Stochastic Properties of Discrete Waves of the *Limulus* Photoreceptor

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ABSTRACT In the dark-adapted photoreceptor of the horseshoe crab, *Limulus*, transient discrete depolarizations of the cell membrane, discrete waves, occur in total darkness and their rate of occurrence is increased by illumination. The individual latencies of the discrete waves evoked by a light stimulus often cannot be resolved because the discrete waves overlap in time. The latency of the first discrete wave that follows a stimulus can be determined with reasonable accuracy. We propose a model which allows us to make an estimate of the distribution of the latencies of the individual light-evoked discrete waves, and to predict the latency distribution of the first discrete wave that follows a stimulus of arbitrary intensity-time course from the latency distribution of the first discrete wave that follows a brief flash of light. For low intensity stimuli, the predictions agree well with the observations. We define a response as the occurrence of one or more discrete waves following a stimulus. The distribution of the peak amplitudes of responses suggests that the peak amplitude of individual discrete waves sometimes has a bimodal distribution. The latencies of the two types of discrete waves, however, follow similar distributions. The area under the voltage-time curve of responses that follow equal energy long (1.25 sec) and short (10 msec) light stimuli follows similar distributions, and this suggests that discrete waves summate linearly.

Much is known about the chemical changes that occur when a visual pigment molecule absorbs a photon (Hubbard, Bownds, and Yoshizawa, 1965). These changes are presumed to cause the flow of electrical currents across the cell membrane of the photoreceptor. The ionic bases of the currents have been studied in both the lateral and ventral eye of the horseshoe crab, *Limulus* (Fuortes, 1959; Kikuchi, Naito, and Tanaka, 1962; Smith, Stell, and Brown, 1968; Millecchia and Mauro, 1969). However, the mechanisms that link the absorption of a photon to the resulting changes in the electrical properties of the photoreceptor membrane are poorly understood. It is known, however, that the photoexcitatory process is stochastic, so that random events must intervene between the absorption of a photon and the flow of current across

the cell membrane (Fuortes and Yeandle, 1964). Therefore, a detailed analysis of the response variability of photoreceptors may prove helpful in specifying the molecular events that make up the photoexcitatory process. The purpose of this paper is to develop some techniques useful for the analysis of response variability in the photoreceptor of the lateral eye of the *Limulus*.

Response variability is a prominent feature of the dark-adapted ommatidium of *Limulus*. Transient depolarizations of the photoreceptor membrane, which have been called "bumps" or "discrete waves," are observed by using an intracellular microelectrode. They occur in total darkness and their rate of occurrence is increased by low levels of steady illumination (Yeandle, 1957, 1958). The noisy response observed at higher intensities of steady illumination can be explained as the superposition of discrete waves (Dodge, Knight, and Toyoda, 1968). If the response achieves sufficient depolarization, nerve action potentials are generated at the eccentric cell axon.

When a sequence of identical brief pulses of light of low energy is presented to the ommatidium, a given pulse in the sequence may or may not evoke a response (one or more discrete waves). The responses observed vary in size, shape, and latency from pulse to pulse. It is sometimes difficult to determine exactly how many discrete waves make up a response, because the discrete waves overlap in time. The probability that a short pulse of light of average energy E evokes a response is given approximately by $1 - \exp(-pE)$ where p is a constant (Yeandle, 1958). Fuortes and Yeandle (1964) found that the number of discrete waves evoked follows a Poisson distribution whose mean was proportional to light intensity. Discrete waves may be evoked by light flashes which deliver an average of 37.5 photons at the surface of the ommatidium (Borsellino and Fuortes, 1968). In the ventral photoreceptor cell of *Limulus*, there may be as few as 5 photon absorptions per discrete wave (Millecchia and Mauro, 1969). Taken together, these findings suggest that a single discrete wave results from a single photon absorption. We accept this suggestion as a working hypothesis. (For a review of the problem up to 1966 see Wolbarsht and Yeandle, 1967.)

We may define the latency of a response as the time between the onset of the stimulus and the first detectable depolarization. For weak stimuli the latency fluctuates from trial to trial as noted above, but for each trial where a response occurs the latency can be measured unambiguously to within 20–30 msec. The latencies of the individual discrete waves that make up a response are often difficult or impossible to resolve. It is these, rather than the response latency, that are of particular interest since the discrete waves probably result from the absorption of single photons and, hence, their latencies reflect the photoexcitatory process more directly than the response latencies. In this investigation we propose a simple stochastic model applicable to low energy light stimuli. The model allows the latency distribution of

individual discrete waves to be determined from the latency distribution of responses. The stochastic process proposed belongs to the class of processes generally called nonstationary or time-dependent Poisson processes. We show here that the model we propose can be used to predict the latency distribution of responses to stimuli of arbitrary time-varying intensities from the latency distribution of the responses to short flashes of light.

We have also examined in less detail the distribution of peak voltage of the first discrete wave following a stimulus, the correlation between peak voltage and the response latency, and the distribution of the areas under the voltage-time curve of the response.

THEORETICAL

Let us assume that each discrete wave we observe is the result of a single photon absorption. Not every photon incident on the ommatidium is absorbed by a photopigment molecule in the receptor and not every photon absorbed necessarily causes a discrete wave. If the average number of photons incident on an ommatidium during a short flash is E, then let pE be the average number of photons that are absorbed and that are effective in producing a discrete wave. The coefficient p is the probability that a photon incident on the cornea will produce a discrete wave and is assumed to be time independent. We call pE the average number of effective absorptions.

Consider a stimulus of finite duration whose intensity, in terms of number of photons per second, varies arbitrarily with time t according to some function $I(t)$. The origin of the time axis is chosen so that $I(t)$ is zero for $t < 0$. The average number of effective absorptions in any small time subinterval Δt at t is $pI(t)\ \Delta t$. Since photons are absorbed according to a Poisson process (Pirenne, 1951), the probability that there will be n effective absortions in this interval Δt is

$$\frac{[pI(t)\Delta t]^n}{n!} \exp[-pI(t)\Delta t].$$

We make two assumptions,

1. The occurrence of a discrete wave associated with a particular photon absorption is statistically independent of the occurrence of a discrete wave associated with any other photon absorption.

2. If a photon is effectively absorbed at time t', the probability that the discrete wave associated with that photon will occur in the interval (t', t) depends on $t - t'$ and not on t'. We call this probability $R(t - t')$, and from its definition $R(0)$ is 0 and $R(\infty)$ is 1. Let $r(t) = dR(t)/dt$, so that $r(t)\Delta t$ is the probability that a photon effectively absorbed at $t = 0$ will produce its discrete wave in a small subinterval Δt at t. $R(t)$ and $r(t)$ are the quantities of greatest interest in this theory.

Let $g_L(t)\Delta t$ be the probability that the first light-evoked discrete wave following a stimulus that begins at $t = 0$ occurs in a small subinterval Δt at t. We assert that

$$g_L(t) = pM(t)\exp[-p\int_0^t M(t')dt'] \tag{1}$$

where

$$M(t) = \int_0^t I(t')r(t-t')dt'. \tag{2}$$

In order to prove this, divide the time axis into many small subintervals each of length Δt. For some time t', $t' < t$, the probability $P(t',t)$ that no discrete wave will occur in the interval (t',t) due to effective absorptions in the small subinterval at t' is

$$P(t', t) = \sum_{n=0}^{\infty}[1 - R(t - t')]^n[pI(t')\Delta t]\exp[-pI(t')\Delta t]/n!$$

This is a consequence of enumerating and summing the probabilities of all the different ways that this can occur. Using the Maclaurin series expansion for the exponential function one sees that

$$P(t', t) = \exp[-pI(t')\Delta tR(t - t')].$$

Let $G_L(t)$ be the probability that the first light-evoked discrete wave occurs in the interval $(0, t)$ due to effective absorption of photons in this interval. Since a photon absorbed in any subinterval preceding t could result in a discrete wave occurring before t, the probability $1 - G_L(t)$ of no light-evoked discrete wave in the interval $(0, t)$ is

$$1 - G_L(t) = \prod_{t'=\Delta t}^{t} \exp[-pI(t')\Delta tR(t - t')].$$

Letting $\Delta t \to 0$, $G_L(t)$ becomes

$$G_L(t) = 1 - \exp[-p\int_0^t I(t')R(t - t')dt']. \tag{3}$$

Since $g_L(t)$ is equal to $dG_L(t)/dt$, and $R(0)$ is zero, differentiating equation (3), and using standard arguments concerning differentiation under the integral sign yield equation (1) and hence equation (1) is proved.

The probability of observing a response evoked by a given stimulus is $G_L(\infty)$, as can be calculated from equation (3) by letting $t \to \infty$. As $R(\infty) =$

1, the integral in equation (3) becomes $\int_0^\infty I(t')dt'$, which is the energy E of the stimulus in terms of total number of photons. Thus,

$$G_L(\infty) = 1 - \exp(-pE). \tag{4}$$

Equation (1) is the density function for the time to the first event for what is generally called a time-dependent or nonstationary Poisson process (see Cox and Miller, 1965, or Blanc-Lapierre and Fortet, 1953). It should be emphasized at this point that the physical model presented here, i.e. that one discrete wave results from one photon absorption, is not the only one which will lead to equation (1). We present it because it seems physically plausible and is consistent with previous work.

Spontaneous discrete waves can be included in the formalism. Previous work (Fuortes and Yeandle, 1964; Adolf, 1964) has shown that the intervals between spontaneous waves follow the exponential distribution. This implies that the probability of no spontaneous wave occurring in the interval (0, t) is $\exp(-M_d t)$ where M_d is the probability per unit time of a spontaneous discrete wave occurring. If we assume statistical independence between spontaneous and light-evoked waves, the probability that the first discrete wave occurs in the interval (0, t) is

$$1 - [1 - G_L(t)] \exp(-M_d t).$$

Differentiating this expression yields

$$g(t) = [pM(t) + M_d]\exp[-p\int_0^t M(t')dt' - M_d t]. \tag{5}$$

The quantity $g(t)\Delta t$ is the probability that the first discrete wave following a stimulus occurs in the subinterval Δt at t, and can be estimated experimentally from the response latencies. We discuss below how $r(t)$ can be calculated from measurements of $g(t)\Delta t$ for short pulses, and then can be used to predict $g(t)$ for stimuli of different time courses. Comparison with experiment can be made, and provides a test of the validity of equations (1) and (5).

METHODS

An intracellular electrode was inserted into a single ommatidium of the horseshoe crab, *Limulus*, using standard techniques, and responses were recorded on a Grass polygraph. The excised eye was bathed in artificial seawater at a pH of approximately 7.8. After successful insertion of a microelectrode, the eye was kept in the dark for 45 min to allow the discrete waves to achieve maximum size. Temperature was continuously monitored and maintained constant to within 0.1 °C.

Sequences of light stimuli were presented in groups or runs. A run consisted of 500 (in some cases 1000) equally spaced light stimuli or trials. In each run there were two

different types of trials, a control stimulus and a test stimulus. The control stimulus was a 10 msec pulse of light whose energy was coarsely adjusted by means of a neutral density wedge in the light path so that between 10 and 90% of the stimuli evoked a discrete response. The test stimulus, although constant for any one run, was selected from one of three types: (*a*) a 10 msec pulse of light with energy about four times that of the control stimulus; (*b*) a pair of pulses of light, each pulse identical to the control stimulus; (*c*) a light pulse 1.25 sec in duration but delivering the same total energy as the control.

Previous work (Fuortes and Yeandle, 1964) had indicated that a time interval between successive stimuli (interstimulus interval) of 5 sec insured a lack of correlation between responses to successive stimuli. This interstimulus interval was adopted here except in a few runs in which it was 6.3 sec.

In any run the control and test stimuli were presented in random order according to instructions on a perforated paper tape. These instructions were punched on the tape according to a table of random numbers before the experiment. The presentation of each stimulus was preceded by the following sequence: the timer that determined the stimulus interval signalled a tape reader to advance the tape to the next instruction which in turn allowed a servomotor to position a neutral density filter in the light beam and also set a switch to select the time course of the stimulus. Before the experiment, the transmission of the neutral filter used was determined in the photostimulator with a photomultiplier.

Both the test and control stimuli were monochromatic and at the same wavelength. We used wavelengths 404, 500, 656 nm in different runs with no substantial differences in results. The results discussed here are based on the study of 53 runs with a total of 31,000 trials recorded in 14 ommatidia.

The records were measured by hand. First the intervals between stimuli were subdivided into a convenient number of subintervals. The subinterval duration, which ranged from 20 to 40 msec, was fixed for any one run, and was determined by the paper speed of the Grass recorder. For each trial the stimulus type and whether a response began within the 5 sec interval following the stimulus were noted. If a response occurred, the number of subintervals intervening between stimulus onset and the beginning of the response was measured (the latency of the response). The peak voltage of the response was also measured, where we defined the peak as the first clearly detectable maximum following the beginning of a response.

In order to illustrate, Fig. 1 shows a sample of a recording sequence for one run. Control and test stimuli appear in random order. The sequence of eight consecutive trials shown in the figure was selected more or less at random from our recordings. With reference to Table I, the trials have been numbered left to right and upper line to lower line. The table indicates the measurements made. Trials 1 and 3 illustrate that it was sometimes difficult to decide whether a response had occurred. We took pains to be consistent in deciding whether a response had occurred. Fig. 1 gives an exaggerated impression of the number of trials for which the decision was difficult. Approximately 5–10% of the trials we observed could be called "possible responses."

Latency histograms were plotted for each run showing the number of stimuli as a

function of response latency (see Figs. 2 and 3). Perusal of these histograms indicates that most of the light-induced responses occurred in the first 2 sec after stimulus onset. We assumed that discrete waves that occurred in the last second of the interstimulus interval were spontaneous. M_d was estimated by counting the number of waves occurring in the last 25 subintervals of the interstimulus interval for all stimuli of the run. In some cases when the subinterval was 20 msec the last 50 subintervals were used.

The responses and conditions were also recorded on four-channel analogue tape and

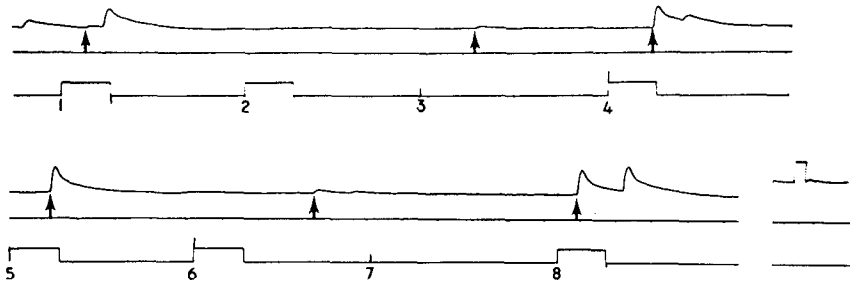

FIGURE 1. 8 consecutive trials from a run of 500 trials in which the test stimulus was 1.25 sec long and of energy equal to the control stimulus (10 msec flash). Top trace DC-coupled, high frequency limited by pen response to about 40 Hz. A 5 mv calibration mark is shown at the end of the eight trials. Middle trace, 1 sec time marker. Bottom trace, light stimuli. (The variable spike at the beginning and end of the light stimulus is an artifact.) The record strips are consecutive and have been separated vertically only as a convenience for display. Temperature 12.0°C, wavelength 500 nm. Arrows indicate beginning of responses.

TABLE I

Trial No.	Stimulus type	Latency	Peak height
		msec	*mv*
1	Test	460	0.6
2	Test	NR	—
3	Control	1120	0.8
4	Test	925	4.8
5	Test	830	5.0
6	Test	2420	0.8
7	Control	NR	—
8	Test	400	5.8

NR indicates no response.

the total area of each response was determined during the 4 sec interval following the onset of the stimulus. By the use of a biasing circuit, electronic clipper, and analogue integrator, the area of the voltage time curve exceeding a fixed reference level, just above the baseline noise, was measured. The location of this reference level was determined visually on an oscilloscope

METHOD OF ANALYSIS

From the experimental data for each run the following parameters were tabulated:

F the ratio of the energy of a control stimulus, E_c, to the energy of a test stimulus, E_t. (As no absolute energy calibrations were made, F was determined from the calibration of the neutral density filters used.)

N_c number of control stimuli in the run

M_c number of control stimuli in the run for which a response was observed in the interstimulus interval

N_t number of test stimuli in the run

M_t number of test stimuli in the run for which a response was observed in the interstimulus interval

T_m number of subintervals in the interstimulus interval

T_d number of subintervals at the end of the interstimulus interval used for estimating the rate of spontaneous discrete waves

S_d the total number of spontaneous discrete waves counted in the last T_d subintervals for all stimuli in the run

$g_c(k)$ the fraction of the number of control stimuli for which the response latency occurred in the kth subinterval after the control stimulus began. (k ranged from 1 to T_m).

$g_t(k)$ the fraction of the number of test stimuli for which the response latency occurred in the kth subinterval after the test stimulus began. (k ranged from 1 to T_m.)

The probability that the response latency is in the kth subinterval following a stimulus (i.e., that the first discrete wave begins in the kth subinterval following a stimulus) can be derived from equations (1) and (5). This results in the following incremental form of equation (5) for the control stimulus:
for $k = 1$

$$g_c(1) = pEr(1) + \bar{M}_d \tag{6 A}$$

for $k > 1$

$$g_c(k) = [pE_cr(k) + \bar{M}_d] \exp\left[-\sum_{j=1}^{k-1} pE_cr(j) - \bar{M}_d(k-1)\right]. \tag{6 B}$$

Equation 6 B is the product of the probability that a discrete wave occurs in the subinterval k and the probability that no discrete wave occurs in the $k - 1$ preceding subintervals. The following estimates were made from the tabulations for each run:

$$\bar{M}_d = S_d/[(N_c + N_t)T_d]$$

$$pE_c = -[\log_e(1 - M_c/N_c) + T_mM_c].$$

Equation 6 A was solved directly for $r(1)$. With $r(j)$ known for all $j < k$, $r(k)$ was determined from equation 6 B. Thus all values of $r(k)$ was determined iteratively for $k > 1$. If during the iteration $r(k)$ became negative for a particular k, $r(k)$ for that k was set equal to zero. (This could and occasionally did happen because of small sample (statistical) fluctuation in the estimates of $g_c(k)$.) The iteration was stopped as soon as $\sum_{j=1}^{k} r(j) > 1$ and the last $r(k)$ adjusted so that this sum equaled one. All subsequent $r(k)$ were set equal to zero.

For the test stimulus equation (2) was written in incremental form and $M(k)$ calculated using the estimate of $r(k)$ derived from the control stimulus. The latency distribution, $g_t(k)$, for the test stimulus was calculated from the incremental form of equation (5):

$$g_t(k) = [pE_tM(k) + \bar{M}_d] \exp\left[-\sum_{j=1}^{k-1} pE_tM(j) - \bar{M}_d(k-1)\right].$$

We used the same estimate of M_d in this equation as we had used in equations 6 A and 6 B, but the pE_t we substituted

$$pE_t = -[\log_e(1 - M_t/N_t) + T_mMd].$$

This insured that the predicted probability of a response during the interstimulus interval was approximately equal to M_t/N_t. As a check on internal consistency we also estimated pE_t by $-[1/F]\ [\log_e(1 - M_c/N_c) + T_mM_d]$ and compared the two estimates.

Fig. 2 shows the latency histogram using a control stimulus. Equations (6 A) and (6 B) were used to obtain an estimate of $r(t)$, which is shown in this figure referenced to the right ordinate scale. For most runs $r(t)$ had a peak slightly shifted to later times and a slower decay than the total response latency distribution. This is expected from the theory. The variability of the estimate of $r(t)$ is inherent in the numerical method we used to obtain it and therefore expected. We would expect that $r(t)$ is a smooth function. As our understanding of the photoexcitatory process improves we might be able to establish a priori arguments for a particular form of $r(t)$. However, the purpose of the experiment reported here was to test the ability of equations 1 and 2 to predict the latency distributions of responses due to stimuli of several different time-varying intensities. Since we were not interested in testing the applicability of a particular form of $r(t)$ we chose to use a numerical estimate.

Fig. 3 shows examples of the latency histograms for the control stimuli and the predicted and observed latency histograms for the three types of test stimuli used. In column 2 of this figure where the test stimulus is a double pulse, the first 500 msec of the test and control histograms represent the response to identical stimuli. Here differences between the two histograms

give an indication of the statistical fluctuation in sampling inherent in the measurement.

Table II summarizes the data used for the runs shown in Fig. 3.

In order to decide whether the predicted and observed histograms differed,

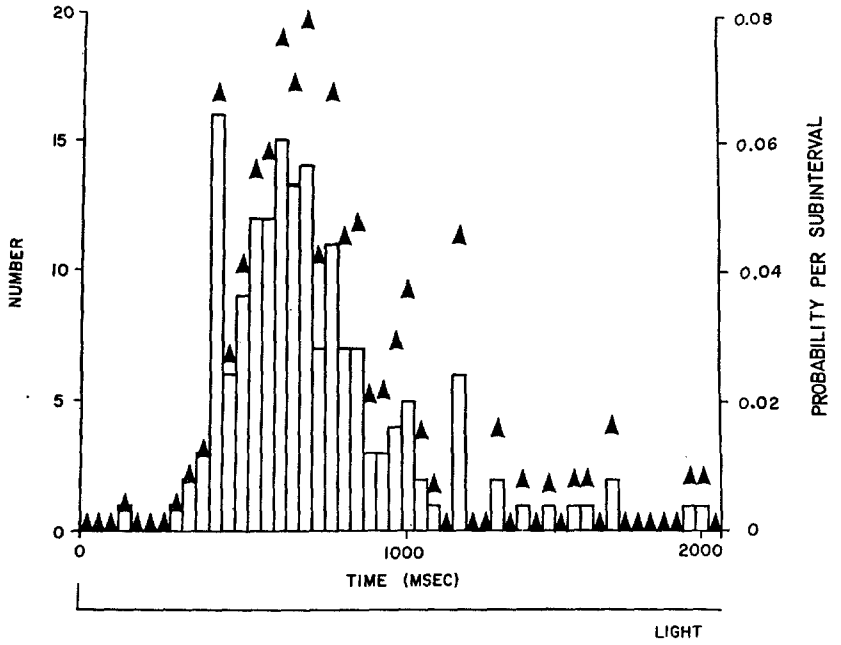

FIGURE 2. Response latency frequency histograms for a 10 msec light pulse and derived function r(t). Bar diagram shows the number of responses, indicated on left ordinate, vs. latency of response, indicated on abscissa. Triangles show derived $r(t)$, indicated on right ordinate, vs. time, indicated on abscissa. The right ordinate of the base of each triangle gives the probability that a discrete wave resulting from an effective photon absorption at zero time occurs in the subinterval whose center coincides with the abscissa of the triangle's center. The length of the triangle's base is the duration of the subinterval. Only the first 2.06 sec are shown although tabulations were carried out to 5 sec. Temperature 9.8°C, wavelength 500 nm. Number of trials in run 330. Number of trials which gave a response 179. Number of subintervals in interstimulus interval 129. Estimated probability per subinterval of a spontaneous wave 0.000333. The sum of the $r(t)$ values shown in this figure equals 0.9572. Four nonzero values of $r(t)$ occurring after 2.06 sec are as follows: 2.16 sec $r(t)$ = 0.008, 2.26 sec $r(t)$ = 0.008, 2.56 sec $r(t)$ = 0.0082, 2.40 sec $r(t)$ = 0.0168. All values of r(t) for $t > 2.4$ sec are zero.

the histogram values were tabulated and grouped into M cells so that the expected number of trials in which a response began was at least 10 for any one cell. A $2 \times M$ contingency table was constructed and a chi-square test applied to test the null hypothesis that the expected and observed frequency distributions were samples of the same population (Dixon and Massey, 1957). In 44 of 53 runs (83%) the null hypothesis could not be rejected. This test

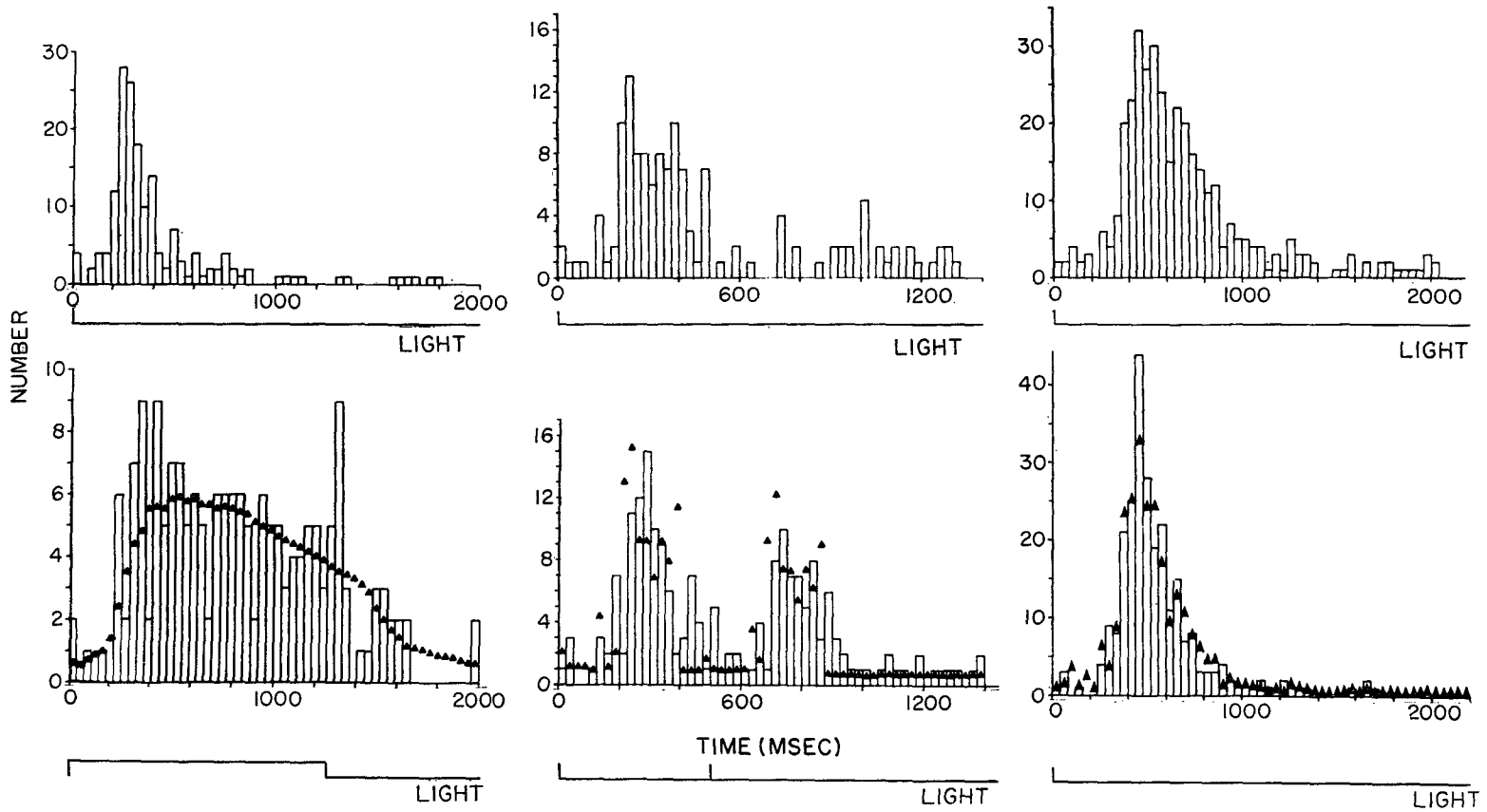

FIGURE 3. Predicted and observed latency frequency histograms for three runs from three different ommatidia. Ordinates, number of responses. Abscissas, time. Below each abscissa is shown the time course of the light stimulus. The histograms are shown only for the first 2 sec following the stimuli. The graphs in each column are from the same run. The top row shows the latency histograms due to the control stimulus (10 msec flash) for each run. The bottom row shows the observed (bars) and predicted (triangles) latency histograms for each test stimulus. See Table II for conditions of each run.

implicitly assumes that the predicted and observed frequencies have comparable variability. This assumption may not be strictly true in our case and seems likely to be violated when the test stimulus is a 1.25 sec flash of light, because of the smoothing inherent in the convolution procedure. (See Fig. 3, column 1.) Thus, runs of this type might yield acceptable fits when they should not. In order to estimate the seriousness of this error, we have calculated a chi-square, goodness-of-fit test for the observed and predicted latency histograms when the test stimulus was 1.25 sec long. Use of this chi-square test

TABLE II

	Column 1	Column 2	Column 3
Control stimulus, *msec*	10	10	10
Test stimulus	1.25 sec pulse equal in energy to control stimulus	Two pulses each identical to control pulse	10 msec duration but energy four times that of control pulse
Temperature, *°C*	14.5	12.5	12.4
Wavelength, *nm*	500	500	500
No. of control stimuli (N_c)	251	508	694
No. of test stimuli (N_t)	248	490	274
Fraction of control stimuli that gave a response	0.757	0.463	0.530
Fraction of test stimuli that gave a response	0.810	0.563	0.938
Duration of interstimulus interval, *sec*	5	5	5
No. of subintervals in the interstimulus interval (T_m)	123	203	126
No. of discrete waves in the last 25 subintervals (for all stimuli)	27	58	60

implicitly assumes that the predicted latency histograms had negligible variability. There were 24 runs of this type, 7 of which produced unacceptable fits to the predictions. The contingency table method on the other hand rejected two of these runs. Since the assumption that the predicted latency frequency histograms had negligible variability is too strong, the method based on this assumption rejected too many runs. Thus we can say that between 71 and 92% of the runs in which the test stimuli were 1.25 sec long, produced acceptable fits to theory. The runs in which the test stimuli were either another short flash or a pair of flashes produced predictions whose variability was comparable to the observed data (see Fig. 3, columns 2 and 3) and, therefore, the assumption of the contingency table test is not seriously violated. There

were 29 runs of these types, 22 of which produced acceptable fits to theory (76%). Moreover, in almost all runs which were rejected these obvious reasons caused the failure: (*a*) insufficient light was used so that the estimate of $r(t)$ was poor; (*b*) the probability of a response was nearly equal to unity.

As noted in the Methods of analysis section two estimates of pE_t were made as a check on the internal consistency. If the assumptions of the proposed model are correct, the estimate of pE_t made from the control stimulus and the value of F, minus the estimate of pE_t made entirely from the test stimulus should have equal probability of being plus or minus. For the runs in which the test stimulus had energy equal to the control, the difference was negative for 18 out of 24 runs which is just significant at the 5% level. However, the difference between the two estimates of pE_t was no more than 10% for any one run. (This small discrepancy may result from the assumption of statistical independence of light-evoked and spontaneous discrete waves.) For the two other types of test stimuli there was no systematic difference between the two estimates of pE_t. There was, however, more variability in the difference between the two estimates than in the case of the equal energy stimuli. The two estimates of pE_t are not equally reliable when $F > 1$ since the numbers N_c, M_c, N_t, and M_t that were used to make the estimates were often of different magnitudes.

Peak voltage distributions were tabulated for each stimulus type and run. Fig. 4 shows the two typical types of distributions we encountered; i.e., unimodal and bimodal. 17 of 53 runs had bimodal peak voltage distributions. 30 had unimodal peak voltage distributions and in the remainder, the occurrence of nerve action potentials prevented meaningful measurements. Correlation coefficients were calculated for the peak voltage against latency for each stimulus type and run. All runs having a bimodal peak voltage distribution had a significant negative correlation coefficient. However, the correlation coefficients were within the range -0.15 to -0.52. 11 of the 30 runs having a unimodal peak voltage distribution also had a significant negative correlation coefficient within the same range. In those runs having a bimodal peak voltage distribution and about equal numbers of responses in the two modes we were unable to detect by visual inspection a difference in the latency histograms plotted for the two modes separately.

Regression of the peak voltage and latency against the serial order number of the response was done by run and stimulus type. (That is, the first response due to, for example, a control stimulus was assigned the serial order number 1, the second response 2, etc. The time elapsed from the beginning of a run is a monotonic function of the serial order number.) The slope of the regression line of peak voltage vs. serial order number was significantly different from zero in 14 runs, about equally likely to be positive or negative, and unrelated to the modal pattern of the peak voltage distribution. The slope

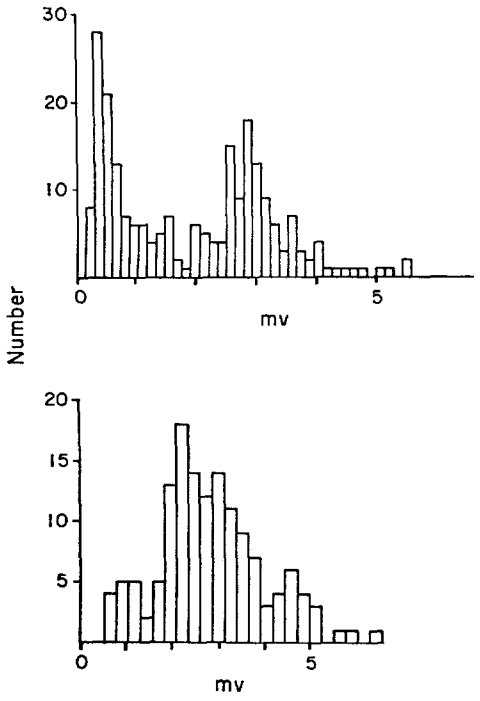

FIGURE 4. Peak voltage frequency histograms. Ordinates, number of responses having a peak voltage indicated by abscissa. (See text for methods.) The top histogram is an example of a bimodal peak voltage histogram. Top, temperature 15.0°C, wavelength 500 nm. Bottom, temperature 12.5°C, wavelength 500 nm. The stimuli were 10 msec flashes in both cases.

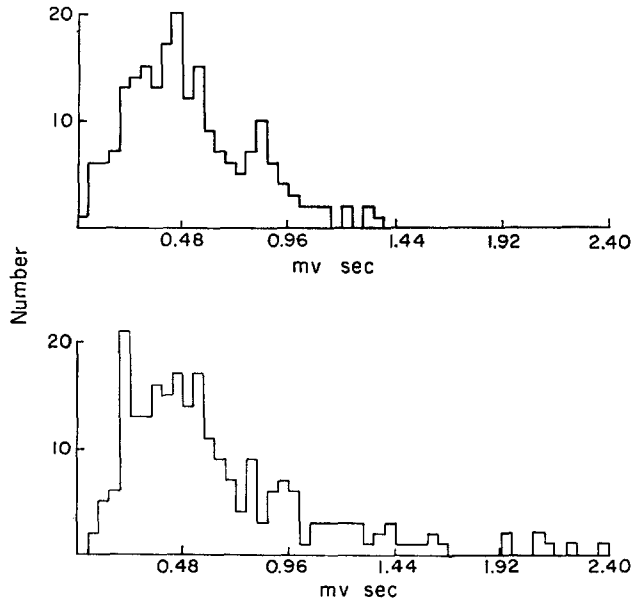

FIGURE 5. Distribution of area under voltage time curve for responses due to a short (10 msec) stimulus (top) and equal energy long (1.25 sec) stimulus (bottom). See text for methods. Ordinates, number of responses having area equal to that shown on abscissas. Trials for which there was no response are not shown. Temperature 15.6°C, wavelength 656 nm.

of the regression line of latency vs. serial order number was significantly different from zero in only one run.

The total area of each response (see Methods) in a 4 sec interval following the onset of the light stimulus, was determined in several runs where the test stimulus was 1.25 sec long and of energy equal to that of the control. Fig. 5 is an example of such an area histogram. Except for a slight tendency of the long pulses to produce larger areas than the short pulses, the two types of stimuli produce similar area histograms.

CONCLUSIONS

The data we have presented support the usefulness of equations (1) and (2) as a general description of the timing of discrete waves. From equation (5) it is seen that the latency distribution behaves as a nonhomogeneous Poisson process with time-dependent rate parameter $(pM(t) + M_d)$. From equation (2) it is seen that $M(t)$ is the convolution of the intensity-time course of the light stimulus with a function $r(t)$. Given that each discrete wave is the result of a single photon absorption, $r(t)$ is the probability density function for the latency of that discrete wave. Any physical (molecular) model that one might consider for the linkage between the absorption of photons and the change in membrane permeability thereby produced, must account for the shape and behavior of $r(t)$. The success of the formulation justifies the use of the methods of linear systems analysis in exploring the properties of $r(t)$.

The above conclusions about the timing of discrete waves are separate from questions about how discrete waves sum. The results shown in Fig. 5 suggest that discrete waves summate linearly regardless of how they are spaced in time. Taken together, the findings suggest that the average response of the ommatidium to stimuli of low light intensity follows linear addition rules.

The origin of the bimodal peak voltage distributions we observed is unclear. Since the larger responses had peak voltages four or five times greater than the smaller ones (see Fig. 4), they probably do not represent coincident small discrete waves. It is more likely that there are two classes of discrete waves. Responses with larger peak voltages also appeared to have a faster rise time, which explains the slight negative correlation between peak voltage and latency that we observed. We did not observe dramatic differences in the latency distributions of the large and small responses and infer that large and small discrete waves have identical latency distributions. It is possible that the large discrete waves came from the same retinula cell in which our electrode was inserted, while small ones were generated in more distant retinula cells. It follows from this hypothesis that the responses sensed by an electrode in the eccentric cell always have a unimodal peak voltage distribution. Since only one eccentric cell was included in this study (that cell did produce unimodal peak voltage distributions), we are unable to test the hypothesis.

The regression analysis indicates that the latency distribution is stable during the course of a run. On the other hand, the peak height is subject to positive and negative drifts. The decoupling of peak height from latency by unknown factors during the course of a run may indicate that the two parameters are controlled by different mechanisms. For example, the timing of a discrete wave may be the result of a sequence of changes in the shape of the rhodopsin molecule that leads to a transient permeability increase of the receptor membrane. These changes might well be temperature-sensitive but, as the temperature was kept constant during a run, the response latencies would not be expected to show significant drifts during the course of a run. The size of a discrete wave, however, may depend not only on permeability changes but also on the concentration gradients across the receptor membrane of some ion, most likely sodium, that carries the electrical current associated with the discrete waves. Since the gradients could not be controlled in these experiments, slow drifts in response size would not be unexpected.

This work was supported by Public Health Service grants NB06046 and NB08537 from the National Institute of Neurological Diseases and Stroke and by the Bureau of Medicine and Surgery, Navy Department Research Task MR005.09-0078.
The opinions or assertions contained herein are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

Received for publication 24 October 1969.

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