Facilitation of Inhibition in the Compound Lateral Eye of *Limulus*

(retina/threshold of inhibition/calcium/synapse)

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ABSTRACT **In the compound eye of *Limulus* the inhibitory effect of a burst of impulses from one group of ommatidia on the response of a neighboring ommatidium is greater when that burst is preceded by another burst of impulses. This facilitation of inhibition decays slowly, with a time constant of several seconds. Facilitation of inhibition accumulates as the number of impulses in the first burst increases, but there is a maximum that it cannot exceed. The facilitation is localized; one group of ommatidia does not facilitate the inhibition exerted by another group. The mechanism of this facilitation may be similar to that which has been postulated for facilitation of excitatory influences at the neuromuscular junction.**

Ommatidia in the compound lateral eye of *Limulus* inhibit one another mutually (1). When several ommatidia are simultaneously active, the total inhibition exerted on any one appears to be the simple arithmetic sum of the separate inhibitory effects exerted on it by each of the others. This study considers a facilitation of inhibition that may be inherent in the summation of sub-threshold inhibitory influences (2–5).

The steady-state responses of n ommatidia interacting with one another may be described, to a good first approximation, by a set of n simultaneous equations—one for each ommatidium—in each of which the supra-threshold inhibitory influences from the other ommatidia are simply summed (2):

$$r_p = e_p - \sum_{j=1}^{n} k_{p,j}\,(r_j - r^0_{p,j})$$

The response r_p (frequency of discharge) of a particular ommatidium p is given by the excitation e_p diminished by the summed inhibitory influences of the other ommatidia j; each inhibitory influence is given by the inhibitory coefficient $k_{p,j}$ multiplied by the response r_j that exceeds the threshold $r^0_{p,j}$.

It is implicit in these equations that there is no sub-threshold summation of inhibitory influences from different ommatidia or, in other words, that there are separate thresholds $r^0_{p,j}$ for each ommatidium j that inhibits ommatidium p rather than one threshold that the sum of the inhibitory activity on p from all ommatidia j must overcome. Although many lines of evidence indirectly support this implicit assumption, it has not previously been tested by direct experiment, except for one preliminary observation (4). The first objective of the work reported here is to make such a test. A second objective is to explore the dynamics of sub-threshold influences. The steady-state equations have now been extended to include the dynamics (3), but as now written this set of dynamic equations is limited to the supra-threshold range. A third objective is to consider possible neural mechanisms for the threshold of inhibition, which has yet to be analyzed in terms of cellular or molecular processes, although there are recent studies on the anatomy of the inhibitory pathways (6).

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METHODS

Compound lateral eyes of adult horseshoe crabs (*Limulus polyphemus*) were used in these studies. The techniques were based on earlier experiments in which activity of single optic nerve fibers was recorded (7) and in which facilitation of inhibition was studied either with light stimuli to elicit activity in neighboring fibers (4) or with electrical stimuli to elicit antidromic activity in them (3, 5). An eye was excised, along with about 1 or 2 cm of the optic nerve, and mounted in the front of a moist chamber partially filled with *Limulus* blood. A small bundle containing a single active axon was dissected from the optic nerve and placed on cotton wick silver–silver chloride electrodes. A single glass optical fiber, 70 μm in diameter, was used to illuminate that ommatidium (the "test" ommatidium) from which the axon arose.

For those experiments done exclusively with light stimulation, either one or two neighboring groups of ommatidia were also illuminated through separate small bundles of glass optical fibers. Each of these bundles was 2 mm in diameter and illuminated a circular group of about 50 ommatidia. The single optical fiber and the two bundles of fibers were illuminated by light from glow modulator tubes (Sylvania R 1131-C) focused on their far ends.

For those experiments in which the activity of neighbors was controlled by electrical stimulation, the whole optic nerve, except for the single axon from the test ommatidium, was placed on three electrodes. The cut end of the nerve was suspended in air by two platinum iridium electrodes, spaced about 2 mm apart. These served as the stimulating electrodes through which current was passed to elicit antidromic volleys of impulses. The remainder of the nerve was immersed in the blood, except for a short loop about midway between the eye and the stimulating electrodes. This loop was placed on a platinum iridium hook, which held it in contact with a cotton wick silver–silver chloride electrode. This electrode recorded the compound action potential, which served as a monitor to ensure that the electrical stimulation of the nerve was equally effective throughout the experiment.

Preset timers controlled the onset and duration of the light stimuli and electrical stimuli. Episodes of stimulation were 10 sec in duration, spaced 120 sec apart. All experiments were done at room temperature (about 19°). Data were recorded by a small computer (CDC 160-A) and stored on digital tape (8). Instantaneous frequencies could be plotted either as the reciprocal of inter-impulse interval at each moment of time (the single records of Fig. 2) or as the number of impulses per 50-msec time bin (the averaged records of Figs. 1, 3, and 4).

RESULTS

The inhibitory effect of a burst of impulses from one group of ommatidia on the response of a neighboring test ommatidium is greater when the burst follows shortly after another burst of impulses than when it occurs alone (4, 5). The thick trace of Fig. 1 shows the inhibition of the test ommatidium's response when its neighbors were stimulated with one short flash of light. The thin trace shows the facilitation of the inhibition when this one short flash was preceded by an identical flash. Increasing the separation of the two flashes decreases the amount of facilitation. However, facilitation often persists at least 10 sec. For a fixed separation of the two flashes, increasing the intensity or duration of the first flash increases the amount of facilitation up to some limit. Over a considerable range the effects of changing intensity and duration of the first flash in opposite directions can cancel

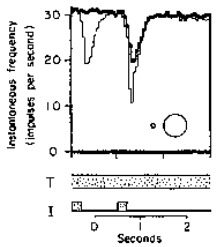

FIG. 1. Facilitation of inhibition. The graphs show the instantaneous frequency of impulses discharged by a single-test ommatidium from the 7th to 10th sec of a 10-sec period of steady illumination. The steady illumination on the test ommatidium is indicated by the *stippled bar* labeled *T* underneath the graph. The *thin-line* graph of instantaneous frequency shows the test ommatidium response when neighboring ommatidia were illuminated by two short (0.2 sec) flashes of light at the 7th and 8th sec. These two short flashes causing inhibition are indicated by the *stippled squares* on the line labeled *I* at the bottom of the figure. The *thick trace* shows the response of the test ommatidium during control trials when neighboring ommatidia were illuminated by one short flash of light at the 8th sec. A 1-sec adapting flash of light illuminated the neighboring ommatidia at the beginning of the 10-sec period of illumination of the test ommatidium. This adapting flash was used so that the responses of the illuminated group of neighbors to both of the later flashes of light would be approximately of the same size. Without the adapting flash, or if the adapting flash is not strong enough, the response of the illuminated group of neighbors to the second of two light flashes will be smaller than its response to the first, thus tending to obscure any facilitation of inhibition. The spatial configuration of illumination is shown by the *two circles*. The *small circle* represents the 0.25-mm diameter facet of the single test ommatidium, the *large circle* represents the 2-mm diameter spot of light on neighboring ommatidia (about 50). Each trace is the average of three episodes.

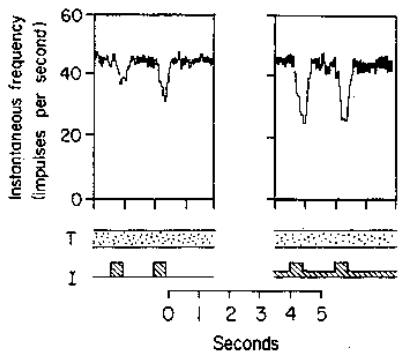

FIG. 2. Effect of a steady background. Symbols are as in Fig. 1, except that the *hatched symbols* on lines labeled *I* indicate antidromic stimulation of neighboring ommatidia. The *left graph* shows the instantaneous frequency of the test ommatidium when its neighbors were stimulated by two bursts of 10 antidromic shocks (at a rate of 25 per sec) that occurred at the 4.6th and 6th sec of the 10-sec period of steady illumination of the test ommatidium. The *right graph* shows the instantaneous frequency of the test ommatidium when its neighbors were stimulated at a steady rate of 10 shocks per sec increased to 25 per sec during the two time periods corresponding to the two bursts in the left graph. Each trace is a single episode. The same effect can be produced by light stimulation.

each other, producing an approximately constant amount of facilitation. In fact, experiments in which the neighbors are stimulated antidromically, and thus their activity is known exactly, show that the amount of facilitation depends only on the total number of impulses discharged by the neighbors during the preceding period of at least several seconds. This integration of preceding activity over several seconds again suggests a long time constant for the underlying process. However, as the duration of a moderately intense first flash is increased, the amount of facilitation does not continue to increase over this period of several seconds; it reaches a maximum at a duration of less than 1 sec. This result suggests that there is a maximum to the amount of facilitation possible.

Such a maximum was demonstrated in the experiment of Fig. 2. Antidromic stimulation of the axons of neighboring ommatidia was used in order to control their activity exactly. The graph on the left shows facilitation of inhibition like that in Fig. 1: The second burst produced more inhibition than the first. The graph on the right of Fig. 2 shows the effect of the same two antidromic bursts when they were imbedded in a background of antidromic stimulation. With this background activity the inhibition caused by both bursts was much greater than that when there was no background, that is, the steady background facilitated the inhibitory effect of the brief bursts. However, the second burst did not cause any more inhibition than the first: The first burst did not facilitate the effect of the second burst. The effect of a background on the inhibition produced by the two bursts is graded. As the background was increased from zero upwards, the response of the test ommatidium gradually changed from that in the left record to that in the right. In the record on the right the steady background was evidently producing the maximum amount of facilitation possible, and therefore the first burst could not contribute any additional facilitation.

The next question considered was whether a burst of nerve impluses coming from one neighbor of the test ommatidium

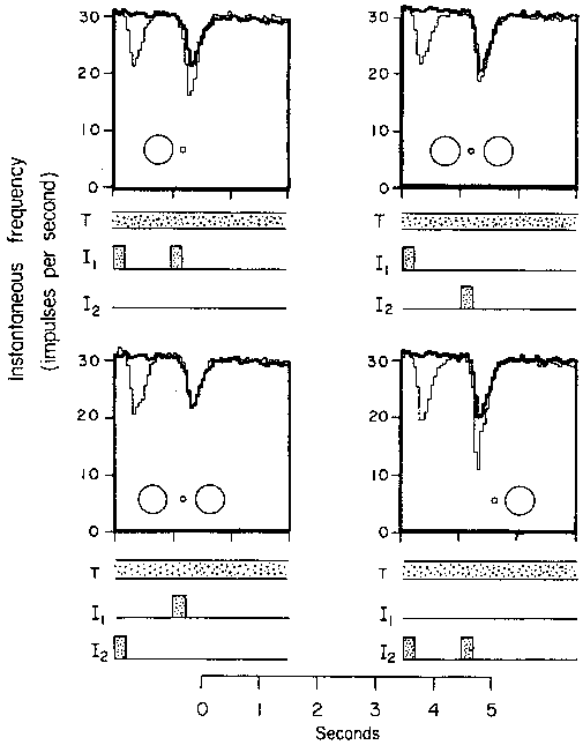

FIG. 3. Localization of facilitation of inhibition. Symbols as in Fig. 1, except that the lines labeled I and I_2 represent the stimulation of two different groups of neighboring ommatidia. The *thin traces* show the instantaneous frequency of the test ommatidium when its neighbors were stimulated by two 0.2-sec flashes of light occurring at the 7th and 8th sec of the 10-sec period of illumination of the test ommatidium. In the *upper left graph* both flashes illuminated the left-hand group of neighbors, and in the *lower right graph* both flashes illuminated the right-hand group of neighbors. In the *upper right* and *lower left graphs* the first flash illuminated one group of neighbors and the second flash illuminated the other. The *thick traces* show the instantaneous frequency when the neighbors were illuminated only once by a flash corresponding in temporal and spatial location to the second of the two flashes used for the thin traces. A 1-sec adapting flash of light illuminated both groups of neighboring ommatidia at the beginning of the 10-sec period of illumination of the test ommatidium. Each trace is the average of three episodes. (The small apparent facilitation in the upper right-hand graph is not significant.)

will facilitate the inhibitory effect of a later burst of nerve impulses coming from another neighbor. (The facilitation described thus far has always resulted from a second stimulus activating the same axons as the first.) Because it is impractical to measure the effect of only one neighbor at a time (the amount of inhibition is too small), two groups of about 50 neighbors each were used in the experiment of Fig. 3. When both flashes of light illuminated the same group of neighbors (graphs at top left and bottom right), there was facilitation of inhibition--the inhibition produced by the first flash increased the inhibitory effect of the second flash. When the two flashes illuminated different groups of neighbors (graphs at bottom left and top right) there was no facilitation.

If there is a maximum facilitation (as indicated by the results illustrated in Fig. 2) and if facilitation is indeed localized (Fig. 3), then a steady background of inhibition produced by the activity of one group of ommatidia should only limit the further facilitation of inhibition produced by members of that same group; it should not affect the facilitation of inhibition by another group of ommatidia. Results of an experiment demonstrating this are shown in Fig. 4. The left side of the figure shows the results of an experiment similar to that in Fig. 2, except that light rather than antidromic stimulation was used. Inhibition of the test ommatidium was elicited from one neighboring area by illuminating it with flashes of light superimposed on a steady background of lower illumination. As in Fig. 2, the first flash produced no facilitation of the inhibitory effect of the second flash. The right side of the figure also shows an experiment similar to that of Fig. 2, except that the steady background light illuminated a different group of ommatidia from that illuminated by the flashes of light. Here there was facilitation. Evidently, when the background illuminated the same ommatidia as those illuminated by the flashes, the background drove the facilitation to its maximum. However, when the background illuminated a different group of ommatidia, it did not affect the mechanisms of facilitation of those ommatidia illuminated by the flashes, and thus, could not drive that facilitation to its maximum.

Each of the above experiments was repeated on several *Limulus* eyes, and qualitatively, the results were always the same. Quantitatively, however, there was considerable variation from eye to eye, and there were occasional preparations where facilitation of inhibition was not found at all.

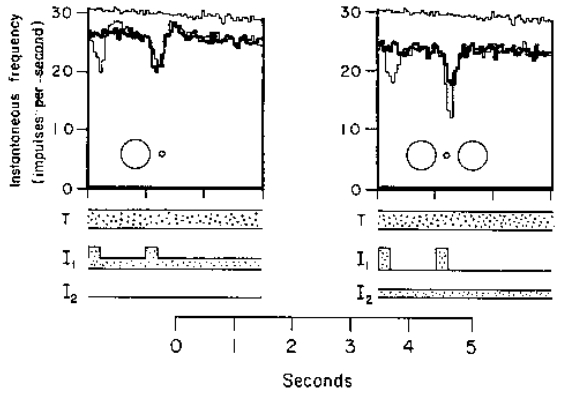

FIG. 4. Localization of the effect of steady background inhibition. The *upper thin line* is the instantaneous frequency of the test ommatidium in response to the steady illumination on it but in the absence of any light on its neighbors. The *lower thin line* is the instantaneous frequency when there were two 0.2-sec flashes of light plus a steady background of light on the neighbors, and the *thick line* is the instantaneous frequency when there was only one 0.2-sec flash plus the background on the neighbors. In the *left graph* the short flashes and the steady background illuminated the same group of neighboring ommatidia; in the *right graph* the short flashes and steady background illuminated groups of ommatidia on opposite sides of the test ommatidium. The group of ommatidia illuminated by the two short flashes (occurring at the 7th and 8th sec of the 10-sec period of illumination of the test ommatidium) was also illuminated by a 2-sec adapting flash at the beginning of the 10 sec.

The absence of facilitation was noticed only in the course of some experiments with light stimuli. In retrospect, it appears that this absence of facilitation may have been due to use of an adapting flash of light that preceded the two short flashes of light by only 4–7 sec in those experiments. It is possible that for some eyes the adapting flash produced maximum facilitation that persisted long enough to prevent any further facilitation in the responses to the two short flashes.

DISCUSSION

The inhibitory effect of nerve impulses from one ommatidium in the *Limulus* lateral eye on the activity of another ommatidium is not constant but is larger (facilitated) when there have been preceding inhibitory impulses. Within limits, the amount of facilitation depends on the integral of the preceding activity (the number of nerve impulses no matter how distributed in time). The decay of facilitation is slow—there is still facilitation after several seconds. There is a maximum that the amount of facilitation cannot exceed. And nerve impulses from one group of ommatidia cannot facilitate the inhibitory effect of nerve impulses from another group of ommatidia, at least not when the groups are rather widely separated.

A nonlinear model of the dynamics of the response of an ommatidium that can account for some aspect of facilitation was proposed earlier (5). This model was an extension of the steady-state equations to nonsteady-state conditions. Each impulse from a neighboring ommatidium j produced an inhibitory influence that lasted for some time (half-time about 500 msec) and added to other inhibitory influences to form a "lateral inhibitory pool." A static threshold for the pool was assumed ($r^0_{p,j}$ is constant) so that the activity of the test ommatidium was inhibited only when the level of the lateral inhibitory pool exceeded the static threshold level. [Whether each neighbor had its own pool or they all contributed to one pool was not an issue in the earlier studies (5 and 6).] This model explained the facilitation in the absence of a steady background (Fig. 1) by assuming that the inhibition due to the second flash is added to residual subthreshold inhibition left in the lateral inhibitory pool by the first flash. However, according to the early model, the presence of steady background inhibition (right half of Fig. 2) should merely raise the absolute level of lateral inhibition without changing the form of the transients due to the two short flashes. In particular, the maximum inhibition in the transient produced by the second flash should still be greater than that in the transient produced by the first flash. Or, in other words, the minimum instantaneous frequency in the transient produced by the second flash should still be less than that in the transient produced by the first flash. That this is not the case can be seen in the right half of Fig. 2.

In order to account for the experimental results in this paper, the earlier model may be revised. Instead of assuming $r^0_{p,j}$ is constant, assume $r^0_{p,j}$ decreases as a result of past activity of the inhibiting ommatidium j and recovers slowly when that activity ceases. In particular, assume that the threshold is highest when there has been no preceding activity and that it gets lower as the amount of immediate past activity increases until at some point the threshold reaches a minimum level (the "steady-state" level). With this assumption, the second of two identical inhibitory pulses from ommatidium j will produce a greater decrement in the test ommatidium's firing rate than the first because the threshold will be lower for the second pulse than for the first. The existence of a minimum level for the threshold will account for the maximum to the facilitation effect, and postulating that past activity is integrated over a long time (seconds) in determining the threshold will account for the long time-constant of the facilitation effect. In the steady state, this model reduces to the original steady-state equations. At present there is no clear interpretation of this model in terms of underlying physiological mechanisms.

In looking for possible physiological mechanisms, it may be worthwhile to consider the facilitation of excitatory influences found at the frog neuromuscular junction. The second of two nerve impulses arriving at the frog neuromuscular junction produces a larger end-plate potential in the muscle than does the first, and the time delay over which this facilitation can last is long compared to the time-course of other synaptic events. The end-plate potential is known to depend on external calcium-ion concentration. This has led to the hypothesis that the release of neural transmitter at the junction synapse can only occur when calcium ions are "bound to critical sites" on the pre-synaptic membrane (9) and that facilitation is due to residual calcium ions left from the first nerve impulse that are still bound to critical sites at the time of the second nerve impulse (10). The cooperative action of about four calcium ions at a critical site is apparently necessary for the release of a neural transmitter packet (11). The hypothesis that facilitation in the frog neuromuscular junction is due to residual calcium ions left bound to critical sites on the pre-synaptic membrane has been tested with some success (12, 13). Qualitatively, at least, this hypothesis could explain many of the characteristics of inhibition in the *Limulus* eye. A threshold for inhibition in the steady state ($r^0_{p,j}$ in the original steady-state equation) could result if there were no post-synaptic effect at all whenever the number of critical sites filled by critical ions was too low. Facilitation of inhibition would result from residual ions left bound to critical sites and its time-course would depend on the time it takes ions to become unbound, which might be long. A maximum to the facilitation effect would result if there were a maximum number of critical sites to be filled. And inhibitory impulses from one group of ommatidia would not facilitate the effect of inhibitory impulses from another group of ommatidia if the impulses from the two groups arrived at different portions of pre-synaptic membrane.

Whether one regards facilitation as the result of an abstract threshold whose level is dependent on the amount of immediate past inhibitory activity or as the result of a concrete threshold-producing mechanism involving ions and membranes such as that hypothesized for the frog neuromuscular junction, the results shown in Figs. 3 and 4 support the assumption of the original steady-state equation that there is a separate threshold for each inhibiting ommatidium j rather than one threshold that the sum of the inhibitory influences on p must overcome. For, in abstract terms, if there were only one threshold, it must be affected by past activity from both groups of ommatidia inhibiting the test ommatidium (since both show facilitation when stimulated alone), and thus, there should be cross-group facilitation effects. Figs. 3 and 4 show that there are none. Likewise, in the more concrete terms of the mechanism postulated for the

frog neuromuscular junction, sub-threshold activity has no inhibitory effect because it does not leave enough ions bound to the critical sites. The results in Figs. 3 and 4 show that the activity of one ommatidium does not contribute to the critical sites associated with another ommatidium.

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