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Variations in the Voltage Response to Single Quanta of Light in the Photoreceptors of *Locusta Migratoria**

J. Howard

Department of Neurobiology, Research School of Biological Sciences,
P.O. Box 475, Canberra City, A.C.T. 2601, Australia

Abstract. Brief, dim flashes of light that produced on average only one response per three to six flashes were presented to dark-adapted locust photoreceptors. The amplitude, latency, and half-duration of the discrete voltage responses showed substantial variation. However all frequency distribution curves were single peaked, indicating that there is only one class of discrete voltage responses in the locust. Correlations between these parameters are low (accounting for less than 7% of the variance) but statistically significant. The low correlations suggest that different processes underlie the generation of amplitude, latency and duration of the bumps.

Key words: Locust photoreceptor – Bump – Quantum response

Introduction

The voltage response to dim light of many invertebrate photoreceptors consists of clearly resolvable discrete events. These events, known as bumps, occur at rhadom and are thought to be responses to single photon absorptions (for references see Lillywhite 1977). Bumps are not stereotyped events, but show variation in amplitude, latency and time-course (locust – Lillywhite 1977; Lillywhite and Laughlin 1979; *Limulus* lateral eye – Borsellino and Fuortes 1968, Wong et al. 1980; *Limulus* ventral photoreceptor – Behbehani and Srebro 1974, Stieve and Bruns 1980). This variation has been termed transducer noise by Lillywhite and Laughlin (1979), and these authors estimate that 50% of the

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variance of the light response is due to transducer noise, the other 50% being due to the random arrival and absorption of photons (Dodge et al. 1968). Wong et al. (1980) found that the Q_{10} of the latency process in the *Limulus* lateral eye photoreceptor is about 5, while the timescale of the bumps has a Q_{10} of only 3, indicating that different mechanisms underlie the latency and time-course of the bumps. The purpose of this paper is to describe accurately the distributions of and the correlations among the amplitudes, latencies and durations of bumps in the locust photoreceptor in order to help establish whether or not these three variables share common underlying mechanisms. The low rate of spontaneous (dark) activity exhibited by locust photoreceptors (Lillywhite 1977) makes the locust preparation most suitable for this purpose.

Methods

Preparation

Adults of the species *Locusta migratoria* were immobilised in wax and a small hole (< 150 μm) cut in the cornea. The hole was subsequently covered with silicon high vacuum grease through which the glass microelectrode was lowered. For these experiments it was important that the bumps be large (average amplitude > 3 mV) and the baseline noise recorded within the cell in the dark be low (s.d. < 0.15 mV). The high signal-to-noise ratio obtained in these experiments ensures that less than 2% of the variance in bump amplitudes can be attributed to baseline noise. Also the number of smaller bumps not resolved in the noise is minimized. The success achieved in meeting these requirements was largely dependent on the quality of the microelectrodes. As a general rule, the higher the electrode resistance the larger the bumps but the greater the noise. Optimal recordings were made using electrodes (filled with 3M K Acetate) that had a resistance of 80 MΩ when measured in the eye.

Animals were prepared in the afternoon. The electrode was inserted into the eye and a photoreceptor penetrated. After the optical axis was located, the animal was dark-adapted for 2–3 h. Every effort was made to exclude stray light from the preparation, and under these conditions the spontaneous bump rate was less than 20 per hour – comparable to that found by Lillywhite (1977). Thus less than 1% of the bumps analysed would be expected to arise from spontaneous activity. Experiments were performed at room temperature (22° C–25° C). Data were rejected if they failed to meet any of the following three criteria for stability. The first involved comparing the average waveforms at various times during the experiment. This subjective method allowed the detection of marked changes in any of the bump parameters (amplitude, latency or duration). A second, more objective criterion involved calculating the statistical correlation of the parameters with time. Data passed this test only if no significant correlation (at the 95% level) was found between either amplitude, latency or half-duration tested against time. The third criterion was that the resting potential in the dark changed by less than 1 mV during the experiment.

Light Stimulus

Light from a 75 W Xenon arc-lamp was collimated, attenuated, filtered by a green interference filter (λ_{max} = 490 nm, halfwidth = 9 nm) and focussed upon the tip of a quartz light-guide, the other end of which was located on the optical axis of the impaled photoreceptor. The light stimulus subtended an angle of less than 2° at the eye. A Uniblitz high-speed electronic shutter delivered flashes of 2 ms duration at a rate of two flashes per second. The short duration of the flashes ensures that less than 2% of the variance observed in bump latency can be attributed to the scatter in photon arrival times at the eye. The light intensity was attenuated so that a response was recorded on average only once per three to six flashes, ensuring that most of the responses are due to single photon absorptions rather than multiple absorptions.

Data Collection and Analysis

A PDP 11/02 minicomputer running a commercial operating system (DAOS 11) under RT11 was used for data collection and storage on floppy disk. 256 samples of the intracellular voltage were taken at 1-ms intervals, starting 5 ms before the beginning of the flash. After the completion of an experiment, each 256-point waveform was displayed and scored (by eye) to be either no response, a single bump, or more than one bump. This rather tedious approach had to be taken as no satisfactory computer program could be written that reliably discriminated between a single bump and a response that was the superposition of two or more bumps (see Fig. 1). When two bumps were not clearly separated in time, an abrupt change in the slope was used as the subjective criterion that the response

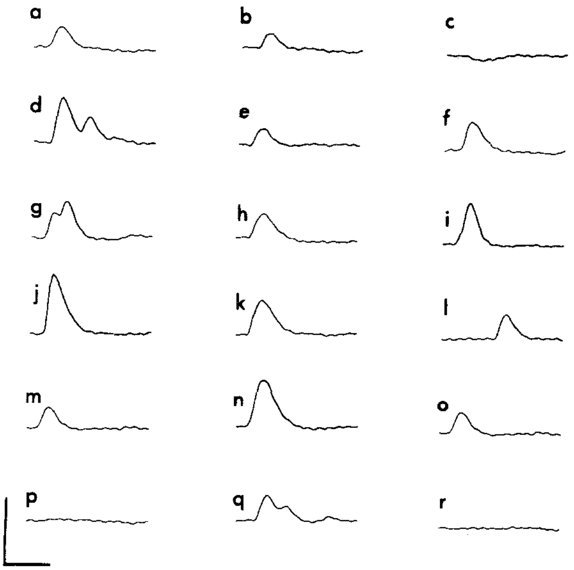

Fig. 1. Responses to the dim, 2 ms flash of light exhibited by cell 1. The flash started 5 ms after the beginning of sampling. Responses c), p), and r) were scored as "no bump", responses d), g), and q) were scored as "multiple bumps" and the rest as being single bumps. The intensity of the flash was such that 66% of the flashes failed to elicit any response. Scale bars represent 10 mV (vertical) and 100 ms (horizontal)

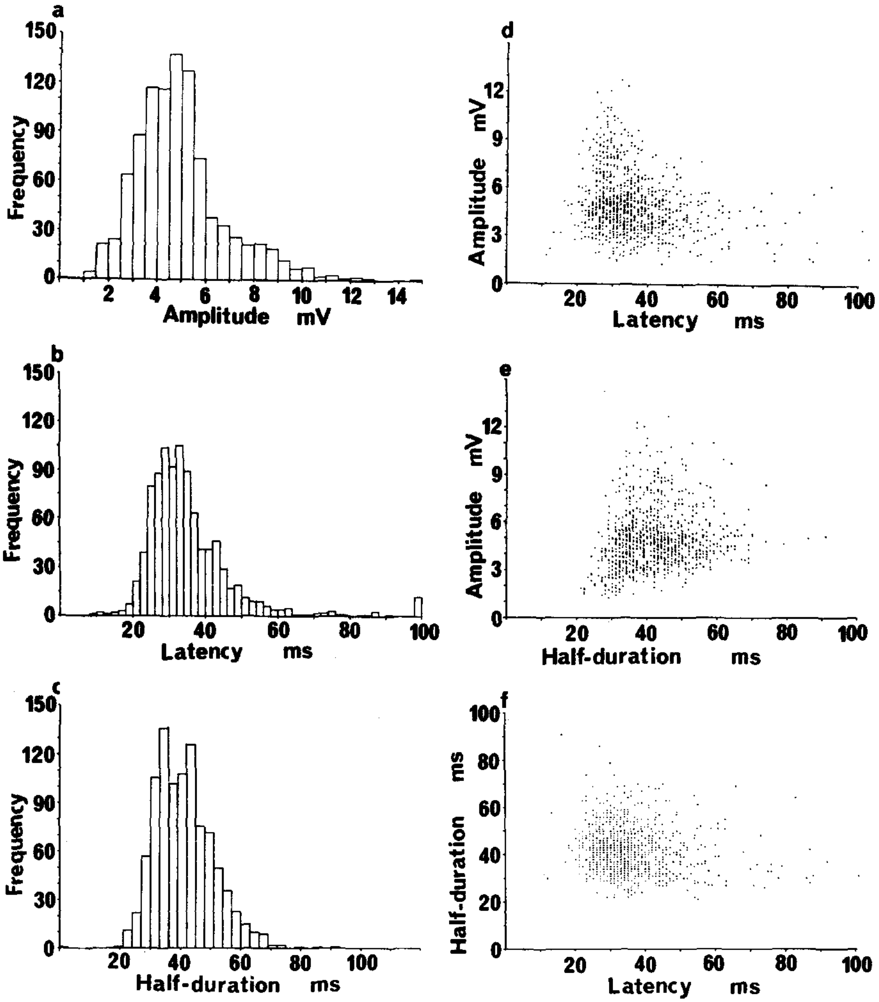

Fig. 2. Bump histograms and scattergrams for cell 1 of Table 1

was caused by the superposition of two or more bumps. If the rising phase of two bumps overlap, then such a discrimination cannot be made as easily and hence a number of "double bumps" were, no doubt, scored as single bumps. The low average bump yield per flash was chosen in order to minimise the number of responses incorrectly scored. If each flash produces on average p bumps, then the probability that no bump occurs is $\exp(-p)$ and the probability of exactly one bump is $p \exp(-p)$. These figures arise from the Poisson distribution governing photon arrival and of course we are assuming each effectively absorbed photon triggers the production of one bump (Lillywhite 1977). By decreasing p, the ratio of multiple bumps to single bumps can be made arbitrarily low, though at the expense of increasing the number of flashes (and thus the duration of the experiment) in order to count a fixed number of bumps. In cell 1 of Table 1 (Figs.

Table 1

Cell number	1	2	3	4
Bumps	967	292	273	153
Flashes	3,000	1,500	1,500	1,000
Amplitude (mV) (a)	4.92 ± 1.85	3.64 ± 1.19	3.70 ± 1.15	3.45 ± 1.22
Latency (ms) (l)	36.3 ± 15.2	39.7 ± 16.8	30.6 ± 14.7	27.9 ± 15.6
Half-duration (ms) (hd)	41.3 ± 9.5	35.4 ± 6.3	29.5 ± 5.0	28.4 ± 4.6
Minimum latency (ms)	11	18	5	9
Correlation a × 1	−0.23[b]	−0.10[b]	−0.09 n.s.	+0.01 n.s.
Correlation a × hd	+0.18[b]	+0.10[b]	+0.07 n.s.	−0.16[a]
Correlation	−0.15[b]	−0.20[b]	−0.25[b]	−0.13 n.s.

[a] Significant at the 5% level
[b] Significant at the 1% level
n.s. not significant at the 5% level

1 and 2), 967 responses were scored as single bumps while 49 responses were scored as multiple bumps out of a total of 3,000 flashes. From the fraction of null responses (1,984/3,000) we calculate $p = 0.41$. The expected number of single bumps is then 820, some 145 short of the number of single bumps scored. Thus it must be concluded that some 15% of the responses scored as single bumps were in fact multiple bumps. Similar arguments applied to the other cells of Table 1, which had larger null-response ratios, indicate that about 9% of these bumps were multiple bumps. The discrimination between no bump and a single bump was comparatively easy because bumps as small as 0.5 mV in amplitude could be resolved above the baseline noise. It is difficult to estimate the number of very small bumps that were not resolved but the general shape of the amplitude frequency histograms (Fig. 2a which was typical) suggests that this fraction is small and probably less than 1%.

Those waveforms that had been scored as being single bumps were analysed in detail on a PDP 11/34 computer. For every bump, the mean of the first 15 samples was used as the zero level. The response maximum and the time to reach the maximum were measured. The times taken to rise and fall to 25%, 50%, and 75% maximum were then measured. The latency was calculated by linear extrapolation from the times to rise to 25% and 50% maximum back to the zero level. The extrapolated latency corresponded closely to the subjective latency obtained by deciding (by eye) when the bump started. The half-duration of a bump was defined as the time to fall to 50% maximum minus the time to rise to 50% maximum.

Results and Discussion

Frequency Histograms

Frequency histograms of the bump amplitude, latency and half-duration from a typical cell are displayed in Fig. 2. All frequency histograms are concave with a single prominent peak. There is no evidence for the existence of two populations of bumps; slow, small ones and fast, large ones as reported by a number of authors working on a number of preparations (*Limulus* lateral eye – e.g., Adolf 1964, Borsellino and Fuortes 1968; *Limulus* ventral photoreceptors – Yeandle and Spiegler 1973, Behbehani and Srebo 1974, Stieve and Bruns 1980; locust – Lillywhite 1978; fly – Dubs et al. 1981). Such a bimodal distribution would be clearly demonstrated in the height-width scattergram (Fig. 2e). Lillywhite (1978) and Dubs et al. (1981) argue that the small bumps originate in neighbouring photoreceptors (in the same ommatidium) that are electrically coupled to the recorded cell. The four cells of Table 1 had the highest signal-to-noise ratio and greatest stability from over 100 intracellular recordings. No clear evidence for the existence of a class of small, slow bumps was seen in any recordings, indicating that electrical coupling between photoreceptors was very low or even non-existent in these experiments. A possible explanation for the difference between Lillywhite's and my own results may be that Lillywhite actively sought cells exhibiting small bumps (Lillywhite 1978, p 23) whereas the objective of the present experiments was to record a large signal-to-noise ratio. The discrepancy between Lillywhite's data and my results raises the possibility that his small bumps were caused by artifactual coupling produced by the glass microelectrode as it passed through the ommatidium. Such an interpretation is consistent with Lillywhite's finding that the recorded cell could be coupled with no other cells or with one or two other cells, though no estimate of the percentage of cells showing small bumps was given.

The frequency distribution of latencies shown for a single cell in Fig. 2b is typical for locust photoreceptors at room temperature. The average latency is 36 ms, but the distribution is skewed, with less than 1% of the bumps having a latency of less than 20 ms and the minimum latency was 11 ms in this cell. In the four cells of Table 1, only four of the 1,685 bumps had a latency of less than 10 ms, suggesting the existence of a minimum delay, possibly attributable to events at the level of the pigments (Hamdorf and Kirschfeld 1980). But clearly the more typical latencies must be caused by processes subsequent to the formation of metarhodopsin, which in insects takes less than 1 ms at room temperature (review Kirschfeld 1981).

Correlations

It is evident from the scattergrams of Fig. 2 that there is little correlation among the three parameters – amplitude, latency and half-duration. Though many of the correlation co-efficients in Table 1 are statistically significant, correlation can account for at most 7% (and often much less) of the variance. A low correlation between bump amplitude and latency has also been found in the *Limulus* ventral photoreceptor (Stieve and Bruns 1982).

It is improbable that variations and correlations in these parameters can be attributed to the cable properties of the retinula cell. If the size and shape of the recorded bumps were dependent on the distance between the site of origin of the bumps and the recording electrode, then one would expect a positive correlation between latency and half-duration; and negative correlations between both amplitude and latency, and amplitude and half-duration. No such pattern of correlations is found. This implies that the cell's length constant is large compared to the length of the photoreceptor. This agrees with Lillywhite's (1978) finding that bump amplitude is independent of wavelength (different wavelengths will cause different light-absorption profiles down the rhabdom).

On the other hand it is probable that correlations are caused by the unavoidable ambiguity as to whether or not a response scored as a single bump (see methods) was in fact the superposition of two or more bumps. This is because the most frequent latency is less than the mean of the latency distribution, and hence temporally overlapping bumps (that are incorrectly scored as being single bumps) would be more common when they occur with shorter latency. These mis-scored bumps will on average be larger and of longer duration than single bumps and thus we expect negative correlations between both amplitude and latency, and half-duration and latency; and a positive correlation between amplitude and half-duration – a pattern of correlations found in three of the four cells in Table 1. The problem of the mis-scoring of bumps is intractable. For example, if the flash intensity were decreased to reduce the probability of mis-scoring bumps to 1%, then the duration of the experiment would increase by a factor of about 15 (in order that the same number of bumps be counted) but in this case the spontaneous bumps would contribute about 5%–10% of the total.

Whether or not the correlations are caused by errors in sampling outlined above, the fact that they are so low implies that the mechanisms underlying the amplitude, latency and duration of bumps are, to a great extent, different. In particular, these findings support both the hypothesis of Wong et al. (1980) that different processes underly the latency and time-course of bumps, as well as the conclusion of Stieve and Bruns (1982) that different processes underly latency and amplification in the ventral photoreceptor of *Limulus*.

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