

Integrative Synaptic Mechanisms in the Caudal Ganglion of the Crayfish

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ABSTRACT A study of activity recorded with intracellular micropipettes was undertaken in the caudal abdominal ganglion of the crayfish in order to gain information about central fiber to fiber synaptic mechanisms. This synaptic system has well developed integrative properties. Excitatory post-synaptic potentials can be graded, and synaptic potentials from different inputs can sum to initiate spike discharge. In most impaled units, the spike discharge fails to destroy the synaptic potential, thereby allowing sustained depolarization and multiple spike discharge following single pulse stimulation to an afferent input. Some units had characteristics which suggest a graded threshold for spike generation along the post-synaptic fiber membrane. Other impaled units responded to afferent stimulation with spike discharges of two distinct amplitudes. The smaller or "abortive" spikes in such units may represent non-invading activity in branches of the post-synaptic axon. On a few occasions one afferent input was shown to inhibit the spike discharge initiated by another presynaptic input.

INTRODUCTION

To extend our understanding of synaptic mechanisms, a study of the characteristics of the central synapses of the crayfish sixth abdominal ganglion has been undertaken using intracellular recording methods. As discussed in the preceding communication, the synapses in this ganglion occur within a dorsally located neuropile which is composed of fibers. These fiber to fiber junctions will be shown to be integrative rather than relay in function.

Available information on central nervous system synaptic events has been obtained chiefly by intracellular recordings in mammals; the spinal motoneuron, and to a lesser extent spinal interneurons, have been the principal target for these investigations. As a result of these studies we have gained considerable information about characteristics of axosomatic synapses (Eccles (2)) but relatively little information exists about the fiber to fiber synapses so common in invertebrate central nervous systems.

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In the past, investigations into the characteristics of invertebrate synaptic structures have been limited principally to giant fiber synapses. Although some giant fibers have properties suggestive of integrative synaptic behavior (Kao and Grundfest (3, 4)), these functions for the most part are relay in nature (1).

A preliminary account of the data presented in this communication has been previously published in abstract form (7).

Methods

The crayfish was prepared as described in the preceding paper (6). The intracellular recording methods were also identical to those previously described. Pairs of stimulating electrodes were placed upon two or more roots of the sixth abdominal ganglion and square wave pulses of 60 to 90 microseconds duration were delivered through these electrodes in order to stimulate the root fibers.

RESULTS AND DISCUSSION

I. Apparent Inexcitability of Ganglion Cell Somata

The micropipette was driven through the ventral surface of the sixth abdominal ganglion and advanced slowly until a unit was impaled. On more than 200 occasions a D.C. potential shift of 60 to 90 mv. occurred within 100 μ of the ventral surface. This steady state potential was maintained as long as the microelectrode position was unchanged. However, neither natural stimuli nor electrical pulses delivered to the nerve roots of the ganglion succeeded in initiating spike discharges or synaptic potentials. Since the first 100 μ of penetration from the ventral surface passes through the layer of monopolar ganglion cell somata, it seems likely that these steady potentials represent the resting membrane potential of such somata. To assure ourselves of this, we obtained on two occasions such potentials which failed to show either spontaneous or evoked transient activity; the ganglion was then stained with methylene blue, with the micropipette left in place. After staining, direct observation indicated that the micropipette tip was in fact located in a large cell soma. These results support the proposition that the ganglion cell somata do not participate in the generation of transient electrical changes.

II. Synaptic Events in the Ganglionic Neuropile

When the micropipette was advanced deeper into the ganglion, *i.e.* into the region of the neuropile with its innumerable fiber to fiber synapses, single units exhibiting transient potentials were readily impaled. The study of this region will be the subject of the remainder of this communication.

A. RELATIONSHIPS BETWEEN SYNAPTIC POTENTIALS AND SPIKE DISCHARGE

Stimulation of the presynaptic input, by means of single square wave electrical pulses, resulted in two potential changes in the impaled unit illustrated in Fig. 1; the records labeled 1 through 5 represent, in that order, responses to stepwise increases in the stimulus strength applied to the presynaptic input. Low intensity stimulation (trace 1, Fig. 1) resulted in a transient slow potential similar in form to the excitatory post-synaptic potentials described in other preparations (2). This synaptic potential increased in amplitude as the stimulus intensity was increased. When the synaptic potential amplitude reached a critical level of depolarization the unit responded with generation of spike discharges. Therefore, in this unit the generation of spike potentials was dependent upon an excitatory post-synaptic potential.

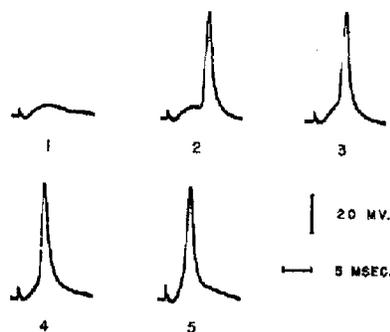


FIGURE 1. Intracellular recording from a single unit within the neuropile of the sixth abdominal ganglion. Traces 1 through 5 represent the responses of this unit to step-wise increases in the stimulus intensity applied to one of the caudal roots.

The unit illustrated in Fig. 1 demonstrates another property seen in many of the units studied during this investigation; namely, the failure of spike discharge to destroy the synaptic potential. In Fig. 2, traces 1 through 6 show the response of another unit to increasing intensities of stimulation. The synaptic potential was again graded in character; it increased with each increase in the stimulus intensity. In trace 2 a spike discharge is initiated on the synaptic potential, and in traces 3 through 6 this discharge occurred at progressively shorter latencies as electrical stimulus and synaptic potential increased in magnitude. The spike discharge had a duration of 1 msec. at half-amplitude and 2 msec. at its base (see inset photo, recorded at a faster oscilloscope sweep speed). The most striking characteristic of this unit was the origin of the spike well out on the falling phase of the synaptic potential at threshold stimulus strength.

This behavior, not unique to this unit, suggests the possibility that the spike discharge did not originate at the synaptic site. If this be true, then the synaptic region must have had a higher threshold for excitation than

did an adjacent area of post-synaptic membrane. Furthermore, on the basis of 20 consecutive observations at gradually increasing stimulus intensity (samples of which are reproduced in Fig. 2), the spike latency gradually decreased as the synaptic potential grew in amplitude until, at maximal stimulus intensity, the spike discharge began on the rising phase of the synaptic potential (trace 6, Fig. 2). Such behavior not only suggests that the synaptic region had a relatively high threshold for spike generation, but also that the post-synaptic membrane had a graded threshold, longitudinally oriented, such as a tapering fiber might display; if so, then the synaptic region would be near the high threshold end of the fiber.

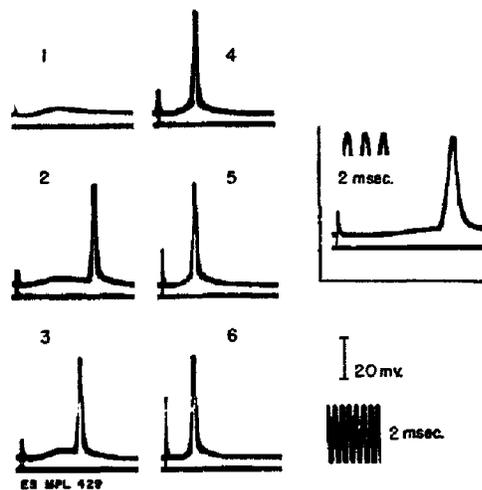


FIGURE 2. A unit responding to afferent stimulation with a synaptic potential and a single spike discharge. Records numbered 1 through 6 were recorded at increasing stimulus strength in that order. The spike originated on the falling phase of the synaptic potential in records 2 and 3. The record in the inset was recorded on a faster time base; the strength of afferent stimulation was approximately that used in trace 4.

An alternative hypothesis would assume several synaptic sites with different transmitter potentialities along a post-synaptic fiber. In such a system the recorded synaptic potential might not be the potential initiating the spike occurring on the falling phase; the responsible synapse might be several space constants from the recording site. Therefore, the effective synaptic potential would not contribute to the recorded activity, but the resulting spike would propagate into the recording site. If this hypothesis were correct the recorded spike latency should either remain constant or decrease in abrupt steps with increasing stimulus intensity. Since the spike latency decreased continuously with increasing stimulus intensity, the former hypothesis seems more probable. In either case, the behavior of the unit in Fig. 2 can be explained most readily by the assumption that the post-synaptic mem-

brane is longitudinally arranged as indeed it must be in fiber to fiber synaptic systems.

The majority of units impaled in the sixth abdominal ganglion gave

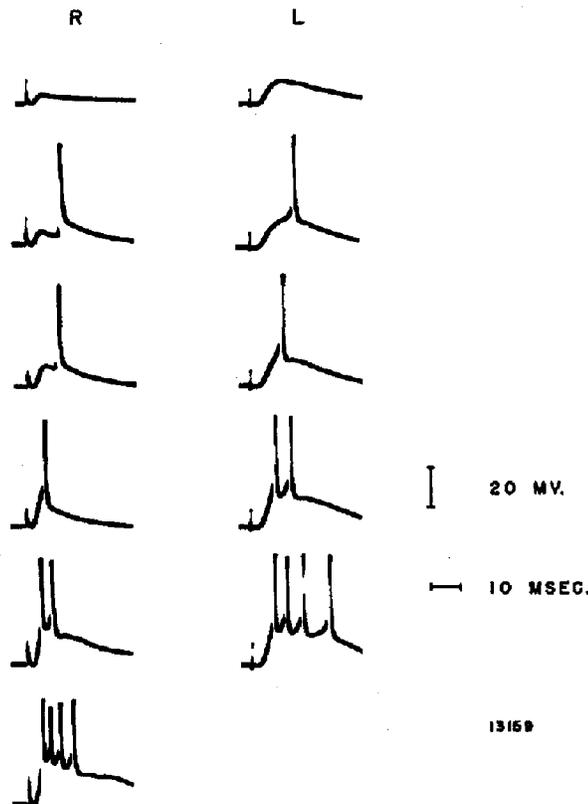


FIGURE 3. Responses of a single unit to stimulation of two different inputs, R (right ventromedial root) and L (left ventromedial root), respectively. The traces in the vertical columns, R and L, were recorded at increasing intensities of peripheral root stimulation from above, downward. This unit responded to single shock afferent stimulation with multiple spike discharge arising from a maintained level of depolarization. Compare the relationship of synaptic potential amplitude to spike discharge in the second record from the top in columns R and L.

multiple spike discharges following a single afferent volley. In general, the spike discharge failed to destroy the synaptic potential in units which showed such repetitive responses. Fig. 3 illustrates responses of a typical unit of this type to stimulation of two different inputs.

The spike discharges initiated in this unit rose out of the synaptic potential but failed to destroy it. Presumably the maintenance of the synaptic potential allows the generation of repetitive spikes. This behavior of post-synaptic units

is clearly integrative in nature, for we have observed that impaled sensory fibers in the ganglionic roots do not fire repetitively in response to single shocks.

The relationship between the active synapse and the site of spike origin in the unit shown in Fig. 3, was presumably different for synaptic excitation by the two inputs since input R, at threshold, resulted in spike generation well out on the falling phase of its synaptic potential while input L did not illustrate such behavior. Furthermore, it is apparent that either the micro-pipette was closer to the synaptic region of L which generated the larger synaptic potential, or the synaptic excitation by R and L was quantitatively different. In the latter case one must assume that the presynaptic input of R was adjacent to a lower threshold spike-generating zone on the post-synaptic membrane than was the synaptic region for input L. Regardless of the mechanisms responsible for such behavior, it is compatible with the longitudinal orientation of a post-synaptic fiber membrane.

B. SYNAPTIC INTERACTION BY AFFERENT VOLLEYS FROM DIVERSE INPUTS

To further elucidate the synaptic mechanisms of the neuropile, afferent volleys from diverse inputs were interacted in time. The results of these experiments demonstrated summation of both subthreshold and suprathreshold excitatory post-synaptic events, as well as inhibitory interaction in a few cases.

Interaction of subthreshold synaptic potentials from two different inputs is illustrated in Fig. 4. Stimulation of a left ventromedial root gave small synaptic potentials which failed to generate spike discharge (line L, Fig. 4). Stimulation of a right ventromedial root yielded larger but still subthreshold synaptic potentials (line R, Fig. 4). However, stimulation of the two inputs together in close temporal proximity yielded summation of synaptic potentials, and spike discharge of the unit in over 50 per cent of the interaction trials. The lower line of traces in Fig. 4 (L and R) shows interaction of the left and right inputs with the absence and presence of spike discharge.

Although over 200 units have been studied to date, we have observed inhibitory interaction only three times in the sixth abdominal ganglion. This paucity of inhibitory interaction may be due to the fact that the caudal extremities of the crayfish usually act as a unit, and therefore may not require extensive reciprocal innervation.

Fig. 5 illustrates records of inhibitory interaction. Stimulation of right ventromedial roots generated a synaptic potential and a spike discharge from the impaled unit (record 1). Stimulation of a left root alone (L, in upper left corner) gave no constant response. However, when the left input was stimulated 2 msec. prior to the right input, the latter failed to generate a spike on its synaptic potential (records 2 and 4 in Fig. 5). Records 1 through 4 were

recorded in sequence with the left input alternately stimulated in combination with the right input. Stimulation of the inhibitory input generated no visible hyperpolarizing potential, characteristic of other inhibitory post-synaptic potentials (Eccles); indeed, we have failed to record such potentials

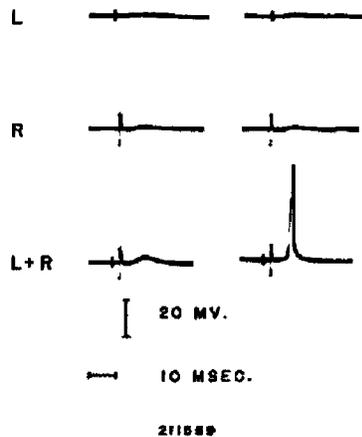


FIGURE 4. Interaction of two different inputs, both producing subthreshold synaptic potentials in the same unit. Horizontal rows L and R illustrate responses of the impaled unit to different inputs. Horizontal row L + R illustrates the results when both inputs were activated in close temporal proximity.

in any of the three units exhibiting inhibitory interaction. The problem of inhibition in the central nervous system of the crayfish will perhaps be more easily analyzed when studies of thoracic ganglia are undertaken.

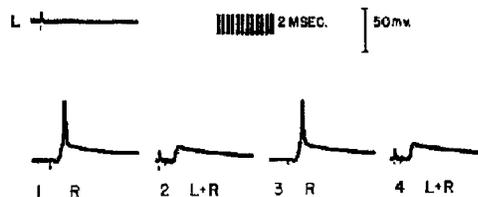


FIGURE 5. This illustration demonstrates inhibitory interaction between two different inputs. Stimulation of input R alone (traces 1 and 3) resulted in an excitatory post-synaptic potential with a single spike discharge. Stimulation of input L (upper left corner) gave no constant response. Temporal interaction of L and R (traces 2 and 4) resulted in failure of the spike discharge seen when R alone was stimulated. See text.

C. UNITS RESPONDING WITH SPIKE DISCHARGES OF TWO AMPLITUDES

Occasionally an impaled unit responded to afferent stimulation with spike-like discharges of two distinct amplitudes (Fig. 6). The responses in Fig. 6A represent an intensity series in which the peripheral root stimulus strength was increased from left to right, first along the top row and then on the second row. The recordings in Fig. 6B are from a similar intensity series in the same unit, but were recorded on a faster time base. At low stimulus intensity a low amplitude spike was generated. These spikes increased in

number as the stimulus intensity was increased, and at a critical stimulus strength high amplitude spikes were initiated. These large spikes occurred in bursts of high frequency when the stimulus strength was further increased. The later spikes of a series were generated on the falling phase of the preceding discharges.

Although the small spikes, when present alone, originated at the same latency after the stimulus artifact as did the large spikes, there was no ob-

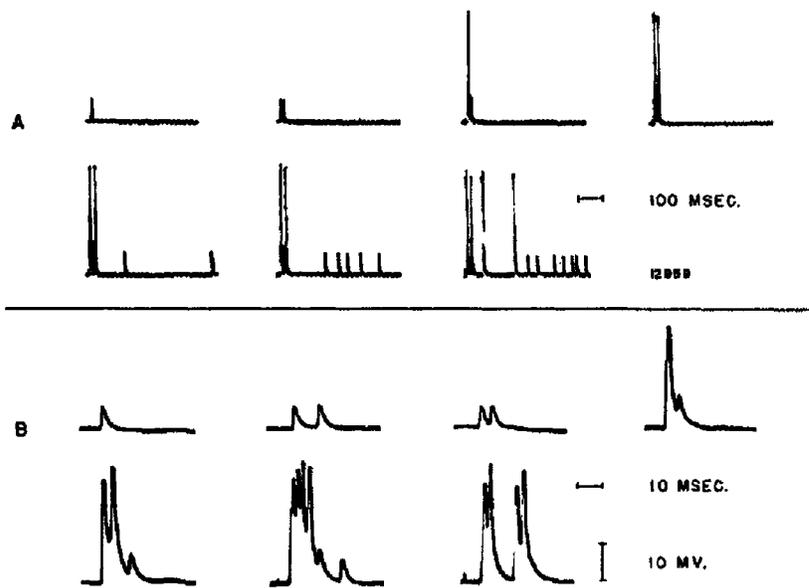


FIGURE 6. Unit responding with spike discharges of two amplitudes following stimulation of a peripheral root. Section A illustrates the responses of this unit to different intensities of root stimulation. The intensity of the stimulus was increased from left to right, first in the top row and then in the bottom row of section A. Section B is a similar intensity series recorded on a faster time base. See text.

vious inflection point on the large spikes which would clearly relate small spike generation to large spike initiation. More suggestive of such a relationship are the results shown in Fig. 3 in the preceding communication (6). It was noted (6) that in many units the spike discharges failed to show overshoot. However, even if the absence of spike overshoot is truly a characteristic of some post-synaptic fibers of the crayfish neuropile, it seems quite unlikely that the same membrane could generate spikes of two distinct amplitudes while in a presumably stable (non-refractory) condition. Since the axons of the neuropile have branches, it would seem more probable that the small amplitude "abortive" spikes may have been the result of discharge in an axon branch, with failure of the impulses to invade the fiber in which the micropipette was located. Such an interpretation would be analogous with

that made by Katz (5) in studies of a peripheral sensory receptor. It might be argued that the small spike was caused by activity in an adjacent fiber which was activated by the same input. Although we cannot refute this possibility in the unit shown in Fig. 6, such reasoning cannot be used to explain the results shown in Fig. 7A. In this series, the intensity of the peripheral root stimulus was gradually increased from left to right on the top row and continued in the bottom row. At low intensity only a synaptic potential was generated. On the top row, third record from the left, an abortive spike was

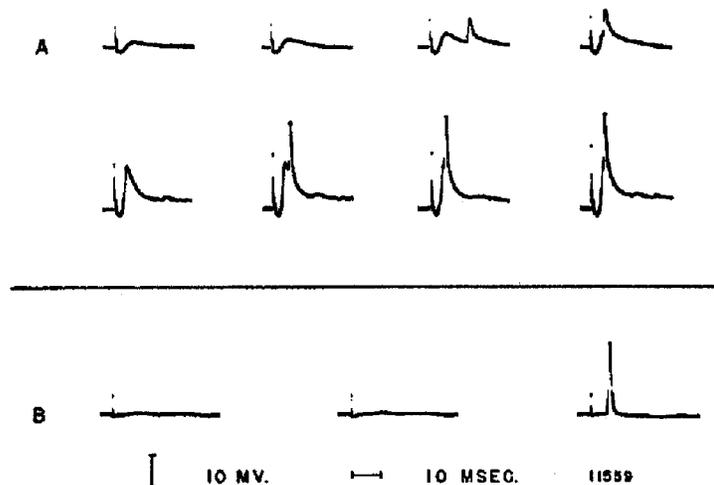


FIGURE 7. Section A, responses of the illustrated unit to stimulation of one input at varying stimulus intensities; the intensity of root stimulation was increased from left to right on the top row and then along the bottom row. At low intensities of stimulation only a graded synaptic potential was produced; at intermediate stimulus strengths a small abortive spike was initiated upon the synaptic potential; at high stimulus intensities a spike arose from the synaptic potential-abortive spike complex. Section B, response of the same unit to stimulation of an input other than the root stimulated in section A. See text.

generated on the synaptic potential. With further increase in stimulus strength (and, thereby, in synaptic potential amplitude), a large spike was generated on top of the abortive spike; the latency of this large spike decreased with further increase in stimulus amplitude. We interpret these results as follows: The presynaptic input was, at least in part, located on a branch of the post-synaptic axon; the synaptic potential generated a spike discharge in this branch which failed to invade the particular branch impaled by our micro-pipette. However, as the synaptic current increased with increased pre-synaptic input, the combined synaptic potential and abortive spike triggered a discharge in the impaled axon. Alternatively, the recorded synaptic potential may have resulted from synaptic input upon the impaled axon, which,

when reaching threshold amplitude, was responsible for large spike generation; the abortive spike may have been activated by the same input, but not causally related to the large spike's initiation. Threshold stimulation of another afferent input of this unit (Fig. 7B) resulted in a synaptic potential which generated the large spike alone. The synaptic potential was of much lower amplitude than the potential generated by the input in Fig. 7A.

DISCUSSION

The results presented in this communication illustrate some characteristics of fiber to fiber synapses in the crayfish neuropile. There can be little doubt that this synaptic system has well developed integrative capabilities. This integrative behavior appears to be accomplished in several ways. The synaptic potentials are graded in character, and excitatory synaptic potentials from different inputs can sum to generate spike discharge. In most units the spike potential does not destroy the excitatory synaptic potential; it may therefore originate outside the synaptic region, and fail to invade it. This would permit sustained depolarization of the synaptic region and repetitive spike generation. In a few cases one afferent input was shown to inhibit spike discharge initiated by another input. Some impaled units had characteristics which suggest a graded threshold for spike generation along the post-synaptic fiber membrane (Fig. 2). Finally, additional integrative behavior is possible by means of synaptic impingement upon the several branches of a post-synaptic axon; at least this conclusion seems to be the best interpretation of our results (Figs. 6 and 7) and is compatible with the anatomy of the neuropile. A possible model of this synaptic system, compatible with both the morphology and the functional characteristics of the crayfish neuropile, is a longitudinally oriented post-synaptic membrane having branches with multiple synaptic input. The post-junctional membrane has a varying threshold for spike initiation along at least part of its course.

Another point of concern is the nature of the presynaptic input upon the post-synaptic membrane; *i.e.*, are all or any of the synaptic potentials which we record the results of direct projection of presynaptic fibers, or are other interneurons interpolated in the pathway? It is impossible to speak unequivocally on this point, for one cannot accurately measure the presynaptic conduction pathway through the tangled fiber mass of the neuropile. The shortest latency recorded from shock artifact to beginning of the synaptic potential was 1 msec.; the stimulating electrodes were approximately 5 mm. from the ganglion.

Since we know nothing of intraganglionic conduction distances or synaptic delays in this system, it is impossible to determine the number of synapses between afferent fiber and the particular post-synaptic unit we had impaled.

However, one fact is clear: many impaled units responded to afferent volleys with a synaptic potential of perfectly smooth contour, as seen in mammalian spinal motoneurons during monosynaptic group 1A afferent excitation. Increasing the afferent volley in many such units did not result in additional synaptic humps, but rather brought about an increase in amplitude of the synaptic wave form without significantly prolonging its duration. This suggests recruitment of additional fibers having approximately equal presynaptic delay. Some units responded to increased afferent stimulation with both an increase in the initial synaptic potential amplitude and appearance of secondary synaptic potentials occurring on the falling phase of the first.

In conclusion, it should be mentioned that an occasional unit fired in response to root stimulation without appreciable delay (less than 0.3 msec.). Such units had a fixed threshold for spike initiation, and no synaptic potentials. Presumably these units were either presynaptic sensory fibers or antidromically activated motor fibers. The properties of such unit responses will be the basis of a future communication.

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