Dispersion of Latencies in Photoreceptors of *Limulus* and the Adapting-Bump Model

FULTON WONG, BRUCE W. KNIGHT, and FREDERICK A. DODGE

From The Rockefeller University, New York, New York 10021. F. Wong's present address is the Marine Biomedical Institute, The University of Texas Medical Branch, Galveston, Texas 77550.

ABSTRACT To light stimuli of very low intensity, *Limulus* photoreceptors give a voltage response with a fluctuating delay. This phenomenon has been called "latency dispersion." If the generator potential is the superposition of discrete voltage events ("bumps"), and if the effect of light upon bump size is negligible, then the latency dispersion and the bump shape completely characterize the frequency response to sinusoidal flicker. For very low light intensities, the latency dispersion of the bumps, the bump shape, and the frequency response are measured. It is found that for data obtained at 20°C, the frequency response can be accounted for completely by the latency dispersion and by the bump shape derived from steady-state noise characteristics. At 10°C, the time scale of the response of the photoreceptor is lengthened. The dispersion of latencies and the bump shape are found not to have the same temperature dependence. However, just as those measured at 20°C, the bump shape and the dispersion of latencies measured at 10°C can predict the frequency response measured under the same conditions. These results strongly suggest that the major mechanisms involved in the generator potential are the latency process and the bump process. At high light intensities, the time scale of the generator potential shortens. The decrease in time scale of the generator potential can be attributed to the decreases in time scales of the bumps and of the latency dispersion process.

INTRODUCTION

Intracellular recordings from *Limulus* photoreceptors suggest that the generator potential arises from a superposition of discrete events ("bumps"), which are triggered by the absorption of photons (Yeandle, 1957; Rushton, 1961; Adolph, 1964). A shot noise model has been proposed to explain the quantitative relationship between the noise in the generator potential observed under steady light and the response to sinusoidally modulated light (Dodge et al., 1968). The basis for such a model is that for some physical systems, the average return toward equilibrium from spontaneous fluctuation of macroscopic variables follows the same physical laws as the relaxation from external perturbation (Stevens, 1972). For *Limulus* photoreceptors, the fluctuations can...
be partially characterized by the autocovariance function $C(\tau)$, which is defined as $C(\tau) = [g(t) - \bar{g}][g(t + \tau) - \bar{g}]$, that is, the average of the lagged products of the instantaneous departure of the signal $g(t)$ from its mean value $\bar{g}$. In this definition, the average of an ensemble of measurements is taken; in the laboratory it may be evaluated by averaging over the time $t$. We note that $C(0)$ is the variance of the signal. The response of a system to external perturbation is conveniently characterized by the response to small sinusoidal variations in a variable of the system. In *Limulus* eccentric cells, the response to a sinusoidally flickering light can be measured. The light-to-voltage (conductance) transduction has been shown to behave much like a linear system (Dodge et al., 1968; Knight et al., 1970). The amplitude and phase of the frequency response (transfer function), defined as the (complex) ratio of the fractional variation $\delta g/\bar{g}$ in excitatory conductance to the fractional variation $\delta I/\bar{I}$ in light intensity, completely characterizes the response of the eccentric cell to small changes in light intensity.

The relationship that Dodge et al. (1968) studied may be expressed as

$$C(\tau) = A \int_0^{\infty} d f \cdot |T(f)|^2 \cos(2\pi f\tau)$$

where $A$ is a constant of proportionality and $|T(f)|$ is the frequency response amplitude. It can be shown that Eq. 1 holds for a Poisson shot noise model (uncorrelated shots) in which the expected rate is perturbed. A more general model, in which the occurrence of a bump may influence the sizes of subsequent bumps, also leads to Eq. 1 (Knight, 1973). Both $C(\tau)$ and $T(f)$ may be measured in the laboratory. The success of Eq. 1 in describing data obtained from *Limulus* eccentric cells supports the idea that even at high light intensities the generator potential is the summation of many bumps triggered by the absorption of photons. At low light intensities, it has been observed that the rate of occurrence of the bumps increases linearly with light intensity (Adolph, 1964; Fuortes and Yeandle, 1964).

In their studies, Dodge et al. (1968) noticed a systematic departure from Eq. 1; the time scale of the autocovariance predicted from the frequency response was often slower than that observed directly, especially at low levels of light. This discrepancy became very prominent when the temperature was lowered from 20° to 4° C. These observations suggested an additional response feature that is relevant when the input is time dependent. Because theory predicted that a dispersion in latencies would lead to such a qualitative discrepancy, and because it was known that the latencies of the bumps may show a large variability (Fuortes and Yeandle, 1964), Dodge et al. (1968) suggested that the dispersion of latencies of the bumps might be the cause of the systematic discrepancy which was observed. The motivation for the work reported here was to test this statement quantitatively.

Theory

**Relationship to be tested** For the purposes of this analysis, we assume that the bumps are underlying “unitary events” in the response of the

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1 Dodge, F. A., B. W. Knight, and T. Toyoda. Unpublished observation.
photoreceptor. It is known that for low levels of light intensity, the responses of the photoreceptors to flashes and to steady illumination can be described in terms of a Poisson process (Fuortes and Yeandle, 1964; Adolph, 1964; Weiss and Yeandle, 1975). In the development of the theoretical basis for this study, we shall assume that the bumps have an average shape and size $B(t)$. This assumption simplifies the presentation of theory without altering the major conclusions, and it removes some mathematical complexities. (Although the amplitude and shape of the individual bumps seen in dim light may vary [Adolph, 1964; Borsellino and Fuortes, 1968], there is no direct evidence to suggest that there are distinctly different populations of bumps above the very dim light levels. In this and the following paper [Wong and Knight, 1980], we will comment on this simplification when the occasion arises.)

From the response of the photoreceptor to dim flashes, it can be seen that the bumps do not occur immediately after a stimulus but may occur with a latency (Fig. 1a) (Table I). The latency varies from trial to trial. If a photon is captured at time zero, we may define a probability density for bump appearance, $D(t)$, such that the probability of a bump's appearing within the interval $dt'$ at $t'$ is $D(t') dt'$. We may descriptively call the probability density $D(t)$ the "dispersion of latencies." The time-course of a bump appearing at time $t'$ will be $B(t - t')$. It follows that the average impulse response $\mathcal{A}(t)$, the average of an ensemble of single bump responses to dim flashes, will be given by weighting $B(t - t')$ according to the probability $D(t') dt'$ that the bump appears within $dt'$, thus

$$\mathcal{A}(t) = \int_0^t dt' D(t') B(t - t'),$$

where $B(t)$ is the conductance time-course of an individual bump. By a Fourier transformation, Eq. 2 yields

$$T(f) = \tilde{D}(f) \cdot \tilde{B}(f),$$

where $T(f)$, $\tilde{D}(f)$, and $\tilde{B}(f)$ are the Fourier transforms of $\mathcal{A}(t)$, $D(t)$, and $B(t)$, respectively. (We use the Fourier transform formula $\tilde{X}(f) = \int_{-\infty}^{\infty} dt X(t) e^{-i2\pi ft}$.)

Eqs. 2 and 3 relate the average dynamic response of the photoreceptor to the dispersion of latencies of the bumps and to the bump shape. To express this relation in terms of quantities measurable in the laboratory, it is more convenient to use Eq. 3 than Eq. 2. The functions in Eq. 3 are complex functions that have amplitude and phase. In the following discussion, it will become clear that the phase of $\tilde{B}(f)$ cannot be determined, but the absolute square of $\tilde{B}(f)$ can be determined from steady-state data. Thus, Eq. 3 yields

$$|T(f)|^2 = |\tilde{D}(f)|^2 \cdot |\tilde{B}(f)|^2.$$

In the special case of a latency that is fixed rather than dispersed, $|\tilde{D}(f)|^2$ is

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2 It is found that, strictly speaking, the transfer function $T(f)$ does not have the minimum phase property, but it is also found that it can be approximated by a function having a minimum phase property and a pure delay. This pure delay corresponds to the offset of the gamma distribution used to fit the distribution of latencies (Fig. 1c).
unity, and Eq. 4 reduces to Eq. 1, the relation tested by Dodge et al. (1968). Because the latency is observed to fluctuate, Eq. 4 is the appropriate relation to be tested to determine whether the model is adequate to describe the response of *Limulus* photoreceptors.

For a particular class of complex functions that frequently arise in practical applications, the amplitude and phase are related in a particular way. That is, the phase part can be calculated from knowledge of the amplitude part, and vice versa. Such complex functions are said to have the minimum phase...
property. In the following paper (Wong and Knight, 1980), we show that the functions in Eq. 3 may be assumed to have the minimum phase property. Although the phase parts of these functions do not explicitly enter into discussion here, they are not totally lost in the step to Eq. 4. In the present analysis, the "transfer function" \( T(f) \) is determined by measuring the response of the photoreceptor to sinusoidally flickering light (Pinter, 1966; Dodge et al., 1968; Knight et al., 1970). The measurement of the latency distribution and the bump shape are described in the following discussion.

DETERMINATION OF THE LATENCY DISTRIBUTION FROM FIRST OCCURRENCES

As can be seen in Fig. 1a, the latencies of the responses to flashes can be seen clearly, but the latencies of the individual bumps are difficult to measure because the bumps overlap in time. However, if a time-dependent Poisson process is assumed for the response of the photoreceptor, a relation between the distribution of first occurrences \( F(t) \) and the dispersion of latencies of the individual bumps \( D(t) \) can be deduced. The derivations presented here and the method of data analysis in the estimation of \( D(t) \) from \( F(t) \) are similar to those used by Srebro and Yeandle (1970).

To show that \( F(t) \) and \( D(t) \) are related, we first recognize that if \( \eta \) is the mean number of bumps in the responses to dim flashes, the probability of getting no bump\(^3\) is \( P_0 = e^{-\eta} \). If \( \eta \) is a function of time, then

\[
P_0 = e^{-\eta(t)},
\]

and further,

\(^3\)Here, to simplify the presentation of the theory, we assume that the occurrence of a bump is due only to light. However, it is well known that bumps can occur spontaneously in the dark. Therefore, a small correction for the occurrence of spontaneous bumps has to be applied to \( P_0 \) in Eq. 5. This correction was used routinely in analyzing the data. Details of the correction are essentially the same as those described by Srebro and Yeandle (1970).
\[ \eta(t) = \frac{I}{I_0} \int_0^t dt' D(t'), \quad (6) \]

where \( I_0 \) is the number of photons needed to elicit one bump on the average and \( I \) is the average number of photons contained in a flash. We also observe that the probability distribution \( F(t) \), for the appearance of the first bump at time \( t \), is normalized to the fraction of responses that have at least one bump, \( \int_0^\infty dt F(t) = 1 - P_0(\infty) \). From \( \int_0^\infty dt F(t) = 1 - P_0(t') \), we get

\[ F(t) = \frac{d}{dt} \left( 1 - e^{-\eta(t)} \right) \]
\[ = \frac{d\eta}{dt} e^{-\eta(t)} \]
\[ = \frac{I}{I_0} D(t) e^{-\frac{t}{I_0} \int_0^\infty dt' D(t')} \quad (7) \]

Eq. 7 shows how \( F(t) \) is related to \( D(t) \). To express \( D(t) \) in terms of \( F(t) \), recall Eq. 5 and 6; we get \( \ln P_0(t) = -\eta(t) \) from Eq. 5. From Eq. 6, we get

\[ \int_0^t dt' D(t') = \frac{-\ln P_0(t)}{I/I_0} \quad (8) \]

Since \( P_0(\infty) = \text{failure rate} = e^{-I/I_0} \), \( I/I_0 = \ln(\text{failure rate}) = -\ln P_0(\infty) \), and recall that

\[ P_0(t) = 1 - \int_0^t dt' F(t'), \quad (9) \]

which may be substituted into Eq. 8 to determine \( D(t) \). Thus, the dispersion of latencies of the individual bumps \( D(t) \) can be determined from the distribution of first occurrences \( F(t) \) and from the failure rate \( P_0(\infty) \), both of which are measurable in the laboratory.

**THE BUMP SHAPE** Important features of a random signal may be obtained from its "power spectrum"—the spectral density of its variance—defined by means of the autocovariance as

\[ S(f) = \int_{-\infty}^{\infty} \int_0^\infty dt \ e^{-i2\pi f t} C(\tau) \];

\[ C(\tau) = \int_{-\infty}^{\infty} df e^{i2\pi f} S(f). \quad (10) \]
If $\tau$ is set at zero, Eq. 11 yields for the signal variance

$$C(0) = \int_{-\infty}^{\infty} df S(f),$$

(12)

which shows that the power spectrum expresses the manner in which contributions at various frequencies sum to yield the total variance of the signal.

The power spectrum may be obtained directly from laboratory data in the following way: from samples of the signal $g(t)$, of an interval $L$, which is long compared with the time over which the autocovariance $C(\tau)$ differs from zero, we may evaluate the "random Fourier coefficient"

$$G(f) = \int_0^L dt \, e^{-i 2\pi ft} g(t).$$

(13)

It is easy to show, by interchange of averaging and integration, that

$$\frac{1}{L} |G(f)|^2 = \frac{1}{L} \frac{G(f)G^*(f)}{S(f)} = S(f).$$

(14)

The autocovariance or the power spectrum gives important information about the statistical structure of the random signal (see Rice [1944]). In particular, if the random signal arises from a Poisson shot noise, the power spectrum will yield information concerning the average shape of the underlying shots. For the sake of concreteness, the following discussion will be based on the Limulus photoreceptor. The aim of the discussion is to show that the bump shape can be estimated from the power spectrum.

If the sample interval in Eq. 13 contained only one bump $B(t - t_j)$ which originated at time $t_j$ well within the interval, then Eq. 13 would give

$$G(f) = e^{-i 2\pi f t_j} \tilde{B}(f).$$

(15)

Inasmuch as $t_j$ is a random time, $G(f)$ is a complex random variable, uniformly distributed on a circle of radius $r = |\tilde{B}(f)|$ on the complex plane. In the realistic case, if the event rate is $\lambda$, in the long interval $L$, the large number approximately $n = \lambda L$ of events, at the random times $t_1, t_2, \ldots, t_n$ will occur, whence Eq. 13 will give

$$G(f) = \sum_{j=1}^{n} \tilde{B}(f)e^{-i 2\pi f t_j}. $$

(16)

The sum from $j = 1$ to $n$ is a sum of vectors, each with length $r = |\tilde{B}(f)|$ and with angles that are randomly distributed because of the Poisson arrival times. The situation is equivalent to a random walk on the complex plane, each step of length $r$, and the classical result for the expected squared distance from the origin at the $n$th step is $nr^2$ (easily derived from the central limit theorem), whence
For a wide class of bump shapes, including those that are reasonable on physiological grounds, the real function $|\tilde{B}(f)|$ determines the full complex function $\tilde{B}(f)$ (see Wong and Knight [1980]). In this manner, the power spectrum $S(f)$ may be used to determine the bump shape $B(t)$.

**METHODS**

**Biological Preparation**

The experiments reported here were performed on *Limulus* photoreceptors. Photoreceptor cells from ommatidia in the lateral eyes were studied. The morphology (Miller, 1957) and the physiology (Hartline et al., 1952; Purple, 1964) of these cells have been described extensively. Thin slices of the compound eye were obtained by cutting perpendicularly to the cornea with a razor blade. Each slice contained several rows of ommatidia. Retinular cells or eccentric cells of ommatidia from the top row were studied. Results from seven eccentric cells of ommatidia from the top row were evaluated, and the results from seven eccentric cells and one retinular cell are reported here. The resting membrane resistance of the eccentric cells studied ranged from 5 MΩ to 10 MΩ. The resting potential ranged from -40 to -55 mV. The resting membrane resistance of the retinular cell was 9 MΩ and the resting potential was -50 mV. The membrane resistance was measured with a bridge circuit similar to the one described by Purple (1964).

The preparation was mounted in a small plastic chamber and bathed in artificial seawater. The artificial seawater contained salts in the following concentrations: NaCl, 435 mM; KCl, 10 mM; CaCl₂, 10 mM; MgCl₂, 20 mM; and MgSO₄, 25 mM. The pH of this solution was adjusted to 7.3 by buffering with tris(hydroxymethyl)aminomethane. The plastic chamber was placed in contact with a brass block. The temperature of the bathing solution was controlled by varying the temperature of water circulating through the brass block from a temperature control unit (model K-2/R, Lauda Div., Brinkmann Instruments, Inc., Westbury, N. Y.). Experiments were performed at either 20° or 10°C, bath temperature. After an eccentric cell was successfully impaled, a small amount of tetrodotoxin (Sigma Chemical Co., St. Louis, Mo.) was added to the bath to abolish the action potentials (Dodge et al., 1968; Knight et al., 1970).

**Recording Instrument**

The responses of the photoreceptor cells to light were measured by monitoring changes in the membrane potential. The membrane potential was measured by inserting a glass capillary microelectrode into the cell soma. The microelectrode was filled with 3 M KCl (DC resistance 15-20 MΩ) which contacted an Ag–AgCl electrode connected to a high input-impedance amplifier. The membrane potential was measured with respect to the bathing solution. Another Ag–AgCl electrode was used as the indifferent electrode. The bath’s potential was taken as zero. The output of the amplifier was displayed on an oscilloscope, recorded on chart paper, and sampled by a computer.

**Stimulus Control**

The light source was a Sylvania glow modulator tube R1131C (GTE Sylvania Inc., Stamford, Conn.). The spectrum extended from 360 to 600 nm. Light from the glow tube was guided into a shielded cage by a 3-ft fiber optic bundle with a tip diameter.
of 2.0 mm (LGM, American Optical Corp., Buffalo, N. Y.). The fiber optic bundle was mounted on a manipulator so that it could be aligned with the optical axis of the ommatidium under study. The glow tube was activated by a fixed voltage (300 V) and could be turned on in 50 μs. When a flash was required, a pulse (300 V, 1 ms) was applied to the glow tube. When a steady light was required, pulses of 300 V and 0.5 ms were applied. This was achieved by a voltage-to-frequency converter. The voltage was set at a value that gave a pulse frequency of 500 per second. The stimulus intensity could be modulated by varying the voltage input to the voltage-to-frequency converter, modulating the frequency of the pulses sent to the glow tube. This technique of pulse modulation was used by Dodge et al. (1968) and Knight et al. (1970). For the experiments described here, frequencies of modulation from 0.1 to 20 Hz were achieved. The amplitude of modulation was ±40% of the steady state. Steady light intensity was controlled by neutral density filters. The unattenuated light intensity at the cornea was 7.5 × 10¹⁴ photons/cm²-s when measured at 520 nm over a 100-nm bandwidth. The turning on and turning off and the modulation of the steady light were under computer control.

Data Collection
For data used to determine the dispersion of latencies, the stimulus control and data collection were achieved by using a digital timer and a PDP 8/e computer (Digital Computer Corp., Maynard, Mass.). Sequences of pulses from the digital timer were presented in episodes. Each episode was 8 s long. At the beginning of each episode, a pulse was sent to start sampling by the computer. The computer sampled and stored the data at 500 samples per second until 1,000 samples had been taken. The data were then stored on magnetic disks for later analysis. 1 s after the computer had started the sampling, the digital timer sent a 1-ms pulse to activate the glow tube driver. This caused the glow tube to give a 1-ms flash. The intensity of the flash was adjusted so that two bumps were elicited per flash on the average. The next episode started immediately after the previous episode had ended. This sequence continued until 240 episodes were completed. In effect, this procedure generated 240 records and each record was 2 s long. The first second contained information about the spontaneous occurrences of bumps (footnote 3) and the base-line value of the intracellular voltage. The rest of the record contained the response to the flash. The sampling rate described above was for experiments performed at 20°C. At 10°C, the sampling rate was at 250 samples per second, and the records were 4 s long. The last 3 s of data in each record contained the response to the flash.

For data used to determine the frequency response and the bump shape, the stimulus control and data collection were achieved by programming a PDP 11/45 computer. The program was written by Mr. David Koscis and Mr. Norman Milkman of our laboratory. Sequences of stimulus patterns were presented in runs. Each run contained 20 episodes. Each episode was 120 s long for high light intensities and was 60 s long for low light intensities. The stimulus in each episode was either a step or a sinusoid superimposed on a step. All the steps were 25 s long. The sinusoids were added on to the step 5 s after the onset of the step (after the response had reached a “steady state”). The stimulus patterns generated by the computer were used to control the frequency of pulses sent to the glow tube. The voltage response of the photoreceptor was sampled at 60 samples per second. The digitized data were stored on disks for later analysis. The stimulus sequence for the 20 episodes was identified by: 0.0, 0.1, 0.0, 0.2, 0.0, 0.3, 0.0, 0.5, 0.0, 0.7, 0.0, 1.0, 2.0, 3.0, 5.0, 7.0, 10.0, 13.0, 17.0, 20.0, where 0.0 indicates no modulation and the other numbers indicate cycles per second. (If the dark interval between stimuli is long enough, any particular stimulus sequence
will not introduce a bias to the response.) Each experiment consisted of one to five runs.

**Experimental Determination of the Dispersion of Latencies**

From the records of responses to flashes (described in the data collection section), the first-occurrence distribution \( F(t) \) was formed. The 2-s-long records were plotted on an oscilloscope. The baseline was calculated from data in the first second of the record. The latency may be defined as the time between the occurrence of the flash and the time when the response started to rise above the baseline. This measurement was done by eye and was accurate to within 10 ms. An example of \( F(t) \) is shown in Fig. 1b.

The failure rate (footnote 3) was calculated by dividing the number of trials that gave no bump by the total number of trials (240 in all cases). The failure rate was also estimated from the average number of bumps in each flash. According to the Poisson hypothesis, \( P_0 = e^{-\eta} \), where \( P_0 \) is the failure rate and \( \eta \) is the average number of bumps in each response. For the experiment presented in Fig. 1, \( \eta \) was estimated to be 2.5, which predicted a failure rate of 0.082. The number of failures directly observed was 20, which gave a failure rate of 0.083. The degree of agreement of these two values is seen to be accidentally close when the uncertainties in resolving the number of bumps in a response are taken into consideration. However, from the results of other experiments performed, these two methods gave values that generally agreed to within 25%, which indicated that the assumption of a Poisson process should not be grossly in error.

From the failure rate, \( I/I_0 \) was calculated. \( P_0(t) \) was calculated from Eq. 9. With these values, \( D(t) \) was calculated from Eq. 8. The calculations were done numerically with a computer program. The deduced \( D(t) \) from \( F(t) \), Fig. 1b, is shown in Fig. 1c.

The deduced \( D(t) \) differs from \( F(t) \) in an expected way. \( D(t) \) shows more bumps with longer latency than does \( F(t) \). This is consistent with the analysis because the formation of \( F(t) \) ignores information about bumps that occurred after the first bump. The distribution \( D(t) \), in contrast, directly furnishes information concerning the arrival of the later bumps.

The shape of \( D(t) \) looks very much like that of a gamma distribution (Fuortes and Yeandle, 1964; Srebro and Yeandle, 1970). The gamma distribution is defined as

\[
\Gamma(t; n, \tau) = \frac{1}{n! \tau} (t/\tau)^n e^{-t/\tau}.
\]

It describes a two-parameter family of curves. By varying \( n \) and \( \tau \), a family of similar looking curves may be generated. The \( D(t) \) shown in Figure 1c was fitted to a particular gamma distribution. There is no compelling physical reason for fitting \( D(t) \) with a gamma distribution; it is done here as a matter of convenience. Therefore, the values of the parameters should be treated simply as numbers chosen to characterize the shape of a reasonable fit.

In fitting \( D(t) \) with \( \Gamma(t; n, \tau) \), two points should be mentioned. (a) To test Eq. 4, the most important measurement of the dispersion of latencies is the width (variance) of the distribution. The details of the rising phase and falling phase of the distribution are relatively unimportant. Considering the nature of the method used to deduce \( D(t) \), values near the two ends of the distribution are not as reliable as those near the mean of the distribution. Therefore, the fit was biased in favor of data within the central half of the distribution. This was achieved by finding the points on the time axis corresponding to one-fourth and three-fourths of the total area under the curve.
These two measurements uniquely determine the gamma distribution that has a given time origin and the same two corresponding area points. (See Appendix.) (b) As shown in Fig. 1 c, the origin of the gamma distribution was offset, in this particular case, by 80 ms with respect to the measured $D(t)$. This procedure was followed because an offset gamma distribution with a low value for $n$ was found to fit the data better than a gamma distribution with a high value for $n$ and started from $t = 0$. In the test of Eq. 4, the offset of a given distribution does not affect the comparison because when $D(t)$ is transformed to the frequency domain, the offset only affects the phase of $\hat{D}(f)$, and it is only the absolute value $|\hat{D}(f)|$ that enters the comparison.

**Experimental Determination of the Transfer Function**

The transfer function, defined as the ratio of the fractional variation $\delta g/g$ in excitatory conductance to the fractional variation $\delta I/I$ in light intensity, can be calculated from data obtained in the way described in the section on Data Collection. Only those episodes in which the light intensity was modulated entered into the determination of the transfer function. Besides storing the raw data in digitized form, the computer performed a binning procedure concurrently with generating the sinusoidal signal. Each complete cycle of the sinusoid was divided into 32 bins. In each cycle the average of the voltage data in each bin was calculated. In each of the 32 bins this number was added to the total on each additional cycle of the sinusoid, until the end of the modulation. The grand average for each of the 32 bins was then computed from the accumulated sums and stored. This was done for each of the modulation frequencies in the run. From the 32 averaged values, the Fourier coefficient of the fundamental frequency was calculated. The squared amplitude of the transfer function was determined from the Fourier coefficients that corresponded to the modulation frequencies. Since in very dim light, about five bumps per second, the generator potential is very noisy ("bumpy"), the values of the transfer function determined this way show considerable scatter from a smooth curve. However, by using a sufficient number of runs, the transfer function could be determined. Fig. 2 is an example of one experiment in which the transfer function was determined from five runs. The smooth curve passing through the points was put in by eye.

**Experimental Determination of the Bump Shape**

From data obtained in the way described in the Data Collection section, the power spectrum was estimated from data in the steady-state (5 s after the onset of light) of those episodes in which no modulation was used (Fig. 3 a). Only the last 20 s of the response to the 25-s step of light was used. The 20-s-long segment was broken into eight overlapping segments, each 4.267 s long (256 samples obtained at 60 samples per second; Welch, 1967). The Fourier coefficients of the fundamental frequency (0.235 Hz) and the harmonics were calculated by the Fast-Fourier-Transform algorithm (Cooley and Tukey, 1965). In each run in which six episodes were steps of light with no modulation, 48 spectra were averaged to obtain the power spectrum. The power spectrum obtained this way was smooth enough to give a good estimation of $|\tilde{B}(f)|^2$. Therefore, no further smoothing was necessary.

**RESULTS**

**In the Time Domain**

The test of Eq. 4 will first be performed in the time domain for comparison with the original work of Dodge et al. (1968). (Although the results of this
particular experiment are presented in the time domain, the comparison with prediction was also done in the frequency domain. The degree of agreement between prediction and observation for this cell is similar to that of the cell shown in Fig. 5). The three quantities in Eq. 4 were measured independently from the same cell. The curves shown in Fig. 4 are normalized so that at $t = 0$ they have value 1. The filled circles in Fig. 4 correspond to the autocovariance function obtained from the Fourier transform of the power spectrum (Fig. 3 b). Because the power spectrum is proportional to $|\hat{B}(f)|^2$, the filled circles correspond to the Fourier transform of $|\hat{B}(f)|^2$ in Eq. 4. The open circles correspond to the Fourier transform of $|T(f)|^2$ in Eq. 4, and they also correspond to the curve predicted without latency dispersion in the analysis of Dodge et al. (1968). It is quite clear that the filled circles do not coincide with the open circles. The general time scale of the curve defined by the open circles is slower than that of the curve defined by the filled circles. This is the "discrepancy" described earlier. In the present extended model, which takes into account the dispersion of latencies, Eq. 4 predicts that the Fourier transform of $|T(f)|^2$ should be equal to the Fourier transform of the product of $|\hat{B}(f)|^2$ and $|\hat{B}(f)|^2$. As can be seen in Fig. 4, this prediction is verified quite well.
The response of a dark-adapted photoreceptor to very dim steady illumination (~6 log units from maximum available light intensity). The discrete nature of the photoresponse is conspicuous. The bump shape was estimated from data such as the record shown here. The procedure of estimating $|\hat{B}(f)|^2$ is described in the text. The power spectrum calculated from data such as that shown in a. The horizontal axis is frequency and the scale is 0.234 Hz per bin. The vertical scale is in arbitrary units for power density.

FIGURE 4. Results from the eccentric cell that yielded Figs. 1, 2, and 3; temperature, 20°C. The curves are normalized so that at $t = 0$ they have value 1. The filled circles show the autocovariance function, which corresponds to the transform of the bump shape $|\hat{B}(f)|^2$ obtained from the power spectrum. The open circles correspond to the transform of $|T(f)|^2$. It can be seen that the time scale of the curve obtained from $|T(f)|^2$ is slower than that obtained from $|\hat{B}(f)|^2$. This observation is typical of all the experiments performed in this project. The difference between these two curves is accounted for well by the dispersion of latencies of the bumps. The continuous line corresponds to the transform of $|\hat{D}(f)|^2$ and $|\hat{B}(f)|^2$, Eq. 4.

In the Frequency Domain

The goodness of fit between experiment and theory can be judged more easily by comparing the quantities in Eq. 4 in the frequency domain. The results shown in Fig. 5 were obtained from a retinular cell under experimental
conditions very similar to those that yielded Fig. 4. The quantities, which were measured independently, are shown with their corresponding labels. The areas under the three curves were normalized to a common value to facilitate visual comparison of the curves. Since the test of Eq. 4 is independent of the absolute amplitudes of the individual functions in it, the normalization does not affect the test of the theory. The comparison according to Eq. 4 is performed in the upper right frame of Fig. 5. The histogram corresponds to $|T(f)|^2$ in Eq. 4. The dots correspond to $|\tilde{D}(f)|^2$, $|\tilde{B}(f)|^2$, and, again, the curve defined by the dots was normalized so that it has an area equal to that of the curve corresponding to $|T(f)|^2$. It can be seen from this comparison that the two curves agree very well. The dots are scattered around the smooth histogram because of the noise inherent in the estimation of $|\tilde{B}(f)|^2$. The frequency components of $S(f)$ are random variables. Each component can be thought of as a random variable that is chi-square distributed (Jenkins and Watt, 1968). The standard error for each component depends on the total length of record used and the length of the record segments used for spectral estimation. For the data shown in Fig. 5, the standard error was estimated to be $\sim 20\%$. Within this error, the two curves shown in the upper right frame of Fig. 5 agree well.

**Effects of Temperature**

The mean latency of the bumps reported by Fuortes and Yeandle (1964) was $\sim 1$ s at $7^\circ C$. This value is more than 6 times the value observed at $20^\circ C$. (The
values are quoted in this section.) Adolph (1968) reported that the time scale of the rising phase and the falling phase of the bumps has a $Q_{10}$ of 2–3. These reports suggest that the time scale of response of the photoreceptor must lengthen with low temperature. At 20°C, the parameters of the deduced dispersion of latencies of the bumps have the following values: mean = 185 ± 27.4 ms, width (defined as 2 times the square root of the variance of distribution) = 90.3 ± 15.7 ms. These values were derived from the results of seven eccentric cells and one retinular cell. At 10°C, the time scale of the dispersion of latencies increases. Fig. 6 shows the measured $F(t)$ and the
deduced $D(t)$ at 10°C from the cell shown in Fig. 1. The mean of the distribution has increased by a factor of 4 (from 160 ms at 20°C to 634 ms at 10°C). The width has increased by a factor of 3.7 (from 94 to 351 ms). The average from three eccentric cells gave an average $Q_{10}$ of 4.7 for the mean and an average $Q_{10}$ of 5.1 for the width. These results suggest that the latency process depends strongly on temperature. The $Q_{10}$ for the time scale of the latency process is ~5 (between 20°C and 10°C).

For the cell that yielded Fig. 4, the same experiment was performed at 10°C. It can be seen from Fig. 7 that the time scale of the bumps has increased by a factor of 2.5. The time scale of the transfer function and the dispersion of latencies has increased. Nevertheless, the prediction of Eq. 4 was confirmed at 10°C. This again suggests that the transfer function is determined by the bump shape and by the dispersion of latencies of the bumps.

\begin{figure}
\begin{center}
\includegraphics[width=\textwidth]{figure6.png}
\end{center}
\caption{The distribution of latencies of the individual bumps at 10°C was estimated from responses of the same cell that yielded Fig. 1. The methods for the estimation were the same as those used to obtain Fig. 1. The latencies of the first occurring bumps are shown in a. The distribution $D(t)$, deduced from $F(t)$, is shown in b. It can be seen that the mean and the width of $D(t)$ increased by a factor of ~4 when the temperature was reduced by 10°C (compare with Fig. 1). The smooth curve shown here is a gamma distribution with $\alpha = 2.25$ and $\tau = 97.3$ ms. (Offset from $t = 0$ by 320 ms.) Here, $D(t)$ has been normalized to the total number of bumps: 240.}
\end{figure}
Effects of Light Intensity

At high light intensity the absolute transfer function was again determined from the voltage response (to flickering light), and the bump shape was again determined from the autocovariance as described, except a small correction was applied to the data to remove a slow trend (Wong and Knight, 1980). The time scales of these two quantities were found to shorten at high light intensities. Furthermore, as shown in Fig. 8, the curves corresponding to the Fourier transforms of $|T(f)|^2$ and $|\check{B}(f)|^2$ show a characteristic undershoot—the curves go through negative values before their eventual return to zero.

This phenomenon reflects the effects of light adaptation and it has been elaborated by Dodge et al. (1968) and Wong and Knight (1980). The point to be emphasized is that the time scale of $|T(f)|^2$ is also systematically slower than that of $|\check{B}(f)|^2$, although the difference between the two curves is less than that shown in Fig. 4. This difference is typical of all the cells studied at high light. If the transfer function is determined by the dispersion of latencies and by the shape of the bumps, $|\check{B}(f)|^2$ can be determined by the departure of $|T(f)|^2$ from $|\check{B}(f)|^2$. In this experiment (Fig. 8) and two others, $|\check{D}(f)|^2$ was deduced by multiplying the measured power spectrum by an appropriate function derived from Eq. 18. By varying the values for $n$ and $\tau$ in Eq. 18, the curve which gave the best fit was determined. Again, this fitting was judged by eye. In this way, the value for the width of the distribution $D(t)$ can be judged to within 15%. The reason for using this approach to determine $|\check{D}(f)|^2$ instead of dividing $|T(f)|^2$ by $|\check{B}(f)|^2$ is that at very high frequencies...
(>15 Hz) the functions have very small values. Large errors would result from
division by these small numbers. The procedure described here was used
because it avoided this problem and allowed the efficient estimation of
$|\hat{D}(f)|^2$. In these three experiments, performed at light intensity $10^5$ times
greater than our lowest light intensity, it was found that the difference between
the transfer function and the deduced bump shape could be reconciled by a
dispersion of latencies whose time scale decreased by ~40% from that directly
measured in low light. That is, the value of $\tau$ did not change but the value for
$\tau$ decreased by 40%. For the cell shown in Fig. 8, the value for $\tau$ was 24.9 ms in low light and 15 ms in $10^5$ times that light intensity.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{example.png}
\caption{The result obtained at 0 log background from an eccentric cell
different from the one that yielded Fig. 4. The filled circles show the transform
of $|\hat{B}(f)|^2$, and the open circles show the transform of $|T(f)|^2$. It can be seen
that the time scales of these curves shorten at high light intensities. Furthermore,
they both show a characteristic undershoot. The point to be emphasized is
that the time scale of $|T(f)|^2$ is slower than that of $|\hat{B}(f)|^2$. Although the
difference is not as obvious as the differences shown in Figs. 4 and 7, this
difference is typical of all the cells studied at high light. The continuous line
corresponds to the transform of $|\hat{D}(f)|^2 - |\hat{B}(f)|^2$, where the parameters of
$|\hat{D}(f)|^2$ are chosen to give the best fit (see text).

In Fig. 1c, it is shown that the gamma distribution used to fit the dispersion
of latencies at low light was offset from $t = 0$ and that the offset described was
measured directly at low light intensity. At high light intensities the procedure
for estimating $|\hat{D}(f)|^2$ does not give information about this offset.
However, at high light intensity, this offset can be estimated from the latency
of the response to a flash superposed on the high light background. Here the
latency may be defined as the difference between the time of the flash and the
time its response rises above base line. Such a measurement made on one cell
at 0 log light background gave a latency of 40 ms. The offset measured from
the same cell at the lowest light intensity was 70 ms, which is a 43% decrease.
These results indicate that the time scale of the latency process decreases by
~40% for an increase of $10^5$ in light intensity. Fig. 9 illustrates the deduced
dispersion of latencies of the bumps in low and in high light.
DISCUSSION

From the results presented here, we conclude that the extended model, Eq. 4, accounts well for the data obtained from *Limulus* lateral eye photoreceptors. This agreement with experiment justifies our simplifying assumption of a single "standard" bump shape. Furthermore, the bump shape calculated from the power spectrum is found to agree well with the average of the directly observed bumps (Wong and Knight, 1980). Because the systematic discrepancy described by Dodge et al. (1968) can be explained quantitatively by the dispersion of latencies of the individual bumps, the result of this analysis indicates that there is no need to postulate any other time-dependent mechanisms to describe the response of the photoreceptor.

![Figure 9](image)

**Figure 9.** The dispersion of latencies $D(t)$ at the highest (0 log) and lowest (~5 log) background are plotted. Both curves are from the same gamma distribution ($n = 2$) but with different time constants (24.9 ms for low background and 15.0 ms for high background). The two curves are normalized to the same area. The vertical scale is an arbitrary number of bumps. The effect of light adaptation is to decrease the time scale of the latency process [$D(t)$ plus the offset] by ~40%.

The time scale of the latency process depends strongly on temperature, $Q_{10} = 5$. The mechanisms underlying the latency process are not known. However, the time scale of the mean latency and the width of the dispersion of latencies both depend in the same way on temperature and on light intensity, which supports the idea that the latency and the dispersion of latencies arise from the same underlying process.

The time scale of the bumps and the latency process depend differently on temperature (Fig. 7 and Adolph, 1968) (although both depend similarly on light intensity), suggesting that the mechanisms underlying the bump and the latency process are different. They are separable by changes in temperature. Nonetheless, the success of Eq. 4 in accounting for the data at 20° and 10°C suggests that the major mechanisms underlying the response of *Limulus* photoreceptors have been correctly isolated: they are the process for the latency and dispersion of latencies and the process for the bumps.
The hypothesis that the latency process and mechanisms underlying the bump are different processes finds further support from the analysis of Drosophila mutants. Pak and his co-workers (1976) have reported an unusually large dispersion of latencies in the mutant Norp A^{H52}. Furthermore, this phenomenon is temperature dependent, that is, at temperatures below \(\sim 17^\circ C\), the response is normal, with very little dispersion of latencies. Above that temperature, the response is abnormal, and the abnormality stems from a large dispersion of latencies. The spectral transitions of the photopigments and the bump shape were shown to be normal. The defect in this mutant must be in the process underlying the dispersion of latencies. This is important because it implies that a macromolecule, presumably a single protein, defectively encoded by the Norp A gene, influences the dispersion of latencies. It is particularly relevant to this discussion because it confirms that the dispersion of latencies and the bump shape are due to different underlying processes. In this mutant, one process is affected and the other is not.

The salient features of the photoreceptor's response to light have been recognized for a long time. Several kinetic models for the phototransduction process are in the literature (Fuortes and Hodgkin, 1964; Levinson, 1966; Borsellino and Fuortes, 1968). The analysis most relevant to this discussion involves the relationship between the change in time scale and sensitivity that occurs when the photoreceptor becomes adapted to light or darkness. In their analysis, Fuortes and Hodgkin (1964) found that for low-intensity flashes, the response of the photoreceptor can be approximated by the impulse response of a cascade of low pass filters. The formal expression that describes the linear impulse response of such a network (without feedback) is the gamma distribution. For low-intensity flashes, and at low temperatures \(\sim 7^\circ C\) Fuortes and Hodgkin (1964) found that the response is fit well by a gamma distribution with \(n = 9\) (ten stages of exponential delay with time constants of about 77 ms for each stage). Some of the techniques used in our project are similar to theirs. The analytical forms used for curve-fitting were the same: for example, for the bump shape (Wong and Knight, 1980) and for the dispersion of latencies, which were well fit by gamma distributions. In this sense, the results of the present study are consistent with those of Fuortes and Hodgkin (1964); furthermore, they make the ten stages of exponential delay in the Fuortes-Hodgkin model physiologically plausible. According to the present model about seven of those stages should be attributed to the dispersion of latencies, whereas the remaining stages should be attributed to the bump shape. The decrease in time scale, due to adaptation of the generator potential, described by Fuortes and Hodgkin can be attributed to the decrease in the time scales of the bumps (Wong and Knight, 1980) and of the dispersion of latencies.

**APPENDIX**

*A Simple Procedure for Determining the Parameters of the Gamma Distribution*

The gamma distribution, Eq. 18, is a two-parameter curve. Our procedure is based on the observation that the parameter \(n\) determines the shape of the curve, while \(\tau \) determines its time scale. Let \(t_{1/4}\) and \(t_{3/4}\) be the particular values of \(t\) marking \(\frac{1}{4}\) and
of the area under the curve, measured from the origin. Evidently $n$ but not $\tau$ will be determined by the ratio $\rho = \frac{t_{3/4}}{t_{1/4}}$. However, once $n$ is known, $\tau$ may be related to the mean of the two times given above, $t_m = \frac{1}{2}(t_{1/4} + t_{3/4})$. Any two gamma distributions with the same value of $n$ but different values of $\tau$ can be related by $\frac{t_m^{(1)}}{t_m^{(2)}} = \frac{\tau^{(1)}}{\tau^{(2)}}$, where the superscripts denote different gamma distributions.

These observations lead to an algorithm that evaluates $n$ and $\tau$ for a particular $D(t)$. The procedure is first to find $t_{1/4}$ and $t_{3/4}$ corresponding to $D(t)$. From these, $\rho$ and $t_m$ are determined. With $\rho$, $n$ can be determined. By comparing $t_m$ obtained from $D(t)$ with the value of $t_m$ calculated from a known gamma distribution with the same $n$ value, the appropriate value of $\tau$ can be determined. These steps may be systematized by empirical formulas. (The formulas are obtained by fitting two constants in “large $n$ asymptotic” results to exact values at modest $n$.) Let

$$\alpha = 0.7016 - 0.022 \times \rho,$$

$$n = \left( \frac{\rho + 1}{\rho - 1} \right)^2 - 0.82,$$

$$\tau = \frac{t_m}{(n + 0.82)}.$$

In the important range between $n = 1$ and $n = 15$, these expressions are accurate to within 0.5%.

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