Electrophysiological Basis for the Spatial Dependence of the Inhibitory Coupling in the Limulus Retina

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ABSTRACT A technique for measuring, with total optical isolation, the inhibition between two individual receptor units in the Limulus lateral eye is described. The extracellular responses of pairs of units were recorded, using light piping microelectrodes. The inhibitory coupling between two units was found to be nonlinear and describable by a simple hyperbolic equation written in terms of saturation rate (S), half saturation (H), and threshold (fT). By plotting reciprocal frequencies, the data could be linearized and compared for different pairs of units. The magnitude of inhibition (in terms of S and H) was found to decrease monotonically as the anatomical distance between receptors increased. An electrical model of the inhibitory system was developed which accounts for many of the properties of the observed inhibitory interactions. Using the equations from the model and the experimental data, it is shown that the "electrical distances" (which are computed in terms of space constants $\lambda$) of the inhibitory synapses from the impulse-generating region of the test unit are directly related to the anatomical distance between receptors. It is also shown that "synaptic strength" is relatively constant with separation. The electrical distances of the inhibitory synapses range from about 0.1$\lambda$ to 0.25$\lambda$ for adjacent units to greater than 0.5$\lambda$ for units seven to nine receptors away. It is concluded that the nonlinear character of the inhibitory coupling is attributable to synaptic effects, and that the decrease of inhibition with distance between receptors is caused primarily by an increase in the electrical distance of the inhibitory synapses from the test unit.

INTRODUCTION

The mutual inhibition between receptor units (ommatidia) in the lateral eye of the horseshoe crab, Limulus polyphemus, has been studied for many years (for reviews see Wolbarsht and Yeandle, 1967; Hartline and Ratliff, 1972). It has been found that the activity of each receptor unit influences (and is influenced by) the activity of nearby receptors and that these mutual interactions are mainly inhibitory. The function relating the magnitude of inhibition to distance between receptor units has been given various descriptions in the literature. Ratliff and Hartline (1959) reported a uniform decrease in inhibition with increasing distance between receptors, Kirschfeld and Reichardt (1964) reported a Gaussian function for the decrease in inhibition, and Barlow (1969) found that the
maximum inhibition was not between adjacent receptors but was at a distance of three to five receptors from the test unit. One common problem encountered by investigators of this lateral inhibitory network is the difficulty in measuring directly the very small inhibitory interactions between two single receptor units. In most cases the summated inhibitory influences from a group of receptors have been measured instead (Barlow, 1969).

The original Hartline-Ratliff equations (Hartline and Ratliff, 1957) which describe the mutual interaction between receptors are piecewise linear equations. Tomita (1958) recognized, as did Lange et al. (1966) and Purple (1964), that there is a distinct saturation of the inhibitory effect. At high firing frequencies of the inhibiting unit the inhibition exerted on a test unit begins to level off to a maximum inhibitory effect. Barlow and Lange (1974) describe another nonlinearity associated with lateral inhibition. They found that the magnitude of inhibition is related to the level of excitation of the test unit. Maximum inhibition was found to occur with the test unit firing at about 26 impulses/s.

Previous work on the Limulus eye has usually been done using two somewhat different preparations. One is to make a cross-sectional slice with a razor blade perpendicular to the cornea through the horizontal axis of the excised eye. A micropipette is used to impale eccentric cells within ommatidia that have been exposed by the slice (Purple and Dodge, 1965). A second method consists of recording from small bundles of nerve fibers or single fibers which have been separated from the optic nerve. Stimulation is accomplished by a focused beam of light or a fiber optic which illuminates, through the cornea, one or more receptor units (Barlow, 1969).

Here we report a method which utilizes light piping microelectrodes to optically stimulate as well as to record the extracellular impulse activity of single receptor units after the cornea and lens system have been removed. Inhibition between specific pairs of receptors was measured, and it was found that the inhibitory coupling between two units is nonlinear and can be fit closely by a simple hyperbolic curve. The saturation of the inhibitory effect, however, was found to occur at lower firing frequencies of the inhibiting unit than previously reported (Lange et al., 1966; Purple, 1964). A possible explanation for this discrepancy is discussed.

In measuring inhibition between pairs of receptors at different interommatidial distances, we found that inhibition decreased monotonically with distance. We could not detect any peaking of the inhibitory field as reported by Barlow (1969). An electrical model is presented which suggests that the magnitude of the inhibitory coupling between units is determined by the "electrical distance" of the inhibitory synapse from the encoder region of the test unit, and that this electrical distance increases monotonically with the spatial separation of receptors.

1 The equations are piecewise linear in that they describe three distinct regions for the inhibitory coupling: a flat subthreshold region, a linear segment between threshold and maximum inhibition, and a flat saturation region.
METHODS

Preparation

All experiments were performed on horseshoe crabs (Limulus polyphemus) supplied by Gulf Specimen Co., Panacea, Fla. or collected at the Duke University Marine Lab in Beaufort, N.C. The animals were of medium size (15-20-cm carapace), and were kept in artificial seawater (Instant Ocean) at room temperature (20-22°C). The animals were selected for clear lateral eyes and reasonably thick shells. They were not fed, but were used within 1 or 2 mo.

The lateral eyes were excised from the animal and fixed to a paraffin dish. With a small scalpel, a shallow cut was made along the perimeter of the cornea, and with fine forceps the entire cornea and lens system was peeled away in a manner similar to opening a can of sardines (Fig. 1). This technique was devised by M. L. Wolbarsht (see Hartline et al., 1956 and Wolbarsht and Yeandle, 1967) and has been successfully applied in previous studies (Cronin, 1973). The eye, with completely exposed retina, was then mounted in an aluminum chamber with a paraffin base and flooded with fresh artificial seawater. The chamber temperature was normally maintained at 20°C.2

Preparation of Light Piping Micropipettes

Micropipettes were pulled from glass capillary tubing 2-mm OD and 1-mm ID. Several days before an experiment the pipettes were painted with at least two coats of Testor silver enamel (Testor Co., Rockford, Ill.). The day of the experiment the tip of the electrode was cut with a pair of dissecting scissors and filled with seawater via a syringe. The tips of the electrodes were 20–40 μm in diameter with a resistance of 1–2 MΩ. 20 mil. (0.5 mm) diameter Crofon light fibers from Edmund Scientific Co., Barrington, N.J. were inserted down the barrel of the electrodes. The fibers were jacketed with opaque heat shrink tubing their entire length, except for the final 2 inches which were in the electrode. The other end of the fiber was illuminated by a 15-W, 6-V tungsten filament lamp. An Ag-AgCl wire was inserted into the electrode next to the light fiber (see Fig. 2).

Extracellular Recording of Impulses

The tip of our light piping micropipette was inserted into the area vacated by the crystalline cone, just distal to an ommatidium (Figs. 1 and 2). Impulses recorded with these pipettes were definitely extracellular as determined by standard criteria (Cronin, 1973).

The amplitude of the recorded impulses was inversely proportional to the frequency of firing. The frequency could be increased to the point where the impulse height decreased into the noise level. The appearance of extracellular impulses is due to their electrotonic spread along the dendrite of the eccentric cell. As light intensity increases, the resistance of the rhabdom decreases and shorts out the eccentric cell dendrite (Borsellino et al., 1965; Wolbarsht and Yeandle, 1967). This shunting along the length of the dendrite would cause less extracellular current to be seen at the tip of the dendrite and hence by our extracellular electrode.

To verify that removing the cornea and lens system was nondamaging to the retina, control experiments were performed in which excitation and inhibition of a single test unit were measured before and after removal. A small bundle of nerve fibers was teased

2 On several occasions the eye was cooled quickly to about 10°C with an almost complete elimination of lateral inhibition (Johnston, 1973). Adolph (1973) in a more thorough investigation of temperature sensitivity found similar results.
from the optic nerve and recorded from using a suction electrode. The cornea was probed with a small optical fiber until impulses from a single receptor unit were isolated. Inhibition of this receptor was obtained by stimulating a nearby group of receptors (six) with a larger optical fiber while monitoring one receptor from the group. The test unit was stimulated until it fired at approximately 5–6 impulses/s, and then the inhibition from the group of receptors was measured. The cornea with lens layer was then carefully removed, as described above, and a light piping micropipette was used to record the extracellular impulse activity of the same or of a nearby receptor unit. Again it was inhibited by stimulating a nearby group of receptors with a large light fiber and monitoring one member of the group. This type of experiment was performed repeatedly, and we never detected any difference in the inhibitory properties of the retina after removing the cornea. Inhibition was obtained with or without the cornea intact; the only detectable difference was that the retina seemed to remain stable and responsive for longer periods of time (up to 24 h) after removing the cornea (Johnston, 1973). These findings are in complete agreement with previous studies using this technique (Cronin, 1973).

Data Collection

After dissecting the eye from the animal and removing the cornea, it was left in the dark for 30–60 min before any attempt was made at extracellular recordings. Impulse amplitude was 20–50 μV before and 50–500 μV after dark adaptation. As stated above, we were recording the electrotonic spread of the impulses up the dendrite of the eccentric cell. One theory for the increase in impulse amplitude with dark adaptation is that suggested by Wolbarsht and Yeandle (1967). They state that the resistance of the rhabdom increases with dark adaptation and causes less shunting of the dendrite.

Barlow (1967) and Barlow and Lange (1974) showed that maximum inhibition was achieved when the test unit was firing at about 26 impulses/s. At this high frequency, which required strong illumination, we found small adaptation effects which caused slight changes in the firing rate of the test unit over long periods of time. We instead chose a firing rate of 5–6 impulses/s for the test unit. Although at these low firing rates there was some instantaneous variability in frequency, the change in the total number of impulses between counting intervals was never more than one or two over a 9-s interval. To measure the inhibitory coupling we first illuminated the test unit until it fired at a stable rate of 5–6 impulses/s. Since inhibition is a function of the level of excitation of the test unit (Barlow and Lange, 1974), it is very important, regardless of the frequency of firing we chose for the test unit, that it be kept constant for all trials in any given experiment (Johnston and Wachtel, 1973, 1974).

Because of the anatomy of the eye with its densely packed pigmented tissue above and surrounding each ommatidium (see Figs. 1 and 2) and because the tip of our electrode
was deep within this area, optic isolation should be very good (Johnston, 1973). In order to verify this, we inspected each electrode before using it to be certain light emerged only from its tip. Next, with a pair of electrodes, we recorded from adjacent ommatidia and stimulated each with a light intensity that produced maximum firing (see Fig. 3). We then

![Diagram of experimental setup](image)

**Figure 2.** A simplified diagram of the experimental setup with one electrode. The electrode is inserted in the area vacated by the crystalline cone (the black hole of Fig. 1). Surrounding the electrode and each ommatidium is densely packed pigmented tissue which prevents light from scattering to neighboring receptor units. Light emerges from the tip of the electrode only. The arrangement for the second electrode is identical (from Johnston and Wachtel, 1973).

![Waveforms](image)

**Figure 3.** A test for light scatter. In this figure a pair of adjacent receptor units are being recorded from using two light piping microelectrodes. In A, unit 1 is illuminated strongly, and unit 2 is left in the dark. In B, unit 2 is illuminated and not 1. There is no firing of the nonstimulated unit, even though stimulation is many times that used during measurements of inhibition. Arrow and artifact on the bottom trace indicate onset of illumination. No amplitude calibration is given because impulses were recorded, through a bandpass filter, on a chart recorder with a limited frequency response.
stimulated the receptors, one at a time, with an intensity many times that which produced maximum firing of the stimulated unit. We never detected any firing of the nonstimulated unit, not even early firing before the time lag of lateral inhibition (Ratliff et al., 1963). Since the intensities with which we tested for light scatter were many times greater than that used in an actual experiment, and since we still detected no excitation of neighboring units during these tests, we can be certain that adequate optical isolation was achieved.

The test unit was illuminated for 15 s. Impulses were counted during a 9-s interval beginning 4.5 s after the onset of the illumination. Because we were interested only in steady-state inhibition, it was necessary to allow all transients to die out before measurements were taken (Barlow, 1967). After 15 s of illumination the test unit remained in the dark for 45 s. The total period of this cycle was therefore 60 s. In other words, for the first 15 s of the cycle the test unit was illuminated, and impulses counted for 9 s; for the remainder of the cycle the unit was left in the dark. The test unit was put through several of these cycles until a very stable firing pattern was obtained. As mentioned above, the total number of impulses during the 9-s counting interval did not vary by more than 1 or 2 impulses from one cycle to the next.

The inhibiting unit was illuminated for 15 s during every other 60-s cycle, with impulses counted during the same 9-s interval as the test unit. The uninhibited firing rate of the test unit was determined by taking the total number of impulses during the 9-s counting interval for the cycles immediately before and after the inhibiting cycle, and calculating the average between these two values. The decrease in firing of the test unit with inhibition ($\Delta f_1$) was the difference between this uninhibited firing rate average and the firing rate with inhibition.

The inhibitory threshold ($f_T$) was defined as the highest frequency of the inhibiting unit at which no inhibition could be measured. When many measurements are taken at inhibiting firing rates near this threshold, it has been reported that the curve becomes nonlinear (Hartline et al., 1961; Johnston, 1973). We did not take data in the frequency range below the above defined threshold and therefore did not include this nonlinearity into our measurements for inhibition. After determining the threshold as defined above, the light on the inhibitory unit was increased slightly and left at this value for at least two runs to be sure a reproducible inhibition was obtained. The light was then increased again. This process was continued until the inhibiting unit was firing at its maximum rate, or until the impulses were too small to count above the noise (usually 40–50 impulses/s). The most consistent results were obtained by increasing the light stepwise on the inhibitory unit. Randomized measurements, however, yielded similar results.

For the purposes of determining interommatidial distances, we defined one distance unit ($D$) as the distance from the center of one receptor unit to the center of its nearest neighbors (see Fig. 1). In most cases we recorded from units along the nearest adjacent pathway. Occasionally, however, it was necessary to record from units which were at opposite ends of the "diamond pattern." To indicate that this distance was greater than between adjacent units, we arbitrarily assigned it a distance of $D = 1.5$ away and, likewise, for $D = 2.5$ away, etc.

**RESULTS**

Experiments were done by taking as many measurements as possible over the full frequency range of the inhibiting unit ($f_2$), paying particular attention to the lower frequencies. Fig. 4 A shows the results of one such experiment. It can be seen that the relationship between $\Delta f_1$ and $f_2$ is clearly nonlinear. The curves seem to saturate, and very little additional inhibition is obtained by increasing $f_2$.
FIGURE 4. (A) Curves for the inhibitory coupling, \( \Delta f_1 \) vs. \( f_2 \) are plotted for one test unit and two inhibiting units at \( D = 2 \) and 6. Data points were obtained over the full range of frequencies of the inhibiting units. The three parameters \( f_T, S, \) and \( H \), which define the hyperbolic nature of the curve, are indicated for the unit at \( D = 2 \). (B) Plot of reciprocal frequencies from A. From this figure saturation, \( S \), and half saturation, \( H \), can be calculated. (Intercept = \( 1/S \) and slope = \( (H - f_P)/S \). The intercept is indicated for the unit at \( D = 2 \). (One data point from each curve in A is off-scale in B.)

Beyond a certain point, forty single receptor-receptor interactions were investigated over the full range of \( f_2 \), and all yielded results similar to those presented in Fig. 4 A; i.e., unit to unit inhibition is nonlinear.

In Fig. 5 A a representative plot of the inhibition between one test unit and three inhibiting units at different distances from this test unit is illustrated. In order to compare the inhibitory coupling between different pairs of receptors at different distances from the test unit, we must be able to characterize the curves on a firm quantitative basis. Visual examination of Figs. 4 A and 5 A suggests that the curves might be hyperbolic. If so, they should be fit by the general hyperbolic equation,
Figure 5. Inhibition vs. distance. (A) Three curves are shown for the inhibition between a single test unit and inhibiting units at \( D = 1.5, 2.5, \) and 6. The maximum or saturation point of the inhibition curves decreases as the distance between receptors increases. (B) The curves from A are linearized by plotting reciprocal frequencies. The slopes are very nearly identical while the intercepts increase with spatial separation of the receptors. The intercepts are \( b = 1/S \) and the slopes are \( m = (H - f_T)/S. \) (Explain in text.) The scale of each axis is reciprocal frequency or seconds per impulse.

\[
y = \frac{a_1x}{x + a_2},
\]

\( a_1 \) and \( a_2 \) are constants. Using our variables and shifting the x axis, this equation becomes

\[
\Delta f_1 = \frac{a_1(f_2 - f_T)}{(f_2 - f_T) + a_2}.
\]

The limit of \( \Delta f_1 \) as \( f_2 - f_T \to \infty \) is the saturation value of \( \Delta f_1 \) which we designate as \( S. \) Taking the limit,
\[ \lim_{f_2 \to f_T} \Delta f = \frac{a_2(f_2 - f_T)}{(f_2 - f_T) + a_2} = a_1 = S. \quad (3) \]

We can then designate the point at which \( \Delta f \) reaches half saturation as \( S/2 \). The frequency, \( f_2 \), at half saturation we will define as \( H \). Referring to Fig. 4 A, at

\[ \Delta f = \frac{S}{2} = \frac{S(H - f_T)}{(H - f_T) + a_2}, \quad (4) \]

or

\[ a_2 = (H - f_T). \quad (5) \]

So our hyperbolic equation can now be written as,

\[ \Delta f = \frac{S(f_2 - f_T)}{(f_2 - f_T) + (H - f_T)}. \quad (6) \]

Taking reciprocals gives,

\[ \frac{1}{\Delta f} = \frac{1}{S} + \frac{(H - f_T)}{S} \frac{1}{(f_2 - f_T)}. \quad (7) \]

This is an equation for a straight line with \( 1/\Delta f \) and \( 1/(f_2 - f_T) \) the dependent and independent variables, respectively. If our assumption that the curves are hyperbolas was correct, then plotting \( 1/\Delta f \) and \( 1/(f_2 - f_T) \) should yield straight lines with slopes and intercepts equal to \( (H - f_T)/S \) and \( 1/S \), respectively. The intercept is solely a function of saturation, \( S \), while the slope is a function of both saturation and the half-saturation point, \( H \). Figs. 4 B and 5 B show the results of plotting reciprocal frequencies. The linearization is very good and this indicates that the inhibitory coupling curves can be approximated by a hyperbolic equation. The inhibitory threshold, \( f_T \), fixes the crossing point on the \( f_2 \) axis. The saturation, \( S \), and half saturation, \( H \), determine the overall shape of the hyperbola. These three parameters, \( f_T \), \( S \), and \( H \), therefore, are necessary and sufficient to completely describe the inhibitory coupling. Saturation can be calculated from the hyperbolic curves by extrapolating to infinite frequency, or from the intercept of the linear plots (\( S = 1/\text{intercept} \)). The inhibitory threshold can be taken from the hyperbolic curves, while the half-saturation point is best calculated from the slope of the linear plots (\( H - f_T = \text{slope} \times S \)).

To determine the relationship between the strength of inhibitory coupling and the spatial separation of receptors, it was necessary to measure the inhibition between one receptor and several inhibiting receptors at different distances from this test receptor (see Fig. 5 A). Distance from the test receptor was measured in the separation units previously defined. For each test receptor a plot can be made of \( S \), \( H - f_T \), and \( f_T \) versus distance for several different inhibiting receptors. All parameters were found to change monotonically with distance between receptors. Saturation (\( S \)) and half saturation (\( H - f_T \)) decreased, while the inhibitory threshold (\( f_T \)) increased. From all our experiments \( f_T \) varied from about 7 impulses/s for immediately adjacent units (\( D = 1 \)) to 15–16...
impulses/s for receptors $D = 7-9$ apart. The inhibitory threshold always increased with increasing separation. Saturation varied from a $\Delta f_i$ of 1.3 impulses/s (for $D = 1$) to about 0.1 impulses/s (for $D = 9$). The highest saturation was for nearest neighbors, and it always decreased monotonically for more distant units. Fig. 6 shows the relationship between saturation and distance for all experiments. It should be mentioned that the inhibition between distant units ($D = 7-9$) was very small and within the variability of our measurements. We were never able to measure inhibition beyond $D = 9$. Half saturation ($H - f_r$) also decreased with distance between receptors. It varied from about 10 impulses/s for nearby units to 4 impulses/s for distant units.

![Figure 6. Saturation, S, vs. distance. Saturation is a measure of the magnitude of the inhibition between two receptor units. The mean (and SD) of S is plotted vs. distance between receptors ($D = 1-9$) for all experiments. The magnitude of inhibition was found to decrease monotonically with distance.](image)

**Discussion**

The results of these experiments are indicative of a nonlinear relationship between the decrease in firing frequency of one unit and the frequency of firing of its inhibiting unit. Our results clearly show a saturation effect for the inhibitory coupling. This saturation has been observed by others (Purple, 1964; Purple and Dodge, 1965; Lange et al., 1966; Tomita, 1958), but was not reported to appear until the inhibiting unit was firing at 30-40 impulses/s (Lange et al., 1966; Tomita, 1958). Our results indicate that saturation can occur earlier, at rates of 20-30 impulses/s. It has been suggested by many (Adolph, 1966; Purple and Dodge, 1965; Wolbarsht and Yeandle, 1967) that inhibition is mediated by chemical synapses and, if so, then this nonlinearity or saturation of the inhibitory effect should not be surprising. An inhibitory synapse cannot hyperpolarize the postsynaptic membrane beyond the equilibrium potential for inhibition, and this sets an upper limit, or saturation level, for the inhibitory effect. Only if one
were operating over a very small range would the postsynaptic voltage change appear linear.

Barlow and Lange (1974) reported a nonlinearity between the excitation of the test unit and the magnitude of inhibition. In their experiments the firing frequency of the test unit was varied while the frequency of the inhibiting unit was held constant. Since the firing frequency of the test unit was held constant throughout our experiments, the nonlinearity reported by Barlow and Lange should not have been involved. However, it is possible that the low level of excitation of the test unit (5–6 impulses/s) used in our experiments may produce saturation at lower inhibiting frequencies than those reported by others. For example, Barlow (1969), in measuring the inhibitory coupling, used uninhibited firing rates of the test unit as high as 26 impulses/s and reported no saturation of the inhibitory effect. In modeling studies of the inhibitory network (Johnston et al., 1975) we have found that the firing frequency of the inhibiting unit at which the inhibition begins to saturate is very dependent on the level of excitation.

The data presented by us for the inhibition between two ommatidia should also be discussed in terms of earlier work by Hartline and Ratliff (1957) in which the inhibitory coupling was described by a "piecewise" linear equation. In Hartline and Ratliff’s (1957) pioneering experiments stimulation was accomplished by focusing a spot of light on the cornea, while recording from a bundle of nerve fibers. When light must pass through the cornea and lens system, scatter to neighboring receptors is difficult to avoid. It is possible that Hartline et al. were not measuring true unit-to-unit inhibition, but a summed effect from several receptors. This would also explain why we found that the decrease in firing of the test unit (Δf1), was generally less than that found by Hartline and Ratliff, for the same range in frequencies of the inhibiting unit (f2). The Hartline-Ratliff equations (Hartline and Ratliff, 1957) for the inhibitory coupling might then be a description of the inhibition from several receptors, while Eq. 6 would describe the inhibition between individual receptors. Again, however, the discrepancy may be due to the difference in level of excitation of the test unit.

As mentioned previously, threshold was defined in our experiments as the highest firing frequency of the inhibiting unit at which little or no inhibition could be measured. This explains why threshold in our results is a sharply defined "break point." Hartline et al. (1961) and Johnston (1973) report that inhibition is not sharply defined, but that the inhibition decreases nonlinearly towards lower firing frequencies of the inhibiting unit. In light of the experiments reported here, this would produce a sigmoidal shape for the total curve describing the inhibitory coupling between two units. Threshold will be discussed again when the electrical model is presented.

In this study we have shown that the inhibitory coupling curves can be fit closely by a hyperbolic equation. The three parameters saturation, S, half saturation, H, and threshold, f0, are necessary and sufficient to describe the exact nature of the inhibition between two units. Half saturation, H, is an arbitrary point on the curve which was chosen in order to solve the hyperbolic equation, and threshold, f0, is necessary for plotting reciprocals and linearizing
the hyperbolic curves. Saturation, however, is the most useful parameter with which to define the magnitude of inhibition. Saturation is easily defined from all the curves, and can be used to compare the inhibition between different pairs of receptors. $H$ and $f_T$ are necessary only if linearization of the curves is desired.

These experiments did not reveal any peaking of the inhibitory coupling with distance between receptors. Inhibition always decreased (and threshold increased) monotonically with distance. This will be discussed more fully in the next section, and possible explanations for the apparent discrepancy with previous work (Barlow, 1969) will be presented in Appendix I.

**Electrical Model**

There has always been the vexing question of why inhibition varies with distance between receptor units. One suggestion has been that the so-called “strength” of the synapses (i.e. postsynaptic conductance change for a particular frequency of firing of the presynaptic fiber) decreases with distance (Barlow, 1969). One way of explaining this might be that the effective area of synaptic contact (which, in turn, might be attributable to the number of synapses) is different for each inhibiting receptor depending on its distance from the test unit. If the effective area of an inhibitory synapse from a distant unit was less than the synaptic area for a closer unit, then this would result in a smaller postsynaptic conductance change and a lesser inhibitory effect. Another explanation is that the synapses mediating inhibition from distant receptors are more remote electrically from the encoder region of the test unit than synapses from nearby receptors. This would also result in a smaller inhibitory effect. In other words, a postsynaptic potential from a synapse at a large “electrical distance” from the impulse-generating region of the test unit would be more attenuated reaching this point than a postsynaptic potential from a synapse which is closer “electrically.” If this were the case, then there might be a direct correlation between this “electrical separation” of the synapses and the spatial separation of ommatidia.

An electrical model of the eccentric cell which accounts for many of the electrophysiological properties of excitation and inhibition has been developed in great detail by Purple (1964) and Purple and Dodge (1965). Purple represented a length of uniform axon by a $\pi$ equivalent resistive network (see Fig. 7). Using cable equations, he has shown that the equivalent internal longitudinal resistance, $R_a$, can be calculated from the equation

$$R_a = R_\infty \sinh \frac{X}{\lambda},$$  \hspace{1cm} (8)

where $X$ is some fraction of the characteristic length, $\lambda$, of the axon. $R_\infty$ is the equivalent shunt resistance of a semiinfinite axon and can be obtained from the equation

$^3$ Electrical distance (or electrotonic length) will be used in this paper to mean the partial length, in terms of the space constant, $\lambda$, of an equivalent uniform axon (Purple, 1964). The electrical distance can be put in terms of actual length by knowing the space constant (e.g. if $\lambda = 1$ mm and the electrical distance of a synapse is 0.5 $\lambda$, then the distance of the synapse from the encoder is actually 0.5 mm).

$^4$ The equations for the elements of the $\pi$ network were developed by Bruce Knight and reported in Purple (1964) and Purple and Dodge (1965).
Treating the steady-state cable properties of the model axon, Purple (1964) showed that the portion, $X$, can be represented by the three-resistor $\pi$ network shown above. The resistor, $R_b$, at either end of the axon model represents the resistance across the membrane of the model (transverse resistance to ground) as "seen" from each end of the cable. The resistor, $R_a$, represents the equivalent internal longitudinal resistance of the uniform axon. The formulae expressing $R_a$ and $R_b$ are given below the diagrams. $R_m$ and $\lambda$ are explained in the text. (Modified from Purple, 1964.)

\[ R_a = R_m \sinh \left( \frac{X}{\lambda} \right) \]

\[ R_b = R_m \frac{1 + \cosh \left( \frac{X}{\lambda} \right)}{\sinh \left( \frac{X}{\lambda} \right)} \]

Figure 7. Equivalent circuit representation of a length of uniform passive axon. The excitatory process (a battery in series with a variable resistance) was shown by Purple to be electrically separated from the impulse-generating region (sum-
ming junction or encoder region). He represented this electrical distance by a
length of axon in the form of a \( \pi \) equivalent network. It is at this summing
junction that the competing processes, namely a depolarizing current from the
excitation of the cell, and a hyperpolarizing current from the various inhibitory
inputs, are summed. The resultant membrane potential determines the firing
frequency of the cell. Purple (1964) and MacNichol (1956) have shown that the
frequency of firing of the eccentric cell is directly proportional to the membrane
potential of the summing junction.

In studying inhibition in the Limulus eye, Purple primarily used antidromic
inhibition. Although this method uses a large population of inhibitory units,
most of the inhibition is attributable to units near the test unit. Purple in
formulating his model, lumped all the inhibitory inputs to the eccentric cell
together and assumed the total inhibition to act at one point along the axon.
Purple noted this limitation in his model, but for his purposes this approxima-
tion was perfectly justified. He also found that the equilibrium (reversal) poten-
tial for antidromic inhibition was always 1-3 mV more negative than for self-
inhibition. This discrepancy, he felt, indicated that the synapses for self-inhibi-
tion lay closer to the summing junction than the synapses for antidromically
produced inhibition. It seems reasonable that the synapses from other units
might also be electrically separated.

Fig. 8 shows our electrical model for inhibition between two receptors. For the
purposes of modeling the inhibitory system, we are not interested in the excita-
tion of the test unit as long as it is constant.\(^5\) We represented the total resistance
to ground at the summing junction, except for one synaptic input, as a single
resistor \( R_e \). This includes the resistance of the soma and dendrite, the resistance
of the length of axon separating the soma from the summing junction, and the
resistance of all collaterals as seen at this point. The membrane potential at the
summing junction, without inhibition, includes the depolarization associated
with the excitation of the eccentric cell. Since this is always constant in our
experiments, and since we are only interested in the incremental voltage change
at the summing junction due to a single synaptic input, we can omit the
excitation of the cell and reference all voltages to the resting potential. We are
interested in the electrical distance of a single synaptic input (i.e. inhibition from
one receptor unit) from the summing junction and can represent this distance by
a \( \pi \) equivalent network, as already discussed. \( V_i \) is the equilibrium potential for
inhibition and \( g_i \) is the conductance of the postsynaptic region. \( g_i \) varies with the
frequency of firing of the inhibiting synapse. Purple (1964) assumed that the
average conductance increase at the synapse is directly proportional to the
frequency of firing of the inhibiting unit, \( g_i = \beta f^2 \), with \( \beta \) a constant. A different
inhibiting frequency, therefore, would be represented by a different \( g_i \). Our
model represents only one inhibiting synapse, but it can readily be modified to
include any number of units (Johnston, 1973; Johnston et al., 1975). Although
Purple's (1964) assumption \( g_i = \beta f^2 \) implies a hyperbolic relationship between
\( ^5 \) Since the resistance of the soma and dendrite changes with the level of excitation, the excitation
must be kept constant to justify representing the soma and dendrite by a fixed resistor. See
Appendix II for further explanation.
FIGURE 8. Electrical model for one inhibitory input. The model represents the length of uniform axon separating one inhibiting 'synapse' from the encoder or summing junction of the test unit. \( V_{s} \) is the inhibitory equilibrium potential, \( g_{i} \) is the synaptic conductance (which is proportional to the frequency of firing of the inhibitory unit), \( V_{e} \) is the voltage at the summing junction (which is proportional to the frequency of firing of the test unit), \( R_{s} \) is the total shunt resistance as seen at the summing junction except for the one synaptic input, and \( R_{a} \) and \( R_{b} \) are elements of the \( \pi \) network from Fig. 7. It can be shown that \( R_{b} \gg R_{a} \) so that resistor \( R_{b} \), which should be in parallel with \( R_{a} \), was omitted. To increase the electrical distance of the inhibiting synapse in this model, resistors \( R_{a} \) and \( R_{b} \) are changed in accordance with Eqs. 8 and 11 in the text.

As stated, the frequency of firing of the test unit is directly proportional to its level of polarization at the summing junction. This means that on the model \( AV_{s} \) is proportional to \( \Delta f \) of the test unit. Since we have defined the voltage without inhibition to be zero, \( \Delta V_{s} = V_{s} \) and \( V_{s} = \alpha \Delta f_{1} \), with \( \alpha \) a constant. Also, as stated above, \( g_{i} = \beta f_{2} \). Thus, in plotting the voltage at the summing junction \( (V_{s}) \) in response to different \( g_{i} \)'s, we are essentially plotting \( \Delta f_{1} \) vs. \( f_{2} \). Fig. 9 shows the results of such an experiment. The form of these graphs is similar to the graphs obtained from actual experiments on the eye.

Comparison of the Curves
When the electrical separations of the inhibiting synapses are increased by changing the \( \pi \) network on the model, Fig. 9 shows that the shape of the resultant curves changes accordingly. The electrical distance of the synapses was varied from about 0.005 \( \lambda \) to 0.5 \( \lambda \). By increasing the electrical distance, the values of \( V_{s} \) at which the curves saturate decreased, and the values of \( g_{i} \) at which the curves reach half saturation also decreased. This is in close agreement with what we found on the eye as we chose units farther away from the test unit. Fig.
Figure 9. (A) "Inhibitory coupling" curves generated from the electrical model. The synaptic conductance, $g_i$, was varied and the voltage at the summing junction was measured for different electrical distances of the synapse. The electrical distance was changed from 0.005 $\lambda$ to 0.5 $\lambda$. $g_i$ represents the frequency of firing of the inhibiting unit and $V_{s_i}$ represents the decrease in frequency of firing of the test unit with inhibition. (B) Linearized curves from the electrical model. By plotting $1/g_i$ vs. $1/V_{s_i}$ from A, a family of straight lines is obtained with constant slope but different intercepts. This confirms that the curves in A are hyperbolas (see text).

10 shows the results of plotting saturation, $S$, as a function of the electrical distance on the model. Both $S$ and $H$ show the same monotonic decrease as was obtained from the experimental data.

It should be noted that the properties of the inhibitory threshold are not represented by the model. In order to make comparisons with the experimental curves, it is therefore necessary to subtract $f_T$ from $f_2$. Since the electrical model only represents a steady-state situation wherein inhibitory postsynaptic potentials (IPSP's) are completely fused, it is not really adequate for predicting behavior.
FIGURE 10. Saturation, S, vs. electrical distance (λ) on the model. S decreases uniformly with the electrical distance of the synapse. The values of S can be obtained from the curves in Fig. 9 A or the linearized curves of 9 B. For the linearized curves $S = 1/\text{intercept}$. S could be plotted in frequency units if one assigned or empirically determined $\alpha$ and $\beta$ (see text for details).

at low values of $f_2$. For the model it was assumed that individual IPSP's temporally summate to give a steady hyperpolarization. At low frequencies of the inhibiting unit, however, there will be an incomplete summation of these IPSP's. The sigmoidal shape of the experimental curves near the threshold frequency (Hartline et al., 1961; Johnston, 1973) is probably due to this nonlinear summation of IPSP's (Johnston and Whisler, in preparation). The model does suggest that the electrical distance of a synapse from the summing junction will cause an attenuation of these summated postsynaptic potentials. A distant synapse must be activated more rapidly than a closer one to produce the same potential change at the summing junction. Consequently, its apparent threshold would increase accordingly.

Linearizations of the Model Equations

Writing the circuit equation for our model, we get

$$V_{si} = V_i \frac{R_sR_e}{1 + \frac{R_a + R_b + R_e}{R_sR_e} + \frac{R_aR_b + R_sR_e}{R_i}}$$

(12)

where $V_{si}$ is the voltage at the summing junction, $V_i$ is the inhibitory equilibrium potential, $R_a$ and $R_b$ are elements of the $\pi$ network (which vary with the electrical distance of the synapse), $R_e$ is the total resistance at the encoder region except for the one synaptic input, and $g_i$ is the synaptic conductance, which is proportional to the frequency of firing of the inhibiting unit. Taking reciprocals of Eq. 12 gives

$$\frac{1}{V_{si}} = \frac{1}{g_i} \frac{R_a + R_b + R_e}{R_sR_eV_i} + \frac{R_a + R_e}{V_eR_e}$$

(13)
This is an equation for a straight line with 1/V, the dependent variable, 1/g, the independent variable, the slope, m, equal to

\[ m = \frac{R_a + R_b + R_e}{R_b R_e V_i}, \]  

(14)

and the intercept, b, equal to

\[ b = \frac{R_a + R_e}{V_i R_e}. \]  

(15)

Rewriting the equations, we have

\[ \frac{1}{V_i} = \frac{m}{g_i} + b. \]  

(16)

It is now obvious that plotting 1/V, vs. 1/g, should yield a straight line with slope m and intercept b. Using Eq. 8, R, the series resistor of the network, ranges from 5 to 500 MΩ as we let the electrical separation vary from 0.005 λ to 0.5 λ. We estimated R, to be 10 MΩ, and we let V, equal 15 mV. Since the intercept is b = (R_a + R_b)/R_e V_i, its value should change greatly with R_a and, therefore, with electrical separation. R_b, the shunt resistor of the π network, is very large. R_b varies from about 400,000 MΩ at 0.005 λ to about 5,000 MΩ at 0.5 λ and, therefore, Eq. 14 can be simplified,

\[ m = \frac{R_a + R_b + R_e}{R_b R_e V_i}, \]

and if R_b >> R_a, R_e, then

\[ m = \frac{1}{R_e V_i}. \]  

(17)

Since we are assuming that R_e and V_i are essentially constant, m will not change with electrical separation. Referring to Fig. 9 B, we see that this is indeed the case. By plotting 1/V, vs. 1/g, from the model, we obtain a family of straight lines with the same slope but with different intercepts. The circuit equation of the model is thus identical in form to the empirical equation to which we fit our experimental curves.

In linearizing the curves from the model and measuring their slopes and intercepts, the intercepts are, as expected, equal to the reciprocals of the saturation points. The slopes are equal to the conductance, g_i, at which their curves reach half saturation divided by the saturation, or m = H/S.

As mentioned before, the voltage at the summing junction is proportional to the change in the frequency of firing of the test unit or V, = αΔf_i. The synaptic conductance is proportional to the frequency of firing of the inhibiting unit, or g_i = β(f_2 - f_τ). Substituting into Eq. 16 yields

\[ \frac{1}{\alpha \Delta f_i} = \frac{m}{\beta (f_2 - f_\tau)} + b. \]  

(18)
will be constant for any one ommatidium or test unit regardless of its synaptic input. It can, therefore, be included with the other constants, and we obtain

\[
\frac{1}{\Delta f_1} = \frac{m'}{\beta} \frac{1}{(f_2 - f_1)} + b'.
\]  

(19)

\(\beta\), on the other hand, is a function of each synapse. If the strengths of the various synapses are different (i.e. different conductance change for the same frequency of firing), then \(\beta\) will reflect this difference. In other words, if an inhibiting unit at \(D = 1\) produces a greater change in synaptic conductance than a unit at \(D = 5\), for the same frequency of firing, then \(\beta_1\) will be greater than \(\beta_5\). The slopes of the linearized curves from the model do not change, because, as we said, \(m\) is essentially a constant. The slopes of the linearized experimental curves, however, are equal to \(m'/\beta\), so that any difference in synaptic strengths would cause a variation in \(\beta\) and in the slope of these straight lines.

The slopes of the linearized experimental curves do not change by more that 16%, while the intercepts change by as much as 300% (see Figs. 4 and 5). This indicates that \(\beta\) is very nearly constant for all synapses, and that the decrease in inhibition with distance is not due to a decrease in synaptic strength. Thus the saturation points (and intercepts) are directly related to the electrical distances of the synapses and vary in a uniform and predictable manner with spatial separation between receptors. It is the electrical separation of the individual synapses from the summing junction of the test unit which determines the electrical attenuation of the IPSP's and hence the magnitude of the inhibitory effect. In turn, this electrical distance is directly related to the anatomical separation of receptors on the eye.

Calculations of the Electrical Distances of the Synapses

Using the equations we have developed in the preceding sections and the slopes and intercepts from the linearized curves, we can calculate the electrical distances of the inhibitory synapses from the impulse-generating or encoder region of the test unit. \(V_h\), the inhibitory equilibrium potential, was given a value of 15 mV for our calculations and assumed to be constant for all synapses (Purple, 1964). The best estimate for \(R_m\), based on anatomical data and on calculations by Purple, is 10 MΩ. Rearranging and combining Eqs. 8 and 15 and using values of \(R_2\) and \(R_m\) from Purple (1964) we obtain

\[
\frac{X}{\lambda} = \sinh^{-1} \frac{b'(15) - 1}{100}.
\]  

(20)

In order to use this equation to calculate the electrical distance of an inhibiting synapse from the encoder of the test unit, we need the saturation point of the hyperbolic curve or the intercept of the linearized plot. For units very close (\(D = 1\) or 2) the electrical distance ranged from about 0.1 \(\lambda\) to 0.25 \(\lambda\). The electrical distance always increased uniformly with spatial separation up to about nine receptors away. Beyond this point the inhibition was essentially too small for our measuring techniques. We calculated the electrical distance to be about 0.5 \(\lambda\) at seven receptors away to more than 1 at \(D = 9\) (see Fig. 11).
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1.0 ≤ z ≤ 0.8, 0.6 ≤ u ≤ 0.4 ≤ 0.2

OMMATIDIAL DISTANCE

FIGURE 11. Electrical distance vs. ommatidial distance. The electrical distance of the inhibiting synapse (in terms of space constant, λ, see text) is plotted against the distance between receptor units for all experiments. The electrical distance is shown to increase monotonically with ommatidial distance. (Curve shows mean and standard deviation.)

Conclusions from the Electrical Model

In conclusion, we infer from our data that the decrease of inhibition with distance may be due to an increase in the electrical distance of the synapses from the impulse-transducing region of the eccentric cell. Miller (1957) using the light and electron microscopes has shown that clumps of neuropile are found to be distributed along the axons of the eccentric cells. The neuropile is thought to be the region of synaptic contacts where inhibitory interactions occur. Within the neuropile there are numerous branches and subbranches from various ommatidia. If the synapses are distributed within these neuropile regions, which are themselves distributed along the eccentric cell axon, it is reasonable to believe that the electrical effect of these synapses upon the summing junction will be different depending on their location. Purple (1964) has shown that the impulse-transducing region of the eccentric cell is somewhat removed from the soma. The electrical distance from this point to a synapse located on a fine collateral could be significant. Even using very conservative figures for the dimensions of the collaterals when calculating elements on our model, we find that this electrical distance cannot be ignored.

The most direct anatomical evidence which supports our hypothesis is the electron microscopy study by Gur et al. (1972). In this study serial sections were made of cores containing one or more ommatidia, and the branching pattern of an ommatidium was traced throughout most of the plexus. They concluded that the collaterals closest to the cell body make contact with themselves (self-inhibition), and hypothesized that the collaterals lying successively farther from the cell body make contact with ommatidia successively farther away. This very orderly arrangement of axon collateral output also suggests a similar arrangement for collateral input, and it seems reasonable to speculate that such an
arrangement might exist. If so, then synapses from distant ommatidia would make contact with the eccentric cell at points distant from the encoder region and exert a lesser inhibitory effect than synapses from nearby ommatidia. Their hypothesis correlates well with our experimental and theoretical data.

The belief is widely held that inhibition in the Limulus eye is synaptically mediated (Miller, 1957; Purple, 1964; Gur et al., 1972; Adolph, 1966). As stated before, it is not at all unreasonable to expect a synaptic process to be nonlinear and approach a final saturation value at the postsynaptic membrane. This final value will be the equilibrium or reversal potential for the inhibiting synapse, if the synaptic conductance is infinite. If the synapse is electrically removed from its site of action on the eccentric cell, then the potential seen at that site (summing junction) will be less than the potential at the postsynaptic membrane. The degree to which this potential is attenuated will depend on the cable properties of the collateral branch or the electrical “remoteness” of the synapse. If all the inhibiting synapses were at the same electrical distance, given that other factors were equal (i.e. the strengths and the reversal potentials for the synapses), then their effects on the eccentric cell would be identical. Since the effects did vary, it suggests that each synapse is at a different electrical distance from its site of action on the eccentric cell.

**APPENDIX I**

Spatial Distribution of Inhibition

In Fig. 6 we plotted the values for saturation, $S$, as a function of distance between receptors. Saturation is a measure of the magnitude of the inhibitory coupling between two units. In all experiments we found a monotonic decrease in inhibition with distance. We never saw any peaking of the inhibitory effect at some distance from the test unit. The strongest inhibition was always from nearest neighbors. This appears to contradict the findings of Barlow (1969) who found maximum inhibition at three to five receptor diameters from the test unit. While it is possible that a more extensive study of individual pairs of units might reveal a peaking of the inhibitory effect as reported by Barlow, it is also possible to suggest a physiological explanation for the discrepancy in results.6

In his experiments Barlow used a group of four receptors for his source of inhibition and divided the inhibitory effect by four to obtain an average coefficient. This approximation becomes less reasonable as the inhibitory group gets closer to the test unit. As the group approaches the test unit, it no longer looks like a point source of inhibition, and the distance between receptors in the group becomes significant. Also the mutual inhibitory interactions among the four receptors in the group cannot be ignored. Certainly, these interactions would affect any measurements for threshold and, quite possibly, would distort calculations for the inhibitory field configuration. In addition to the inhibitory interactions among the units in a cluster, there is a possibility that their individual synapses on the test unit may load each other when mutually activated. In other words, the linear summation of inhibiting inputs (Hartline and Ratliff, 1958) may not hold for inhibiting units adjacent to the test unit. Shunting effects may produce a decrease in inhibition for groups closer than three to five receptors from the test unit. At a distance

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6 All our data, except for one experiment, were obtained with the distance between receptors increasing in the anterior-posterior or horizontal direction. We, therefore, can make no statements concerning any difference which might exist with the decrease in inhibition between the vertical and horizontal directions.
greater than three to five receptors, the approximation of a point source of inhibition may be justified, so that the total inhibition can be divided by four to obtain an average coefficient. The configuration of the inhibitory fields reported by Barlow at distances greater than three to five receptors is similar to that presented here. Although the spatial extent of measurable inhibition reported by Barlow was greater than our findings (about 13 receptor diameters vs. about 9), this is readily explainable because of the stronger inhibitory input used by Barlow. The larger the number of inhibiting receptors, the greater the change in firing frequency of the test unit. In our experiments inhibition from units beyond \( D = 9 \) were below the variability of our measurements, but if the inhibiting effects from several receptors had been summed (as Barlow did), then a detectable inhibition might have been obtained beyond this distance.

**APPENDIX II**

*Inhibition vs. Level of Excitation*

Barlow (1967) and Barlow and Lange (1974) present data to indicate that the inhibitory coupling between two units varies with the level of excitation of the test unit. Barlow (1967) showed that maximum inhibition occurs with the test unit firing at approximately 26 impulses/second. Using the electrical model presented in previous sections, this result is both reasonable and fully expected.

In order to use the model to represent excitation at the soma of the test unit, it must be modified by adding a battery in series with a variable conductance. This branch is electrically separated from the summing junction by a \( \pi \) network (Purple, 1964). The battery represents the equilibrium potential of the excitatory process and its associated series conductance. A larger series conductance represents a stronger excitation of the eccentric cell (Purple, 1964). With this arrangement the voltage at the summing junction, without inhibition, will be nonzero and will vary with the value of the series conductance. Fig. 12 illustrates the behavior of the model when "inhibition" is measured as a function of "excitation."

![Figure 12](image)

**Figure 12.** Additional nonlinear behavior of the model. Excitation was added to the model of Fig. 8 as described in the text. \( V_{ij} \) is the voltage at the summing junction with no inhibition. This voltage represents the excitation of the test unit. \( \Delta V_{ij} \) is the decrease in voltage at the summing junction when inhibition is applied. By letting the inhibition remain constant (fixed \( g_i \)) and varying the excitation, a nonlinear relationship between excitation and inhibition results. (See Appendix II, Purple, 1964, and Barlow and Lange, 1974.)
Inhibition was held constant by using a fixed g_i while excitation was increased. The decrease in voltage at the summing junction (ΔV_e), with inhibition, was plotted against V_o, without inhibition. ΔV_e represents the decrease in firing of the test unit due to inhibition, while V_o represents the uninhibited firing rate of the test unit. As shown in Fig. 12, the magnitude of inhibition increases up to a certain level of excitation and then, as the resistance of the soma decreases further, it begins to shunt the inhibitory effect. A more thorough description is given in Purple (1964).

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