Electrophysiological Measurement of the Number of Rhodopsin Molecules in Single *Limulus* Photoreceptors

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ABSTRACT Two partly independent electrophysiological methods are described for measuring the number of rhodopsin molecules (R) in single ventral photoreceptors. Method 1 is based on measurements of the relative intensity required to elicit a quantal response and the relative intensity required to half-saturate the early receptor potential (ERP). Method 2 is based on measurements of the absolute intensity required to elicit a quantal response. Both methods give values of $R \cong 10^9$. From these and other measurements, estimates are derived for the surface density of rhodopsin (8,000/μm^2), the charge movement during the ERP per isomerized rhodopsin (20×10^{-21} C), and the half-time for thermal isomerization of rhodopsin (36 yr).

INTRODUCTION

We describe here two electrophysiological techniques for measuring the number of rhodopsin molecules in single photoreceptors of the *Limulus* ventral eye. In conjunction with other measurements, determination of this number allows us to estimate the charge displacement in rhodopsin during the early receptor potential, the surface density of rhodopsin in the plasma membrane, and the rate of spontaneous isomerization of rhodopsin.

Recordings of the early receptor potential (ERP) indicate that *Limulus* rhodopsin is a typical invertebrate visual pigment. Light converts rhodopsin to a stable photoproduct, metarhodopsin; absorption of light by metarhodopsin photoregenerates rhodopsin (Lisman and Sheline, 1976). The chromophore of the visual pigment in *Limulus* ventral eye has not been identified but is probably the same as that in the lateral eye which has been identified as vitamin A_1 aldehyde (Hubbard and Wald, 1960). Microspectrophotometry of single ventral photoreceptors indicates that the λ_{max} of the visual pigment is 529 ± 5 nm (Murray, 1966).

The methods described here for determining the number of rhodopsin molecules per cell depend on measurements of two types of electrical responses: the early receptor potential (ERP) and the late receptor potential (LRP), both of which have previously been studied in *Limulus* photoreceptors. The ERP in both vertebrates and invertebrates is thought to be generated by charge movements associated with conformational changes in the visual pigment

molecules (for a review see Cone and Pak, 1971). A short-latency response to bright flashes recorded from *Limulus* photoreceptors has been identified as an ERP on the basis of its membrane origin (Smith and Brown, 1966), its spectral sensitivity (Brown et al., 1967), and its resistance to ionic changes and fixation (Hillman et al., 1973). The LRP in *Limulus* photoreceptors is a graded depolarization generated by an increase in membrane conductance to sodium and potassium (Millecchia and Mauro, 1969*a*,*b*; Brown and Mote, 1974). At very low light intensities the LRP consists of randomly occurring discrete waves (quantum bumps), the frequency of which increases linearly with intensity. Statistical analysis of these waves indicates that single light-evoked discrete waves are initiated by single photon absorptions (Fuortes and Yeandle, 1964; Yeandle and Spiegler, 1973).

In this paper we describe a method of computing the number of rhodopsin molecules per cell (R) from measurements of the relative intensity needed to elicit a discrete wave and the relative intensity needed to half-saturate the ERP (Method 1). R is also computed by a second method (Method 2) which requires measurement of the absolute intensity required to evoke a discrete wave. Methods 1 and 2 are based, in part, on independent assumptions; however, both depend on the assumption that isomerization of rhodopsin results in discrete waves with a probability close to one. Both methods indicate that ventral photoreceptors contain approximately 1 billion rhodopsin molecules. Furthermore, by measuring cell capacitance we can estimate the area of the plasma membrane and thus, by purely electrophysiological means, arrive at a value for the surface density of visual pigment. A preliminary report of our findings has been presented (Lisman and Bering, 1973).

MATERIALS AND METHODS

The methods for dissection and recording are as previously described (Lisman and Sheline, 1976). Cells were bathed in artificial seawater containing 423 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 22 mM $MgCl_2$, 26 mM $MgSO_4$, 2.2 mM $NaHCO_3$, and 15 mM Tris buffer at pH 7.8.

Two different light sources were used. The first was a tungsten lamp used to visualize the photoreceptors while positioning the microelectrodes. The second (xenon flash) was a Strobonar 880 (Honeywell, Inc., Test Instruments Div., Denver, Colo.) used for evoking both the ERP and discrete waves.

Light from the two sources was combined with a thin plate of glass and then directed to the microscope condenser which focused the light from below onto the preparation. The light sources were arranged so that the glass plate transmitted the light from the flash source and partially reflected the light from the tungsten source.

Method 1 (described in Results) required the computation of the effective density (D^*) of each neutral density filter for stimulation of a 529-nm pigment with white light from the xenon source. It was necessary to use white rather than monochromatic light in Method 1 because monochromatic flashes were not bright enough to saturate the ERP. D^* was computed as follows:

$$\text{let } F = 10^{-D^*}, \text{ where}$$

$$F = \frac{\sum_{\lambda = 400\ nm}^{600} P(\lambda)A(\lambda)f(\lambda)}{\sum_{\lambda = 400\ nm}^{600} P(\lambda)A(\lambda)},$$

and $f(\lambda) = 10^{-D(\lambda)}$, where $D(\lambda)$ is the optical density of the filter at wavelength λ measured in a Cary spectrophotometer. $P(\lambda)$ is the photon flux at λ relative to that at 529 nm and was computed from the relative energy output of a xenon light source (Wyszecki and Stiles, 1967). $A(\lambda)$ is the relative absorption of a dilute 529-nm pigment computed from the Dartnall (1972) nomogram. The filters calibrated in this way were absorption filters. The difference between D^* and D (529 nm) was typically less than 0.1 ND per density unit. Even after such corrections, there was an uncertainty in the density of each filter which we estimated to be about ±0.015 ND per density unit.

Method 2 required measurement of the absolute light intensity delivered at the photoreceptor surface by stimuli from the xenon flash. Stimuli were made monochromatic with a 530-nm narrow-band (10-nm width at half-height) interference filter. To determine the absolute intensity of the beam at the lower surface of the olfactory nerve, a calibrated pinhole (Edmund Scientific Co., Barrington, N. J.) was placed in the position normally occupied by the nerve. The energy of the light passing through the pinhole was measured with a calibrated United Detector Technology (Santa Monica, Calif.) PIN 10 photodiode. The attenuation of light due to passage through the entire thickness of the olfactory nerve was measured in a microspectrophotometer in two preparations and was about 0.5 log unit at 530 nm. The ventral photoreceptors used in our experiments were embedded in the upper half of the olfactory nerve. The light intensity reaching the lower surface of the photoreceptor depended on the thickness of the nerve beneath it and on the light-scattering properties of the nerve, both of which varied from animal to animal. Our calculations are based on the assumption that, on the average, the light reaching the lower surface of the photoreceptor was about 50% of that incident on the lower surface of the nerve. Light intensities were measured at a light level about 6 log units above that which evoked discrete waves. The absolute light intensity at lower light levels (Table I) was computed by using the measured absorbances of the neutral density filters.

RESULTS

The number of rhodopsin molecules in single ventral photoreceptors was determined by two partly independent methods discussed below, first qualitatively and then quantitatively. The cells used in all experiments had been dissected under bright white light. Rhodopsin and metarhodopsin were therefore in photoequilibrium at the start of the experiments (Lisman and Sheline, 1976). Experiments on other invertebrates indicate that only photons absorbed by rhodopsin contribute to excitation of the LRP (Hamdorf et al., 1973; Rosner, 1975).

Method 1, Qualitative

A flash of intensity I_1 isomerizes one rhodopsin molecule in a cell. The number of molecules isomerized by a flash rises linearly with flash intensity over a wide intensity range. Therefore, if the intensity is raised by a factor of y so that the flash is just sufficient to isomerize all the rhodopsin molecules R, then R is approximately equal to y. We have used the occurrence of discrete waves to estimate the relative intensity at which one isomerization of rhodopsin occurs, and the saturation of ERP to estimate the relative intensity at which all rhodopsins are isomerized.

Quantitative Derivation of Method 1

An equation for the net number of rhodopsin molecules isomerized by a flash

was derived by Williams (1964) and was shown to be applicable to the vertebrate ERP (Cone, 1964). The equation (Eq. [1]) takes into account the fact that unstable intermediates formed during the flash may absorb a second photon during the flash and be reconverted to rhodopsin. Such photoregeneration (Hubbard and Kropf, 1958) accounts for the observation (Hagins, 1965) that a brief flash, no matter how bright, can never bleach more than approximately half the rhodopsin present. A quantitatively similar limitation on the isomerization produced by a flash has been found for *Limulus* rhodopsin (Lisman and Sheline, 1976, Fig. 3). The value 0.5 in Eq. (1) represents the maximum fraction of rhodopsin that can be bleached by a single flash.

$$N(I) = 0.5\,R\,(1 - e^{-\beta I}) \tag{1}$$

I is flash intensity, R is the total number of rhodopsin molecules, $N(I)$ is the net number of rhodopsin molecules isomerized by the flash, and β is a constant proportional to the photosensitivity of the pigment. If I_1 is the flash intensity at which one rhodopsin is isomerized, then from Eq. (1) (and given that $\beta I, \ll 1$)

$$1 = 0.5\,R\,(1 - e^{-\beta I_1}) \cong 0.5\,R\,\beta I_1. \tag{2}$$

If I_2 is the intensity of the flash that isomerizes half of the maximum number of rhodopsin molecules that can be isomerized per flash (i.e. $0.25\,R$), then from Eq. (1)

$$N(I_2) = 0.5\,(0.5\,R) = 0.5\,R\,(1 - e^{-\beta I_2}). \tag{3}$$

Rearranging terms,

$$0.5 = (1 - e^{-\beta I_2})$$

$$0.5 = e^{-\beta I_2}$$

$$\ln 2 = \beta I_2$$

$$\beta = \frac{\ln 2}{I_2}, \tag{4}$$

substituting 4 into 2 and rearranging terms,

$$R \cong \frac{2}{\ln 2}\frac{I_2}{I_1} \cong 2.9\frac{I_2}{I_1}. \tag{5}$$

Since R depends on the ratio of intensities, only the relative intensities KI_1 and KI_2 need to be measured in order to compute R (K is an arbitrary constant).

Results of Method 1

Cells were penetrated with a single microelectrode and then dark adapted. Flashes of white light were attenuated with neutral density filters until there were, on an average, 1–2 discrete waves per flash. The average number of light-induced discrete waves per flash was computed by subtracting from the total number of discrete waves per flash the average number of spontaneous discrete waves (Adolph, 1964) which occurred during an equal period in the dark. Since the relationship between light intensity and the frequency of

discrete waves is linear, the relative flash intensity (KI_1) which evoked an average of one light-induced discrete wave could be computed (Table I).

The cell was then stimulated with bright white flashes at a series of different intensities. The amplitudes of both the positive and negative components of the ERP increased with intensity at low intensities and saturated at higher intensities (Fig. 1). The positive and negative components saturated at the same intensity (the average difference in six cells was 0.008±0.08 log units). Response-intensity curves were fitted by eye with a template curve having the form of Eq. (1). The relative intensity at which the amplitude of the negative component

TABLE I

NUMBER OF RHODOPSIN MOLECULES (R) PER CELL DETERMINED BY TWO METHODS

Cell no. . . .	1	2	3	4	5	6	Average ± SD
Method 1							
KI_1 (relative flash intensity that evokes an average of one discrete wave) ($-\log_{10}$ relative intensity)	9.96	9.84	9.83	9.71	9.91	9.87	
KI_2 (relative flash intensity that half saturates the ERP) ($-\log_{10}$ relative intensity)	1.12	1.12	1.07	1.18	1.02	0.86	
$R \cong 2.9 \frac{KI_2}{KI_1}$	2.0×10^9	1.5×10^9	1.7×10^9	0.98×10^9	2.3×10^9	2.9×10^9	$1.9\pm0.67\times10^9$
Method 2							
I_1 (flash intensity that evokes an average of one discrete wave) (quanta (530 nm) per cm^2 per flash)	6.1×10^6	8.0×10^6	7.3×10^6	9.6×10^6	5.9×10^6		
$R = \frac{1.0\times10^{16}}{I_1}$	1.6×10^9	1.3×10^9	1.4×10^9	1.0×10^9	1.7×10^9		$1.4\pm0.27\times10^9$
Average of Methods 1 and 2							1.65×10^9

was half of its maximum was taken to be KI_2 (Table I). Values for R derived by Method 1 (Eq. [5]) range from 0.98×10^9 to 2.9×10^9 (average ±SD is $1.9\pm0.67 \times 10^9$) (Table I).

Method 2, Qualitative

The number of rhodopsin molecules isomerized per flash depends on the absolute light intensity, the number of rhodopsin molecules present, the molar extinction of the pigment, and the quantum efficiency of isomerization. In Method 2, we used the discrete waves to estimate the number of isomerizations caused by flashes of known absolute intensity and assumed values for the molar extinction and quantum efficiency of isomerization.

Quantitative Derivation of Method 2

Optical density (OD) is defined by the Beer-Lambert Law

$$\text{OD} = \log_{10} \frac{I_i}{I_t} = \epsilon CL, \tag{6}$$

in which I_i and I_t are the incident and transmitted intensities, respectively (defined here in units of photons per square centimeter per flash), ϵ is the molar extinction, C is the molar concentration, and L is the path length in centimeters. I_a is the effective intensity of the absorbed photons $I_a = I_i - I_t$. If

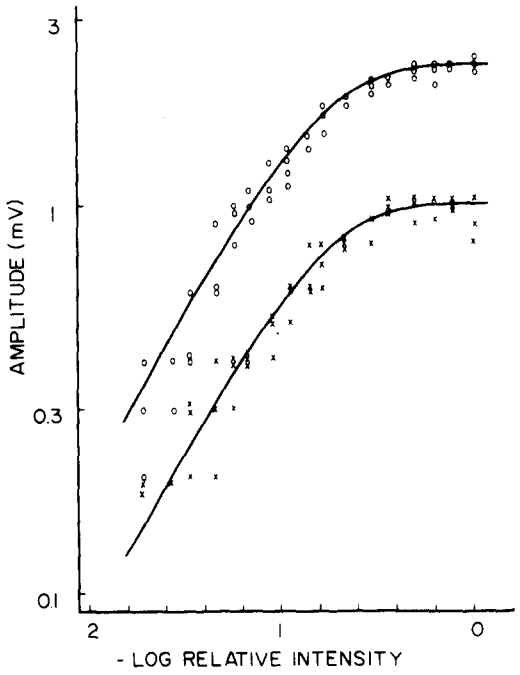

FIGURE 1. Response-intensity curve for the initial positive component (×) and the subsequent negative component (○) of the ERP. The solid curves have the form of Eq. (1) and were fit by eye to the data points. The amplitudes were measured from the base line before the flash to the peak of each component. All the data are from the same cell. Response waveforms were as shown in Lisman and Sheline (1976). To ensure that data points were taken under identical initial conditions, we stimulated the cell with several saturating flashes 10 s or more before each data point was taken.

the solution is dilute, Eq. (6) becomes

$$\frac{I_a}{I_i} = 2.3\ \epsilon CL. \tag{7}$$

This approximation is valid for the ventral photoreceptor since its visual pigment is so dilute that the cells are virtually colorless. Eq. (7) applies only if the chromophores are randomly oriented with respect to the light beam. In most invertebrate photoreceptors this is not the case both because the microvilli are uniformly oriented in the cell and because rhodopsin is probably oriented in the plane of the villus membrane (Goldsmith and Wehner, 1977). However,

Eq. (7) is probably valid for *Limulus* ventral photoreceptors because the villi appear to be randomly oriented (Clark et al., 1969).

The total number of rhodopsin molecules (R), the volume (V) in cubic centimeters, and Avogadro's number (Z) are related to C by

$$C = \frac{R10^3}{ZV}. \tag{8}$$

The volume equals L times the area (A), so

$$\frac{I_a}{I_i} = 2.3\frac{\epsilon R10^3}{AZ}. \tag{9}$$

The number of photons (N_A) absorbed by rhodopsin in area A is

$$N_a = I_a A. \tag{10}$$

From Eq. (9) and (10)

$$N_a = 2.3\frac{\epsilon R10^3}{Z} I_i. \tag{11}$$

The number of isomerizations (N_i) is

$$N_i = \alpha N_a, \tag{12}$$

where α is the quantum efficiency of isomerization. From Eq. (12) and (11)

$$N_i = 2.3\alpha\epsilon\frac{R}{Z} I_i 10^3. \tag{13}$$

If a flash, I_1, produces on average one isomerization, as determined by the presence of an average of one light-evoked discrete wave, then $N_i = 1$ and from Eq. (13)

$$R = \frac{Z10^{-3}}{2.3\alpha\epsilon I_1}. \tag{14}$$

$Z = 6 \times 10^{23}$, and taking $\alpha = 0.65$ (Dartnall, 1972) and a value of $\epsilon = 40{,}000$ computed by Hubbard and Wald (1960) for the visual pigment of *Limulus* lateral eye, then

$$R = \frac{1.0 \times 10^{16}}{I_1}. \tag{15}$$

Results of Method 2

The flash intensity that evoked an average of one discrete wave was determined as in Method 1. The absolute intensity (I_1) of the stimulus at the lower surface of the nerve was measured as described in Materials and Methods and corrected for the average absorbance of the nerve (0.3 density units) through which the stimulus passed before reaching the photoreceptor. Values of R computed by Method 2 (Eq. [15]) range from 1.0×10^9 to 1.7×10^9 (average ± SD is $1.4 \pm 0.27 \times 10^9$) (Table I).

DISCUSSION

Number of Rhodopsin Molecules Per Cell

The average number of rhodopsin molecules per cell is 1.9×10^9 as measured by Method 1 and 1.4×10^9 as measured by Method 2. The estimated maximum error of these measurements is 0.4–0.5 log (a factor of ~3). The principle source of error (0.3 ND) in Method 1 was in the calibration of the neutral density filters. This estimate was arrived at by assuming a calibration error of 0.03 ND per density unit and making the worst case assumption that all errors were of the same sign. In addition, there was error inherent in the sampling of a limited number (~100) of discrete waves, and there were counting errors which occurred when two discrete waves overlapped to such a degree that they could not be resolved and were counted as a single event. Method 2 suffers from further error in the correction for the nerve's absorbance (see Materials and Methods).

Both methods yield values for the number of rhodopsin molecules per cell which are lower limits. This is because we have assumed that every isomerized rhodopsin molecule produces a discrete wave, i.e. that the quantum efficiency of isomerization equals the quantum efficiency of excitation. If one judges from experiments on squid photoreceptors, this assumption is not likely to lead to large errors. In squid (Hagins, 1965) the maximum measured quantum efficiency of excitation is 0.3. This number is probably low since measurements were made on slices of squid retina which were likely to be partially damaged by the preparative procedure. If one assumes that the quantum efficiency of isomerization in squid is 0.65 as in other rhodopsins (Dartnall, 1972), at least one of every two isomerized rhodopsins must excite the squid photoreceptor.

What we have measured is the amount of rhodopsin present, but the cells also contain metarhodopsin. Due to the bright dissecting lights, rhodopsin and metarhodopsin were in photoequilibrium at the start of the experiments (Lisman and Sheline, 1976). The nearly equal half-saturation intensity for the negative (rhodopsin) and positive (metarhodopsin) components of the ERP (Fig. 1) indicates that rhodopsin and acid-metarhodopsin have nearly equal photosensitivities (see Eq. [1]). Therefore the total number of visual pigment molecules (rhodopsin plus metarhodopsin) in cells dissected under white light must be about double the number of rhodopsin molecules (i.e. 3.3×10^9 molecules).

It is of interest to compute the expected absorption of rhodopsin from our measurements and compare it to spectroscopic data (Murray, 1966). Given the flash intensity (530 nm) that evokes an average of one discrete wave (7.4×10^6 photons/cm^2, Table I), the area of the cell (6×10^{-5} cm^2), and the asumption that the quantum efficiency for evoking a discrete wave is 0.65, it can be computed that rhodopsin absorbs one of every 282 photons incident on the photoreceptor. This is the case for cells dissected under lights bright enough to establish a photoequilibrium between rhodopsin and metarhodopsin (Lisman and Sheline, 1976). If the cells had been dissected under red light, the probability of evoking a discrete wave would have been approximately five

times higher (Lisman and Sheline, 1976). Therefore, in cells dissected under red light, the expected absorption due to rhodopsin would be 0.018 and the expected absorbance 0.0077. This can be compared to the maximum light-induced absorbance decrease of 0.015 measured by Murray (1966) in single ventral photoreceptors dissected under red light.

The discrepancy between Murray's measurements and the absorbance predicted by our calculations may not be real given the assumptions and approximations we have made, however it is instructive to consider how such discrepancies might arise. One possibility, suggested by the observation that sensitivity to light is nonuniform over the cell surface (Yeandle and Spiegler, 1973), is that rhodopsin is nonuniformly distributed in the cell. Since Murray used a measuring beam (diameter 20 μm) which only partly covered the cell (50 × 150 μm), and since he reported only his largest values, the average absorbance of the whole cell is likely to be lower than 0.015. A second possibility stems from the inherent differences in measurement techniques. Our methods measure only those pigment molecules which have the potential to excite the cell. Photoreceptors may contain visual pigment precursors or pigments having other roles in the visual process. For example, squid photoreceptors contain a second pigment, retinochrome, of unknown function (Hara and Hara, 1965). Such a pigment would contribute to the absorbance of the cell, but would not be detected by our technique since it is not directly involved in the excitation process.

The methods for measuring the number of rhodopsin molecules developed in this paper are applicable to other photoreceptors. Method 1 requires no absolute calibration of light intensity and should be useful in situations where it is difficult to estimate the absolute intensity at the transducing membrane. Method 1 is insensitive to the presence of a filter (for instance a screening pigment) interposed between the light source and the membrane, provided the density of the filter is the same when low- and high-intensity stimuli are presented. Since the number of quantal events can be estimated from the response noise (Dodge et al., 1968), Method 1 should be applicable even in cells which produce no observable discrete waves.

Surface Density, Charge Movement, and Thermal Isomerization of Rhodopsin

The surface density of visual pigment in the plasma membrane could be estimated if the membrane area were known. Since the specific capacity of cell membranes is close to 1 μF/cm^2 (Cole, 1968), an estimate of membrane area can be derived from measurements of cell capacitance.

The capacitance of *Limulus* ventral photoreceptors was determined by injecting hyperpolarizing current pulses (1–7 nA) through one intracellular microelectrode while monitoring the change in membrane potential (less than −10 mV) with another. The initial portion (2–5 ms) of the charging curve was linear; its slope defined $\frac{dv}{dt}$. Capacitance (C) was computed from the equation $C = i/\frac{dv}{dt}$ (see Gorman and Mirolli, 1972, for the advantages of determining C

by this method). The average capacitance as determined from five cells was $4.2 \pm 1.4 \times 10^{-9}$ F, a value in reasonable agreement with the results of Millecchia and Mauro (1969*a*). This implies a total membrane area of 4.2×10^{-3} cm^2. If the photoreceptor were not invaginated, its surface area would be 5×10^{-4} cm^2 (Millecchia and Mauro, 1969*a*). Thus most of the plasma membrane in the ventral photoreceptor is in the microvilli. If each villus has a length of 1 μm and a diameter of 0.07 μm (Clark et al., 1969), then there are approximately 1 million villi per cell.

From the above figure for membrane area and the value for the number of pigment molecules per cell, we calculate a surface density of pigment molecules of approximately 8,000/μm^2. This density is in fair agreement with a value of 10,000–20,000 derived from absorbance measurements in crayfish rhabdomes (Goldsmith and Wehner, 1977). Our estimate of pigment density is close to the density of membrane particles revealed by freeze-fracturing of the rhabdomes of crayfish (4,000/μm^2; Fernandez and Nickel, 1976) (8,000/μm^2; Eguchi and Waterman, 1976) and of *Drosophila* (4,214/μm^2; Harris et al., 1977). Because the particle density in *Drosophila* rhabdomes is substantially lowered by vitamin A deprivation, the particles are thought to be visual pigment (Harris et al., 1977). However, it is possible that the particles are aggregates of rhodopsin rather than single rhodopsin molecules. The fact that the disk membrane of vertebrate rods has a particle density (5,000/μm^2; Chen and Hubbell, 1973) which is substantially lower than the rhodopsin density (20,000/μm^2; Blaisie et al., 1965; Daemen, 1974) suggests that the particles in vertebrate rods may contain more than one rhodopsin molecule.

The charge flow during each component of the ERP can be calculated from the voltage changes (ΔV) and cell capacitance (C) by the formula $q = \Delta V \cdot C$. The amplitude (ΔV) of the positive component is typically 1 mV. The negative going response (from the peak of the positive component to the peak of the negative component) is typically 4 mV (Lisman and Sheline, 1976). The average number of rhodopsin (and of metarhodopsin) molecules is 1.65×10^9, about half of which are isomerized by a saturating flash. Given an average capacitance of 4.2×10^{-9} F, the charge flow per isomerized molecule during the positive component is 5.1×10^{-21} C and, during the negative component, 20×10^{-21} C. In one case C, R, and ΔV were measured in the same cell; the charge flow was 7.8×10^{-21} during the positive component and 27×10^{-21} during the negative component. These transmembrane charge movements are less than a single electronic charge (1.6×10^{-19} C). One way by which charge movements this small could occur is for a single electronic charge to move only a fraction of the distance through the membrane thickness (3% for the positive component; 14% for the negative component). This is of the same order as the 6% value calculated for rat rods (Hagins and Rüppel, 1971) and the 12% value for red-sensitive turtle cones (Hodgkin and O'Bryan, 1977). What we have measured is the charge flow during the first few milliseconds of pigment transitions that require hundreds of milliseconds for completion (Fein and Cone, 1973). Therefore the inequality of the measured positive and negative charge movements need not imply that there is a net charge flow during the entire visual cycle.

The discrete waves recorded from *Limulus* photoreceptors in the dark are thought to represent excitation due to the thermal isomerization of rhodopsin (Srebro and Behbehani, 1972). Since there is about one spontaneous discrete wave per second and about 1.65×10^9 rhodopsin molecules, the half-time for the thermal isomerization of rhodopsin must be greater than 37 yr. Neither spontaneous nor light-evoked discrete waves can be measured in vertebrate photoreceptors (Fain, 1975). However, Barlow (1956) used psychophysical measurements of "dark noise" to estimate that the half-time for thermal isomerization of human rhodopsin is between 31 and 257 yr.

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