Light-Evoked and Spontaneous Discrete Waves in the Ventral Nerve Photoreceptor of *Limulus*

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ABSTRACT Discrete waves, recorded from the ventral nerve photoreceptor, occur in the light and in the dark. Spontaneous waves, on the average, are smaller than light-evoked waves. This suggests that not all spontaneous waves can arise from spontaneous changes in the visual pigment molecule identical to changes induced by photon absorption. Spontaneous and light-evoked waves are statistically independent of each other. This is shown by determination of frequency of response as a function of pulse energy for short pulses and determination of the distribution of intervals between waves evoked by steady lights. The available data can be explained by two models. In the first each photon produces a time-dependent excitation that goes to zero the instant the wave occurs so that the number of effective absorptions from a short light pulse equals the number of waves produced by the light pulse. In the second the excitation produced by photon absorption is unaffected by the occurrence of the waves so that the number of waves produced from a short light pulse may be different from the number of effective absorptions. Present results do not allow a choice between the two models.

In darkness the visual system can send signals to the central nervous system. The origin of these signals is not clearly understood. One hypothesis is that such signals result from spontaneous thermal configurational changes in the visual pigment molecules that are identical to the changes induced by photon absorption (Denton and Pirenne, 1954; Barlow, 1956).

We present evidence suggesting that the explanation of spontaneous signals, at least for one type of photoreceptor, the ventral nerve receptor of *Limulus*, is more complicated than this hypothesis.

It is possible to observe units of membrane depolarization in single dark-adapted photoreceptors of arthropods (Yeandle, 1958; Scholes, 1965; Kirschfeld, 1965; DeVoe and Small, 1970). In the *Limulus* lateral eye, these units of depolarization can sum, so that if a threshold level of depolarization is ex-

ceeded, a propagated nerve impulse is produced (Yeandle, 1958). These units of depolarization have been variously called quantum bumps, discrete waves, and slow potential fluctuations. If one assumes that the absorption of a single photon can evoke a measurable depolarization with a probability p, then one can prove theoretically that the probability of a short pulse of light evoking a measurable depolarization as $1\text{-}\exp(-fpE)$. E is the average number of photons incident on the receptor, and f is the fraction of E absorbed by the visual pigment (see Pirenne, 1951). Discrete waves of depolarization occur in the dark. Their existence is consistent with the suggestion that the photoreceptors themselves are a source of noise in the visual system (Hecht, 1945).

One of the early observations on discrete waves is that there is a latency between the absorption of light and the occurrence of light-evoked waves. Furthermore, this latency fluctuates within a certain time interval after light absorption (Fuortes and Yeandle, 1964; Srebro and Yeandle, 1970). There is evidence from the *Limulus* lateral eye that under steady illumination the spontaneous waves occur randomly in time, i.e., follow a time-independent Poisson process (Fuortes and Yeandle, 1964; Adolph, 1964). If the spontaneous waves are statistically independent of light-evoked waves, and if the probability of a light pulse evoking one or more waves is $1\text{-}\exp(-pfE)$, then one can show that the probability, P, of one or more waves occurring after a short pulse of light is

$$P = 1 - \exp(-fpE - k\tau), \tag{1}$$

where τ is the time interval after the light pulse where light-evoked discrete waves occur and k is the probability per unit time of spontaneous waves occurring. Agreement of Eq. (1) with experimental data on the frequency of response to weak pulses of light is consistent with, but does not prove, the hypothesis that the absorption of a single photon can evoke with probability p a discrete wave.

A few years ago, peculiar photoreceptors were discovered in a nerve on the ventral side of *Limulus* (Clark et al., 1969). These receptors are not organized into an eye, and no propagated nerve impulses have been observed originating from them upon light stimulation. Their function is not understood. Despite this, their response to light is very similar to that found in the retinular cells of the *Limulus* lateral eye and in other arthropod receptors. The ease of penetrating these cells with intracellular microelectrodes and the simplicity of their structure have made them a good model system for studying the initial steps of the transduction of light by a receptor into an electric current.

Millecchia and Mauro (1969) have shown that discrete waves can also be observed in the ventral nerve receptor. In this paper it will first be shown that for the ventral receptor Eq. (1) correctly predicts the probability of oc-

currence of discrete waves after a short pulse of light. This suggests that these waves represent single photon absorptions. It will then be shown that the statistical properties of waves occurring in the dark are different for those occurring in the light and that spontaneous waves and light-evoked waves are very likely independent of each other. This suggests that not all the spontaneous waves result from the same changes in the visual pigment molecules that produce light-evoked waves.

METHODS

The ventral nerve was excised from young specimens of *Limulus* (4–5 inches across the carapace), desheathed, and pinned to the bottom of a petri dish whose bottom had been coated with a transparent rubbery potting compound. The nerve was bathed with artificial seawater made from a commercial preparation called Seven Seas Marine Mix (Utility Chemical Co., Paterson, N. J.). The dish was placed on a peltier thermoelectric cooler, one of the receptors was impaled with an intracellular microelectrode, and a thermistor was placed near the impaled receptor. This arrangement allowed temperature control within 0.1°C.

The preparation was stimulated with a photostimulator consisting of a 6 V, 18 A tungsten bulb powered by a constant current supply, a shutter capable of producing pulses from 10 ms to infinity, and various lenses arranged to focus the bulb filament on the shutter and project a spot of light on the preparation. The wavelength of the light was fixed at 5400 Å by an interference filter with 130 Å band pass placed in the light beam. The output of the stimulator was constant to within 0.2%. The intensity was controlled by neutral density wedges placed in the light beam. The relative transmissions of the wedges were calibrated with a photomultiplier. An absolute calibration of the photostimulator with wedges set for maximum transmission was obtained by measuring the current from a PIN diode (United Detector PIN 10 diode, United Detector Technology, Inc., Santa Monica, Calif.) placed at the output of the photostimulator. An Eppley (Eppley Laboratory, Inc., Newport, R.I.) standard of spectral irradiance was used to calibrate the diode in terms of photons per second of input flux per microampere of output current.

Responses were recorded on a Grass Polygraph (Grass Instrument Co., Quincy, Mass.) with a bandpass of DC to 40 Hz. All records were measured by hand with a ruler and all calculations were done using computer programs written in BASIC.

RESULTS

The experimental protocol to test Eq. (1) was as follows. A cell, after it had been impaled with an intracellular electrode, was allowed to dark adapt for about 45 min to 1 h. In most dark-adapted preparations discrete waves were observed. In a few preparations no discrete waves were observed after a long period of adaptation even though a relatively large receptor potential (about 40 mV) could be evoked by an intense light pulse. If discrete waves occurred, a low intensity flashing spot of light of about 5–10 μm in diameter was moved over the receptor to determine the region of the cell where light stimulation

evoked discrete waves. The spot of light was then placed well within the sensitive region of the preparation. A train of 50 ms light pulses, spaced 5 s apart, was presented and the pulse intensity was varied until discrete waves were evoked by about ½ of the pulses. Four or five additional pulse intensities were chosen spanning a range between ½ and 1 log units of intensity. The intensity of pulses evoking responses with about probability ½ was approximately at the midpoint of this range. The preparation was allowed to remain in the dark for about 15–30 min. If there were no discernable change in the resting membrane potential, sequences of pulses were presented to the preparation. Each sequence contained 80–100 pulses. The intensity of all pulses in any one sequence was the same, and was chosen from among the four or five values determined above. The pulse duration and interpulse interval were 50 ms and 5 s, respectively.

As has been previously reported for receptors of the *Limulus* lateral eye, the latency to the first discrete wave is a random variable. The same is true for the ventral nerve receptors. (Fig. 1). For each experiment the latency distribution was measured to determine the interval of time after the light pulse where most of the discrete waves occurred. For all experiments in this study, this interval was within 1 s. The last second of the interpulse interval was treated as a pulse of zero intensity. For each experiment we determined the number of pulses in each sequence where discrete waves occurred in the first second after the pulse and the number of interpulse intervals where a response occurred in the last second.

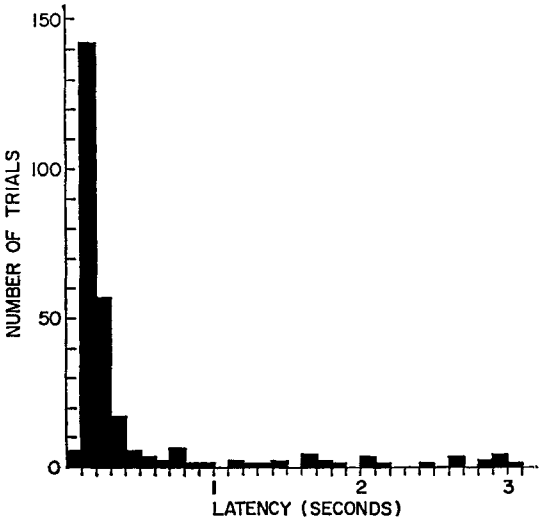

FIGURE 1. Distribution of latency to the first response after a short pulse of light. Pulse duration, 50 ms. Fraction of pulses producing response in first second was 0.58, temperature 18°C. Time between successive pulses is 5 s.

A total of six experiments were done on three cells. In four of the experiments, the pulse intensities of the odd numbered sequences were the same and the pulse intensities of the even numbered sequences were different. In two of the experiments there were two sequences at each intensity. For both of these protocols there was no statistical difference in the fraction of pulses evoking discrete waves between sequences of the same pulse intensity. In each experiment a total of 600–1000 pulses were presented to the preparation.

For two of the cells one experiment was done with a large spot that illuminated most of the active region of the cell and the other with a small spot 5–10 μm in diameter concentric with the large spot. For one of the cells one experiment was done with the 5–10 μm spot illuminating one region of the cell and the other experiment with an identical spot about 50 μm from the first spot.

To determine if the data were consistant with Eq. (1), we used the following statistic:

$$\chi^2 = \sum_{i=1}^{n} \frac{(N_i P_i - M_i)^2}{P_i(1 - P_i)N_i}, \tag{2}$$

where N_i is the total number of pulses at the ith intensity, M_i is the number of pulses at the ith intensity which evoked discrete waves, and n is the total number of intensities used in the experiment. P_i is given by

$$P_i = 1 - \exp(-fpE_i - k\tau), \tag{3}$$

where τ is 1 s. E_i is the average number of photons incident on the receptor in the pulses of the ith intensity. fp and k have the same meaning as before and were chosen to make χ^2 a minimum. The resulting values of fp and k were used as the best estimates of these quantities. Under these conditions χ^2 is distributed as χ^2 with $n - 2$ degrees of freedom.

This is because the random variables $(N_iP_i - M_i)/(N_iP_i[1 - P_i])^{1/2}$ are approximately normally distributed with zero mean and unit variance and are statistically independent of each other. General statistical theory says that the sum of squares of n such variables are distributed according to the χ^2 distribution with $n - m$ degrees of freedom where m is the number of subsidiary equations involving the parameters. Since two parameters were adjusted to minimize χ^2 this was equivalent to two added equations so that the number of degrees of freedom was $n - 2$. A table of χ^2 can then give a measure of how well the data fit the theory.

Fig. 2 shows the results of one experiment. Table I summarizes the other experiments by showing the values of the χ^2 statistic, the estimates of pf and k, and the extent of illumination. For each experiment, the χ^2 statistic was nonsignificant at the 5% level. The fit of Eq. (1) to the data appears to be un-

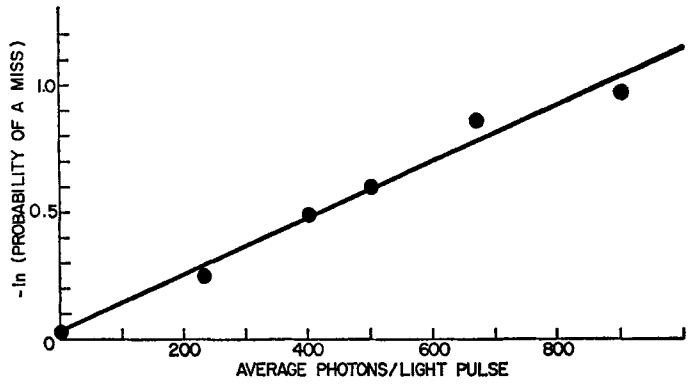

FIGURE 2. Plot of experimental and theoretical frequency of no response (miss) occurring after a light pulse (ordinate) vs. average number of photons in a pulse (abscissa). The probability of a miss within 1 s after a light pulse in exp $(-fpE_i - k)$. Points are experimental frequencies and line is fitted to above expression by method discussed in text. Temperature 14.9°C. The data upon which the graph is based are:

Number of pulses	Number of pulses failing to evoke a response	Intensity of photons per pulse
98	37	899
95	40	674
94	52	503
387	238	403
95	74	235
769	744	0

TABLE I

RESULT OF FITTING EXPERIMENTALLY DETERMINED CURVES OF FREQUENCY OF NO RESPONSE VS. AVERAGE NUMBER OF PHOTONS PER PULSE TO EQ. (1)

Cell no.	Temperature	Experiment no. and extent of illumination	pf	k	χ^2	Degrees of freedom	Level of significance
	°C						%
		(1) 10 μm spot	0.00189	0.244	2.928	3	30–50
1	18	(2) 10 μm spot 50 μm from 1	0.00221	0.264	2.520	3	30–50
		(3) Large spot	0.00102	0.067	4.440	4	30–50
2	14.7	(4) 10 μm spot concentric with large spot	0.00105	0.184	3.260	4	50–70
		(5) Large spot	0.00111	0.0327	1.289	4	80–90
3	14.9	(6) 10 μm spot concentric with large spot	0.00106	0.0155	2.857	4	50–70

Units of pf are reciprocal photons and of k reciprocal seconds. One may add the χ^2's and degrees of freedom for each experiment to obtain a total χ^2. Total χ^2 is 17.29 and total degrees of freedom 22.

affected by the extent to which the cell was illuminated. For cell no. 2, the estimates of k for the two experiments are quite different. There was a lapse of time of about an hour between the two experiments and the large difference between the two values of k may mean that the value of k for the preparation drifted.

To gain a better idea of the validity of Eq. (1) we added the values of χ^2 and the degrees of freedom for all the experiments. This total χ^2 was non-significant at the 5% level of significance for the total number of degrees of freedom. The fit of the data to Eq. (1) is quite good and is consistant with the hypothesis that, as has been previously suggested for the *Limulus* lateral eye (Yeandle, 1957, 1958; Fuortes and Yeandle, 1964; Borsellino and Fuortes, 1968), the locust eye (Scholes, 1965), and the fly eye (Kirschfeld, 1965), these waves represent single photon absorptions.

To study the differences between light-evoked and spontaneous waves, recordings were taken in darkness and upon exposure of the preparation to steady lights.

Fig. 3 shows segments of records taken in darkness and at three steady light intensities. Defining the wave height to be the difference between the base line potential and the maximum potential of a wave, we measured the height of all waves recorded in darkness and at the three light intensities and plotted the height distributions. The results are shown in Fig. 4. If the waves superimpose linearly then this definition of height may be incorrect for a wave that begins on the upper part of the falling phase of another wave. The fourth and

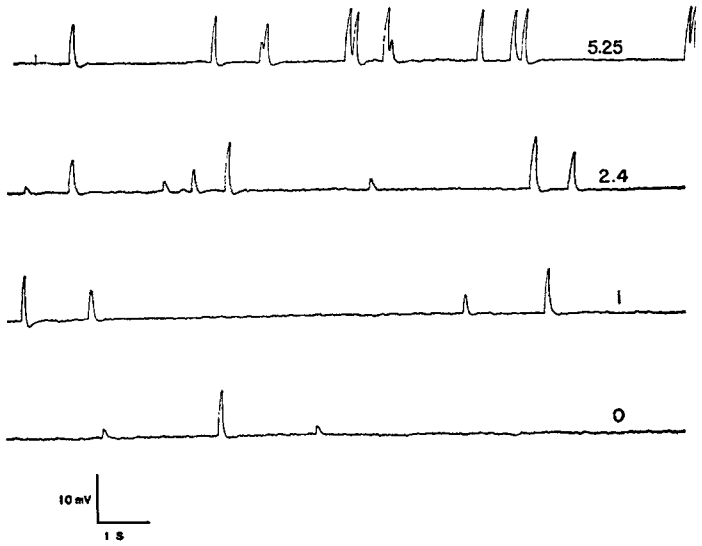

FIGURE 3. Records taken from three intensities and dark. Relative values of the intensities listed on records. Voltage and time scale shown on dark record applies to other records.

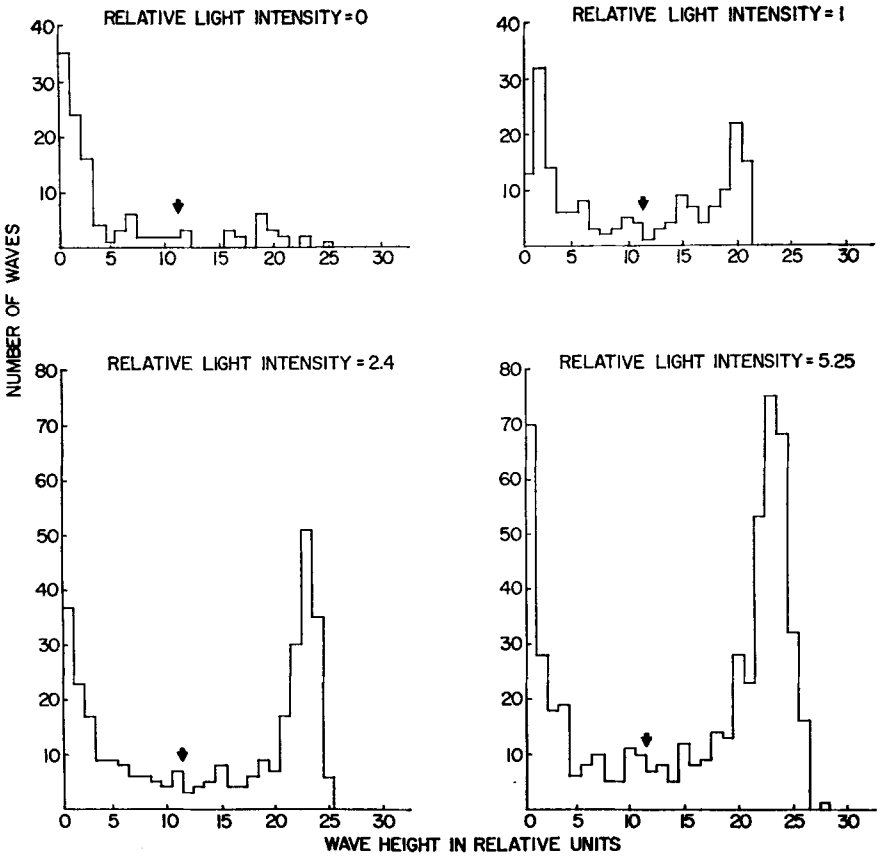

FIGURE 4. Histograms of wave heights in relative units evoked by steady illumination for experiment shown in Fig. 3. One relative unit equals 0.55 mV and is the smallest potential difference that could be measured from our records. The arrow on each graph indicates the point of separation between big and little waves. A wave was classified as big if its height equaled or exceeded 12 units, otherwise, it was classified as small. The illumination was continuous for 9.50, 9.63, 9.89, and 9.60 min for relative intensities, 0, 1, 2.4, and 5.25, respectively. Temperature of experiment 25.5°C.

last waves in the top record of Fig. 3 are examples of this difficulty. This particular length of record has an unusual number of such double waves. In the records analyzed in detail this difficulty occurs at most for about 5% of the wave. These distributions, because of their bimodal character in the presence of light, enabled us to make a somewhat arbitrary classification into big and little waves. The arrows in the figure indicate the choice of the separation point between big and little waves. In general, increasing the intensity of steady illumination increased the proportion of big waves to little waves as shown in Fig. 5, where the average rate of big, little, and all waves are plotted against steady light intensity. The small range of intensities used this relation

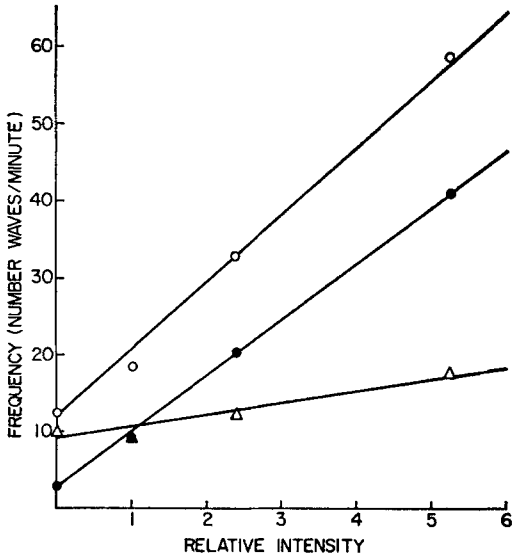

FIGURE 5. Plots of frequency vs. relative intensity for experiment shown in Figs. 3 and 4. Open circles represent frequencies of all waves, closed circles frequency of big waves, and triangles frequency of little waves. The ordinates of the open circles are the sums of ordinated for the closed circles and triangles.

is linear. In some experiments, not illustrated, the steady rate for small waves did not change with steady light intensity.

The question that immediately arises is to what extent the increase in the proportion of big waves with increasing light intensity results from overlap of waves due to increase of average rate of waves. The following argument shows that such overlap is probably of minor importance and is an unlikely explanation of the results observed.

It has been shown in the lateral eye, under steady illumination with weak lights, that the occurrence of discrete waves can be represented by a time-independent Poisson process. We will show that this is also true for the ventral nerve receptors. For a time-independent Poisson process the probability density function, $g(t)$, for the intervals between successive waves is

$$g(t) = \lambda \exp(-\lambda t), \tag{4}$$

where λ is the average rate of waves and t is the interval between successive waves.

The interval between two successive waves was defined as the time from the beginning of the rising phase of one wave to the beginning of the rising phase of the following wave. At the steady intensities used the intervals between successive waves were measured. All waves, both big and little, were included in the analysis. However, the first 2 seconds after the onset of illumination

were excluded from the analysis for the following reason. Because of fluctuation in the latency between photon absorption and occurrence of discrete waves there is a period of time immediately after the onset of illumination when the rate of discrete wave occurrence is not constant with time. As the latency distributions in the cells studied did not exceed 1 s we judged that a 2 second wait after the onset of illumination was sufficient to insure that the cell had achieved a steady state. Letting T be the duration over which the intervals between successive waves were measured and N the total number of waves in time T, λ was estimated by N/T.

The intervals were sorted into bins by a computer program such that the expected number of intervals in any one bin was as small as possible without being less than 5, a generally accepted lower bound for a χ^2 goodness of fit test. The upper and lower bounds of each bin were determined by the precision of the interval measurements and the number of intervals measured. Let t_i be the upper bound of bin i and the lower bound of bin $i + 1$. t_o is the lower bound of bin 1 and is set equal to zero. The expected number of intervals in each bin, EX_i, is

$$N \int_{t_{i-1}}^{t_i} \lambda \exp(-\lambda x)\, dx,$$

where N is the total number of waves. The standard goodness of fit χ^2 is

$$\sum_{i=1}^{n} (EX_i - OB_i)^2/EX_i$$

where n is the total number of bins and OB_i is the observed number of intervals in bin i. For all interval histograms on the two preparations subjected to detailed analysis this χ^2 statistic was not significant at the 5% level. A typical record of experimental values for an interval distribution is shown in Fig. 6 along with the theoretical curve derived from Eq. 4.

For a Poisson process each member of a pair of successive intervals should be independent of the other member. The probability $h(x,y)dxdy$ that the first interval of a pair lies in the range x, $x + dx$ and the second in the range y, $y + dy$ is

$$h(x, y)\, dxdy = \lambda^2 \exp(-\lambda [x + y])\, dxdy.$$

In the same records for which interval histograms had been constructed, pairs of intervals were set off as follows: interval 1 and interval 2 comprised the first pair, interval 3 and interval 4 comprised the second pair, and so on. The pairs of intervals were divided into bins so that the expected number of pairs of intervals in each bin was as small as possible without being less than 5. Each bin was characterized by two numbers, i and j. t_{i-1} and t_i are the

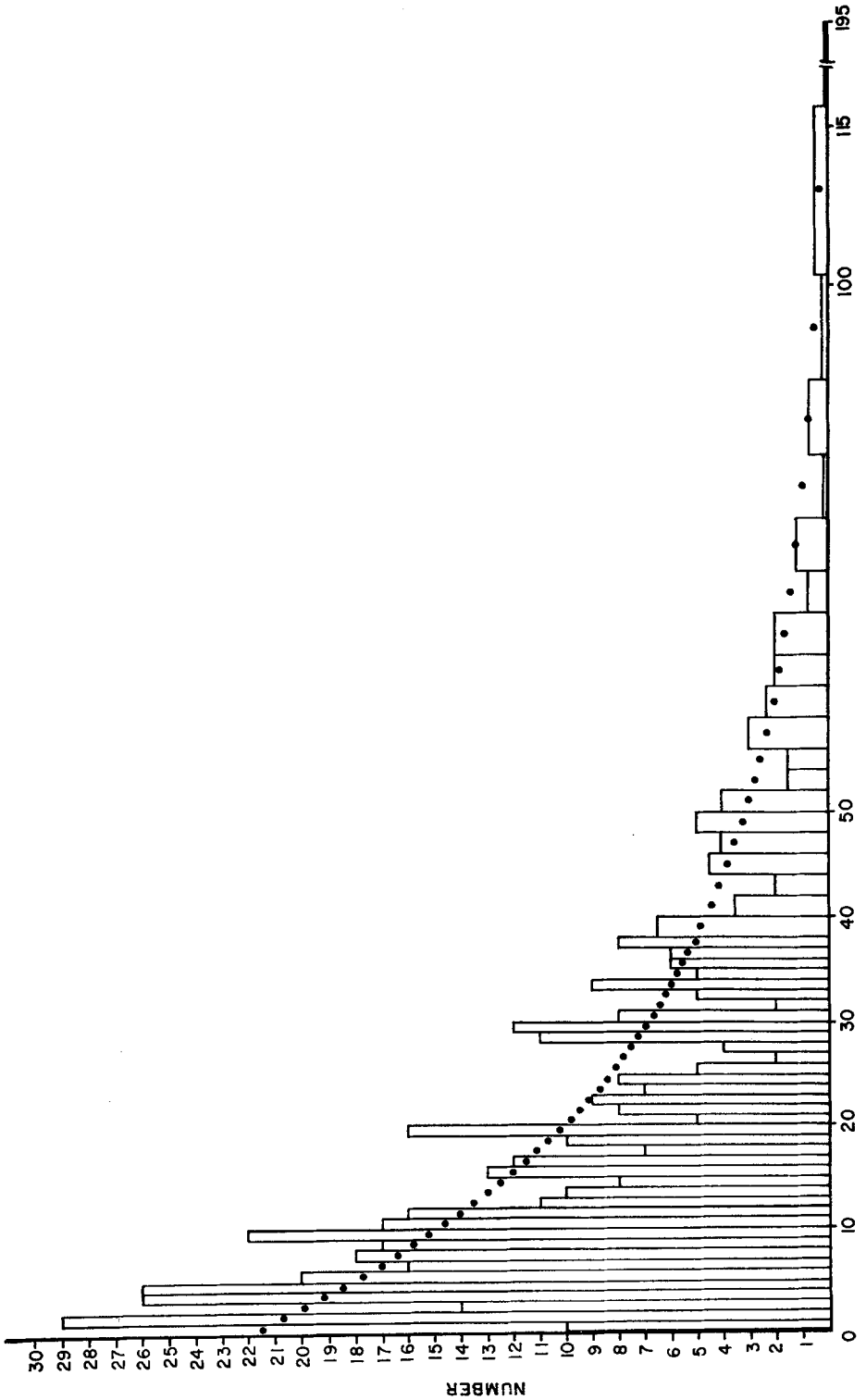

FIGURE 6. Time intervals between waves. Intervals taken from run of relative intensity 5.25 of the experiment shown in Fig. 3. For each bin, the expected and observed number of intervals are represented by a filled circle and a bar, respectively. The area of each bar is equal to the observed number of intervals. The ordinate of a filled circle, whose abscissa is the midpoint of the base of a bar, is equal to the expected number of intervals divided by the base of the bar. Probability per unit time of a wave occurring is about 1 per second. Time on abscissa is in relative units. Each relative unit is 40 ms and is the smallest interval that could be measured from our records.

lower and upper bounds for the first interval, and t_{j-1} and t_j are the lower and upper bounds for the second interval for all pairs of intervals in bin ij. The expected number in bin ij is

$$N\lambda^2 \int_{t_{i-1}}^{t_i} \int_{t_{j-1}}^{t_j} \exp(-\lambda[x + y])\, dxdy, \tag{5}$$

where N is total number of pairs of intervals.

Table II illustrates the results for the run of relative intensity 5.25 of the experiment of Fig. 3.

TABLE II

EXPECTED AND OBSERVED NUMBER FOR BINS OF INTERVAL PAIRS

Second interval limits	First interval limits					
	0,4	5,9	9,15	15,23	23,34	34,195
0,4	5.89 4	6.18 8	5.99 5	6.08 9	5.79 2	10.77 13
4,9	6.18 5	6.48 5	6.28 10	6.38 9	6.07 11	11.29 18
9,15	5.99 10	6.28 4	6.08 7	6.18 11	5.88 5	10.93 8
15,23	6.08 4	6.38 5	6.18 4	6.27 3	5.97 3	11.11 12
23,34	5.79 6	6.07 5	5.88 4	5.97 4	5.68 5	10.57 14
34,195	10.77 9	11.29 12	10.93 8	11.11 13	10.57 9	19.66 17

Data taken from same record used in Fig. 6. 281 pairs of intervals were tabulated. The left and upper margins of the table indicate in arbitrary time units the lower and upper bounds of the first and second interval defining each bin. An arbitrary time unit is 40 ms. The upper and lower number of each entry in the table are, respectively, the expected and observed number of interval pairs in the bin indicated in the table margins. The value of the χ^2 statistic for this experiment is 35.54 with 34 degrees of freedom which is nonsignificant at the 5% level.

Using the standard χ^2 goodness of fit statistic, all the runs analyzed in this manner showed at the 5% level of significance no evidence against the hypothesis that the intervals of successive pairs were independent.

A test was made to see if the height of a wave depended on the height of the previous wave. Using the criterion of big and little waves indicated in Fig. 4, we determined the proportion of big and little waves. The waves were grouped in pairs in the same manner as for the interval pair analysis just described. We divided the pairs into four groups based on the size category

of the first and second wave, respectively. Let P and $1 - P$ be the proportion of little and big waves, respectively, and N be the total number of pairs in the record. The four groups and the theoretical values of the expected number in each group, on the assumption of statistical independence between heights of successive waves, are shown below.

Size of first wave	*Size of second wave*	*Expected number of pairs*
Little	Little	NP^2
Little	Big	$NP(1-P)$
Big	Little	$NP(1-P)$
Big	Big	$N(1-P)\ (1-P)$

Table III shows the results of the analysis indicated above for the experiment illustrated in Fig. 4. The results are nonsignificant at the 5% level of significance. This rather crude test shows no evidence of dependence of heights of successive waves.

TABLE III A

DATA COLLECTED FROM HISTOGRAMS OF FIG. 4, AND CALCULATED PROPORTIONS OF BIG AND LITTLE WAVES

Relative intensity	Number of little waves	Number of big waves	P	$1-P$
Dark	97	22	0.815126	0.184874
1.0	96	82	0.539326	0.460674
2.4	131	189	0.409375	0.590625
5.25	191	374	0.338053	0.661947

The results suggest that under steady illumination there is more than one time-independent process occurring in these receptors and that these processes are independent of each other.

As the waves appear to obey a time-independent Poisson process, it is extremely simple to estimate the fraction of waves that are the overlap of two waves. If a wave begins on the rising phase or maximum of another wave, the two waves will most likely be identified as one wave. By examining the duration of the rising phase and maximum of a number of waves, we estimated this duration to be no more than 40 ms. For the brightest light used in the experiment of Fig. 4, the average rate of wave occurrence was about 1/s. Thus, about 4% of the waves may be the result of this kind of wave overlap. If a wave begins on the falling phase of another wave it will be detected, as illustrated by the fourth, sixth, eighth, and last waves of the top record of Fig. 3, but there is some doubt as to whether the smallest of the small waves will be visible if they occur on the most rapidly falling part of a large wave. Some small waves of this sort were probably missed. However, if, at the intensities used in this study, an appreciable number of large waves were due to

TABLE III B

EXPECTED NUMBER OF PAIRS COMPARED WITH OBSERVED NUMBER

Relative intensity	Size of first wave	Size of second wave	Expected number of pairs	Observed number of pairs	χ^2
	Little	Little	39.20	38	
Dark	Little	Big	8.89	9	1.051
	Big	Little	8.89	11	
	Big	Big	2.02	1	
	Little	Little	25.89	25	
1.00	Little	Big	22.11	23	0.143
	Big	Little	22.11	23	
	Big	Big	18.89	18	
	Little	Little	26.81	33	
2.40	Little	Big	38.69	30	4.410
	Big	Little	38.69	35	
	Big	Big	55.81	62	
	Little	Little	32.23	36	
5.25	Little	Big	63.10	56	1.340
	Big	Little	63.10	63	
	Big	Big	123.57	127	

χ^2 for each intensity calculated as follows: $\sum$ (expected number pairs–observed number pairs)^2/(observed number pairs) where the sum is taken over the four possible pairs of heights. As one parameter was estimated from the data, the degrees of freedom for each χ^2 is 2. None of the above χ^2's is significant at the 5% level.

wave overlap then it would be difficult to see how there could be both a linear relation between light intensity and the rates of big and small waves and an exponential distribution of intervals.

The histograms of Fig. 4 show that the percentage of big waves in the dark is 19% and in the most intense light used is 67%. The difference between these two numbers is too great to conclude anything other than that there is a real difference between light-evoked and spontaneous waves.

DISCUSSION

If one interprets the fit of Eq. (1) to the experimental frequency of response vs. pulse intensity to mean that single photons trigger discrete waves, then one must conclude that not all the spontaneous waves represent thermal changes in the visual pigment molecules identical to light-evoked changes. If all spontaneous waves and light-evoked waves were caused by the same changes in the visual pigment molecule, then they should be identical because the rest of the excitatory mechanism would have no means of distinguishing between different causes of the changes in the visual pigment molecule that evoke waves.

There are two possible classes of explanations for the observed difference between light-evoked and spontaneous waves. The first is that the visual pigment molecules can undergo thermal changes that evoke discrete waves, which are different from light-induced changes. The second is that changes in molecules, other than visual pigment molecules, evoke spontaneous waves. The latter possibility seems the more likely of the two. The spontaneous waves may represent the opening of gates in the receptor membrane that trigger some kind of regenerative conductance change. This conductance change is responsible for the discrete wave.

In single receptors of the lateral eye of *Limulus* the rate of spontaneous waves decreases with decreasing temperature (Fuortes and Yeandle, 1964; Adolph, 1968) We have noticed the same phenomenon in the ventral nerve receptors, although we have not studied this in detail. Srebro and Behbehani (1972), after an analysis of the rate of spontaneous waves in the lateral eye as a function of temperature, claim their results are consistant with the hypothesis that these waves represent spontaneous decomposition of visual pigment molecules.

Our results do not rule out the possibility that some of the spontaneous waves may represent conformational changes in the visual pigment molecule identical to those induced by photon absorption. We feel that it is very unlikely that all spontaneous waves are caused in this way.

There may be a difference in the mechanism of discrete wave production in the lateral eye receptor and the ventral nerve receptor. The discrete waves recorded from the two receptors are not identical. It is clear from the records in Fig. 3 that waves from the ventral nerve receptors show negative afterpotentials which, at least in our experience, have not been observed in the lateral eye.

The results indicate that the spontaneous and light-evoked waves are statistically independent of each other. In Eq. (1) the units of E are photons so that the units of fp must be reciprocal photons. In these units the values of fp that we have obtained range from 1/452 to 1/952. What is of physical interest is the value of p, for this is related to the mechanism of excitation. However, to know p, one must know f, and to know f one must have good spectrophotometric measurements of f which at this writing do not exist for the ventral nerve cell. Murray (1966) has measured the difference spectrum of ventral nerve cells with a microspectrophotometer. The spectrum he obtained looks very much like a rhodopsin spectrum and is similar to the action spectrum of the late receptor potential. His work indicates that about ½–1.5% of the incident light at a wavelength equal to the peak of the rhodopsin difference spectrum is absorbed by the cell's rhodopsin. However, he did not monitor the electrical activity of the cells on which the spectrophotometric measurements were done. In our experience we often have found specimens

where most of the ventral receptors show normal resting membrane potentials but no response to light.

There is the possibility that the cells with which he worked did not produce receptor potentials, and that the amount of visual pigment may be lower than normal in nonviable cells. This suspicion is strengthened by the recent work of Fein and DeVoe (1973) on the rate of visual pigment recovery in cells showing normal late receptor potential during dark adaptation after exposure to intense adapting lights. They used the early receptor potential to monitor visual pigment changes. Their results suggest that during the first second after a bleaching light flash the visual pigment changes extremely rapidly in viable cells. Murray's instrument was not a fast spectrophotometer and it could very well be that if the cells he measured were functioning normally, he would not have observed a rhodopsin difference spectrum. For the sake of the following argument we take Murray's measurements as a lower bound for the percentage of light absorbed whose wavelength is at the peak of the rhodopsin spectrum, although we realize this may not be true.

Solutions of bovine rhodopsin dissolved in 1% Emulphogene (General Aniline & Film Corporation, New York) at pH 6.5 and viewed with transmitted white light look practically colorless when they are sufficiently dilute so as to absorb no more than 3–5% of the incident light at the peak of the rhodopsin difference spectrum. All ventral nerve cells which we have impaled appeared colorless when viewed with transmitted light after being in the dark for some time. Although bovine and *Limulus* rhodopsins are different it is probably safe to take 5% as the upper limit for the percent of light removed whose wavelength is at the peak of the rhodopsin difference spectrum. If one takes these estimates in conjunction with the fairly precise determination of fb in the present work, one arrives at a value of p ranging from about $\frac{1}{2}$ reciprocal photons to $1/50$ reciprocal photons. We will now show why more precise knowledge of this quantity is of some importance.

When analyzing their data, most people who work on quantum responses in arthropod receptors have either implicitly or explicitly assumed that each photon absorbed produces one discrete wave with probability p and no response with probability $1 - p$. The excitation associated with a particular photon disappears when the discrete wave occurs. This assumption has led to models which agree with the data. Let us construct a model that replaces this assumption with another.

Assume each photon absorbed gives rise to a time-dependent function $M(t)$. If n photons are absorbed by the visual pigment from a short pulse of light, the probability of a wave occurring in the interval t, $t + \Delta t$ is $nM(t)\Delta t$. We assume that the n photons absorbed initiate a time-dependent Poisson process so that the presence or absence of a wave at a particular time does not influence the value of $M(t)$ at later times. A possible physical interpretation

is the following. There might be gates in a membrane and the opening of one gate suffices to trigger a wave. The probability of a gate opening may be proportional to the concentration of a transmitter substance released upon the absorption of a photon. This model has already been discussed by Srebro and Yeandle (1969) for the limiting case where the number of waves per absorbed photon was small. Here we will not impose this restriction.

Implicit in this model is the possibility of a single photon making more than one wave. The probability of this happening increases with increasing $M(t)$. Let us assume that the limit as t approaches ∞ of $\int_0^t M(x)dx$ exists and is equal to c. (The transmitter released by the photon absorption does not stay around forever).

It can be shown that the probability of m waves after a light pulse from which the receptor has absorbed exactly n photons, under the above assumptions is $(cn)^m \exp(-cn)/m!$. Since the probability of exactly n photons being absorbed from the pulse is $(fE)^n \exp(-fE)/n!$, the probability $P_a(m)$ of exactly m waves occurring after the pulse is the compound Poisson distribution (see Feller, 1968, for a discussion of compound Poisson distributions).

$$P_a(m) = \sum_{n=0}^{\infty} \frac{(fE)^n \exp(-fE)(cn)^m \exp(-cn)}{n!\,m!}. \tag{6}$$

If one assumes that the mechanism of discrete wave production is such that each photon absorbed can evoke at most one wave with probability c then the number of waves after a pulse obey the Poisson distribution.

$$P_b(m) = (cfE)^m \exp(-cfE)/m!. \tag{7}$$

From Eqs. (6) and (7), it can be shown that the probability of getting no response for the first model is $\exp(-fE[1 - \exp(-c)])$ and for the second model is $\exp(-cfE)$. Notice these expressions have the same form so that the determination of frequency of response vs. pulse intensity offers no help in differentiating between these two broad classes of models.

The means of both $P_a(m)$ and $P_b(m)$ can be shown to be both equal to cfE. It can also be shown in the limit as c approaches zero, $P_a(m)$ and $P_b(m)$ approach each other. Direct calculation shows that c does not have to be very small for the difference between these distributions to be small. Table IV shows a comparison between $P_a(m)$ and $P_b(m)$ for $c = 0.05$, 0.1, 0.2, and 0.5 for a fixed value of cfE of 1.2.

Previously published data from the *Limulus* lateral eye on the distribution of the number of waves after a pulse agree with the Poisson distribution. (Fuortes and Yeandle, 1964; Srebro and Behbehani, 1971). In the present work we worked with preparations with rather narrow latency distributions so that wave overlap was appreciable after a pulse. This made the determina-

TABLE IV

COMPUTED VALUES OF POISSON AND COMPOUND POISSON DISTRIBUTION FOR VALUES OF c AND CONSTANT cfE OF 1.2

c	fE	M	Compound Poisson	Poisson
		0	0.310212	0.301194
		1	0.354099	0.361433
		2	0.210950	0.216860
0.05	24.0	3	0.087148	0.086743
		4	0.028007	0.026023
		5	0.007450	0.006245
		6	0.001705	0.001249
		0	0.319195	0.301194
		1	0.346583	0.361433
		2	0.205490	0.216860
0.10	12.0	3	0.087495	0.086743
		4	0.029813	0.026023
		5	0.008610	0.006245
		6	0.002183	0.001249
		0	0.337019	0.301194
		1	0.331113	0.361433
		2	0.195767	0.216860
0.20	6.0	3	0.088069	0.086743
		4	0.032969	0.026023
		5	0.010797	0.006245
		6	0.003182	0.001249
		0	0.388942	0.301194
		1	0.283086	0.361433
		2	0.173791	0.216860
0.50	2.4	3	0.088299	0.086743
		4	0.039791	0.026023
		5	0.016387	0.006245
		6	0.006281	0.001249

See text for definition of symbols.

tion of this distribution more difficult than in the previous work and we did not attempt it. If one extrapolates the results obtained in the lateral eye to the ventral eye, then one sees that a determination of the fraction of incident light absorbed becomes of interest. If such determination were to result in a large value c, then a determination of the distribution of a number of waves after a pulse should give information on the excitatory mechanism.

If the number of waves per absorbed photon was a low number then, in the experiments designed to test Eq. (1), there might be many photon absorptions per pulse. The possibility exists that visual pigment molecules that have absorbed a photon might interact with each other. Such interactions would be expected to increase inversely with area of the cell over which the

photons are absorbed and, if the interaction were sufficiently great, might be of such a nature as to cause the frequency of response vs. light intensity to deviate from Eq. (1). Since the fit of Eq. (1) was independent of the area of the cell illuminated such interaction is unlikely and the absorbed photons most probably act independently of each other.

One could imagine that when a wave occurs $M(t)$ is modified in some other way, but it does not appear worthwhile at present to work out the consequences of such a complication.

If the trigger that initiates $M(t)$ is a specific change in the visual pigment molecule, then the same reasoning presented at the beginning of the discussion should apply no matter what controls the eventual form of this function. So despite our inability to gain very specific information about the visual excitatory process from present data, we can at least conclude that in the *Limulus* ventral nerve receptors more than one kind of molecular change can trigger discrete waves.

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