Voltage Noise in Limulus Visual Cells

Abstract. *Intracellular recordings from* Limulus *eccentric cells suggest that the generator potential arises from the superposition of numerous discrete fluctuations in membrane conductance. If this is so, a relation between frequency response to flickering light and noise characteristics under steady light may be predicted. This prediction is verified experimentally. If a discrete fluctuation model is assumed, the data indicate that increased light has two major effects: (i) the discrete events are strongly light-adapted to smaller size, and (ii) the time course of each event becomes briefer.*

The eccentric cell in the compound eye of *Limulus polyphemus,* the horseshoe crab, is particularly well suited for investigation of the generator potential which apparently underlies visual sense perception. Here we present evidence from our observations on this cell in support of three suggestions concerning the nature of the generator potential.

1) The generator potential arises from a superposition of discrete voltage "shot" events or "bumps," which are triggered by the absorption of light.

2) The average size of the bumps decreases markedly as the ambient light intensity is increased, and this is the major mechanism for light adaptation.

3) The improved time-resolution of visual response that occurs with increasing level of ambient light can be attributed to two factors—a more rapid rate at which the average bump size adjusts to light intensity and a small decrease in the duration of an individual bump.

Rushton (*1*) has discussed the possibility that the generator potential is the summation of bumps and has outlined the essential ideas embodied in the suggestions cited above. Yeandle (*2*), Fuortes and Yeandle (*3*), and Adolph (*4*) have extensively investigated several properties of the bumps which are resolved as discrete events in the dark-adapted ommatidium. Some important results of these studies are that the rate at which bumps occur increases proportionally to light intensity (*2–4*), that the times of occurrence appear to be random and independent (*3*, compare with *4*), and that the statistics of the bumps elicited by brief flashes is consistent with the idea that a bump is triggered by absorption of a single photon (*3*).

Physically, the bumps are discrete transitory increases in the membrane conductance of the visual cell (*4*). These bumps differ from the analogous quantal conductance changes underlying the miniature end-plate potentials of the neuromuscular junction (*5*) in that the duration of a bump is long compared to the membrane time constant and that the average amplitude of the bumps varies markedly as a consequence of normal function. In a study of the electrical equivalent circuit of the eccentric cell, Purple (*6*) has shown that the equilibrium potential associated with the excitatory conductance change is about 50 mv above the resting potential. Because the amplitude of the generator potential can be an appreciable fraction of this equilibrium potential, we have taken into account the nonlinear relation between membrane potential and conductance in the analysis of our data, in the way routinely used in the analysis of end-plate potentials (*7*).

Figure 1a shows a sequence of generator potentials measured at several different light intensities from an eccentric cell (action potentials of the nerve were suppressed by a minimal amount of tetrodotoxin in the bathing solution). In examining these records we note: (i) in darkness, the spontaneous bumps are recorded as discrete events; (ii) in response to dim light, the very noisy generator potential appears to be the superposition of more frequent "dark" bumps; (iii) the mean amplitude of the generator potential does not increase proportionally with the light intensity, but increases more

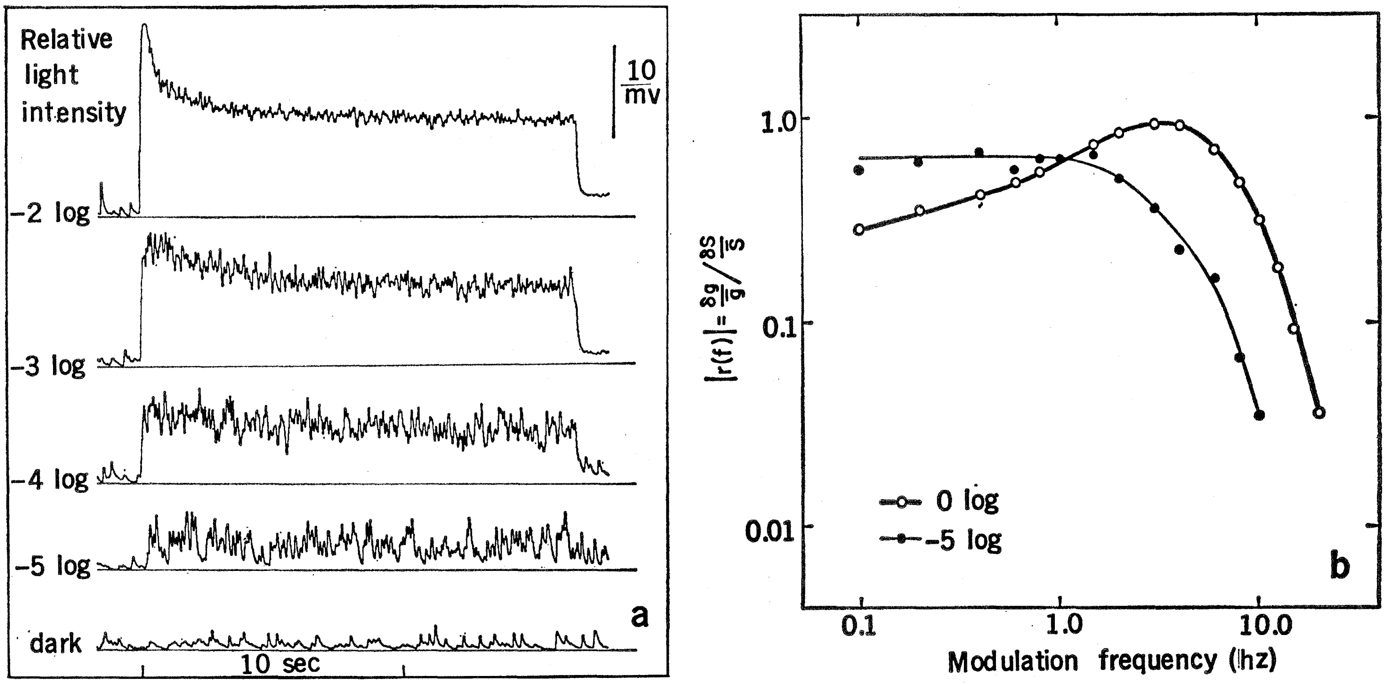

Fig. 1. (a) Representative records of generator potentials at different light intensities measured from an eccentric cell in which the nerve spikes were blocked by tetrodotoxin. (b) Transfer functions for the modulation of the generator potential in response to sinusoidal modulation of the light intensity, normalization described in text. The mean amplitude of generator potential was 25 mv for 0 log and 4.0 mv for —5 log.

nearly as its logarithm; (iv) the amplitude of the noise in the generator potential decreases with increasing light intensity; and (v) no large bumps are seen immediately following a bright light. All these remarks are qualitatively consistent with suggestions 1 and 2.

A quantitative relation between the noise observed under steady light and the response to a sinusoidally flickering light will be utilized below. We have measured the flicker response by a method similar to that of Pinter (*8*) except that our measurements were made on eccentric cells, rather than retinular cells, and that our frequency responses were refined by a narrow-pass digital filter (*9*). In these experiments the peak-to-peak modulation of the light intensity was about 40 percent of the mean, and the linearity of response was excellent, as checked by the absence of harmonics. Typical results are illustrated in Fig. 1b by plots of the amplitude of the frequency response measured at two greatly different mean light intensities. For this plot, the measured voltage changes (modulated component and mean value) were normalized so that the ordinate is the ratio of the fractional variation $\delta g/\bar{g}$ in excitatory conductance to the fractional variation $\delta S/\bar{S}$ in light intensity. For the very low mean light intensity, the frequency response shows simply the steep high frequency cutoff, which we ascribe to the shape of the bumps. For the high mean light intensity, the frequency response also shows a prominent low frequency cutoff, which we ascribe to the readjustment of the average bump size in response to the variation in light intensity.

Important temporal features of a (stationary) shot-noise process are conveniently given by its autocovariance function, that is, the time average of the lagged products of the instantaneous departure of the signal from its mean value:

$$C(\tau) = \overline{[g(t) - \bar{g}]\;[g(t + \tau) - \bar{g}]} \quad (1)$$

where the average is taken over the time t. Thus $C(0)$ is the variance of the signal, and roughly speaking $C(\tau)$ gives a picture of how the signal remembers its past. For the same cell that yielded Fig. 1b, we have computed the autocovariance functions according to Eq. 1 from records of the response to constant light at the same two intensities (50 seconds of data were used for the dim light and 130 seconds for the bright). The results, normalized to $C(0)$, are plotted as the points in Fig. 2a.

For a wide variety of shot-noise phenomena, a relation can be deduced between the autocovariance of the steady-state response and the expected response to small sinusoidal variations in a parameter of the system. The relation may be expressed as

$$C(\tau) = A \int_0^\infty |\, r(f) \,|^2 \cos\,(2\pi f \tau)\, df \quad (2)$$

where A is a constant of proportionality and $|\ r(f)\ |$ is the frequency response amplitude, as shown in Fig. 1b. Equation 2 is easily demonstrated for an inhomogeneous Poisson (uncorrelated) shot noise (*10, 11*) where the parameter is the expected rate. More general models, in which the occurrence of a bump may influence the sizes of subsequent bumps (as suggestion 2 would imply), also lead to Eq. 2 (*12*). We have evaluated the integral in Eq. 2 by the Cooley-Tukey fast Fourier algorithm (*13*), using the data of Fig. 1b, and the results are plotted as the solid lines in Fig. 2a. We emphasize that the points and lines in Fig. 2a have been generated directly by the two kinds of data; there has been no fitting of parameters.

The degree of agreement in Fig. 2a encourages us to accept suggestion 1 provisionally and to examine suggestions 2 and 3 by deducing how the parameters of the shot noise depend on light intensity.

The mean and variance of a shot-noise signal are related by a pair of expressions of the form:

$$\bar{g} = \lambda\, T\, \alpha \quad (3)$$

$$\overline{(g - \bar{g})^2} = \lambda\, T\, \alpha^2 \quad (4)$$

where λ is the shot rate; T, the effective shot duration; and α, the effective shot amplitude. Equations 3 and 4, together with rigorous expressions for T and α, are known as Campbell's theorem (*10*). (If the shots, of which a shot noise is composed, are of constant amplitude for a finite duration, Eqs. 3 and 4 are satisfied if the amplitude is α and the dura-

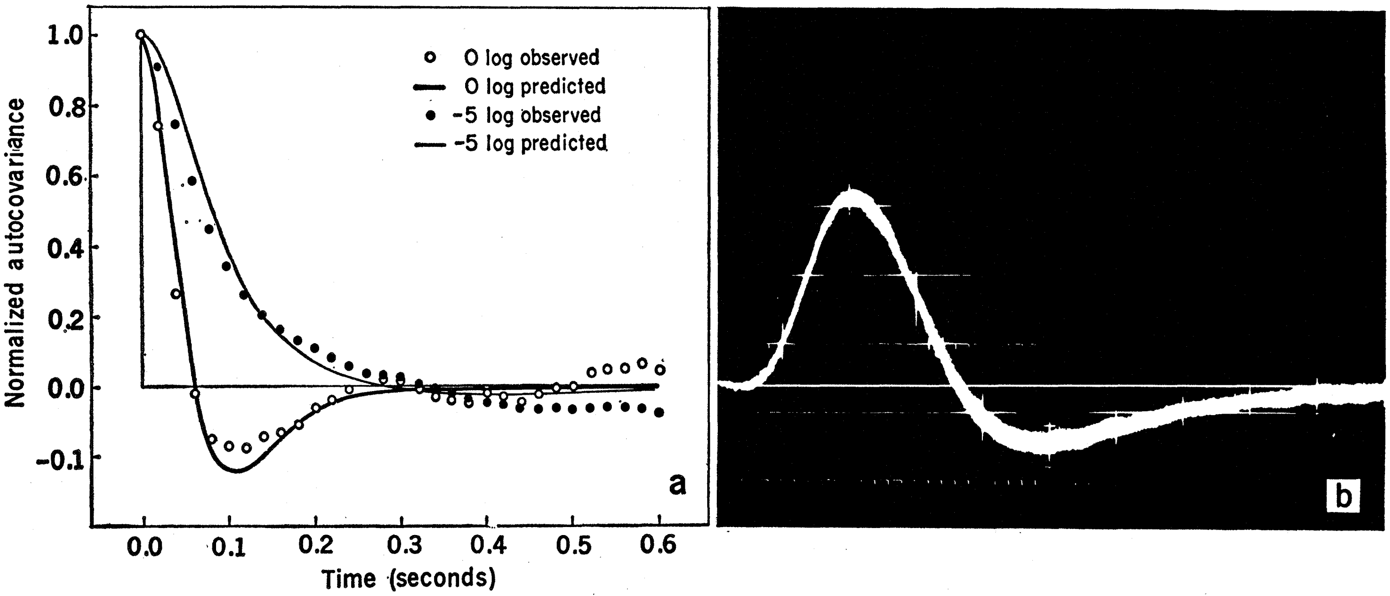

Fig. 2. (a) Autocovariance functions, normalized to the variance, at two mean light intensities measured from generator potentials (points) and predicted from the transfer functions (curves); same cell as Fig. 1b. The variance $(\text{mv})^2$/mean amplitude (mv) was .0032/25 for 0 log; .17/4.0 for −5 log. (b) Direct measurement of the response to a brief (40-msec) flash superimposed on a steady bright background. Sweep duration, 0.5 second; response amplitude, about 5 mv; and generator potential amplitude, 16 mv.

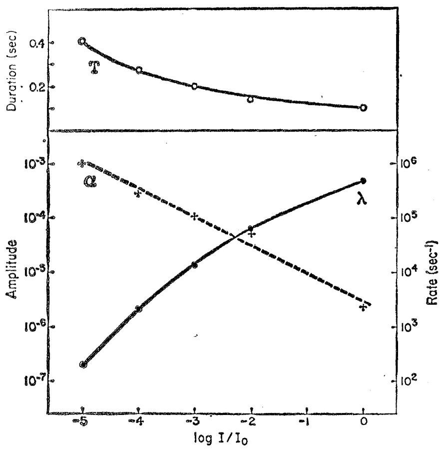

Fig. 3. Dependence of bump parameters on light intensity deduced from the noise on the generator potential. The effective bump amplitude (α) is given as the fraction of the conductance of the cell in the resting state. Same cell as in Figs. 1b and 2a. At intermediate light intensities the variance $(mv)^2$/mean amplitude (mv) was .016/16 for −2 log; .032/12 for −3 log; and .077/7.9 for −4 log.

tion is T.) If the effective duration T is known, then Eqs. 3 and 4 may be solved together for the rate λ and the amplitude α from measured values of the mean and variance. [This has previously been done by Hagins (*14*) for voltages recorded in the squid retina and by Adolph (*4*) for the *Limulus* generator potentials under very dim illumination.] Even for correlated shot processes, following from suggestion 2, the effective duration T may be evaluated rigorously from the frequency response of Fig. 1b (*12*). We have evaluated these parameters over a broad span of light intensities, with results shown in Fig. 3. Several features of these results deserve comment. (i) Over the span of a factor of 10^5 in light intensity the effective duration decreases by a factor of 4, of which about a factor of 2 results from the correlation of bump size (as indicated by attenuation of low frequencies in Fig. 1b), and the remainder may be attributed to a shortening in the time scale of the underlying bump as implied by the shift of the high frequency cutoff by about a factor of 2 in Fig. 1b. (ii) With increasing light intensity the rate departs from strict proportionality to light intensity, which is indicative of a reduced quantum efficiency. (iii) The steady-state bump size decreases continuously, approximately as the inverse square root of the light intensity.

Suggestion 2 implies that even a momentary flash of light should cause a slight readjustment of bump sizes toward smaller values. A flash has been superimposed on steady bright light and the result is shown in Fig. 2b. It is seen that, after an initial voltage surge in response to the flash, the voltage drops briefly below its steady-state value, as predicted. Similar experiments, in which the cell resistance was measured simultaneously, have shown that there is a definite minimum of the conductance at the minimum of the potential change. This observation speaks against the alternative interpretation that the underlying bumps might be diphasic at high light intensities, as high conductance at the minimum of Fig. 2b would be implied.

The undershoot of the response to a flash superimposed on a bright background (Fig. 2b) is reflected in the prominent negative phase of the corresponding autocovariance function (Fig. 2a). This implies that a positive fluctuation predisposes the generator potential to undergo a subsequent negative fluctuation. If the individual bumps are monophasic, this lends additional support to suggestion 2, with the further implication that it is the rate of bump occurrence that regulates the sizes of subsequent bumps.

Fuortes and Hodgkin (*15*) have investigated the quantitative relation between the changes in sensitivity and time scale that occur when the eye of *Limulus* is light- or dark-adapted. A major conclusion from their study is that a 200-fold reduction in the sensitivity is associated with a halving of the time constant of the response. If these responses were the summation of many quantum bumps triggered nearly synchronously by the test flash, their conclusion might be readily interpreted in terms of the results summarized by Fig. 3, in which the adaptation of the bump size by a factor of 200 was also associated with a shortening of the time scale of the bump by about a factor of 2.

A difficulty, which precludes the quantitative comparison of the two types of experiments, is our ignorance of how the dispersion in the time of bump occurrence, observed by Fuortes and Yeandle (*3*), depends on the past history of light intensity. Pinter (*8*) has pointed out that the formal model developed by Fuortes and Hodgkin (*15*) to describe the time course of the response to a flash is quantitatively consistent with the high frequency cutoff seen in the frequency response of the generator potential.

We close with two additional comments. First, not every eccentric cell shows the precision of agreement seen in Fig. 2a. The time scale of the autocovariance predicted from flicker is often a bit slower than that directly observed, especially at low levels of light. This suggests an additional response feature that is relevant only when the input is time-dependent, and a good presumptive candidate is the time dispersal of bumps triggered by light (*3*). Second, the close qualitative correspondence between our Fig. 1b, the wolf-spider retinograms of DeVoe (*16*), and the frequency responses measured psychophysically by deLange (*17*) for human subjects raises the possibility that the mechanisms discussed here may have applicability to other visual systems.

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18. Partially supported by research grant B864 from the National Institute of Neurological Diseases and Blindness and grant GB-6540X from the National Science Foundation. We thank H. K. Hartline and D. Lange for major help with this project.

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24 November 1967