Mutation That Selectively Affects Rhodopsin Concentration in the Peripheral Photoreceptors of *Drosophila melanogaster*

DENIS C. LARRIVEE, SHERRY K. CONRAD,
ROBERT S. STEPHENSON, and WILLIAM L. PAK

From the Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907. Dr. Larrivee's present address is the Department of Biology, Yale University, New Haven, Connecticut 06520.

ABSTRACT A *Drosophila* mutant ($ninaA^{P228}$) that is low in rhodopsin concentration but identical to the wild-type fly in photoreceptor morphology has been isolated. R1–6 photoreceptors of the mutant differ from those of wild type in that (*a*) the prolonged depolarizing afterpotential (PDA) is absent, (*b*) concentrations of rhodopsin and opsin are substantially reduced, and (*c*) intramembrane particle density in the membranes of the rhabdomeres is low. Each of these traits is mimicked by depriving wild-type flies of vitamin A. The $ninaA^{P228}$ mutation differs from vitamin A deprivation in that in the mutant (*a*) the rhabdomeric membrane particle density is reduced only in the R1–6 photoreceptors and not in R7 or R8, (*b*) the PDA can be elicited from the R7 photoreceptors, and (*c*) photoconversion of R1–6 rhodopsin to metarhodopsin by ultraviolet (UV) light is considerably more efficient than in vitamin A-deprived flies. The absorption properties of the mutant rhodopsin in the R1–6 photoreceptors appear to be identical to those of wild type as judged from rhodopsin difference spectra. The results suggest that the mutation affects the opsin, rather than the chromophore, component of rhodopsin molecules in the R1–6 photoreceptors. The interaction between the chromophore and R1–6 opsin, however, appears to be normal.

INTRODUCTION

Because of its basic importance to the understanding of sensory receptor function and probably also of neuronal excitation processes in general, the mechanism of phototransduction has been of intense interest to many investigators. In recent years, many of these investigators have focused their attention on subcellular and molecular mechanisms of the process (see Hubbell and Bownds [1979]; Pober and Bitensky [1979]; Shichi and Rafferty [1980]). The basic mechanisms of phototransduction, however, remain largely unknown. For example, although several different kinds of protein are likely to

be involved in phototransduction, the only proteins unequivocally known to be involved in it are the visual pigment proteins, rhodopsins. Moreover, in spite of a considerable body of information on rhodopsin that now exists in the literature (e.g., Yoshizawa and Tokunaga [1979]; Shichi and Rafferty [1980]), to date the only role that can be clearly attributed to rhodopsin is that of light capture.

Most of the existing information on rhodopsin comes from extensive studies on vertebrate rhodopsins (e.g., Kropf [1972]; Ebrey and Honig [1975]; Ostroy [1977]). Available evidence suggests that invertebrate rhodopsins have properties basically similar to those of vertebrates. Invertebrate rhodopsins, for example, have 11-cis retinals as their chromophores (Hubbard and St. George, 1958; Hara and Hara, 1967; Paulsen and Schwemer, 1972), which isomerize to the all-trans form on illumination, and have a molecular weight of ~40,000 (Paulsen and Schwemer, 1973; Hagins, 1973; Ostroy, 1978; Stein et al., 1980). Moreover, the rhodopsin proteins apparently comprise a major fraction of membrane proteins in both the vertebrate photoreceptor outer segments (Hall et al., 1969; Bownds et al., 1971; Robinson et al., 1972; Heitzman, 1972), and the invertebrate rhabdomeres (Hagins, 1973; Boschek and Hamdorf, 1976; Harris et al., 1977). Thus, available information is consistent with the interpretation that rhodopsin plays basically the same role in both vertebrate and invertebrate photoreceptors.

One of the most direct ways to assess the role of rhodopsin is to alter the molecular composition and structure of opsin or to eliminate opsin molecules entirely from the photoreceptor and to see what effects such manipulations have on the physiology and biochemistry of the living photoreceptor, but it has not been possible to manipulate rhodopsin in this way. Molecular manipulations of rhodopsin can be achieved, however, if there are organisms available that carry a mutation in the structural gene for opsin, i.e., the gene that codes for the amino acid sequence of opsin. The mutant we describe in this report is of considerable interest in this respect because it appears to carry a lesion in either the structural gene for opsin or some other gene closely associated with opsin function.

We have isolated in the past few years a number of mutants with drastically reduced rhodopsin content (Pak, 1979; Pak et al., 1980). These mutations fall into five complementation groups, three on the second chromosome and two on the third. We describe in this paper the properties of one of these mutations, $ninaA^{P228}$, in some detail. A striking feature of the $ninaA^{P228}$ mutation is that it affects the concentration of rhodopsin in one particular class of photoreceptors (R1–6), but not that of rhodopsin in the other classes of photoreceptors (R7 or R8).

MATERIALS AND METHODS

Materials

All experiments were performed on the fruit fly *Drosophila melanogaster*. The flies used in this work include: wild-type flies of the Oregon R strain, vitamin A-deprived wild-

type flies, the second-chromosome mutant $ninaA^{P228}$ (neither inactivation nor after-potential A; allele designation, P228; see Pak [1979]), and the first chromosome mutant sev^{LY3} (sevenless; see Harris et al. [1976]). Nearly all flies used had their eye color pigments removed genetically using the mutation white (w). We found no evidence that removal of the eye color pigments affected either the concentration or absorption properties of the rhodopsin photopigments. Eliminating the eye-color pigments removed extraneous absorbance that otherwise would have interfered with absorbance measurements of fly rhodopsin. Moreover, the removal of the eye color pigments facilitated the induction of the prolonged depolarizing afterpotential (PDA).

Vitamin A-deprived flies were produced by raising wild-type flies for one generation on Sang's medium (1956), which does not contain carotenoids. Penicillin G (6.3 mg/ 100 ml of medium), streptomycin sulfate (60 mg/100 ml of medium), propionic acid (0.15 ml/100 ml of medium), and methyl parahydroxybenzoate (0.15 g/100 ml of medium) were emulsified with polysorbate 80 (2.6 ml/100 ml of medium) and added to the medium to prevent the growth of microorganisms, which are capable of synthesizing vitamin A.

The second-chromosome recessive mutant $ninaA^{P228}$ was isolated by chemically mutagenizing the Oregon R wild-type strain and screening for defects in the electro-retinogram (ERG) (Pak, 1979). It is named for its characteristic electrophysiological phenotype. Various properties of the mutant, including its ERG, are detailed in Results. The mutation $ninaA^{P228}$ was mapped on the second chromosome between aristaless (2–0.01) and dumpy (2–13.0), using multiply marked second chromosomes (N. J. Scavarda and F. Wong, unpublished data). Cytological mapping placed the mutation within the limits of the deficiency Df(2L)S3 (N. E. Kremer and F. Wong, unpublished data), which has break points at 21D2-3 and 21F2-22A1 (Lindsley and Grell, 1968).

The sex-linked recessive mutant sev^{LY3} was obtained from the Benzer laboratory, California Institute of Technology, Pasadena, Calif. According to Harris et al. (1976), the mutation eliminates the rhabdomeres of R7 photoreceptors without affecting other classes of photoreceptors. According to Campos-Ortega et al. (1979), however, the entire R7 cells are missing in the mutant.

Rhodopsin, Opsin, and Membrane Particle Analyses

The procedures for the extraction and measurement of rhodopsin, the extraction and electrophoretic analysis of opsin, and freeze-fracture electron microscopy and the determination of membrane particle densities have been described in detail elsewhere (Larrivee, 1979).[1] Briefly, to obtain an extract of rhodopsin for absorbance measurements, flies were dark-adapted overnight and frozen. Their heads were removed, homogenized, and extracted into a 2% digitonin solution (Ostroy, 1978). Absorption spectra were obtained from the extract with a Cary 14 spectrophotometer (Varian Associates, Inc., Palo Alto, Calif.). After taking an initial spectrum from an unillu-minated sample, the sample was subjected alternately to intense blue or red illumi-nation. An absorption spectrum was taken after each blue or red illumination. From a series of absorption spectra so obtained, difference spectra were constructed by taking the difference between each two successive absorption spectra. The mean spectrum was calculated for each sample from several such difference spectra.

Opsin extracts were prepared as described above, except that the digitonin extracts

---

[1] Larrivee, D. C., R. Schinz, S. E. Ostroy, and W. L. Pak. A biochemical analysis of membrane particles in the rhabdomeres of *Drosophila melanogaster*. Manuscript in preparation.

were subsequently treated with 5% sodium dodecyl sulfate (SDS) and then subjected to electrophoresis on 1% SDS, 10% polyacrylamide, cylindrical gels.

To examine the rhabdomeric, intramembrane cytostructure, the eyes were frozen in liquid nitrogen and then fractured with a Balzers freeze etch apparatus (model BA 360M; Balzers High Vacuum Corp., Santa Ana, Calif.). Rhabdomeric membrane particles were visualized with a Philips 300 electron microscope (Philips Electronic Instruments, Inc., Mahwah, N. Y.). The membrane-particle densities were obtained by counting the particles observed on the protoplasmic surface of the microvillar membrane within a defined area.

Electroretinogram (ERG)

The ERGs were recorded with glass microelectrodes pulled from 1.0-mm outer diameter pyrex capillary tubing on a Narishige vertical electrode puller (Narishige Scientific Instrument Laboratory, Tokyo, Japan) and filled by cooling them in Hoyle's saline. The recording electrode was positioned with its tip just puncturing the cornea, and the reference electrode was placed near the base of the proboscis. Voltage signals were amplified and recorded by means of a high-impedance microprobe amplifier (model 725; W-P Instruments, Inc., New Haven, Conn.), a Tektronix 502A oscilloscope (Tektronix, Inc., Beaverton, Oreg.), and a Brush 220 strip chart recorder (Gould Inc., Instruments Div., Cleveland, Ohio). The light source was a Bausch & Lomb xenon lamp (Bausch & Lomb Inc., Rochester, N. Y.), filtered by broad band filters (Corning Glass Works, Corning, N. Y.).

M-potential

The M-potential is a biphasic response that arises from photoexcitation of metarhodopsin and can be observed in the initial portion of the ERG response to intense orange light (Pak and Lidington, 1974) (Fig. 9 A). It is composed of a small, corneal-negative deflection (M_1), followed by a much larger, corneal-positive deflection (M_2). The M_1 is a true early receptor potential (ERP), whereas the M_2 arises from transsynaptic excitation of second-order neurons by the M_1 (Stephenson and Pak, 1980; Minke and Kirschfeld, 1980). Because the M-potential is small in ninaA or vitamin A-deprived flies, we were able to measure only the larger, M_2 component accurately. Though not an ERP, this component is related in an approximately linear manner to the amount of metarhodopsin photoexcited by the M-potential-eliciting flash, or to the amount of metarhodopsin present in the eye before the flash (Pak and Lidington, 1974; Minke and Kirschfeld, 1980). Stephenson and Pak (1978 and 1980) have shown that in the presence of a PDA the M_2 is reduced, causing a departure from linearity. Because both ninaA and vitamin A-deprived flies lack the PDA, however, this error affected only the measurements from wild-type flies on a normal diet and then only for large amounts of blue or UV pre-illumination. Comparison of the M_2-based plot (Fig. 9 B) from such flies with a similar plot based on the M_1, moreover, showed that this error had only a slight effect on the slope of the graph.

The recording conditions for the M-potential were similar to those described above except for precautions taken to prevent photoartifacts. The orange stimulus flash (0.5-ms duration) originated from a 60-J photographic strobe lamp (Strobonar 65C; Honeywell, Inc., Denver, Colo.) and was delivered to the eye by means of a bifurcated fiber optics light guide (Galileo Electro-Optics Corp., Sturbridge, Mass.). Light from the strobe lamp passed through two heat filters (KG-1, Klinger Scientific Corp., Richmond Hill, N. Y.) and a sharp-cut orange filter (Corning CS 3-67). The intensity of the unattenuated orange strobe flash was 1.5 × 10^{16} photons/cm^2, as measured

with a calibrated photodiode (Lite Mike; Edgerton, Germeshausen & Grier, Inc., Boston, Mass.) and a monochrometer (model 33-86-02; Bausch & Lomb Inc.).

The adapting light used in the photoconversion efficiency measurements came from the xenon lamp filtered by 480-nm blue or 361-nm UV interference filters. Unattenuated intensities were 2×10^{16} and 4×10^{14} photons$\cdot$cm^{-2}s^{-1} for blue and UV, respectively, although during the experiments the blue light was attenuated 100-fold by a neutral density filter. Intensities were measured after each experiment at the level of the fly's eye, using a 0.4-mm pinhole and a radiometer (model J16; Tektronix, Inc.). The efficiency of the radiometer for the UV light (relative to its efficiency for blue light) was determined by comparison with a photomultiplier (model 700-24; Gamma Scientific, Inc., San Diego, Calif.) of known spectral response.

The basic experimental procedure was to start with a fly that had been orange-adapted, expose the fly to a blue adapting light of short duration, and measure the M-potential elicited from the fly by an orange strobe flash. The fly was then subjected to a series of five orange flashes to convert any remaining metarhodopsin back to rhodopsin. This procedure was repeated for successively longer periods of blue illumination, and the M-potential was elicited after each blue illumination until the M-potential amplitude reached a saturated level. To complete an experimental run, the procedure was continued in the reverse order with progressively shorter periods of blue illumination. For each fly, the entire run was repeated once more using blue pre-illuminations and twice using UV pre-illuminations.

The M-potential data were analyzed according to the method described by Kirschfeld et al. (1977) and Minke and Kirschfeld (1979). The differences between the saturated M₂ amplitude and its amplitudes after varying amounts of blue or UV pre-illumination (*ordinate*) were plotted against the amount (intensity × duration) of pre-illumination (*abscissa*) in a semi-log plot (Fig. 9 *B–D*). Before each pre-illumination, the eye was orange-adapted, converting virtually all the visual pigment to rhodopsin. Therefore, the amplitude of the M-potential after a given pre-illumination corresponds to the net amount of rhodopsin photoconverted to metarhodopsin by the pre-illumination, saturated amplitudes occurring when the visual pigment had attained a photoequilibrium with respect to the pre-illumination. The difference between the saturated M-potential amplitude and the amplitude of the M-potential elicited after a given amount of blue or UV pre-illumination is linearly related to the amount of rhodopsin remaining after the pre-illumination. The graphs described above (Fig. 9 *B–D*), therefore, plot the relative amount of rhodopsin remaining after varying amounts of blue or UV illumination against the amount of pre-illumination used. A steeper slope in such a semi-log plot corresponds to a faster approach to equilibrium (or relaxation) of the photopigment.

Prolonged Depolarizing Afterpotential (PDA)

The compound eye of *Drosophila* and other muscoid flies contains three anatomically distinct classes of photoreceptor: the peripheral photoreceptors R1–6, central photoreceptor R7, and central photoreceptor R8. These classes differ with respect to the PDA.[2] Intense blue or UV stimuli induce a PDA in R1–6 photoreceptors of wild-type

---

[2] According to Harris et al. (1976), the rhodopsin of the central photoreceptor R7 of *Drosophila* absorbs maximally at 370 nm and photoconverts to a metarhodopsin absorbing maximally at 470 nm, whereas the rhodopsin of R8 absorbs maximally at about 490 nm, but cannot be converted to a spectrally distinguishable metarhodopsin. In *Musca*, microspectrophotometric measurements indicate that the difference spectrum of R7 UV cells shows a peak at ~470 nm,

flies (Fig. 1, *top*), whereas orange or red stimuli cancel it. Photoreceptor R8, on the other hand, shows no PDA at all (Harris et al., 1976). In the case of R7, UV light induces a PDA, whereas blue light cancels it (Stark et al., 1976). In the closely related fly *Calliphora*, intracellular recording has revealed two types of R7 (Hardie, 1979). 30% of R7 photoreceptors are of the UV (or 7p) type. In these R7 photoreceptors, UV light induces, and blue light depresses a PDA, whereas in the remainder (designated
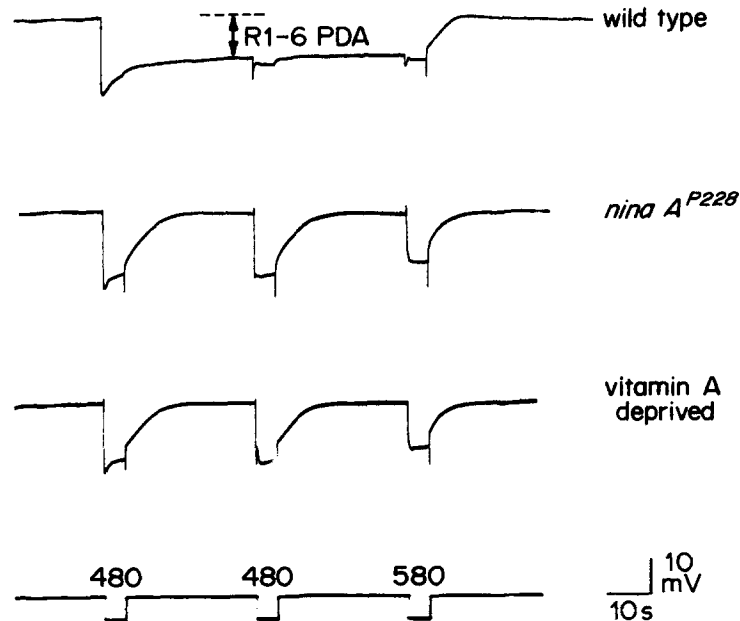

FIGURE 1.   ERG recordings from wild-type (raised on either a normal or vitamin A-deficient medium) and mutant *Drosophila*. In the wild-type fly, the initial blue stimulus (Corning filter CS4-104; 5.3×10^{15} photons·cm^{-2} s^{-1}) induces a long-lasting PDA in the peripheral photoreceptors (R1–6), whereas a second blue stimulus elicits a response from the central photoreceptors alone. An orange stimulus (Corning CS 2-73; 5.5×10^{16} photons·cm^{-2} s^{-1}) abolishes the PDA. In the *ninaA*^{P228} mutant and in the vitamin A-deprived fly, the blue-induced response quickly returns to the baseline. ERGs were recorded from 4–7-d-old flies at 17°C.

UT, UB, or 7y cells), blue light induces and green light depresses the PDA (Hardie et al., 1979). If, as appears likely, a similar situation prevails in *Drosophila*, then the UV-induced PDA mentioned above reflects only the R7 UV cells.

The afterpotential that results in the ERG when a wild-type *Drosophila* eye is illuminated with blue light (Fig. 1, *top*) reflects primarily the PDA in the R1–6

---

presumably corresponding to the metarhodopsin peak. In the case of R7 UT cells, the corresponding peak is shifted toward longer wavelengths by ~50 nm. In both cases, the difference spectra are nearly zero at 580 nm (Kirschfeld, 1979). The predominant rhodopsin, however, is that of R1–6, which in *Drosophila* absorbs maximally at 480 nm and photointerconverts with a thermostable metarhodopsin absorbing maximally at ~580 nm (Ostroy et al., 1974; Harris et al., 1976).

photoreceptors. We shall refer to this as an R1–6 PDA, notwithstanding that (*a*) it is recorded extracellularly and (*b*) cells other than R1–6, such as R7 UT and pigment cells, may contribute to it. The scheme we used to observe the PDA in the R7 UV photoreceptors in extracellular recordings is illustrated in Fig. 6. In this method, the eye was constantly illuminated with a 600-nm orange light (6 × 10^{15} photons/cm^{-2}· s^{-1}), and blue (480 nm) and UV (Corning CS 7-51) stimuli were presented alternately, separated by 30-s intervals. After each stimulus, the orange background quickly killed any PDA generated in R1–6 but did not affect the R7 UV photoreceptors, because its metarhodopsin absorbs in the blue. The increment in the ERG after a UV stimulus was thus due primarily to a PDA in R7 UV cells. We shall refer to this increment as an R7 PDA, recognizing as before the liberty we are taking in doing so. We measured the R7 PDA 30 s after the end of the UV stimulus. Because the amplitude of the R7 PDA was only a few millivolts at most, it was measured at least three times in each fly and the values were averaged. The UV stimulus was sufficiently bright that in 1 s it induced a nearly full R1–6 PDA.

RESULTS

The mutant *ninaA* was initially isolated on the basis of its ERG (Pak, 1979). Under our experimental conditions, an unattenuated blue stimulus, ≥0.1 s in duration, will induce a PDA in the wild-type fly (Fig. 1, *top tracing*). Subsequent blue stimuli elicit only small ERG responses from the central photoreceptors (R7 and R8), superimposed on the PDA (Minke et al., 1975). In contrast to the wild-type fly, a stimulus of comparable intensity and duration does not induce a PDA in the mutant (Fig. 1, *middle tracing*), and subsequent blue stimuli elicit responses that are similar in amplitude and wave form to the initial response. Similarly, vitamin A deprivation also causes the loss of the PDA (Fig. 1, *bottom tracing*), as was reported previously (Stark and Zitzmann, 1976).

We have measured the relative rhodopsin concentration in the mutant using extracted preparations. Although *Drosophila* has at least three different visual pigments contained in three anatomically distinct classes of photoreceptors,[2] the predominant rhodopsin is that of the peripheral photoreceptors, since these are larger and more numerous than the central ones. Because of this and the spectral properties of the photopigments in the central photoreceptors,[2] difference spectra of extracted preparations almost exclusively reflect absorbance changes of the photopigment in the R1–6 photoreceptors. Fig. 2 shows the difference spectra obtained from wild-type and mutant flies (see Materials and Methods). The similarity of the shape of these spectra suggest that the absorption spectra of both rhodopsin and metarhodopsin of the mutant photopigment are similar to those of the wild-type photopigment. However, the absorbances obtained from the mutant pigment are considerably smaller in magnitude than those obtained from the wild-type pigment, indicative of the lower concentration of R1–6 rhodopsin in the mutant. The relative amount of rhodopsin present can be obtained by taking the ratio of mutant to wild-type absorbance changes at 580 nm. (It is generally preferable to measure the pigment concentration at the metarhodopsin peak rather than the rhodopsin peak because this measurement uses the relatively larger

absorbance of metarhodopsin and avoids the possible contribution to absorption measurements by R7 metarhodopsin).[2] Table I shows the results of absorption measurements of wild-type, mutant, and vitamin A-deprived (A⁻) flies at 578 nm and the rhodopsin concentrations of the mutant and A⁻ flies relative to that of the wild-type fly obtained from the measurements. The
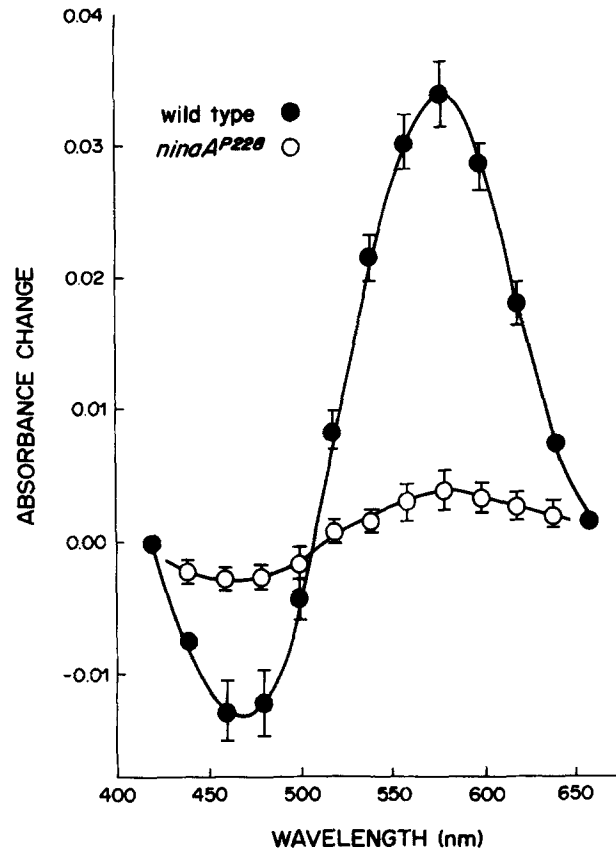

FIGURE 2. In vitro rhodopsin difference spectra obtained from digitonin extracts of 1,000 heads of wild-type and mutant flies. The absorbance of a dark-adapted sample was recorded between 400 and 660 nm with a Cary 14 spectrophotometer at 10°C. The sample was subsequently illuminated alternately for 5 min with blue light and for 5 min with orange light and scanned after each illumination. Each scan of the sample was subtracted from the preceding scan at 20-nm intervals to construct a series of difference spectra. These difference spectra were averaged to yield a single mean difference spectrum for each sample. The spectra shown in this figure are the average of mean difference spectra from several samples (see Table I). Error flags, standard deviations.

mutant $ninaA^{P228}$ has only ~11% the amount of rhodopsin contained in the wild-type fly. The absorption measurements for A⁻ flies are below the noise level, and the corresponding rhodopsin concentration was estimated to be <3% of wild type.

Vitamin A deprivation has been shown to cause the number of rhabdomeric membrane particles in *Drosophila* photoreceptors to decrease by about fourfold (Harris et al., 1977). To determine whether the mutation *ninaA*^{P228} also causes a reduction in membrane particle density, freeze-fracture electron microscopy was carried out on the mutant photoreceptors. Fig. 3 displays high-magnification photographs of freeze-fractured replicas of the R1–6 rhabdomeres of wild-type, mutant, and A$^-$ flies. The freeze-fractured rhabdomere surface typically appears striated with alternating bands of rough and smooth faces. These striations arise as a result of the fracture plane passing through and exposing, alternately, protoplasmic (rough) and exoplasmic (smooth) faces of neighboring microvilli. Numerous particles can be seen on the protoplasmic faces of the microvillar membranes of the wild-type rhabdomere (Fig. 3 *A*). In contrast to wild-type flies, there are considerably fewer particles on the

TABLE I

ABSORBANCE DIFFERENCES Δ*A* OF WILD-TYPE, *ninaA*^{P228}, AND VITAMIN A-DEPRIVED FLIES MEASURED AT 578 nm, AND RHODOPSIN CONCENTRATIONS RELATIVE TO THAT OF THE WILD-TYPE FLY

Type	Number of extracts examined	Δ*A* at 578 nm	Percent of wild type
		mean ± SD	
Wild type	5	0.034±0.003	100±9
ninaA^{P228}	5	0.004±0.0015	11±4
A$^-$	4	<0.001	<3

The analyses were performed on extracts containing 1,000 heads/ml. The path length was 1 cm.

protoplasmic faces of the rhabdomeres of the mutant and A$^-$ fly (Fig. 3 *B* and *C*). We have examined nearly 40 rhabdomeres (9 animals, 3–16 d of age) from peripheral photoreceptors (R1–6) of the mutant. All of them had substantially reduced membrane particle density when compared with wild-type peripheral photoreceptor rhabdomeres.

Harris et al. (1977) have demonstrated that vitamin A deprivation reduces the rhabdomeric particle density of both the peripheral (R1–6) and central (R7 and R8) photoreceptors of *Drosophila*. Our results also show that both the peripheral and central cells are affected by vitamin A deprivation (Table II). By contrast, we found that the mutation *ninaA*^{P228} affects only the peripheral photoreceptors. Fig. 4 displays a replica of a cross-fractured retinula of the mutant fly. In this photograph, four peripheral rhabdomeres (rh_p) surround a central rhabdomere (rh_c), most likely that of R7,[3] seen at the bottom of the

[3] It is possible to distinguish the rhabdomeres of the peripheral photoreceptors from those of the central photoreceptors with a fair degree of certainty. It is much more difficult to distinguish unambigously the rhabdomeres of the two central retinular cells (R7 and R8) from each other. To determine the effect of *ninaA* on R8 rhabdomeres, therefore, Schinz et al. (manuscript in
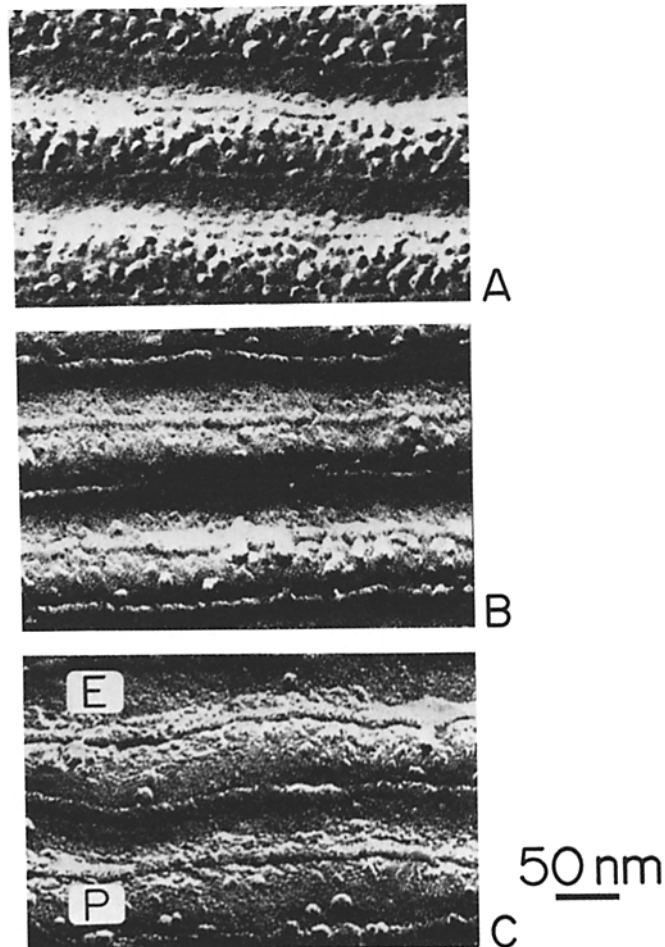
FIGURE 3. Freeze-fracture replicas of rhabdomeres from (A) wild type, (B) the $ninaA^{P228}$ mutant, and (C) vitamin A-deprived flies. Each eye was fractured and then coated with platinum-carbon vapor at a temperature of $-109°C$ and at an angle of $48°$ from the specimen surface. The protoplasmic (P) and exoplasmic (E) microvillar surfaces alternate in most freeze-fractured preparations of rhabdomeres. Numerous particles may be seen on the protoplasmic surface of the wild-type fly. The protoplasmic membrane particle densities of $ninaA^{P228}$ and vitamin A-deprived flies are substantially lower than that of the wild-type fly.

figure. The figure shows clearly that the protoplasmic surfaces of the central photoreceptor microvilli of the mutant contain numerous particles, whereas those of the peripheral photoreceptor microvilli contain substantially fewer

preparation) made use of the mutation *sev* (sevenless) (see Materials and Methods). They constructed double mutant flies carrying both *ninaA* and *sev* and examined the membrane particle density in the R8 rhabdomeres (the only remaining central cell rhabdomeres in these flies) by freeze-fracture electron microscopy. They found that the particle density in R8 rhabdomeres is normal, suggesting that *ninaA* affects neither of the two central retinular cells.

particles. Fig. 5 illustrates a replica of another central photoreceptor rhabdomere at higher magnification and shows the high density of intramembrane particles. We have displayed in Table II the particle density measurements obtained from the peripheral and central photoreceptors of both wild type and the mutant. It can be seen that the rhabdomeric particle density in the peripheral photoreceptors of the mutant is significantly lower than that of either the mutant central photoreceptors or the wild-type photoreceptors of either type, peripheral or central. The rhabdomeric particle density of the mutant central photoreceptors, on the other hand, does not differ significantly from that of either wild-type central or wild-type peripheral photoreceptors. Thus, unlike vitamin A deprivation, the mutation *ninaA* appears to have a specific effect on the peripheral photoreceptors.[3]

The *ninaA*^{P228} mutation is also specific in its effect on the PDA, as shown in Figs. 6 and 7. Fig. 6 is an ERG from the mutant showing the procedure used to measure the R7 PDA (see Materials and Methods). The additional negative-going potential that follows the UV stimulus, indicated by the *arrows*, is the

TABLE II

PARTICLE DENSITIES IN RHABDOMERES OF CENTRAL AND PERIPHERAL PHOTORECEPTORS OF WILD-TYPE AND *ninaA*^{P228} FLIES

Type of fly	Receptor class	Number of cells studied	Particles/μm^2 (mean ± SD)	Percent of wild-type peripheral photoreceptor	Statistical significance*
Wild type	Peripheral	20	3,160±380	100±12	
	Central	10	2,900±440	92±14	No
ninaA^{P228}	Peripheral	37	1,480±310	47±10	Yes
	Central	11	3,060±400	96±13	No
A$^-$	Peripheral	42	1,020±250	32±8	Yes
	Central	6	1,160±220	37±7	Yes

* The particle densities of the *ninaA* photoreceptors (both peripheral and central) and of the wild-type central photoreceptors were compared with those of the wild-type peripheral photoreceptors by means of Cochran's (1964) application of the Student's *t* test at the confidence level of 99%.

R7 PDA. The magnitude of this R7 PDA, 1.2 mV after 30 s, is typical of wild-type flies. Fig. 7 plots the amplitude of the R7 PDA against that of the R1–6 PDA for wild-type flies, the mutant *ninaA*^{228}, the mutant *sev*^{LY3} (sevenless), and wild type deprived to varying degrees of vitamin A. Each point in Fig. 7 represents a single fly. Vitamin A deprivation reduces both R1–6 and R7 PDAs, although the R1–6 PDA appears more sensitive to mild deprivation than the R7 PDA. The mutation *ninaA*^{P228}, on the other hand, specifically reduces the R1–6 PDA. The mutation *sev*^{LY3} is known to eliminate specifically the R7 rhabdomeres (Harris et al., 1976) or R7 cells (Campos-Ortega et al., 1979), and thus reduces the R7 PDA without affecting that in R1–6. The small amount of R7 PDA remaining in this mutant may indicate that *sev*^{LY3} does not eliminate the PDA in 100% of R7 photoreceptors. On the other hand, it may also indicate, as noted in Materials and Methods, that what we have referred to as the R7 PDA contains a small contribution from cells other than R7.
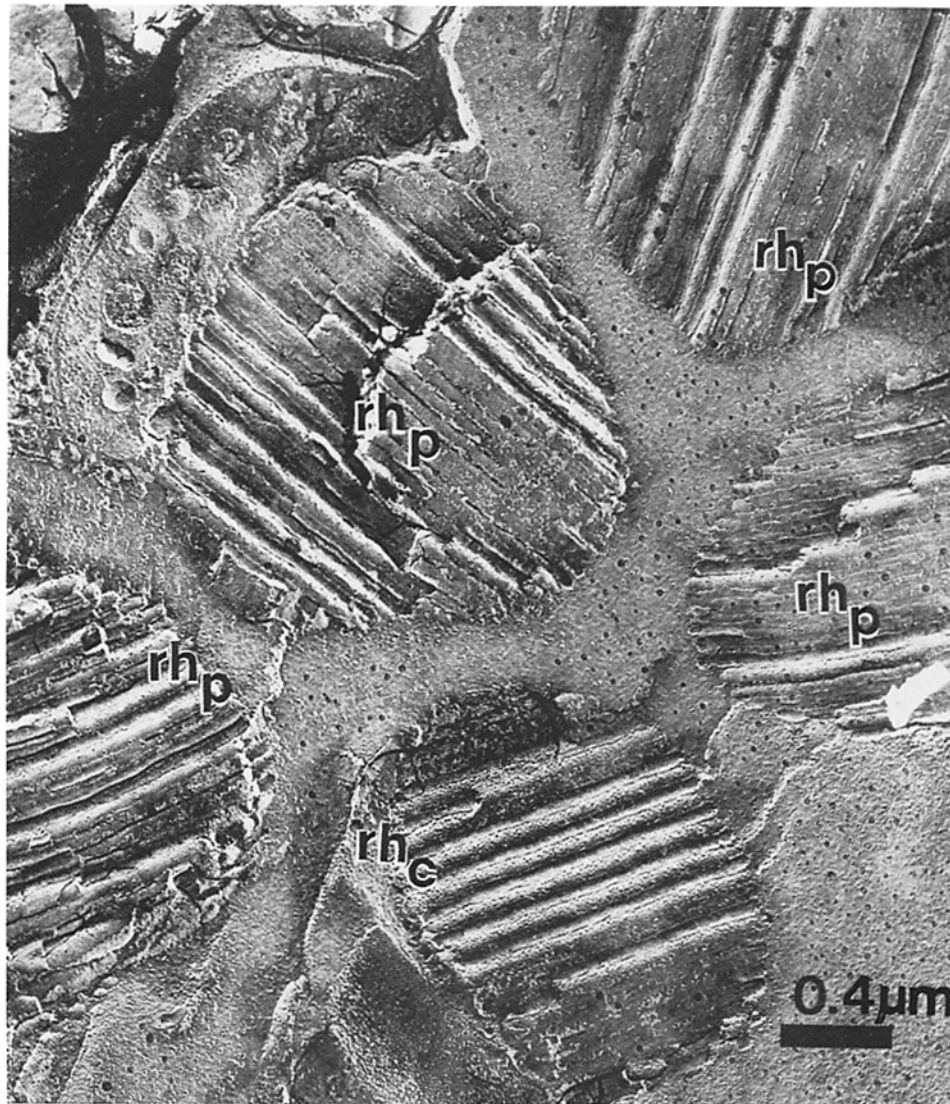
FIGURE 4. Freeze-fracture replica of a retinula from the mutant $ninaA^{P228}$. The rhabdomere of a central cell (rh_c) is surrounded by the rhabdomeres of four peripheral cells (rh_p). Two other peripheral rhabdomeres lie outside the field of view. Note the high density of protoplasmic rhabdomeric membrane particles of the central cell in comparison with the low densities in the peripheral cells.

A reduction in either spectrally determined rhodopsin concentration or membrane particle density does not necessarily indicate a reduction in opsin concentration, because opsin molecules that lack chromophores do not contribute to spectral measurements of rhodopsin. Therefore, we made independent determinations of the mutant opsin concentration. Fig. 8 displays gel scans of extracts from wild-type, mutant, and A⁻ flies. Both the mutant and

the A^- fly scans are superimposed on scans taken from their respective wild-type controls. Eight electrophoretic runs were made of each class of fly, and in all instances the opsin peaks of both the mutant and the A^- fly were substantially less than that of the wild-type control. Thus, the results suggest
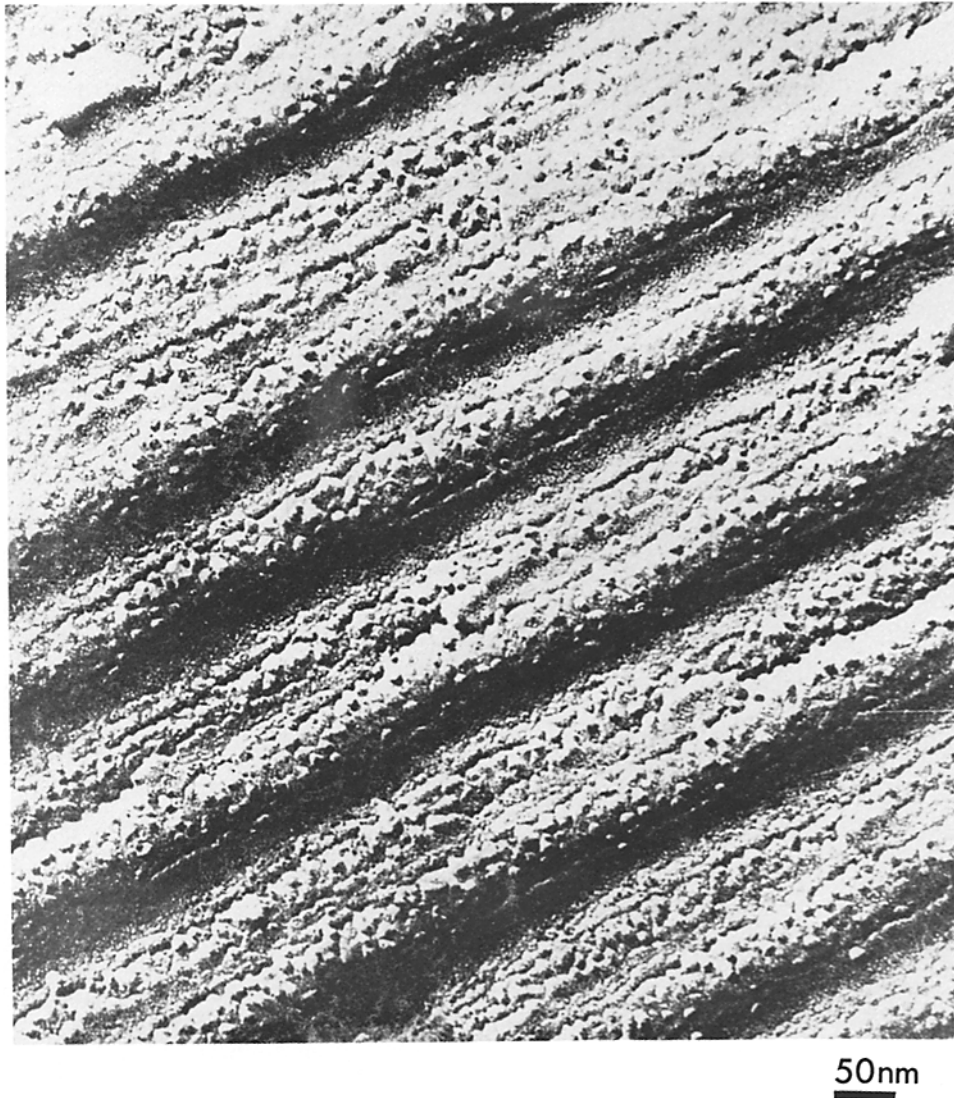

50nm

FIGURE 5.   A replica of another central photoreceptor rhabdomere from the mutant $ninaA^{P228}$ at a higher magnification than the previous photograph.

that the mutant is deficient in not only the retinal chromophore but also the opsin protein.

Previous investigators have shown that concurrent with a reduction in rhodopsin concentrations caused by vitamin A deprivation (Razmjoo and
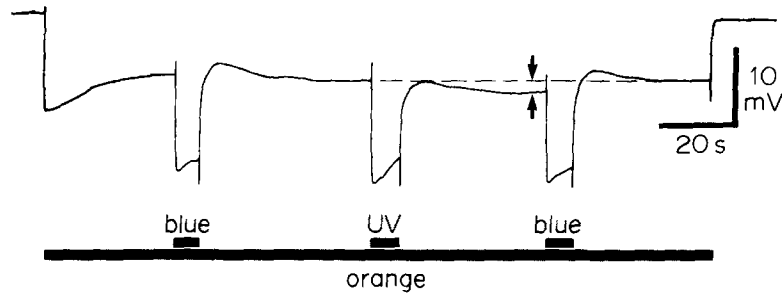
Figure 6.   ERG from the mutant ninaA^{P228} demonstrating the R7 PDA. The UV (Corning CS 7-51 filter) and blue (480 nm) stimuli alternately induce and cancel a PDA in R7 photoreceptors, respectively, whereas the orange background cancels any PDA induced by these stimuli in R1–6. The arrows indicate the difference in potential due to the R7 PDA.
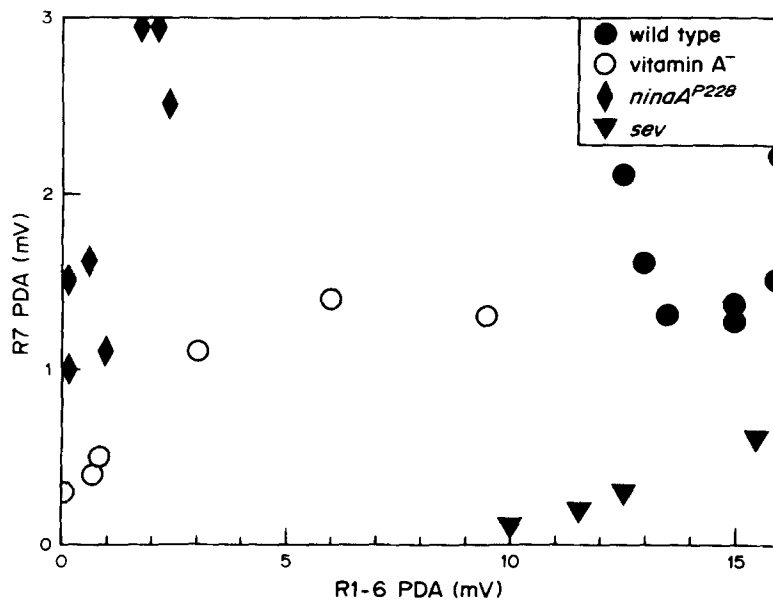

Figure 7.   Scattergram plotting R7 PDA against R1–6 PDA for wild type, wild type partially deprived of vitamin A, and the mutants ninaA^{P228} and sev^{LY3}. Each point represents a single fly. Both R1–6 and R7 PDAs were measured 30 s after the end of the PDA-inducing stimulus.

Hamdorf, 1976; Harris et al., 1977), there is a reduction in visual sensitivity in diptera (Goldsmith et al., 1964; Zimmerman and Goldsmith, 1971; Stark and Zitzmann, 1976). The reduction in sensitivity is greater in the UV than in the blue (Stark et al., 1977), because the efficiency with which rhodopsin is converted to metarhodopsin by UV light decreases more than that by blue light (Kirschfeld et al., 1977). We have examined what effect the ninaA lesion
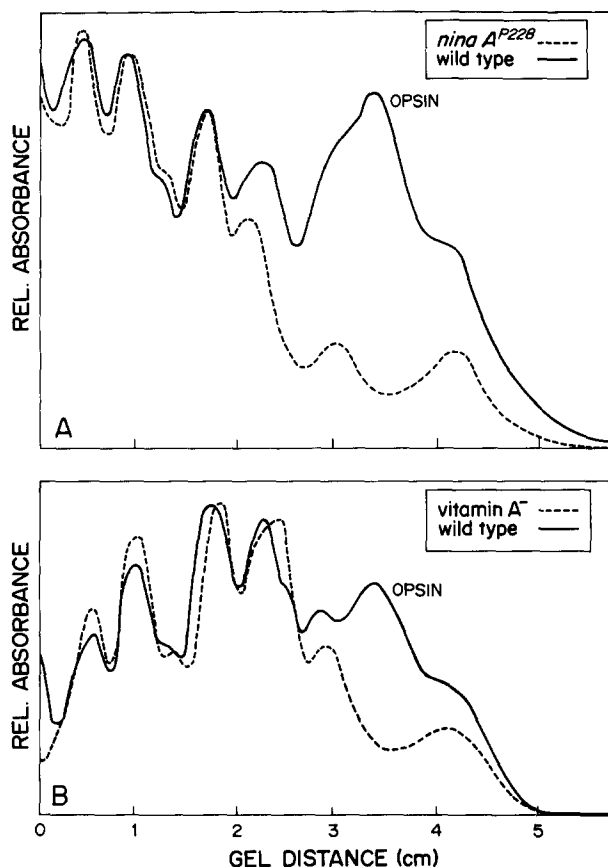
Figure 8.   Densitometric scans of gels from the eyes of (A) $ninaA^{P228}$, (B) vitamin A-deprived flies, and their wild-type controls after staining with Coomassie Brilliant Blue. Each figure shows a set of two gel scans obtained in a given electrophoretic run consisting of a control extract (wild-type flies raised on a normal medium) and a test extract ($ninaA$ or vitamin A-deprived flies) subjected to electrophoresis simultaneously on the same apparatus. The control and test gels obtained in the same run were then stained and destained under nearly identical conditions. The densitometric scan of the gel from the mutant or vitamin A-deficient fly was then superimposed on the wild-type control obtained in the same run. It can be seen that although the protein patterns obtained in different runs vary, those obtained in the same run are very similar except for the opsin peak.

might have on the efficiency of UV-induced photoconversion of R1–6 rhodopsin. To measure the photoconversion efficiency, we assayed the metarhodopsin concentration by means of the M_2 potential (Fig. 9 A) after exposing the fly eye to a known quantity of either UV or blue light (see Materials and Methods). Fig. 9 B–D illustrates the results of these measurements. In the wild-type fly raised on normal media, more than twice as much blue light as

A

12.5×10^{15} photons/cm²   M₂   M₁   on-transient

8.3×10^{15} photons/cm²

2.8×10^{15} photons/cm²

2 mV   4 ms
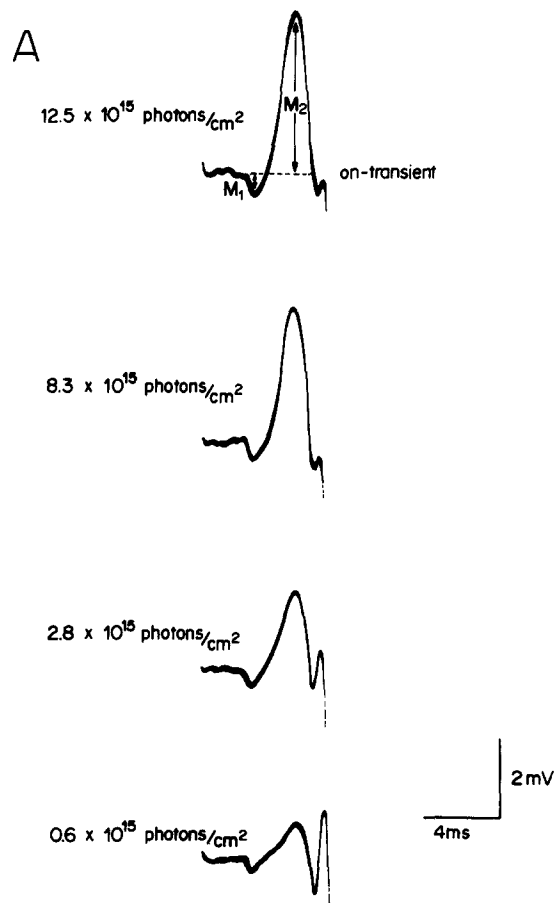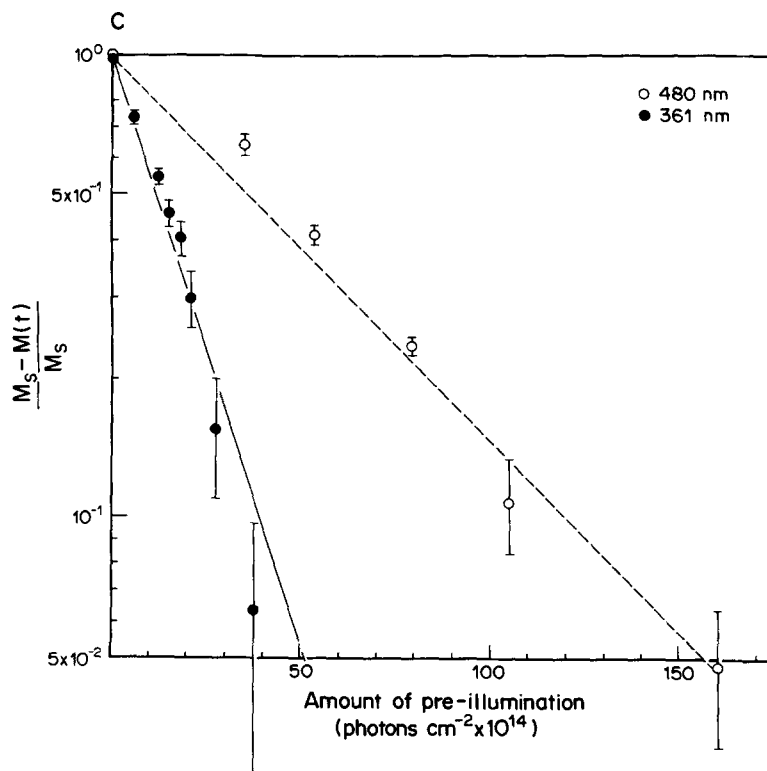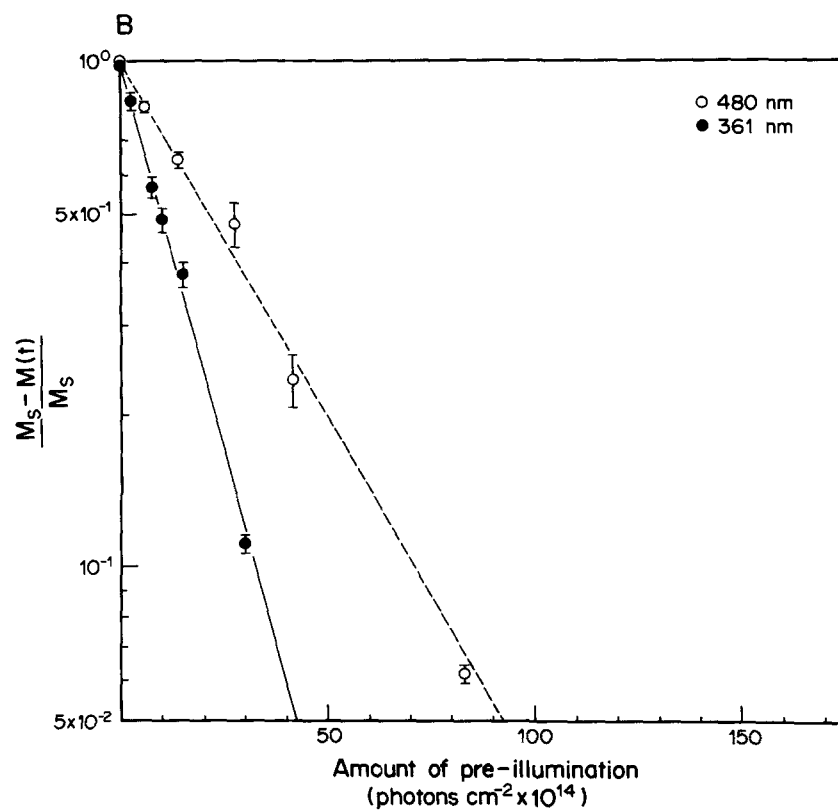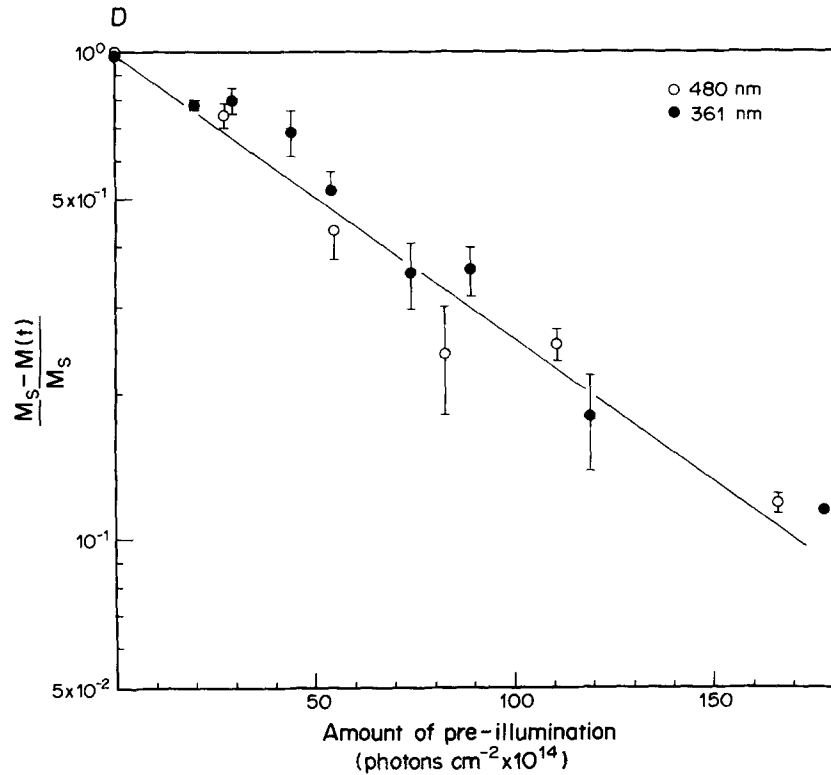
0.6×10^{15} photons/cm²

FIGURE 9. (A). The M-potentials obtained from wild-type flies (placed on white eye background) as a function of the amount of preadapting blue (480 nm) light. The M-potential is seen in the initial portion of the ERG response to an intense orange flash. It consists of a small, initial, corneal-negative deflection, the M_1, and the much larger corneal-positive deflection, M_2. To obtain each of these ERG tracings, the eye was subjected sequentially to the following treatments: (a) four orange strobe flashes to insure that most of the visual pigment was initially in the rhodopsin state, (b) a blue adapting light of the amount shown to the left of each trace, and finally (c) an intense, orange strobe flash. (B–D) Rhodopsin "relaxation curves" showing the effectiveness of UV (361 nm) or blue (480 nm) light in photoconverting R1–6 rhodopsin to metarhodopsin (see Materials and Methods). The ordinate plots the difference between the saturated M_2 potential amplitude (M_s) and that obtained after exposing the animal to various amounts of UV or blue light $[M(t)]$, normalized to the saturated amplitude. The abscissa plots the amount of pre-illumination or adapting light (UV or blue) used to photoconvert rhodopsin to metarhodopsin. A steeper slope corresponds to a more rapid approach to photoequilibrium. All flies used had white eyes because of the mutation white (w) used to eliminate the screening pigments. B, 7-d-old wild-type flies (data from two flies); C, 7-d-old $ninaA^{P228}$ mutants (five flies); D, 7-d-old vitamin A-deprived flies (two flies). At least three complete sets of measurements were obtained from each fly. The data from each fly were then normalized to the saturated M_2 amplitude, M_s, for that fly and combined with data from other flies of the same type. Error flags, standard errors.

536

B

$\dfrac{M_S - M(t)}{M_S}$

Amount of pre-illumination
(photons cm^{-2} x 10^{14})

○ 480 nm
● 361 nm

C

$\dfrac{M_S - M(t)}{M_S}$

Amount of pre-illumination
(photons cm^{-2} x 10^{14})

○ 480 nm
● 361 nm

UV light (photons/cm^2) was required to convert a given amount of rhodopsin to metarhodopsin (Fig. 9 *B*). That is, UV light converted rhodopsin to metarhodopsin more efficiently than blue light in these flies. In the case of the mutant, the ratio of UV photoconversion efficiency to that for blue light remained, within measurement errors, approximately the same as in wild type (Fig. 9 *C*). In fact, the ratio was somewhat greater than that for wild type in the flies tested (Fig. 9 *B* and *C*). By contrast, vitamin A deprivation, while decreasing the slopes of relaxation curves for both UV and blue lights, reduced the slope of the UV curve much more than that for blue light, so that the ratio of UV to blue photoconversion efficiencies became considerably smaller than that for normal, wild-type flies (Fig. 9 *D*). These results for A⁻ flies are consistent with those reported by Kirschfeld et al. (1977) and Minke and Kirschfeld (1979).[4] It may be recalled, however, that the R1-6 rhodopsin

[4] Minke and Kirschfeld (1979) have shown previously that in wild-type houseflies, the photoconversion efficiency for UV is greater than that for blue. Thus, our results for wild-type *Drosophila* are in agreement with theirs. They found, however, that in vitamin A-deprived *Drosophila*, the photoconversion efficiency for blue becomes greater than that for UV, whereas we have found that UV and blue photoconversion efficiencies are about the same (Fig. 9 *D*). The difference apparently arises from the fact that we deliberately selected partially vitamin A-deprived flies, as described in the text, whereas Minke and Kirschfeld (1979) presumably used more thoroughly deprived flies. In the case of the Kirschfeld et al. (1977) paper, the "control"

concentration is somewhat greater in the $ninaA^{P228}$ mutant than in A^- flies (~11% vs. <3% of wild type; Table I). Therefore, a possibility exists that the above difference in photoconversion efficiencies between the mutant and A^- flies is due to the difference in R1–6 rhodopsin concentration. To avoid this difficulty, we used in our measurements those A^- flies that had M-potentials exceeding the $ninaA$ M-potentials in amplitude. Presumably, these A^- flies were only partially vitamin A deprived and had R1–6 rhodopsin levels at least as great as that of $ninaA^{P228}$. In fact, even when these A^- flies were put back on vitamin A-rich media for 2–4 d, the ratio of UV to blue photoconversion efficiency remained substantially less than that for $ninaA$ (data not shown). These observations suggest that the difference in UV photoconversion efficiency between A^- and $ninaA^{P228}$ flies does not arise simply from a difference in R1–6 rhodopsin concentration.

DISCUSSION

Wild-type fruit flies raised on a vitamin A-deficient diet for a generation retain <1% of the photopigment observed in flies raised on a vitamin A-rich medium (Harris et al., 1977). Moreover, these flies lack the PDA in their photoreceptor responses (Stark and Zitzmann, 1976) and display a greatly reduced intramembrane particle density in their rhabdomeric membranes (Harris et al., 1977). Our results with vitamin A-deprived flies confirm these findings (Figs. 1–3; Tables I and II). Recently, Paulsen and Schwemer (1979) have shown that in the blowfly *Calliphora erythrocephala*, vitamin A deprivation leads to a reduction not only of rhodopsin concentration but also of opsin concentration. Consistent with these findings, we find that the opsin concentration is reduced in vitamin A-deprived *Drosophila* as well (Fig. 8). Moreover, the reductions in the two quantities parallel each other so that the quantities of opsin and rhodopsin remaining in vitamin A-deprived flies are nearly the same.[1]

Many of the results that have been obtained for the $ninaA^{P228}$ mutant (Figs. 1–3, 8; Table I) are similar to those of the vitamin A-deprived fly. Thus, one of the main effects of the $ninaA^{P228}$ mutation is apparently to reduce the rhodopsin concentration in R1–6 photoreceptors. Other effects of the mutation are probably secondary to this main effect. For example, the reduction in membrane particle density can be understood in terms of a reduction in concentration of opsin (or rhodopsin) proteins, which would contribute to membrane particles. The reduction in rhodopsin concentration is probably responsible also for the lack of PDA in the mutant, because the PDA has been shown to be absent in vitamin A-deprived flies (Stark and Zitzmann, 1976).

---

photopigment relaxation curve shows the UV photoconversion efficiency to be about the same as the blue photoconversion efficiency, in apparent disagreement with both the Minke and Kirschfeld results and ours. A careful examination of the figure legend reveals, however, that the "control" flies were raised on a partially vitamin A-deprived medium. Thus, all photoconversion results to date are in qualitative agreement with each other: for wild type raised on a vitamin A-rich medium, the UV photoconversion efficiency is greater than the blue efficiency; for partially vitamin A-deprived flies, UV and blue efficiencies are about the same; and for more completely vitamin A-deprived flies, the UV efficiency becomes less than that for blue.

There are, however, three notable differences between the effects of the mutation $ninaA^{P228}$ and those of vitamin A deprivation: (a) the mutation reduces the rhabdomeric intramembrane particle density only in R1–6 photoreceptors (Fig. 4; Table II), whereas vitamin A deprivation reduces particle density in all photoreceptors (Harris et al., 1977; and Table II); (b) the mutation does not significantly alter the ratio of UV to blue photoconversion efficiencies, whereas vitamin A deprivation substantially reduces this ratio (Fig. 9); and (c) the mutation affects the PDA only in R1–6 photoreceptors, and not in R7 (Figs. 6 and 7). These results strongly suggest that the mutation $ninaA^{P228}$ affects the protein (opsin) portion, rather than the chromophore portion, of R1–6 rhodopsin.

First, the results of freeze-fracture electron microscopy show that the mutation reduces rhabdomeric membrane particle density only in the R1–6 photoreceptors (Fig. 4; Table II),[3] which contain one particular class of rhodopsin.[2] Because the rhodopsin molecules have been found to be the main contributors in the formation of rhabdomeric membrane particles (Boschek and Hamdorf, 1976; Harris et al., 1977; Brown and Schwemer, 1977; Schinz et al., 1977), the freeze-fracture results suggest that the mutation specifically reduces the concentration of that class of rhodopsin contained in the R1–6 photoreceptors. Consistent with this interpretation, the spectrophotometrically determined concentration of R1–6 rhodopsin is low in the mutant (Fig. 2; Table I). Although spectrophotometric measurements of rhodopsin concentrations in the R7 and R8 photoreceptors of the $ninaA^{P228}$ mutant have not yet been carried out, it seems highly unlikely that the mutation would decrease the concentrations of R7 and R8 rhodopsins without also decreasing the rhabdomeric membrane particle density in R7 and R8. The existence of a normal R7 PDA in the mutant (Figs. 6 and 7) is further evidence that $ninaA^{P228}$ does not affect R7 rhodopsin level. Inasmuch as all visual pigments studied to date contain the same 11-cis retinal as their chromophores, a specific reduction in one class of rhodopsin would presumably have to be brought about through alterations in the opsin portion of rhodopsin.

Another argument in support of the above interpretation comes from the results of the rhodopsin photoconversion efficiency experiment (Fig. 9). Earlier investigators have shown that the spectral sensitivities of the ERG of wild-type flies reared on a normal medium display two peaks, one at ~480 nm and another in the near UV (Goldsmith and Fernandez, 1968; Pak et al., 1970; McCann and Arnett, 1972; Minke et al., 1975; Rosner, 1975; Stark et al., 1977). Vitamin A deprivation, although it depresses the sensitivity throughout all visible wavelengths, has a much stronger effect on the UV peak (Stark et al., 1977). Corresponding effects are found in the efficiency with which rhodopsin is photoconverted to metarhodopsin by either blue or UV light (Kirschfeld et al., 1977; Minke and Kirschfeld, 1979) (see explanations in Materials and Methods, and Results). According to our results, in flies reared on a normal medium, it requires less than one-half as much UV light as blue light to photoconvert a given amount of rhodopsin to metarhodopsin (Fig. 9 B). Vitamin A deprivation reduces the efficiency of rhodopsin photoconver-

sion by both UV and blue lights, but it affects the UV photoconversion efficiency much more (Fig. 9D).[4]

Kirschfeld et al. (1977) have suggested that photoconversion of visual pigment by UV light in R1–6 photoreceptors is mediated through a sensitizing pigment of carotenoid origin, which absorbs light in the UV and transfers the absorbed energy to rhodopsin. Recent observations of a UV-induced fluorescence in R1–6 photoreceptors appear to be consistent with such a sensitizing pigment hypothesis, since the UV sensitizing pigment presumably is responsible for part of the fluorescence (Stark et al., 1979; Franceschini et al., 1981; Franceschini and Stavenga, 1981). In addition, Gemperlein et al. (1980) demonstrated the presence of a fine structure in the UV peak of the blowfly spectral sensitivity, leading them to suggest that a short polyene is responsible for the UV peak. Their results, thus, also appear to be consistent with a sensitizing-pigment hypothesis. According to this hypothesis, vitamin A deprivation, which reduces both the sensitizing pigment and rhodopsin concentrations, would depress the UV photoconversion efficiency more strongly than the blue photoconversion efficiency because the former depends on concentrations of both pigments, whereas the latter depends only on rhodopsin concentration.

In the case of the mutant $ninaA^{P228}$, however, the ratio of UV to blue photoconversion efficiencies is roughly the same as in wild type (Fig. 9 C). One might attempt to attribute this difference between $ninaA^{P228}$ and vitamin A-deprived flies to the higher concentration of R1–6 rhodopsin found in $ninaA^{P228}$ (Table I). This explanation is untenable because we have used partially vitamin A-deprived wild-type flies that have at least as much R1–6 rhodopsin as $ninaA^{P228}$, as judged by the M-potential amplitude. One can explain the difference between $ninaA^{P228}$ and vitamin A-deprived flies in terms of the sensitizing pigment hypothesis; however, if one assumes that the $ninaA^{P228}$ mutation, unlike vitamin A deprivation, affects only the rhodopsin concentration and not the concentrations of other carotenoid pigments that might be present. Such specific alterations of a given class of carotenoid pigment again suggest that $ninaA^{P228}$ affects the protein portion, rather than chromophore portion, of the rhodopsin molecule in R1–6 photoreceptors.

The defect in $ninaA^{P228}$, however, apparently does not alter the interaction between the chromophore and R1–6 opsin. If a faulty interaction were present, the defect should manifest itself in abnormal absorption properties of R1–6 rhodopsin of $ninaA^{P228}$. As can be seen in Fig. 2, there are no obvious differences between wild type and $ninaA^{P228}$ in the shape of their rhodopsin difference spectra.

One possible explanation for the observed specificity of the $ninaA^{P228}$ mutation for R1–6 opsin is that the *ninaA* gene is the structural gene for R1–6 opsin, i.e., it codes for the amino acid sequence of R1–6 opsin protein. We have been attempting to test this hypothesis by means of two approaches. One of them is to see whether independently isolated alleles of *ninaA* produce electrophoretic variants of R1–6 opsin (see O'Brien and MacIntyre [1978] and references cited therein), and the other is to see whether the amount of R1–6

rhodopsin varies with doses of the *ninaA* gene (see Stewart and Merriam [1974], and Hall and Kankel [1976], and references cited therein). Because no definitive data have been obtained to date, no conclusive statement can yet be made regarding this hypothesis. (See, however, the preliminary data on the lack of *ninaA* gene dosage effect on R1-6 rhodopsin concentration (Pak et al. [1980]).

Regardless of whether *ninaA* is the structural gene for R1-6 opsin or not, it interests us because of its specificity for a particular class of rhodopsin. If *ninaA* is the R1-6 opsin structural gene, some of its alleles (which can be isolated with a reasonable amount of effort) would produce R1-6 opsins with altered molecular structure. Such molecular variants of R1-6 opsin should be extremely valuable in probing the functional properties of R1-6 rhodopsin. On the other hand, if *ninaA* turns out not to be the structural gene for R1-6 opsin, the existence of this class of a rhodopsin-specific gene(s) may indicate that the synthesis of rhodopsin or its insertion into membrane requires not only the coding information contained in the opsin structural gene but also other rhodopsin-specific information contained in the gene(s) of this type. If so, mutants such as *ninaA* should prove useful in elucidating the nature of such rhodopsin-specific information.

REFERENCES

BOSCHEK, C. B., and K. HAMDORF. 1976. Rhodopsin particles in the photoreceptor membrane of an insect. *Z. Naturforsch. Sect. C Biosci.* **31c**:763-764.

BOWNDS, D., A. GORDON-WALKER, A.-C. GAIDE-HUGUENIN, and W. ROBINSON. 1971. Characterization and analysis of frog photoreceptor membranes. *J. Gen. Physiol.* **58**:225-237.

BROWN, P. K., and J. SCHWEMER. 1977. Insect visual pigments. *Invest. Ophthalmol. Visual Sci.* **16**(Suppl.):93-94. (Abstr.).

CAMPOS-ORTEGA, J. A., G. JÜRGENS, and A. HOFBAUER. 1979. Cell clones and pattern formation: studies on sevenless a mutant of *Drosophila melanogaster. Wilhelm Roux's Arch. Dev. Biol.* **186**:27-50.

COCHRAN, W. 1964. Approximate significance levels of the Behrens-Fisher test. *Biometrics.* **20**:191-195.

EBREY, T. G., and B. HONIG. 1975. Molecular aspects of photoreceptor function. *Q. Rev. Biophys.* **8**:129-184.

FRANCESCHINI, N., K. KIRSCHFELD, and B. MINKE. 1981. Fluorescence of photoreceptor cells observed *in vivo. Science (Wash. D. C.).* In press.

FRANCESCHINI, N., and D. G. STAVENGA. 1981. The ultra-violet sensitizing pigment of flies studied by *in vivo* microspectrophotometry. *Invest. Ophthalmol. Visual Sci.* **20**(Suppl.):111. (Abstr.).

GEMPERLEIN, R., R. PAUL, E. LINDAUER, and A. STEINER. 1980. UV fine structure of the spectral sensitivity of flies visual cells. *Naturwissenschaften.* **67:**565–566.

GOLDSMITH, T. H., R. J. BARKER, and C. F. COHEN. 1964. Sensitivity of visual receptors of carotenoid-depleted flies: a vitamin A deficiency in an invertebrate. *Science (Wash. D. C.).* **146:**65–67.

GOLDSMITH, T. H., and H. R. FERNANDEZ. 1968. The sensitivity of housefly photoreceptors in the mid-ultraviolet and the limits of the visible spectrum. *J. Exp. Biol.* **49:**669–677.

HAGINS, F. M. 1973. Purification and partial characterization of the protein component of squid rhodopsin. *J. Biol. Chem.* **248:**3298–3304.

HALL, J. C., and D. R. KANKEL. 1976. Genetics of acetylcholinesterase in *Drosophila m. Genetics.* **83:**517–535.

HALL, M. O., D. BOK, and A. D. E. BACHARACH. 1969. Biosynthesis and assembly of the rod outer segment membrane system. Formation and fate of visual pigment in the frog retina. *J. Mol. Biol.* **45:**397–406.

HARA, T., and R. HARA. 1967. Rhodopsin and retinochrome in the squid retina. *Nature (Lond.).* **214:**573–575.

HARDIE, R. C. 1979. Electrophysiological analysis of fly retina. I. Comparative properties of R1–6 and R7 and R8. *J. Comp. Physiol.* **129:**19–33.

HARDIE, R. C., N. FRANCESCHINI, and P. D. McINTYRE. 1979. Electrophysiological analysis of fly retina. II. Spectral and polarization sensitivity in R7 and R8. *J. Comp. Physiol.* **133:**23–39.

HARRIS, W. A., D. F. READY, E. D. LIPSON, A. J. HUDSPETH, and W. S. STARK. 1977. Vitamin A deprivation and *Drosophila* photopigments. *Nature (Lond.).* **266:**648–650.

HARRIS, W. A., W. S. STARK, and J. A. WALKER. 1976. Genetic dissection of the photoreceptor system in the compound eye of *Drosophila m. J. Physiol. (Lond.).* **256:**413–439.

HEITZMANN, H. 1972. Rhodopsin is the predominant protein of rod outer segment membranes. *Nat. New Biology.* **235:**114.

HUBBARD, R., and R. C. C. ST. GEORGE. 1958. The rhodopsin system of the squid. *J. Gen. Physiol.* **41:**501–528.

HUBBELL, W. L., and M. D. BOWNDS. 1979. Visual transduction in vertebrate photoreceptors. *Annu. Rev. Neurosci.* **2:**17–34.

KIRSCHFELD, K. 1979. The function of photostable pigments in fly photoreceptors. *Biophys. Struct. Mechanisms.* **5:**117–128.

KIRSCHFELD, K., N. FRANCESCHINI, and B. MINKE. 1977. Evidence for a sensitizing pigment in fly photoreceptors. *Nature (Lond.).* **269:**386–390.

KROPF, A. 1972. The structure and reactions of visual pigments. *In* Physiology of Photoreceptor Organs. M. G. F. Fuortes, editor. Vol. VII/2. Springer-Verlag, Berlin. 239–278.

LARRIVEE, D. C. 1979. A biochemical analysis of the *Drosophila* rhabdomere and its extracellular environment. Ph.D. Thesis, Purdue University, Lafayette, Ind.

LINDSLEY, D., and E. H. GRELL. 1968. Genetic variations of *Drosophila melanogaster.* Carnegie Institute of Washington, Washington, D. C.

McCANN, G. D., and D. W. ARNETT. 1972. Spectral and polarization sensitivity of the dipteran visual system. *J. Gen. Physiol.* **59:**534–558.

MINKE, B., and K. KIRSCHFELD. 1979. The contribution of a sensitizing pigment to the photosensitivity spectra of fly rhodopsin and metarhodopsin. *J. Gen. Physiol.* **73:**517–540.

MINKE, B., and K. KIRSCHFELD. 1980. Fast electrical potentials arising from activation of metarhodopsin in the fly. *J. Gen. Physiol.* **75:**381–402.

MINKE, B., C.-F. WU, and W. L. PAK. 1975. Isolation of light induced responses of the central retinula cells from the electroretinogram of *Drosophila. J. Comp. Physiol.* **98:**345–355.

O'BRIEN, S. J., and R. J. MacINTYRE. 1978. Genetics and biochemistry of enzymes and specific proteins of *Drosophila. In* The Genetics and Biology of Drosophila. M. Ashburner and T. R. F. Wright, editors. Academic Press, Inc., London. 2a:396–551.

OSTROY. S. E. 1977. Rhodopsin and the visual process. *Biochim. Biophys. Acta.* 463:91–175.

OSTROY. S. E. 1978. Characteristics of *Drosophila* rhodopsin in wild type and *norpA* vision transduction mutants. *J. Gen. Physiol.* 72:717–732.

OSTROY, S. E., M. WILSON, and W. L. PAK. 1974. *Drosophila* rhodopsin: photochemistry, extraction, and differences in the *norpA^{P12}* phototransduction mutant. *Biochem. Biophys. Res. Comm.* 59:960–966.

PAK, W. L. 1979. Study of photoreceptor function using *Drosophila* mutants. *In* Neurogenetics: Genetic Approaches to the Nervous System. X Breakefield, editor. Elsevier North-Holland, Inc., New York. 67–99.

PAK, W. L., S. K. CONRAD, N. E. KREMER, D. C. LARRIVEE, R. H. SCHINZ, and F. WONG. 1980. Photoreceptor function. *In* Development and Neurobiology of Drosophila. O. Siddiqi, P. Babu, L. M. Hall, and J. C. Hall, editors. Plenum Publishing Corp., New York. 331–346.

PAK, W. L., J. GROSSFIELD and K. ARNOLD. 1970. Mutants of the visual pathway of *Drosophilia melanogaster. Nature (Lond.).* 227:518–520.

PAK, W. L., and K. J. LIDINGTON. 1974. Fast electrical potential from a long-lived, long-wavelength photoproduct of fly visual pigment. *J. Gen. Physiol.* 63:740–756.

PAULSEN, R., and J. SCHWEMER. 1972. Studies on the insect visual pigment sensitive to ultraviolet light: retinal as the chromophoric group. *Biochim. Biophys. Acta.* 283:520–529.

PAULSEN, R., and J. SCHWEMER. 1973. Proteins of invertebrate photoreceptor membranes: characterization of visual-pigment preparations by gel electrophoresis. *Eur. J. Biochem.* 40: 577–583.

PAULSEN, R., and J. SCHWEMER. 1979. Vitamin A deficiency reduces the concentration of visual pigment protein within blowfly photoreceptor membranes. *Biochim. Biophys. Acta.* 557:385–390.

POBER, J. S., and M. W. BITENSKY. 1979. Light-regulated enzymes of vertebrate retinal rods. *In* Advances in Cyclic Nucleotide Research. P. Greengard and G. A. Robison, editors. Raven Press, New York. 265–301.

RAZMJOO, S., and K. HAMDORF. 1976. Visual sensitivity and the variation of total pigment content in the blowfly photoreceptor membrane. *J. Comp. Physiol.* 105:279–286.

ROBINSON, W. E., A. GORDON-WALKER, and D. BOWNDS. 1972. Molecular weight of frog rhodopsin. *Nat. New Biol.* 235:112–114.

ROSNER, G. 1975. Adaptation und Photoregeneration im Fliegenauge. *J. Comp. Physiol.* 102:269–295.

SANG, J. H. 1956. The quantitative nutritional requirements of *Drosophila melanogaster. J. Exp. Biol.* 33:45–72.

SCHINZ, R., S. E. OSTROY, and W. L. PAK. 1977. Freeze-fracture study of the *Drosophila* rhabdomeric membrane. *Invest. Ophthalmol. Visual Sci.* 16(Suppl.):146. (Abstr.).

SHICHI, H., and C. N. RAFFERTY. 1980. Yearly review: the molecular aspects of visual photoreceptors. *Photochem. Photobiol.* 31:631–639.

STARK, W. S., A. M. IVANYSHYN, and R. M. GREENBERG. 1977. Sensitivity and photopigments of R1–6, a two peaked photoreceptor in *Drosophila, Calliphora* and *Musca. J. Comp. Physiol.* 121: 289–305.

STARK, W. S., A. M. IVANYSHYN, and K. G. HU. 1976. Spectral sensitivities and photopigments in adaptation of fly visual receptors. *Naturwissenschaften.* 63:513–518.

Stark, W. S., D. G. Stavenga, and B. Kruizinga. 1979. Fly photoreceptor fluorescence is related to UV sensitivity. *Nature (Lond.).* **280:**581–583.

Stark, W. S., and W. G. Zitzmann. 1976. Isolation of adaptation mechanisms and photopigment spectra by vitamin A deprivation in *Drosophila. J. Comp. Physiol.* **105:**15–27.

Stein, P., J. D. Brammer, and S. E. Ostroy. 1980. Renewal of opsin in photoreceptor cells of the mosquito. *J. Gen. Physiol.* **74:**565–582.

Stephenson, R. S., and W. L. Pak. 1978. Metarhodopsin potential of *Drosophila*: corneal-positive component due to second-order neurons. *Biophys. J.* **21:**172a. (Abstr.).

Stephenson, R. S., and W. L. Pak. 1980. Heterogenic components of a fast electrical potential in *Drosophila* compound eye and their relation to visual pigment photoconversion. *J. Gen. Physiol.* **75:**353–379.

Stewart, B. R., and J. R. Merriam. 1974. Segmental aneuploidy and enzyme activity as a method for cytogenetic localization in *Drosophila melanogaster. Genetics.* **76:**301–309.

Yoshizawa, T., and F. Tokunaga. 1979. Yearly review: vision. *Photochem. Photobiol.* **29:**197–202.

Zimmerman, W. F., and T. H. Goldsmith. 1971. Photosensitivity of the circadian rhythm and of visual receptors in carotenoid depleted *Drosophila. Science (Wash. D. C.).* **171:**1167–1169.