INHIBITORY INTERACTION OF RECEPTOR UNITS IN THE EYE OF LIMULUS*

By H. K. HARTLINE AND FLOYD RATLIFF

(From The Rockefeller Institute for Medical Research)

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In the lateral eye of the horseshoe crab, *Limulus*, the visual receptor units exert an inhibitory influence mutually upon one another. The discharge of impulses in any one optic nerve fiber, generated in the sensory structure of the particular ommatidium from which that fiber arises, is determined principally by the intensity of the light stimulus to the ommatidium and the state of adaptation of this receptor unit. However, the ability of an ommatidium to discharge impulses is reduced by illumination of the ommatidia in neighboring regions of the eye: its threshold to light is raised, and the frequency of the discharge that it can maintain in response to steady suprathreshold illumination is decreased. This inhibitory action is exerted reciprocally between any two ommatidia in the eye that are separated by no more than a few millimeters. As a result of inhibitory interaction among neighboring receptors, patterns of optic nerve activity are generated which are not direct copies of the patterns of external stimulation, but are modified by this integrative action that takes place in the eye itself.

These basic features of inhibition in the eye of *Limulus* have been described in detail in a recent paper (Hartline, Wagner, and Ratliff, 1956). In that paper it was shown that the anatomical basis for the inhibitory interaction is a plexus of nerve fibers lying just back of the layer of ommatidia, connecting them together. Furthermore, a direct experiment demonstrated mutual inhibitory action between two ommatidia whose respective optic nerve fibers were placed on the recording electrodes together. It is the purpose of the present paper to analyze the inhibitory interaction of receptor units in the eye of *Limulus*, and to describe quantitative properties of receptor activity that arise as a consequence of this interaction.

Method

The experiments reported in the present study are based on the measurement of the frequency of the discharge of nerve impulses from two receptor units simultane-

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ously, enabling the exact description of their interaction. In each experiment, a lateral eye of an adult *Limulus* was excised with 1 to 2 cm. of optic nerve and mounted in a moist chamber (maintained at 18°C.). Two small strands were dissected from the optic nerve and each placed over a pair of wick electrodes connected to its own separate amplifier and recording system. In some experiments we dissected each strand until only a single fiber remained, as evidenced by the uniformity and regularity of the action potential spikes elicited in response to illumination of the eye. In other experiments, bundles containing many active fibers were used and the isolation of single units was accomplished by coating the eye with opaque wax (a heavy suspension of lampblack in paraffin wax) and then removing the coating carefully from a very small region, exposing the corneal facet of just that one ommatidium from which it was desired to record impulses. The black wax evidently prevents internal reflections inside the cornea of the eye, for by this method perfect isolation of single units can often be obtained,—a result rarely achieved merely by focussing a small spot of light on the facet by means of a lens.

The receptors of the eye were illuminated by the same optical system that was described in the paper mentioned above. Small spots of light were projected on the eye, their sizes controlled by diaphragms and their intensities by neutral wedges. The direction of incidence of each beam could be adjusted for maximal effectiveness by a system of mirrors. A separate system was employed for each spot of light, to avoid scatter in a common optical path.

Oscillograms of the amplified action potentials in the nerve fibers were recorded photographically in some experiments. Often, however, it was preferable to measure directly the frequency with which impulses were discharged in each nerve fiber. This was done by leading the output of each amplifier through a pulse-shaper into an electronic counter. The threshold of the pulse-shaper was calibrated, and could be set to discriminate between the action potential spikes and amplifier noise with perfect reliability (in *Limulus* optic fibers, spikes can usually be obtained that are many times greater than any fluctuations of potential due to noise); the uniform output of the pulse-shaper insured perfect operation of the counting circuits. Each counter was "gated" by an electronic timer, so that only those nerve impulses occurring within a specified interval of time (usually several seconds) were registered. The gating timer was activated by a delaying timer, which permitted the counting period to be started at any desired time (usually 1 or 2 seconds) after the onset of illumination to the eye.

To obtain maximum precision in the measurement of frequency of discharge, we displayed the gating voltages and the pulses to the counter from both recording channels on a dual trace oscilloscope. We then estimated for each channel that fraction of an interval between impulses that occupied the time between the onset of the counting period and the occurrence of the first counted impulse, at the beginning of the counting period, and the corresponding fraction between the last counted impulse and the cessation of the counting period. These fractions were added to the total number of intervals registered. For greater convenience in some experiments the delaying timer, instead of activating the counter gate directly, was arranged to sensitize an electronic "trigger" which, upon the occurrence of the next nerve impulse, activated the gate to the counter. Thus the counting period always started

at the occurrence of an impulse, and only the fractional interval at the end of the gated counting period needed to be estimated. The precision gained by these methods was necessary when the counting period was short (1 or 2 seconds), and was desirable for the longer periods usually employed (7 to 10 seconds). Measurement to within about one-quarter interval was warranted by the regularity of the discharge found in many preparations, and the reproducibility of the frequencies observed.

In the experiments reported in this paper, we have confined our attention to the frequency of the discharge of impulses that is maintained at a more or less steady level during steady prolonged illumination of the eye. The transient changes in frequency that occur during the first second or two after light is turned on or off were excluded from the measurements. Exposures were fixed in duration (usually less than 10 seconds), and were repeated at fixed intervals of 2 to 5 minutes, with longer periods of rest interspersed, to minimize cumulative effects of light adaptation.

RESULTS

In the recent paper to which we have referred, direct evidence was given that receptor units in the *Limulus* eye may inhibit one another mutually. Recording the action potentials in a nerve strand containing two active fibers, it was shown that the discharge of impulses in either of the fibers was slowed when the other was brought into activity by illuminating the ommatidium from which it arose. A similar experiment is illustrated in Fig. 1, in which two strands dissected from the optic nerve, each containing a single active fiber, were placed on separate recording electrodes so that the action potentials of each of them were recorded separately. A small spot of light was centered on each of the ommatidia (designated "A" and "B") in which the fibers originated. The oscillograms show the effects of illuminating each of these small regions of the eye separately and together. The steady frequency of discharge of impulses in each fiber was less when both receptor units were active than when they were stimulated singly.

In the experiment shown in Fig. 1 the spots of light centered on the respective ommatidia were each made large enough to illuminate several receptors immediately adjacent to them in order to make the slowing of the discharges large enough to be apparent at a glance. Strictly mutual inhibition of the individual units, however, was exerted by these receptors, for similar (though less pronounced) slowing of the discharge was produced when each was illuminated by a spot so small as to be confined to the facet of its ommatidium, except for slight amounts of light that may have been scattered in the eye. In many of the experiments to be reported below, we took precautions to ensure that we were dealing with the mutual interaction of only two ommatidia by using opaque wax to effect complete optical isolation, as described in the section on Method.

From our previous study, we know that the inhibition of a receptor unit, measured by the decrease in the frequency of its discharge, is greater the

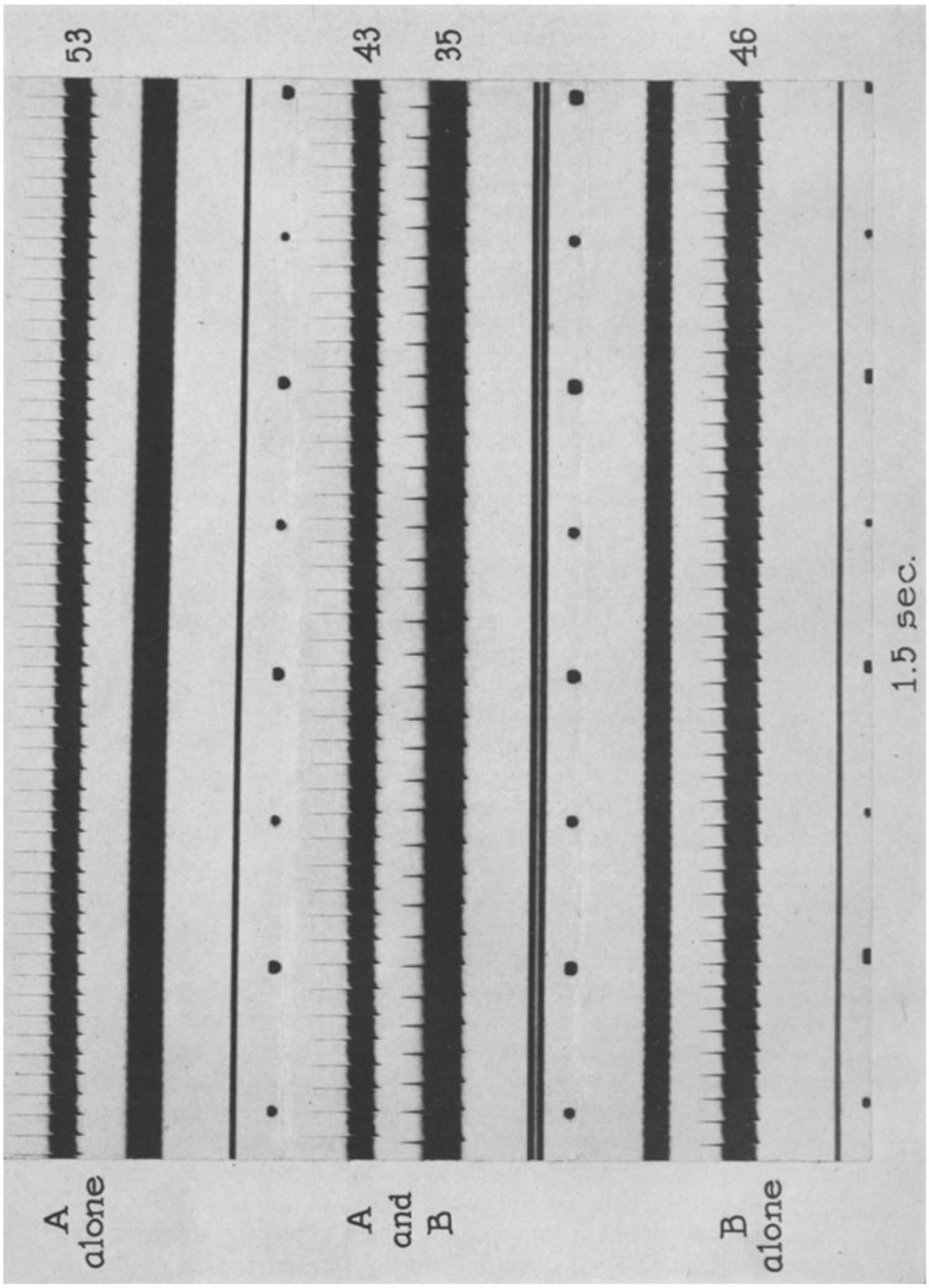
53
43
35
46
A alone
A and B
B alone
1.5 sec.

FIG. 1. Oscillograms of action potentials recorded simultaneously from two optic nerve fibers of a lateral eye of *Limulus*, showing the discharge of nerve impulses when the respective ommatidia in which these fibers originated were illuminated singly and together. In the top record, one ommatidium ("A," nerve fiber activity recorded by upper oscillographic trace) was illuminated by itself at an intensity that elicited the discharge of 53 impulses (as indicated at the right) in the period of 1.5 seconds covered by the records. In the bottom record, the other ommatidium ("B," activity recorded by lower trace) was illuminated by itself at an intensity that elicited the discharge of 46 impulses in 1.5 seconds. In the middle record, both ommatidia were illuminated together, each at the same intensity as before; ommatidium A discharged 43 impulses, ommatidium B 35 impulses, in 1.5 seconds. For A, the decrease in frequency of 10 impulses per 1.5 seconds is taken as the magnitude of the inhibition exerted upon it while B was discharging at the rate of 35 impulses per 1.5 seconds; for B, the decrease of 11 impulses per 1.5 seconds measures the inhibition exerted upon it while A was discharging at the rate of 43 impulses per 1.5 seconds. Two separate optical systems were used, each focusing a small spot of light (approximately 0.5 mm. in diameter) on the eye, one centered on ommatidium A, the other centered on ommatidium B. The spots were 1 mm. apart, center to center. Each spot illuminated about 5 ommatidia in addition to A and B. For each record, the light had been turned on 7 seconds before the start of that portion of the record shown in the figure. Time marked in one-fifth seconds; black bands above time marks are the signals of the stimulating illumination.

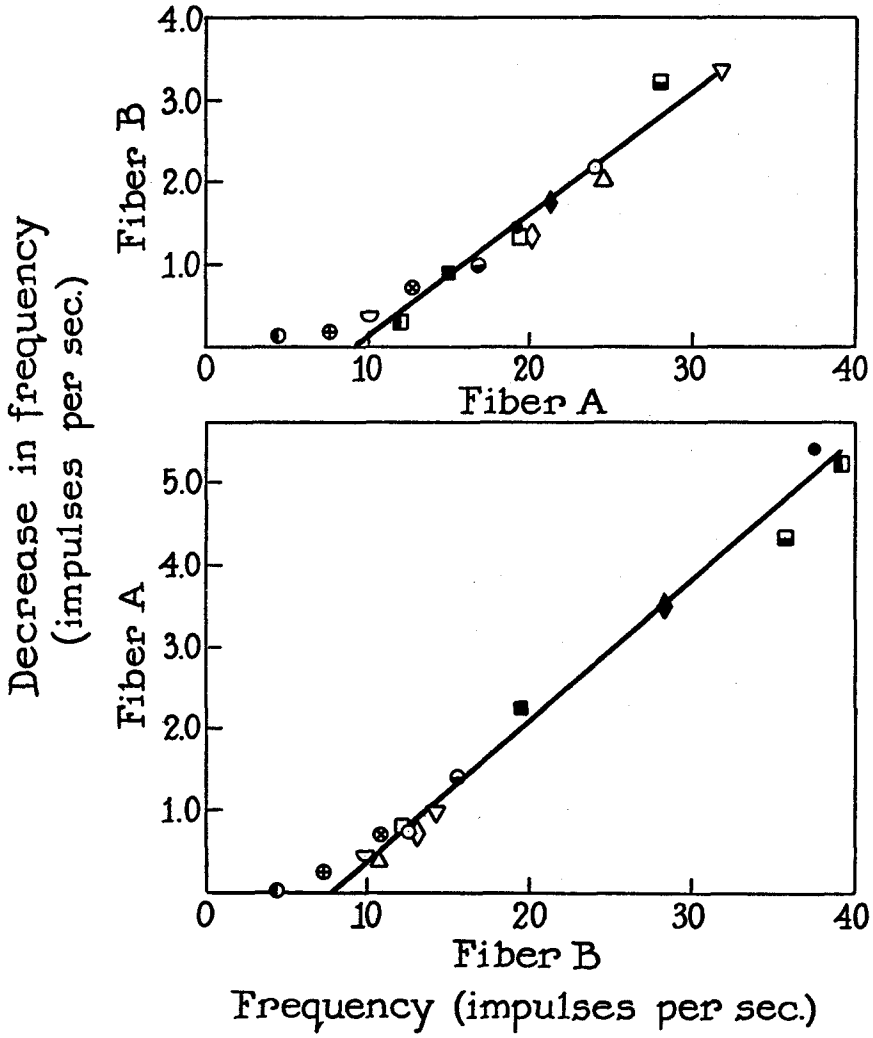

FIG. 2. Graphs showing mutual inhibition of two receptor units in the lateral eye of *Limulus*. In each graph the magnitude of the inhibition of one of the ommatidia is plotted (ordinate) as a function of the degree of concurrent activity of the other (abscissa). Sets of optic nerve fiber responses similar to those shown in Fig. 1 were analyzed as explained in the legend of that figure, each set yielding a point in the upper graph (inhibition of ommatidium B by ommatidium A) and a corresponding point (designated by the same symbol) in the lower graph (inhibition of ommatidium A by ommatidium B). The different points were obtained by using various intensities of illumination on ommatidia A and B, in various combinations.

Frequencies were determined by counting the number of impulse intervals during

stronger the stimulus to receptors that inhibit it. Experiments similar to that illustrated in Fig. 1 make it possible to show quantitatively how the amount of inhibition exerted on a receptor varies with the degree of activity of a nearby receptor unit that exerts this inhibition; the mutual interaction of two receptors can be analyzed by stimulating each of them at different intensities singly and in combination. The result of such an experiment is shown in Fig. 2. In this experiment, the frequencies of discharge of each of two ommatidia were measured, for various intensities of illumination, when each was illuminated alone and when both were illuminated together. The decrease in the frequency of discharge of each has been plotted as ordinate against the frequency of the concurrent discharge of the other as abscissa. The upper graph shows the amount of inhibition exerted upon ommatidium B by ommatidium A, as a function of the degree of activity of A; the lower graph shows the converse effect upon A of the activity of B. Both sets of points are adequately fitted by straight lines. In each case there was a fairly distinct threshold for the inhibition; each ommatidium had to be brought to a level of activity of 8 or 9 impulses per second before it began to affect the discharge of the other. Above this threshold, the frequency of discharge of B was decreased by 0.15 impulse per second for each increment of 1 impulse per second in the level of activity of A; the corresponding coefficient of the inhibitory action in the reverse direction (A acted on by B) was 0.17.

We have performed many similar experiments. Six of them, including the one just described, were done with "optical isolation," employing large nerve bundles that had exhibited activity of many fibers before the application of

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the last 5 seconds of a 7 second exposure to light (so that only the steady discharge was measured). To obtain each pair of points in the two graphs two such counts were made for each of the following conditions of illumination: A alone, B alone, A and B together, presented in an order designed to minimize systematic errors. The averages of such duplicate determinations of the magnitude of the inhibition are the values plotted in the graph. From the distribution of the differences between the individual measurements in each duplicate determination the standard error of the points in the graph was calculated to be 0.12 impulse per second. The straight lines were fitted by the method of least squares. In the upper graph the line has a slope of 0.15, which is the value of the "inhibitory coefficient" $K_{B,A}$ (effect of A on B); in the lower graph the slope is 0.17 ($= K_{A,B}$, the coefficient of the effect of B on A). The intercept of the line on the axis of abscissae is 9.3 impulses per second for the upper graph, 7.8 for the lower. Disregarding a possible "toe" at the bottom of each plot, these give the values, respectively, of the thresholds of the inhibitory effect of A acting on B, designated later in the text as r_A^0, and of B acting on A (r_B^0).

In this experiment illumination was restricted to the two ommatidia from which activity was recorded by masking the rest of the eye with opaque wax (see text). These ommatidia were 1 mm. apart.

opaque wax to the eye to mask all but those two receptor units singled out for observation. In such experiments we could be quite certain that not any of the receptors adjacent to those under observation were excited by scattered light (since no nerve impulses from them were observed). In these experiments, therefore, the observed inhibitory effects were entirely those exerted mutually by the two receptor units upon one another. In other experiments we could be less certain about possible contributions from adjacent receptors excited by scattered light, although the scattered light was never very strong, and its effects were probably below threshold in most cases. All these experiments have shown features similar to those exhibited in Fig. 2. All showed a linear relation between the magnitude of the inhibition of one receptor (measured by the decrease in its frequency) and the degree of concurrent activity of the other (measured by its frequency). Nearly all experiments showed a "threshold" frequency below which no inhibitory effect was detected. The threshold was usually about as distinct as that shown in Fig. 2—a slight "toe" at the bottom of the curve was often noted. Although the values of the two thresholds were nearly identical in the experiment of Fig. 2, in other experiments they were not always the same for both members of an interacting pair. Likewise the slopes of the two curves often differed more than was the case in the experiment we have figured, sometimes by as much as a factor of 2.

The key to the analysis of the mutual interaction in the eye of *Limulus* lies in the correlation between the magnitude of the inhibition of a receptor and the degree of concurrent activity of the receptors that inhibit it. The degree of activity of any one of these receptors, however, depends not only on the stimulus to it but also on whatever inhibitory influences it may be subjected to in turn. It is the resultant level of activity of a receptor unit that determines the strength of the inhibition it exerts on a neighboring receptor. We have direct experimental evidence for this. Fig. 3 shows a small portion of the upper graph of Fig. 2; the points plotted as open symbols are measurements of the inhibition of ommatidium B produced by illuminating ommatidium A at two different intensities (two points at each intensity), with B illuminated at a low intensity. At the higher of the two intensities on A, which elicited a discharge in fiber A of approximately 24 impulses per second, the response of B was decreased by a little more than 2.0 impulses per second; at the lower intensity (A discharging at the rate of approximately 20 impulses per second) the discharge of B was reduced by about 1.3 impulses per second, following the trend of the solid line, which is a portion of that drawn through all the experimental points in the upper graph of Fig. 2. For these points plotted as open symbols, the activity of B itself was small (11 to 12 impulses per second); consequently the inhibition that B exerted back on A was also small (a little less than 1 impulse per second). This is

indicated by the short dotted arrows; the "tails" of these arrows are plotted at the abscissae that represent the values of the frequency obtained when ommatidium A was illuminated alone. The two points marked by the solid symbols, on the other hand, were obtained with B illuminated at higher in-

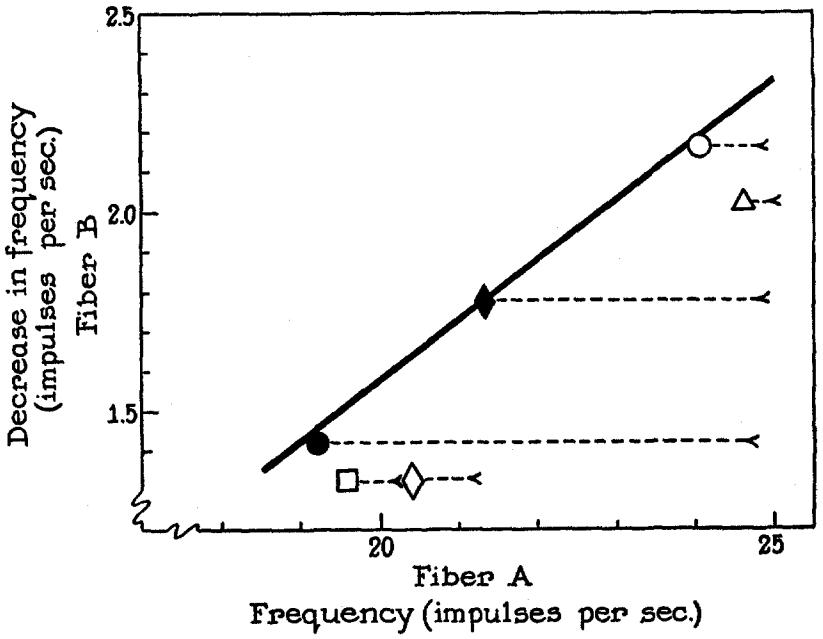

FIG. 3. Portion of the upper graph of Fig. 2. Inhibition exerted on ommatidium B is correlated with the degree of activity of ommatidium A. For the open symbols, B was illuminated at low intensity and exerted very little inhibition back on A. This is shown by the short lengths of the dotted lines, the right hand ends of which are plotted at the abscissae which give the frequency of A when it was illuminated alone. For the solid symbols, B was illuminated at high intensity and exerted strong inhibition back on A, as shown by the long lengths of the dotted lines associated with these points. For the solid symbols, ommatidium A was illuminated at the higher of the two intensities used for the open symbols. The solid line is a portion of that plotted in Fig. 2. The symbols are the same as those used for these same points in Fig. 2.

tensities. As a consequence of the resulting higher levels of activity of B (28 and 37 impulses per second), the discharge rates of ommatidium A (which for these points was illuminated at the higher of the two intensities used before) were much reduced, as can be seen by the lengths of the dotted arrows associated with these points. Corresponding to the reduced activity of A, the magnitude of the inhibition it exerted on B was smaller. This also

followed the trend of the solid line. Thus any change in the frequency of A, whether brought about directly by changing the intensity of its stimulating light, or indirectly by changing the amount of inhibition exerted upon it as a consequence of altering the level of activity of B, resulted in comparable changes in the amount of inhibition it in turn exerted upon ommatidium B. Other sets of points can be found illustrating this same principle, both in other parts of this same graph, and in the other graph of this same experiment (effect of ommatidium B on the response of A). We have performed other experiments as well, that verify this principle for the interaction of a pair of receptor units. All the observations show that an alteration in the activity of a receptor unit, whether produced by changing the intensity of light shining on it, or by changing the inhibition exerted upon it by the other member of the pair (by changing the degree of activity of the latter), results equally in an alteration of the amount of inhibition it in turn exerts upon the other member of the pair. This result sometimes has been obscured by the scatter of the points, but usually there has been good agreement (as in Fig. 3), and we have never observed a case in which this principle was violated.

In the analysis we have just made we assumed that when the intensity on ommatidium B was increased, so that it discharged impulses at a higher rate, the ensuing diminution of the inhibition on this receptor unit was solely the result of the lowered discharge rate of ommatidium A. Our interpretation is based on the experimental finding described in the previous paper (Hartline, Wagner, and Ratliff, 1956) that the magnitude of the inhibition of a receptor unit, when measured by the absolute decrease in its frequency of impulse discharge, is independent of its own level of activity. This basic result, however, was established only as an approximation; indeed, it was noted in that paper that as the level of excitation of a "test" receptor was raised, the reduction in its frequency resulting from a fixed illumination of nearby ommatidia did in fact decrease slightly but significantly, in most experiments. This was attributed to an appreciable inhibition of the nearby ommatidia by the test receptor, just as we have done in the present case. But it might be argued alternatively that the measure of inhibition we have adopted has the inherent property that it yields a smaller value as the frequency of discharge of the test receptor is increased, and that the quantitative correlation of this measure of inhibition of one receptor unit with degree of activity of the other is only fortuitous in the present experiments. Independent experimental evidence is required to establish our interpretation more firmly.

Such independent evidence is furnished by experiments in which a third spot of light has been introduced to provide additional inhibitory influences that could be controlled independently of the two interacting receptor units whose activity was being measured. We have made use of the fact that the

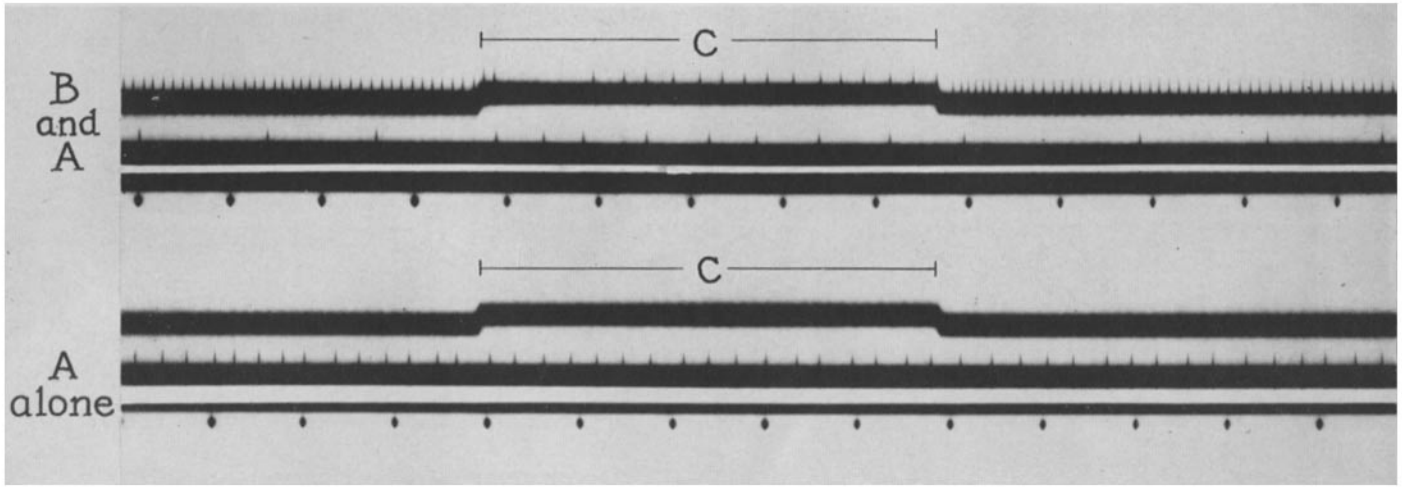

FIG. 4. Oscillograms of the electrical activity of two optic nerve fibers, showing disinhibition. In each record, the lower oscillographic trace records the discharge of impulses from ommatidium A, stimulated by a spot of light 0.1 mm. in diameter confined to its facet. The upper trace records the activity of ommatidium B, located 3 mm. from A, stimulated by a spot of light 1 mm. in diameter, centered on the facet of B, but that also illuminated approximately 8 to 10 ommatidia in addition to B. A third spot of light ("C"), 2 mm. in diameter, was directed onto a region of the eye centered 2.5 mm. from B and 5.5 mm. from A (B approximately midway between A and C); exposure of C was signalled by the upward offset of the upper trace. Lower record; activity of ommatidium A in the absence of illumination on B, showing that illumination of C had no perceptible effect under this condition. Upper record; activity of ommatidia A and B together, showing (1) lower frequency of discharge of A (as compared with lower record) resulting from activity of B, and (2) effect of illumination of C, causing a drop in the frequency of discharge of B and concomitantly an increase in the frequency of discharge of A, as A was partially released from the inhibition exerted by B.

Time marked in one-fifth seconds. The black band above the time marks is the signal of the illumination of A and B, thin when A was shining alone, thick when A and B were shining together.

inhibitory influence becomes weaker with increased separation between an affected receptor and the region of the eye used to inhibit it (Hartline, Wagner, and Ratliff, 1956). Consequently, it is often possible to find a region on the eye that is too far from the first of the two receptor units under observation to affect that one directly by an appreciable amount, but that is near enough to the second to inhibit it markedly. We then observe the effect that the altered frequency of discharge from this inhibited receptor has on the response of the first ommatidium. Fig. 4 shows oscillograms of the activity recorded simultaneously from two receptor units, showing the effects of illuminating regions of the eye in the manner just described. When one of these receptor units (A) was illuminated alone (lower trace, lower record) its activity was not appreciably affected by illuminating a distant region of the eye (C) (signalled by the upward displacement of the upper trace). When ommatidium A was illuminated together with a small region centered on ommatidium B, which was intermediate in position between A and C, the discharge of impulses by A was markedly slower than when A was illuminated alone (lower trace, upper record). This result is attributable to the vigorous activity of ommatidium B and the receptors stimulated with it, evidenced by the discharge of impulses in B's optic nerve fiber (upper trace, upper record). Then when C was turned on, the discharge rate of A actually increased, concomitantly with a decrease in frequency of discharge from B. When C was turned off, the discharge rate of B rose again and that of A fell. We interpret this result to mean that as the receptors in the region that included ommatidium B were inhibited by illumination of region C, the decrease in their activity partially released ommatidium A from the inhibition they exerted upon it. The amount of inhibition exerted on A by region B, measured by the difference in frequency of A between the lower record (A alone) and the upper (A with B) was less when C was being illuminated than when it was not; this diminished inhibition paralleled the lessened degree of activity recorded in fiber B.

The parallelism between the degree of activity of a receptor subjected to inhibition and the inhibition it in turn exerts on its neighbors is quantitative. This is shown in Fig. 5, drawn from data obtained from the same experiment as Fig. 4, except that the spot of light centered on ommatidium B was reduced in size, so that it was confined to that ommatidium. Consequently, the inhibition exerted by B may be correlated strictly with the activity recorded in its axon. In Fig. 5, the inhibition (decrease in frequency) of ommatidium A is plotted as a function of the frequency of discharge of ommatidium B; the open symbols are for two different values of light intensity on ommatidium B with no illumination on region C. The solid symbols are for a high intensity on B, but with the addition of light on the region C. The effect that C had on the discharge of B is represented by the length of the

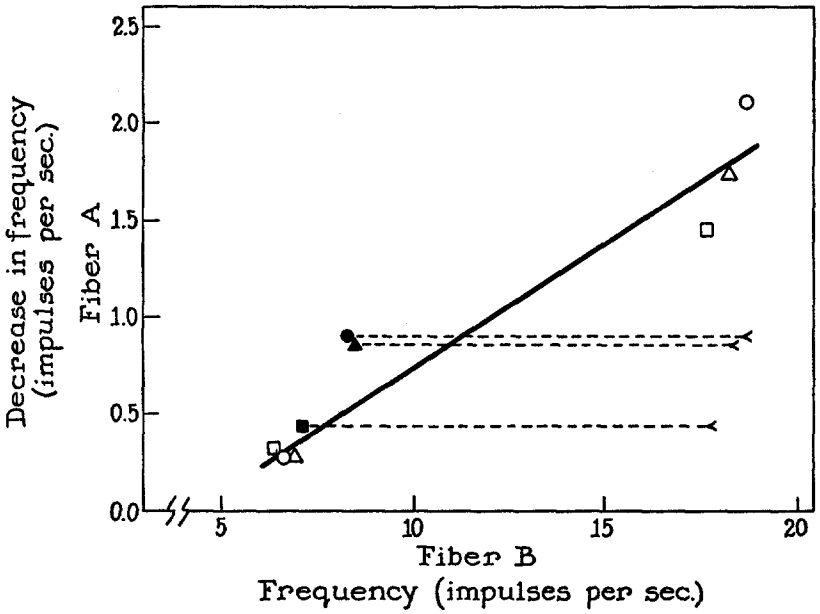

FIG. 5. Decrease of the inhibition exerted on one receptor unit (A) by another (B), as a result of inhibiting the activity of the second by illuminating a region of the eye (C) close to it. From the same experiment as that of Fig. 4 (see legend), but with the spot of light on ommatidium B reduced to 0.2 mm. diameter. The inhibition of ommatidium A was measured by the difference between its frequency when illuminated alone and when it was illuminated together with ommatidium B; this has been plotted as ordinate against the frequency of B as abscissa (as in Figs. 2 and 3). Three points were determined for a high intensity and three for a low intensity on B, when there was no illumination on C (open symbols). Three points were similarly determined for a high intensity on B when the nearby region of the eye, C, was illuminated (see legend of Fig. 4). These points are designated by the solid symbols. The lengths of the dotted lines associated with these points show the amount of reduction in the frequency of discharge of ommatidium B, as a result of the inhibition exerted upon it by C. Corresponding to this reduction in the activity of B, the inhibitory effect it in turn exerted on A was reduced, by an amount that is in quantitative agreement with the reduction obtained by lowering the intensity on B, as given by the solid line drawn through the open symbols. Illumination of the region C with no light shining on ommatidium B had very little effect on the activity of A: a reduction in frequency of 0.3 impulse per second was the maximum observed (the region C must have contributed even less than this amount to the total inhibition, since the receptors in it were also subject to inhibition by the activity of B).

Determination of the frequencies was made as described in the legend of Fig. 2.

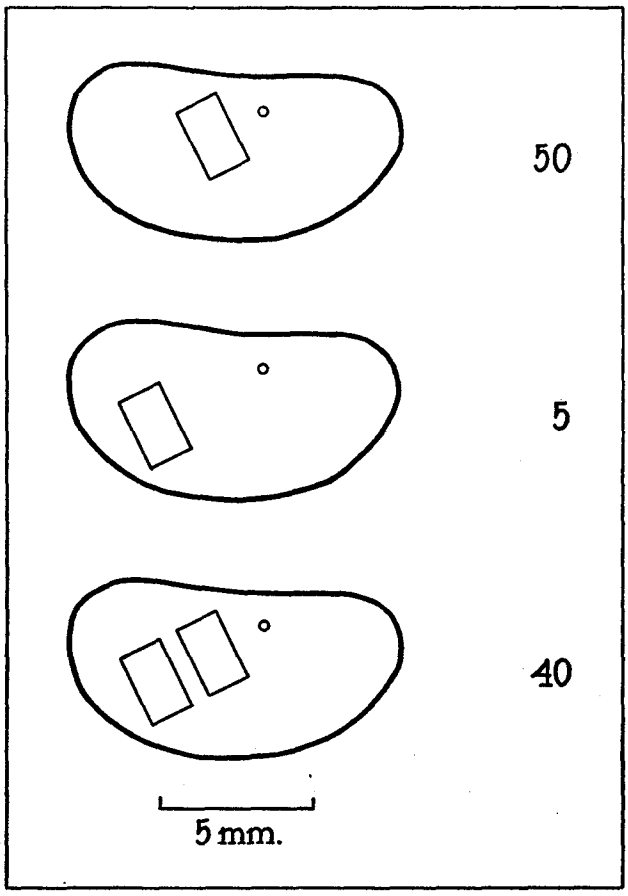

FIG. 6. A schematic diagram of patterns of light on a lateral eye of *Limulus* in an experiment illustrating disinhibition. The heavy lines are sketches of the outer margins of the eye. A small spot of light marked "o" was centered on the facet of a "test" ommatidium whose activity was measured by recording the action potentials in the optic nerve fiber arising from it. This spot was small enough to illuminate only the ommatidium on which it was centered. For each measurement the small spot of illumination was turned on for 12 seconds at a constant intensity. The number of impulses discharged by the test ommatidium in the last 10 seconds of such exposure was determined when the ommatidium was illuminated alone, and again when it was illuminated together with rectangular patches of light on other regions of the eye, as shown in the three sketches. The difference in the counts (decrease in frequency, in impulses per 10 seconds) measures the magnitude of the inhibition exerted on the test ommatidium by the receptors in the regions illuminated by the rectangular patches of light; these differences are given at the right for the respective parts of the experiment. Upper sketch, a rectangular patch of light near the test omma-

dotted lines. This experiment shows that the diminution in activity of ommatidium B had the same effect in reducing the inhibition exerted on A, whether that diminution was the result of inhibition of B by illumination of region C, or the result of reduction in the intensity of the light stimulus to B. Thus the degree of activity of a receptor unit does indeed determine, quantitatively, the strength of the inhibition it exerts on another receptor unit. Our analysis of the interaction between two receptor units illuminated together is therefore substantiated.

The release of a receptor unit from the inhibiting effects of others, by causing those others to be inhibited by yet a third group of receptors, is interesting physiologically. Such "disinhibition" is not difficult to obtain, though it may require some pains to show a strong effect. We have performed one other experiment similar to that of Figs. 4 and 5, recording from two fibers and using a third spot of light to inhibit one and disinhibit the other. It is considerably easier to show disinhibition when recording from only one receptor, for then it is possible to choose a favorable combination of locations for the spots of light that serve to inhibit and to disinhibit this test receptor. We have done many such experiments. An example is given in Fig. 6; the experiment is explained in its legend. Instead of focussing spots of light in various locations on the surface of the eye, disinhibition can also be demonstrated by using sources of light in various places in the external visual field, where the directional sensitivity of the ommatidia determines the location in the eye of the groups of receptors stimulated by the respective light sources. Dr. William Miller, in our laboratory, has also demonstrated disinhibition of receptors in the median eye of *Limulus* (a simple eye), using light sources in the external visual field.

Disinhibition simulates facilitation: illumination of a distant region of the eye results in an increase in the activity of the test receptor. In the *Limulus* eye the dependence of such an effect on the stimulation of receptors in an intermediate region of the eye (to produce the original inhibition) makes it

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tidium produced a decrement of 50 impulses in 10 seconds. Middle sketch, a similar rectangular patch of light farther away from the test ommatidium produced a decrement of 5 impulses in 10 seconds. Lower sketch, both patches of light shining together produced a decrement of only 40 impulses in 10 seconds.

Thus in the last case the distant patch, rather than adding to the inhibition exerted by the near one, caused a disinhibition of 10 impulses per 10 seconds. As established by the experiments of Figs. 4 and 5, this was the result of the inhibition of the receptors in the near patch by the activity of those in the distant one, with the consequence that they in turn exerted less inhibition on the test ommatidium. This release of the test ommatidium from the inhibition exerted by the receptors in the near patch was greater than the slight inhibitory effect exerted directly on the test ommatidium by the receptors in the distant patch.

easy to recognize the mechanism involved. But if such a group of intermediate inhibiting elements were active spontaneously, or through uncontrolled influences, it might be difficult to recognize the true nature of a disinhibiting action. Perhaps the most significant aspect of these experiments showing disinhibition is the principle that they reveal, that indirect effects may extend considerably beyond the limit of the direct inhibitory connections among the receptors of the eye. Indeed, no member of the population of receptors is completely independent, under every condition, of the activity in any part of the eye. This is a direct consequence of the principle of interaction that we have established: the inhibiting influence exerted by a receptor depends on its activity, which is the resultant of the excitatory stimulus to it and whatever inhibitory influences may in turn be exerted upon it.

The principles that we have established experimentally may be conveniently summarized in a simple algebraic expression. The activity of a receptor unit—its response (r)—is to be measured in the present case by the frequency of the discharge of impulses in its axon. This response is determined by the excitation (e) supplied by the external stimulus to the receptor, diminished by whatever inhibitory influences may be acting upon the receptor as a result of the activity of neighboring receptors. The excitation of a given receptor is to be measured by its response when it is illuminated by itself, thus lumping together the physical parameters of the stimulus and the characteristics of the photoexcitatory mechanism of the receptor. Each of two interacting receptor units inhibits the other to a degree that depends (linearly) on its own activity. The responses of two such units are therefore given by a pair of simultaneous equations:

$$r_A = e_A - K_{A,B}(r_B - r_B^0)$$

$$r_B = e_B - K_{B,A}(r_A - r_A^0)$$

in which the subscripts are used to label the respective receptor units. In each of these equations, the magnitude of the inhibitory influence is given by the last term, written in accordance with the experimental findings as a simple linear expression. The "threshold" frequency that must be exceeded before a receptor can exert any inhibition is represented by r^0. The "inhibitory coefficient," K, in each equation is labelled to identify the direction of the action: $K_{A,B}$ is the coefficient of the action of receptor B on receptor A; $K_{B,A}$ *vice versa*. It is to be clearly understood that the equations do not apply in the ranges of responses for $r < r^0$ in either case: negative values of inhibition must be excluded since they are never observed. Also, r and e, by their nature (being measured by frequencies), cannot be negative. Appropriate changes must be made in the equations in those ranges of the variables where these restrictions apply: if, for example, $r_B < r_B^0$, the first equation must be replaced by $r_A = e_A$; if, to choose another example, r_B is

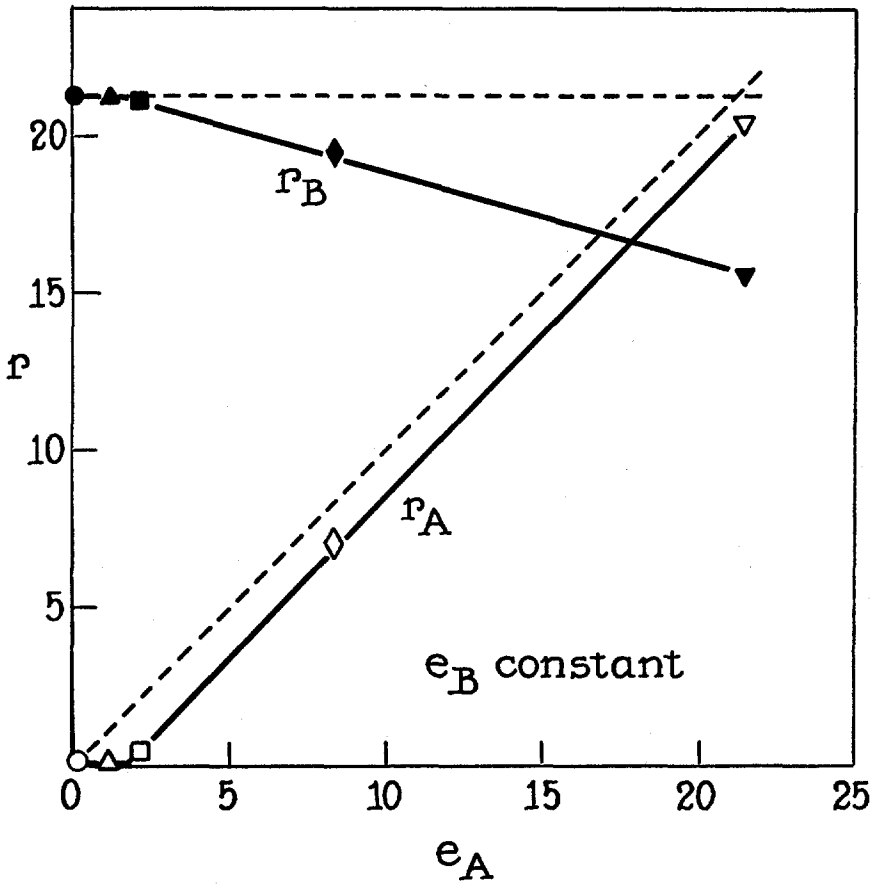

FIG. 7. Graph showing the relation of the responses of two interacting receptor units. One ommatidium (B) was illuminated at a fixed intensity; the intensity on the other (A) was varied. Responses (r) were measured by the frequency of the steady discharge of nerve impulses (last 7 seconds of a 10 second exposure to light). When both A and B were illuminated together, r_A refers to the response of ommatidium A (open symbols), r_B refers to the response of ommatidium B (solid symbols). The excitation of A, designated by e_A, is measured by the response of this ommatidium when it was illuminated alone; the excitation of B, designated by e_B, is measured by the response of B when it was illuminated alone. For each set of exposures (A alone, B alone, A and B together) with a given intensity for ommatidium A, the values of r_A and r_B obtained have been plotted (ordinates) against the value of e_A (abscissa). Values obtained for e_B were consistent within 0.1 impulse per second for all the sets of exposures; their average (21.2 impulses per second, shown by the horizontal dotted line) has been used in the calculations. The solid lines are the solutions of the simultaneous equations given in the text; the calculations and values of the constants are given there. The vertical distance from each dotted line ($r = e_A$, and $r = e_B$) to the corresponding solid line beneath it shows the amount of the inhibition for each receptor at that value of e_A.

so great that $e_A < K_{A,B}(r_B - r_B^0)$, then the first equation must be replaced by $r_A = 0$.

In Fig. 7 we give the results of an experiment using two receptor units to illustrate the solutions of the simultaneous equations governing their responses. A small spot of light centered on ommatidium B was maintained at a fixed intensity, such as to give a frequency of discharge (when it was shining alone) of 21.2 impulses per second in the optic nerve fiber from this receptor unit. This is the value of e_B. Another small spot, centered on ommatidium A, was set at various intensities of illumination; at each intensity the steady frequency of discharge of this receptor unit was determined when the spot illuminating it was shining alone. These measurements give the value of e_A, for the corresponding stimulus intensities. The frequencies of the discharges obtained when the two receptor units were illuminated together are plotted for each one (r_A and r_B) as functions of e_A. On another graph (not shown) similar to Fig. 2 we plotted the decrease in frequency ($e - r$) for each unit as a function of the corresponding frequency (r) of the other, obtaining plots which, when fitted by straight lines, gave values of the intercepts $r_A^0 = 0$ (an unusual value, in our experience), $r_B^0 = 4.0$, and slopes $K_{A,B} = 0.09$, $K_{B,A} = 0.26$. The solid lines of Fig. 7 are drawn as determined by these values of the constants in the solutions of the simultaneous equations given above. For low intensities of illumination on ommatidium A (small values of e_A), activity of this receptor was prevented ($r_A = 0$) by the strong inhibition exerted by the activity produced by illumination of ommatidium B, and therefore, since no inhibition was exerted on B, the activity of B was the same as when it was illuminated alone ($r_B = e_B$). At the intensity for which the excitation e_A just overcame the effects of the inhibition exerted by B ($e_A = K_{A,B}[e_B - r_B^0] = 1.6$), receptor A began to respond; as e_A increased, the frequency of its discharge increased linearly with a slope of $1.024 \left(= \frac{1}{1 - K_{A,B} K_{B,A}} \right)$. At this same value of e_A (since $r_A^0 = 0$), the inhibition by A on B began to be exerted, and r_B decreased linearly with increasing e_A, the slope being $-0.27 \left(= \frac{-K_{B,A}}{1 - K_{A,B} K_{B,A}} \right)$. (The dotted lines show the form the graphs would have taken had there been no inhibition.) In this experiment each spot of light actually illuminated about 8 or 9 other ommatidia in the immediate vicinity of the one on which it was centered and from which activity was recorded. For purposes of illustration, it is permissible to treat the results as though individual units were interacting, although in actuality it was each small group. That the principles involved hold rigorously when only two single receptor units are actually used is inherent in the treatment, for these principles were derived from the experi-

ment of Fig. 2 (and those like it), in which strict optical isolation of individual ommatidia was employed.

The mutual interdependence of two receptor units responding to steady illumination is thus concisely and accurately described by a pair of simultaneous equations. Similar equations hold for the responses of any two ommatidia that are close enough together in the eye to interact. When more than two interacting elements are activated, similar relations apply simultaneously to the responses of all of them. In addition, however, each receptor unit is subjected to inhibitory influences from all the others, and the degree to which its response is decreased is known to be greater, the greater the number of neighboring ommatidia that are stimulated (Hartline, Wagner, and Ratliff, 1956). The simultaneous equations governing the responses of more than two ommatidia therefore must contain terms expressing the inhibition contributed from all the active elements, combined according to some law of spatial summation. Experiment shows that simple arithmetic addition of such terms is adequate to describe spatial summation of inhibitory influences in the eye of *Limulus*. In a paper that will follow (see also Hartline and Ratliff, 1954 and Ratliff and Hartline, 1956) we will describe the experiments that establish this law of spatial summation and will illustrate some of the effects that are obtained when more than two receptor units in the eye inhibit one another mutually.

SUMMARY

The inhibition that is exerted mutually among the receptor units (ommatidia) in the lateral eye of *Limulus* has been analyzed by recording oscillographically the discharge of nerve impulses in single optic nerve fibers. The discharges from two ommatidia were recorded simultaneously by connecting the bundles containing their optic nerve fibers to separate amplifiers and recording systems. Ommatidia were chosen that were separated by no more than a few millimeters in the eye; they were illuminated independently by separate optical systems.

The frequency of the maintained discharge of impulses from each of two ommatidia illuminated steadily is lower when both are illuminated together than when each is illuminated by itself. When only two ommatidia are illuminated, the magnitude of the inhibition of each one depends only on the degree of activity of the other; the activity of each, in turn, is the resultant of the excitation from its respective light stimulus and the inhibition exerted on it by the other.

When additional receptors are illuminated in the vicinity of an interacting pair too far from one ommatidium to affect it directly, but near enough to the second to inhibit it, the frequency of discharge of the first increases as it is partially released from the inhibition exerted on it by the second (disinhibition).

Disinhibition simulates facilitation; it is an example of indirect effects of interaction taking place over greater distances in the eye than are covered by direct inhibitory interconnections.

When only two interacting ommatidia are illuminated, the inhibition exerted on each (decrease of its frequency of discharge) is a linear function of the degree of activity (frequency of discharge) of the other. Below a certain frequency (often different for different receptors) no inhibition is exerted by a receptor. Above this threshold, the rate of increase of inhibition of one receptor with increasing frequency of discharge of the other is constant, and may be at least as high as 0.2 impulse inhibited in one receptor per impulse discharged by the other. For a given pair of interacting receptors, the inhibitory coefficients are not always the same in the two directions of action. The responses to steady illumination of two receptor units that inhibit each other mutually are described quantitatively by two simultaneous linear equations that express concisely all the features discussed above. These equations may be extended and their number supplemented to describe the responses of more than two interacting elements.

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