

# Physiology of Photoreceptor Neurons in the Abdominal Nerve Cord of the Crayfish

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**ABSTRACT** Nerve fibers which respond to illumination of the sixth abdominal ganglion were isolated by fine dissection from connectives at different levels in the abdominal nerve cord of the crayfish. Only a single photosensitive neuron is found in each connective; its morphological position and pattern of peripheral connections are quite constant from preparation to preparation. These cells are "primary" photoreceptor elements by the following criteria: (1) production of a graded depolarization upon illumination and (2) resetting of the sensory rhythm by interpolated antidromic impulses. They are also secondary interneurons integrating mechanical stimuli which originate from appendages of the tail. Volleys in ipsilateral afferent nerves produce short-latency graded excitatory postsynaptic potentials which initiate discharge of one or two impulses; there is also a higher threshold inhibitory pathway of longer latency and duration. Contralateral afferents mediate only inhibition. Both inhibitory pathways are effective against both spontaneous and evoked discharges. In the dark, spontaneous impulses arise at frequencies between 5 and 15 per second with fairly constant intervals if afferent roots are cut. Since this discharge rhythm is reset by antidromic or orthodromic impulses, it is concluded that an endogenous pacemaker potential is involved. It is postulated that the increase in discharge frequency caused by illumination increases the probability that an inhibitory signal of peripheral origin will be detected.

## INTRODUCTION

If the sixth abdominal ganglion of the crayfish is illuminated, either directly or through the translucent exoskeleton which overlies it, a tonic increase in the level of impulse activity may be recorded from the whole ventral nerve cord. This observation, first made by Prosser in 1934, was the first demonstration of "natural" photosensitivity in neurons not normally associated with visual function, and it has been followed by a number of others, especially in molluscs (Arvanitaki and Chalazonitis, 1949, 1961; Kennedy, 1961).

More detailed analysis of the various properties of this sensory system in crayfish became possible upon the development of a technique for recording the activity from isolated nerve cords, in which the competing spontaneous

activity was reduced or eliminated by bathing the excised cord in cold Ringer's solution. From such preparations, discharges could be obtained from the photosensory fibers virtually in isolation. The experiments (Kennedy, 1958 *a*, 1958 *b*) showed (1) that the discharge pattern is tonic, exhibiting very little adaptation (following an initial peak frequency) to a constant stimulus; (2) that the critical duration for adherence to the reciprocity relation (intensity  $\times$  duration = constant) is very long, about 1 second; (3) that threshold latency for stimuli of long duration may be as long as 10 seconds; (4) that subthreshold, brief flashes leave in their wake an excitatory state which can persist and sum with a second flash as much as 10 seconds later, and that this facilitation can also be demonstrated when the conditioning exposure is above the threshold for impulse discharge; and (5) that only a few (two or possibly more) neurons are involved in the production of discharges in response to light. Other experiments (Bruno and Kennedy, 1962) showed that the neurons exhibit a spectral sensitivity peak at 500 m $\mu$ , resembling in form the absorption spectra of rhodopsins from the compound eyes of other decapod crustacea. It seems fair to conclude that these sensory elements are equipped with a photochemical system not radically unlike those of other light-sensitive cells, but that the over-all time constant of the receptor neurons by a number of different criteria is about 10 times as long as that, for example, of the single *Limulus* ommatidium (*cf.* Hartline *et al.*, 1952).

Because of the gross nature of the recording situation, however, it was impossible in these experiments to specify anything about the anatomical localization or central connections of the receptor units. The work of Wiersma and his associates (Wiersma, 1958; Hughes and Wiersma, 1960), which clearly demonstrated the feasibility of identifying functional single entities within the crayfish central nervous system, lent encouragement to the possibility that the photoreceptor neurons might be isolated as single fibers from the intact ventral nerve cord. This paper describes the results of recording from such single-fiber preparations. Some of the more tentative conclusions reached earlier have been confirmed and some new information about the nature and origin of the sensory discharge has been obtained. In addition, however, the discovery that the photoreceptor neurons are also second order interneurons relaying tactile stimuli from the tail has made possible an analysis of the interaction between presynaptic excitation, inhibition, and autogenic activity. Finally, it is shown that only a single photoreceptor neuron occurs in each symmetrical half of the nerve cord; thus sensitivity to light affords a unique label which may be used for identification and analysis of the same individual neural element in different preparations. This is an important advantage in a preparation where each central neuron engages in unique relationships with others, and in which assignment of properties cannot be made

on the basis of population responses. This paper is the first in a series of analyses of the functional connections of individually identifiable interneurons in the crayfish.

#### METHODS

Crayfishes (*Procambarus clarkii* Girard) were collected locally the year round from streams, and maintained in large cement tanks in the laboratory or outdoors. In general, animals of body length between 9 and 11 cm (rostrum to telson) were used.

The animals were pinned out rigidly ventral side up in a sculptured paraffin mold within a lucite chamber and covered with van Harreveld's solution. The ventral nerve cord was exposed for the length of the abdomen, the dissection being extended laterally to expose each set of ganglionic roots. The third pair of roots from each ganglion is exclusively motor, and supplies ventral flexor muscles; these were cut on ganglia 2 through 5 to reduce movement. The ventral blood vessel and associated connective tissue was dissected away from the surface of the nerve cord and then the connective between the third and fourth or fifth and sixth ganglion (depending upon the site of recording) was desheathed. The solution was then pipetted off to a level flush with the surface of the nerve cord and a small pool of oil introduced over the recording region. Fibers were isolated by picking up fine strands from the anterior end of the connective, peeling them back, and lifting the end up into the oil layer over a fine silver hook. In this procedure the fiber (or small bundle of fibers) was separated from the connective all the way back to the posterior ganglion, and the recording site was never more than 2 mm from that ganglion. It has been possible, under magnification of 40 times with good lateral illumination, to achieve very consistent isolation of single fibers in this way: many interneurons in the nerve cord have diameters of 10 to 40  $\mu$ , and they separate very easily if the connective has been properly desheathed. Usually bundles containing several active fibers could be split again to isolate the desired unit.

The electrode carrying the fiber was connected to one input grid of a high gain a.c. preamplifier, usually used with a coupling time constant of 1 second. The bath was grounded through an indifferent electrode. Conventional means of oscilloscope display and recording were used. Stimulating electrodes were pairs of fine platinum wires carried on micromanipulators, maneuvered under intact roots or connectives and then raised so as to exert some tension on them. Volleys in groups of presynaptic fibers were evoked by brief pulse stimulation (0.1 msec. duration); since the roots were left intact, natural modes of stimulation (moving of appendages or tactile hairs) were also available.

Illumination was provided by the focused beam of a 6 volt tungsten filament lamp, passed through a 5 cm water cell to remove infrared radiations. The luminous flux incident upon the sixth ganglion in the few experiments on light-evoked discharge was approximately 100 ft-c. In general, since these experiments were primarily concerned with aspects other than the response to illumination, photic responses were used mainly as a means of identification of the units.

## RESULTS

1. *Location and Number of the Photoreceptor Elements*

Single neurons which showed light-evoked discharges similar in form and sensitivity to those reported previously from whole nerve cords (Kennedy, 1958 *a*, 1958 *b*) were isolated over 40 times in the present study, and on 35 of these occasions the single fibers were analyzed in detail in recording periods ranging from 1 to 7 hours. Isolation was achieved usually in the connectives between ganglia 5 and 6, but frequently also between 3 and 4 or 2 and 3. In three preparations, photosensitive units were found successively in both halves of the cord in the same segment, and once (out of only two attempts) in two successively lower connectives (2-3 and 4-5) on the same side. Despite a large number of experiments in which systematic fraying and sequential



FIGURE 1. Responses from a whole excised nerve cord to steady illumination of the sixth ganglion. Duration of the record is approximately 3 seconds. The discharge is clearly composed of impulses from two nerve fibers which fire at slightly different frequencies.

recording from small bundles was performed on a particular connective, two of the photoreceptor units have never been found in the same connective on the same side. Records from whole cords (see Fig. 1) nearly always show a discharge pattern of light-evoked spikes in which two units clearly participate; this can be seen from the manner in which their frequencies, which are slightly different, produce “beats” of voltage addition in the record. Finally, photoreceptor fibers have always been found in a defined geographical region of the nerve cord, corresponding to areas 83 and 84 of Hughes and Wiersma (1960). Taken together, this evidence shows clearly that the caudal photoreceptor system consists of only one pair of cells, each traveling rostrally in a particular region of its own connective.

2. *Peripheral Connections*

Also relevant to the question of identification of the photoreceptor units is the fact that they can be driven presynaptically by fibers which enter the sixth ganglion through the several roots of both sides. They thus belong to a population of interneurons engaged in the integration of mechanical stimuli originating in the sixth and other abdominal segments. The response patterns and identification of such neurons in the abdominal nerve cord have been the subject of considerable investigation (Hughes and Wiersma, 1960; Kennedy and Preston, 1960; and current experiments in this laboratory).

All this work has served to establish that the population of central interneurons is sufficiently small to permit individual recognition of the responses of specific entities in the nerve cord. Considerable experience with such response patterns is thus available to support the statement that the photoreceptor neurons are *uniquely definable members of this interneuron population* on the sole basis of their connections with sensory fibers.

In every case investigated, these connections have proved to be of the following sort. On the ipsilateral side, afferent fibers exert both excitation and inhibition of the photoreceptor neuron, the former effect showing shorter latency and a lower voltage threshold for brief-pulse electrical stimulation of the presynaptic nerve roots. The effect of an electrically evoked ipsilateral presynaptic volley delivered to a photoreceptor fiber which is discharging (either "spontaneously" or as the result of illumination) is a short-latency discharge of one to three impulses, followed by a more sustained suppression of the preexisting activity. The contralateral afferent fibers, however, produce only inhibition. This inhibition is effective both against "spontaneous" activity and against impulses evoked through ipsilateral nerve stimulation.

Natural stimuli have been routinely employed in an attempt to classify the afferent fiber population mediating these postsynaptic responses. Marked modulation of spontaneous or light-evoked activity can be achieved by touching the ventral surface of the uropods and telson, or by manipulating the joints of these appendages. As one would expect with an afferent pathway exerting mixed excitatory and inhibitory effects, the result of such stimulation is to modify the preexisting discharge pattern into one of pronounced bursts. This happens even with the exclusively inhibitory contralateral side, since inhibition may be followed by rebound excitation. It has not been possible to decide whether the responses are mediated by joint receptors, tactile hairs, or both. This is a recurrent problem with this preparation (Wiersma, 1958; Kennedy and Preston, 1960). In current work on other interneuron systems in crayfish, we are able to achieve a definite modality identification only about 70 per cent of the time. The difficulty is that crustacean joint receptors (Wiersma and Boettiger, 1959) and tactile hairs (Mellon and Kennedy, 1962) both show considerable gradations in time constant, both phasic and tonic types of ending being encountered within each modality. Both, too, may show considerable sensitivity; so the fact that light touch near the base of uropods and telson is effective does not eliminate either afferent source from consideration. It well may be that both kinds of input converge upon the photoreceptor neurons.

A. AFFERENT EXCITATION OF PHOTORECEPTOR FIBERS As Figs. 2 and 3 show, stimulation of the ipsilateral nerve roots results in the generation of short-latency impulse activity in the photoreceptor fibers. Usually only two impulses are discharged at the maximum stimulus intensities employed, but

on occasion the maximum discharge number has been three. When recordings are made from photoreceptor neurons isolated near the sixth abdominal ganglion, it is possible to demonstrate (Fig. 3) that the impulses arise from

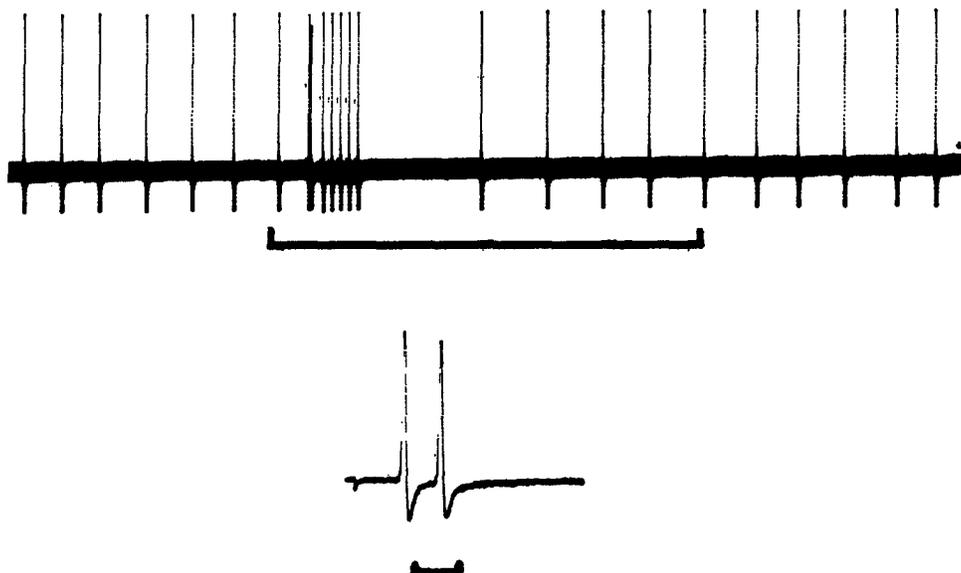


FIGURE 2. Responses of a single photoreceptor neuron to stimulation of presynaptic fibers in the ipsilateral sixth ganglion roots. Upper record: six high intensity stimuli at a frequency of 50 per second interrupt the endogenous dark discharge; the driven responses are followed by inhibition of the spontaneous activity. Lower record: a single stimulus at faster sweep speed. Time calibrations, 1 sec. and 5 msec., respectively.

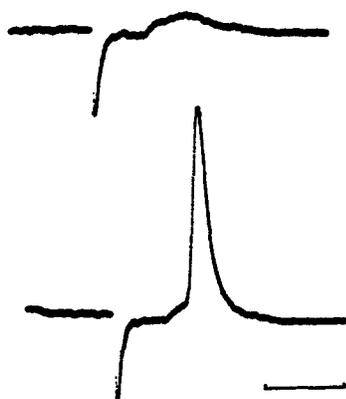


FIGURE 3. Responses of a single photoreceptor neuron to single ipsilateral nerve volleys near threshold. The response in the upper record shows a subthreshold excitatory postsynaptic potential; in the lower record, in which a slightly higher stimulus intensity was used, a spike rides on the synaptic potential. Time calibration, 5 msec.

graded excitatory postsynaptic potentials (EPSP's). The two records in Fig. 3 are responses to stimuli just below and just above threshold for spike initiation respectively; the EPSP can be seen alone in the first record and identified in the second as a step underlying the spike.

Since the recording conditions are not those usually associated with recording the graded postsynaptic potentials of single neurons, some justification will be given for the assertion that the EPSP is solely that of the isolated neuron and not a focal potential involving contributions from neighboring elements in the ganglion. Such EPSP's have been recorded from a number of neurons using this technique, and several different methods have been used to show that they are seen *only when the ganglion nearest to the point of isolation* is the locus of initiation of the spikes. Large and well isolated fibers have been picked up close to a ganglion which happens to lie in a segment from which no impulses can be evoked in the fiber by natural stimulation. Under these conditions, massive electrical stimuli delivered to the ganglionic roots produce no potential change in the recorded cell, even though they are obviously producing a high level of focal activity in the neuropile of that ganglion. Thus the presence of EPSP's is invariably associated with the presence of functional input to the recorded cell in the ganglion nearest the recording site.

In Fig. 3, the latency of onset of the EPSP is about 3.0 msec. The stimulating cathode was located approximately 2 mm from the ganglion on the presynaptic roots, and the recording site was 2 mm from the ganglion. Conduction time in the postsynaptic fibers, assuming a conduction velocity of 3.0 m/sec. (see below), would equal 0.7 msec; presynaptic conduction time would approximate 1.0 msec. if a conduction velocity of 2.0 m/sec. for the sensory fibers were assumed. Thus a central delay of approximately 1.3 msec. remains, the synaptic component of which would be reduced by an unknown amount owing to the tendency of fibers to thin out and take a tortuous course in the neuropile. In short, only by assuming fairly high conduction velocities for the sensory fibers could one make the value for central delay large enough to allow for polysynaptic connections between excitatory afferents and the photoreceptor interneurons. Monosynaptic connections, on the other hand, are also favored by the smooth time course and short duration of the EPSP. Such connections have always been assumed to mediate synaptic transfer within a single ganglion in arthropods; but with only a few of the largest interneurons in crayfish is it possible to show unequivocally central delays of 0.7 msec. or less. It appears likely that the tangled pathways of the neuropile impose larger conduction times than one would usually assign, and in the present case and most others the assumption of monosynaptic transfer is probably the correct one.

**B. AFFERENT INHIBITION** The inhibitory input to the photoreceptor fibers has a far longer time course than the excitatory one. When single shocks of increasing intensity are applied to the ipsilateral sixth ganglion nerves, excitatory driving appears at the lowest intensity; at higher intensity, suppression of background spontaneous activity can be seen to follow the

driven impulses, and the duration of the inhibition increases with further increases in stimulus strength. Tetanizing the pathway can prolong the inhibition still further, for a matter of half a second or more (Fig. 2). The inhibition is clearly an active process, and not the result of postexcitatory depression of randomly occurring input, since (1) light-evoked or synaptically evoked discharges are influenced by it as well, and (2) the contralateral inhibitory pathway, which produces no early discharge and therefore lacks an excitatory component, shows identical behavior. Prolonged inhibition following excitatory depolarizations has been reported by Tauc (1960) in *Aplysia* neurons.

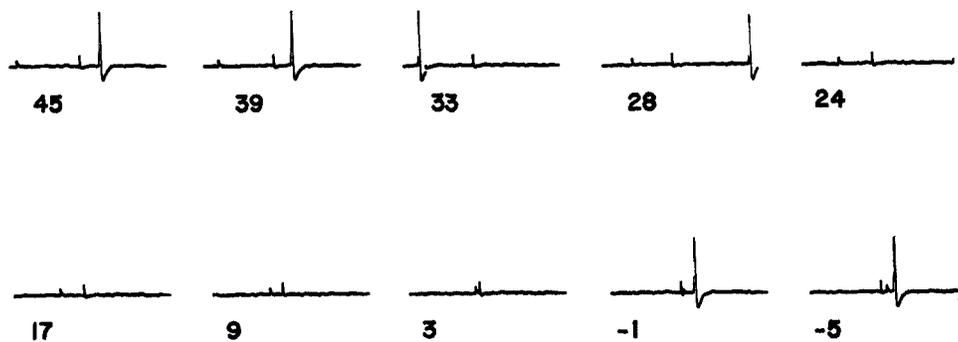


FIGURE 4. Interaction between excitatory and inhibitory presynaptic events in a single photoreceptor fiber. A barely threshold ipsilateral volley initiates a single impulse in the first two records; it is preceded by a stimulus to the contralateral nerves. In successive records, the contralateral volley occurs later in the sweep; the interval between the two stimulus artifacts in milliseconds is given beneath each record. In the last two sweeps, the contralateral volley follows the ipsilateral one. Note that inhibition of the ipsilateral spike occurs at from 33 to 3 msec. intervals. The early and late impulses in the records marked 33 and 28 respectively are of spontaneous origin; the fiber showed the usual dark discharge.

Fig. 4 shows interaction between threshold stimuli for excitation *via* the ipsilateral nerves and inhibitory input from the contralateral nerves. In the figure, the inhibitory stimulus is scanned from the beginning of the sweep and the excitatory stimulus remains fixed in time. Inhibition of the ipsilateral root response occurs only if the contralateral stimulus precedes it. This can be accounted for by the fact that the excitatory afferents have demonstrably lower voltage thresholds and therefore presumably faster conduction velocities than inhibitory ones, and also by the fact that the conduction distance through the neuropile is slightly longer for the contralateral fibers. Inhibitory interaction occurs at a maximum interval of about 33 msec. in this series.

Inhibition is effective also against spontaneous and light-evoked activity. Fig. 5 shows comparisons of both actions; in each column, single volleys

resulting from contralateral stimulation have been interpolated into trains of spontaneous or light-evoked impulses. The results of inhibitory input are similar in the two cases: lengthening of the succeeding one to two intervals is usually followed by a transient rebound frequency increase. In this case also, the duration of the inhibitory effect from a single volley is long.

When the contralateral roots are stimulated repetitively, tonic inhibition is

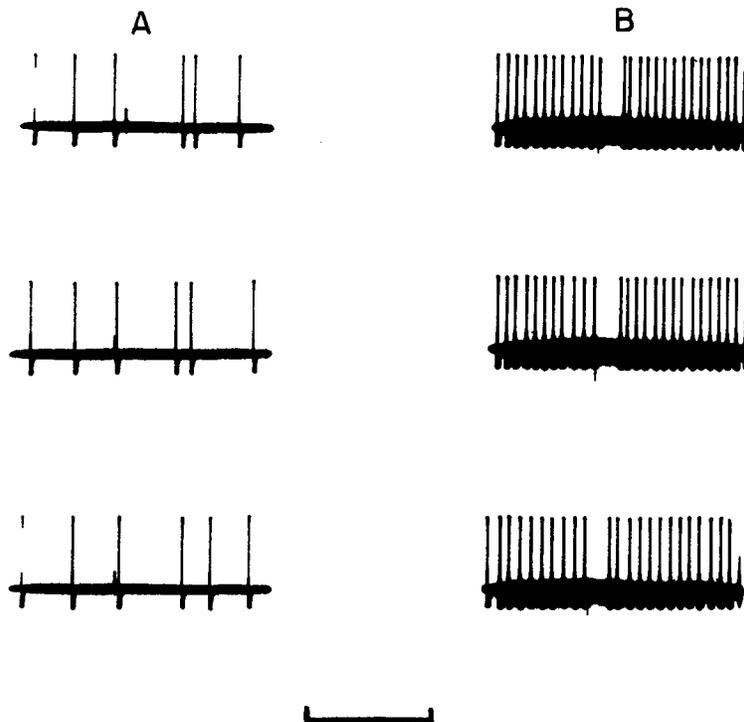


FIGURE 5. The inhibitory effect of contralateral nerve volleys upon ongoing activity. Column A, in the dark; Column B, sixth ganglion illuminated. Stimuli are delivered in the middle of each sweep. Note that the inhibitory pause in both cases is followed by some rebound excitation. Time calibration, 500 msec.

exerted upon the photoreceptor fiber, and at high frequencies of stimulation such inhibition may suppress spontaneous activity for several hundreds of milliseconds after the cessation of stimulation. Conversely, if the intensity and frequency of inhibitory nerve stimulation are adjusted so that they are barely adequate to prevent firing during the first 200 msec. or so of stimulation, it can be shown that the effectiveness of stimulation declines during the tetanus and that spontaneous impulses "escape" with progressively greater frequency. Fig. 6 shows such a situation. Background activity here is provided by random impingement of sensory impulses in the intact preparation. In the lower

record, high intensity stimulation of contralateral roots at 100 per second completely inhibits the discharge of the cell, whereas in the upper record, where a lower stimulus intensity was used, escape into the inhibitory train occurs with increasing frequency.

Most of the records illustrated were made with fibers isolated between the third and fourth ganglia. Attempts have been made with several isolations near the sixth ganglion to record electrical signs of inhibitory nerve stimulation

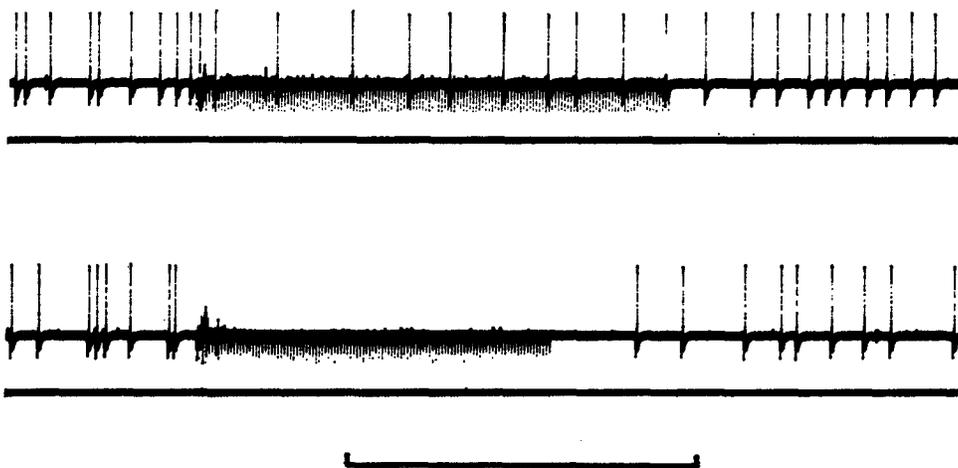


FIGURE 6. The inhibitory effect of repetitive contralateral nerve volleys upon ongoing activity. In this case the peripheral connections of the sixth ganglion were left intact, and the spontaneous activity is irregular because of random impingement of afferent activity. Stimulation of the contralateral nerves at 100 per second (upper record) causes incomplete tonic inhibition of activity; note that the "escape" of impulses occurs with increasing frequency. In the lower record, the intensity of the stimuli was increased but the frequency remained constant; inhibition is complete during the entire period of stimulation. Time calibration, 1 sec.

with methods like those used for recording the EPSP's shown in Fig. 3. In no case were such responses found; this is hardly surprising in view of the large number of cases in which inhibition is unaccompanied by hyperpolarization (including at least some inhibitory junctions in crayfish neuropile; see Preston and Kennedy, 1960).

### 3. "Spontaneous" Activity

In early experiments with chilled, excised cords, the photoreceptor units were found to be electrically silent in the dark. When recorded from in the intact preparation, however, the single fibers show a brisk asynchronous discharge in the dark which occasionally appears to have a modal frequency of 5 to 15 per second. Excitatory and inhibitory influences of presynaptic origin play

upon this discharge, converting it to a pattern of bursts and silent periods corresponding to periods of stimulation. It is easy to demonstrate that asynchronous elements in the spontaneous pattern drop out when the afferent nerve supply is interrupted by severing all roots to the sixth ganglion (Fig. 7);

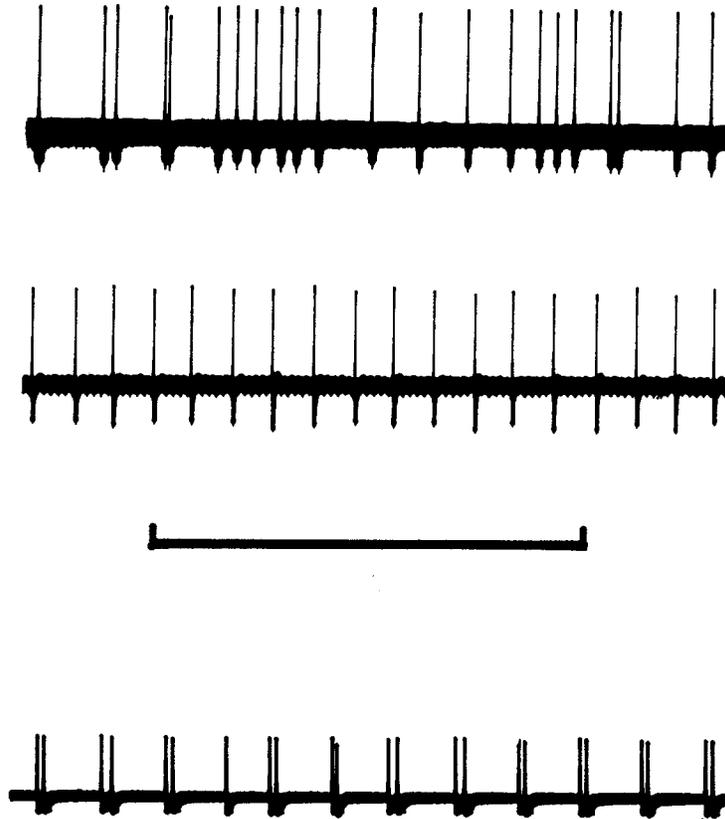


FIGURE 7. Forms of spontaneous activity under different conditions. The upper two records are from the same neuron: the first was made with the innervation of the sixth ganglion intact, the second after cutting all afferent pathways to the ganglion. The bottom record, from another preparation, illustrates the paired discharge occasionally observed in the photoreceptor neuron (isolated ganglion). All records taken in the dark. Time calibration, 1 sec.

what remains is a highly regular discharge with a frequency in the dark ranging between 5 and 15 impulses per second. In occasional preparations, the spikes occur in closely spaced pairs instead of singly; and very rarely, usually in old preparations, the discharge will be absent altogether.

Evidence has been presented previously that in other crayfish interneurons (Preston and Kennedy, 1962) having such activity patterns, the discharge is autogenic and thus that the cells showing them can be classified as pacc-

makers. The spontaneous discharge of the photoreceptor neuron isolated from random afferent bombardment clearly is in that category; the following evidence establishes the relationship between this discharge and other events in the cell. Interpolated antidromic impulses, evoked by stimulating the

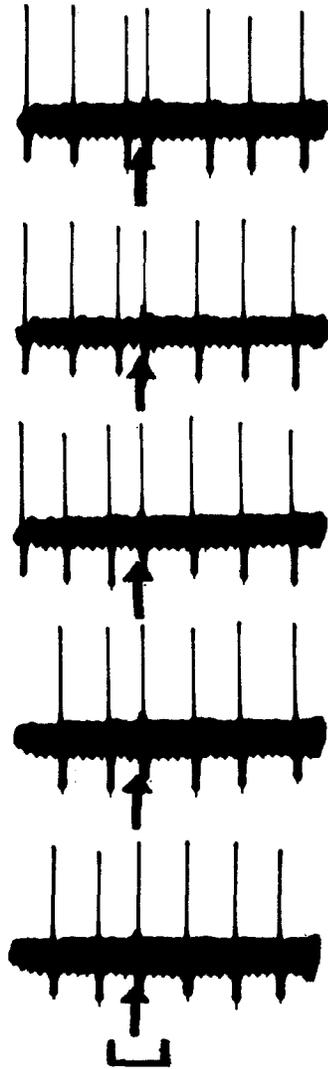


FIGURE 8. Resetting of spontaneous activity rhythm by antidromic impulses. The arrows indicate impulses interpolated by stimulation of the nerve cord in segment 5-6; recording was between 3 and 4. The succeeding interval is never shorter than the normal spontaneous intervals, but is longer than normal if the interpolation occurs early in the cycle. The records show successively later interpolations from top to bottom. Time calibration, 100 msec.

nerve cord between the fifth and sixth ganglia at an intensity just supra-threshold for producing a spike in the photoreceptor fiber, reset the spontaneous discharge. Such an experiment is illustrated in Fig. 8. It has been ascertained in all such experiments that there is no effect from stimulating the whole nerve cord unless a spike is evoked in the recorded cell, so the resetting of the discharge rhythm can only be attributed to the presence of an

antidromic impulse. If the extra discharge is interpolated early in a spontaneous cycle, the interval following the evoked response is somewhat longer than if the interpolation is timed to occur late in the cycle. Presumably, the extra duration (which amounts at most to a 40 per cent increase in the cycle

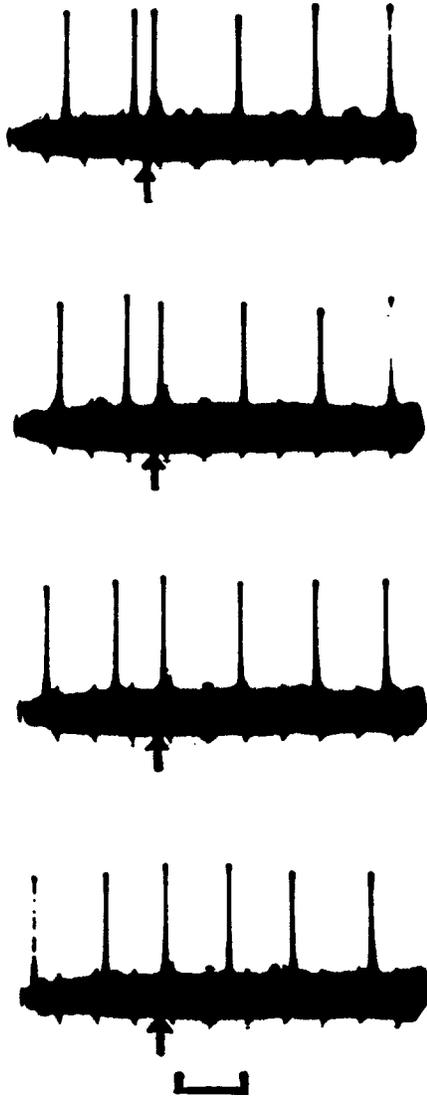


FIGURE 9. Conditions identical with those of the preceding figure, except that the interpolated spikes are generated by threshold stimulation of the ipsilateral nerves. Resetting of the spontaneous interval occurs just as with antidromic impulses. Time calibration, 100 msec.

length) indicates that pairs of closely timed impulses somehow accomplish a more complete abolition of the pacemaker depolarization than does one alone. When impulses evoked by threshold presynaptic stimulation are interpolated into the spontaneous cycle, identical results are obtained; Fig. 9 shows records from such an experiment. In this case also, intervals longer



when the *preceding* interval has been nearly a full cycle. Both orthodromic and antidromic spikes also reset the rhythm of light-evoked discharge.

These results establish that the photoreceptor neurons show a regular fluctuation of excitability of the relaxation-oscillation type, in which the

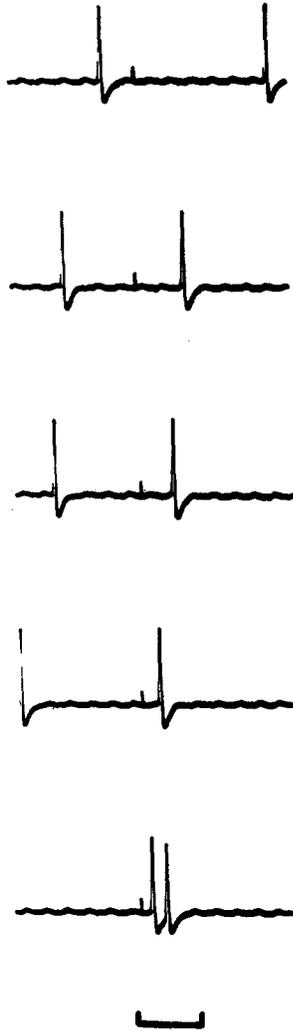


FIGURE 11. Excitability changes during the spontaneous activity cycle. A barely threshold stimulus to the ipsilateral nerves is delivered in the middle of the sweep, marked by the stimulus artifact; from the top record down, it falls progressively later in the interval between spontaneous impulses. The latency for the synaptically evoked spike grows progressively shorter; in the last record, two impulses are discharged. Time calibration, 25 msec.

occurrence of a spike restarts the cycle. Though recordings of the pacemaker depolarization preceding spontaneous firing have not been obtained from these particular neurons, indirect evidence for the existence of such a pacemaker potential has been established by the following experiment. A pre-synaptic volley evoked by electrical stimulation of the ipsilateral uropod nerves is adjusted in intensity so that when it falls just after a spontaneous

spike it barely fails to evoke an orthodromic impulse. The timing of the volley with respect to the spontaneous cycle is then varied. A typical series of records is illustrated in Fig. 11. When the stimulus follows a spontaneous spike closely (top record), the next impulse is clearly a spontaneous one. As the preceding interval is progressively lengthened, however, the latency of the evoked spike becomes shorter and shorter; finally (bottom record), when the stimulus is very late in the spontaneous cycle it is sufficiently effective to evoke a pair of impulses. The term "evoked" may be justifiably applied to the second impulse in traces 2 through 5 since the preceding interval in each case is much shorter than the spontaneous interval (trace 1). Assuming only that latency

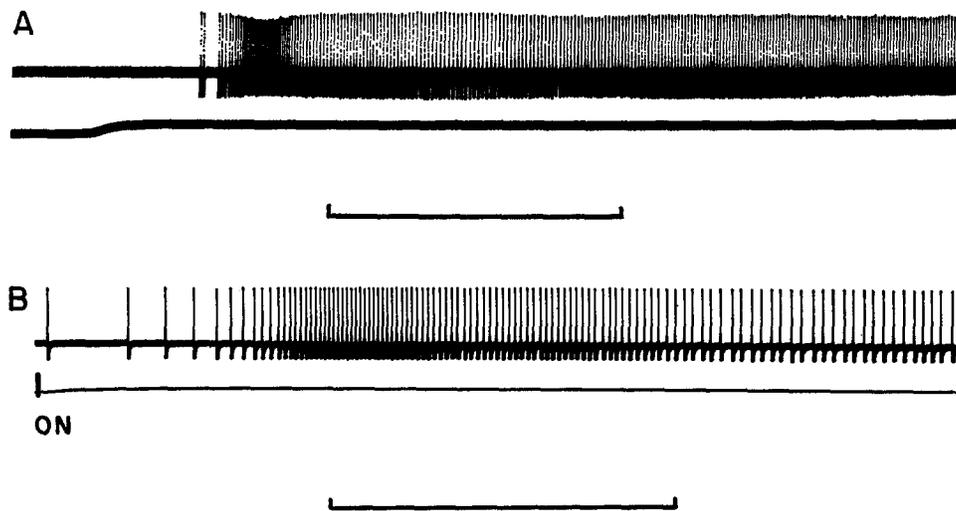


FIGURE 12. Responses to illumination. A, a preparation in which the spontaneous activity had been lost; B, a more typical case. The first interval following illumination is within the range of spontaneous intervals measured in the dark. Time calibrations, 1 sec.

is a satisfactory inverse measure of synaptic efficacy, the results of such experiments show that the EPSP's produced by orthodromic stimulation are being "boosted" toward the firing level of the neuron by a level of depolarization which is increasing during the spontaneous cycle.

#### 4. Sensory Discharge

The light-evoked discharge of the photoreceptor neurons has been previously analyzed (Kennedy, 1958 *a*, 1958 *b*) and only some special aspects will be discussed here. Fig. 12 shows typical responses to light from two different experiments; in A the neuron was not firing spontaneously, and in B it was. In both cases, the sixth ganglion was isolated by root section. Though the stimulus intensity in A was quite high, there is still a latency of some 200

msec. before the onset of firing. In B, however, the background of pacemaker discharge makes it possible to show that the onset of illumination results in gradually increasing reduction of subsequent impulse intervals. Thus the long latencies observed in the previous experiments, done on silent units, must be attributed not to a long time course of photochemical action but to a generator potential of prompt onset but slow rise time. Illumination also results in reduction of the threshold for spike initiation by ipsilateral nerve volleys.

Such generator potentials have been previously recorded with intracellular microelectrodes in the neuropile of the sixth abdominal ganglion. An example is found in Fig. 7 of Kennedy and Preston (1960). At that time, such potentials—slow depolarizations with superimposed spikes—were interpreted as the postsynaptic responses of interneurons connected with the primary ganglionic photoreceptor cells. This interpretation was deemed preferable to the alternative of assuming that they were from the photoreceptor cells themselves, because the few units encountered showed asynchronous spontaneous activity in the dark and had higher thresholds than photoreceptor cells in the previously investigated whole cord preparation. The reasons for this are now clear. First, the microelectrode studies were conducted on intact preparations, in which such spontaneous discharge is entirely usual. Second, there is a good deal of individual sensitivity variation in the photoreceptor neurons, and dark adaptation may not have been complete in the units studied. Finally, the localizations accomplished in the present work show that only a single neuron in each half of the cord can be driven by light. Thus the original interpretation was incorrect: the intracellular responses must have been from the same cells described herein. The generator potential differs from those reported from a variety of other receptors only in that it has an unusually long time course: following the cessation of the stimulus, the cells show maintenance of the depolarization, and concomitant firing, for up to 30 seconds at moderate light intensities.

Since a photoreceptor neuron is located in each half of the sixth ganglion, the possibility has been considered that they interact with one another through commissural cross-connections, in a manner established for the lateral giant fibers (Watanabe and Grundfest, 1961) and for some non-giant interneurons with bilateral receptive fields (Hughes and Wiersma, 1960). It is clear that such an interaction between photoreceptor fibers could not be excitatory, for the following reasons. (1) Illumination of one half of the caudal ganglion causes light-evoked activity only in the fiber ascending the ipsilateral half of the cord; contralateral illumination produces only the very weak acceleration of discharge to be expected from weak internal scattering of light. The fibers are thus uncrossed and do not excite one another. (2) If the interconnections were excitatory one would expect excitation from *both* ipsilateral and contra-

lateral roots, whereas in fact only the ipsilateral ones are excitatory. On the other hand, inhibitory cross-connections are consistent with the results, and would in fact provide a mechanism by which contralateral root inhibition might be mediated. This possibility was considered for another reason: at relatively high intensities of illumination, the initial light-evoked discharge peak is followed by a decline in frequency and then a secondary ascent to a stable value (Fig. 13). In some cases, this postburst depression may actually take the form of a silent period (Kennedy, 1958 *a*). Though such depressions may actually reflect the conformation of the generator potential in some instances, they have been shown in the case of the *Limulus* eye (Hartline *et al.*, 1961) to result from activity in neighboring elements which make inhibitory connections with the recorded unit.

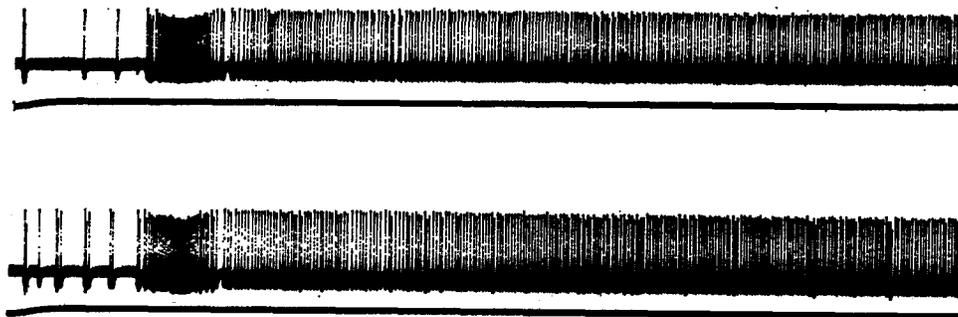


FIGURE 13. Responses to illumination of ipsilateral half of the ganglion alone (upper record) and both halves together at the same intensity (lower record). The depression following the initial burst is prominent in both cases, and therefore cannot be explained by inhibitory interaction. Time calibration, 1 sec.

This possibility was tested by recording the response in a single fiber to illumination of the ipsilateral half of the sixth ganglion with and without illumination (at the same intensity) of the contralateral half. If the postburst depression did depend upon the arrival of inhibitory impulses due to activity in the partner fiber, then the response to illumination of the ipsilateral ganglion alone should have lacked the phase of depression. As Fig. 13 shows, however, the responses to the two sorts of stimulation were identical, and there are thus no grounds for proposing cross-connections between the fibers at the level of the third ganglion or below.

##### 5. Conduction Velocity Measurements

The ready means of identifying these particular neurons provides an opportunity for indirect evaluation of the constancy of their morphological features

from preparation to preparation. In particular, it was of interest to determine whether the differentiation of these specific neurons is under such precise control that units having unique properties show consistent size, as evaluated by conduction velocity. Only a few preliminary data are available. All were obtained by simply measuring the conduction time of impulses initiated by stimulation between fifth and sixth ganglia to a single-fiber recording site between the third and fourth ganglia—a distance which ranged from 10 to 12 mm in the different experiments. In five measurements of this kind, values of 2.1, 2.6, 3.1, 3.4, and 3.5 m/sec. were obtained. The considerable variance in these measurements, in animals of very nearly the same size, suggests a considerable variability in the diameter of the photoreceptor neurons. In one case, velocity measurements were made for different segments (5–6 and 4–5) in the same fiber, and the value for the rostral segment was nearly 50 per cent higher than that for the caudal one. The fibers may well show significant diameter changes in their course through the cord, tapering toward the sixth ganglion; and the differences in diameter between individuals may reflect different rates of taper.

## DISCUSSION

### 1. *Interaction between Routes of Impulse Initiation*

It is evident from the results that the neurons under discussion show activity of three different kinds—each familiar from other systems, but not usually combined with the other two. Activity may arise through (1) pacemaker depolarizations of endogenous origin, which may be reset by the interpolation of antidromic or orthodromic spikes; (2) light-induced depolarization; and (3) orthodromic activation by presynaptic impulses. In addition, inhibitory influences from afferent sources can attenuate or block all three kinds of activity. It appears probable that a single spike-generating region in the neuropile of the sixth ganglion is involved in impulse origin by all three routes of activation, for the following reasons. First, initiation thresholds for orthodromic spikes vary through the pacemaker cycle in a way which strongly indicates that postsynaptic potentials summate with the pacemaker depolarization. Second, the threshold for orthodromic activation is markedly reduced by illumination, indicating interaction between light-induced depolarization and EPSP's. Third, illumination does not suddenly change the pattern of pacemaker discharge, but rather results in a systematic and progressive decrease in interval, as would be expected from simple addition of depolarizing current at a single site of impulse discharge. The origins of pacemaker and photoreceptor depolarizing currents must, furthermore, be located in regions capable of invasion by spikes of antidromic or orthodromic origin, since both pacemaker and sensory discharges are fully reset by interpolated discharges

of either kind. Such resetting would not be expected unless the interpolated impulse effectively short-circuited (through increased conductance) the entire membrane resistance between current source and sink.

## 2. *Location of the Photoreceptor Site*

It should be emphasized that none of the evidence cited proves that the locus of photochemical events is within the neurons studied—that, in other words, the latter are strictly “first order” sensory elements. Some of the classical elements of proof for such a statement are present: the demonstration of a “generator potential,” the resetting of the sensory discharge rhythm by antidromic stimuli, etc. But the problem of proving that the site of photochemical activation is within the spike-generating cell has recently become more difficult with the clear demonstration that the eccentric cells of the *Limulus* ommatidium, though they show generator potentials, are in fact secondarily activated by the retinula cells (see for example Fuortes, 1959). The present evidence therefore shows only that light-evoked spikes are driven by a slow depolarization and are not evoked individually by arriving presynaptic impulses. Recently two reports of photoreceptor structures in the neuropile have appeared (Uchizono, 1961; Hama, 1961). They are assumed to have photoreceptive function only on the basis of their lamellar ultrastructure; and those reported by Hama are found in various regions of the nerve cord associated with the giant fibers. Their position therefore is completely inappropriate for a relationship with the sensory system described here.

## 3. *Function of the Photoreceptor Neurons*

Unfortunately, an insufficient knowledge of the connections made by these cells at other levels of the nervous system, and of their influence upon behavior, prevents a reasonable conjecture as to their function. It is known that illumination of the caudal ganglion can initiate leg movements (Welsh, 1934), and that the caudal photoreceptor can substitute for the compound eyes in regulating the diurnal activity cycle of crayfish on a normal program of 12 hours of light alternating with 12 hours of darkness (Chapple, 1960). No unique function has been associated with the system, but it seems at present most reasonable to suppose that it operates in the general regulation of activity level as a function of brightness. The present experiments show that the same units performing this primary sensory transduction are also interneurons integrating mechanical stimuli from the tail, and the intimacy of the relationship between these two sources of stimuli suggests that tactile information may be modified as a signal by the level of tonic (light-induced) activity against which it must be pitted on the same communication channel. When the fiber shows relatively low levels of activity, as in the dark, natural excitation from the ipsilateral uropods seems to take the form of brief clusters of

impulses; when it is driven at a high rate by illumination, inhibitory pauses become (at least to the experimenter) the most discernible sign of natural mechanical manipulation. This fact may well account for the prominence of inhibitory input to these cells as opposed to most crayfish interneurons (*cf.* Kennedy and Preston, 1960): since the cells are frequently showing high rates of endogenous discharge, the *interruption* of discharge is apt to be the most effective means of communicating a superimposed signal. The combination of inhibitory and excitatory presynaptic action might thus produce quite different effects in the light and in the dark—excitation being prominent in the latter case, and inhibition in the former. Since inhibition is the most prominent and powerful sign of afferent activity, the significance of light sensitivity may be a novel one: by increasing the level of background activity, it could *improve* the discrimination of impinging inhibitory signals. There is in any event little opportunity for confusion between the two sensory modalities communicated by the same fiber, since one is highly phasic and the other tonic.

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