

# Independence of Location of Light Absorption and Discrete Wave Latency Distribution in *Limulus* Ventral Nerve Photoreceptors

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**ABSTRACT** Discrete waves of depolarization evoked by dim pulses of light in dark-adapted ventral nerve photoreceptors in *Limulus* show fluctuation in their latency. To a resolution of 5–10  $\mu\text{m}$  the latency distribution function appears to be independent of where in the receptor light is absorbed. Also, there is apparent local adaptation to bright light pulses.

## INTRODUCTION

In a number of arthropod receptors discrete waves of depolarization have been observed. (See Wolbarsht and Yeandle, 1967, for a review of this phenomenon.) These discrete waves have also been called quantum bumps, quantized responses, and spontaneous slow potential fluctuations. Evidence exists suggesting that each bump is triggered by a photon absorption with a certain probability but calculations done recently have shown that other models may fit the available data (Yeandle and Spiegler, 1973).

Ever since the discovery of the quantum bumps it has been known that the latency between light absorption and quantum bump occurrence fluctuates. This has led to a number of attempts to model the latency fluctuations (Borsellino and Fuortes, 1968; Srebro and Yeandle, 1970; Srebro and Behbehani, 1971). All the models proposed so far assume that the cause of the latency fluctuation lies in the intervening processes between the absorption of a photon by a visual pigment molecule and the change in membrane permeability responsible for the discrete depolarization. In all experiments done to date the entire receptor was illuminated. Since each bump might be triggered by a single photon, and since the photon could be absorbed anywhere, the possibility exists that the observed latency fluctuations reflect differences in the properties of the receptor in different regions and not stochastic properties

of the process responsible for the bump. In this report an attempt will be made to show to within a resolution of 5–10  $\mu\text{m}$  that the latencies of bumps evoked by light falling on different regions of the receptor are the same.

#### METHODS

Desheathed ventral eye nerves, excised from young horseshoe crabs, *Limulus polyphemus*, (carapace 4–6 inches wide) were pinned to a transparent elastomeric silicon potting compound coating the bottom of a Petri dish filled with a solution of artificial seawater buffered at pH 7.5. The Petri dish was placed on a Cambion peltier cooler (Cambridge Thermionic Corp., Cambridge, Mass.) that had a hole in the center to allow a narrow beam of light from a photostimulator to pass through the bottom of the dish and illuminate a single receptor. Small glass microhooks, attached to micromanipulators, were used to roll the nerve so that the beam of light illuminated the receptor being studied without traversing the nerve. This receptor was impaled by an intracellular microelectrode coupled to a microelectrode preamplifier (Bioelectronics NF1, Bioelectric Instruments, Farmingdale, N. Y.) whose output led to a Grass model 5 polygraph (Grass Instrument Co., Quincy, Mass.) of frequency response DC to about 40 Hz. By regulating the current through the peltier cooler the temperature could be maintained to within 0.1°C at any temperature between 0° and 27°C. The temperature was monitored by a thermistor probe placed near the impaled receptor.

The Petri dish, cooler, and micromanipulators holding the hooks and microelectrode were mounted on a stage that was moved in the horizontal plane by micrometer drives. These drives were either turned manually or remotely by electric motors. The drives were also coupled by precision potentiometers to an X-Y plotter in order to record the position of the light beam relative to the preparation. This apparatus allowed positioning of the stage to within 2  $\mu\text{m}$  of any desired position.

The photostimulator is illustrated in Fig. 1. The light source was a General Electric 6-V 18-A coiled filament projection lamp type CPR (General Electric Co., Nela Park, Cleveland, Ohio) powered by a well-regulated 15-A constant current power supply, stable to within 0.02%. Lens  $L_1$ , focal length 10 cm, imaged the filament ( $f$ ) with unit magnification on an electromagnetic shutter  $S_1$  made from the pen motor of a Grass recorder. Lens  $L_2$ , focal length 17 cm, imaged lens  $L_1$  at the aperture plane

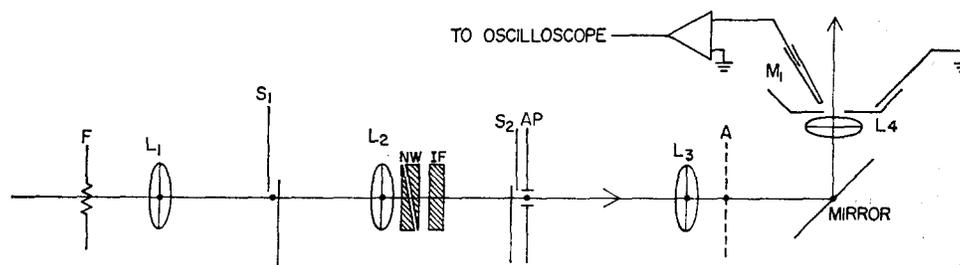


FIGURE 1. Diagram of apparatus.  $M_1$  refers to microelectrode, T refers to thermistor probe. See text for explanation of symbols that refer to optical stimulator.

(AP) where one could place either an iris diaphragm or a special mounting that allowed two holes to be independently positioned. A doubly demagnified image of the aperture plane was projected on a single photoreceptor by camera lens  $L_3$ , focal length 5 cm, which imaged the aperture plane at plane A, and microscope objective  $L_4$ , focal length 1.6 cm, which imaged A on the preparation. A vane  $S_2$ , attached to a Grass recorder pen motor, allowed either hole to be covered so that either spot could be projected onto the receptor. The intensity of the light was controlled by two circular neutral density wedges (NW) and the color by interference filters (IF) of about 40-Å band width. In all experiments either white light or monochromatic light of 5,461 Å was used. At any wedge setting the output of the photostimulator did not fluctuate by more than 1% over several hours. Because of scattered light in the receptor, it was found that the smallest size spot capable of being projected onto the preparation was between 5 and 10  $\mu\text{m}$  in diameter.

#### RESULTS

After penetrating a receptor we plotted regions of equal sensitivity by observing the response to a flashing light spot 5–10  $\mu\text{m}$  in diameter as the preparation was moved relative to the light beam. The flashing spot was a train of identical pulses of about 50-ms duration occurring once a second and the pulse energy was just below that necessary to saturate the late receptor potential at the most sensitive region of the cell. We positioned two 5- to 10- $\mu\text{m}$  spots of light about 40  $\mu\text{m}$  apart on either side of the most sensitive region. We used the presence or absence of local adaptation, first reported by Hagins et al., 1962, in squid photoreceptors, to gain some idea of the degree of optical isolation between the two spots. Before discussing local adaptation let us consider the cell response to repetitive light pulses. If a receptor, that has been in the dark for some time, is stimulated by a train of short bright pulses spaced equally apart in time, the amplitude of the response evoked by the first pulse is always the largest while that of the second is frequently the smallest. The amplitude of the response is defined to be the difference in the potential at the beginning of the response from the potential at maximum depolarization. If a similar set of stimuli is presented to a receptor immediately after cessation of intense illumination the response amplitudes increase as a function of pulse number until a relative steady value is reached.

Stimulation with a moderately intense light of one part of the ventral nerve photoreceptor *apparently* adapts only that part and not neighboring parts as is illustrated by the following experiment (see Fig. 2). Consider two small discrete portions (A and B) of the photoreceptor approximately 40  $\mu\text{m}$  apart and of nearly equal sensitivity. A set of 21 identical stimuli was presented at area A. The stimuli occurred at approximately 1-s intervals and each was a white light pulse 50 ms in duration. The spot diameter was 5–10  $\mu\text{m}$ . After an interval of 21 s without stimulation, the same set of stimuli was again presented at point A with similar results. An identical pattern of stimulation

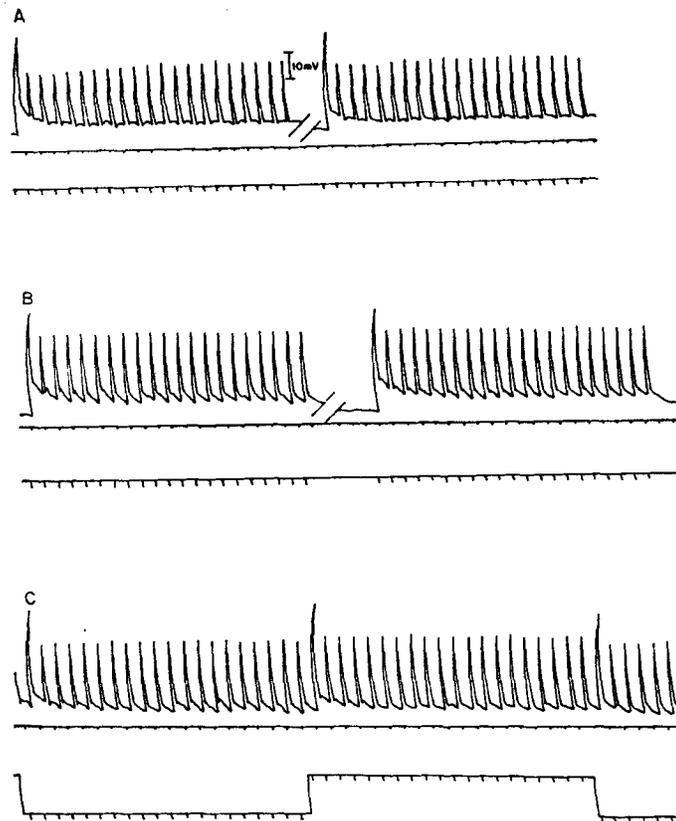


FIGURE 2. Intracellular recordings from ventral nerve photoreceptor, temperature 24°C. In records A, B, and C upper trace shows recording from intracellular recording electrode, middle trace indicates 1-s time marks, and bottom trace indicates occurrence of light pulses by a downward spike. (A) Receptor potentials evoked by bright light pulses presented at region A for a certain time period alternately with an equal period of no stimuli. 10-mV calibration in A applies also to B and C. (B) Same as A except stimulation occurs at region B approximately 40  $\mu\text{m}$  from region A. (C) Receptor potentials evoked by regions A and B being stimulated alternately by the same sequence of light pulses. Level of base line in bottom trace indicates which region is being stimulated. Upper level indicates region A, lower level region B.

was presented to area B with the same results. If the set of stimuli were alternately presented to area A and B without any quiescent period, the responses evoked by the stimuli at A were little influenced by stimuli at B and vice versa. This experiment indicated that at these particular intensities, the responses evoked by stimuli at one area in the receptor were little influenced by previous stimulation of another area.

Not all preparations showed local adaptation when the above experiment was performed. Such preparations were rejected for further study of possible spatial effects on the latency distribution of discrete waves because the ab

sence of local adaptation was judged as evidence of excessive light scatter from the light spots. The results reported below were obtained from preparations showing marked local adaptation as defined by the above experiment. Two types of experiments were performed in order to discover if the response latency fluctuation to very dim flashes of light was a function of the location of photon absorption.

(a) In the first type two kinds of stimuli were used, a small flashing spot, 5–10  $\mu\text{m}$  in diameter, projected onto the region of greatest sensitivity and a large flashing spot, concentric with the small spot, that illuminated the entire receptor. In both cases the stimulus duration was 20 ms and the interval between successive stimuli was 5 s. The intensity for each kind of stimulus was adjusted so that the frequency of a response occurring was between 50 and 60%. This resulted in the intensity of the large spot being less than the intensity of the small spot. Sequences of 100 small-spot stimuli and sequences of 100 large-spot stimuli were alternately presented to the receptor until a total of about 200–400 of each kind was presented. The latency and heights of the responses when they occurred were measured according to the definitions given below.

When a response occurs after a dim pulse of light, the wave form is variable. Sometimes it is difficult to resolve the individual discrete waves in the response. Therefore, the latency of the response was defined to be the time interval between the stimulus onset and the first detectable depolarization. This question is discussed in detail elsewhere (Srebro and Yeandle, 1970).

Under our experimental condition there was a clearly observable base line. The height of the response was defined to be the maximum depolarization of the wave form measured from the base line.

(b) The protocol for the second type of experiment was identical to that of the first type except that the contrasting stimuli used were two small flashing spots 5–10  $\mu\text{m}$  in diameter and separated at the receptor by a distance of about 40  $\mu\text{m}$  (center-to-center). The latency and height distributions were measured as before.

Figs. 3 and 4 show plots of the latency and height histograms for the two spatially separated spots. When the two stimuli were a large and small light spot the latency and height distributions were also similar for the contrasting stimuli.

The latency histograms reveal that the latency to the first discrete wave after a short flash is a random variable. Approximately 95% of the first discrete waves occur within the first second after the stimulus. Thus almost all light-evoked discrete waves occur within the first second of the stimulus onset and those occurring later most probably represent spontaneous waves. Therefore, the two contrasting stimuli latency distributions were compared, in 100-ms intervals, only for the first second after the stimulus. One cell was studied by method one and six cells were studied by method two.

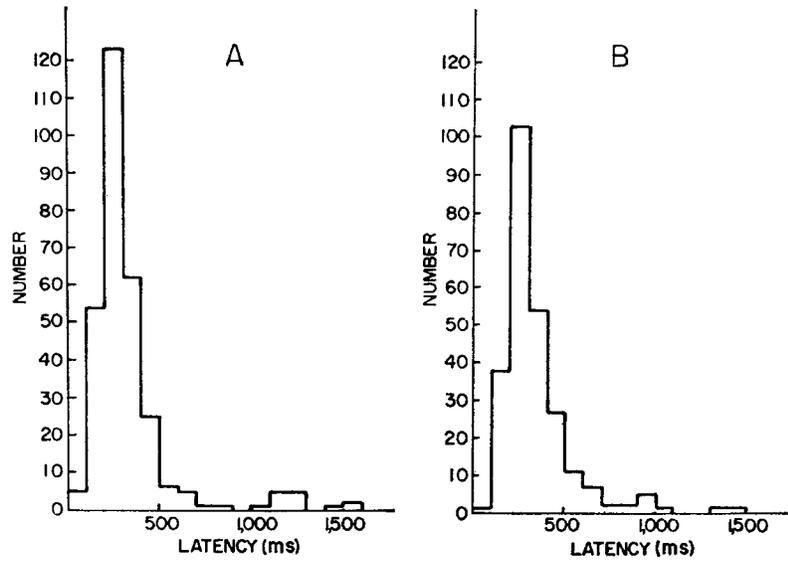


FIGURE 3. Latency histogram of the first discrete wave following a 50-ms low intensity light pulse. Temperature 12°C. (A) Spot size 5–10  $\mu\text{m}$  at region A. (B) Spot size 5–10  $\mu\text{m}$  at region B about 40  $\mu\text{m}$  from region A. Probability of response for stimulus at region A is 0.650 and for region B is 0.640.

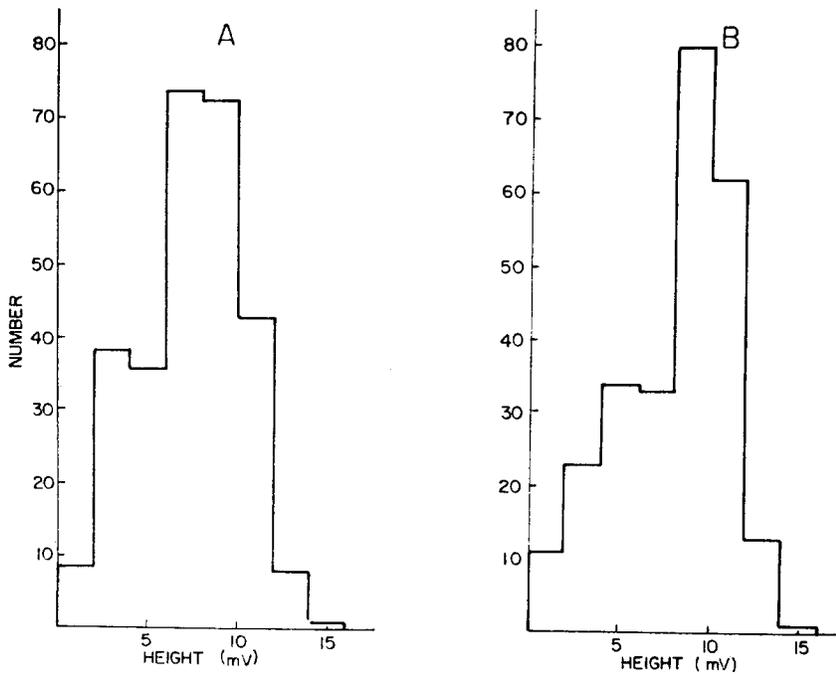


FIGURE 4. Height histograms for same experiment illustrated in Fig. 3. (A) Height histogram of responses evoked by stimulus at A. (B) Height histograms of responses evoked by stimulus at B.

The results are shown in Table I. For the data from each experiment a chi square contingency test was performed. The chi square statistic, degrees of freedom, extent of illumination, and temperatures for the seven cells are listed.

If the expected number of discrete waves in a 100-ms interval was less than five, it was added to one or more contiguous intervals until their sum was

TABLE I  
COMPARISON OF HEIGHT AND LATENCY HISTOGRAMS

Cell number	Temperature °C	Approximate spot size μm	Response frequency	Latency		Height	
				X <sup>2</sup>	df	X <sup>2</sup>	df
1	14.25	5-10	214/290=0.733	9.47	7	22.26	14
		5-10	177/287=0.616				
2	12.5	60	265/433=0.613	7.26	9	33.69	29
		5-10	230/427=0.538				
		Concentric with first spot					
3	15	5-10	119/197=0.604	1.26	5	5.5	7
		5-10	112/169=0.662				
4	25	5-10	122/279=0.438	2.71	5	6.50	4
		5-10	142/351=0.405				
4	20	5-10	123/310=0.396	16.0	6	6.82	5
		5-10	175/347=0.504				
6	12	5-10	259/395=0.650	4.29	5	24.25	6
		5-10	280/437=0.640				
7	18	5-10	202/389=0.519	16.59	4	22.07	11
		5-10	239/408=0.585				
Totals				57.58	41.0	121.08	76.0

Table I shows the results of comparing height and latency histograms for two small separated spots and two different sized concentric spots. In the column labeled response frequency the denominator and the numerator in each entry are, respectively, the total number of trials and the number of trials evoking bumps. With the exception of cell 2 all experiments involve two small spots separated about 40 μm. For cell 2 the two stimuli were a large and small spot concentric with each other. The total chi square statistic for the contrasting latency distributions is not significant at the 5% level, while that of the height distributions reveal a significant difference at the 0.1% level.

equal to or greater than five, a generally accepted lower bound for a chi square contingency bin. In Fig. 3 A and B the number of discrete waves in the first 100-ms interval was grouped with those of the second interval while those found in the last four intervals were grouped so that their sum would be greater than five.

The chi square statistic used was

$$\chi^2 = n \left( \sum_{i,j} \frac{\nu_{ij}^2}{\nu_{i.} \nu_{.j}} - 1 \right),$$

where  $j$  is equal to 1 or 2 and refers to one of the two spots of light.  $i$  refers to a latency interval and ranges from 1 to the number of latency intervals determined as above.  $\nu_{ij}$  is the number of events in the  $i$ th interval associated with the  $j$ th light stimulus.  $\nu_{i.} = \sum_j \nu_{ij}$  and  $\nu_{.j} = \sum_i \nu_{ij}$  (see Cramér, 1946 or any text on mathematical statistics for explanation of contingency tables).

At the 5% level, the null hypothesis was not rejected in five of the seven experiments. Adding the chi square statistic and the degree of freedom of all the contrasting latency distributions showed that the total chi square statistic was not significant at the 5% level. Although the height distributions were similar for the contrasting stimuli, the total chi square statistic revealed a significant difference between the two at the 5% level.

The presence of a significant chi square for two of the seven cells studied is an improbable event for the hypothesis tested and suggests that if more experiments were performed the total chi square might be significant at the 5% level. We feel, however, that if there is a spatial contribution to the latency distribution's upon illumination of the entire receptor that such a contribution is very likely to be minor.

One important contingency should be discussed. In our experiments the microspot traversed the receptor so that both regions near the surface of the cell and regions in the interior of the cell were stimulated. There may be a dependence of discrete wave latency on distance from the cell surface of the excited visual pigment molecule causing the discrete wave. Our experiments give no information on this possibility.

Since stimuli of low energy light pulses to different regions of the receptor produce similar latency distributions, at least to within a resolution of 5–10  $\mu\text{m}$ , fluctuation in latency when the entire photoreceptor is illuminated cannot be entirely attributed to differences in the latency for different areas of the cell.

#### DISCUSSION

The phenomenon of local adaptation in the ventral eye is consistent with a similar effect observed by Ratliff (1965) in the lateral eye. He presented adapting light to different regions of the ommatidium and measured resulting changes in the number of nerve impulses evoked by a test spot in the nerve fiber leading from the ommatidium. He suggested a number of explanations for the effect he observed, one of which was melanin pigment migration, a phenomenon commonly observed in the lateral eye. Since there is no masking pigment in the ventral receptors, other mechanisms must be sought. Perhaps the local adaptation we observed is caused by a temporary depletion of sodium ions in extracellular spaces at the place where the light falls.

There are two main hypotheses that have been proposed for the latency fluctuation. That of Borsellino and Fuortes (1968) postulates a sequence of

chemical reactions in which the number of molecules involved in each step is small enough to allow significant fluctuations in the concentrations of the reacting substances. That of Srebro and Behbehani (1971) postulates a stochastic process initiated by photon absorption that controls the opening of gates in the membrane, and when a sufficient number of gates are opened a discrete wave results. Our results are consistent with both ideas. Our technique would not reveal differences in the receptor properties over distances less than 5  $\mu\text{m}$ . Such differences may exist. Our results, however, are consistent with the notion that the latency fluctuations do represent some stochastic process in the receptor.

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#### REFERENCES

- BORSILLINO, A., and M. G. F. FUORTES. 1968. Response to single photons in visual cells of *Limulus*. *J. Physiol. (Lond.)* **123**:417.
- CRAMÉR, H. 1946. *Mathematical Methods of Statistics*. Princeton University Press, Princeton, N. J.
- HAGINS, W. A., H. V. ZONANA, and R. C. ADAMS. 1962. Local membrane current in the outer segments of squid photoreceptors. *Nature (Lond.)* **194**:844.
- RATLIFF, F. 1965. Selective adaptation of local regions of the rhabdom in an ommatidium of the compound eye of *Limulus*. In *The Functional Organization of the Compound Eye*. C. G. Bernhard, editor. Pergamon Press, Inc., Elmsford, N. Y.
- SREBRO, R., and M. BEHBEHANI. 1971. A stochastic model for discrete waves in the *Limulus* Photoreceptor. *J. Gen. Physiol.* **58**:267.
- SREBRO, R., and S. YEANDLE. 1970. Stochastic properties of discrete waves in the *Limulus* photoreceptor. *J. Gen. Physiol.* **58**:267.
- WOLBARSH, M. L., and S. S. YEANDLE. 1967. Visual processes in the *Limulus* eye. *Annu. Rev. Physiol.* **29**:531.
- YEANDLE, S., and SPIEGLER, J. B. 1973. Light-evoked and spontaneous discrete waves in the ventral nerve photoreceptor of *Limulus*. *J. Gen. Physiol.* **61**:552.