Dynamics of Excitation and Inhibition in the Light-Adapted *Limulus* Eye *in situ*

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ABSTRACT   The dynamics of spike discharge in eccentric cell axons from the *in situ* lateral eye of *Limulus*, under small sinusoidal modulation of light to which the eye is adapted, are described over two decades of light intensity and nearly three decades of frequency. Steady-state lateral inhibition coefficients, derived from the very low-frequency response, average 0.04 at three interommatidial spacings. The gain vs. frequency of a singly illuminated ommatidium is described closely from 0.004 to 0.4 cps by the linear transfer function $s^{0.25}$; this function also accounts approximately for the measured phase leads, the small signal adaptation following small step inputs, and for Pinter's (1966) earlier low-frequency generator potential data. We suggest that such dynamics could arise from a summation in the generator potential of distributed intensity-dependent relaxation processes along the dendrite and rhabdome. Analysis of the dynamic responses of an eccentric cell with and without simultaneously modulated illumination of particular neighbors indicates an effect equivalent to self-inhibition acting via a first-order low-pass filter with time constant 0.42 sec, and steady-state gain near 4.0. The corresponding filters for lateral inhibition required time constants from 0.35 to 1 sec and effective finite delay of 50–90 msec.

INTRODUCTION

Although the lateral eye of *Limulus* has been studied for a number of years (see, e.g., Hartline, Ratliff, and Miller, 1961), investigation of its dynamic performance is fairly recent. Now several studies of the spike output of eccentric cells (Fuortes and Hodgkin, 1964; Lange, Hartline, and Ratliff, 1966 *a, b;* Ratliff, Hartline, and Lange, 1966), the generator potential response (Pinter, 1966), or both (Purple and Dodge, 1966; Ratliff, Knight, Toyoda, and Hartline, 1967; Knight, Toyoda, and Dodge, 1970) to various programs of light input have characterized the rate-dependent events underlying the

response of the eye to light. The experiments described here add to this picture in three main ways: (a) the eye was left *in situ*, (b) all experiments were done with the eye well-adapted to light, and (c) the range of frequencies used was broad, particularly at the low-frequency end of the spectrum.

The first two of these factors provide controls on previous work. In most experiments so far the eye has been excised and placed for many hours in a recording chamber; the *in situ* preparation also interferes with the physiological system (e.g., the nerve is exposed and the ocular blood vessel surrounding it is opened) but the eye itself and its immediate surroundings are not touched. More importantly, much of the recent "steady-state" data (see, e.g., Kirschfeld and Reichardt, 1964; Ratliff et al., 1967; Knight et al., 1970) has been obtained within 20 sec of a transition from dark to light. The light-adapted condition is not only of interest with respect to the normal physiology of the animal, but it also might be expected to facilitate preliminary analysis of the dynamics of the system, since some of the complicated nonlinear disturbances associated with the large step from zero to some level of light are avoided. Although an understanding of the full nonlinear behavior is desirable, characteristic rate limitations within the receptor may be more readily apparent in the small-signal case. For example, the small-signal dynamics of work production by insect fibrillar flight muscle have suggested the current view that they correspond to the predicted fluctuations in the number of cross-bridges linking actin and myosin filaments (Thorson and White, 1969). The large-signal behavior on the other hand remains comprehensible in terms of related fluctuations, but modified and altered in aspect by specific nonlinearities.

Low input frequencies were possible in these experiments both because of the light-adapted condition of the eye, and because spike discharge rate was used as an output variable (thus avoiding the danger of artifacts due to electrode processes which is present during long-term intracellular recording). We could thus characterize the low-frequency behavior well enough to justify comparison with a theory of its origin. The associated disadvantage is that the discharge rates obtainable under light-adapted conditions limited the usable range of frequencies at the upper end. There is, however, sufficient overlap between these results and those of other investigators to enable useful comparison. In particular we confirm several of the quantitative interpretations which Knight et al. (1970) make from analysis of data for the excised eye.

METHODS

A. *Equipment*

The light source was a 6 v 15 w tungsten filament (Carl Zeiss, 38-01-77). By means of a system of lenses a pattern of holes in a metal disc was focused on the eye; the light pattern at the eye surface was a horizontal row of three circular spots, a central one of 100 μ diameter flanked by two spots with diameters of 500 μ. The distance between

the edges of the central and lateral spots was 150 μ. Thus with the side spots covered, illumination was confined primarily to a single ommatidium (see controls in section B). Removing both the covers illuminated two additional groups of about seven ommatidia each without appreciably changing the illumination of the test ommatidium; the nearest inhibiting ommatidium was separated by about one ommatidial diameter from the test ommatidium. Ommatidia selected for experiment were those in the center of the eye the optical axes (i.e., direction of maximum sensitivity) of which did not diverge by more than 10° from the axis of the focused light passing through the central hole.

Light intensity was modulated by a pair of crossed polaroid filters, one of which was driven by a motor-cam-lever system so that it was displaced with sinusoidal rotational motion about an average angle of 45° with respect to the other polaroid. This procedure produced a nearly sinusoidal modulation of the light intensity about a mean value equal to approximately half the intensity entering the polaroids, up to more than 20% modulation. (20% modulation produced adequate modulation of spike frequency and was used in the experiments.) Thus the mean intensity could be varied while keeping the per cent modulation constant. Waterman (1954) has shown that change in the angle of polarization per se does not produce a change in response.

Light modulation was measured with a photomultiplier via fiber optics projecting into an unused part of the beam. The absolute light flux was calculated from measurements with a Gossen luxmeter (No. 10.62-1175) at the final exit of the optical system. This provided an estimate of the maximum light flux available at the eye; the effective intensity at the rhabdome was, of course, less owing to scattering, absorption, and slight misalignment with respect to the direction of maximum sensitivity of the test ommatidium.

B. Experimental Procedure

Limulus polyphemus were shipped by air freight from Woods Hole, Massachusetts, were kept at 15°C in circulating, filtered artificial seawater, and were occasionally fed fresh mussels. Under these conditions they remained apparently healthy for several months: mortality was about 5% over a 6 month period. During an experiment the animals were clamped to a table and the optic nerve was exposed via a hole about 1 cm in diameter in the carapace; after exposure of the optic nerve the edges of the hole were packed with cotton and the small chamber thus formed was filled with freshly mixed artificial seawater. Small fiber bundles were dissected from the nerve and placed on wire and soaked-cotton electrodes. This in situ preparation maintained good condition of the eye, as judged by the following criteria: (a) Single visual units continued firing at constant mean rate with regular discharge (for example, SD of interval/mean interval = 0.1 at 4.5 spikes per sec) for at least half an hour under constant illumination of the eye. Comparable values, e.g. 0.05 at five spikes per sec, were found by Ratliff, Hartline, and Lange (1968) for the spike trains measured during 20 sec test stimuli. (b) Temporal pairing or "galloping" of the units was observed in only 1 of 29 eyes, and firing in the dark did not occur; both these phenomena are generally regarded as evidence of poor condition (although they may be reversibly induced by certain conditions of temperature and light intensity: Lange, 1969, personal communication). (c) Lateral inhibition was always present.

The data analyzed in this paper are from 11 such fiber preparations, each of which produced acceptable records for 90–120 min. Air temperature in the room varied from 20° to 26°C in different experiments but was constant within ±0.5°C during an experiment on a given fiber. Before each frequency response curve was measured the ommatidium or ommatidia were exposed for at least 5 min to a steady light of the mean intensity and input geometry to be used. Measurements of the time course of the decay in discharge rate following illumination of dark-adapted eyes indicated that adaptation to the levels of intensity used in these experiments was virtually complete within 5 min.

Although the illuminating spot was smaller than an ommatidial diameter, neighboring ommatidia might have received effective scattered light or have been struck by the slightly off-axis light beam after it had passed through the test ommatidium. Therefore the following test was employed to determine whether the supposed singly illuminated ommatidia were in fact free of lateral inhibition. The response of each ommatidium was determined for different displacements of the light spot away from the maximum sensitivity position in the horizontal and vertical directions (Fig. 1). If the test ommatidium no longer responded when the light was centered on the adjacent ommatidia, it is fair to assume that the reverse would also be true. In fact, the response of the test ommatidium usually vanished with displacements of the spot less than one interommatidial spacing as in Fig. 1.

C. Data Analysis

The recorded impulse trains were photographed and the data analyzed in the following way (illustrated in Fig. 2): The period of the light modulation cycle was divided
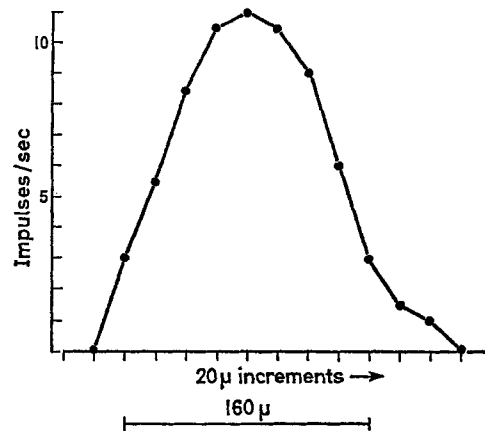

FIGURE 1.   An example of the response of an ommatidium as the central small spot of light is moved across its surface. At the peak response position the 100 μ spot is presumably centered on the ommatidium. As it is displaced from this position the discharge rate of the unit declines, reaching zero at a displacement of 100 μ to the left and 120 μ to the right. 160 μ is the average center-to-center distance between adjacent ommatidia. Assuming that the response vs. displacement curves of neighboring ommatidia are similar, we infer that the firing rate of adjacent ommatidia is probably very low or zero when the light spot is centered on the test ommatidium.
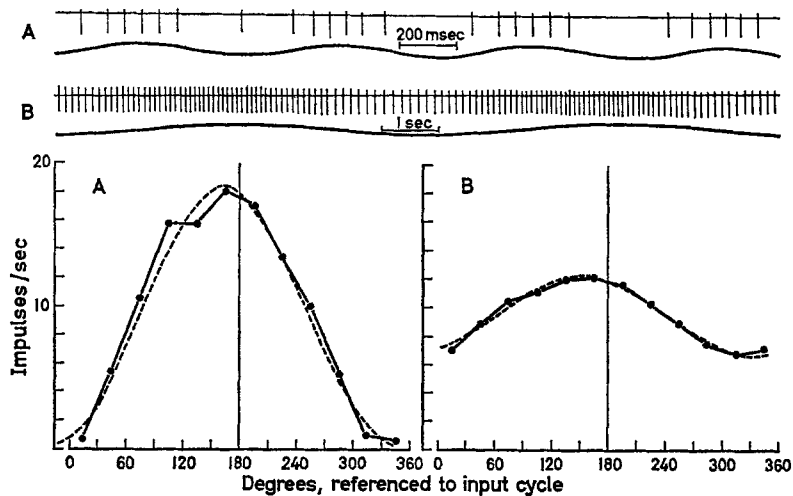
Figure 2.   The method of representing a train of spikes in terms of the amplitude of a continuous sine wave. The number of spikes occurring during each of 12 divisions of the input cycle is counted (for light modulation frequencies of 1.5 cps in A and 0.07 cps in B). The average number of spikes occurring during the time interval represented by each bin is expressed as impulses per second and plotted vs. position of the bin on the input cycle (dots connected by solid lines). Template sinusoids of different amplitudes are compared with this plot; the one which matches best in each case (dashed curves) is taken as an estimate of the amplitude of modulation of the spike train, and its position on the abscissa provides an estimate of phase lead or lag (peak occurring to the left or right, respectively, of the 180° line).

into 12 equal sections and the number of spikes occurring during each section was counted and divided by the time in seconds occupied by $\frac{1}{12}$ cycle at that modulation frequency; this gave a measure, in impulses/second, of the average discharge rate of the fiber during that portion of the input cycle. The 12 values so obtained were then plotted as estimates of instantaneous frequency at the centers of the appropriate time periods and matched by eye to one of a set of template sinusoids of different amplitudes. At the lowest frequency used (0.004 cps) data from just one cycle were taken; otherwise the spike counts in the corresponding sections of several successive cycles were averaged. From 2 to 20 cycles were averaged in each case.

RESULTS

A. *Linearity*

The response of a singly illuminated (uninhibited) ommatidium to sinusoidally modulated light is shown for different degrees of modulation in Fig. 3, for the modulation frequency 0.07 cps. The modulation of spike discharge rate is about linearly related to the light modulation up to at least 40%. Above that point the discharge rate modulation begins to fall off, and the wave form begins to deviate from a sinusoid in the way that would be expected at the output of a logarithmic operator: the curve becomes somewhat flattened at

the top and pointed at the bottom. (Beyond 30–40% modulation, the input drive system introduces further nonlinearity.)

A check for the kind of nonlinearity noted by Hughes and Maffei (1966) in sinusoidal driving of cat retinal ganglion cells was also made. This non-
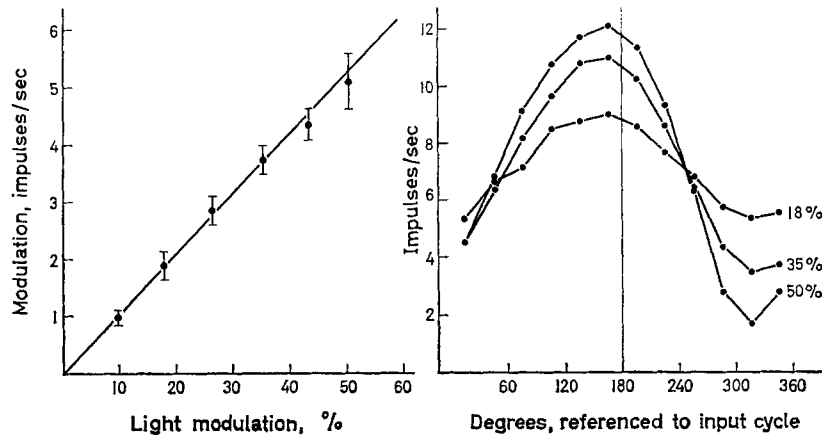

FIGURE 3. The left-hand graph shows the linear relation between the modulation of the spike output and that of the light input for a singly illuminated ommatidium, up to 40% modulation. At greater modulations, the relation begins to depart from linearity. This departure can be seen in the shape of the output wave form (right-hand plot), which is approximately sinusoidal at 18 and 35% modulation, but at 50% modulation becomes rounded at the top and pointed at the bottom.
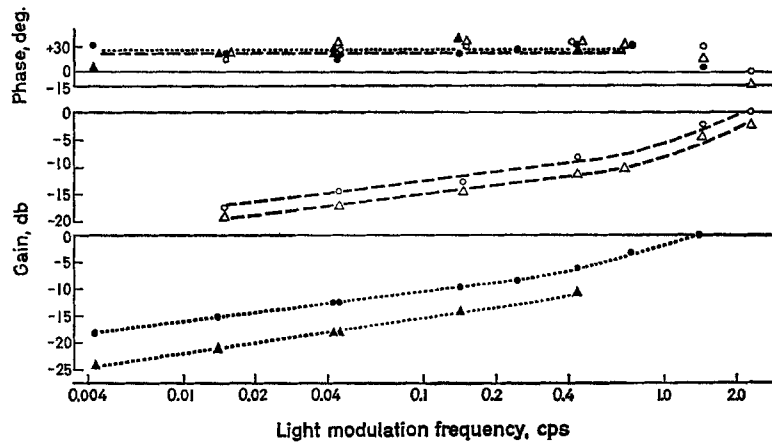

FIGURE 4. Phase and gain responses of two different ommatidia at different intensities. Only the test ommatidium was illuminated in each case. Circles indicate the response at high intensity (610 lumens/m²), triangles that at lower intensity: for the open triangles, intensity was reduced by a factor of 10 (average discharge rate reduced from 14 to 9 spikes/sec) and for the solid triangles, by a factor of 100 (average discharge rate reduced from 10 to 4 spikes/sec). In both cases the gain curve is shifted downward without change of shape or of slope, and the average phase lead remains about the same.

linearity amounts to the average discharge rate of the cell varying with the frequency of the light modulation. Although some variation in average discharge rate occurred over the course of the experiment (about $\pm 10\%$ in all but two cells and never more than $\pm 20\%$), no correlation of this drift with frequency of light modulation was noted.

B. *Frequency Response*

The Bode plots (gain and phase of the spike discharge rate response with respect to frequency of 20% sinusoidal modulation of light) obtained for different singly illuminated ommatidia are very similar. Examples are shown in Fig. 4. They are characterized by a low-frequency straight region with faster rising gain at intermediate frequencies. With the average discharge rates obtained in these experiments, 2–4 cps was the highest practical driving frequency. The slope of the low-frequency straight region in the 10 different ommatidia for which it was measured ranged from 1.1 to 1.6 db/octave. There was a relatively constant phase lead at low frequencies. At higher frequencies the phase lead usually tended to increase slightly, and above 1 cps there was a decrease in phase lead in all units and occasionally a phase lag at the highest frequencies studied.

The effect of intensity upon the frequency response of singly illuminated ommatidia was also measured in two units (Fig. 4). In the region studied, gain was decreased at lower intensities (0.1 and 0.01 times the maximum intensity) without appreciable change in the shape of the curve.

For eight ommatidia it was possible to obtain complete frequency response data both with and without simultaneous modulated illumination of neighboring ommatidial groups. One of these experiments is illustrated in Fig. 5. The effect of inhibition applied in this way is to decrease the gain uniformly at low frequencies, and at high frequencies to diminish it less or not at all. Usually the phase lead during inhibition is greater than in the noninhibited case at frequencies of 0.4 cps or higher. This is qualitatively the kind of effect which would be expected if the inhibition were acting via a low-pass filter. Transfer functions for these effects are derived in the next section.

C. *Step Response*

A test of the linearity of a system is provided by comparison of its step response with that predicted from a transfer function which represents the frequency response. When a 25% step increase or 20% decrease in light was given, the discharge rate first changed in the appropriate direction and then drifted back toward the original rate. A double-logarithmic plot of this drift is shown in Fig. 6.
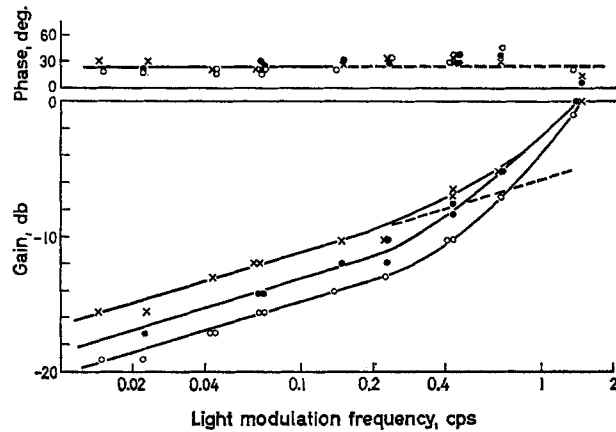
FIGURE 5.   The Bode plot of a visual unit when only the test ommatidium is illuminated (×'s), compared with that obtained during simultaneous illumination of one additional group (closed circles) and two additional groups (open circles) of seven ommatidia. At low frequencies the responses in all cases fall approximately on a straight line; above about 0.2 cps the gain increases more rapidly and the phase lead first increases and then decreases (compare with dashed extensions of the straight lines). These changes in gain and phase vs. frequency are more marked, the greater the number of stimulated ommatidia (i.e., the greater the amount of lateral inhibition).
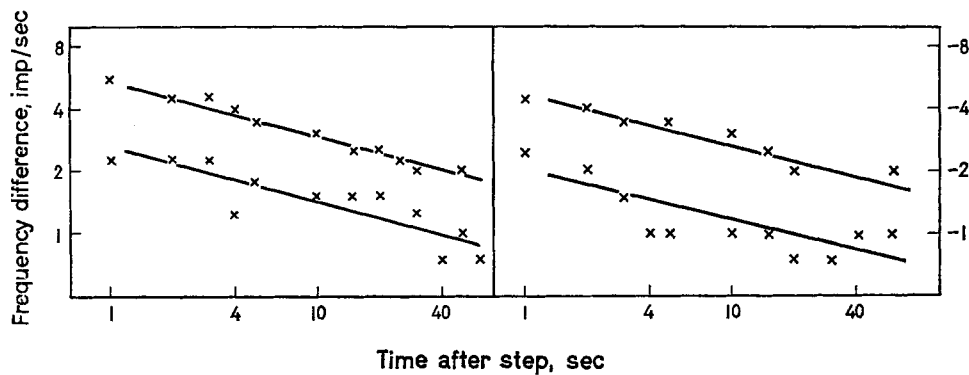

FIGURE 6.   Discharge rate of an ommatidium after a 25% step increase (left) or 20% step decrease (right) of light intensity. The ordinate shows the increase or decrease, respectively, in discharge rate, with respect to the rate of discharge before the step. In each case the upper set of points shows the response when only the test ommatidium is illuminated; for the lower set, two nearby groups of seven ommatidia were also illuminated and received the same step change in light intensity as the test ommatidium.

ANALYSIS AND DISCUSSION

A. *Dynamics of the Ommatidium at Low Frequencies*

The long straight region of the frequency response curves of both inhibited and noninhibited ommatidia can be described by the linear transfer function

s^k, $0 < k < 1$, where s is the Laplace variable and k is the ratio between the observed slope and a slope of 6 db/octave. This transfer function requires a phase lead of $k\pi/2$ radians, which is approximately the amount of phase lead observed in this frequency region. The value of k ranged from 0.18 to 0.27 in 10 units, with an average of 0.23. The step response associated with this linear transfer function requires that the discharge rate change as t^{-k}, after the transients associated with the high-frequency response have settled out (see, e.g., Chapman, 1963). The general trend of such step responses conforms to this prediction, as is illustrated in Fig. 6 for the ommatidium of Fig. 5. The straight lines represent the time function $t^{-0.27}$, predicted from the $s^{0.27}$ fit of the low-frequency portion of the Bode plot for this unit in Fig. 5. Events reflected in the high-frequency range of the frequency response curve affect primarily the early events in the step response; their influence is not well resolved in the step experiments.

Next we should rationalize the result that even at the very low frequencies used, the gain of the receptor appears to continue to fall with frequency. Since the ommatidium apparently has some appreciable DC (zero frequency) response, the frequency response curve ought to become flat at low enough frequencies. This effect can be described by expression of the dominant low-frequency transfer function as $(s + a)^k$ rather than s^k. From estimates of the DC component of the very long-term step response it is possible to estimate the sinusoidal modulation frequency below which the gain should be level. For one ommatidium (not illustrated) with a straight (s^k-like) frequency response down to 0.0045 cps, the response without inhibition to a 25% step increase was followed until an apparently steady value was reached; this DC gain was 0.5 spike per sec per 25% change in illumination. The absolute maximum gain in that experiment (6.9 spikes/sec per 25% modulation at 1.4 cps) was 22.8 db above this value. Therefore the Bode plot should flatten out at 22.8 db down; the extrapolated line obtained in this experiment reaches that value at 0.0007 cps, so that it is not surprising that there is no evidence of flattening at 0.0045 cps or higher. Strictly, therefore, the small-signal light adaptation (though as an asymptotic process it is never in principle "complete") requires on the order of half an hour following a small step change of light.

Pinter (1966) measured the frequency response of the generator potential of singly illuminated ommatidia to sinusoidally modulated light. He found that the high-frequency response could be described by the model of Fuortes and Hodgkin (1964) but that the model showed constant gain and zero phase at sufficiently low frequencies. Pinter's low-frequency gain data fall nearly on a straight line with about the same slope as those which fit the low-frequency responses of our units, but he proposed a "linear lead network" of integral order (as opposed to the linear lead transfer function of fractional order describing the data of this paper) to describe the low-frequency attenuation and phase lead. The response of the integral order network shows systematic

misfits to the data in both frequency response and step response plots. The gain vs. frequency data for 30% modulation in the range from 0.02 to 0.2 cps and the response to a 60% step increase in light (his Fig. 9, replotted on logarithmic coordinates) are fitted as well or better by straight lines with slope 0.15 to 0.2. This interpretation of his data shows that the generator potential low-frequency response may be as well or better described by a transfer function of the form s^k.

Transfer functions of this form have been found to describe the "slow adaptation" of several receptor preparations (Pringle and Wilson, 1952; Chapman, 1963; Thorson, 1966; Brown and Stein, 1966; Barth, 1967). Cole and Curtis (1936) considered a related function to describe their data on nerve and muscle membrane impedance, and suggested that polarization impedances in electrolytes and certain statistical distributions of rate constants could be associated with s^k dynamics.

Our finding here that similar dynamics apply to the *Limulus* photoreceptors suggests that light adaptation may involve distributed effects along the long rhabdome and dendrite of the eccentric cell, which produce small-signal adaptation of the form s^k. This idea is supported by our reinterpretation of Pinter's data, which demonstrates that this process precedes the generator potential. In a separate paper (Thorson and Biederman-Thorson, in preparation) we show that one can in fact account approximately for these s^k-like frequency response curves in terms of plausible intensity-dependent dynamics of photon absorption and conductance change; that is, the local dynamics vary along the rhabdome and dendrite as photon intensity varies, and these effects are summated in the generator potential.

B. Dynamics of Self-Inhibition

Above the low-frequency region just discussed, the response of a singly illuminated ommatidium departs from the s^k line, rising more steeply with increasing frequency. Since a single eccentric cell is considered (Purple, 1964; Stevens, 1964) to exert an inhibitory influence upon itself, a transfer function including a self-inhibitory component is a candidate for description of this region.

Consider the steady-state equation

$$f = I - hf \tag{1}$$

or

$$f = \frac{I}{1 + h} \tag{2}$$

where f is the output of the test ommatidium, I is the equivalent driving excitation (i.e., the output which would be obtained without inhibition), and h is

the inhibitory coefficient of the ommatidium acting on itself. This is the self-inhibitory equivalent of the Hartline-Ratliff (1957) equation for lateral inhibition. If the operations defined by this equation are taken as linear, a dynamic version[1] may be written in the Laplace domain as

$$f(s) = I(s)G(s) - hQ(s)f(s)$$

where $f(s)$ and $I(s)$ are the Laplace transforms of the quantities f and I defined in (1), which are now considered to vary with time. The fact that the inhibitory process may be rate-dependent is described by $Q(s)$; $G(s)$ describes the net dynamic relation between effective input $I(s)$ and $f(s)$ in the absence of any inhibition, and therefore includes generator potential dynamics. In order that this equation reduce to equation (1) in the steady state, the transfer functions $Q(s)$ and $G(s)$ have DC gains of unity. The time-dependent version of equation (2) becomes

$$f(s) = I(s)G(s) \cdot \frac{1}{1 + hQ(s)}. \tag{3}$$

This casts the generator potential and inhibitory dynamics in the form of linear equivalent series (multiplicative) transfer functions (the two factors on the right side of [3]). Multiplication of transfer functions is equivalent to addition of their log magnitudes (and of their phase angles). Therefore, the response of the ommatidium without inhibition would be described by $G(s)$, and the difference, in decibels, between that and the response with inhibition is described by $1/(1 + hQ(s))$.

In our experiments it was not possible to eliminate the influence of self-inhibition upon the spike discharge. However, the frequency response of the generator potential was found by Pinter (1966) to fall approximately on a straight line with slope about the same as ours at low frequency; at higher frequencies the two sets of data diverge (Fig. 7). If we assume that this divergence represents the differential response due to self-inhibition (lateral inhibition was excluded in both cases by confining the light to one ommatidium), the inhibitory dynamics can be estimated. For example (replacing s by $j\omega$ to determine frequency response), if

$$Q(j\omega) = \frac{1}{1 + j\omega\tau},$$

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[1] Several of the transfer functions in this and the following section, although derived independently, are formally similar to expressions published by Knight et al. (1970) in their related analysis. In order to acknowledge the development of the ideas underlying such expressions, we should note that Hassenstein and Reichardt (1956) studied lateral interaction of receptors via low-pass filters 15 ago in connection with movement perception. Explicit replacement of the inhibitory constants of the Hartline-Ratliff equation with low-pass filter dynamics is found in analyses as early as those of Stevens (1964), Lange (1965), Lange et al. (1966 *b*), Thorson (1965, 1966), and Purple and Dodge (1966). These analyses were, in turn, much influenced by private discussions among these authors as well as with Dr. Knight.

a first-order low-pass filter, then the predicted differential response is

$$R(j\omega) = \frac{1}{1+h} \cdot \frac{1 + j\omega\tau}{1 + j\omega \dfrac{\tau}{1+h}} \qquad (4)$$

which can be plotted directly from ordinary engineering nomograms (see, e.g., D'Azzo and Houpis, 1966).
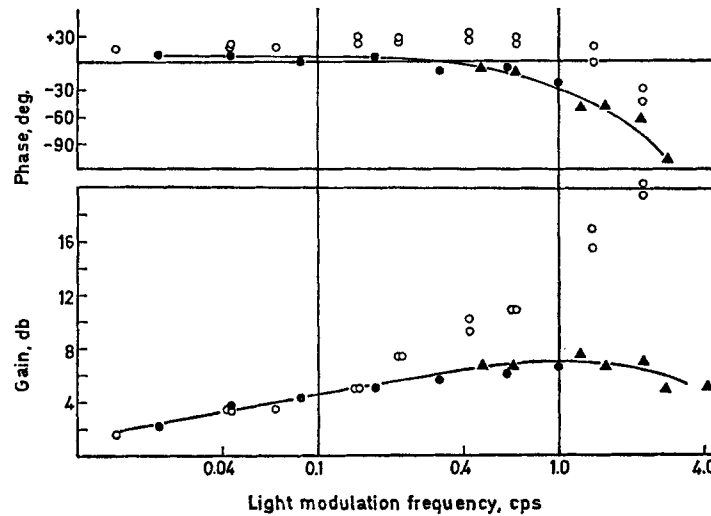

Light modulation frequency, cps

FIGURE 7. Bode plot comparing the spike discharge response of an ommatidium with a typical generator potential response curve obtained by Pinter (1966). The open circles show the phase and gain of the spike response in our experiment, with illumination by a single small spot containing 1.6×10^{-6} lumens ($= 200$ lumens/m², 20% sinusoidal modulation); generator potential response is shown by the solid circles (9500 lumens/m², 30% sinusoidal modulation) and triangles (136 lumens/m², 40% modulation). The position on the ordinate of the generator potential gain curve has been chosen so as to coincide with that for the spikes, for ease of comparison. The generator potential data are taken from Pinter's Figs. 2, 3, and 9.

Response curves $R(j\omega)$ were computed with $Q(j\omega)$ taken tentatively as both first- and second-order low-pass filters, with finite delay ranging from zero to 150 msec. The value of h can be determined by comparing the gain at high and low frequency, on the assumption that the gain would not continue to increase beyond the highest frequencies we were able to measure. This assumption is partially justified by (a) the fact that in three units the slope of gain vs. frequency decreased sharply at the highest frequencies, and (b) comparison with the results of Ratliff et al. (1967), where the frequency response to a small spot of sinusoidally modulated light reached a peak at frequencies near 2 or 3 cps. All predictions based on a second-order filter, or using delay, rose too

rapidly with frequency as compared with the data. The best correspondence with the lumped gain data (see Fig. 8) was obtained with a transfer function having $h = 4.0$ and $Q(j\omega)$ a first-order low-pass filter with time constant $= 0.42$ sec. The phase response of this transfer function also corresponds approximately to that of the data, except at the highest frequencies, where the data are least reliable. The transfer functions which best fitted the differential responses of individual units had h in the range 3.0 to 4.5 and time constant in the range 0.31 to 0.59 sec.
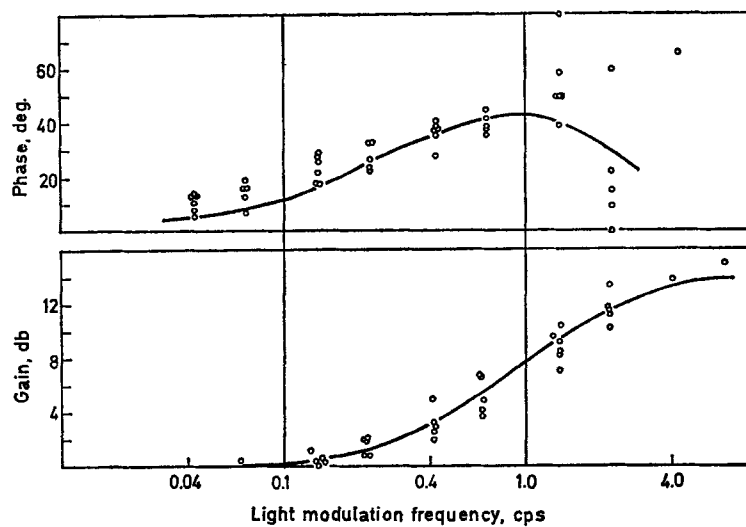

Figure 8. Differential response curve for self-inhibition. This plot contains data from six singly illuminated ommatidia, the low-frequency response of which had the same slope as do the generator potential data of Pinter (1966) shown in Fig. 6. (Five other units gave response curves of similar shape, but the low-frequency slope was somewhat steeper or shallower, so that comparison with the generator potential curve was not as straightforward; these units are not included here.) The difference, in decibels or phase angle, between each data point (for each of the six units) and the value of the generator potential curve at the corresponding frequency was measured and plotted vs. frequency of light modulation. The smooth curves drawn through the data points show the predicted response for a unit with self-inhibition having the characteristic of a first-order low-pass filter with time constant 420 msec and $h = 4.0$.

Knight et al. (1970) have approached the question somewhat differently, using short light stimuli to a dark-adapted eye isolated in an experimental chamber, and comparing the response of generator potential to light, spike frequency to modulation of generator potential by imposed current, and spike frequency to light. They predicted the third response on the basis of the first two sets of measurements, under various assumptions about self-inhibitory dynamics. Their findings that the assumption of linearity is justified, that the

self-inhibitory coefficient in their data averaged 3.4 and the time constant 0.57 sec (14 experiments on 12 units) are in remarkable agreement with ours considering the differences in method.

The notion that lateral and self-inhibition might have the dynamic characteristics of a first-order low-pass filter was suggested by Lange (1965) and shown to be qualitatively compatible with the responses of ommatidia to step changes in the frequency of antidromic stimulation of the optic nerve. He assumed that each axonal impulse produced a quantal increase in an inhibitory "pool" which then decayed exponentially. The envelope of response to a step change in rate of a sequence of such quanta is an exponential with time constant equal to that of the decay of the quanta. Thus the finding of a low-pass filter characteristic with a certain time constant might reflect synaptic transmission by i.p.s.p.'s with a time constant for voltage decay in that range. In fact, Purple and Dodge (1966) observed transient changes in membrane conductance of *Limulus* ommatidia, and produced evidence that these were associated with self-inhibitory synaptic processes. The time constant of decay of the slow changes in their figure was about 300 msec, while a time constant of 500 msec produced a model output similar to their data. Our estimate of time constant = 0.42 sec is within this range, and that of Purple and Dodge (0.85 sec) is not far from it.

C. Dynamics of Lateral Inhibition

By a derivation similar to that discussed in the previous section, the Hartline-Ratliff equations including lateral inhibition may be written in dynamic form. In this case the steady-state equation

$$f_i = I_i - h_i f_i - \sum_{\substack{j=1 \\ j \neq i}}^{n} h_j f_j$$

becomes

$$f_i(s) = I_i(s)G_i(s) - h_i Q_i(s)f_i(s) - \sum_{\substack{j=1 \\ j \neq i}}^{n} h_j Q_j(s)f_j(s)$$

where h_j is the actual inhibitory coefficient of the jth ommatidium with respect to the ith one, and $Q_j(s)$ describes the rate dependence of the lateral inhibition. The coefficient h_j in this equation, of course, does not have the value of the apparent inhibitory coefficient as usually defined experimentally, but is larger by the factor $(1 + h_i)$; that is, the actual lateral inhibition, expressed as a proportion of the inhibiting ommatidium's rate, necessary to produce a given reduction in spike output of a test ommatidium is greater, the larger the amount of self-inhibition assumed to be present. This equation, like that for self-inhibition alone, may be rearranged so as to express the transfer functions as series (multiplicative) elements, under the rather restrictive assumption

that the system of ommatidia is homogeneous with respect to input, inhibitory coefficients, and dynamic behavior of the inhibitory interactions. The predicted differential response for lateral inhibition (the difference between the Bode plots for a singly illuminated, self-inhibited ommatidium and for that ommatidium illuminated together with its neighbors by light with the same modulation) is then

$$R_{\text{lat}}(j\omega) = \frac{1 + hQ_{\text{self}}(j\omega)}{1 + hQ_{\text{self}}(j\omega) + HQ_{\text{lat}}(j\omega)}$$ (5)

where

$$H = (n - 1)h_j.$$

However, the assumption of homogeneity is certainly not met in practice. Barlow (1969) has shown that the inhibitory influence of an illuminated group of 4 ommatidia was greatest upon ommatidia about 5 ommatidial diameters removed from the source of inhibition; nearer and more distant ommatidia were inhibited less, and at a distance of approximately 13 ommatidial diameters inhibition was not detected. Although in our experiments we reduce these effects by inhibiting with 2 small patches of ommatidia separated from the test ommatidium, average intensity of light at the 15 ommatidia varies somewhat with the direction of their optical axes and the amount of the surface actually illuminated. Equation (5) therefore represents only a first estimate of the average behavior of the inhibitory elements. The average value of the lateral inhibitory coefficient is given by the constant difference between the inhibited and noninhibited curves at low frequencies. For different units in our experiments it ranged from 0.012 to 0.06 (average 0.036), for individual ommatidia two to four ommatidial diameters away from the test ommatidium. This is to be compared with the most recent estimates of 0.02 for nearest neighbors (Kirschfeld and Reichardt, 1964) and 0.06 ± 0.02 (Barlow, 1969) for the maximum effect, which Barlow showed to be exerted by ommatidia four to five ommatidial diameters away from the test one. Since the center of our inhibiting group was only three diameters away, the agreement between our results and his is excellent.

We have compared the predicted differential response (with vs. without lateral inhibition) $R_{\text{lat}}(j\omega)$, as in equation 5, with our corresponding differential response data. The values for h and $Q_{\text{self}}(j\omega)$ for each unit are computed as described in the preceding section, and H is computed by multiplying the apparent lateral inhibitory coefficient by the factor $(1 + h)$. When $Q_{\text{lat}}(j\omega)$ was taken as a first-order low-pass filter, no approximate fit to the data could be found: any curve with time constant chosen to put it in the right frequency range rose more gradually with frequency than did the data. Introduction of a finite delay in $Q_{\text{lat}}(j\omega)$ (i.e., multiplying $Q_{\text{lat}}(j\omega)$ by $e^{-j\omega\tau_d}$) provides a better approximation, as shown for two units in Fig. 9.

Ratliff et al. (1967) suggested that the 150 msec delay in response to steps of light observed by Hartline et al. (1961) might account for the rapid rise of the lateral inhibition differential frequency response. The delays we infer are shorter, in the region 50–90 msec for different units. This finding is at least in keeping with Lange's (1965) demonstration that some of the delays observed in step experiments can include time required to reach the threshold for inhibition; firing rates of ommatidia in our experiments were such that inhibition ought to have exceeded this threshold at all times. Ratliff, Knight, and
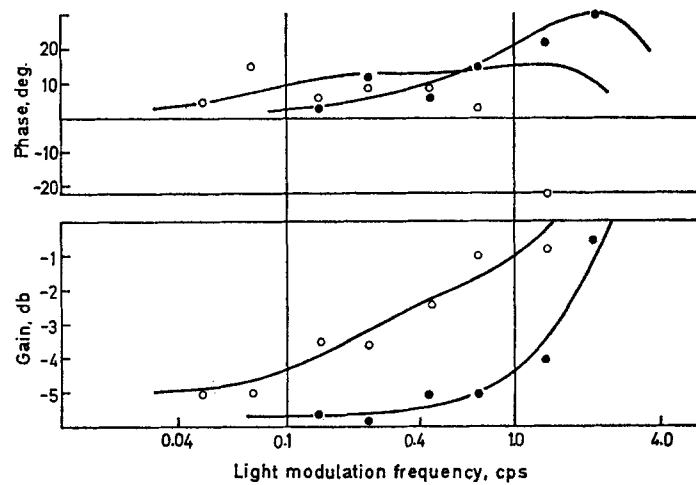

FIGURE 9.   Differential response curve for lateral inhibition. The data are taken from two test ommatidia for each of which the frequency response was measured both with and without lateral inhibition. In each case values for h and τ were selected to give a good match between equation 4 and the response without lateral inhibition. Thus in fitting the differential response data for lateral inhibition with equation 5, h, H, and the time constant for self-inhibition were fixed, and only the lateral inhibitory time constant and delay were varied. The constants which produced the "near-fit" curves shown here are (open circles) $h = 4.5$, $H = 4.3$, τ (self) $= 0.41$ sec, τ(lat) $= 1.00$ sec, delay $= 90$ msec; (solid circles) $h = 3.0$, $H = 3.7$, τ(self) $= 0.34$ sec, τ(lat) $= 0.35$ sec, delay $= 50$ msec.

Graham (1969) also found that delays of 100 msec, with a lateral inhibitory time constant of 0.3 sec, permitted approximation of the enhancement of frequency response via lateral inhibition reported by Ratliff et al. (1967).

Finally, it should be clear that our description of the dynamics of excitation and inhibition over the entire frequency response range in terms of linear transfer functions and finite delays, although facilitating comparison and heuristic to mechanism, does not rule out their realization via nonlinear or higher order transfer functions. For example, the apparent delays could in principle arise from an approximately 100 msec transient depolarization

which appears under some conditions to precede the inhibiting hyperpolari-
zation, as suggested by Toyoda, Knight, and Dodge (1969) and shown by
Knight et al. (1970) to be compatible with the frequency response of the
system.

SUMMARY

1. Measurements of the spike discharge in eccentric cell axons from om-
matidia of the *Limulus* lateral eye, light-adapted and *in situ*, have been
made with small sinusoidal variations of light input. Analysis permits
estimates of the steady-state inhibitory coefficients, the nature of the
low-frequency adaptation, and the dynamics associated with both
lateral inhibition and presumed self-inhibition.

2. A novel and particularly convenient measure of the steady-state lateral
inhibition coefficients is derived in terms of the depression of the very
low-frequency portion of the frequency response of an ommatidium
when particular neighbors are illuminated with identically modulated
light. Coefficients average 0.04 at three interommatidial spacings, in
remarkable agreement with the recent fiber-optic measurements of R.
Barlow—despite our study of the eye *in situ* and our use of the low dis-
charge rates associated with light adaptation to physiological levels of
illumination.

3. Small signal adaptation of a single ommatidium at low frequencies is
characterized closely from 0.004 to about 0.4 cps by the linear transfer
function s^k with k about 0.25. Phase leads (near $k\pi/2$) and step responses
(varying as t^{-k}) are consistent with this dynamic description. We in-
terpret these dynamics, now found in a variety of receptors under small
perturbations of input, as suggestive of a summation, via the generator
potential, of spatially distributed relaxation processes, the individual
rate constants for which depend upon the local stimulus intensity along
the eccentric cell dendrite and the rhabdome.

4. Departure of the frequency response of a singly illuminated ommatidium
from the estimated generator potential response at high frequency, if
interpreted as the failure-to-follow of self-inhibition via a low-pass
filter, implies that self-inhibition has a steady-state gain of about 4.0
and a time constant of about 0.42 sec. This estimate for the *in situ*
preparation differs from that made in earlier studies (Purple and
Dodge, 1966) but is compatible with the recent results of Knight et al.
(1970) for excised eyes.

5. Given the self-inhibitory dynamics, we derive a method for estimating
the lateral inhibitory dynamics from the frequency response with and
without illumination of specific neighbors. The lateral inhibitory coeffi-
cient averaged 0.036 for an average spatial separation of three omma-

tidial diameters. In contrast to the situation for self-inhibition, low-pass filter characteristics alone were not sufficient for the lateral effects. Inclusion of finite delays of 50–90 msec, with filter time constants from 0.35 to 1 sec, accounts approximately for the light-adapted *in situ* data.

REFERNCES

BARLOW, R. B. 1969. Inhibitory fields in the *Limulus* lateral eye. *J. Gen. Physiol.* **54**:383.

BARTH, F. G. 1967. Ein einzelnes Spaltsinnesorgan auf dem Spinnentarsus: seine Erregung in Abhängigkeit von den Parametern des Luftschallreizes. *Z. vergl. Physiol.* **55**:407.

BROWN, M. C., and R. B. STEIN. 1966. Quantitative studies on the slowly adapting stretch receptor of the crayfish. *Kybernetik.* **3**:175.

CHAPMAN, K. M. 1963. Transfer functions in sensory receptor analysis. *Bol. Inst. Estud. Med. Biol. Univ. Nac. Mex.* **21**:141.

COLE, K. S., and H. J. CURTIS. 1936. Electric impedance of nerve and muscle. *Cold Spring Harbor Symp. Quant. Biol.* **4**:73.

D'AZZO, J. J., and C. H. HOUPIS. 1966. Feedback Control System Analysis and Synthesis. McGraw-Hill Book Co., New York.

FUORTES, M. G. F., and A. L. HODGKIN. 1964. Changes in time scale and sensitivity in the ommatidia of *Limulus*. *J. Physiol. (London).* **172**:239.

HARTLINE, H. K., and F. RATLIFF. 1957. Inhibitory interaction of receptor units in the eye of *Limulus*. *J. Gen. Physiol.* **40**:357.

HARTLINE, H. K., F. RATLIFF, and W. H. MILLER. 1961. Inhibitory interaction in the retina and its significance in vision. *In* Nervous Inhibition. E. Florey, editor. Pergamon Press, New York. 241.

HASSENSTEIN, B., and W. REICHARDT. 1956. Systemtheoretische Analyse der Zeit-, Reihenfolgen- und Vorzeichenauswertung bei der Bewegungsperzeption des Rüsselkäfers *Chlorophanus*. *Z. Naturforsch.* **11b**:513.

HUGHES, G. W., and L. MAFFEI. 1966. Retinal ganglion cell response to sinusoidal light stimulation. *J. Neurophysiol.* **29**:333.

KIRSCHFELD, K., and W. REICHARDT. 1964. Die Verarbeitung stationärer optischer Nachrichten im Komplexauge von *Limulus*. *Kybernetik.* **2**:43.

KNIGHT, B. W., J.-I. TOYODA, and F. A. DODGE, JR. 1970. A quantitative description of the dynamics of excitation and inhibition in the eye of *Limulus*. *J. Gen. Physiol.* **56**:421.

LANGE, G. D. 1965. Dynamics of Inhibitory Interactions in the Eye of *Limulus*: Experimental and Theoretical Studies. Ph.D. Thesis. The Rockefeller University, New York.

LANGE, D., H. K. HARTLINE, and F. RATLIFF. 1966 *a*. Inhibitory interaction in the retina: techniques of experimental and theoretical analysis. *Ann. N. Y. Acad. Sci.* **128**:955.

LANGE, D., H. K. HARTLINE, and F. RATLIFF. 1966 *b*. The dynamics of lateral inhibition in the compound eye of *Limulus*. II. *Funct. Organ. Compound Eye. Proc. Int. Symp.* **7**:425.

Pinter, R. B. 1966. Sinusoidal and delta function responses of visual cells of the *Limulus* eye. *J. Gen. Physiol.* **49**:565.

Pringle, J. W. S., and V. J. Wilson. 1952. The response of a sense organ to a harmonic stimulus. *J. Exp. Biol.* **29**:220.

Purple, R. L. 1964. The Integration of Excitatory and Inhibitory Influences in the Eccentric Cell in the Eye of *Limulus*. Ph.D. Thesis. The Rockefeller University, New York.

Purple, R. L., and F. Dodge. 1966. Self-inhibition in the eye of *Limulus*. *In* The Functional Organization of the Compound Eye. Stockholm Symposium. Pergamon Press, Oxford. 451.

Ratliff, F., H. K. Hartline, and D. Lange. 1966. The dynamics of lateral inhibition in the compound eye of *Limulus*. I. *Funct. Organ. Compound Eye. Proc. Int. Symp.* **7**:399.

Ratliff, F., H. K. Hartline, and D. Lange. 1968. Variability of interspike intervals in optic nerve fibers of *Limulus*; effects of light and dark adaptation. *Proc. Nat. Acad. Sci. U. S. A.* **60**:464.

Ratliff, F., B. W. Knight, and N. Graham. 1969. On tuning and amplification by lateral inhibition. *Proc. Nat. Acad. Sci. U. S. A.* **62**:733.

Ratliff, F., B. W. Knight, J.-I. Toyoda, and H. K. Hartline. 1967. Enhancement of flicker by lateral inhibition. *Science* (*Washington*). **158**:392.

Stevens, C. F. 1964. A Quantitative Theory of Neural Interactions: Theoretical and Experimental Investigations. Ph.D. Thesis. The Rockefeller University, New York.

Thorson, J. 1965. Small-Signal Analysis of a Visual Reflex in the Desert Locust. Ph.D. Thesis. University of California, Los Angeles.

Thorson, J. 1966. Small-signal analysis of a visual reflex in the locust. II. Frequency dependence. *Kybernetik.* **3**:53.

Thorson, J., and D. C. S. White. 1969. Distributed representations for actin-myosin interaction in the oscillatory contraction of muscle. *Biophys. J.* **9**:360.

Toyoda, J., B. W. Knight, and F. A. Dodge. 1969. Time course of inhibitory synaptic potentials in the eccentric cell of the *Limulus* photoreceptor. Abstracts of the Biophysical Society 9th Annual Meeting. Los Angeles, California. 244.

Waterman, T. 1954. Polarized light and angle of stimulus incidence in the compound eye of *Limulus. Proc. Nat. Acad. Sci. U. S. A.* **40**:258.