SPATIAL SUMMATION OF INHIBITORY INFLUENCES IN THE EYE OF LIMULUS, AND THE MUTUAL INTERACTION OF RECEPTOR UNITS*

By H. K. HARTLINE and FLOYD RATLIFF

(From The Rockefeller Institute for Medical Research)

(Received for publication, December 13, 1957)

ABSTRACT

The inhibitory influences exerted mutually among the receptor units (ommatidia) of the lateral eye of *Limulus* are additive. If two groups of receptors are illuminated together the total inhibition they exert on a "test receptor" near them (decrease in the frequency of its nerve impulse discharge in response to light) depends on the combined inhibitory influences exerted by the two groups. If the two groups are widely separated in the eye, their total inhibitory effect on the test receptor equals the sum of the inhibitory effects they each produce separately. If they are close enough together to interact, their effect when acting together is usually less than the sum of their separate effects, since each group inhibits the activity of the other and hence reduces its inhibitory influence. However, the test receptor, or a small group illuminated with it, may interact with the two groups and affect the net inhibitory action. A variety of quantitative effects have been observed for different configurations of three such groups of receptors. The activity of a population of n interacting elements is described by a set of n simultaneous equations, linear in the frequencies of the receptor elements involved. Applied to three interacting receptors or receptor groups equations are derived that account quantitatively for the variety of effects observed in the various experimental configurations of retinal illumination used.

The inhibition that is exerted mutually among the ommatidia of the lateral eye of *Limulus* depends on the degree of activity of each of these receptor units. It also depends on the number and location of units interacting: the discharge of nerve impulses by a given ommatidium is slowed to an extent that is greater the larger the number of other ommatidia that are illuminated in its vicinity and the closer they are to the ommatidium in question (Hartline, Wagner, and Ratliff, 1956). When many receptor units are active in an eye—each one affecting and affected by its neighbors—the resulting pattern of activity is determined by a set of simultaneous relationships that expresses not only

* This investigation was supported by a research grant (B864) from the National Institute of Neurological Diseases and Blindness, Public Health Service, and by Contract Nonr 1442(00) with the Office of Naval Research. Reproduction in whole or in part is permitted for any purpose of the United States government.

the distribution of external stimulating light over these elements, but also the magnitudes of the inhibitory influences exerted mutually among them and the way in which the influences from many elements combine to affect the activity of each one.

In a preceding paper (Hartline and Ratliff, 1957) we dealt specifically with interaction between pairs of receptor units. We showed that a pair of simultaneous linear equations is required to describe the frequency of the discharge of nerve impulses from two ommatidia in the eye, illuminated independently of one another. When more than two interacting receptor units are activated simultaneously, so that each is subjected to inhibition from more than one other, the set of simultaneous equations must also describe how the inhibitory influences from several receptor units combine in exerting their net inhibition upon any given receptor unit. It is the purpose of this paper to present experimental results establishing the law of spatial summation of inhibitory influences in the eye of *Limulus*, to proceed with the construction of the set of simultaneous equations governing the action of a number of interacting ommatidia, and to show some of the consequences of the mutual inhibitory interaction when more than two receptors are illuminated simultaneously at various intensities.

Method

In each of the experiments reported here, we recorded the discharge of impulses in a single optic nerve fiber from the lateral eye of *Limulus* when the ommatidium in which it originated was illuminated. We then determined the inhibitory effects of illuminating nearby regions of the eye. The ommatidium from which activity was recorded was stimulated by a spot of light of constant intensity, usually so small as to be confined to its facet. The inhibitory effect on this "test receptor," when other receptor units in its vicinity were being illuminated, was measured by taking the difference between the frequency of discharge of the test receptor when it was illuminated by itself and its frequency when it was illuminated together with the other receptors. It has already been shown that the magnitude of the decrease in frequency produced by a constant inhibitory influence is independent of the level of activity of the test receptor (Hartline, Wagner, and Ratliff, 1956).

The receptors whose inhibitory influences were to be studied were illuminated by patches of light, usually circular and about 1 to 2 mm. in diameter, centered several millimeters from the facet of the test ommatidium. Approximately 10 to 20 ommatidia would be illuminated uniformly by such patches of light. The several groups of receptors and the test receptor were illuminated through separate optical systems to minimize the effects of scattered light. The amplified action potential spikes were either recorded oscillographically or registered by an electronic counter suitably "gated" for a desired interval of time. Frequency determinations were always made 2 or 3 seconds after the onset of any illumination to permit the transient changes in frequency to subside before impulses were counted; the counting intervals were 5 to 10 seconds long. Thus the present paper, like the preceding one, deals only with the

steady levels of the receptor discharge and the steady inhibition exerted upon it. The exposures were made at regular intervals, usually 2 minutes or more, to minimize cumulative effects of light adaptation. All measurements required for each determination of an inhibitory effect were made at least in duplicate, in an order designed to minimize systematic errors. Details of our method are described in the previous papers already cited.

RESULTS

We have analyzed the spatial summation of inhibitory influences by measuring the inhibition exerted on a test receptor separately by each of two small groups of ommatidia near it, and then by these two groups together. Since ommatidia close to each other in the eye inhibit one another mutually it may be anticipated that in general the results of such an experiment will depend on the amount of interaction between the two groups. We will begin with a case in which there was little or no interaction. This could easily be achieved experimentally, since the interaction between ommatidia is less the greater their separation (Hartline, Wagner, and Ratliff, 1956; Ratliff and Hartline, 1957); consequently it was possible to choose two regions of the eye, on either side of the test receptor, that were too far apart to affect each other appreciably, but that still were close enough to the test receptor to inhibit it significantly.

The results of such an experiment were quite simple, as shown in Fig. 1: the inhibitory effect on the test receptor produced by the groups of receptors on either side of it, when both were acting together, was equal to the sum of the inhibitory effects produced by these groups acting separately. Measurements of the discharge frequency of the test receptor were made for several different intensities of light on the inhibiting receptor groups, in various combinations. For the points at the upper end of the graph, both receptor groups were illuminated at high intensity; for those at the lower end, both were illuminated at low intensity. For the intermediate points, some were obtained by equal illumination of the two groups of receptors at intermediate intensities, others by illuminating one group at high intensity and the other at low intensity, and still others with these unequal intensity relations interchanged. A line has been drawn through the origin with a slope of unity, representing equality between ordinates and abscissae. Most of the points lie as close to this line as is in accord with the reproducibility of the measurements. The fact that some of them fall slightly above the line will be discussed below. No systematic effects of different combinations of intensities were noted in the data. Many other less extensive experiments gave similar results; some of these will appear below.

It is our suggestion that the experiment of Fig. 1, and those like it, establish the law of spatial summation of inhibitory influences in the lateral eye of *Limulus*, for the steady levels of response to steady illumination: the total

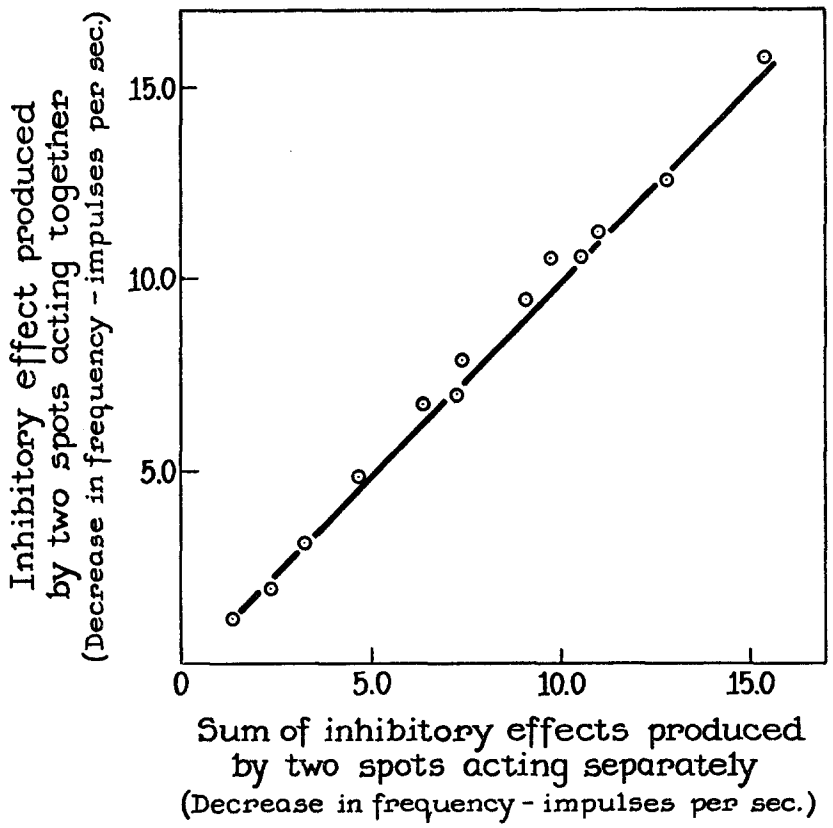

FIG. 1. The summation of inhibitory effects produced by two widely separated groups of receptors. The sum of the inhibitory effects on a test receptor produced by each group acting separately is plotted as abscissa; the effect produced by the two groups of receptors acting simultaneously is plotted as ordinate. The solid line is not fitted to the experimental points, but instead is drawn through the origin with a slope of 1.0 (equality of ordinates and abscissae); a line fitted to the points by the method of least squares would have the equation $y = 1.030x - 0.11$.

The two spots of light used to stimulate the two groups of receptors were each 1.0 mm. in diameter, each illuminating about a dozen receptors, and were 4.6 mm. apart on the eye. The test receptor, located midway between these two spots of light, was illuminated by a third small spot of light of constant intensity confined to its facet. Several intensities of illumination were used for the two larger spots, in various combinations (see text).

Exposures were for a period of 8 seconds; 2 seconds after onset, the counter registering the number of impulses from the test receptor was gated for a period of 5 seconds. Frequency measurements obtained when the test receptor was exposed alone were interspersed between measurements obtained when it was illuminated together with one or the other or both of the inhibiting spots. Two such series of measurements were made for each combination of intensities on the inhibiting regions, and the corresponding frequencies averaged.

inhibitory influence exerted by more than one group of receptor units is equal to the sum of the inhibitory influences exerted by each group. We will show how this simple law can explain a variety of experimental results.

When the regions illuminated to inhibit a test receptor were not widely separated, their combined influences produced an effect that was no longer equal to the sum of their separate effects. An example is shown in Fig. 2. Spots of light were projected onto the eye in three different locations near a test receptor, singly and in combination. The locations of these small regions were chosen to produce inhibitory effects that were nearly the same for each when illuminated singly. Two of these locations were close together, the third was some distance away from these two. Each panel of Fig. 2 is a map of the region of the eye in the vicinity of the test receptor (marked X) showing the locations of the spots of light and the decrease their exposure produced in the number of impulses discharged by the test receptor in 8 seconds (numbers at the right). The three panels on the left show the inhibitory effects of each of the three spots exposed singly, the three on the right show the effects when they were exposed in pairs. For the upper two panels on the right, the most widely separated pairs of spots were used. These two cases resemble the experiment of Fig. 1, just described. In each of these cases the decrease in frequency produced by the two spots together was almost equal to the sum of the decreases produced by each one of them alone (40 compared with 22 + 22, and 42 compared with 22 + 23). The bottom panel on the right shows that the two spots close to each other together produced an inhibitory effect (35) considerably less than the sum of the effects they produced singly (22 + 23). This experiment illustrates results we have obtained invariably in many experiments: simultaneous illumination of receptor groups that were close together produced an inhibitory effect on a test receptor in their neighborhood that was less than the sum of the separate effects produced by illumination of each group singly.

Our interpretation of this experimental result is based on the fact that the inhibitory influence exerted by a receptor unit depends on its activity, which is the resultant of the excitation provided by the stimulating light and whatever inhibition may in turn be exerted upon it by other receptor units in its neighborhood (Hartline and Ratliff, 1957). In the experiment of Fig. 2, the spots of light to the right of the test receptor illuminated receptor groups that were close enough together to inhibit one another. As a result, the amount of receptor activity produced in each group, and hence the inhibitory influence exerted by each group, must have been less when both groups were illuminated together than when each was illuminated separately. Consequently, the inhibitory effect produced by the combined influences of these two groups on the test receptor when both spots of light were shining should have been less than the sum of the inhibitory effects produced by each receptor group illuminated alone. This is what was observed.

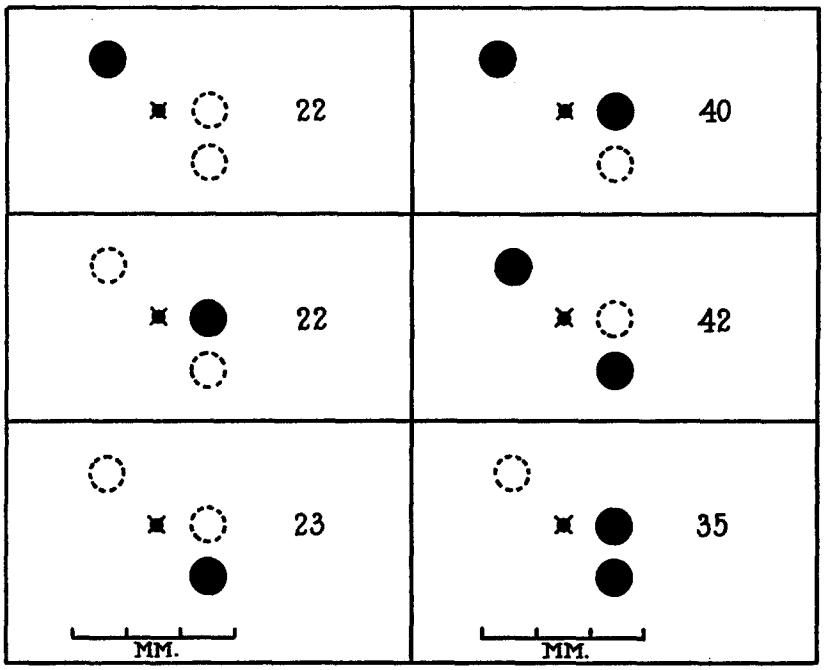

FIG. 2. The summation of inhibitory influences exerted by two widely separated groups of receptors and by two groups of receptors close together. Each panel in the figure is a map of the same small portion of the eye. The test receptor, location indicated by the symbol X, was illuminated steadily by a small spot of light confined to its facet. Larger spots of light could be placed singly in any of three locations, as shown in the three panels on the left side of the figure, or in pairs, as shown in the three panels on the right. The filled circles indicate the spots actually illuminated in each case; the other locations (not illuminated) are indicated in dotted outline merely for purposes of orientation. The number of impulses discharged from the test receptor in a period of 8 seconds was decreased upon illumination of the neighboring spot or spots by the amount shown at the right in each panel. Thus for the upper left hand panel, the test receptor when illuminated alone discharged 252 impulses in an 8 second period beginning 2 seconds after the onset of steady illumination on its facet. This is the mean of 39 determinations taken over a 2 hour period (σ_m = 0.4). When the test receptor was illuminated together with the group of receptors indicated in the panel as being above it and to its left, it discharged 230 impulses in a correspondingly timed period. This is the mean of 6 determinations, ranging from 228 to 232, interspersed among the above controls and the determinations recorded in the other panels. The other determinations were made similarly. See text for discussion of results.

It is the essential feature of this interpretation that the law of spatial summation itself is not called into question; indeed, it is assumed that the inhibitory influences exerted on any given receptor by other receptors in its neighborhood always add according to the simple law stated above. The

mutual inhibition among receptors, however, affects the quantitative outcome in any configuration of interacting elements. This interpretation is supported by the analysis of the following experiments.

We have made quantitative determinations of the inhibitory effects produced by the combined influences from two interacting regions of the eye, exerted on a test receptor (X) near them, for various intensities of illumination upon them. For these experiments we have considered it sufficient to vary the intensity on only one of the regions (A), holding constant the intensity on the other (B). We have presented the results in terms of A's effects on the response of X when A was illuminated together with B, expressed as a function of the amount of inhibition exerted on X by A alone.

These determinations were made by measuring the frequency of discharge of nerve impulses from the test receptor, over the last 10 seconds of a 15 second exposure, in response to illuminating it alone and again when it was illuminated together with region A. The difference between the two frequencies is the measure of the inhibition exerted on X by A alone; we designate it $I_{X(A)}$ and have used it as the abscissa of the point to be plotted. The frequency of discharge was next measured when the test receptor was illuminated together with region B; the difference between this frequency and the frequency of the test receptor illuminated alone is designated $I_{X(B)}$. Finally, the frequency of X was measured with A and B illuminated together, yielding $I_{X(A+B)}$. The difference between these last two measurements, $(I_{X(A+B)} - I_{X(B)})$, is the amount of inhibitory effect produced by A and B together in excess of the amount produced by B alone. This difference has been plotted as ordinate (y) at the abscissa already determined. This procedure yielded graphs with coordinates similar to Fig. 1, but with the origin shifted to the point at which both ordinate and abscissa equal the inhibitory effect of B alone (effect of A equal to zero). Regions between which there was no interaction would yield points lying on a line of slope $+1$, as in Fig. 1 (provided the influence of the test receptor's activity is negligible). This line has been dotted in the graphs we will show.

Fig. 3 shows the results of several experiments of the kind just described; points from a particular experiment are identified by the same symbol. All the points in Fig. 3 fall below the diagonal (dotted) line; *i.e.*, in all cases the total inhibitory effect of A and B acting together was less than the sum of their separate effects. In the experiment designated by the open circles, the points are only slightly below the dotted line; in this experiment the regions A and B were on opposite sides of the test receptor, about 4.0 mm. apart, and, as was the case in the experiment of Fig. 1, evidently interacted very little. The other experiments showed varying degrees of failure of the total effect to equal the sum of the separate effects. For the most part, the degree of such failure could be correlated with the separation on the eye of the regions A and B in the various experiments: the less the separation the farther the points fell below the diagonal line. But, as we shall see, the spatial relations of all three illuminated regions affect the graphs.

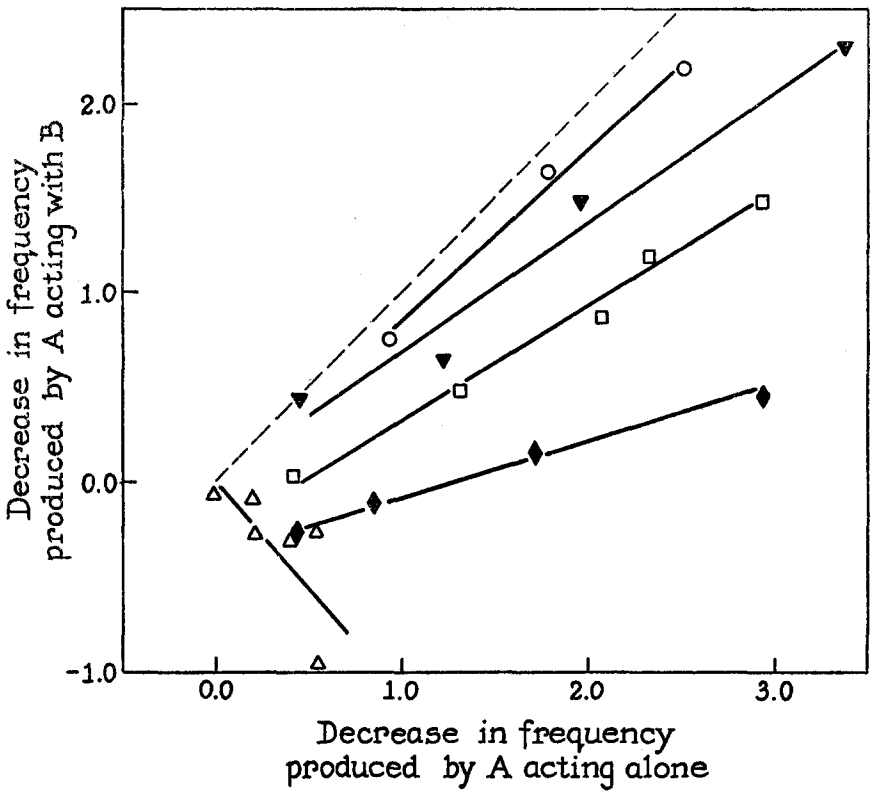

FIG. 3. The summation of inhibitory influences exerted on a test receptor (X) by two groups of receptors at various distances from one another and from X. Each of the graphs was obtained from an experiment on a different preparation. In each case B refers to a spot held at fixed intensity and A refers to a spot illuminated at various intensities. As abscissa is plotted the magnitude of the inhibition (decrease in frequency of response of the test receptor in impulses per second) resulting from illumination of A alone. In the text this quantity is designated $I_{X(A)}$. As ordinate, y, is plotted the change in frequency produced by A when it acted with B; that is, the decrease in frequency produced by illumination of spots A and B together less the decrease produced by illumination of spot B alone. In the text this quantity is designated $(I_{X(A+B)} - I_{X(B)})$.

For each frequency measurement the impulses in the discharges were counted over the last 10 seconds of a 15 second exposure; these measurements were made in duplicate and averaged for each determination of both ordinate and abscissa of each point. The standard error of the determination was of the order of 0.1 impulse per second for each point (see the legend of Fig. 2 in our previous paper for description of the procedure comparable to that used in these experiments).

The upper graph (open circles) was obtained in an experiment in which the two spots A and B were each centered 2 mm. from the test receptor, one on either side.

The results of any one experiment in Fig. 3 are adequately described by a linear relation between the variables that have been used. This relation is a consequence of two factors. The first is the linearity of the inhibitory influence exerted by each receptor as a function of its degree of activity, established in our preceding paper; the second is the simple law of spatial summation of inhibitory influences from more than one receptor, established by the experiment of Fig. 1 and those like it. We will show this in a theoretical section to be given below. We will also show that usually the stronger the interaction between two regions, the greater should be the depression of the line below the diagonal of the graph, and the smaller its slope, as is shown experimentally in Fig. 3.

In one of the experiments of Fig. 3 (points marked by open triangles), region A was located on the opposite side of region B from the test receptor, so far away from the latter that it exerted only slight inhibition on it when acting alone. In this case illumination of A together with B resulted in a decrease instead of an increase in the net inhibitory effect—the ordinates of these points on the graph are all negative. This is a case of disinhibition, discussed in our preceding paper, and is in fact taken from the experiment described in Fig. 6 of that paper. Disinhibition illustrates with especial force the need to consider the mutual interaction of the receptors in analyzing the effects of inhibitory influences in the eye.

Up to this point we have considered only how the inhibition of a test receptor by groups of receptors in its neighborhood is modified by the inhibitory interaction between these groups. We have neglected the influence that

---

A was 1 mm., B 1.5 mm. in diameter. The average value of $I_{X(B)}$ was 2.55. The equation of the line is: $y = 0.903\ I_{X(A)} - 0.057$. For the second graph (filled triangles), A and B were on the same side of the test receptor, equidistant from it (centered 1.25 mm. from X, 1.9 mm. apart); they were each 1.75 mm. in diameter. Average $I_{X(B)} = 2.72$. Equation of line: $y = 0.670\ I_{X(A)} + 0.043$. For the third graph (open squares) A and B were rectangular patches of light 2.5 mm. long, 0.75 mm. wide long edges parallel, the adjacent edges being 0.2 mm. apart. The test receptor was 0.75 mm. from one end of B, on the prolongation of its center line. Average $I_{X(B)} = 2.72$. Equation of line: $y = 0.588\ I_{X(A)} - 0.253$. For the fourth graph (filled diamonds), B was a spot 1.1 mm. in diameter centered 1.0 mm. from the test receptor; A was a rectangular patch (approximately 2 mm. × 3.5 mm.) on the opposite side of B from the test receptor, centered 2 mm. from the center of B. Average $I_{X(B)} = 2.66$. Equation of line: $y = 0.288\ I_{X(A)} - 0.359$. The fifth graph (open triangles) was obtained from the experiment described in Fig. 6 of our previous paper (Hartline and Ratliff, 1957). As in the fourth graph, A was on the opposite side of B from the test receptor, but the patches of light were more widely separated. Average $I_{X(B)} = 4.97$. Equation of line: $y = -1.12\ I_{X(A)} + 0.05$. All lines were fitted to the points by the method of least squares. For all cases, the frequency of discharge of the test receptor when illuminated alone (e_X) was of the order of 20 impulses per second.

the test receptor itself may have on the activity of these groups, and how this might be reflected in the inhibition they exert. It is true that this influence must have been comparatively small in the experiments we have

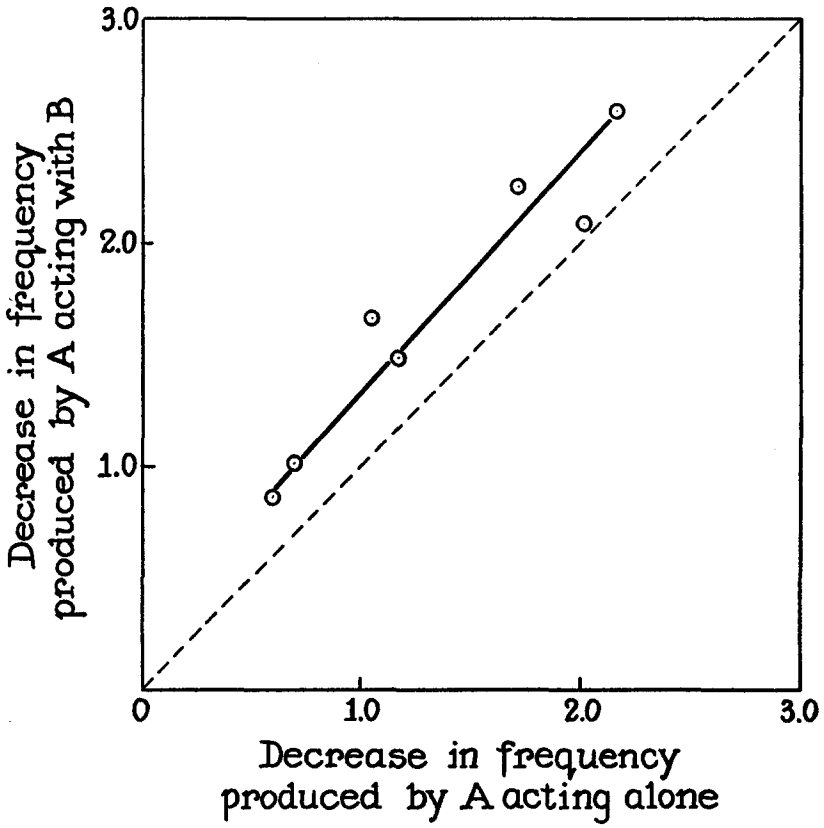

FIG. 4. The summation of inhibitory influences exerted by two widely separated groups of receptors upon a test receptor within a third active group of receptors. Spots A and B were located on either side of the test receptor. They were each approximately 1.0 mm. in diameter and were centered about 2.0 mm. from the test receptor. Unlike the previous experiments, the illumination on the test receptor was not confined to its facet: the spot of light used was about 1.0 mm. in diameter and illuminated some 8 or 9 receptors in addition to the one in the center of the group from which the discharge of impulses was recorded. Abscissae and ordinates as in Fig. 3. The positions of the points above the dotted diagonal reflect the influence of the test receptor group, as discussed in the text. Because of the variability of the points in this experiment the slope of the line that should be drawn through them cannot be determined with precision. The line that has been drawn is in accordance with plausible assumptions concerning the constants of the interacting system as given in the text of the section on Theory. Average $I_{X(B)} = 1.55$. The equation of this line is: $y = 1.13\ I_{X(A)} + 0.20$.

reported thus far, for the test receptor region was illuminated by a spot of light confined to just that one ommatidium from which impulses were recorded, while the illumination on each of the adjacent regions usually covered 10 to 20 ommatidia. Nevertheless, the test receptor is a member of the interacting system and its influence on the other receptor units must be included in a complete description of this system.

The influences exerted by the test receptor region can be augmented be enlarging the spot of light projected on it, so that several other ommatidia are illuminated in addition to the one from which impulses are recorded. The effects of this group that includes the test receptor are most clearly seen in experiments in which the other two regions, A and B, are widely separated, so that they do not interact with one another. It is easy to predict the result of such an experiment: the activity of the ommatidia in groups A and B will be reduced by the inhibitory action of the group containing the test receptor; consequently the amount of inhibition they in turn exert back on the test receptor group will be less than if no such action took place. Since the activity of the test receptor and the others in its group will be less when both the region A and the region B are illuminated together than when only one of them is illuminated, the receptors in each of these regions will be subject to less inhibition from the test receptor group when they act together than when one or the other of them acts alone. Consequently, the inhibitory effect of A and B together will actually be greater than the sum of their separate effects.

Fig. 4 confirms this expectation; the experimental points fall above the diagonal line of the graph by a significant amount. Likewise, in Fig. 1 some of the points fell above the diagonal of the graph; evidently the test receptor had an effect in this experiment even though we had confined the spot of light to its facet alone. It should be realized, of course, that the test receptor also must have exerted its influences in the other experiments we have described (Fig. 3), affecting the positions and slopes of the lines. The theoretical treatment developed in the next section will clarify and render more exact the understanding of the diverse effects that result from the interaction of all three receptor groups under different experimental conditions.

THEORY

In our preceding paper, we showed that the activity of two interacting receptor units may be described by a pair of simultaneous linear equations:

$$\begin{aligned} r_A &= e_A - [K_{AB}\ (r_B - r^0_{AB})] \\ r_B &= e_B - [K_{BA}\ (r_A - r^0_{BA})] \end{aligned} \tag{1}$$

In each equation, the response (r) of the receptor to which that equation applied was put equal to the excitation (e) of the receptor minus a term representing the inhibition exerted on it by the other receptor. This inhibitory

term was written in accordance with the experimental findings, as a linear function of the response of the other receptor.

When three receptors (A, B, and X) are active, three simultaneous equations will be required. Each equation will contain two inhibitory terms similar to those just mentioned, combined by simple addition as required by the law of spatial summation that we have established experimentally in the present paper. These equations are:

$$\begin{aligned} r_A &= e_A - [K_{AB}(r_B - r^0_{AB}) + K_{AX}(r_X - r^0_{AX})] \\ r_B &= e_B - [K_{BX}(r_X - r^0_{BX}) + K_{BA}(r_A - r^0_{BA})] \\ r_X &= e_X - [K_{XA}(r_A - r^0_{XA}) + K_{XB}(r_B - r^0_{XB})] \end{aligned} \tag{2}$$

In these equations, the notation is that adopted in our preceding paper. The response, r, of a particular receptor unit, designated by an appropriate subscript, is measured by the steady frequency of the discharge of impulses in its optic nerve fiber, elicited by steady illumination of its corneal facet at a specified intensity, under whatever conditions of neighboring illumination may also be specified. The excitation, e, of this unit is defined as the receptor's response to this same intensity when it is illuminated by itself. The subscripts serve to identify the respective receptor units: r_A is the response of ommatidium A, etc. Each inhibitory term is written to express the experimental facts, established in our preceding paper, that for each receptor unit there is a "threshold" frequency (represented by the constant r^0) below which it exerts no inhibition on a particular neighboring unit, and that the magnitude of inhibitory influence it exerts on that particular neighbor is directly proportional to the amount by which its frequency exceeds this threshold. The constant of proportionality, K, in each term is labelled with subscripts to identify the receptor units interacting. These subscripts are ordered to indicate the element acted upon and the element exerting the influence. Thus K_{AB} is the coefficient of the inhibitory action exerted on ommatidium A by ommatidium B.

Unfortunately for the simplicity of the treatment, the threshold constants as well as the Ks must also be labelled so as to distinguish the receptor units involved in the inhibitory action. For it has turned out (experiments not yet published) that the threshold frequency for the action of one receptor on a second is not necessarily the same as the threshold for the action of the first receptor on a third (*e.g.*, $r^0_{BA} \neq r^0_{XA}$), and in our previous paper we showed that thresholds for the mutual inhibition of two receptors are often different for the two directions of action (*e.g.*, $r^0_{AB} \neq r^0_{BA}$).

Equations (2) apply only in the range of conditions for which their solutions yield values of r such that none of the quantities $(r - r^0)$ is less than zero.

The above equations are meant to apply strictly to individual interacting receptor units; however, it is reasonable to extend their meaning to apply to small groups of receptors, such as have been studied in the present experiments. This extension can be made rigorously if it is assumed that every receptor in a given group has the same properties and that each is subject to equal influences from every other member of that group, and furthermore

that each receptor within a given group is subject to equal influences from every receptor in any other particular group. Even if the properties of the receptors and the influences exerted are not exactly uniform in this sense, it is plausible to assume that this extension of the equations will yield a useful approximation.

With this extension understood, a response, r, in any equation of a given set refers to the frequency of discharge of a typical receptor in the group specified by the subscript attached to r when that group was illuminated together with the other groups in the given experimental configuration. Similarly an excitation, e, will be understood to refer to the response of a typical receptor in the group specified by the attached subscript when that group was illuminated alone. Each coefficient, K, will be understood to refer to the coefficient of the inhibitory action exerted on each receptor in the group specified by the first subscript of K, by the receptors acting together in the group specified by the second subscript. Thus K_{AB} would be given by the decrease in frequency of a typical receptor in group A per unit increment in frequency of a typical receptor in group B.

In any given configuration of illumination on the receptor mosaic the total inhibition exerted on a receptor in a particular group by the other groups of receptors will be given by one of the expressions in square brackets in the set of equations appropriate to the configuration. It is convenient to designate it by a single term, I, labelled so as to identify the interacting groups. Thus the entire expression in the square brackets of the third equation of (2) will be designated $I_{X(A+B)}$. It represents the total inhibition exerted on the test receptor (one of the group X) by groups A and B acting together. For the measurements in which the test receptor group was illuminated together with A alone, and for those with B alone, two pairs of equations similar to (1) are required, appropriately labelled. The inhibition measured in these two cases will be designated respectively $I_{X(A)}$ and $I_{X(B)}$. It is these quantities, I $(= e - r)$, that are needed in the discussion of the experiments, for they are determined from measurements of frequencies for the uninhibited and inhibited conditions taken in such order as to minimize effects of drift and systematic errors on their averages.

In the experiments we are discussing in this paper each experimental point is obtained from determinations of $I_{X(A)}$, $I_{X(B)}$, and $I_{X(A+B)}$ (see section on Results). The three sets of equations yielding these quantities can be solved for them in terms of the es, the Ks, and the r^0s. The solutions can be combined, and after appropriate eliminations yield $I_{X(A+B)}$ as a linear function of $I_{X(A)}$ and $I_{X(B)}$:

$$I_{X(A+B)} = MI_{X(A)} + NI_{X(B)} + R \tag{3}$$

in which $$M \equiv (1/D)\,(1 - K_{XA}K_{AX})\,(1 - K_{BA}K_{XB}/K_{XA})$$

$$N \equiv (1/D)\,(1 - K_{XB}K_{BX})\,(1 - K_{AB}K_{XA}/K_{XB})$$

$$R \equiv (1/D)\,[K_{BA}\,(K_{XB} - K_{XA}K_{AB})\,(r^0_{BA} - r^0_{XA}) + K_{AB}\,(K_{XA} - K_{XB}K_{BA})\,(r^0_{AB} - r^0_{XB})]$$

$$D \equiv 1 - K_{XA}K_{AX} - K_{XB}K_{BX} - K_{AB}K_{BA} + K_{AX}K_{XB}K_{BA} + K_{XA}K_{BX}K_{AB}$$

In the experiments that were described in Fig. 3, we varied the intensity on only one of the spots of light (A), holding that on B constant, and found it convenient to plot as ordinate (y) the quantity ($I_{X(A+B)} - I_{X(B)}$). This may be described as A's effect in the presence of B. (This practice permits several experiments, for which $I_{X(B)}$ had widely different values, to be represented in a single figure.)

Equation (3) thus accounts for the linearity of the graphs in Fig. 3. The slope and position of each graph yield an experimentally determined value of M and of the intercept y_0. The kind of experiments reported in this paper cannot provide enough information to evaluate separately the six coefficients, K, and the four thresholds, r^0, that occur in equation (3). Therefore, the particular values of these constants that occur in combination in the expressions for M and y_0 may be chosen with considerable latitude, although consideration of the sizes and separations of the interacting groups narrows this choice. We will show, for each experiment in Figs. 3 and 4, that plausible choices of the constants can be made to account for the observed values of the slopes and positions of the graphs. The theory may thus be used to account for the diverse effects obtained by various configurations of interacting groups of receptors. Special cases for which simplifying assumptions can be made will be considered first.

In most experiments the group (X) contained the "test" receptor alone; the influence of a single receptor on larger groups is comparatively small, and may be neglected in a first approximation (K_{AX}, $K_{BX} \cong 0$). To begin with, we may note that if the groups of receptors A and B exert no inhibition on each other ($K_{AB} = K_{BA} = 0$), then $I_{X(A+B)} = I_{X(A)} + I_{X(B)}$. This was essentially the situation in the experiment of Fig. 1, when A and B were on opposite sides of X, too far apart to affect one another.

The consequence of interaction between A and B is clearly seen if we consider a symmetrical configuration in which these groups are of equal size, and are equally distant from X. Because of the symmetry, A and B may usually be assumed to have equal coefficients of action on each other, ($K_{AB} = K_{BA} \equiv \bar{K}$), and on X, ($K_{XA} = K_{XB}$). Equation (3) (neglecting R) then yields $I_{X(A+B)} = \dfrac{1}{1+\bar{K}}\,(I_{X(A)} + I_{X(B)})$; the net effect of A and B acting together should thus be less than the sum of their separate effects, as experiments have shown. Moreover, the greater the interaction (the closer A and B are to one another) the greater should be the amount by which the net effect falls below this sum. In the experiments that provided the data for the upper three curves of Fig. 3 the configurations of the illuminated groups

were approximately symmetrical. On the assumption that the inhibitory coefficients were indeed symmetrical, the slopes of these lines would be accounted for by values of $\bar{K}$ of 0.11, 0.50, and 0.70 (top to bottom, respectively).

If the influences are not exerted symmetrically by the groups A and B on the test receptor or on each other, the slope M of the line in a plot like Fig. 3 is affected. Thus, if the receptor group on which the intensity is being varied (A) has a smaller coefficient of action on the test receptor than the other group (B) (so that $K_{XB}/K_{XA} > 1$), the slope M may be much reduced, even though the interaction between A and B is only moderate (K_{AB} and K_{BA} small). This was the case in the experiment whose graph in Fig. 3 is next to the bottom (diamonds). The numerical value of the slope of this line can be accounted for by assuming that $K_{AB} = K_{BA} = 0.30$, but that $K_{XB} = 2.5\ K_{XA}$ (since B was closer to X than was A).

A closer consideration of the experiments represented by the upper three graphs of Fig. 3 suggests that in these experiments also the influences were probably not strictly symmetrical. For the uppermost graph (open circles) the spot B was about twice the size of A; if the influences each exerted on the other and on X were in this ratio, the observed value of the slope M could be accounted for by the assumptions $2K_{BA} = K_{AB} = 0.10$; $2K_{XA} = K_{XB}$. For the third graph from the top (squares) A and B were equal in size but B was closer to X than was A, and might be expected to have affected X more strongly than did A. The assumptions $K_{BA} = K_{AB} = 0.27$; $K_{XB} = 1.7\ K_{XA}$ yield the observed value of M. For the second graph from the top (solid triangles) there is some reason to prefer the assumption that the coefficients of the action on X were also unequal even though the geometrical configuration was symmetrical. The assumptions $K_{BA} = K_{AB} = 0.15$; $K_{XB} = 2.3\ K_{XA}$ yield the observed value of M for this experiment.

A sufficiently great inequality of coefficients, with A exerting comparatively little direct influence on X, can even result in a negative slope ($K_{BA}K_{XB}/K_{XA} > 1$), as in the lower graph of Fig. 3 (open triangles). This is the case of disinhibition, which we have already discussed. The set of assumptions $K_{BA} = K_{AB} = 0.30$; $K_{XB} = 6.7\ K_{XA}$ is not implausible and yields the numerical value of M that was observed.

If the inequality of the coefficients of the inhibitory action exerted on the test receptor is in the opposite direction, so that $K_{XA} > K_{XB}$, the slope of the line will be greater than if the coefficients are equal: it can equal or even exceed 1 even though A and B interact ($K_{XB}/K_{XA} < K_{AB}$). We have performed one experiment in which A (the spot whose intensity was varied) was closer to the test receptor than was B, and exerted a stronger inhibition on it. This experiment yielded a line with a slope of 0.97.

To account for the position of each line of Fig. 3, an appropriate value of R (Equation 3) is required. Values of the individual constants that appear in the expression

for R may be assumed with some latitude, to yield the value required to fit the data. However, consideration of the known properties of the thresholds of inhibitory effects restricts this choice, and these properties may manifest themselves directly in the experimental results. One example is the graph in Fig. 3 next to the lowest (diamonds). In the experiment that provided the data for this graph, the region A was closer to the region B than to the test receptor. Consequently, it might be expected (on the basis of experiments reported elsewhere, Ratliff and Hartline, 1957) to have reached the threshold of its inhibitory action on B at a lower level of activity than that at which it began to inhibit X. At low levels, therefore, A would first produce an indirect effect on X, releasing it partially from B's inhibition before its direct inhibitory action on X began. The graph should therefore begin at a negative value of y, as is indeed the case. The value of R we have given for this graph is negative (-0.26), reflecting the condition $r^0_{BA} < r^0_{XA}$ (one may assume $r^0_{AB} \cong r^0_{XB}$, since B was roughly equidistant from A and X). It should be added that the necessity to find a suitable value of R affected the choice of the particular values of the Ks needed to account for the slope M. Similar considerations applied to the other experiments but the details need not be pursued here, for the principles are better illustrated by more informative experiments in which representative receptor activity is recorded simultaneously from more than one of the interacting groups.

We may now turn to a consideration of the effect that the test receptor itself (or the group X including it) has on these relations. The simplest case to consider is a symmetrical configuration in which the two spots A and B are on opposite sides of the test receptor, too far apart to interact ($K_{AB} = K_{BA} = 0$; from the symmetry, $K_{XA} = K_{XB}$; $K_{AX} = K_{BX}$). Then $M = N = \frac{1 - K_{XA}K_{AX}}{1 - 2K_{XA}K_{AX}}$. Thus in this case the slope of the line relating $I_{X(A+B)}$ to $(I_{X(A)} + I_{X(B)})$ is greater than unity: the two regions together produce an inhibitory effect that is greater than the sum of their separate effects, as has already been explained (Fig. 4). The assumptions $K_{AX} = K_{BX} = K_{XA} = K_{XB} = 0.32$; $K_{AB} = 0$, $K_{BA} = 0$, account for the line that has been drawn through the points of Fig. 4. Turning to Fig 1, a reasonable value of $K_{XA} = K_{XB} = 0.5$ would require only the small value of $K_{AX} = K_{BX} = 0.06$ to account for the slope of a line fitted to the points by the method of least squares, which would be slightly greater than 1. It is evident that the effects of the test receptor, though small, probably never are entirely negligible, and must have been present in all the experiments of Fig. 3.

The theory presented in this section is a logical development based on the experiments reported in our previous paper, taken together with the experiments in this paper that demonstrate the additivity of inhibitory influences. These basic experiments dealt with the interaction of carefully isolated single receptor units, or at most with the interaction of small groups of receptors. To extend the theory to larger groups, we assumed a certain uniformity of action among the receptors of the groups. With this assump-

tion the theory is successful in providing a quantitative interpretation of the responses of a "test receptor" subject to influences of two nearby groups of illuminated ommatidia in a variety of configural relations. If correct, the theory should be capable of interpreting fuller experiments than those reported here, such as can be done by measuring the responses of more than one receptor unit. Indeed, simultaneous measurements of the discharges of impulses in three optic nerve fibers, one from each of three small groups of receptors, could furnish a complete illustration of the principles that have been discussed, and should provide a crucial test of the theory. Preliminary attempts have shown that such experiments are feasible.

The establishment of the law of spatial summation of inhibitory influences permits the theory to be extended to describe the activity of any number of interacting elements. The set of simultaneous equations for n interacting receptors may be constructed by writing n equations, each with n-1 inhibitory terms combined by simple addition:

$$r_p = e_p - \sum_{j=1}^{n} K_{pj}\,(r_j - r_{pj}^0) \qquad \begin{matrix} p = 1, 2, \ldots n \\ j \neq p \\ r_j \not< r_{pj}^0 \end{matrix} \qquad (4)$$

The same restrictions apply to this set of equations that have been stated previously: only positive values of e, r, K, and r^0 are permitted; the terms in the summation for which $j = p$ are to be omitted; this set of equations applies only in the range of conditions for which no r is less than the associated r^0 in any term.

DISCUSSION

It is our basic interpretation of the experiments described in this paper that the inhibitory influences exerted on any ommatidium in the lateral eye of *Limulus* by other ommatidia always combine by simple addition. As we have shown, this does not mean that the net inhibitory effect produced by two ommatidia, or two groups of ommatidia, when they act simultaneously on a third, is necessarily equal to the sum of the effects which they each produce when acting alone. Indeed, we have shown that the net effect may range from values greater than the sum of the two separate effects to values less than that of one of the separate effects alone. Such results are entirely consistent with our basic interpretation, and reflect merely the consequences of the mutual interaction of the receptor units.

Such a variety of effects obtained with only a few small groups of interacting receptor units presages the complexity that would be encountered in analyzing the pattern of responses of a large population of interdependent elements. But in principle we now have available the theoretical means for

expressing the simultaneous relations describing the activity of the entire population of receptors in the eye, and predicting how their mutual interactions would operate to affect the pattern of optic nerve activity for any configuration of interacting elements. Even when extended to a large number of elements, the theory should remain manageable, thanks to the linearity of the inhibitory terms in the equations, and the simple additive law of combination of the terms; different degrees of interaction are fully expressible by the different values of the inhibitory coefficients and the thresholds for the inhibitory effects.

In the mosaic of receptors that constitutes the sensory layer of the eye, the amount of inhibition exerted mutually between any two single receptor units is less the farther they are apart. We do not yet know the exact form of this dependence of the inhibitory influence on the separation of the interacting elements, or whether it can be expressed in any but statistical terms. Nevertheless, it is clear that this strong dependence of the inhibitory coefficients and the thresholds on distance introduces into the system a geometrical factor that must give to the inhibitory interaction special significance in retinal function. As a consequence, for example, the brightness contrast that retinal inhibition can engender must be accentuated in the neighborhood of sharp gradients and discontinuities of illumination in the retinal image.

Because of the inhibitory interaction and its dependence on the spatial relations of the stimulated elements of the retinal mosaic, the degree of activity of each element is affected by the responses of all the others and by their spatial distribution. The pattern of optic nerve activity is more than a reproduction of the pattern of the various stimulus intensities distributed over the receptor mosaic; it is modified by the inhibitory interaction so as to accentuate various significant features of the configuration of light and shade in the retinal image.

BIBLIOGRAPHY

Hartline, H. K., Wagner, H. G., and Ratliff, F., Inhibition in the eye of *Limulus*, *J. Gen. Physiol.*, 1956, **39**: 651.

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

Ratliff, F., and Hartline, H. K., Fields of inhibitory influence of single receptor units in the lateral eye of *Limulus* (abstract), *Science*, 1957, **126**, 1234.