Spontaneous Activity in Crustacean Neurons

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ABSTRACT Single units which discharged with regular spontaneous rhythms without intentional stimulation were observed in the ventral nerve cord by intracellular recording close to the sixth abdominal ganglion. These units were divided into two groups: group A units in which interspike intervals varied less than 10 msec.; group B units in which interspike intervals varied within a range of 10 to 30 msec. Group A units maintained "constant" interspike intervals and could not be discharged by sensory inputs, while the majority of group B units could be discharged by appropriate sensory nerve stimulation. Both group A and B units discharged to direct stimulation when the stimulating and recording electrodes were placed in the same ganglionic intersegment, and directly evoked single spikes reset the spontaneous rhythm. In group B units, presynaptic volleys reset the spontaneous rhythm of some units; but in others, synaptically evoked spikes were interpolated within the spontaneous rhythm without resetting. The phenomenon of enhancement could also be demonstrated in spontaneously active units as a result of repetitive stimulation. It is concluded that endogenous pacemaker activity is responsible for much of the regular spontaneous firing observed in crayfish central neurons, and that interaction of evoked responses with such pacemaker sites can produce a variety of effects dependent upon the anatomical relationships between pacemaker and synaptic regions.

INTRODUCTION

During the course of previous studies on the properties of the ganglionic neuropile of crayfish (Kennedy and Preston (9), Preston and Kennedy (14)) we were impressed with the abundance of spontaneously discharging neurons, and with the varying patterns of such activity in different units. In this paper, we will describe experiments which were designed to determine some of the properties of spontaneously active cells and the factors which influence their discharge. The results of this analysis demonstrate that spontaneously active units in crayfish ganglia possess many of the properties which characterize other "pacemaker" cells, including ganglion cells of other invertebrates

(Hagiwara and Bullock (7), Bullock and Terzuolo (3), Arvanitaki (1)) as well as the pacemaker cells of the vertebrate heart which have been so extensively studied over the past 50 years (Engelmann (5), Lewis (10), West (22), Trautwein and Zink (20)).

METHODS

Crayfish were prepared as previously described (Kennedy and Preston (9), Preston and Kennedy (14)). The ventral nerve cord between fifth and sixth abdominal ganglia, the sixth abdominal ganglion, and its roots, were exposed from the animal's ventral surface, and ganglionic roots prepared for stimulation so that as many as five pairs of stimulating electrodes could be arranged for peripheral stimulation of afferent fibers. In many preparations, the roots were left intact so that "natural" stimuli could also be utilized to evoke activity (Kennedy and Preston (9)). In addition to the root-stimulating electrodes, one pair of stimulating electrodes was placed on the ventral nerve cord in the same interganglionic segment as the recording micropipette. This electrode pair thus served for *direct stimulation*, without intervening synapses, of the fiber impaled by the micropipette.

Since this procedure involved stimulating the entire ventral nerve cord, it could be argued that the results obtained by *direct* stimulation were due not to direct excitation of the impaled unit but to excitation of adjacent fibers which influenced the impaled unit through synaptic connections. However, the types of behavior to be illustrated subsequently could be produced only when a spike was generated in the impaled fiber as a result of *direct* stimulation. When the direct or nerve cord stimulus was subthreshold for spike discharge in the fiber impaled by the recording micropipette, none of the phenomena to be described could be produced. Furthermore, direct stimuli which were suprathreshold for spike initiation in the impaled unit did not modify or add to behavior seen with threshold stimuli. Therefore, the results to be presented would appear to be the result of stimulation of the impaled fiber, and not of other fibers within the ventral nerve cord. When a spike was generated in the impaled fiber, the latency of onset following stimulation was too brief to be attributable to any synaptic events. Furthermore, synaptic potentials were never observed in an impaled fiber as a result of such direct stimulation, although they were frequently seen when afferent systems were stimulated. Root-stimulating electrodes were used to evoke presynaptic volleys so that synaptic activation of impaled ventral cord fibers (often from a number of peripheral sources) could be studied.

Fibers between the fifth and sixth abdominal ganglia were observed by micropipette penetration in a desheathed portion of the ventral nerve cord. The capillary micropipettes had tip diameters of less than a 1 μ and resistances between 10 and 30 megohms. All units reported in this study maintained stable properties for a minimum of 15 minutes, usually for 1 hour, and on some occasions for as long as 2 hours. The illustrations in this paper do not give voltage calibrations for the illustrated units, because (*a*) we were interested only in isolation of activity from small fibers and not in absolute levels of membrane potential and response height, and (*b*) the D C recording system used had a frequency response which was flat only to 1000 c/s with the micro-

pipette resistances which we employed. This prohibits a meaningful interpretation of recorded spike amplitude. Transmembrane potentials varied from 20 to 90 millivolts. Spike discharges in some units failed to overshoot the resting potential, and in other units overshoot was present. Hagiwara and Bullock (7) have previously noted failure of overshoot in crustacean neurons. The level of "resting" transmembrane potential and spike amplitude bore no relationship to the types of responses to be described.

RESULTS AND DISCUSSION

I. *Classification of Spontaneous Activity*

During this study 102 units were impaled in which evoked activity and spontaneous activity (when present) remained constant for the duration of study. Of this population, fifty-eight units showed no spontaneous activity. The forty-four units which did exhibit such activity were classified into three arbitrary groups (Fig. 1). In units of group A, the spontaneous rhythm was quite regular. The frequency ranged, in different units, from 5 to 20 impulses/second, and in any given unit the intervals between impulses varied by less than 10 milliseconds. In units of this type impaled very near the caudal ganglion, the spike discharges were preceded by prepotentials characteristic of pacemaker cells in other systems. Fig. 1A shows the activity of two group A units. Traces 1 and 2 are continuous; trace 3 is from another cell which was penetrated within the neuropile. The prepotential is clearly present in this trace. Of the forty-four spontaneously active units studied, nine units could be placed in group A. These units with "locked" frequency were not discharged by afferent stimuli, whether initiated by electrical pulses to the roots of the caudal ganglion or by natural stimuli delivered to the uropods and telson. This observation suggests that some neurons within the caudal ganglion are isolated from afferent neural influence and act as pacemakers. It may well be that temperature (Prosser (16)) and/or humoral mechanisms are capable of altering the discharge frequency in these units but as yet we have no direct information on this question. Ozbas and Hodgson (13) have evidence to suggest that in some insects, material secreted by the corpora cardiaca can alter levels of spontaneous activity in the central nervous system.

Responses from units belonging to the second group are illustrated in section B of Fig. 1. Neurons of group B, which numbered twenty-one, were similar to those of group A in that the spontaneous discharge rate was regular, but the intervals between spontaneous responses showed greater variation in the B group. The majority of units in group B had spontaneous discharge rates between 5 and 20 impulses/second and the intervals between responses varied over a range of 20 to 30 milliseconds. Furthermore, group B units could as routine be discharged by presynaptic afferent stimulation. Since a major purpose of our investigation was to determine the influence of both

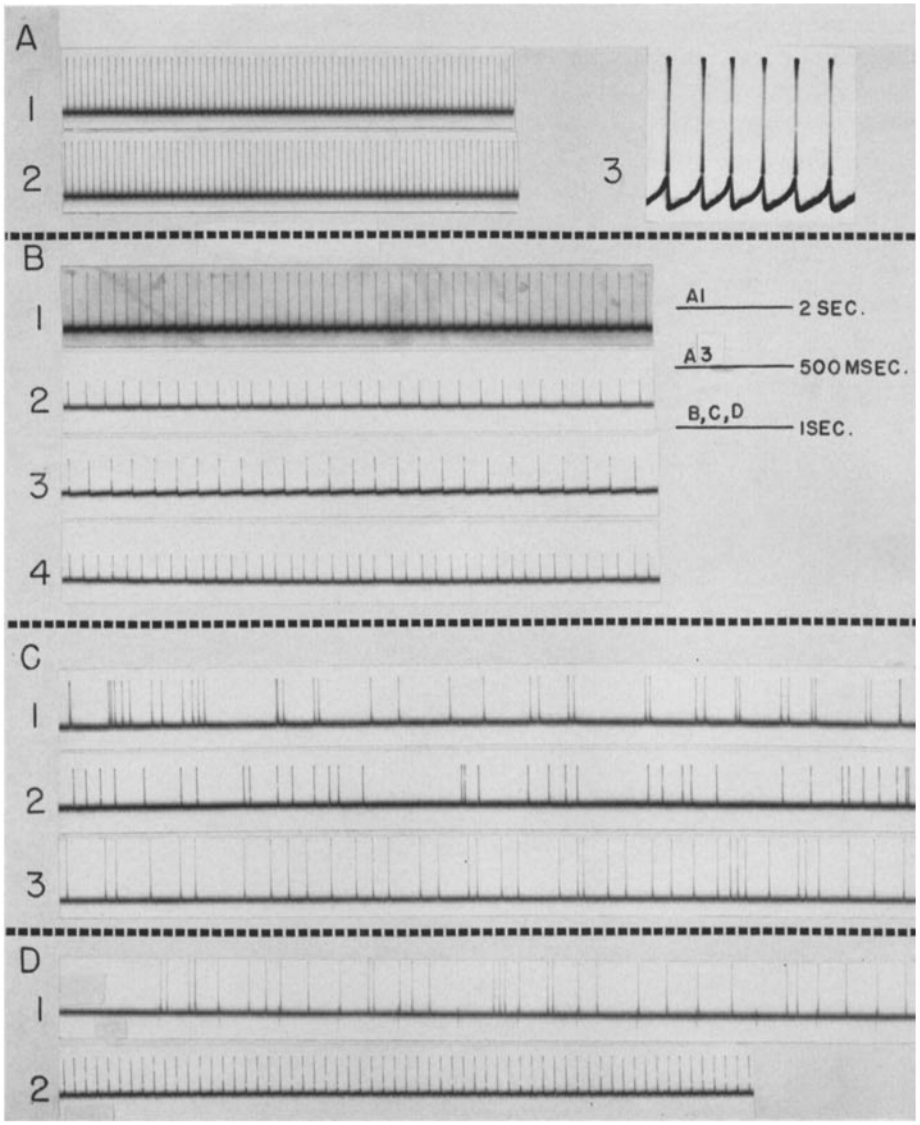

FIGURE 1. Arbitrary classification of spontaneous activity recorded from crayfish central nervous system. A, traces 1 and 2 taken from same unit in ventral nerve cord, trace 3 recorded from sixth ganglion neuropile. B, traces 1, 2, 3, and 4 taken from four different units impaled in ventral nerve cord. C, traces 1, 2, and 3 represent activity of three different units impaled in ventral nerve cord. D, trace 1 illustrates driven activity in a unit without spontaneous discharge; a presynaptic input was stimulated repetitively at a rate of two stimuli per second; trace 2 illustrates the response of another silent unit to presynaptic driving at a rate of ten stimuli per second. See text.

direct and synaptic activation on the discharge pattern of spontaneously active units, we necessarily restricted the analysis to group B units (Fig. 1B).

The final group, C, included cells whose discharge pattern was highly irregular. Traces 1, 2, and 3 in Fig. 1C illustrate three different units in the C group. Although these neurons responded to presynaptic as well as to direct stimulation, rather wide variation of intervals between spontaneous responses prohibited the sort of analysis to be described in this paper.

We have used the phrase "spontaneously active" here to imply only that an impaled unit discharged repetitively without purposeful direct or synaptic activation. The phrase should not be construed to imply that the cell necessarily shows endogenous pacemaker activity. Although we believe that such pacemaker activity does indeed exist in the crayfish nervous system, we cannot in every case decide whether a particular unit is a pacemaker *per se*, or discharging as a result of extrinsic bombardment. The need for caution in the definition of spontaneously active is demonstrated in section D of Fig. 1. Trace 1 illustrates the activity in a fiber which was impaled in the ventral nerve cord to the sixth abdominal ganglion. This unit did not fire spontaneously and discharged only upon afferent or direct stimulation. A few hairs on the uropod were displaced by mechanical stimulation twice every second. The resulting discharge pattern was one of continuous, irregular activity similar to that of the units we have classified as group C. Trace 2 in section D illustrates the results obtained from another silent unit in the ventral nerve cord when tactile stimuli were delivered to the uropod at a frequency of 11/second. This neuron followed the stimulation 1:1, and would, had it been spontaneously active, have been classified as an A or B unit. These examples make it clear that uncontrolled continuous afferent stimulation resulting from the conditions of the experimental set-up could conceivably be responsible for some of the activity patterns we have recorded.

However, we have also recorded, by means of single fiber dissection of the nerve cord as well as by micropipette punctures of single units, spontaneous activity from the neuropile and adjacent ventral nerve cord in the completely isolated caudal ganglion, thus confirming the conclusion of Prosser (15) that spontaneous activity can occur without ongoing peripheral afferent drive.

II. *Influence of Direct and Presynaptic Stimulation on Spontaneous Rhythms*

The influence of evoked spikes on the rhythms of regularly discharging units was determined for responses initiated by direct stimulation of the impaled unit as well as by stimulation of peripheral afferent pathways. Evoked responses initiated by direct stimulation reset the spontaneous rhythm. An example of this phenomenon is illustrated in Fig. 2 by traces 1, 2, and 3, all of which were recorded from the same unit. When the evoked direct spike

fell within the time period between spontaneous responses, the spontaneous response following the evoked impulse did not occur at the expected time, but was reset so that the interval between evoked and subsequent spontaneous response fell within the range of the spontaneous intervals.

In the same unit, the influence of single afferent volleys initiated by brief mechanical displacement of a few hairs on the uropod is illustrated in traces

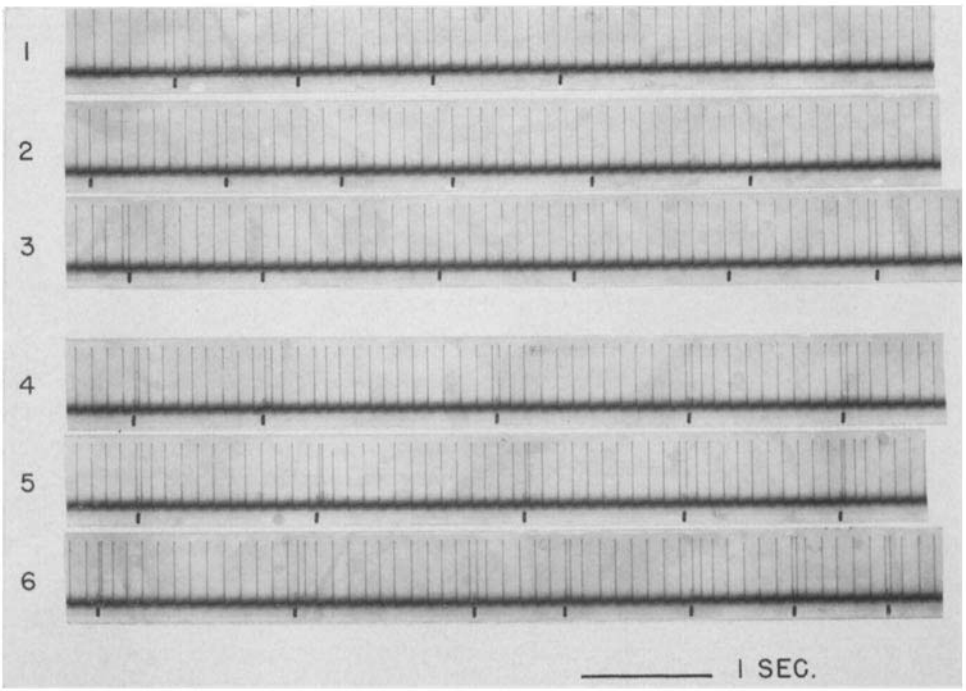

FIGURE 2. Influence of direct and synaptically evoked discharges on the discharge pattern of a spontaneously active fiber in ventral nerve cord. Traces 1, 2, and 3 illustrate effects of direct stimuli. Marks below base line indicate single pulse stimulus application. Traces 4, 5, and 6 demonstrate influence of single presynaptic evoked discharges on this unit's spontaneous rhythm. See text.

4, 5, and 6 of Fig. 2. The spike discharge evoked by afferent stimulation failed to reset the rhythm and was interpolated between spontaneous responses. However, the interpolated evoked response influenced the spontaneous rhythm, since the interval between the two spontaneous responses following an evoked response was frequently shorter than the intervals between spontaneous discharges in the absence of evoked activity. This shortening of the spontaneous intervals following presynaptic evoked activity bore a relationship to the position of the interpolated response within a spontaneous cycle; the later the evoked response occurred in a cycle, the greater was the shortening of subsequent intervals between spontaneous responses.

Failure of synaptically evoked spikes to reset the spontaneous rhythm, as illustrated in Fig. 2, does not apply to all units studied in our experiments. Fig. 3 illustrates a spontaneously active unit in which traces 1, 2, 3, and 4

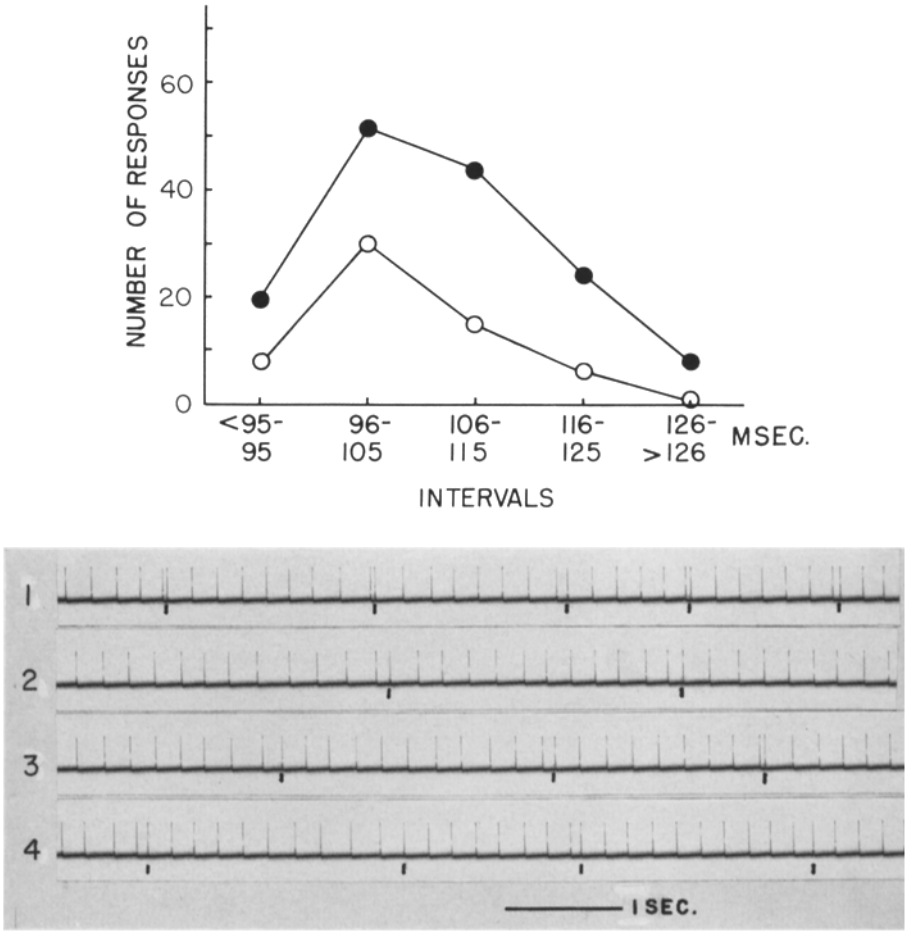

FIGURE 3. Results in a spontaneously active unit of evoked spike activity initiated by presynaptic single pulse stimulation. Traces 1 to 4 illustrate the effect of presynaptic-evoked spikes on spontaneous rhythm. Presynaptic spike is indicated by black markers below base line. Graph at top of illustration relates interval between responses to number of responses falling within the interval ranges listed on abscissae. Closed circles give distribution of spontaneous intervals; open circles give distribution of interval following evoked response to next spontaneous discharge.

are one continuous recording. The evoked responses, indicated by the inked marks below the base line, resulted from peripheral root stimulation. The latency from stimulus to response, the shift of this latency with changes in stimulus intensity, and the presence of synaptic potentials following sub-

threshold stimuli clearly demonstrated the presynaptic nature of the stimulated pathway. Although the evoked spikes were the result of synaptic activation, it is obvious from Fig. 3 that they reset the spontaneous rhythms. The graph at the top of the figure compares the frequency distribution of intervals between spontaneous responses with the frequency distribution of the in-

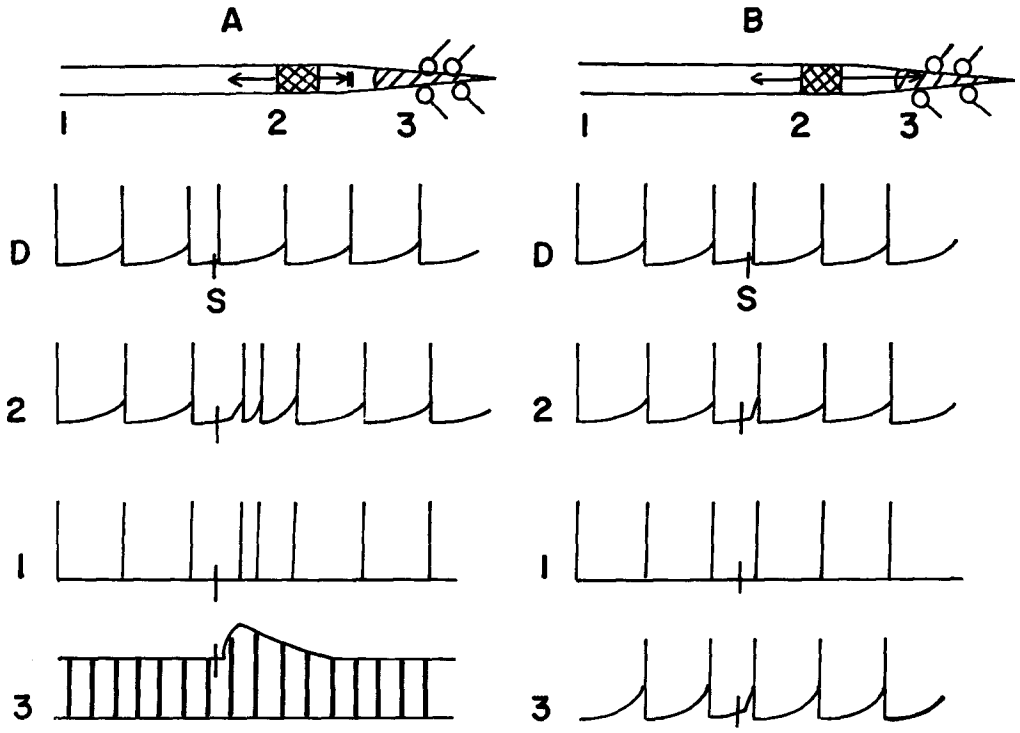

FIGURE 4. Vertical columns A and B represent expected results in two hypothetical models which can explain the two types of results observed following presynaptic activation of spontaneously active units. The details of these models are described in the text.

tervals between evoked and subsequent spontaneous response. The distribution of intervals following evoked responses parallels that for spontaneous intervals, and resetting has thus clearly been produced by the presynaptically evoked responses.

Resetting of the spontaneous rhythm by presynaptic volleys was observed in a number of units when the afferent volley was initiated either by electrical pulse stimulation to the roots or by tactile stimuli delivered to hairs fringing the uropods. There thus exist two different patterns of influence of presynaptic excitation on a spontaneously active unit: first, interpolation of a presynaptically evoked response within the spontaneous rhythm without resetting and second, resetting of the spontaneous rhythm.

The vertical columns A and B in Fig. 4 represent two hypothetical systems which can explain the two different effects observed when a spike discharge of synaptic origin occurs within the spontaneous discharge pattern. The diagram at the top of column B represents a postsynaptic fiber. The cross-hatched zone near the center of the fiber is the low threshold spike-initiating zone, and the arrows emerging from this zone imply that an initiated spike is propagated away from it to the end of the fiber in both directions. The hatched zone at the right end of the fiber diagram represents the area of synaptic contact, with presynaptic terminals indicated on it. The numbers 1, 2, and 3 indicate three possible micropipette recording positions. Position 1 would be in the ventral nerve cord at some distance from the ganglion, whereas positions 2 and 3 would be found in the ganglionic neuropile. The important feature of this model is that active spike invasion of the synaptic region occurs. Trace D illustrates the effects of a directly evoked spike on the spontaneous rhythm. The direct stimulus can be assumed to have been delivered in the region of position 1 and the recording micropipette to have been located at position 2. The evoked response is indicated by a stimulus labeled S which precedes the direct spike. Since the direct spike invades the entire fiber, including the pacemaker area, the prespike slow depolarization phase of the spontaneous rhythm is wiped out, and the rhythm is therefore reset with a new cycle of depolarization beginning after the evoked spike. Traces 2, 1, and 3 depict the expected results when the discharge is evoked *via* a presynaptic input, with the recording micropipette in position 2, 1, and 3. Since the synaptically evoked spike actively invades the entire fiber, including the synaptic region itself, the synaptic potential as well as the existing prespike depolarization of the pacemaker system is wiped out, and the spontaneous rhythm is reset just as with direct activation. This pattern of behavior is shown by the unit in Fig. 3. The only differences among the traces 2, 1, or 3 result from attenuation of the slow potential in distance due to the space constant of the fiber. In this model endogenous pacemaker zones could be placed either at position 2 or 3, or both, without affecting the outcome. If the pacemaker were sufficiently close to the synaptic zone, the discharge frequency would be modulated to a greater degree by subthreshold synaptic activity; indeed, such proximity could account for the spontaneous discharge interval variations seen in group B units.

The diagram at the top of column A is identical in format with that in column B, except that spikes do not invade the synaptic region. Although in this system the pacemaker could be endogenous and located in the region of the spike initiation zone, the generating depolarization could be the result of high intensity, asynchronous synaptic bombardment, which would provide a relatively constant source of current. Since in this model the synaptic zone is not invaded by the spikes thus generated, a sustained depolarization would

be maintained across the spike-initiating zone. The "spontaneous" firing frequency would thus be determined by the rate of depolarization of the spike-initiating zone, the postspike recovery processes taking place there, and the accommodation properties of the firing region. This model resembles the situation found in certain peripheral sensory neurons, such as the crustacean muscle receptor organ (Edwards and Ottoson (4)) in which resetting of rhythmic discharge by antidromic spikes also appears to occur (Eyzaguirre and Kuffler (6), Fig. 7).

The results to be expected from such a system are illustrated in the four traces below the model diagram in column A. Each trace depicts the results of stimulation and recording, as in the corresponding traces of column B. The directly evoked spike (line D) resets the rhythm by invading the spike-initiating zone. This zone will then undergo a period of slow depolarization following spike discharge, either (*a*) if the zone itself is an endogenous pacemaker or (*b*) if the zone is driven by steady depolarizing current from an area of the fiber not invaded by an action potential (*i.e.*, position 3). When the neuron is excited by a synchronous volley of presynaptic origin, the synaptically initiated spike will be generated in the spike-initiating zone but will fail to invade the synaptic region. This behavior is perfectly analogous to that previously reported for units within crustacean neuropile (Preston and Kennedy (14), Maynard (12)). The evoked synaptic potential in this case would not be destroyed by spike invasion, and therefore would add to the depolarization already present.

Trace 3 in column A represents the expected result when recording from the synaptic zone. In this case, the depolarizing drive is assumed to derive from asynchronous synaptic bombardment. The membrane potential is shown to be depolarized from the resting position as indicated by the parallel lines separated by vertical bars and maintained at that level during spike discharge because active invasion does not occur. The synaptic potential is shown as a simple addition to this level of depolarization; it follows an uninterrupted time course of decay since spike invasion does not occur.

The addition of a synaptic potential to the existing level of depolarization would result in a temporary increase in the rate of discharge (*i.e.*, a decrease in spontaneous intervals). The duration of the frequency increase would depend upon the time course of the synaptic potential. This is precisely the behavior seen in the unit illustrated in Fig. 2, traces 4, 5, and 6. The decrease in spontaneous intervals would be expected to be more pronounced when the synaptic potential is generated late in a spontaneous cycle than when it occurs early in the cycle.

The graph in Fig. 5 illustrates this phenomenon for the unit shown in Fig. 2. Intervals between spontaneous responses (SP_0) and subsequent evoked responses (*EV*) are plotted against the intervals between the two spontaneous

responses (SP_1 and SP_2) following an evoked response. Evoked responses were initiated by brief tactile stimuli delivered mechanically to a few hairs on the uropod. The graph shows that when an evoked response is initiated early in the interval between two spontaneous responses there is no significant shortening of the intervals between subsequent spontaneous responses (*i.e.*, no frequency increase), since the normal spontaneous intervals varied be-

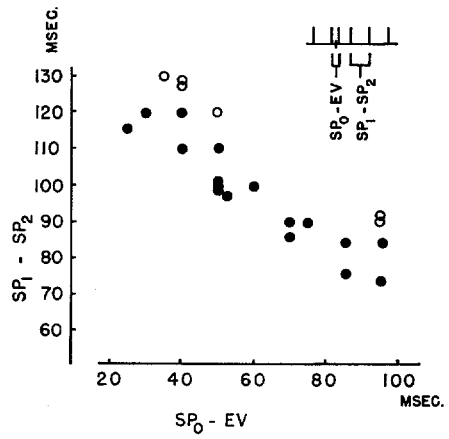

FIGURE 5. Plot of relationship between (1) the time that an evoked spike of presynaptic origin was interpolated within a spontaneous cycle (abscissae) and (2) the interval between two subsequent spontaneous responses (ordinates). The open circles represent a few measurements taken after 65 minutes of recording when the intervals between spontaneous responses increased from 115–120 milliseconds to 125–130 milliseconds. The interval between spontaneous responses was 115 to 120 milliseconds during the time the data represented by the closed circles were recorded. An abscissa represents time between the preceding spontaneous response and the presynaptically evoked response. From left to right on the abscissae an evoked response occurs later in the spontaneous cycle. The diagram in the upper right demonstrates the intervals measured for this plot. See text for discussion.

tween 115 and 135 milliseconds. As the evoked response occurs later and later in the spontaneous response interval, the interval between the spontaneous responses following the evoked response is progressively shortened. This is to be expected if the synaptic potential responsible for the evoked response is not destroyed by spike invasion; for the later the synaptic potential occurs within a spontaneous cycle, the greater the depolarization amplitude remaining during initiation of the subsequent spontaneous responses. The curve plotted in Fig. 5 has a contour which suggests that the synaptic activation was reasonably synchronous with a smooth curve of decay.

The data thus far presented illustrate some of the properties of spontaneously active units within the crayfish nervous system. The regular discharge pattern of many of these neurons suggests that endogenous pacemaker activity

plays an important role in the regulation of central excitability. Perhaps the most convincing evidence comes from neurons which were impaled in the neuropile of the sixth abdominal ganglion. These units exhibited the slow phase of prespike depolarization which characterizes pacemaker activity, and many of these cells were unresponsive to electrical stimulation of the sixth ganglion roots as well as to a variety of natural stimuli. Such behavior suggests a special function for these neurons *not* involved with the transmission of specific information from the periphery.

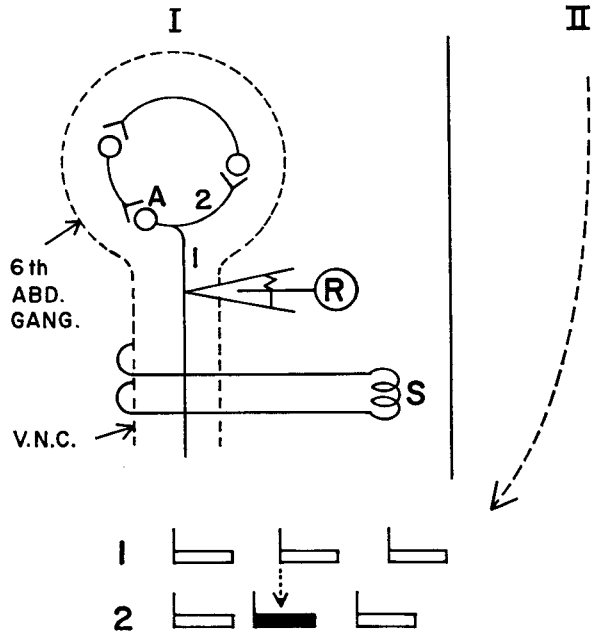

FIGURE 6. Diagram illustrating how resetting of a spontaneous rhythm can occur in a closed loop neuronal system. *I* represents the hypothetical system. Letter *A* indicates neuron with a branch (2) to a closed loop and a branch (1) to the ventral nerve cord (V.N.C.); *S* is stimulating electrode pair, and *R* is recording micropipette. *II* illustrates results which would occur in this system when a direct spike is evoked by V.N.C. stimulation. Discussion and further explanations in text.

Many units with regular activity did respond to presynaptic volleys (group B). However, the fact that the discharge rhythm of these units is reset by direct stimulation suggests that the regular rhythm is being generated within the impaled unit and not as a follower to another pacemaker. If such neurons were being discharged by a presynaptic oscillator, then resetting would not be expected to occur as a result of initiating a response by direct stimulation. Instead, the direct response would either be interpolated within the spontaneous rhythm; or, if postspike depression were of sufficient duration, a

compensatory pause would occur in which the direct spike would simply prevent the occurrence of the subsequent spontaneous discharge without resetting the rhythm. The resetting phenomenon thus supports the position that autogenic activity is a fundamental property of many cells within the crayfish neuropile.

It is, however, sometimes overlooked in discussions of spontaneous neural activity that resetting by direct excitation, as performed in these experiments, does not force the conclusion of an endogenous pacemaker site in the excited unit. It would be possible to reset a rhythm which exists on the basis of the "circus" phenomenon (17, 18) in a closed loop neuronal system. Fig. 6 illustrates this phenomenon. Section I represents a model of a closed loop system. Unit A has a branching axon; one branch (2) propagates to the next neuron of the loop, while branch 1 enters the ventral nerve cord. Stimulation and recording sites on branch 1 are arranged to follow the format of our experimental situation. If a direct response is evoked at the stimulating site at a time when the fiber has recovered from refractoriness, the evoked direct spike will enter the loop and propagate. If the ongoing spontaneous spike within the loop returns to neuron A at a time when it is refractory or depressed from the evoked activity, then the spontaneous response will be blocked. The evoked direct spike would circle the loop and continue the spontaneous rate. Since the discharge rate in the system is determined by loop propagation time, it is clear that the direct spike will return to its point of origin, *i.e.* neuron A, in the time interval characteristic of the ongoing spontaneous rate, which will thus have been reset. Section II demonstrates this more clearly. The horizontal line 1 shows three spontaneous discharges, represented by the vertical lines, from cell A. The horizontal bars represent postspike refractoriness or depression. Line 2 shows the results when a direct spike is evoked in neuron A as illustrated (filled bar). As a result of the unresponsive period set up by the evoked discharge, the subsequent spontaneous response (dotted line) is unable to fire neuron A. Therefore, cell A does not fire again until the direct spike propagates around the loop, and this interval is equal to the time required for *any* response circulating in the loop.

Resetting thus does not unequivocally show that a direct spike has invaded an endogenous pacemaker site within a single neuron. The resetting mechanism illustrated in Fig. 6, however, demands for its operation a long period of synaptic unresponsiveness in a unit following direct spike generation. Resetting by direct spike invasion occurred in most units even when the direct spike was generated in the first 20 milliseconds of a 200 millisecond spontaneous cycle. This would permit 180 milliseconds' recovery before the next synaptically evoked discharge would be due if ongoing activity were due to propagation about a closed loop system. Since we have not found the shortest possible interval between synaptic responses to be more than 20 to

30 milliseconds in the sixth ganglion neuropile, the resetting phenomenon we have observed probably results from invasion of an endogenous pacemaker site rather than from loop invasion.

Therefore we believe that our data can be best explained on the basis of endogenous pacemaker potentiality or autorhythmicity within single units. Another property of units with regular spontaneous rhythms supports this thesis of endogenous pacemaker activity or autorhythmicity; this property is exemplified by the phenomenon of enhancement. This term describes prolonged modifications of the spontaneous activity of a neuron induced by periods of imposed activity. In these experiments, enhancement is most readily detectable in units showing regular discharge; the phenomenon occurs following a period of direct (or presynaptic) stimulation at a fre-

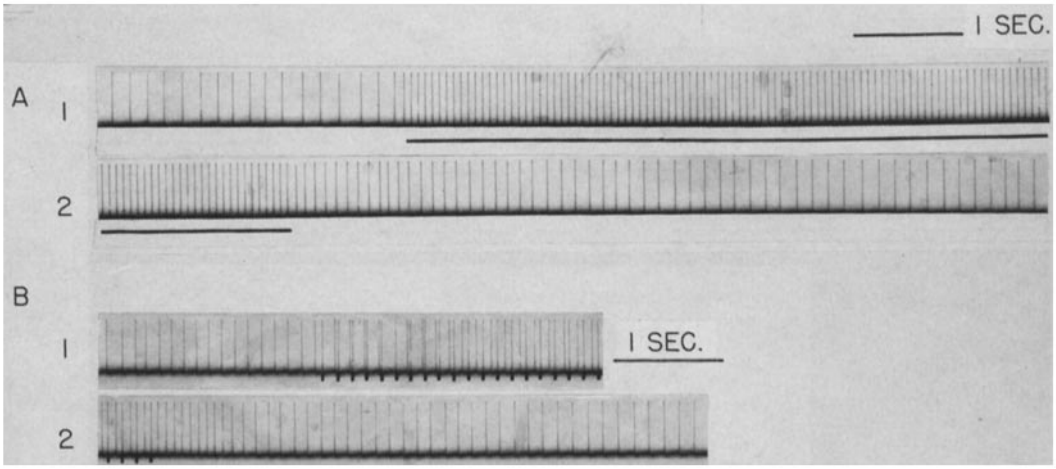

FIGURE 7. A, traces 1 and 2 are continuous. Spontaneously active unit in ventral nerve cord. Unit is driven by direct evoked responses during time indicated by black line under traces. The spontaneous rhythm is momentarily increased following direct driving. See text. B, illustrates a similar phenomenon but direct driving is at a rate which fails to capture spontaneous rhythm. The increased discharge during stimulation (marks below base line) results from evoked responses plus spontaneous discharges. Following stimulation the frequency of spontaneous discharge increases to a rate exceeding the original spontaneous rhythm as well as exceeding the stimulus driving frequency. See text.

quency higher than the spontaneous discharge, and is characterized by *increased* spontaneous frequency which gradually returns to the previous value. Fig. 7A gives a typical example of such behavior. Direct stimuli are given at a frequency of 15/second; the evoked responses control the discharge pattern and no spontaneous impulses escape into it. At the end of stimulation, the spontaneous activity continues at a higher level, gradually declining to its former value over a period of some 5 seconds.

Fig. 7B shows a special feature of enhancement encountered in some units. Direct spike responses were initiated at a frequency of 7/seconds; this frequency is too low to capture the discharge, and spontaneous impulses escape into it throughout. A higher discharge frequency follows the period of stimulation; but its extent is clearly related to the over-all frequency during stimulation, and not to the frequency of the directly evoked impulses alone.

CONCLUSIONS

The data presented in this paper suggest to us that much of the ongoing regular spontaneous activity in crayfish central nervous systems depends on the inherent property of autorhythmicity within single nerve cells. Bremer (2), in particular, has put forth the thesis that autorhythmicity is a property of vertebrate neurons, and his studies on the influence of strychnine on vertebrate spinal cord support this view. The recent studies of Wall (21), as well as those of Strumwasser and Rosenthal (19), also suggest that the property of autorhythmicity exists to varying degrees in vertebrate neurons.

We do not yet know what role spontaneous activity plays within the crayfish neuropile. The fact that spontaneous rhythms can be reset by synaptic inputs suggests that rephasing phenomena have some importance as neural integrating mechanisms. Resetting occurred regularly as a result of direct stimulation. This effect of direct activation would have little meaning for crayfish unless two way propagation normally exists within the non-giant fibers of the ventral nerve cord from which we were recording. Unpublished data from our laboratory and the studies of Hughes and Wiersma (8) have demonstrated that some of these small fibers extend over several segments. These fibers receive threshold synaptic inputs in each of several segments, and two way propagation has been recorded by stimulating presynaptic inputs above and below the recording site in the ventral nerve cord. Therefore, direct invasion in the sense that we have used the term does exist in some units, and events of integrative significance could result from the interplay between endogenous pacemaker sites and evoked spikes.

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BIBLIOGRAPHY

1. ARVANITAKI, A., AND CHALAZONITIS, N., Action potential of the giant neuronic soma (*Aplysia*), *Arch. sc. physiol.*, 1955, **9**, 115.
2. BREMER, F., Some Problems in Neurophysiology, University of London Athlone Press, 1953, 16–25.

3. BULLOCK, T. H., AND TERZUOLO, C. A., Diverse forms of activity in the somata of spontaneous and integrating ganglion cells, *J. Physiol.*, 1957, **138**, 341.
4. EDWARDS, C., AND OTTOSON, D., The site of impulse initiation in a nerve cell of a crustacean stretch receptor, *J. Physiol.*, 1958, **143**, 138.
5. ENGELMANN, T. W., Über den Ursprung der Herzbewegung und die physiologischen Eigenschaften der grossen Herznerven des Frosches, *Arch. ges. Physiol.*, 1896–97, **65**, 109.
6. EYZAGUIRRE, C., AND KUFFLER, S. W., Further study of soma, dendrite, and axon excitation in single neurons, *J. Gen. Physiol.*, 1955, **39**, 121.
7. HAGIWARA, S., AND BULLOCK, T. H., Intracellular potentials in pacemaker and integrative neurons of the lobster cardiac ganglion, *J. Cell and Comp. Physiol.*, 1957, **50**, 25.
8. HUGHES, G. M., AND WIERSMA, C. A. G., Neuronal pathways and synaptic connexions in the abdominal cord of the crayfish, *J. Exp. Biol.*, 1960, **37**, 291.
9. KENNEDY, D., AND PRESTON, J. B., Activity patterns of interneurons in the caudal ganglion of the crayfish, *J. Gen. Physiol.*, 1961, **43**, 655.
10. LEWIS, T., The Mechanism and Graphic Registration of the Heart Beat, New York, Paul B. Hoeber, 1921, 62, 221.
11. LORENTE DE NO, R., Analysis of the activity of the chains of internuncial neurons, *J. Neurophysiol.*, 1938, **1**, 207.
12. MAYNARD, D. M., Forms of activity within the neuropile of the spiny lobster, *Anat. Rec.*, 1960, **138**, 369.
13. OZBAS, S., AND HODGSON, E. S., Action of insect neurosecretion upon central nervous system *in vitro* and upon behavior, *Proc. Nat. Acad. Sc.*, 1958, **44**, 825.
14. PRESTON, J. B., AND KENNEDY, D., Integrative synaptic mechanisms in the caudal ganglion of the crayfish, *J. Gen. Physiol.*, 1960, **43**, 671.
15. PROSSER, C. L., Action potentials in the nervous system of the crayfish. I. Spontaneous impulses, *J. Cell. and Comp. Physiol.*, 1934, **4**, 185.
16. PROSSER, C. L., Action potentials in the nervous system of the crayfish IV. Influence of temperature on nerve impulses arising "spontaneously" in abdominal gaglia, *J. Gen. Physiol.*, 1935, **19**, 65.
17. ROSENBLUETH, A., AND GARCIA RAMOS, J., Studies on flutter and fibrillation. II. The influence of artificial obstacles on experimental auricular flutter, *Am. Heart J.*, 1947, **33**, 677.
18. ROSENBLUETH, A., AND GARCIA, RAMOS J., Estudios sobre el flutter y la fibrilacion, *Arch. Inst. Cardiol.*, Mexico, 1947, **17**, 441.
19. STRUMWASSER, F., AND ROSENTHAL, S., Prolonged and patterned direct extracellular stimulation of single neurons, *Am. J. Physiol.*, 1960, **198**, 405.
20. TRAUTWEIN, W., AND ZINK, K., Über Membran- und Aktionspotentiale einzelner Myokardfasern des Kalt- und Warmblüterherzens, *Arch. ges. Physiol.*, 1952, **256**, 68.
21. WALL, P., Repetitive discharge of neurons, *J. Neurophysiol.*, 1959, **22**, 305.
22. WEST, T. C., Ultramicroelectrode recording from cardiac pacemaker, *J. Pharmacol. and Exp. Therap.*, 1955, **115**, 283.