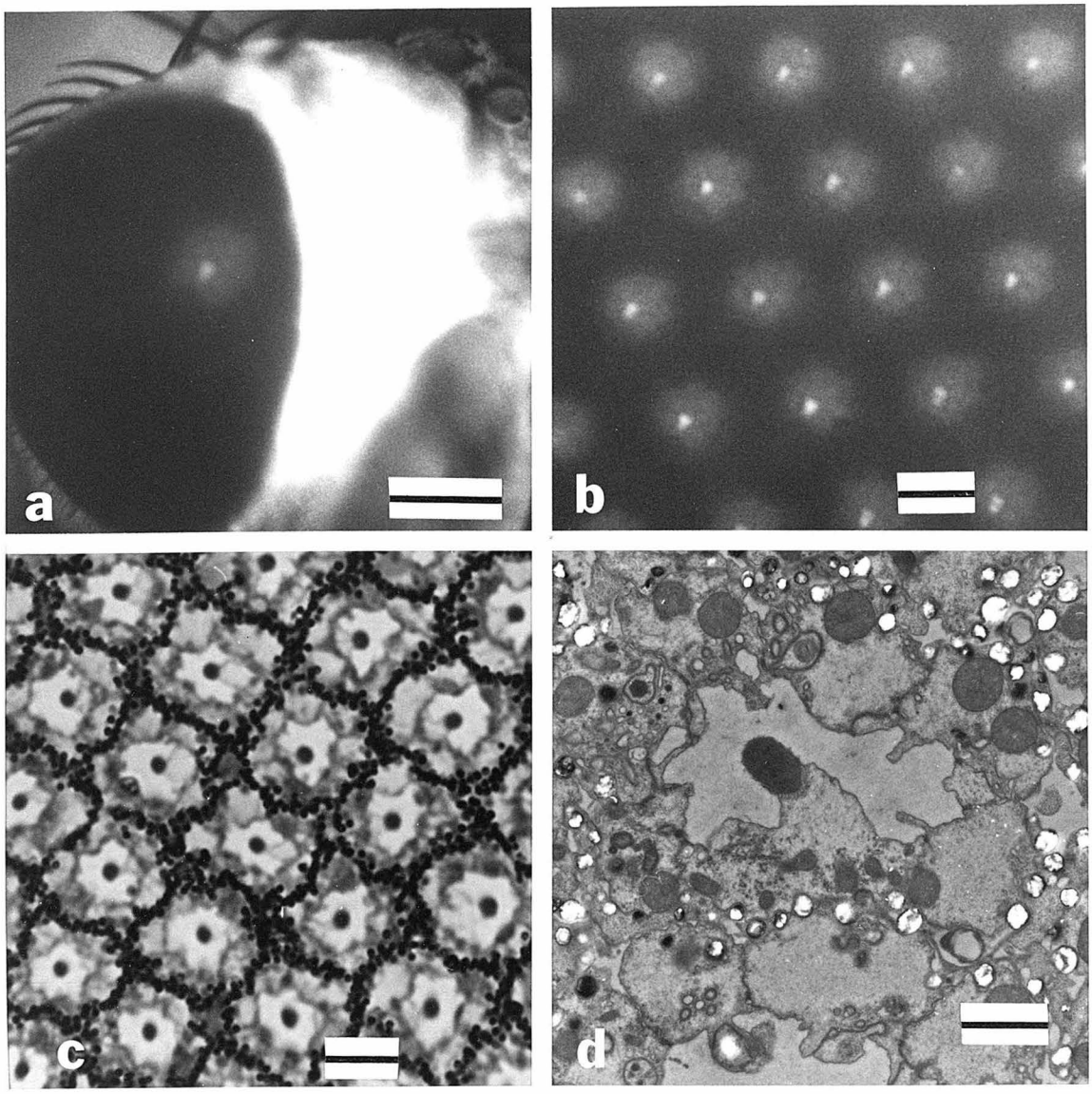

a
b
c
d

Plate 4



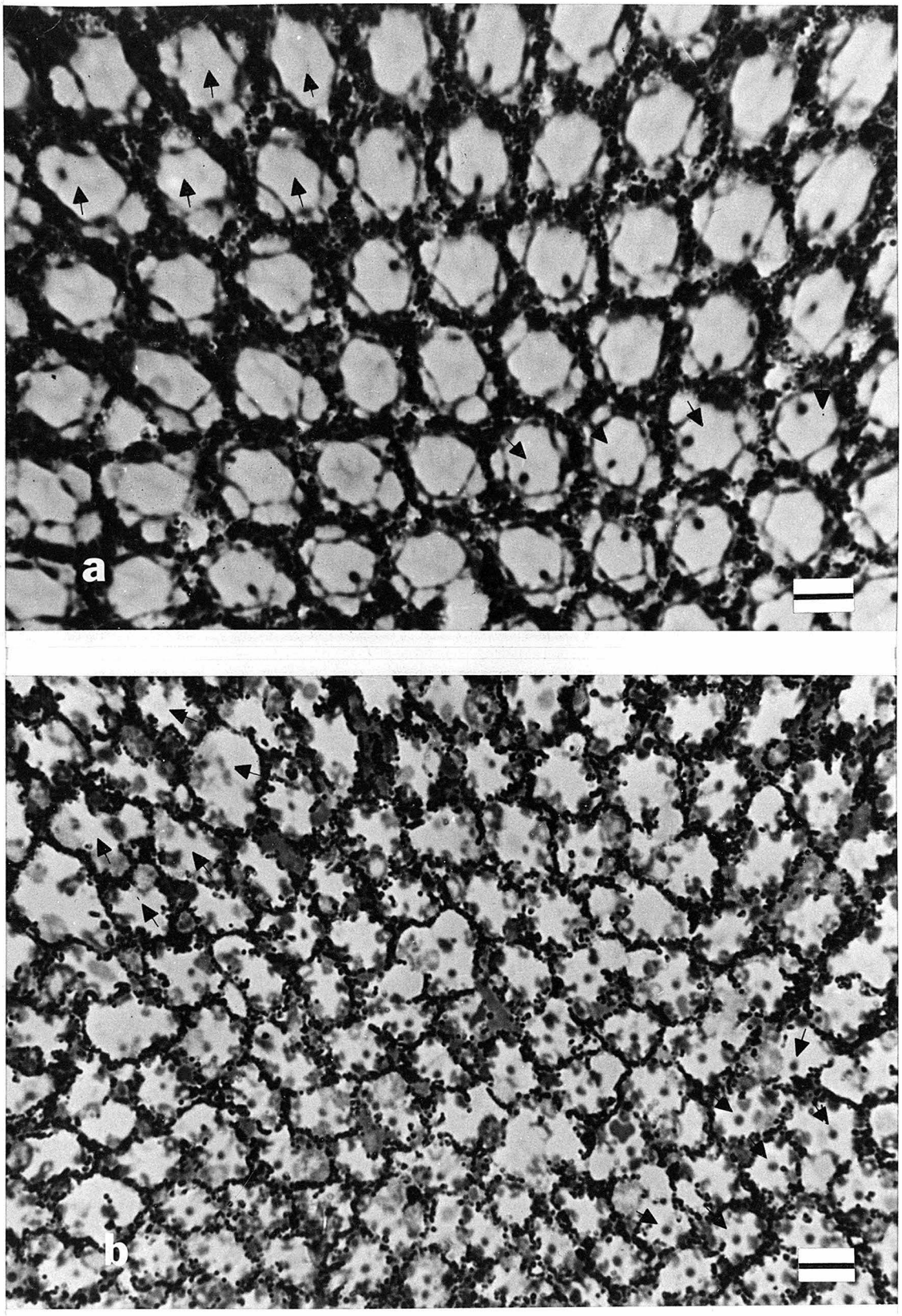
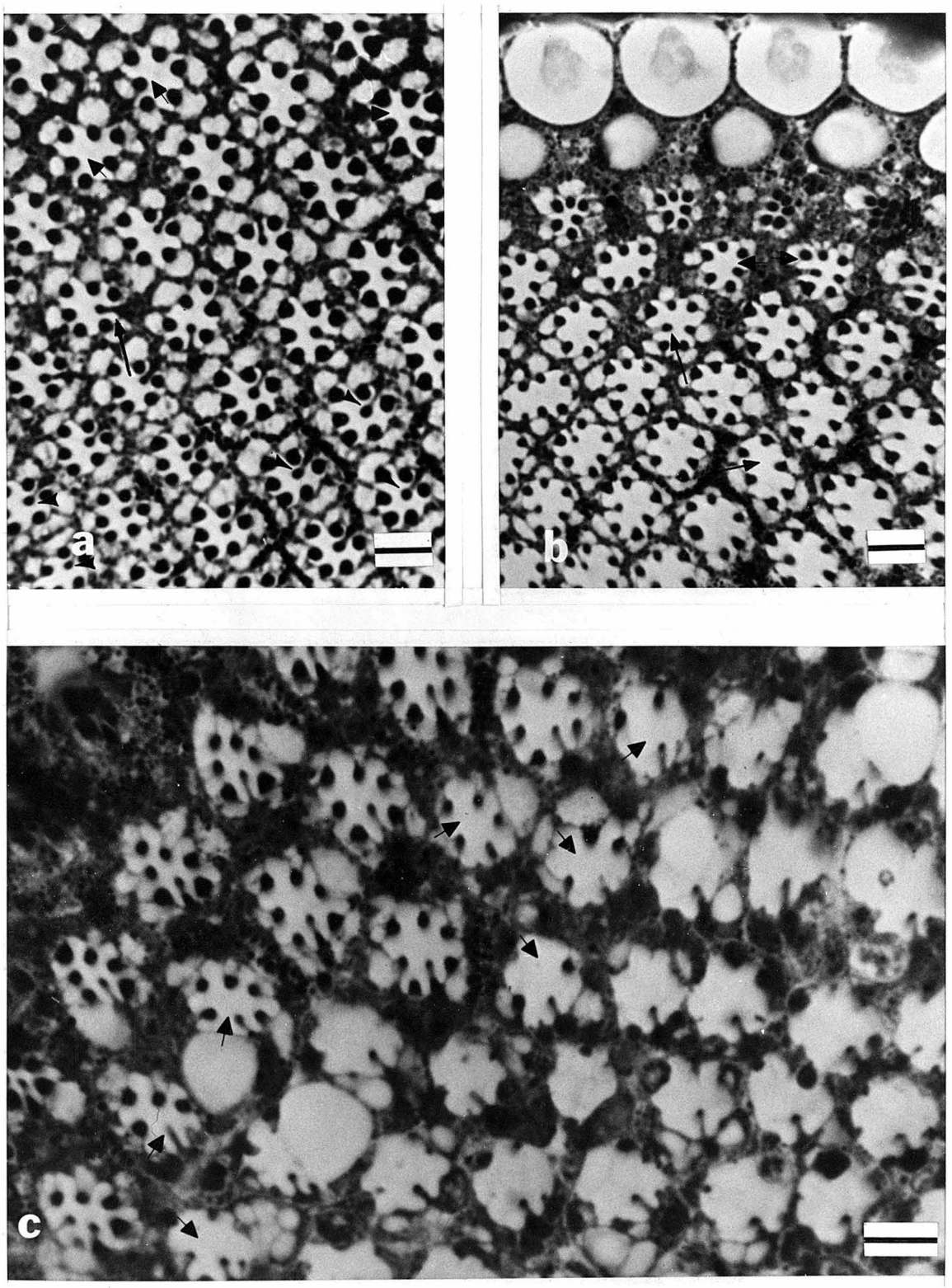

a
b

Plate 6



APPENDIX TO SPECTROPHOTOMETRIC METHODS

Table 1 shows the absolute intensities used to bleach the photopigments in the "Light Induced Absorption Change" experiments. These were measured by a Hewlett-Packard 8330A Radiant Flux Meter and 8334A Radiant Flux Detector at the level of the sample with all the optics in place. For measurements on the R1-6 rhodopsin a 5 sec bleach was used, while for the R7 rhodopsin a 10 sec bleach was used.

Table 2 shows the maximum intensities of the measuring lights that were used. Iris adjustments and neutral density filters usually cut down these intensities by up to an order of magnitude. These intensities did not seem to cause significant bleaching of the sample.

Tables 3 - 6 show the changes in O.D. produced in single typical experiments on R1-6 rhodopsin-metarhodopsin and R7 rhodopsin-metarhodopsin conversions.

In a typical experiment such as the one presented in Tables 3 and 4 and diagramed in Figure 1, after balancing the measuring lights a maximal amount of photopigment was converted to the R state by ten seconds of unattenuated 591 nm actinic light, then a 5 sec bleach of 350 nm (attenuated by 0.5 O.D. neutral density filter) was used to convert some R $\longrightarrow$ M. Then 10 sec of unattenuated 469 nm was used to drive a maximal amount of R $\longrightarrow$ M. Following this, 5 sec of 648 nm

(0.5 O.D.) was given to drive some M $\longrightarrow$ R. This was followed by 10 sec of unattenuated 591 nm, etc.

Figure Legend

Figure 1.

This shows the beginning of the experiment presented in Tables 3 and 4. Arrows up indicate 10 sec unattenuated 591 nm bleaches; arrows down indicate 10 sec unattenuated 469 nm bleaches. A, B, C, D, E, and F are respectively 350, 648, 374, 630, 390 and 611 nm bleaches (all 5 sec and unattenuated by a 0.5 O.D. neutral density filter). Dotted line represents the diminution of the maximal response which probably reflects photopigment denaturation. Absorbance increases at 470 relative to 580 nm are shown as upward deflections.

Fig. 1

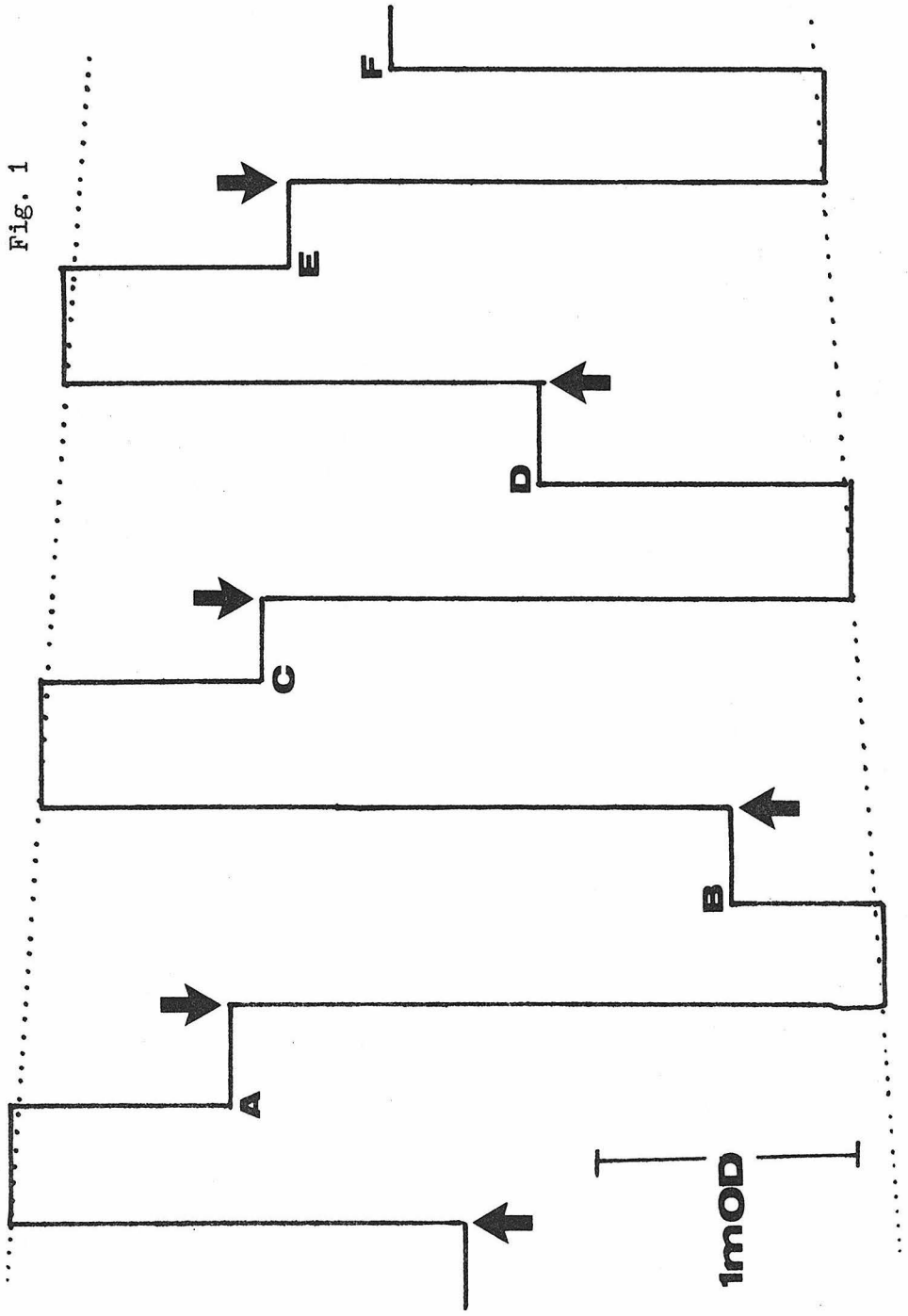


TABLE 1: INTENSITY OF BLEACHING LIGHTS

λ (cm)	(ergs/cm^2 sec)x10^3	(photons/cm^2 sec)x10^{15}
350	5.1	0.9
374	9.2	1.7
390	9.5	1.9
411	12	2.5
431	10	2.2
451	10	2.3
469	8.9	2.1
490	8.9	2.2
509	8.6	2.2
530	8.9	2.4
550	6.3	1.8
571	8.2	2.3
591	8.9	2.6
611	7.6	2.4
630	8.2	2.6
648	6.7	2.2

TABLE 2: INTENSITY OF MEASURING LIGHTS

λ(nm)	(ergs/cm^2 sec)x10	(photons/cm^2·sec)
370	0.5	9.5×10^{11}
470	3.0	7.1×10^{12}
580	7.0	2.0×10^{13}

TABLE 3: R1-6 R → M

Bleaching λ(nm)	Response (Δm.O.D.)	Correction Factor for Pigment Degradation	Ratio of intensities 451 nm to variable Λ	Corrected Response (ΔmO.D.)
350	0.69	1.00	2.10	1.46
374	0.91	1.08	1.10	1.08
390	0.78	1.12	1.05	0.92
411	1.04	1.16	0.84	1.01
431	1.17	1.22	0.97	1.39
451	1.26	1.28	1.00	1.61
469	1.30	1.33	1.14	1.97
490	1.17	1.41	1.14	1.88
509	0.91	1.44	1.19	1.56
530	0.48	1.56	1.14	0.85
550	0.04	1.65	1.61	0.12
571	0.00	1.72	1.24	0.00

TABLE 4: R1-6 M → R

Bleaching λ(nm)	Response (ΔmO.D.)	Correction Factor for Pigment Degradation	Ratio of intensities 451 nm to variable Λ	Corrected Response (ΔmO.D.)
469	0.00	1.61	1.14	0.00
490	0.17	1.50	1.14	0.30
509	0.43	1.40	1.19	0.72
530	0.91	1.39	1.14	1.44
550	1.30	1.28	1.61	2.67
571	1.48	1.25	1.24	2.29
591	1.56	1.20	1.14	2.14
611	1.48	1.14	1.34	2.25
630	1.13	1.12	1.24	1.54
648	0.52	1.01	1.52	0.80

TABLE 5: R7 R → M

Bleaching λ (nm)	Response (ΔmO.D.)	Correction Factor for Pigment Degradation	Correction Factor for Pigment Intensity	Corrected Response (ΔmO.D.)
350	0.09	1.00	2.10	0.18
374	0.26	1.13	1.10	0.32
390	0.13	1.29	1.05	0.18
411	0.00	1.29	0.84	0.00

TABLE 6: R7 M → R

Bleaching (nm)	Response (mO.D.)	Correction Factor for Pigment Degradation	Ratio of intensities 451 nm to variable Λ	Corrected Response (mO.D.)
411	0.00	1.80	0.84	0.00
431	0.09	1.50	0.97	0.13
451	0.21	1.29	1.00	0.28
469	0.26	1.13	1.14	0.34
490	0.21	1.13	1.14	0.27
509	0.11	1.13	1.19	0.15
530	0.09	1.00	1.14	0.09
550	0.00	1.00	1.61	0.00

Chapter III

CONDITIONED BEHAVIOR IN *DROSOPHILA MELANOGASTER*

by William A. Quinn*, William A. Harris and Seymour Benzer

This chapter has been published
in *Proceedings of the National Academy of Sciences*
Volume 71, page 708-712, 1974.

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Princeton, New Jersey, 08540.

INTRODUCTION

Because the hereditary mechanics of Drosophila melanogaster are understood in detail, the behavioral repertoire of this organism and the neural system which specifies it are amenable to genetic analysis. Many flies of identical genotype are readily produced, so that behavioral measurements can be made on populations rather than individuals, yielding instant statistics. If a mutation is found in a gene affecting behavior, methods using genetic mosaics exist for localizing the site of the gene's action to a specific region ("focus") in the fly (Hotta & Benzer, 1972). Anatomical or biochemical changes at the foci of various mutants may then be correlated with alterations in behavior.

One aspect of behavior which so far has been inaccessible to this form of analysis is learning. Conditioning experiments in Drosophila and other dipterans are fraught with complications, and most such studies have been inconclusive. A major problem is pseudoconditioning, in which the training schedule non-specifically alters the state of the organism, producing changes in behavior which can be misinterpreted as associative learning. An example is the "central excitatory state" (Dethier, Solomon, & Turner, 1965) in the blowfly Phormia regina; exposure of a hungry fly to sucrose solution arouses it so that afterward it extends its proboscis in response to a variety of unrelated stimuli. This probably accounts (Dethier, 1966) for the results of Frings

(1941). The proper control for pseudoconditioning is to disassociate the reinforcement in time from the stimulus; if the response results from true learning it should depend on simultaneous or near-simultaneous presentation of stimulus and reinforcement.

Another pitfall is the possibility of odor cues laid down by the flies. Our early experiments indicated that a stimulus, presumably an odor, was left in the apparatus by flies when shocked and later used by them as a cue for avoidance. The presence of odor trails may have affected the results of Murphey (1967) on T-maze learning by Drosophila; these have recently been contradicted by Yeatman and Hirsch (1971).

Habituation is the decrease in a response on repeated presentation of the same stimulus. Although it can be considered a rudimentary form of learning, in some cases it occurs at the sensory receptors (Roeder, 1963), so it is not necessarily related to higher learning in the central nervous system. Exposure of Drosophila larvae to odor altered their behavior as adults (Thorpe, 1939). This was interpreted as associated learning (Hershberger & Smith, 1967), but has since been shown to result from habituation (Manning, 1967).

Nelson (1971) has published a convincing report of classical conditioning in the blowfly Phormia regina, training and testing individual flies with taste cues. In the present study we have sought to demonstrate learning

unequivocally in Drosophila and to devise a paradigm suitable for mutant isolation, in which flies can be trained and tested en masse. All our experiments are variants of one experimental design. During training, flies are exposed to two different stimuli -- either two odorants or two colors of light -- one of which is associated with a negative reinforcement, such as electric shock. The flies are then removed and tested in a new apparatus, similar to the training arrangement but without reinforcement, and their avoidance of each of the two stimuli is measured. The reciprocal experiment is also done using a second group of flies, with shock coupled to the other stimulus. In each case the flies selectively avoid the stimulus which had been associated with shock during their training.

Using this type of experiment and related controls, we have been able to demonstrate olfactory and visual discriminative learning in Drosophila, eliminating the complications discussed above as explanations for our results. The selective avoidance behavior has the properties expected of conventional learning; it is extinguishable or reversible by later training and is an individual rather than a collective property of the flies.

Recently K. G. Götz (personal communication) has trained individual Drosophila to turn toward light or dark portions of the visual field. H. C. Spatz, A. Emanns, and H. Reichert (personal communication) have also found visual discriminative

learning with populations of Drosophila.

MATERIALS AND METHODS

D. melanogaster of the Canton-Special (C-S) wild-type strain were used. A mutant, yellow^2, was the second strain in mixed-population experiments. To make the genetic background of the mutant similar to the normal C-S strain, crosses were done to replace the autosomes and about 50% of the X-chromosome with C-S material. Stocks were maintained as usual (Benzer, 1967). Three-day-old flies were transferred to fresh food bottles to allow them to clean themselves for 10-30 min before training.

Olfactory learning. An apparatus originally designed for behavioral countercurrent distribution (Benzer, 1967) was used in these experiments (Fig. 1). Polystyrene test tubes, 17 x 100 mm (#2017, Falcon Plastics; Oxnard, Cal.) were aired for a week and used for only one experiment. "Rest" tubes had about 20 perforations at the closed end, made with a hot 26-gauge wire before airing. Grids for shocking flies had alternately connected copper strips 1 mm wide, 1 mm apart on an epoxy backing (Fig. 1b). They were made from 0.0025-inch printed-circuit material (Mica Corp., Century City, Cal.), using DCR photoetch materials (Dynachem Corp. Santa Fe Springs, Cal.). Grids were cleaned before experiments by two 24 hr washes in 95% ethanol. They were rinsed in water, ethanol, and ethyl ether and aired for at

least 12 hr.

3-octanol #16449, 4-methylcyclohexanol #16954, cis 4-methylcyclohexanol #25155, and trans-4-methylcyclohexanol #25168 were from K & K Laboratories, Hollywood, Cal. Stearic acid #2733 and quinine sulfate #6970 were from Matheson, Coleman, and Bell, Inc., Norwood, Ohio). Solutions of the odorants in ether were 1 ml:100 ml (1 g:100 ml for stearic acid). 0.2 ml of odorant solution was spread over a grid surface and the ether allowed to evaporate (1 min). Such grids were usable for at least two hours.

The shock reinforcement on the grids was 90 volts A.C., 60 Hz. When quinine sulfate was used as a negative reinforcement, the dry powder was applied to the grids with a #3 artist's brush and the excess tapped off, leaving 8-10 mg on each grid. The conditioning experiments were carried out in a darkened room at 22°C. A 15-watt fluorescent lamp, General Electric cool white, F15T8-CW, was the light source for phototaxis. Flies were etherized and counted after each experiment was completed.

Visual learning. The apparatus was a black lucite Y-maze (Fig. 5). Entry was from a 17 x 100 mm polystyrene test tube covered with black masking tape. The arms were polystyrene tubes with the closed ends cut off and replaced with epoxy-cemented glass cover slips for more uniform illumination. For experiments with ultraviolet light, unaltered tubes were used. The arms contained standard grids

without odorants. Quinine sulfate, when used, was applied as above. The only illumination was by white fluorescent light filtered through Balzers interference filters (half-width 10 nm). Intensity was adjusted with Wratten neutral density filters. The experiments were carried out at 22°C.

Statistical significance levels were determined using Wilcoxon signed-rank tests (Colquhoun, 1971) (one-tailed distribution). Confidence limits, where given, were determined by computing the relevant index for each experiment of a series and calculating the variance of the distribution of these values. The limits given here are standard errors of the mean. The experiments reported were run in consecutive series, with all the experiments in a series included.

RESULTS

Olfactory learning

Basic paradigm and controls. The paradigm required the flies to discriminate between an odor coupled with shock and another odor presented without reinforcement. The apparatus in Fig. 1a has two arrays of tubes which slide past each other so that a tube in one array can abut any tube in the other. For each experiment, appropriate tubes are fitted with grids. Approximately 40 flies are placed in the starting tube. A run is started by holding the apparatus vertical and shaking the flies to the bottom of the start tube, by tapping the apparatus on a rubber pad. The start tube is

shifted into register with the proper grid tube. The apparatus is then laid horizontally before a fluorescent lamp which induces the phototactic response; the flies run from the start tube towards the grid.

In the basic paradigm (Fig. 2) tube 1 is a "rest" tube with holes at the end to allow odor to escape. Tubes 2-5 contain grids with odorants: tubes 2 and 4 each have 3-octanol on their grids; tubes 3 and 5 have 4-methylcyclohexanol. Tubes 2 and 3 are used for training, tubes 4 and 5 for testing. Voltage is applied to tube 2 only. Using separate tubes for training and testing removes the flies from any odors they may have left on the grids during training, so that during testing the chemical odorants are the only possible cues for selective avoidance.

For training the sequence of runs was: rest tube (60 sec), tube 2 (15 sec), rest tube (60 sec), tube 3 (15 sec). This cycle was repeated three times. (A tendency to avoid tube 2 was already evident by the second cycle.) The flies then were tested, in the same sequence, with tubes 4 and 5 instead of 2 and 3. The number of flies avoiding the grid on each run was counted visually. More flies avoided tube 4 than tube 5. Tube 4 contained 3-octanol, which had been presented simultaneously with shock during training.

As a control for odor bias, the paradigm was repeated using a second population of flies, but with the voltage on tube 3. This time, on testing, more flies avoided grid 5.

Table 1 shows the pooled results from a series of 10 such experiments. The data given are for the first test run to each odor. In all 20 cases, the flies selectively avoided the shock-associated odor. The difference in avoidance was significant ($p < 0.001$) for both reciprocal halves of the experiment.

The experimental design rules out pseudoconditioning as an explanation for the results, since the second part of the experiment serves as a control for the first and vice versa. To eliminate experimenter bias, the experiments in this series were run blind. The order of the training tubes and the order of the testing tubes were determined by separate coin tosses, and the experimenter did not known the odor on each tube. In about half the experiments, the sequence of odors during testing was the reverse of that during training. Thus the flies' behavior cannot be explained by a stereotyped order of responses or by nonspecific excitatory effects.

Not all odors work. Of 40 tested, only five gave consistently good results. The 4-methylcyclohexanol used in Table 1 was a mixture of <u>cis</u> and <u>trans</u> isomers. Either isomer can be used for training against 3-octanol or against the other isomer.

The results so far could be explained in terms of sensory habituation instead of learning, if one assumes that the flies are nonspecifically sensitized by shock to avoid

all odors. During training they progressively avoid the tube with grid voltage; therefore, they spend more time in the presence of the control odorant. They might become habituated to it and avoid it less during the subsequent testing. However, if this were true the flies would avoid the control odor used in their training less than an entirely new odor. In fact, this is not so (Table 2). Temporal association of an odor with shock is necessary for avoidance.

Learning index. It is convenient to define a quantitative index of the specific odor avoidance attributable to learning. A simple measure is the fraction of the population avoiding the shock-associated odor minus the fraction avoiding the control odor. For example, in a typical experiment, of 33 flies trained to avoid 3-octanol, 17 (a fraction 0.51) avoided 3-octanol, while 2 flies (a fraction 0.06) avoided 4-methylcyclohexanol. Therefore, the learning index (λ_a) for this trial is 0.45. Similarly, for the reciprocal half of the experiment the index λ_b was 0.27. The learning index (Λ) for the experiment is defined as the average of the values for the two halves (0.36 in this case). Its theoretical range is $-1 \leq \Lambda \leq 1$. If flies always avoid the shock-associated odor, never the control ("perfect learning"), $\Lambda=1$. If association with shock does not affect the flies' odor preference (no learning), $\Lambda=0$. If the population runs preferentially to the shock-associated odor ("masochism"), $\Lambda=0$. For the ten experiments in Table 1, the average value

$\overline{\Lambda}$ was 0.34 ± 0.02.

Extinction, reversal and persistence. Extinction of the selective avoidance behavior becomes evident when the odor cues are presented without shock reinforcement. In each of the experiments of Table 1 the flies were actually tested three times. The selective avoidance response decreased in successive tests: $\overline{\Lambda}_1$=0.34 ± 0.02; $\overline{\Lambda}_2$=0.23 ± 0.03; $\overline{\Lambda}_3$=0.13 ± 0.03. The differences are significant: $p(\overline{\Lambda}_2 \geq \overline{\Lambda}_1) < 0.01$; $p(\overline{\Lambda}_3 \geq \overline{\Lambda}_2) < 0.01$. This decreased response was not due to diffusion or degradation of the odor cues in the tubes, since the flies could be retrained in the same apparatus. Nor was it the result of a lessened "alertness" in the population due to lack of shock; shock alone in the absence of odor cues did not restore selective avoidance.

It is also possible to reverse the flies' odor preference with extinction followed by reverse training (Fig. 3).

If not extinguished by testing, the learned behavior persists longer. Separate groups of flies were trained as in the basic paradigm, but kept undisturbed in the rest tube for various times up to one hour before testing. Figure 4 shows the results. It is clear that memory persists for an hour, although some decay is evident. If the usual training procedure is repeated four times at 2 hour intervals, some selective avoidance behavior is demonstrable 24 hours after the last training session. (Six experiments: $\overline{\Lambda}$=0.12 ± 0.02; $p(\overline{\Lambda} \leq 0) < 0.001$).

Independence and homogeneity. Experiments are necessary to test whether the selective avoidance is a property of individual flies or a collective "stampede" effect. One way to decide this is to train two populations to avoid different odors, mix the flies, then see whether they separate according to their different training experiences. Approximately 50 yellow mutant flies were trained to avoid 3-octanol, and about 50 normal flies were trained simultaneously to avoid 4-methylcyclohexanol. The two groups were then mixed in one of the start tubes and run to a rest tube for 60 seconds. The mixed population was then run into a fresh grid tube containing 3-octanol. After 15 seconds the tube array was shifted so that flies which entered the grid tube were separated from those which avoided it. Flies of each class were collected, etherized, and their genotypes scored. The entire procedure was repeated with two fresh groups of flies of the same two genotypes, except that they were tested with a grid tube containing 4-methylcyclohexanol.

To rule out any effect of genotype on odor preference, a reciprocal pair of procedures was also carried out. yellow flies were trained to avoid 4-methylcyclohexanol, normal flies to avoid 3-octanol. Five complete experiments were performed. In 17 of the 20 test runs, the flies which avoided the grid tube were enriched in the genotype which had experienced that odor simultaneously with shock ($p < 0.01$). The pooled results are shown in Table 3. Both genotypes showed selective avoidance, but the learning index for each was smaller than that in the basic paradigm:

($\overline{\Lambda}$ [yellow] = 0.17 ± 0.08; $\overline{\Lambda}$ [normal] = 0.23 ± 0.06). This reduction indicates that there is some stampede effect, with flies of one persuasion tending to drag along those of the other. Nevertheless, the fact that the two types will separate shows that the information for the proper choice resides in the individual flies.

In the basic paradigm (Table 1), the difference in avoidance corresponding to learning represents only a third of the population. Does this "fractional learning" arise from some inhomogeneity in the population, or is it due to a stochastic component in the behavior of all the flies? To answer this question, flies which avoided the shock-associated odor were separated from those which did not, and each group was retrained and retested 24 hours later (half to the same odor, half to the other). The performance of both groups was the same, [$\overline{\Lambda}$ (avoiders) = 0.31 ± 0.02; $\overline{\Lambda}$ (non-avoiders) = 0.34 ± 0.05]. This suggests that the expression of learning is probabilistic in every fly. There is no evidence for an "intelligent" subset of the population.

Learning with quinine reinforcement. It was found that flies tend to avoid surfaces coated with fine quinine sulfate powder. Accordingly, flies were trained and tested in the usual manner, but with quinine sulfate replacing shock as the aversive reinforcement on one of the training grids. On testing, the flies selectively avoided the odor previously associated with quinine. (Ten experiments: $\overline{\Lambda}$=0.24 ± 0.03).

This demonstrates that the flies' learning is not restricted to a single mode of reinforcement. It also rules out artifacts due to electric shock.

Visual learning

To determine whether a sensory modality other than olfaction can be used, we developed a paradigm based on different colors of light. In addition, it requires a choice by the flies rather than simple avoidance. Figure 5 shows the apparatus. Forty to one hundred flies were placed in a stoppered plastic test tube coated with black tape. After 60 seconds, the tube was placed at the entrance to a Y-maze; one arm was illuminated with 610 nm red light, the other with 450 nm blue light. Light intensities were balanced so that naive flies ran equally to both arms. During training, negative reinforcement was administered in one of the arms by coating the grid with quinine sulfate powder. The flies were allowed 30 seconds to run phototactically into the arms, then shaken back into the start tube, which was removed, stoppered, and kept in darkness for 60 seconds. This training procedure was repeated twice more. After the final 60-second rest, the flies were tested in a second Y-maze, identical to the training maze but without quinine. After 30 seconds the start tube was removed and a foam stopper pushed up to the fork of the maze, holding the flies in the arms that they had chosen. The flies in each arm were etherized

and counted. To rule out induced color bias unrelated to learning, a second population was trained to avoid the other color. Twenty such reciprocal pairs were done. On testing, the flies selectively avoided the light of the color which had been associated with quinine. Figure 6 shows the influence of training on color choice.

The learning index used here is analogous to that for olfactory learning. λ_a is the fraction of flies entering the arm with the control color minus the fraction entering the arm with the quinine-associated color. The reciprocal experiment gives λ_b. The learning index Λ for the experiment is defined as the average of λ_a and λ_b. In 19 of the 20 cases, Λ was positive. $\overline{\Lambda} = 0.09 \pm 0.01$; $p(\Lambda \leq 0) < 0.001$.

Control experiments ruled out left-right and brightness discrimination as explanations for these results (Table 4). Electric shock, when used instead of quinine, was also effective. (Ten experiments, $\overline{\Lambda}=0.09 \pm 0.03$; $p(\overline{\Lambda} \leq 0) < 0.01$.) Flies were successfully trained with another pair of colors 350 nm (ultraviolet) and 470 nm (blue), corresponding to the sensitivity maxima of the two photoreceptor systems in the Drosophila eye (Snyder & Pask, 1973). (Ten experiments, $\overline{\Lambda}=0.08 \pm 0.02$; $p(\overline{\Lambda} \leq 0) < 0.01$.)

DISSCUSSION

Drosophila can be trained to avoid specific olfactory or visual cues. This behavior has many of the characteristics

of learning. It can persist for a day, but is rapidly extinguished or reversed by retraining. Various possible effects have been controlled for in the olfactory paradigm. While these might be relevant under other conditions, their influence on the experiments described here is negligible. Although the visual experiments are less extensive, their symmetrical design and their similarity to the olfactory paradigm make explanations other than learning improbable.

The learned behavior shown by the flies is fairly sophisticated, requiring sensory discrimination and (in the visual situation) choice. However, the effect is not strong. Under the most favorable conditions, only a third of the population demonstrates learning. Nevertheless, all the flies have the same apparent capability. It is likely that we have not yet found the optimal cues or the most suitable task. Conditioning has been demonstrated using two sensory modalities, sight and smell, and two forms of reinforcement, electric shock and guinine sulfate. These can be used in the four possible combinations to give similar avoidance behavior. Therefore, it is plausible that the association of stimulus and reinforcement occurs in the central nervous system.

This work may be useful in the analysis of Drosophila's sensory systems, since discriminative learning proves that the stimuli in question can be distinguished by the fly. The visual learning paradigm provides an example. Anatomical,

physiological, and indirect behavioral experiments have shown that Drosophila has two visual receptor systems, with maximum sensitivities at different wavelengths (Snyder & Pask, 1973). The present experiments suggest that the fly uses the color information it is equipped to detect.

The demonstration of conditioned behavior in Drosophila and the development of procedures in which flies can be trained and tested in populations may permit the isolation of mutants with altered abilities to learn, consolidate, or remember. This would permit the genetic techniques available in Drosophila to be applied to these problems.

TABLE LEGENDS

TABLE 1. (No legend)

TABLE 2. 3-octanol, 4-methylcyclohexanol, and stearic acid were the odors used in this series. Each was used as shock-associated, new, or control odor in different experiments, with the six possible permutations equally represented. Nine experiments (with reciprocal halves).

TABLE 3. Two populations of different genotype, trained to avoid different odors, were mixed and tested against one of the odors. Those flies which entered the odor tube were separated from those which avoided it. Each class was etherized and scored for genotype. The ratios given here are normalized; they represent the fraction of the normal population in the specified class (e.g., avoiders) divided by the fraction of the yellow population in the same class. All the enrichment ratios are in the direction to be expected if flies of each genotype express their learned behavior independently. (OCT, 3-octanol; MCH, 4-methylcyclohexanol).

TABLE 4. Flies are trained in a Y-maze, using quinine sulfate as reinforcement in one of the arms and the stimulus pairs listed. The flies discriminated successfully between both light wavelength pairs (experiments A and B). In

experiments C and D, white light was used on both arms. Intensities were equal in C, 10:3 in D.

TABLE 1: Olfactory avoidance learning.

(ten experiments - pooled data)

specific odor paired with shock in training	total flies	number avoiding		fraction avoiding	
		OCT	MCH	OCT	MCH
3-octanol (OCT)	369	210	68	0.57	0.18
4-methylcyclohexanol (MCH)	397	73	193	0.18	0.49

TABLE 2: Avoidance of odors by trained flies

odor	fraction of flies avoiding
shock-associated	0.40 ± 0.04
new	0.12 ± 0.02
control	0.15 ± 0.02

TABLE 3. Separation of normal and yellow flies with different training.

Aversive Training	Ratio on testing (normal/yellow). OCT on test grid Entering	OCT on test grid Avoiding	MCH on test grid Entering	MCH on test grid Avoiding
normal vs. OCT, yellow vs. MCH	0.69	1.65	1.17	0.65
normal vs. MCH, yellow vs. OCT	1.41	0.55	0.68	3.18

TABLE 4. Visual discrimination learing

Training and test choice in Y-maze	Number of cases $\Lambda > 0$	$\Lambda < 0$
A. 450 nm vs. 610 nm	19	1
B. 350 nm vs. 470 nm	9	1
C. Left vs. right - control	9	11
D. Bright vs. dim light - control	11	9

FIGURE LEGENDS

FIGURE 1.

A. Apparatus used in the olfactory learning experiments. Two plastic blocks can be slid past each other on a dovetail joint. Holes running through each block are fitted with teflon O-rings, to hold plastic tubes.

B. Printed circuit grid for shocking flies. The grid is rolled up and inserted into a plastic tube, which is plugged into the apparatus. Conductive tabs for applying voltage are bent around the tube rim to the outside.

FIGURE 2.

Basic Olfactory paradigm. Tube 1 is the rest tube, 2 and 3 are for training, 4 and 5 are for testing. Tube 6 is the start tube. Horizontal stripes in tubes indicate grids. A and B denote odorants 3-octanol and 4-methylcyclohexanol respectively. "V" indicates voltage on the grid. See text for training and testing sequences.

FIGURE 3.

Extinction and reversal of the learned response. A population of 36 flies was trained to avoid 3-octanol, then tested repeatedly without reinforcement. They were reverse-trained to avoid 4-methylcyclohexanol and retested, then reverse-trained once more and tested again.

FIGURE 4.

Persistence of memory. Between training and testing the flies were left in the rest tube for times up to 60 minutes. Each point represents 3-7, experiments with reciprocal halves. The flies performed erratically on their first run after the long rest; therefore, each population was run once to 3-octanol before testing was begun.

FIGURE 5.

Apparatus for visual training.

FIGURE 6.

Visual learning histograms showing distributions of Y-maze choices.

120

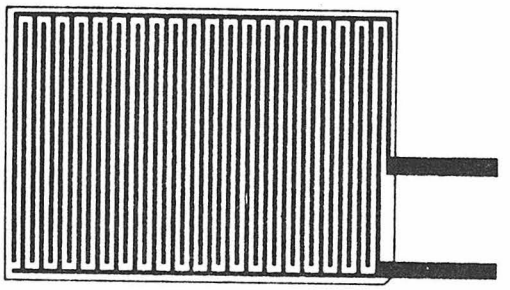
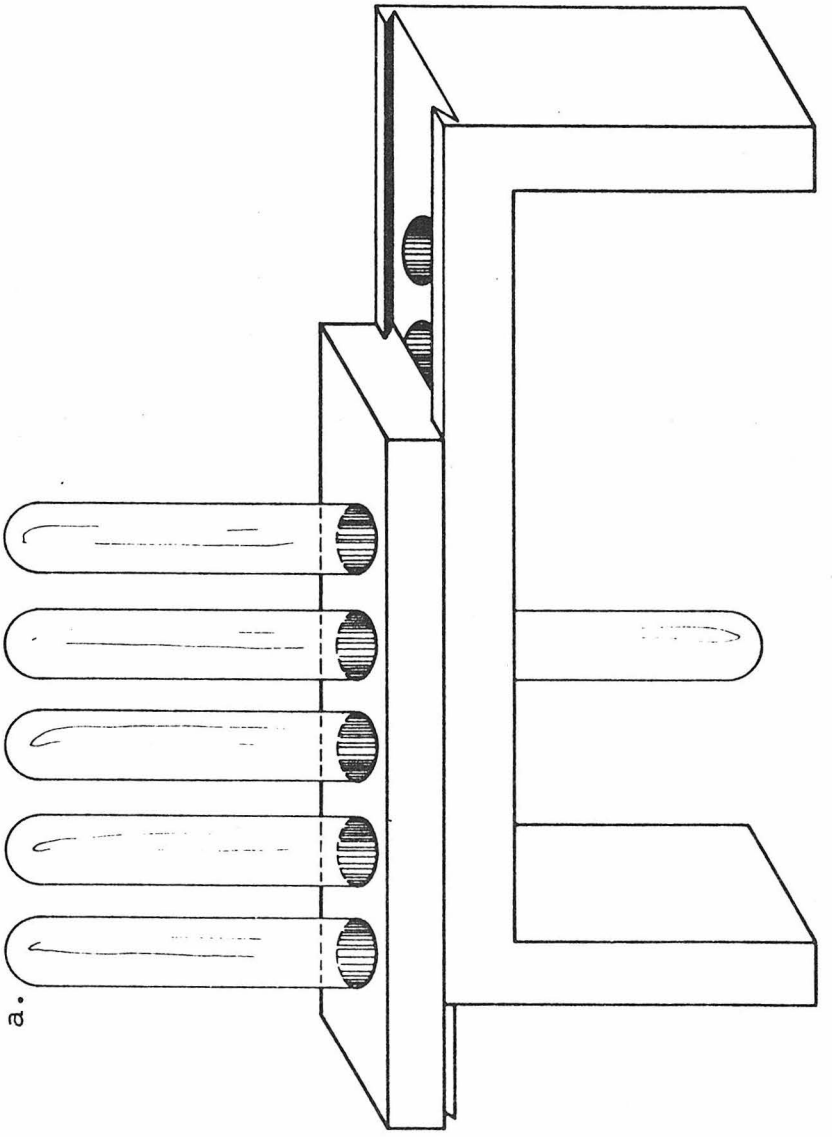


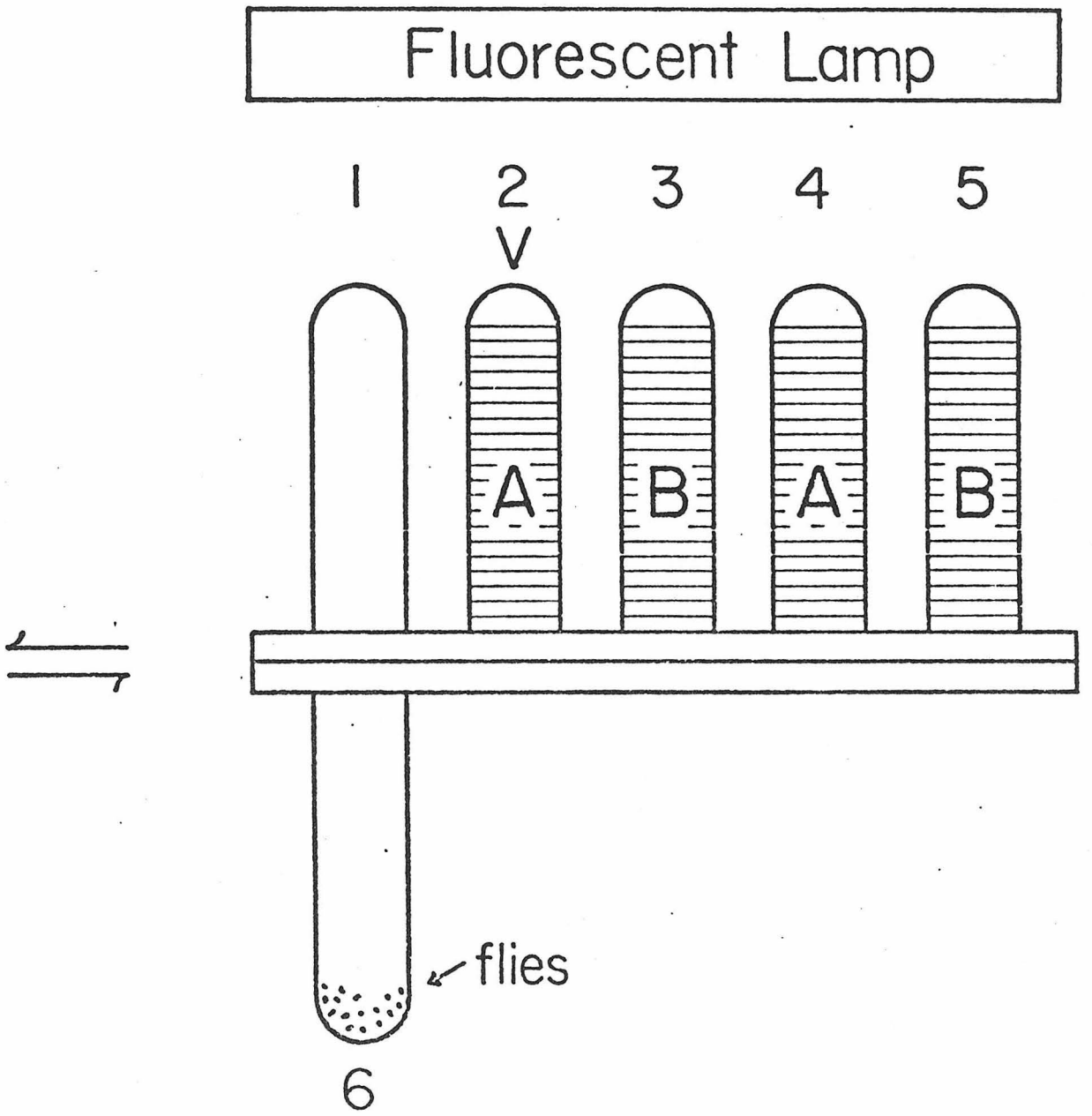
Fig. 1

b.



a.

Fig. 2



NUMBER AVOIDING
3-OCTANOL MINUS
NUMBER AVOIDING
4-METHYLCYCLOHEXANOL
-0.40
-0.20
0
0.20
0.40
TRAINED TO AVOID
3- OCTANOL
TEST NUMBER
1 2 3 4 5 6 7
RETRAINED TO AVOID
4-METHYL- CYCLOHEXANOL
TEST NUMBER
1 2 3 4 5 7
RETRAINED TO AVOID
3- OCTANOL
TEST NUMBER
1 2 3 5 6 7

Fig. 3

Fig. 4

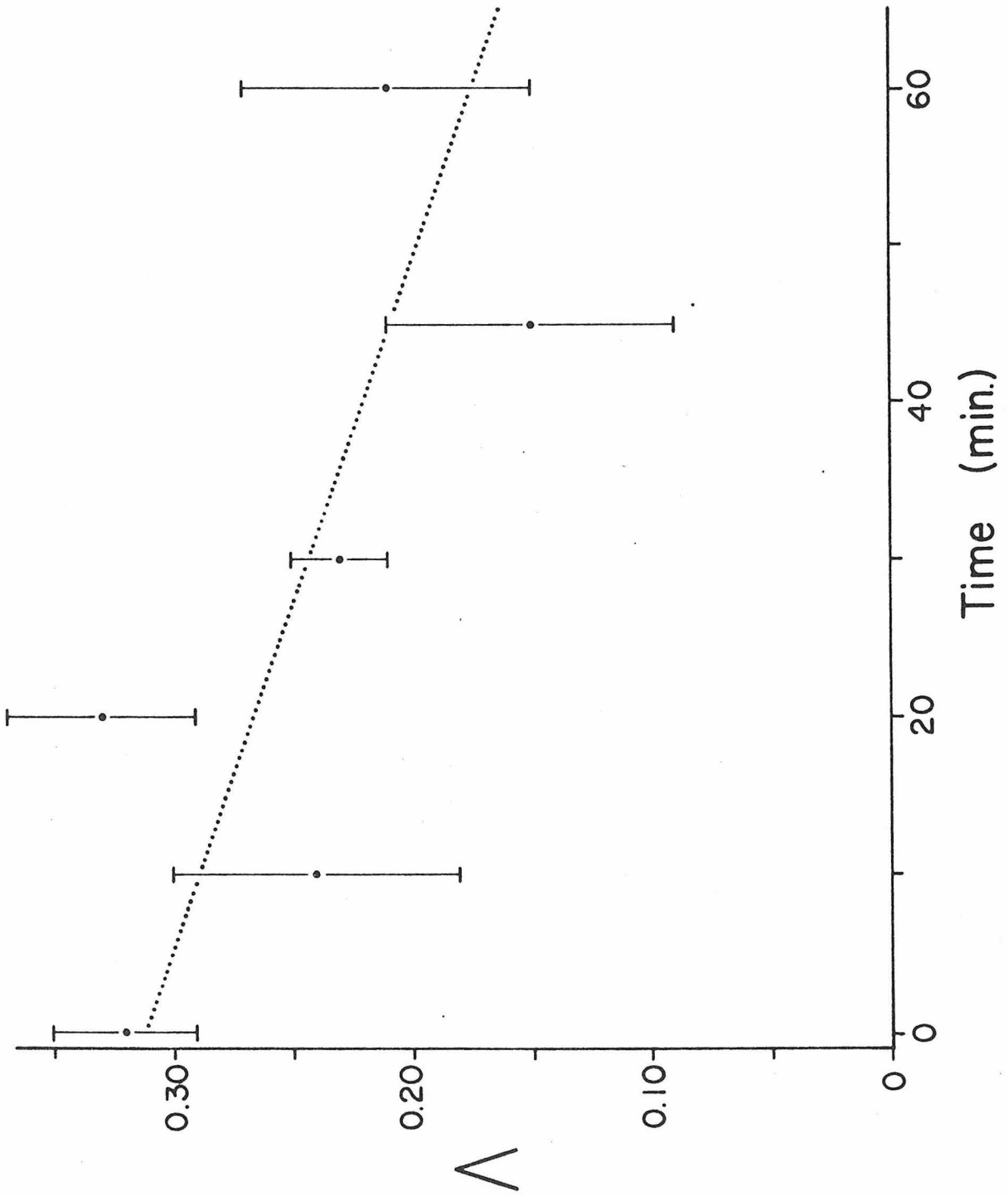


Fig. 5

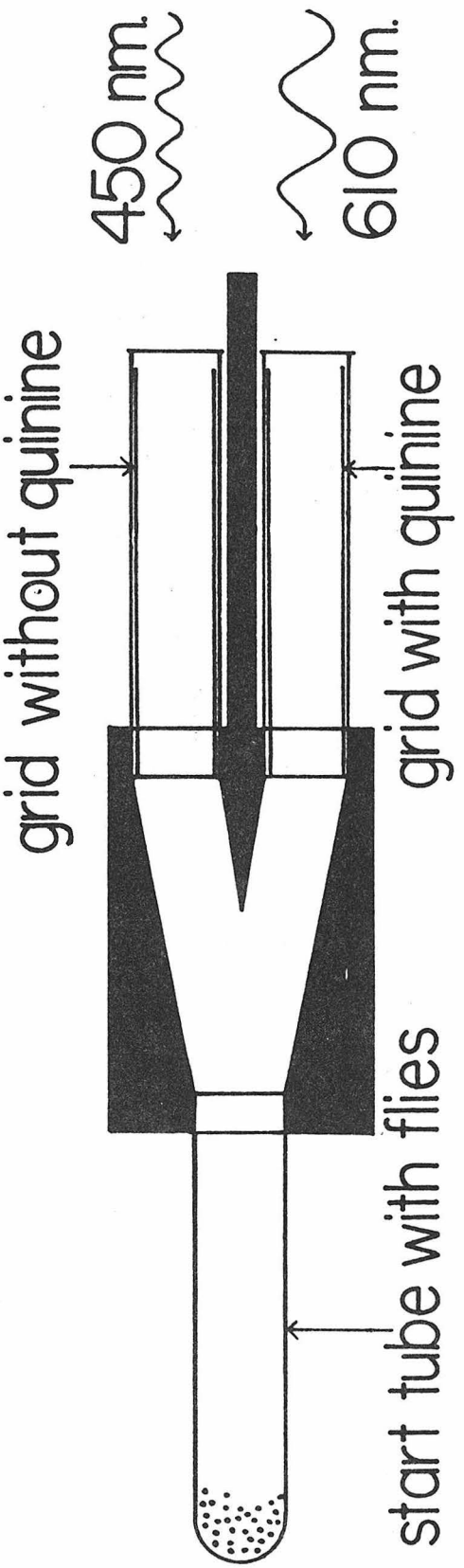
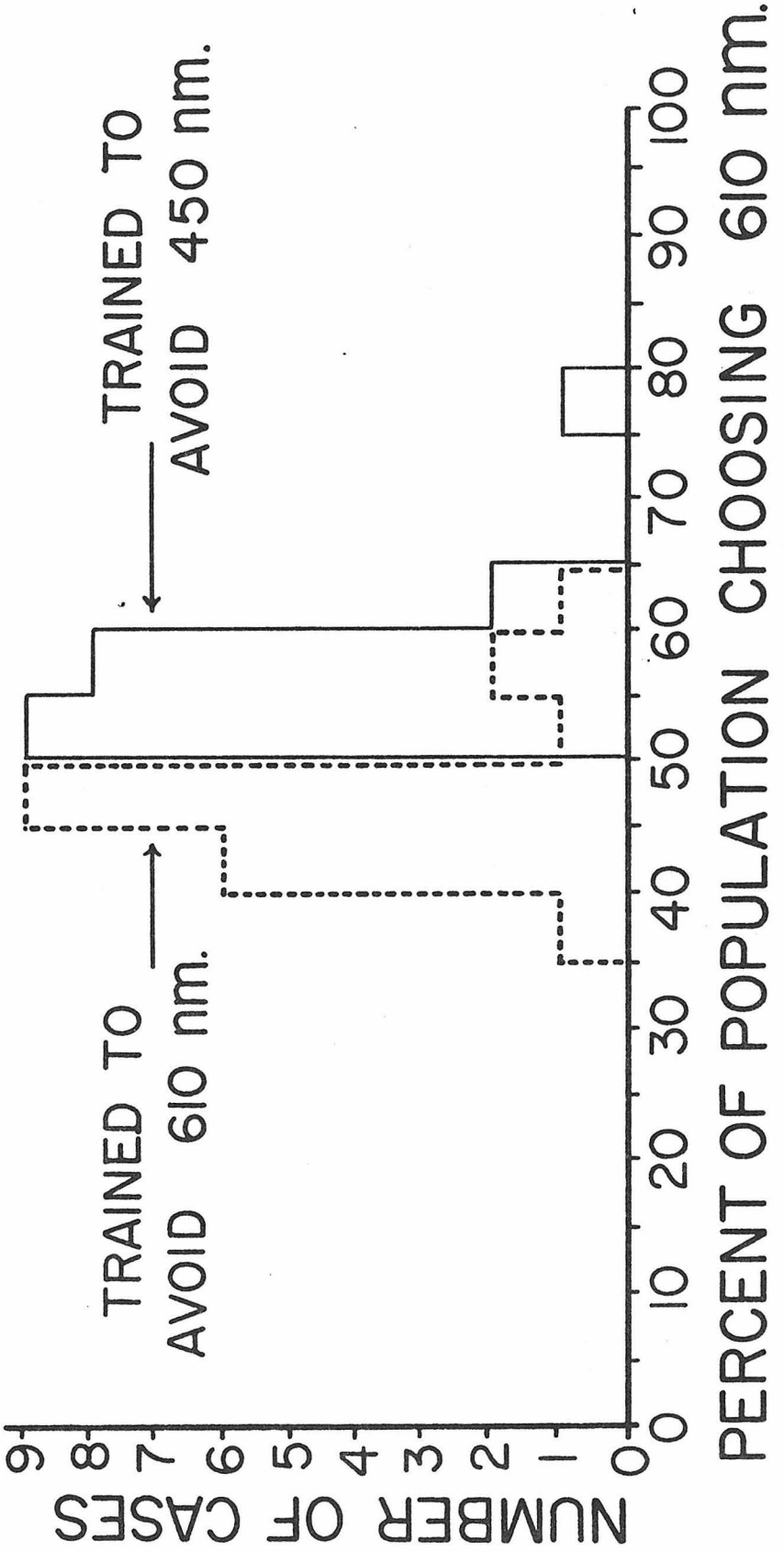

450 nm.
610 nm.
grid without quinine
grid with quinine
start tube with flies

Fig. 6


NUMBER OF CASES
0
1
2
3
4
5
6
7
8
9
PERCENT OF POPULATION CHOOSING 610 nm.
0
10
20
30
40
50
60
70
80
90
100
TRAINED TO
AVOID 610 nm.
TRAINED TO
AVOID 450 nm.

Chapter IV

GENERAL DISCUSSION

The goal of this work was to integrate behavioral, neural, and photochemical aspects of color vision in an invertebrate. It may be of interest to compare what is now known about color vision in Drosophila with that in the bee. In bees, the behavioral experiments, for example, Daumer's (1956) colorimetric experiments on bees showing trichromatic vision, are as impressive as the comparable psychophysical experiments on humans (Maxwell, 1860), and far surpass any yet done on Drosophila even at the present time. The learned discrimination task described in this thesis presents a case for color vision in Drosophila. Single-cell electrophysiological evidence (Autrum & von Zwehl, 1964), and morphological evidence by selective adaptation of three photoreceptor classes in the bee (Gribakin, 1969) have not yet been obtained in Drosophila. Nevertheless, single cell recordings from a very similar fly, Calliphora, have shown three photoreceptor classes (Burkhardt, 1962; Meffert & Smola, 1976), while selective adaptation ERG studies (Minke, Wu & Pak, 1975a; Stark, 1975) show that three classes of photoreceptors occur in Drosophila. Furthermore, the genetic dissection of the Drosophila photoreceptor system described in this thesis

demonstrates which photoreceptor cell in each ommatidium belongs to which spectral sensitivity class. In the bee the state of knowledge concerning neural connections between the retina, lamina, medulla, lobula, and brain is in a state of relative infancy compared to the state of fly visual neuroanatomy (Braitenberg, 1967; Boschek, 1971; Campos-Ortega & Strausfeld, 1973; Strausfeld & Campos-Ortega, 1973; Strausfeld, 1976). The phototactic study presented in this thesis shows that each class of receptor cells has an input into the behavioral response of the fly, and that these inputs do not appear to be simply additive. These and similar results from phototactic studies on normal flies (Schümperli, 1973) suggest neural wiring mechanisms that keep the input of the different photoreceptor classes separate. Optomotor studies with flies (Eckert, 1971; Heisenberg, 1972) also indicate such neural mechanisms. Finally, very little is known about bee visual pigments, while the work presented in this thesis shows that, in Drosophila, there are three spectrally different photopigments which match up one to one with the different spectral sensitivities of the photoreceptors, and that one type of photopigment is located in each photoreceptor class.

While the evidence presented in this thesis that Drosophila makes learned discriminations between colors (not based on intensity) is, perhaps, the strongest evidence for color vision in this animal, it is important to point out that this is not definitive proof of color vision. The

screening pigments between ommatidia are not equally transparent to all wavelengths; they are especially transparent to red. It may therefore be argued that the Drosophila eye sees a red point source as a blur over several ommatidia and a blue point source as a point. The discriminations between 510 and 450 nm could thus have been made on the basis of pattern, rather than wavelength. It is harder, but still possible, to make this argument about the 350 and 470 nm discrimination, because the screening pigments absorb in vivo almost equally at these two wavelengths (Stark, 1973b). To prove unequivocally that flies have color vision, it will probably be necessary to do colorimetric experiments based on conditioning as was done with bees (Daumer, 1956). Only such experiments would indicate if the trichromatic retinal input is used by the fly to discriminate colors.

That flies are capable of learning is a significant finding in itself, for until recently the state of fly learning was a parallel to the state of invertebrate color vision before von Frisch. Several experiments have now demonstrated learning in flies (Nelson, 1971; Quinn, Harris & Benzer, 1974; Spatz, Emanns & Reichert, 1974). Drosophila has been shown to be capable of learning several things simultaneously (Dudai, unpublished 1976) and to have two memory phases: a short term memory which is sensitive to anesthesia, and a long term memory which is not (Quinn & Dudai, unpublished 1976). Recently, a mutant was isolated which is incapable of learning in the

standard paradigm, but seems behaviorally normal in other tests (Dudai, Jan, Byers, Quinn & Benzer, 1976). The isolation of other learning mutants and the application of genetic, biochemical, and electrophysiological techniques to them, will hopefully lead to an understanding of the learning process in the fly, which may turn out to have general validity for other organisms.

According to Mazokhin-Porshnyakov (1966), flies have a red-receptor with a λ_{max} near 620 nm. Burkhardt (1962) and Goldsmith (1965) argued, however, that there actually is no red-receptor but the appearance of one is an artifact due to the transparency of the screening pigments at long wavelengths. The results in this thesis also reveal the absence of a red-receptor. Selective elimination of photoreceptor classes, one at a time, shows that none of the photoreceptors in the fly's eye is either sensitive to red, or contains a red photopigment, confirming the work of Burkhardt (1962) and Goldsmith (1965).

The three photoreceptor classes found in the Drosophila eye by genetic dissection are not quite the same as the three Burkhardt (1962) found by intracellular recording in Calliphora. He found green (λ_{max} ~490), blue (λ_{max} ~470 nm), and yellow (λ_{max} ~520 nm) receptors, all with secondary peaks in the ultraviolet (350 nm). The results in this thesis are in accord with Schümperli (1973), Minke, Wu and Pak (1975a), Stark (1975) all with Drosophila and Meffert and Smola (1976)

with Calliphora who find that of the three classes of photoreceptors two are blue sensitive (λ_{max} near 480 nm) and one is UV sensitive (λ_{max} near 350 nm).

Horridge and Mimura (1975) and Rosner (1975) claimed two visual pigments in the outer six rhabdomeres of Calliphora, one sensitive to blue and one to UV. Horridge and Mimura (1975) suggested that the rhabdomeres may be twisted and contain the UV photopigment at a different level from the blue photopigment. This would explain their result that the spectral sensitivity of a peripheral photoreceptor is a function of polarization. The results from Drosophila, presented in this thesis, are quite different. Only one photopigment was found in the peripheral cells, and this photopigment, in the rhodopsin state, has peaks in both the UV and the blue. Blue or UV light is effective in converting this rhodopsin to a metarhodopsin with a peak near 580 nm. This does not represent two rhodopsins which convert to two metarhodopsins, both of which absorb maximally near 580 nm since 470 nm light is the most effective in diminishing the amount of UV-sensitive photopigment while UV light is also very effective in diminishing the abount of blue-sensitive photopigment. Furthermore in neither Drosophila nor Musca is there any morphological evidence of rotation in the direction of the rhabdomere microvilli (Waddington & Perry, 1960; Kirschfeld, 1969; Hanson, Ready & Harris, unpublished observations).

The findings of an ultraviolet photopigment located in R7 is in agreement with the microspectrophotometric data of Stavenga, Zantema and Kuiper (1973) which was later retracted by Stavenga (1974). While other research has only been able to find one photopigment in the fly eye, the one from R1-6 (Hamdorf, Paulsen & Schwemer, 1973; Ostroy, Wilson & Pak, 1974), genetic elimination of photoreceptor classes revealed others. Microspectrophotometry on single rhabdomeres of Musca (Langer & Thorell, 1966) was used to demonstrate a difference between the central and peripheral receptor photopigment, and very recent unpublished microspectrophotometry on Musca (Kirschfeld & Franceschini, personal communication) shows three different photopigments, two in the two central rhabdomeres and one in the peripheral.

The waveguide theory of spectral sensitivity proposed by Snyder and Miller (1972) and Snyder and Pask (1973) is in striking agreement with the results presented here. They claimed from the diameter and refractive index of the rhabdomeres that R1-6 should be most effective at absorbing near 490 nm with a secondary peak near 350 nm. R1-6 does contain a rhodopsin with spectral characteristics very similar to this. They claimed R7 should be a UV receptor (λ_{max} near 340 nm). R7 does contain a UV rhodopsin. They claimed that R8 should have a sensitivity like R7 but with the UV peak knocked down, thus it should be a blue receptor (λ_{max} near 470 nm). This is also shown to be the case. They did not

claim that each rhabdomere class had a specific rhodopsin they simply showed that each rhabdomere theoretically absorbed certain wavelengths maximally. That the photopigments found have these wavelengths as λ_{max}'s shows that in the evolution of Drosophila photoreceptors the diameter of the rhabdomeres and the photopigments were matched.

The mutants used in this study especially ora and sev, offer the exciting possibility of the purification of the three photopigments. These mutants eliminate the rhabdomeres of specific photoreceptors and also eliminate their photopigment. In fact, it is entirely conceivable that the ora and sev genes code for the structural protein part (the opsin) of the R1-6 rhodopsin and the R7 rhodopsin respectively, and that absence of these proteins prevents formation of corresponding rhabdomeres. Even if this is not so, identification of bands missing from electrophoretic, chromatographic, or isoelectric focusing patterns of mutant versus normal retinas should aid in the purification of the various rhodopsins.

Ostroy, Wilson & Pak (1974) have shown that, in detergent a photoproduct with the spectral characteristics of retinal is released from the 470 nm (R1-6) rhodopsin. Better characterization of the chromophoric group would be desirable. This released substance should be tested with antimony trichloride, which produces the Carr-Price blue substance, with hydroxylamine which changes retinal to retinal oxime, and

with potassium borohydride which reduces retinal to vitamin A. The probability is nevertheless small that retinal is not the chromophoric group of this visual pigment, because of both the Ostroy, Wilson and Pak (1974) results just mentioned and the effect of vitamin A deprivation on retinal sensitivity (Zimmerman & Goldsmith, 1971; Stark and Zitzmann, 1976). What is of more interest is the verification that the R7 and R8 photopigment use retinal as the chromophoric group. That the R7 photopigment does so is suggested by the increased threshold of R7 induced by vitamin A deprivation (Stark, personal communication). The photochemical tests for retinal described above should also be done on flies in which R1-6 and R1-7 have been genetically eliminated. Only this would prove that all three photopigments use retinal.

It would also be of interest to do thin layer chromatography of thermally denatured visual pigments in the fly, to determine whether the 11-cis to all-trans configuration change occurs when light converts the rhodopsin to metarhodopsin. Combining released chromophore with cattle opsin would also help demonstrate this. Using appropriate mutants, this could be checked for all three photopigments. Indirect evidence that the 11-cis to all-trans change occurs in the chromophore of both R1-6 rhodopsin and R7 rhodopsin comes from the ratio of extinction coefficients of rhodopsin and metarhodopsin. The change from 11-cis to all-trans retinal characteristically leads to increase in extinction coefficient.

This has been shown to occur in the metarhodopsin of squid (Hubbard & St. George, 1958) and Ascalaphus (Paulsen & Schwemer, 1972). The three pigments from Deilephila all have metarhodopsins with higher extinction coefficients than their respective rhodopsins (Höglund, Hamdorf, Langer, Paulsen & Schwemer, 1973). Besides having an extinction coefficient comparable to bovine rhodopsin, fly R1-6 rhodopsin has a metarhodopsin with a higher extinction coefficient (by a factor of 1.3), and R7 rhodopsin also has a metarhodopsin with a higher extinction coefficient (by a factor of 1.8). This is, however, weak evidence for the 11-cis to all-trans isomerization of retinal.

The acid-metarhodopsin alkaline-metarhodopsin scheme which has been shown to exist in the squid (Hubbard & St. George, 1958) and in the insect Ascalaphus (Gogala, Hamdorf & Schwemer, 1970) might also occur in flies, but this has yet to be demonstrated. It would be of particular interest to try this for the R8 photopigment. Since, at neutral pH, there appears to be no difference in spectral sensitivity of the R8 rhodopsin and its meta form, a much better characterization of this pigment could be obtained if it could be converted to a metarhodopsin which absorbs maximally at a different wavelength.

Isolation of the UV photopigment from R7 could permit the investigation of how the chromophore is attached to the protein. If retinal is the chromophoric group, this would

constitute a second example of a rhodopsin isolated with a hypsochromic shift, as in Ascalaphus. It would be of great interest to know whether the UV pigments from Ascalaphus and Drosophila are identical or similar in this linkage.

Aside from their application to the genetic dissection of the chromatic system, mutants that cause selective lesions are of interest in their own right. That ora and sev are rhodopsin genes has been suggested, but what these genes really code for and their role in the development of normal photoreceptors is a subject that warrants investigation. An effort has been made to understand the defect in the rdgB mutant (Harris & Stark, 1975) because hereditary retinal dystrophy in man is not well understood. In Drosophila there is evidence that the rdgB gene codes for a product that is involved as an intermediate in the phototransduction process (Stark & Harris, 1976).

In conclusion, the use of mutants such as these, which eliminate specific classes of photoreceptors, provides a method for genetic dissection of the chemistry, physiology, neural circuitry, and behavioral input of the various types of photoreceptor in the Drosophila retina.

REFERENCES

Abrahamson, E. W. & Fager, R. S., 1973, "The Chemistry of Vertebrate and Invertebrate Visual Photoreceptors", Current Topics in Bioenergetics 5, pp. 125-200.

Adolf, A. R., 1964, "Spontaneous Slow Potential Fluctuations in the Limulus Photoreceptor", J. Gen. Physiol. 48, pp. 297-322.

Alawi, A. A., 1972, "Electrophysiological Investigation of Genetically Dissected Visual Processes in Drosophila melanogaster", Ph.D. Thesis, Purdue University.

Alawi, A. A., Jennings, V., Grossfield, J. & Pak, W., 1972, "Phototransduction Mutants of Drosophila melanogaster", In: Advances in Experimental Medicine and Biology. Vol. 24, ed Arden, G. B., Plenum Press, New York, pp. 1-9.

Alawi, A. A. & Pak, W. L., 1971, "On-transient of Insect Electroretingram: Its Cellular Origin", Science 172, pp. 1055-1057.

Arden, G. B., Ikeda, H. & Siegal, I. M., 1966, "New Components of the Mammalian Receptor Potential and their Relation to Visual Photochemistry", Vision Res. 6, pp. 373-384.

Autrum, H., Autrum, I. & Hoffman, C., 1961, "Komponenten im Retinogramm von Calliphora und ihre Abhängigkeil von

der Spektralfarbe", Biol. Zentralbl. 80, pp. 513-547.

Autrum, H. & Gallwitz, M., 1951, "Zur Analyse de Belichtungspotentiale des Insectenauges", Z. Vergl. Physiol. 33, pp. 407-435.

Autrum, H. & Hoffman, C., 1957, "Die Wirkung von Pikrotoxin und Nikotin auf das Retinogram von Insekten", Z. Naturforsch. 12B, pp. 752-757.

Autrum, H. & Hoffman, C., 1960, "Diphasic and Monophasic Responses in the Compound Eye of Calliphora", J. Insect Physiol. 4, pp. 122-127.

Autrum, H. & Kolb, G., 1968, "Spectrale Empfindlichkeit Einzelner Sehzellen de Aeschniden", Z. Vergl. Physiol. 60, pp. 450-477.

Autrum, H. & von Zwehl, V., 1964, "Die Spektrale Empfindlichkeit Einzelner Sehzellen des Bienenanges", Z. Vergl. Physiol. 48, pp. 357-384.

Bennett, R. R., Tunstall, J. & Horridge, G. A., 1967, "Spectral Sensitivity of Single Retinula Cells of the Locust", Z. Vergl. Physiol. 55, pp. 195-206.

Benzer, S., 1967, "Behavioral Mutants of Drosophila Isolated by Countercurrent Distribution", Proc. Nat. Acad. Sci. 58, pp. 1112-1119.

Bernard, G. D., 1971, "Evidence for Visual Function of Corneal Interference Filters", J. Insect. Physiol. 17, pp. 2287-2300.

Bernard, G. D. & Miller, W. H., 1968, "Interference Filters in the Corneas of Diptera", Investve. Ophth. 7, pp. 416-434.

Bernard, C. G., 1942, "Isolation of Retinal and Optic

Ganglion Responses in the Eye of Dytiscus", J. Neurophysiol. 5, pp. 32-48.

Bertholf, L. H., 1931, "The Distortion of Stimulative Efficiency in the Ultraviolet Spectrum for the Honey-bee", J. Agric. Res. 43, pp. 703-709.

Bertholf, L. M., 1932, "The Extent of the Spectrum for Drosophila and the Distribution of Stimulative Efficiency in it", Z. Vergl. Physiol. 18, pp. 32-64.

Bishop, L. G., 1974, "An Ultraviolet Photoreceptor in a Dipteran Compound Eye", J. Comp. Physiol. 91, pp. 267-275.

Boschek, G. B., 1971, "On the Fine Structures of the Peripheral Retina and Lamina Ganglionaris of the Fly, Musca domestica", Z. Zellforsch. N8, pp. 369-409.

Braitenberg, V., 1967, "Patterns of Projection in the Visual System of the Fly; 1) Retina-Lamina Projections", Exp. Brain. Res. 3, pp. 271-298.

Brammer, J. D. & White, R. H., 1969, "Vitamin A Deficiency: Effect on Mosquito Eye Ultrastructure", Science 163, pp. 821-823.

Brown, F. A. & Hall, V. B., 1936, "The Directive Influence of Light Upon Drosophila melanogaster Meig and some of Its Eye Pigments", J. Exp. Zool. 74, pp. 205-220.

Brown, J. E. & Lisman, J. E., 1972, "An Electrogenic Sodium Pump in Limulus Ventral Photoreceptor Cells", J. Gen. Physiol. 59, pp. 720-733.

Brown, K. T., & Murakami, M., 1964, "A New Receptor Potential in the Monkey Retina with No Detectable Latency", Nature (Lond.) 201, pp. 626-628.

Brown, P. K. & Brown, P. S., 1958, "Visual Pigments of the Octopus and Cuttlefish", Nature (Lond.) 182, pp. 1288-1290.

Brown, P. K. & White, R. H., 1972, "Rhodopsin of the Larval Mosquito", J. Gen. Physiol. 59, pp. 401-414.

Bruckmoser, P., 1968, "Die Spektrale Empfindlichkeit Einzelner Sehzellen des Ruckenschwimmers Notonecta glauca L.", Z. Vergl. Physiol. 59, pp. 187-204.

Burkhardt, D., 1962,"Spectral Sensitivity and Other Response Characteristics of Single Visual Cells in the Arthropod Eye", Symp. Soc. Exp. Biol. 16, pp. 86-109.

Burkhardt, D. & Autrum, H., 1960, "Die Belichtungspotentiale Einzelner Sehzellen von Calliphora erythrocephala Meig.", Z. Naturforsch. 15B, pp. 612-616.

Cajal, S. R. y. & Sánchez, D., 1915, "Contribucion al Conocimento de los Centros Nerviosos de los Insectos", Trab. Lab. Invest. Biol. Univ. Madrid 13, pp. 1-164.

Campos-Ortega, J. A. & Strausfeld, N. J., 1973, "Synaptic Connections of Intrinsic Cells and Basket Arborizations in the External Plexiform Layer of the Fly's Eye", Brain Res. 59, pp. 119-136.

Carpenter, F. W., 1905, "The Reactions of the Pomace Fly (Drosophila ampelophila Loew) to Light, Gravity and Mechanical Stimulation", Amer. Nat. 39, pp. 151-171.

Carricaburu, P. & Chardenot, P., 1967, "Spectres d'Absorption de la Cornee de quelques Arthropodes", Vis. Res. 7, pp. 43-50.

Colquhoun, D., 1971, Lectures on Biostatistics, Clarendon Press, London.

Cone, R. A., 1964, "Early Receptor Potential of the Vertebrate Retina", Nature (Lond.) 204, pp. 736-739.

Cone, R. A., 1967, "Early Receptor Potential: Photoreversible Charge Displacement in Rhodopsin", Science 155, pp. 1128-1131.

Cosens, D. & Briscoe, D., 1972, "A Switch Phenomenon in the Compound Eye of the White-Eyed Mutant of Drosophila melanogaster", J. Insect Physiol. 18, pp. 627-632.

Cosens, D. J. & Manning, A., 1969, "Abnormal Electroretinogram from a Drosophila Mutant", Nature (Lond.) 224, pp. 285-287.

Cosens, D. & Wright, R., 1975, "Light Elicited Isolation of the Complementary Visual Input Systems in White-eyed Drosophila", J. Insect Physiol. 21, pp. 1111-1120.

Daumer, K., 1956, "Reizmetrische Untersuchung des Farbensehens der Biene", Z. Vergl. Physiol. 38, pp. 413-478.

Dethier, V. G., 1966, "Insects and the Concept of Motivation", Nebr. Symp. Motiv. 14, pp. 105-136.

Dethier, V. G., Solomon, R. L. & L. Turner, L. H., 1965, "Sensory Input and Central Excitation and Inhibition in the Blowfly", J. Comp. Physiol. Psychol. 60, pp. 303-313.

Dietrich, W., 1909, "Die Facetten-Augen der Dipteran", Z. Wiss. Zool. 92, pp. 465-535.

Dobzhansky, T., Judson, C. L. & Pavlovsky, O., 1974, "Behavior in Different Environments of Populations of Drosophila pseudoobscura Selected for Phototaxis and Geotaxis", Proc. Nat. Acad. Sci. 71, pp. 1974-1976.

Dobzhansky, T. & Spassky, B., 1967, "Effects of Selection and Migration on Geotactic and Phototactic Behavior of Drosophila", Proc. Roy. Soc.Lond. B 168, pp. 27-47.

Dodge, F. A., Knight, B. W. & Toyoda, J., 1968, "Voltage Noise in Limulus Visual Cells", Science 160, pp. 88-90.

Dudai, Y., Jan, Y. N., Byers, D., Quinn, W. G. & Benzer, S., 1976, "dunce, A Mutant of Drosophila Deficient in Learning", Proc. Nat. Acad. Sci., (in press).

Eakin, R. M., 1963, "Lines of Evolution of Photoreceptors", in: General Physiology of Cell Specialization, eds. Mazia, D. & Tyler, A., McGraw-Hill, New York, pp. 393-425.

Eakin, R. M., 1972, "Structure of Invertebrate Photoreceptors", Handbook of Sensory Physiology VII/1, pp. 625-684.

Ebrey, T. G. & Honig, B., 1975, "Molecular Aspects of Photoreceptor Function", Quart. Rev. Biophys. 8, pp. 129-184.

Eckert, H., 1971, "Die Spektrale Empfindlichkeit des Komplexa ges von Musca (Bestimmung aus Messungen de Optomotorischen Reaction", Kybernetik 9, pp. 145-156.

Eguchi, E., 1971, "Fine Structure and Spectral Sensitivities of Retinular Cells in the Dorsal Sector of the Compound Eyes in the Dragonfly Aeschna", Z. Vergl. Physiol. 71, pp. 201-218.

Eguchi, E. & Waterman, T. H., 1967, "Changes in Retinal Fine Structure Induced in the Crab Libinia by Light and Dark Adaptation", Z. Zellforsch. 79, pp. 209-229.

Eguchi, E., Waterman, T. H. & Akiyama, J., 1973, "Localization of the Violet and Yellow Receptor Cells in the Crayfish Retina", J. Gen. Physiol. 62, pp. 355-374.

Eichenbaum, D. M. & Goldsmith, T. H., 1968, "Properties of Insect Photoreceptor Cells Lacking Synapses", J. Exp. Zool. 169, pp. 15-32.

Exner, S., 1891, Die Physiologie der facettierten Augen von Krebsen und Insecten, Franz Deuticke, Vienna.

Fingerman, M., 1952, "The Role of the Eye Pigments of Drosophila melanogaster in Photic Orientation", J. Exp. Zool. 120, pp. 131-164.

Fingerman, M. & Brown, F. A., 1952, "A 'Purkinje Shift' in Insect Vision", Science 116, pp. 171-172.

Fingerman, M. & Brown, F. A., 1953, "Color Discrimination and Physiological Duplicity of Drosophila Vision", Physiol. Zool. 26, pp. 59-67.

Franceschini, N., 1972, "Pupil and Pseudopupil in the Compound Eye of Drosophila", in: Information Processing in the Visual Systems of Arthropods. ed. Wehner, R., Springer-Verlag, Berlin, pp. 75-82.

Franceschini, N., 1975, "Sampling of the Visual Environment by the Compound Eye of the Fly: Fundamentals and Applications", in: Photoreceptor Optics, eds. Snyder A. W. & Menzel, R., Springer-Verlag, Berlin, pp. 98-125.

Franceschini, N. & Kirschfeld, K. 1971a, "Etude Optique in vivo Eléments Photorécepteurs dans l'Oeil Composé de Drosopila", Kybernetik 8. pp. 1-13.

Franceschini, N. & Kirshfeld, K., 1971b, "Les Phénomènes de Pseudopupille dans L'Oeil Composé de Drosophila", Kybernetik 9, pp. 159-182.

Frings, H., 1941, "The Loci of Olfactory End-Organs in the Blowfly, Cynomyia Cadaverina Desvoidy", J. Exp. Zool. 88, pp. 65-93.

Fuge, H., 1967, "Die Pigmentbildung im Auge von Drosophila melanogaster und ihre Beeinflussung durch de white^{+}-locus", Z. Zellforsch, 83, pp. 468-507.

Fuortes, M. G. F. & O'Bryan, P. M., 1972, "Generator Potentials in Invertebrate Photoreceptors", Handbook of Sensory Physiology, VII/2, pp. 279-319.

Fuortes, M. G. F. & Yeandle, S., 1964, "Probability of Occurence of Discrete Potential Waves in the Eye of Limulus", J. Gen. Physiol. 47, pp. 433-463.

Gogala, M., 1967, "Die Spektrale Empfindlichkeit der Doppelaugen von Ascalaphus macaronius Scop. (Neuroptera, Ascalaphidae)", Z. Vergl. Physiol. 57, pp. 232-243.

Gogala, M. K., Hamdorf, K. & Schwemer, S., 1970, "UV-Sehfarbstaff bei Insekten", Z. Vergl. Physiol. 70, pp. 410-413.

Goldsmith, T. H., 1958, "The Visual System of the Honeybee", Proc. Nat. Acad. Sci. 44, pp. 123-126.

Goldsmith, T. H., 1960, "The Nature of the Retinal Action Potential and the Spectral Sensitivities of the Ultra-Violet and Green Receptor Systems of the Compound Eye of the Worker Honeybee", J. Gen. Physiol. 43, pp. 775-799.

Goldsmith, T. H., 1965, "Do Flies Have a Red Receptor?", J. Gen. Physiol. 49, pp. 265-287.

Goldsmith, T. H., 1972, "The Natural History of Invertebrate Visual Pigments", Handbook of Sensory Physiology VII/1, pp. 685-719.

Goldsmith, T. H., 1974, "Metarhodopsin and the Visual Cycle of Arthropods", A.R.V.O. Sarasota (abstract).

Goldsmith, T. H., Barker, R. J. & Cohen, C. F., 1964, "Sensitivity of Visual Receptors of Carotenoid-Depleted Flies: a Vitamin A Deficiency in an Invertebrate", Science 146, pp. 65-67.

Goldsmith, T. H. & Bernard, G. D., 1974, "The Visual System of Insects" in: The Physiology of Insecta II, second edition, ed. Rockstein, M., Academic Press, New York, pp. 165-272.

Goldsmith, T. H. & Fernández, H. R., 1966, "Some Photochemical and Physiological Aspects of Visual Excitation in Compound Eyes", In: The Functional Organization of the Compound Eye, ed. Bernhard, C. A., Pergamon Press, Oxford, pp. 125-143.

Goldsmith, T. H. & Fernández, H. R., 1968, "The Sensitivity of Housefly Photoreceptors in the Mid-Ultraviolet and the Limits of the Spectrum", J. Exp. Biol. 49, pp. 669-677.

Goldsmith, T. H. & Warner, L. T., 1964, "Vitamin A in the Vision of Insects", J. Gen. Physiol. 47, pp. 433-441.

Götz, K. G., 1970, "Fractionation of Drosophila Populations According to Optomotor Traits", J. Exp. Biol. 52, pp. 419-436.

Grenacher, G. H., 1879, Untersuchungen über das Sehorgan der Arthropoden, insbesondere de Spinin. Insecten und Crustaceen, Vanderhoek u. Ruprecht, Göttingen.

Gribakin, F. A., 1969, "Cellular Basis of Colour Vision in the Honey Bee ", Nature (Lond.) 223, pp. 639-641.

Gribakin, F. G., 1972, "The Distribution of the Long Wave Photoreceptors in the Compound Eye of the Honey Bee as Revealed by Selective Osmic Staining", Vis. Res. 12,

pp. 1225-1230.

Hadler, N. M., 1964, "Heritability and Phototaxis in Drosophila melanogaster", Genetics 50, pp. 1269-1277.

Hagins, W. A. & McGaughy, R. E., 1967, "Molecular and Thermal Origins of Fast Photoelectric Effects in the Squid Retina", Science 157, pp. 813-816.

Hamdorf, K., Paulsen, R., Schwemer, J. & Täuber, U., 1972, "Photoreconversion of Invertebrate Visual Pigments", in: Information Processing in the Visual Systems of Arthoropods, ed. Wehner, R., Springer-Verlag, Berlin, pp. 97-108.

Hamdorf, K. & Rosner, G., 1973, "Adaptation und Photoregeneration in Fliegenauge", J. Comp. Physiol. 86, pp. 281-292.

Hamdorf, K., Schwemer, J. & Täuber, U., 1968, "Der Sehfarbstaff, die Absorption der Receptoren und die Spektrale Empfindlichkeit der Retina von Eledone moschata", Z. Vergl. Physiol. 60, pp. 375-415.

Hamdorf, K., Schwemer, J. & Gogala, M., 1971, "Insect Visual Pigment Sensitive to Ultraviolet Light", Nature (Lond.) 231, pp. 458-459.

Hamilton, W. F., 1922, "A Direct Method of Testing Color Vision in Lower Animals", Proc. Nat. Acad. Sci. 8, pp. 350-353.

Hara, T. & Hara, R., 1967, "Rhodopsin and Retinochrome in the Octopus Retina", Nature (Lond.) 214, pp. 572-573.

Harris, W. A. & Stark, W. S., 1975, "Light Sensitive Hereditary Photoreceptor Degeneration in Drosophila", A.R.V.O. Sarasota (abstract).

Hecht, S. & Wald, G., 1934, "The Visual Acuity and Intensity Discrimination of Drosophila", J. Gen. Physiol. 17, pp. 517-547.

Heisenberg, M., 1971, "Separation of Receptor and Lamina Potentials in the Electroretinogram of Normal and Mutant Drosophila", J. Exp. Biol. 55, pp. 85-100.

Heisenberg, M., 1972, "Comparative Behavioral Studies on Two Mutants of Drosophila", J. Comp. Physiol. 80, pp. 119-136.

Heisenberg, M. & Götz, K. G., 1975, "The Use of Mutations for the Partial Degradation of Vision in Drosophila melanogaster", J. Comp. Physiol. 98, pp. 217-241.

Hershberger, W. A. & Smith, M. P., 1967, "Conditioning in Drosophila melanogaster", Anim. Behav. 15, pp. 259-262.

Hertz, M., 1939, "New Experiments on Color Vision in Bees", J. Exp. Biol. 16, pp. 1-8.

Hillman, P., Dodge, F. A., Hochstein, S., Knight, K. B. & Minke, B., 1973, "Rapid Dark Recovery of the Invertebrate Early Receptor Potential", J. Gen. Physiol. 62, pp. 77-86.

Hillman, P., Hochstein, S. & Minke, B., 1972, "A Visual Pigment with Two Physiologically Active Stable States",

Science 175, pp. 1486-1488.

Hirsch, J., 1962, "Individual Differences in Behavior and their Genetic Basis", in: Roots of Behavior, ed. Bliss, E. L., Hafner, Hoeber, N. Y., pp. 3-23.

Hirsch, J. & Boudreau, J. C., 1958, "Studies in Experimental Behavior Genetics. I. The Heritability of Phototaxis in a Population of Drosophila melanogaster", J. Comp. Physiol. Psychol. 51, pp. 647-651.

Hochstein. S., Minke, B. & Hillman, P., 1973, "Antagonistic Components of the Late Receptor Potential in the Barnacle Photoreceptor Arising from Different Stages of the Pigment Process", J. Gen. Physiol. 60, pp. 105-128.

Hoffmann, C. & Langer, H., 1961, "Die Spektrale Augenempfindlichkeit der Mutante "chalky" von Calliphora enthrocephala", Naturwissen 48, pp. 605.

Höglund, G., Hamdorf, K., Langer, H., Paulsen, R. & Schwemer, J., 1973, "The Photopigments in an Insect Retina", in: Biochemistry and Physiology of Visual Pigments, ed. Langer, H., Springer-Verlag, New York, pp. 167-174.

Horridge, G. A., 1969a, "The Eye of the Firefly Photuris", Proc. Roy. Soc. B 171, pp. 445-463.

Horridge, G. A., 1969b, "Unit Studies on the Retina of Dragonflies", Z. Vergl. Physiol. 62, pp. 1-37.

Horridge, G. A., 1971, "Alternative to Superposition Images

in Clear-Zone Compound Eyes", Proc. R. Soc. Lond. B 179, pp. 97-124.

Horridge, G. A., 1975, in: Photoreceptor Optics, ed. Snyder, A. W., & Menzel, R., Springer, Berlin, cited by Duncan, G., 1976, Nature (Lond.) 260, pp. 201.

Horridge, G. A. & Mimura, K., 1975, "Fly Photoreceptors I. Physical Separation of Two Visual Pigments in Calliphora Retinula Cells 1-6", Proc. R. Soc. Lond. B

Horridge, G. A., Mimura, K. & Tsukuhara, Y., 1975, "Fly Photoreceptors II. Spectral and Polarized Light Sensitivity in the Drone Fly Eristalis", Proc. R. Soc. Lond. B 190, pp. 225-237.

Hotta, Y., & Benzer, S., 1970, "Genetic Dissection of the Drosophila Nervous System by Means of Mosaics", Proc. Nat. Acad. Sci. 67, pp. 1156-1163.

Hotta, Y. & Benzer, S., 1972, "Mapping of Behaviour in Drosophila Mosaics", Nature (Lond.) 240, pp. 527-535.

Hubbard, R., 1958, "Bleaching of Rhodopsin by Light and by Heat", Nature (Lond.) 181, pp. 1126.

Hubbard, R. & Kropf, A., 1958, "The Action of Light on Rhodopsin", Proc. Nat. Acad. Sci. 44, pp. 130-139.

Hubbard, R. & St. George, R. C. C., 1958, "The Rhodopsin System of the Squid", J. Gen. Physiol. 41, pp. 501-528.

Isle, D., 1949, "Colour Discrimination in the Dronefly, Eristalis tenax", Nature (Lond.) 163, pp. 255-256.

Jahn, T. J. & Wulff, V. J., 1942, "Allocation of Electrical Responses from the Compound Eye of Grasshoppers", J. Gen. Physiol. 26, pp. 75-88.

Järvelehto, H. & Zettler, F., 1970, "Micro-localization of the Lamina-located Visual Cell Activities in the Compound Eye of the Blowfly Calliphora", Z. Vergl. Physiol. 69, pp. 134-138.

Järvelehto, M. & Zettler, F., 1971, "Localized Intracellular Potentials from Pre- and Postsynaptic Components in the External Plexiform Layer of an Insect Retina", Z. Vergl. Physiol. 75, pp. 422-440.

Kalmus, H., 1943, "The Optomotor Responses of Some Eye Mutants of Drosophila", J. Genet. 45, pp. 206-213.

Kirschfeld, K., 1966, "Discrete and Graded Receptor Potentials in the Compound Eye of the Fly, Musca", in: The Functional Organization of the Compound Eye, ed. Bernhard, C. G., Pergamon Press, Oxford, pp. 291-307.

Kirschfeld, K., 1967, "Die Projection der Optischen Umwelt auf das Raster der Rhabodmere im Komplexauge von Musca", Exp. Brain Res. 3, pp. 248-370.

Kirschfeld, K., 1969, "Optics of the Compound Eye", in: Processing of Optical Data by Organisms and by Machines, ed. Reichardt, W., Academic Press, New York, pp. 144-166.

Kirschfeld, K. & Franceschini, N., 1968, Optishe Eigenschaften der Ommatidien im Komplexauge von Musca",

Kybernetik 5, pp. 47-52.

Kirschfeld, K. & Franceschini, N., 1969, "Ein Mechanisms zur Steuerung des Lichtflusses im den Rhabdomeren de Komplexauges von Musca", Kybernetik 6, pp. 13-22.

Koenig, J. & Merriam, J. R., 1975, "Autosomal ERG Mutants", Dros. Info. Service (in press).

Kolb, G., Autrum, H. & Eguchi, E., 1969, "Die Spektrale Transmission des Dioptrischen Apparates von Aeschna cyanea Müll", Z. Vergl. Physiol. 63, pp. 434-439.

Kropf, A., Brown, P. K. & Hubbard, R., 1959, "Lumi and Meta-Rhodopsins of Squid and Octopus", Nature (Lond.) 183, pp. 446-450.

Kühn, A., 1927, "Über den Farbensin der Bienen", Z. Vergl. Physiol. 5, pp. 762-800.

Kühn, A., Pohl, R., 1921, "Dressurfahigkelt der Bienen auf Spektrallinien", Naturwissen. 9, pp. 738-740.

Langer, H., 1972, "Metarhodopin in Single Rhabdomes of the Fly, Calliphora erythrocephala" in: Information Processing in the Visual Systems of Arthropods, ed. Wehner, R., Springer Verlag, Berlin, pp. 109-113.

Langer, H., 1973, "Discussion on Stavenga, Zantema and Kuiper, 1973" in: Biochemistry and Physiology of Visual Pigments, ed. Langer, H., Spring-Verlag, New York, pp. 179-180.

Langer, H. & Thorell, B., 1966, "Microspectrophotometry of Single Rhabdomeres in the Insect Eye", Exp. Cell. Res. 41,

pp. 673-677.

La Vail, M. M. & Mullen, R. J., 1974, "Analysis of the Pigment Epithelium in Mice with Inherited Retinal Degeneration Using Experimental Chimeras", A.R.V.O. Sarasota (abstract).

Lindsley, D. L. & Grell, E. H., 1968, Genetic Variations of Drosophila melanogaster, Carnegie Institution of Washington Publication, Oak Ridge.

Lisman, J. E., & Brown, J. E., 1972, "The Effects of Intracellular Iontophoretic Injection of Calcium and Sodium Ions on the Light Response of Limulus Ventral Photoreceptors", J. Gen. Physiol. 59, pp. 701-719.

Lisman, J. E. & Brown, J. E., 1975a, "Light-Induced Changes of Sensitivity in Limulus Ventral Photoreceptors", J. Gen. Physiol. 66, pp. 473-488.

Lisman, J. E. & Brown, J. E., 1975b, "Effects of Intracellular Injection of Calcium Buffers on Light Adaptation in Limulus Ventral Photoreceptors", J. Gen. Physiol. 66, pp. 489-506.

Lutz, F. E. & Richtmyer, F. K., 1922, "The Reaction of Drosophila to Ultraviolet", Science 55, pp. 519.

Manning, A., 1967, "'Pre-imaginal Conditioning' in Drosophila", Nature (Lond.) 216, pp. 338-340.

Marak, G. E., Gallik, G. J. & Cornesky, R. A., 1970, "Light Sensitive Pigments in Insect Heads", Ophthal. Res. 1, pp. 65-71.

Maxwell, J. C., 1860, "On the Theory of Compound Colours, and the Relations of the Colours of the Spectrum", Phil. Trans. Roy. Soc. (Lond.) 150, pp. 57-84.

Mazokhin-Porshnyakov, G. A., 1959, "Green, Yellow and Orange Colour Discrimination by Bees", Biophysics 4, pp. 47-54.

Mazokhin-Porshnyakov, G. A., 1960a, "Colormetric Study of the Properties of Colour Vision of Insects as Exemplified by the House-Fly", Biophysics 5, pp. 340-349.

Mazokhin-Porshnyakov, G. A., 1960b, "System of Colour Vision of the Fly Calliphora", Biophysics 5, pp. 790-797.

Mazokhin-Porshnyakov, G. A., 1966, "'Red' Light Receptor of Flies and Colour Vision of Drosophila melanogaster", Biophysics 11, pp. 1184-1192.

McCann, G. D. & Arnett, D. W., 1972, "Spectral and Polarization Sensitivity of the Dipteran Visual System", J. Gen. Physiol. 59, pp. 534-558.

McEwen, R. S., 1918, "The Reactions to Light and to Gravity in Drosophila and its Mutants", Z. Exp. Zool. 25, pp. 49-106.

McReynolds, J. S. & Gorman, A. L. F., 1970, "Photoreceptor Potentials of Opposite Polarity in the Eye of the Scallop, Pecten irradians", J. Gen. Physiol. 56, pp. 376-391.

Meffert, P. & Smola, U., 1976, "Electrophysiological Measurements of Spectral Sensitivity of Central Visual Cells in Eye of Blowfly", Nature (Lond.) 260, pp. 342-344.

Millechia, R. & Mauro, A., 1969, "The Ventral Photoreceptor Cells of Limulus. II The Basic Photoresponse", J. Gen. Physiol. 54, pp. 310-330.

Minke, B., Hochstein, S. & Hillman, P., 1973a, "Early Receptor Potential Evidence for the Existence of Two Thermally Stable States in the Barnacle Visual Pigment", J. Gen. Physiol. 62, pp. 87-104.

Minke, B., Hochstein, S. & Hillman, P., 1973b, "Antagonistic Process as Source of Visible-Light Suppression of Afterpotentials in Limulus UV Photoreceptors", J. Gen. Physiol. 62, pp. 787-791.

Minke, B., Wu, C.-F. & Pak, W. L., 1975a, "Isolation of Light-Induced Response of the Central Retinula Cells from the Electroretinogram of Drosophilia", J. Comp. Physiol. 98, pp. 345-355.

Minke, B., Wu, C.-F. & Pak, W. L., 1975b, "Induction of Photoreceptor Voltage Noise in the Dark in Drosophila Mutant", Nature (Lond.) 258, pp. 84-87.

Mote, M. I. & Goldsmith, T. H., 1970, "Spectral Sensitivities of Color Receptors in the Compound Eye of the Cockroach Periplaneta", J. Exp. Zool. 173, pp. 137-146.

Muijlser, H., Leutscher-Hazelhoff, J. T., Stavenga, D. A. & Kuiper, J. W., 1975, "Photopigment Conversions Expressed in Receptor Potential and Membrane Resistance of Blowfly Visual Sense Cells", Nature (Loud.) 254, pp. 520-522.

Murphey, R. M., 1967, "Instrumental Conditioning of the Fruit Fly, Drosophila melanogaster", Anim. Behav. 15, pp. 153-161.

Naka, K. I., 1961, "Recording of Retinal Action Potentials from Single Cells in the Insect Compound Eye", J. Gen. Physiol. 44, pp. 571-584.

Naka, K. I. & Eguchi, E., 1962a, "Spike Potentials Recorded from the Insect Photoreceptor", J. Gen. Physiol. 45, pp. 663-680.

Naka, K. I. & Eguchi, E., 1962b, "Effect of Background Illumination on the Retinal Action Potential", Science 136, pp. 877-879.

Naka, K. I. & Kuwabara, M., 1959, "Electrical Responses from the Compound Eye of Lucilia", J. Insect Physiol. 3, pp. 41-49.

Nelson, M. C., 1971, "Classical Conditioning in the Blowfly (Phormia Regina): Associative and Excitatory Factors", J. Comp. Physiol. Psychol. 77, pp. 353-368.

Nolte, N. J., 1961, "The Pigment Granules in the Compound Eyes of Drosophila", Heredity (Lond.) 16, pp. 25-38.

Nolte, J. & Brown, J. E., 1972, "Ultraviolet-Induced Sensitivity to Visible Light in Ultraviolet Receptors of Limulus", J. Gen. Physiol. 59, pp. 186-200.

Nolte, J., Brown, J. E. & Smith, T. G. Jr., 1968, "A Hyperpolarizing Component of the Receptor Potential in the Median Ocellus of Limulus", Science 162, pp. 677-679.

Ostroy, S. E., Wilson, M. & Pak, W. L., 1974, "Drosophila Rhodopsin: Photochemistry, Extraction and Differences

in the norpA^{P12} Phototransduction Mutant", Biochem. Biophys. Res. Com. 59, pp. 960-966.

Pak, W. L., 1965, "Some properties of the Early Electrical Response in the Vertebrate Retina", Cold Spring Harbor Symp. Quant. Biol. 30, pp. 493-499.

Pak, W. L., 1976, "Mutations Affecting the Vision of Drosophila melanogaster", in: Handbook of Genetics, Vol. 3, ed. King, R. C., Plenum, New York, pp. 703-733.

Pak, W. L. & Boes, R., 1967, "Rhodopsin: Responses from Transient Intermediates Formed During its Bleaching", Science 155, pp. 1131-1133.

Pak, W. L., Grossfield, J. & White, N. V., 1969, "Nonphototactic Mutants in a Study of Vision of Drosophila melanogaster", Nature (Lond.) 222, pp. 351-354.

Pak, W. L. & Liddington, K. J., 1974, "Fast Electrical Potential from a Long-Lived, Long-Wavelength Photoproduct of Fly Visual Pigment", J. Gen. Physiol. 63, pp. 740-755.

Paulsen, R. & Schwemer, J., 1972, "Studies on the Insect Visual Pigment Sensitive to Ultraviolet Light: Retinal as the Chromophoric Group", Biochim. Biophys. Acta. 283, pp. 520-529.

Payne, F., 1911, "Drosophila ampelophila Loew Bred in the Dark for Sixty-Nine Generations", Biol. Bull. 21, pp. 297-301.

Perry, M. M., 1968a, "Further Studies on the Development of the Eye of Drosophila melanogaster I. The Ommatidia", J. Morph. 124, pp. 227-248.

Perry, M. M., 1968b, "Further Studies on the Development of the Eye of Drosophila melanogaster II. The Interommatidial Bristles", J. Morph. 124, pp. 249-262.

Poodry, C. A. & Schneiderman, H. A., 1970, "The Ultrastructure of the Developing Leg of Drosophila melanogaster", Wilhelm Roux' Archiv. 166, pp. 1-44.

Quinn, W. G. & Dudai, Y., 1976, "Memory Phases in Drosophila", (submitted for publication).

Quinn, W. G., Harris, W. A. & Benzer, S., 1974, "Condtioned Behavior in the Drosophila melanogaster", Proc. Nat. Acad. Sci. 71, pp. 708-712.

Reynolds, E. S. Jr., 1963, "The Use of Lead Citrate at High pH as an Electron Opaque Stain in Electron Microscopy", J. Cell. Biol. 17, pp. 208-212.

Roeder, K. D., 1963, Nerve Cells and Insect Behavior, Harvard Univ. Press, Cambridge, Massachusetts.

Rosner, G., 1975, "Adaptation und Photoregeneration in Fliegenauge", J. Comp. Physiol. 102, pp. 269-295.

Ruck, P., 1961, "Photoreceptor Cell Responses and Flicker Fusion Frequency in the Compound Eye of the Fly, Lucilia Sericata (Meigen)," Biol. Bull. 120, pp. 375-383.

St. George, R. C. C. & Wald, G., 1949, "The Photosensitive

Pigment of the Squid Retina", Biol. Bull. 97, pp. 248.

Scholes, J., 1965, "Discontinuity of the Excitation Process in Locust Visual Cells", Cold Spring Harbor Symp. Quant. Biol. 30, pp. 517-527.

Scholes, J., 1969, "The Electrical Responses of the Retinal Receptors and the Lamina in the Visual System of the Fly, Musca", Kybernetik 6, pp. 149-162.

Schümperli, R. A., 1973, "Evidence for Color Vision in Drosophila melanogaster through Spontaneous Phototactic Choice Behavior", J. Comp. Physiol. 86, pp. 77-94.

Scott, J. P., 1947, "Effects of Single Genes on the Behavior of Drosophila", Am. Nat. 77, pp. 184-190.

Shaw, S. R., 1967, "Simultaneous Recording from Two Cells in the Locust Retina", Z. Vergl. Physiol. 55, pp. 183-194.

Shoup, J. B., 1966, "The Development of Pigment Granules in the Eyes of Wild-Type and Mutant Drosophila melanogaster", J. Cell. Biol. 29, pp. 223-249.

Smith, T. G. & Brown, J. E., 1966, "A Photoelectric Potential in Invertebrate Cells", Nature (Lond). 212, pp. 1217-1219

Smola, U. & Meffert, P., 1975, "A Single-peaked UV-Receptor in the Eye of Calliphora erythrocephala", J. Comp. Physiol. 103, pp. 353-357.

Snyder, A. W. & Miller, W. H. 1972, "Fly Color Vision Vision Res. 12, pp. 1389-1396.

Snyder, A. W. & Pask, C., 1973, "Spectral Sensitivity of Dipteran Retinula Cells", J. Comp. Physiol. 84, pp. 59-76.

Spatz, H. C., Emanns, A. & Reichert, H., 1974, "Associative Learning in Drosophila melanogaster", Nature (Lond.) 248, pp. 359-361.

Stark, W. S., 1973a, "The Effect of Eye Color Pigments on the Action Spectrum of Drosophila", J. Insect Physiol. 19, pp. 999-1006.

Stark, W. S., 1973b, "Studies of the Transient and Steady-State Components of the Electroretinogram of Drosophila", Ph.D. Thesis, University of Wisconsin.

Stark, W. S., 1975, "Spectral Sensitivity of Visual Response Alterations Mediated by Interconversions of Native and Intermediate Photopigments in Drosophila", J. Comp. Physiol. 96, pp. 343-356.

Stark, W. S. & Harris, W. A., 1976, "Physiological, Genetic, and Vitamin A Deprivation Studies of Retinal Degeneration and Abnormal Adaptation in Drosophila", A.R.V.O. Sarasota (abstract).

Stark, W. S. & Wasserman, G. S., 1974, "Wavelength-Specific ERG Characteristics of Pigmented and White-Eyed Strains of Drosophila", J. Comp. Physiol. 91, pp. 427-441.

Stark, W. S. & Zitzmann, W. G., 1976, Isolation of Adaptation Mechanisms and Photopigment Spectra by Vitamin A Deprivation in Drosophila", J. Comp. Physiol. 105,

pp. 15-27.

Stavenga, D. G., 1974, "Visual Receptor Optics, Rhodopsin and Pupil in Fly Retinula Cells", Ph.D. Thesis, University of Groningen.

Stavenga, D. G., Zantema, A. and Kuiper, J. W., 1973, "Rhodopsin Processes and the Function of the Pupil Mechanism in Flies", in: Biochemistry and Physiology of Visual Pigments, ed. Langer, H., Springer, Berlin, pp. 175-179.

Strausfield, N. H., 1976, Atlas of an Insect Brain, Springer Verlag, Berlin.

Strausfeld, N. J. & Campos-Ortega, J. A., 1973, "The L4 Monopolar Neurone: A Substrate for Lateral Interaction in the Visual System of the Fly Musca Domestica (L.)", Brain Res. 59, pp. 97-117.

Thorpe, W. H., 1939, "Further Studies on Pre-imaginal Olfactory Conditioning in Insects", Proc. Roy. Soc. Lond. B 127, pp. 424-433.

Trujillo-Cenóz, O., 1965, "Some Aspects of the Structural Organization of the Intermediate Retina of Dipterans", J. Ultra. Res. 13, pp. 1-33.

Trujillo-Cenóz, O. & Melamed, J., 1966, Electron Microscope Observations on the Peripheral and Intermediate Retinas of Dipteras", in: The Functional Organization of the Compound Eye, ed. Bernhard, C. G., Pergamon Press, Oxford, pp. 339-361.

Tsuneki, K., 1953, "On Color Vision in Two Species of Ants, with Special Emphasis on Their Relative Sensibility to Various Monochromatic Lights", Jap. J. Zool. 11, pp. 187-221.

von Frisch, K., 1914, "Der Farbensinn und Formensinn der Beien", Zool. Jb. Physiol. 35, pp. 1-188.

Waddington, C. H. & Perry, M. M., 1960, "The Ultrastructure of the Developing Eye of Drosophila", Proc. R. Soc. B. 153, pp.155-178.

Wald, G., 1941, "Vitamin A in Invertebrate Eyes", Amer. J. Physiol. 133, pp. P479-P480.

Wald, G. & Brown, P. K., 1953, "The Molar Extinction of Rhodopsin", J. Gen. Physiol. 37, pp. 189-200.

Wald, G. & Burg, S. P., 1957, "The Vitamin A of the Lobster", J. Gen. Physiol. 40, pp. 609-625.

Walther, J. B., 1958, "Changes Induced in Spectral Sensitivity and Form of Retinal Action Potential of the Cockroach Eye by Selective Adaptation", J. Insect Physiol. 2, pp. 142-151.

Walther, J. B. & Dodt, E., 1959, "Die Spektrale Sensitivitat von Insecten Komplexaugen im Ultraviolet bis 290 mμ", Z. Naturforsch. 14b, pp. 273-278.

Washizu, Y., 1964, "Electrical Activity of Single Retinular Cells in the Compound Eye of the Blowfly, Calliphora erythrocephalis (Meig.)", Comp. Biochem. Physiol. 12, pp. 369-387.

Wehner, R. & Toggweiler, F., 1972, "Verhaltensphyiologischer Nachweis de Farbensehens bei Cataglyphis bicolor (Formicidae, Hymenoptera)", J. Comp. Physiol. 77, pp. 239-255.

White, R. H., 1967, "The Effect of Light and Light Deprivation upon the Ultrastructure of the Larval Mosquito Eye: II. The Rhabdom", J. Exp. Zool. 166, pp. 405-425.

White, R. H. & Lord, E., 1975, "Diminution and Enlargement of the Mosquito Rhobdom in Light and Darkness", J. Gen. Physiol. 65, pp. 583-598.

Wolbarsht, M. L., Wagner, H. & Bodenstein, D., 1966, "Origin of Electrical Responses in the Eye of Periplaneta americana", in: The Functional Organization of the Compound Eye, ed. Bernhard, C. G., Pergamon Press, Oxford, pp. 207-217.

Wolken, J. J., 1971, Invertebrate Photoreceptors, Academic Press, New York.

Wolken, J. J., 1975, Photoprocesses, Photoreceptors and Evolution, Academic Press, New York.

Wolken, J. J., Bowness, J. M. & Scheer, I. J., 1960, "The Visual Complex of the Insect: Retinene in the Housefly", Biochim. Biophys. Acta. 43, pp. 531-537.

Woolf, C. M., 1972, "Genetic Analysis of Geotactic and Phototactic Behavior in Selected Strains of Drosophila pseudoobscura", Behav. Genet. 2, pp. 93-106.

Wu, C.-F. & Pak, W. L., 1975, "Quantal Basis of Photoreceptor Spectral Sensitivity of Drosophila melanogaster", J. Gen. Physiol. 66, pp. 149-168.

Yeandle, S., 1957, "Studies on the Slow Potential and the Effects of Cations on the Electrical Responses of the

Limulus Ommatidium", Ph.D. Thesis, Johns Hopkins University.

Yeatman, F. R. & Hirsch, J., 1971, "Attempted Replication of, and Selective Breeding for, Instrumental Conditioning of *Drosophila melanogaster*", *Anim. Behav.* *19*, pp. 454-462.

Yoshizawa, T. & Wald, G., 1964, "Transformations of Squid Rhodopsin at Low Temperatures", *Nature (Lond.)* *201*, pp. 340-345.

Zimmerman, W. F. & Goldsmith, T. H., 1971, "Photosensitivity of the Circadian Rhythm and of Visual Receptors in Carotenoid-Depleted *Drosophila*", *Science* *171*, pp. 1167-1169.