

Neural Basis of a Simple Behavior: Abdominal Positioning in Crayfish

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ABSTRACT Crustaceans have been used extensively as models for studying the nervous system. Members of the Order Decapoda, particularly the larger species such as lobsters and crayfish, have large segmented abdomens that are positioned by tonic flexor and extensor muscles. Importantly, the innervation of these tonic muscles is known in some detail. Each abdominal segment in crayfish is innervated bilaterally by three sets of nerves. The anterior pair of nerves in each ganglion controls the swimmeret appendages and sensory supply. The middle pair of nerves innervates the tonic extensor muscles and the regional sensory supply. The superficial branch of the most posterior pair of nerves in each ganglion is exclusively motor and supplies the tonic flexor muscles of that segment. The extension and flexion motor nerves contain six motor neurons, each of which is different in axonal diameter and thus produces impulses of different amplitude. Motor programs controlling each muscle can be characterized by the identifiable motor neurons that are activated. Early work in this field discovered that specific central interneurons control the abdominal positioning motor neurons. These interneurons were first referred to as “command neurons” and later as “command elements.” Stimulation of an appropriate command element causes a complex, widespread output involving dozens of motor neurons. The output can be patterned even though the stimulus to the command element is of constant interval. The command elements are identifiable cells. When a stimulus is repeated in a command element, from either the same individual or from different individuals, the output is substantially the same. This outcome depends upon several factors. First, the command elements are not only identifiable, but they make many synapses with other neurons, and the synapses are substantially invariant. There are separate flexion-producing and extension-producing command elements. Abdominal flexion-producing command elements excite other flexion elements and inhibit extensor command elements. The extension producing elements do the opposite. These interactions insure that interneurons of a particular class (flexion- or extension-producing) synaptically recruit perhaps twenty others of similar output, and that command elements promoting the opposing movements are inhibited. This strong reciprocity and the recruitment of similar command elements give a powerful motor program that appears to mimic behavior. *Microsc. Res. Tech.* 60:346–359, 2003. © 2003 Wiley-Liss, Inc.

INTRODUCTION

This article reviews one aspect of the neurobiology of crustaceans. It deals almost entirely with crayfish and lobsters in which abdominal positioning is a prominent feature of their overall behavior. Crustacean neurobiology has an interesting history, recently captured with skill and insight by E. Florey (1990), one of the pioneers in this field. The featured animal models in the field of crustacean neurobiology, the crayfishes and lobsters, were selected because of their availability, their large size, their large and identifiable neurons, their ability to withstand surgery, and the viability of the preparations. They will continue to be valuable for arthropod research.

Our present knowledge of the control of abdominal positioning rests upon several advances in technique and upon a general understanding of crustacean neurology. The discovery of stains and whole-cell staining of neurons led to the concept of functional parts of neurons and to the concept of identifiable neurons

(Bethe, 1897; Bullock, 2000; see also Leonard, 2000). A series of studies by Wiersma and his colleagues mapped many dozens of identified sensory interneurons and primary sensory neurons in the connectives of the crayfish CNS.

Wiersma (1958) showed that it is possible to tease out small bundles of axons from the interganglionic connectives. He recorded evoked sensory activity in each bundle in response to a thorough sequence of stimulation. He first stimulated the surface of each animal with a small artist's brush, meanwhile keeping records of fields where stimulation was successful. Ad-

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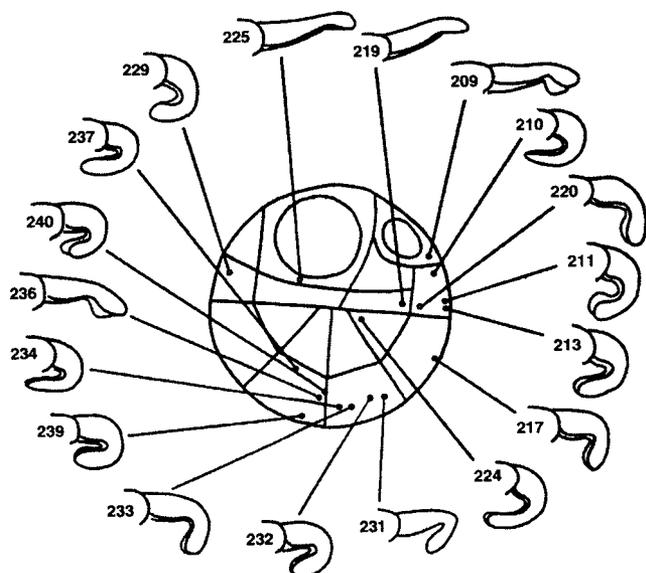


Fig. 1. Abdominal movements generated by stimulating fine bundles in the circumesophageal connectives of crayfish (see text). Typically, one also provokes forward and backward walking, swimmeret beating, turning, escape, etc., as well as abdominal movements by stimulating fine circumesophageal bundles. The data here were obtained by sampling one animal and selecting the behaviors involving abdominal positioning. The images were taken on 16-mm movie film at 8 frames per second and analyzed using a stop-frame projector. Traces were made of selected frames. The approximate location of the axon of each command element in the circumesophageal cord is mapped on the cross-section of the cord as suggested by Wiersma (1958). A similar map was suggested for the abdominal cord (see Wiersma and Hughes, 1961). Reproduced from Bowerman and Larimer (1974a) with permission of the publisher.

ditional stimulation was applied to the joints of appendages. When recording from the circumesophageal bundles, the visual system was stimulated with light flashes, while tactile and proprioceptive stimulation was directed to the surface carapace and the appendages. Each fiber bundle was marked on a cross-section map of the connective (Fig. 1). This allowed the experimenter to go to the same map location and, in some instances, to find the same neuron in another individual of the species. Identification of the same neuron in different individuals depended not only on the location of the axon in the cord but also upon its sensory modality, the size and position of the receptive field, and other characteristics. The thorough stimulus routine not only revealed the receptive field of an interneuron and its sensory modalities, but whether its input was ipsi-, bi-, or contralateral. This decade-long study by Wiersma and Mill (1965) provided a map of many of the sensory interneurons in the CNS of crayfish. This work greatly accelerated the study of motor control in the abdomen by adding new methods as well as new morphological and functional information.

EARLY STIMULATION EXPERIMENTS ON CRUSTACEAN NEURONS

Wiersma (1947) first discovered the phasic escape responses by stimulating the giant fibers in the nerve cords of crayfish. There are two sets of giant fibers in

the CNS of crayfish and lobsters as well as some other crustaceans. Subsequent workers have separated the functions of the two sets of giant fibers and, in some cases, have discovered their controls. Since this review is devoted to abdominal positioning and since the topics of escape behaviors have been reviewed very recently (Edwards et al., 1999), the details of escape behavior will not be considered further.

THE MOTOR NEURONS UNDERLYING THE TONIC MUSCLES FOR ABDOMINAL POSITIONING IN CRAYFISH

An understanding of the tonic muscles of the abdomen and how they are controlled insured rapid progress in studying the neural basis of abdominal positioning behavior (Kennedy and Takeda, 1965). There are three sets of roots off each of the abdominal ganglia, except A_6 . The most anterior set of roots carries sensory axons for the segment and motor axons to the swimmeret muscles. The second set of roots carries sensory and motor axons of the tonic extensor muscles. Finally, the superficial branch of root 3 in each of the abdominal ganglia (except A_6) is a pure motor root supplying the tonic flexor muscles. All 2nd and superficial 3rd roots carry five tonic excitator motor axons and a single peripheral inhibitor. Several additional characteristics of the tonic flexor and extensor control system are of importance. We have already mentioned that motor axons innervating the flexor and extensor muscles are separated in different roots in each ganglion. The motor program emerging from each root would, therefore, reveal the underlying action even when examining an isolated cord. Further characterization of motor programs is made possible by examining the impulse amplitudes. For example, if one is recording from a whole root, the smallest action potential will arise from axon 1 while the largest will come from axon 6. Impulses from axons 2–5 will be of intermediate amplitude (Fig. 2). The fifth largest amplitude belongs to the peripheral inhibitor; all of the other motor neurons are excitatory. Knowing which motor neurons are firing, as well as knowledge of the intended movement (i.e., flexion or extension), will refine our knowledge of the intended motor output. For example, if we initiated a motor program by the stimulation of a “command neuron” taken from the same site in two different preparations, or stimulation of the same command neuron twice in succession, we could ask whether the two motor programs are substantially the same or even identical (Fig. 3).

Please note that, in earlier publications, the abdominal ganglia were referred to as G_1 , G_2 , etc. In later publications, the terminology was switched to A_1 , A_2 , etc. While the more recent terminology is used throughout the text, both labels may be found in the figures.

COMMAND NEURONS EMERGE

Wiersma and Ikeda (1964) did a series of stimulation experiments on small bundles of neurons teased from the ventral nerve cord of crayfish. They located five bundles in each connective that evoked movement of the swimmerets. Each bundle was stimulated at 30 Hz, which was considered a normal frequency, and the effects on the swimmerets were noted. The excitatory

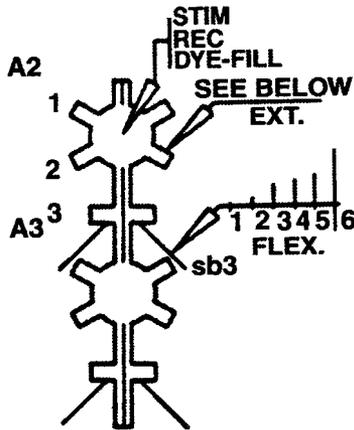


Fig. 2. Drawing of part of the isolated ventral nerve cord of crayfish. Two ganglia are drawn, A_2 and A_3 . A_1 , A_4 , and A_5 are similar. A_6 is formed by the fusion of two ganglia. Motor functions of the abdomen and higher centers are controlled by different ganglia and roots. Root 1 is mixed motor and sensory and controls the swimmerets. Root 2, also mixed, contains the tonic extensor motor neurons of the local abdominal segment. The superficial branch of root 3 (sb3) is all motor and contains the tonic flexor motor neurons of the local segment. Roots 2 and sb3 each contain six motor neurons, five extensors and one inhibitor (root 2), and five flexors and one inhibitor (sb3). Each motor neuron within a set of six has a different impulse amplitude (shown for sb3). If one notes the root of origin and the size of impulses, one can characterize a motor program for abdominal positioning. Reproduced from Larimer (1988) with permission of the publisher.

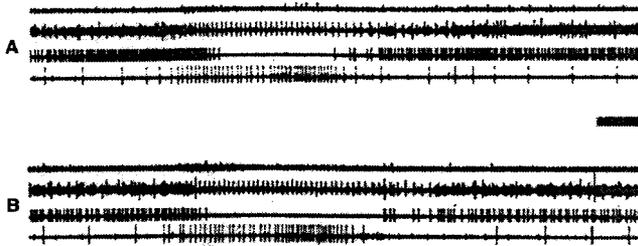


Fig. 3. A complex and patterned motor program that is produced by an unpattered stimulation of a command element before (A) and after (B) deafferentation. The three top traces in each record were obtained from roots 6, 3, and 1 of A_6 , which innervate the uropods and telson. The bottom trace in each record was obtained from root 2 of A_5 , which controls abdominal extension. Calibration bar = 200 msec. Note that the two records are almost identical even though they were produced by stimulation of the left, then the right command elements. Additionally, the bottom record was obtained after deafferentation; thus the complexity and pattern of the motor program does not depend upon sensory feedback nor upon a complex pattern of stimulation of the command elements. Reproduced from Larimer and Kennedy (1969) with permission of the publisher.

interneurons were found to drive the swimmerets in a "normal" metachronous rhythm, with the cycle beginning in ganglion A_5 and moving forward to A_1 , then repeating at A_5 . The location of each bundle was then mapped on the cord cross-section. Several inhibitory neurons that suppressed swimmeret activity also were encountered. Note that these neurons were identifiable and thus could be located in the connectives of different individuals. The authors gave the excitor and inhibitor cells the name "command neurons."

Following the observations of Wiersma and Ikeda (1964), there was a large output of work not only on

crustaceans but many other invertebrates such as annelids, insects, and mollusks, showing the existence of "command neurons" in their CNS. For an introduction to these early articles, see the review by Larimer (1976) as well as many others.

Some of the most important and interesting questions concerning command neurons were posed by the early workers, especially Evoy and Kennedy (1967), Kennedy et al. (1966), Larimer (1976), and Larimer and Kennedy (1969). More recently, Kupfermann and Weiss (1978) led the discussion on "what is the definition of a command neuron?" An examination of some of these questions focuses well on the workings of "command neurons."

It was noted early that the stimulus train required to drive a command neuron was generally simple and of relatively low frequency. The resulting motor output, however, could be widespread and complex (Fig. 3). This implied that the command signal itself simply "permitted" the output and did not directly "instruct" it. The complexity seemed to reside in the CNS elements and their connections. Several persons, including Evoy and Kennedy (1967), remarked on the strong reciprocity that exists between antagonistic muscles such as the tonic flexors and extensors of the abdomen of crayfish and lobsters. They suggested that this reciprocal output was inherent in the ganglionic organization. When studied several years later using microelectrodes, the reciprocal circuits were found to be prevalent. For example, in the abdominal positioning system, the stimulation of a flexion command neuron activated the inhibitor neuron to the extensor muscles in the segment as well as the excitatory neurons to the flexor muscles.

An important question at that time was whether a single command neuron could provide for the complex and widely distributed motor programs that often accompanied the extracellular stimulation of a "bundle" of unknown composition. Kennedy et al. (1966) devised an experiment to insure that they were examining only one command fiber at a time. This procedure became known as a "double header" experiment. It was known that axons of many command neurons course through several ganglia in the abdominal nerve cord. Kennedy et al. (1966) used the location of the axon in the cord cross-section and the motor output it evoked to isolate both ends of one command fiber. They stimulated the caudal and the rostral ends alternately while recording from the opposite end. The stimulus strength was set to recruit only one impulse in the cord. The motor output was seen to appear only when the cord display showed the one impulse. This process insured that only one fiber was examined. The results showed a widely spread motor output including reciprocity.

Does this mean command neurons operate one at a time? What accounts for the widespread output? It was speculated that the command neuron had several "driver" neurons between it and the motor neurons to furnish the needed excitation and inhibition in the circuit. Many of these observations were confirmed by early intracellular experiments while numerous other points of clarification have followed more recently. It is now believed that the drivers are, at least in part, newly recruited command elements. This seems reasonable since the flexor and extensor command ele-

ments that were directly excited by the initiating command element could amply supply the required synaptic activity to recruit others. These results are summarized below.

WHAT IS THE ROLE OF SENSORY FEEDBACK?

Several observers in the 1960s noted that the command elements seemed to function independently of the sensory systems. Larimer and Kennedy (1969) studied this phenomenon directly using a de-afferentation procedure. A command system (see definition below) involving a complex cyclic behavior was chosen for this study. More recent workers have found that this interesting system contains a command neuron, the caudal photoreceptor. Simon and Edwards (1990a,b) showed that strong illumination of the caudal photoreceptor evoked walking behavior. The circuit surrounding the caudal photoreceptor was studied by Moore and Larimer (1987, 1988, 1993). At the time Larimer and Kennedy studied the caudal photoreceptor, however, it was not known to be a command neuron.

Brief electrical stimulation in Wiersma's area 85 turns on the command system, yielding a complex cyclic behavior (Fig. 3). In our reduced preparation, the motor output manifests itself as cyclic movements of the telson and uropods. Figure 3 contains records from the motor neurons to the tail fan muscles on opposite sides. In Figure 3A, the motor program is the result of stimulation of the command fiber in area 85 right. Figure 3B shows the corresponding motor program after de-afferentation and stimulation of the command neuron in area 85 left. The absence of much of the animal's sensory feedback results in no apparent motor deficit. This observation is puzzling because it is well known, for example, that joint receptors, muscle receptors, and vision influence motor output in crustaceans as well as in the vertebrates.

A NEED TO DEFINE "COMMAND NEURON"

For more than a decade since Wiersma and Ikeda (1964) first suggested the use of "command neuron," the term had been applied to almost any neuron that was capable of evoking a motor output when stimulated. Should sensory cells (components of reflexes) or cells that are electrically coupled to motor neurons be included? Kupfermann and Weiss (1978) pointed out that the name itself implied that the "command cells" have some critical function in the release of behavior. In an effort to clarify and limit the term "command neuron," Kupfermann and Weiss offered the following definition. A cell should be considered a command neuron only if its activity is both *necessary* and *sufficient* to initiate a behavior. Between the two, the necessity criterion is most difficult to establish. The authors suggested that a putative command neuron might be better judged to be necessary if a behavior could be temporarily interrupted. This could be done in some instances by functional removal of the cell of interest by hyperpolarization. If the behavior failed to appear after removing the cell, it might be classified as a command neuron. As explained below, in the abdominal positioning system, a group of cells is ordinarily recruited by most command-like cells to drive a behavioral output. No single neuron within one of these groups is a "com-

mand neuron." However, Kupfermann and Weiss (1978) provided for these examples by suggesting the term "command system" for the group and "command element" for individual interneurons within the group.

EMPHASIS ON BEHAVIORS INITIATED BY COMMAND ELEMENTS

Bowerman and Larimer (1974a,b) surveyed the movements induced by stimulation of bundles teased from the circumesophageal connectives of semi-intact crayfish. Since these interneurons were emerging from the brain, we expected their outputs to be complex. We used cinephotography to record the movements produced by interneuronal stimulation. The visual data were gathered on 16-mm film at 8 frames/sec and synchronized with the neuronal activity on the oscilloscope through a photodiode that was recorded on both the movie film and on the oscilloscope. The behavioral data were analyzed using a time-and-motion study projector equipped with a frame counter. Access to the circumesophageal connectives was through a hole in the dorsal cephalothorax. The hepatopancreas and stomach were removed, the cavity was thoroughly washed with saline, and the connectives were desheathed. Otherwise, the animals were intact and the appendages were free to move. Small bundles of axons were teased from the circumesophageal connectives and stimulated. Each bundle was marked on a cross-sectional map of the connective for later use (see Fig. 1). A Lucite post was glued to the cephalothorax to hold the animal. Its feet touched the rim of a 23-cm light-weight, low-friction walking wheel. The animal was able to walk forward or backwards and turn the wheel. The preparation was immersed in cold, oxygen-enriched saline (van Harreveld, 1936). A front surface mirror allowed the animal to be photographed from both the dorsal and side perspectives. In some instances, the animal outlines were traced from the film for presentation purposes. The small bundles were subdivided if possible. However, even after reduction, they probably contained more than one axon. Sometimes, a single axon could be "isolated" by carefully setting the stimulus strength. Most bundles were stimulated at 75 Hz.

Stimulation of bundles from the circumesophageal connectives yielded what appeared visually as some partial and some complete behaviors. This could be explained in several ways. It is possible that more than one neuron is needed to drive the full behavior and the full complement was not recruited. Or perhaps the preparation lacked viability and the stimulated neuron failed to produce the full behavior. Also, the cord locations of axons whose activity affected a single "behavior" or movement were sometimes clustered tightly (general promotion of the limbs) or loosely clustered (as in cheliped lift, Fig. 4). Although the behaviors in these two cases are similar, it is not known whether the neurons are identical or whether the scatter resulted from error in locating the axons in the cord. Many command elements seemed to evoke forward walking. These particular axons fell into three clusters, perhaps representing three command elements, but this is not known. Command elements releasing backward walking were also numerous. However, the axons were located in a large cluster and a second, smaller cluster of only two axons located on the opposite side of the

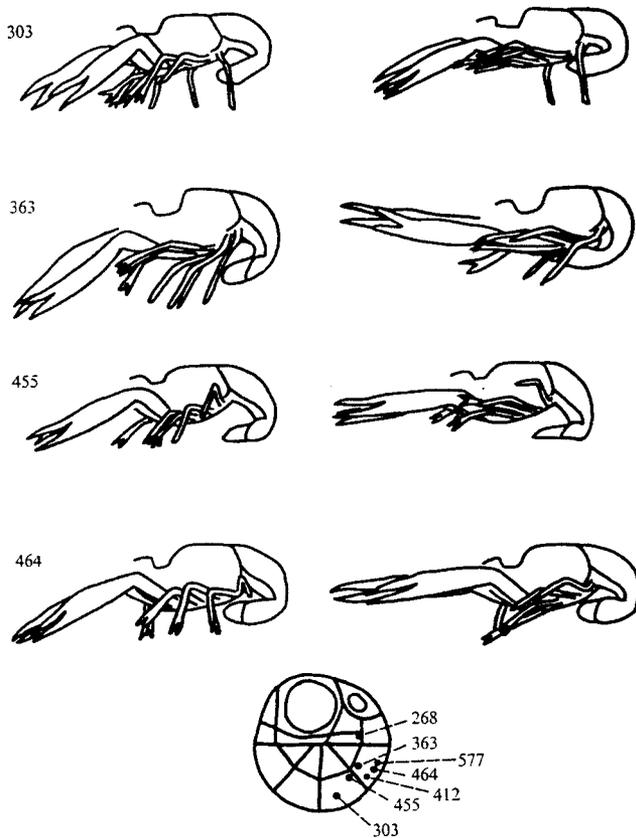


Fig. 4. Behavior-like movements of minimally dissected crayfish in response to stimulation of fine bundles of axons dissected from the circumesophageal connectives. The column of diagrams on the left contains outlines of the starting positions. Diagrams on the right represent the end positions. Locations of the axons are shown on the map proposed by Wiersma (1958). Since the cluster is small, it may represent only one or a few fibers of this type that were located in seven different individuals. Flexion of the abdomen and general promotion of the limbs is also seen in behaving, unstimulated animals. Overall positions reminiscent of these also accompany backward escape and swimming. Reproduced from Bowerman and Larimer (1974a) with permission of the publisher.

connective. Circumesophageal fibers that released escape-swimming behavior were of some interest: many more of these were found (16) than expected and not all of these showed limb promotion that streamlines the animal for speed.

There are several other behavior-like movements of interest that were evoked by the stimulation of circumesophageal bundles. For example, there are sites that, when stimulated, release a feeding-like behavior. The feet are moved about in rapid motion and occasionally brought toward the mouth. Stimulation at other sites evokes scratching-like behavior that may be related to cleaning of the carapace. In addition to abdominal flexion and extension, the animals were seen to stop their movements in characteristic positions providing a particular abdominal geometry. These behaviors were very similar to many observed by Kennedy et al. (1967). Some of the most interesting behavior-like movements obtained from stimulation of circumesophageal bundles included turning, righting, and de-

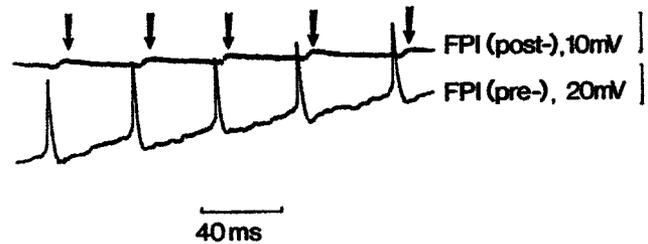


Fig. 5. An apparently monosynaptic connection between a pair of FPIs (flexion producing interneurons or flexion command elements). Stimulation of the cell in the lower trace excited it to a slow impulse train. Each impulse in the train appears to evoke an EPSP with constant latency in the other cell. Impulse traces have been retouched for clarity. Reproduced with permission from Jellies and Larimer (1985) with permission of the publisher.

fense postures. Finally, the stimulation of two bundles gave a striking freezing behavior resembling "thanatosis." It was particularly well represented in one preparation, which was otherwise undergoing spontaneous movements. A brief stimulation of the bundle stopped all motion without regard to the position of the abdomen or limbs. Upon cessation of stimulation, the spontaneous movements resumed. They were again stopped motionless by a brief stimulation of the bundle. It would be of great interest to know if these interneurons are involved in generating the natural behaviors in intact animals.

THE INTRACELLULAR APPROACH TO STUDYING THE OUTPUTS AND SYNAPTIC INTERACTIONS OF COMMAND ELEMENTS

The switch from largely extracellular to an intracellular approach involved the stimulation, recording, and dye filling of the command elements involved. These methodological changes added some important dimensions to our understanding of postural control. For example, (1) we could not only examine impulses in the impaled cells but low level signals such as synaptic potentials (Fig. 5); (2) with intracellular stimulation, we were assured of activating one command element at a time; and (3) we could visualize the dye-filled cell and trace or photograph it for the record. Armed with all these data, it was possible to consider, with some degree of confidence, whether a cell was identifiable.

In an effort to relate the intracellular studies to the earlier extracellular experiments, we combined bundle stimulation with the intracellular approach. Our intracellular experiments were performed predominantly on isolated abdominal nerve cords (Miall and Larimer, 1982a,b); that is, cords including abdominal ganglia A_1 – A_6 . In order to match earlier experiments that involved conventional command elements, fine bundles were teased from the anterior abdominal ganglia. They were treated in the same fashion as in the earlier studies, and stimulated extracellularly. Motor programs for abdominal flexion or extension were measured by recording from ganglionic roots 2 (extension) and the superficial branch of root 3 (flexion). Once found, the bundle was set aside for later use. Meanwhile, A_4 was desheathed for probing with a Lucifer-filled microelectrode (Stewart, 1978). The intracellular

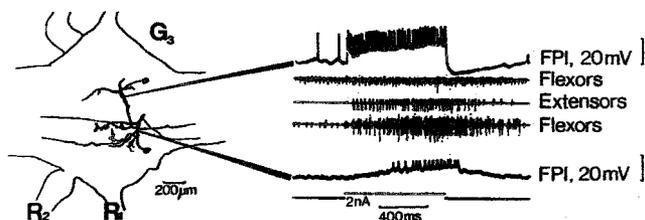


Fig. 6. A unidirectional synapse between two FPIs (flexion producing interneurons or flexion command elements) located in A_3 . Depolarizing current was passed into the upper cell, which recruited the lower cell. The unidirectional interaction is not shown. The flexor traces show impulses of different amplitude while the extensor motor neurons show mainly one amplitude. This is interpreted as the output of the extensor inhibitor motor neuron. Stimulation of the interneuron evoked a stronger flexion in A_5 than in A_3 (top and bottom extracellular traces, respectively). Extension was recorded from A_4 . Traces have been retouched. Reproduced from Jellies and Larimer (1985) with permission of the publisher.

probe was used to detect impulses and synaptic potentials and also to inject dye. The simultaneous use of intracellular and extracellular techniques yielded an unanticipated understanding of the underlying circuits. Both the bundle stimulation and the stimulation of impaled neurons revealed cells with properties of command elements. For the first time, it was possible to detect synaptic interactions between the command elements themselves (Fig. 6) or between command elements and motor neurons.

It soon became apparent that there was little difference between the neurons examined by the intra- and extracellular techniques. Most of the recorded interneurons possessed the characteristics of command elements. The most striking characteristic of the series was the discovery that there are numerous synaptic interactions, especially between pairs of flexion elements. It was noted that the synaptic interactions between command elements were occasionally strong enough to recruit the second cell to impulse activity. Our first impression was that the strong stimulation of a command element could recruit new command elements to drive a movement. Because it appeared that these command elements did not operate alone, they did not fit the definition of "command neurons" according to Kupfermann and Weiss (1978). Jellies and Larimer (1985) pursued this further (see below).

Miall and Larimer (1982a,b) showed that the tonic flexor motor neurons also received extensive synaptic input, mostly from unknown sources, but it was apparent they received both poly- and monosynaptic inputs from the command elements. In fact, the group of synaptically recruited command elements may be the most important organizer of these movements whereas the motor neurons themselves seem to serve as recipients of the control system.

This study was pursued further using two Lucifer-filled microelectrodes (Jellies and Larimer, 1985). Using the isolated nerve cord of the crayfish, a search was made in two or rarely one desheathed ganglion, for two command elements (Fig. 7). The two were interacted by first stimulating one by current passage while examining the other for synaptic activity. We then stimulated the other and looked for activity in the first. We inter-

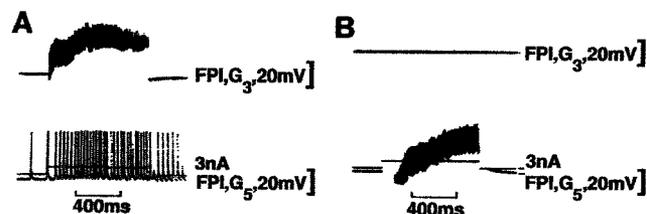


Fig. 7. Two flexion producing interneurons were impaled with microelectrodes, one in A_3 , and the other in A_5 . **A:** The effect on the FPI (flexion producing interneuron or flexion command element) in A_5 of stimulating the FPI in ganglion A_3 . Their synaptic connections are very strong. When the inverse procedure was used, the FPI in A_5 failed to influence the FPI in A_3 (**B**). It is a common occurrence for synapses in this system to be unidirectional. The FPI in A_3 is said to recruit the FPI in A_5 . Reproduced from Jellies and Larimer (1985) with permission of the publisher.

acted flexion elements with other flexion elements, flexion and extension elements, and extension elements with other extension elements. A clear picture of the interactions of command elements emerged. Pairs of flexion command elements often were found to be connected by excitatory synapses, thereby providing a means for one agonistic command element to recruit others. The interactions were both polysynaptic and apparently monosynaptic. As a rule, the synaptic actions were one-way (Fig. 8). Exactly what these unidirectional interactions accomplish is not known. However, mutual excitation would be expected to lead to positive feedback, which tends to be unstable. As with the flexion elements, the interactions of two extension command elements also tended to be excitatory. In contrast, when extension and flexion elements interacted, they were usually inhibitory.

This two-microelectrode study involved the use of 111 pairs of command elements. Only 16% showed no observable synaptic interactions. This percentage of non-interacting interneurons may not be entirely accurate. For example, the great majority of these interneurons are projection types with axons extending throughout much of the CNS, offering the opportunity for synaptic interactions at sites remote from the measuring microelectrode.

In procedures where command elements for abdominal positioning were located in abdominal ganglia with dye-filled microelectrodes in concert with stimulation of axons in the circumesophageal connectives, it became clear that extensive synaptic interactions occurred at remote rostral sites (Larimer and Moore, 1984). It was concluded that synaptic recruitment of groups of interneurons could occur at any level in the nervous system. Similarly, such synaptic interactions of command elements at local and remote sites could coordinate parts of complex behaviors.

The crayfish and lobster tailfan appendages (i.e., the uropods and the telson) undergo complex and independent movements vis-à-vis the abdomen proper. These steering movements are driven by a number of interneurons that course in both directions into and out of A_6 (Nagayama et al., 1993). Sensory stimulation of the telson can affect not only movements of the telson and uropods directly, but the abdominal segments as well.

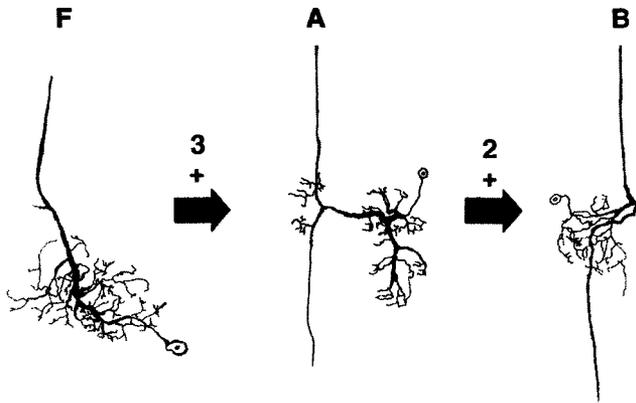


Fig. 8. Dye-filled microelectrodes were used to sample pairs of flexion command elements for the presence of synaptic interactions. Multiple sampling from several preparations revealed three flexion producing interneurons (FPIs) that were encountered several times. Neuron F possesses a cell body in A_6 whereas neurons A and B have their cell bodies in A_3 . All of their axons were ipsilateral. Neuron F was impaled with neuron A three times, and A with B twice. Each interaction was excitatory and always in the same direction. They form what has been called here a small "functional group" that, when stimulated via F, evokes a characteristic abdominal flexion movement. Such a group also demonstrates that the synapses are invariant. Reproduced from Jellies and Larimer (1985) with permission of the publisher.

CYCLIC ABDOMINAL MOVEMENTS IN CRAYFISH

In addition to their participation in myriad postural adjustments of the abdomen, the tonic flexor and extensor muscles also are engaged during a complex, rhythmic behavior. During backward terrestrial walking (a major component of the animal's behavioral repertoire), the abdomen alternately flexes and extends, permitting the tail to contact and lift away from the substrate with an average period of about 1.5 seconds in intact animals (Kovac, 1974a). This cyclic motor program was first studied by Larimer and Kennedy (1969), who found that it could be released by unpatterned, extracellular stimulation of a single command element teased from either the right or left ventrolateral margin of the abdominal connectives, in area 85 (Wiersma and Hughes, 1961). Larimer and Kennedy (1969) determined a number of fundamental properties of the cyclic motor program, including: (1) the involvement of promotion/remotion and rotation of the tailfan as well as abdominal flexion and extension; (2) its continuation in the absence of sensory feedback, thus qualifying it as a centrally generated pattern; (3) equivalent, symmetrical motor outputs driven by stimulation of either of the bilaterally homologous command elements; and (4) strengthened motor activity in response to simultaneous stimulation of both the right and left command elements, indicating a convergence of outputs from both elements. Altering the firing patterns of the tonic flexor and extensor motor neurons fails to reset the phase of the motor rhythm, providing evidence that the tonic motor neurons are driven by, but are not part of, the oscillatory mechanism (Kovac, 1974b). In order to account for the motor program's widespread multisegmental effects and complex phase

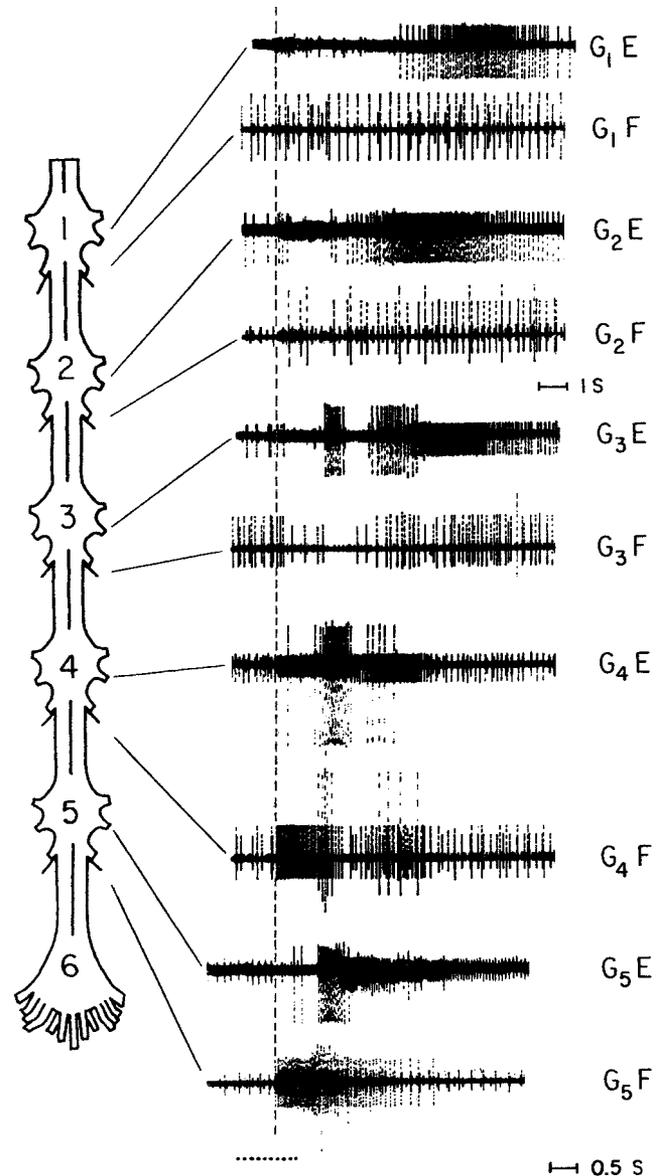


Fig. 9. Tonic flexion (F) and extension (E) outputs of the cyclic motor program recorded from abdominal ganglia 1 through 5 (G_1 - G_5). The complex pattern was generated in response to unpatterned stimulation (1 sec, 10 Hz) of small axon bundles teased from the ventrolateral margins of the interganglionic connectives. This representation is a composite, constructed from several preparations, using the onset of tonic flexion in G_5 (G_5F) as a time reference. The motor pattern was highly consistent, both within the same preparation and among different preparations. Note the complexity of the responses, including significant overlap of extension and flexion in each ganglion as well as phase differences from ganglion to ganglion. Time calibration: 0.5 sec (G_3 , G_4 , and G_5), 1 sec (G_1 and G_2). Reproduced from Moore and Larimer (1988) with permission of the publisher.

relationships, it was suggested that there must be one or more large populations of interconnected "driver" interneurons between the command elements and the motor neurons (Larimer and Kennedy, 1969). The complexity of the cyclic motor program is illustrated in Figure 9.

In an attempt to find interneurons other than command elements that could participate in the cyclic motor program, Kovac (1974b) made en passant recordings from desheathed interganglionic connectives at area 85 and discovered a unit, termed the "flexor driver" (FD). FD units were located in both the right and left hemiconnectives, rostral and caudal to ganglion A_5 . Short bursts of activity delivered to any one of the FD interneurons produced nearly synchronous bursts in the other three units. In response to command element stimulation, the FD interneuron produced a 600-msec burst that typically preceded a 600-msec burst of tonic flexion in ganglion A_5 by about 100 msec. Tonic flexion and extension were observed to alternate in A_5 ; intracellular recordings from the extensor motor neurons showed that they were hyperpolarized during the flexion burst, but fired their own burst upon recovery from inhibition. Stimulation of the FD unit during cyclic activity reset the rhythm, suggesting that it may be a part of the oscillatory mechanism. Based on these results, Kovac (1974b) proposed a model to explain the cyclic motor program: a mutually reinforcing network of FD interneurons that, when activated by the command element, would simultaneously excite the tonic flexor motor neurons and inhibit the tonic extensor motor neurons, thereby producing the alternating abdominal flexion and extension.

Building upon the previous, largely extracellular studies, Moore and Larimer (1987) used Lucifer-filled microelectrodes to probe the neuropilar regions of the abdominal ganglia for elements involved in cyclic pattern generation. No FD units (Kovac, 1974b) were found. However, an extensive sampling of ganglia A_4 , A_5 , and A_6 revealed two populations of interneurons with axons coursing through area 85 of the interganglionic connectives: the pattern-initiating (PI) cells and the partial pattern-initiating (PPI) cells. In response to unpatterned stimulation of the command element in the A_1 - A_2 connective, both the PI and PPI interneurons produced a complex burst of impulses beginning just before the patterned motor activity in the ganglia. Driven to spiking by injection of depolarizing current, PI interneurons elicited a full cyclic motor program throughout the abdominal nerve cord, indistinguishable from that generated by extracellular stimulation of the command element (Fig. 10). In contrast, depolarization of the PPI interneurons to spiking resulted in recognizable, though much weaker, cyclic motor patterns (Fig. 11) of the same periodicity and with the same phasing of abdominal flexion and extension. Suggesting an extensive, electrically coupled, interganglionic network, Lucifer iontophoresis of either PI or PPI interneurons always revealed a number of dye-coupled neurons, with axons closely associated in area 85, in addition to the impaled cell. The dye-coupling was consistent: Lucifer iontophoresis of identified PI or PPI interneurons in different preparations revealed the same group of dye-coupled cells. At least one PPI interneuron apparently was dye-coupled to an identified PI interneuron. Also, consistent with the existence of an electrically coupled network, the bursts recorded from the PI and PPI interneurons typically contained multiple spike amplitudes.

Functional removal of PI interneurons by hyperpolarization during cyclic pattern generation produced no

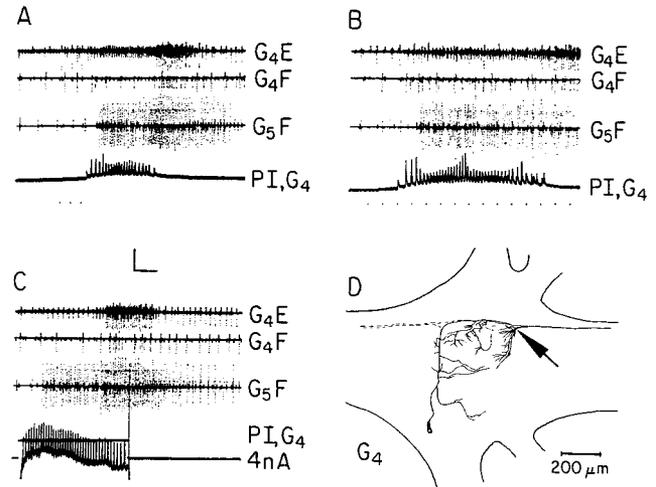


Fig. 10. Records from pattern-initiating (PI) interneuron with cell body in abdominal ganglion 4 (G_4). The PI interneuron was impaled in G_4 . **A,B:** Depict a fast and a slower sweep speed, respectively. The cyclic motor pattern was initiated by extracellular stimulation (dots below traces represent individual stimulus pulses) of an axon bundle in the G_1 - G_2 connective. Note the extension (E) and flexion (F) motor outputs in the top three traces. The intracellular recording (bottom trace) is characterized by the presence of multiple spike amplitudes. **C:** The cyclic motor program was elicited by intracellular stimulation of the PI interneuron; note similarity with outputs in **A**. **D:** The Lucifer yellow-filled PI interneuron, dye-coupled to two additional axons (dotted lines) projecting in the rostral direction as well as an axon terminal (arrow). Calibration: **A** and **C**, 20 mV, 0.2 s; **B**, 20 mV, 0.1 s. Reproduced from Moore and Larimer (1987) with permission of the publisher.

observable deficits in the motor program, indicating a high degree of redundancy in these units, whereas hyperpolarization of PPI interneurons caused significant reductions in motor outputs (Fig. 11). One-to-one, constant latency spikes recorded from PI interneurons in response to high frequency stimulation of command elements in the interganglionic connectives suggested that axons of the PI network probably are the command elements. From these results, Moore and Larimer (1987) concluded that initiation of the cyclic motor program was the responsibility of a distributed command system rather than a single command element. They proposed that the command system consists of a large number of PI interneurons presynaptic to a smaller number of PPI interneurons that, in turn, impinge on the (as yet undiscovered) central oscillator circuitry.

Several lines of evidence provide further support for the hypothesis that cyclic pattern generation is initiated by a widely distributed, interganglionic command system. First, the cyclic motor program can be evoked by stimulation of the ventrolateral margin (area 85) of any of the abdominal hemiconnectives (Moore and Larimer, 1993). Second, the strength of the oscillatory motor output in each ganglion depends upon the simultaneous activity of the command elements in all of the hemiconnectives leading to that ganglion. Elimination of any input to the ganglion reduces the cyclic motor output (Kovac, 1974b). Third, as demonstrated in a series of hemiconnective resection experiments (Moore and Larimer, 1993), there is extensive lateral signal

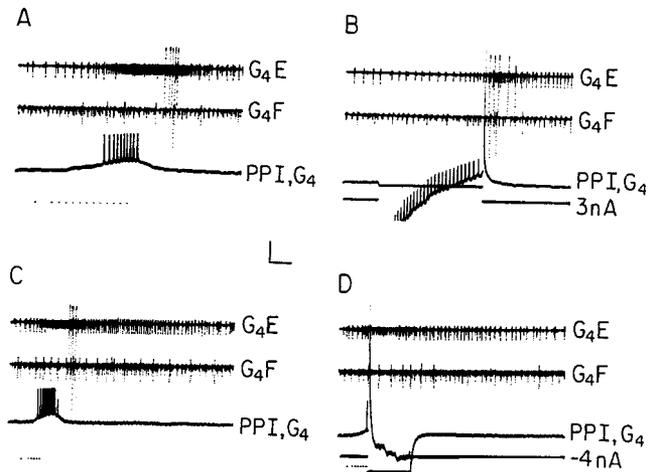


Fig. 11. Recordings from a partial pattern-initiating (PPI) interneuron impaled in abdominal ganglion 4 (G_4). Tonic extension (E) and flexion (F) motor outputs are indicated in the top two traces and the intracellular recording is the third trace. **A:** Intracellular response of PPI interneuron to cyclic pattern initiation via extracellular stimulation of an axon bundle in the G_1 – G_2 connective (stimulus pulses are depicted below the traces); note also the extension and flexion motor outputs. Depolarizing current injection (**B**) excites the PPI interneuron to spiking but results in an incomplete motor output (compare with **A**). **C:** The cyclic motor pattern is again elicited by extracellular stimulation. Impulse formation in the PPI interneuron is eliminated by injection of hyperpolarizing current (**D**) during extracellular stimulation of the cyclic pattern, resulting in a significant reduction of motor outputs (compare with **C**). Calibration: **A** and **B**, 20 mV, 0.2 s; **C** and **D**, 20 mV, 0.5 s. Reproduced from Moore and Larimer (1987) with permission of the publisher.

transfer across all of the abdominal ganglia, but a pronounced caudal bias for descending conduction following the transfer. Finally, contrary to earlier results (Kovac, 1974b), the cyclic motor program (although very weak) can be elicited from an individually isolated ganglion (Moore and Larimer, 1993). Therefore, the cyclic motor system contains both central oscillators and a distributed network of command elements.

The cyclic pattern-initiating network may be activated by illumination of the caudal photoreceptor (CPR) located in the terminal abdominal ganglion (Edwards, 1984). The CPR typically fires a burst of spikes in response to bright light and, if the burst frequency is greater than 60 Hz, the cyclic motor pattern is released (Simon and Edwards, 1990b). The interaction between the CPR and the PI network appears to occur rostral to the abdominal nerve cord but below the brain, possibly at the level of the subesophageal ganglion (Simon and Edwards, 1990b).

Because the tonic and cyclic postural movements rely upon activation of the same superficial flexor and extensor muscles, a reasonable hypothesis is that some premotor interneurons are shared between the two behavioral control systems. Could the tonic flexion- and extension-producing command elements responsible for abdominal positioning also contribute to the alternating flexion and extension in the cyclic motor program? Moore and Larimer (1988) tested this possibility by recording from individual command elements of the tonic postural system during activation of the cyclic

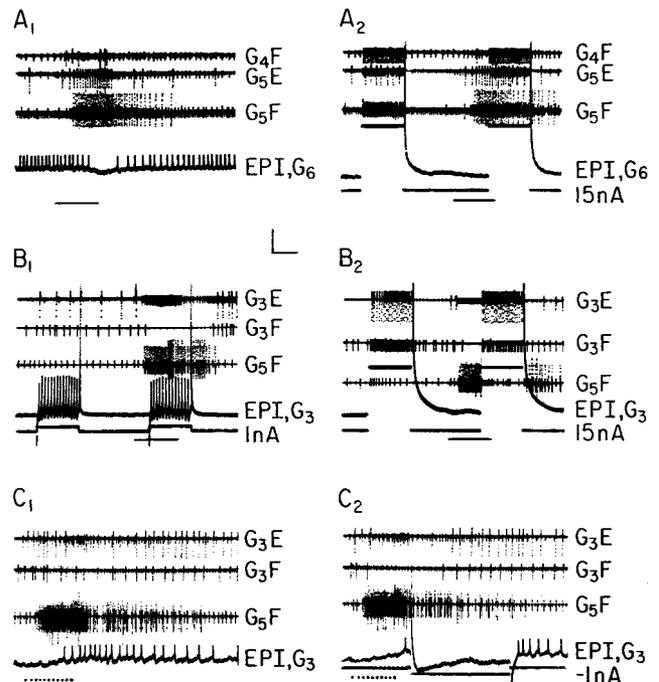


Fig. 12. Three different extension producing interneurons (EPIs) illustrate a diversity of command element interactions with cyclic pattern generation. In all records, the top three traces indicate the extension (E) and flexion (F) motor outputs in various abdominal ganglia (G_3 , G_4 , G_5), as indicated. The cyclic motor program was initiated by extracellular stimulation (indicated by dots or bars beneath traces) of interganglionic axon bundles. The first EPI, impaled in G_6 , was hyperpolarized in response to cyclic pattern generation (**A**₁). When strongly depolarized by current injection (unbalanced bridge; **A**₂, left), it evokes strong tonic flexion and extension motor outputs. However, when driven during cyclic pattern generation (**A**₂, right), the EPI's tonic outputs mask the cyclic motor program for G_4 F, the cyclic program masks the tonic outputs for G_2 E, and both tonic and cyclic outputs are present for G_5 E. The second EPI, impaled in G_3 , shows no change in firing rate during cyclic pattern generation (**B**₁). When strongly depolarized (**B**₂, unbalanced bridge), the EPI's tonic outputs completely override the cyclic pattern. Although the third EPI is depolarized to spiking during the cyclic motor program (**C**₁), eliminating its impulse formation by hyperpolarizing current injection has no effect on the cyclic motor outputs (**C**₂). Calibration: 20 mV, 0.5 s. Reproduced from Moore and Larimer (1988) with permission of the publisher.

pattern. A total of 79 flexion, 29 extension, and 20 inhibitory command elements of the tonic postural system were monitored via intracellular recordings. During cyclic pattern generation, about 27% of the command elements were inhibited and about 38% showed no response. Only about 35% were recruited, and none of these were found to contribute to the cyclic motor outputs. Hyperpolarizing current delivered to these recruited command elements produced no measurable deficits in the cyclic motor program (Fig. 12C).

When the tonic and cyclic motor programs were simultaneously activated, a bewildering diversity of interactions between the two behavioral subsystems was found (Moore and Larimer, 1988). In some cases, the motor outputs of the tonic command elements completely masked the cyclic pattern (Fig. 12B) or were able to override the cyclic activity in some ganglia, but not in others (Fig. 12A). In all such recordings of tonic

disruption of cyclic activity, the cyclic pattern continued without phase shifts after termination of the tonic activity. In other cases, the cyclic pattern completely overwhelmed the output of the tonic command elements. These results strongly suggest that the command elements of the tonic postural system do not participate in generating the cyclic motor program.

Why should the tonic and cyclic postural control systems not share the same premotor interneurons? Stimulation of tonic command elements produces a characteristic reciprocity (i.e., excitation of agonist and inhibition of antagonist motor neurons) resulting in nearly uniform flexion or extension in several neighboring abdominal ganglia (Miall and Larimer, 1982; Larimer and Jellies, 1983; Larimer and Moore, 1984; and others). As shown in Figure 9, not only are the cyclic motor outputs different in each abdominal ganglion but there also is a great deal of overlap between flexion and extension. Therefore, activation of the postural command elements produces motor outputs that are inappropriate to the cyclic pattern. To create the cyclic pattern from tonic command element outputs would require an extensive network of coordinating interneurons to continuously modify the tonic motor outputs during pattern generation, with a unique pattern in each ganglion. Although the tonic and cyclic postural behaviors employ the same motor neurons, perhaps having two separate premotor populations is a more efficient solution than using one population of premotor interneurons in concert with a complex network of coordinating interneurons. However, the premotor elements of the cyclic system have not yet been found.

The cyclic postural motor program in crayfish contains features not typical of other crustacean oscillatory circuits. Unlike crayfish swimmeret movements (Heitler, 1978, 1981), crab scaphognathite beating (Simmers and Bush, 1983), and the pyloric and gastric mill rhythms in the lobster stomatogastric ganglion (Maynard, 1972; Maynard and Selverston, 1975), motor neurons are not components of the oscillator mechanism (Kovac, 1974b). Similar to other rhythmic systems, the pattern-initiating input signal in the crayfish cyclic postural system appears not to have any instructional features. However, in contrast to the lobster swimmeret (Davis and Kennedy, 1972) and the crayfish abdominal positioning (Evoy and Kennedy, 1967) systems, changes in pattern-initiating input frequency have no effect on output frequencies or on the strength or sequence of motor neuron recruitment. In the swimmeret (Davis and Kennedy, 1972) and tonic postural (Jellies and Larimer, 1986) systems, different behaviors are governed by the activation of different groups of command elements. In contrast, stimulation of a single PI interneuron in the cyclic postural system is sufficient to elicit the entire motor program. The extensive coupling within the PI and PPI network (Moore and Larimer, 1987) may account for this property. Finally, unlike many other oscillatory systems, the cyclic postural motor program must have continuous stimulation or it will terminate at the end of each cycle. This property suggests that the behavior makes use of the oscillatory mechanism but exerts an unusually robust control over its activation and termination.

INTERNEURONS INFLUENCING MORE THAN ONE BEHAVIOR

Several workers involved in crustacean neurobiology have observed that two or more behaviors may occur simultaneously. Williams and Larimer (1981), for instance, noted that lifting a crayfish off the substrate frequently resulted in abdominal extension accompanied by swimmeret beating. It is generally accepted that partial behaviors, related behaviors, or behaviors that occur in sequence are somehow organized in a proper order by the nervous system (Evoy and Kennedy, 1967). For example, it was noted that in freely behaving crayfish, the swimmerets are coupled to the position of the abdomen. Kotak and Page (1986) showed that sensory structures on the swimmerets influence the motor control for abdominal positioning. Abdominal extension is often correlated with swimmeret beating and with forward walking (Cattaert et al., 1992). In the course of their extracellular study, Evoy and Kennedy (1967) found command elements that affected both the swimmerets and abdominal positioning. In another extracellular study of command elements and their influence on various movements in crayfish, Williams and Larimer (1981) mapped six abdominal cord loci containing six major extension command elements. In the course of this study, they teased 23 extension fibers from locus 3. Eighteen of these 23 fibers (78%) affected both extension and swimmeret beating. Furthermore, the two behaviors could not be separated by additional subdivision of the bundles nor by grading the voltage used in stimulation. Murchison and Larimer (1990) gave further evidence that the resulting behaviors were driven by single dual-output commands by isolating the command elements with dye-filled microelectrodes.

Evoy and Kennedy (1967) made observations on another dual-output set of abdominal positioning command elements. They were the first to note that movements of the telson and uropods are controlled by some command elements operating independently of other behaviors but also by other command elements that coupled tailfan movements with abdominal movements. In the course of their intracellular study of interneurons that affect more than one behavior, Burdohan and Larimer (1995) confirmed and extended the observations of Evoy and Kennedy (1967): 64% of the abdominal positioning cells examined also increased the activity of the uropod motor neurons while 24% of the command elements for abdominal positioning also influenced the swimmerets (Fig. 13).

The use of command elements in different contexts of behavior, as the few examples above suggest, helps us to understand that animals with a limited number of command elements can accomplish a wide behavioral repertoire. Indeed, one species may not have any more command elements than another species, yet the two may exhibit species-specific behavioral responses.

Swimmeret and abdominal posture behaviors can be evoked by the stimulation of a single command element (Evoy and Kennedy, 1967; Murchison and Larimer, 1992; Williams and Larimer, 1981). The command neurons affecting both swimmeret movements and abdominal extension tend to be synaptically interactive. Like the flexion-flexion or extension-extension command el-



Fig. 13. Motor program showing that one command element can influence several motor systems. (Op) uropod opener; (Cl) uropod closer; (Sw) swimmeret; (Fl) flexor of abdomen; (Ex) extensor of abdomen; (In) the command element. The opener and closer of the uropod were held in position by the command element's activity. The swimmerets moved to the forward position, the abdomen was flexed, and the peripheral inhibitor of abdominal extension became activated. The abdominal motor control thus showed reciprocity. Only one fiber was stimulated at constant frequency; yet the output was bursty and involved motor neurons widely separated. The command element is said to be more permissive than instructive. Reproduced from Burdohan and Larimer (1995) with permission of the publisher.

elements described above, these command elements tend to recruit each other (Murchison and Larimer, 1992). Single command elements have also been found that affect selected movements of the uropods, as well as the position of the abdomen (Burdohan and Larimer, 1985; Takahata and Hisada, 1986). In both crayfish and lobster, walking behavior stimulates, especially in the first few steps, the beating of the swimmerets (Cattaert and Clarac, 1983).

DO ACTIVITIES IN COMMAND ELEMENTS UNDERLIE ANIMAL-INITIATED MOVEMENTS?

One of the most difficult tasks in neuroethology is to demonstrate the neural basis of a behavior. Stimulation of a neuron may appear to release the behavior under study. This apparent simplicity is deceptive, however. What if the command element(s) cannot be secured when the animal moves? Or, what does one do if movement destroys the axon? What if several neurons cooperate to drive a movement and the best available technique allows only one electrode to be used? An even more typical problem might be that the animal "preparation," though minimally dissected, persistently fails to behave. Thus, the neuron(s) underlying the behavior do not fire. One can only try to capture the elusive behavior and correlate it with the even more elusive neural basis. When we did the experiments on the abdominal positioning system, we obtained, as expected, only partial success.

Two series of experiments were performed. In the first set, we used a dissected preparation, placed in the upside down position, and employed one Lucifer-filled microelectrode. We relied upon touch stimulation or spontaneous activity of a behavioral act to study the impaled command element's activity during movement (Jellies and Larimer, 1986). In the second set of experiments, the animals were minimally dissected and placed in an upright position. The animal was placed in a retaining box filled with saline (van Harreveld, 1936)

and a moveable, solenoid-driven platform was positioned under its feet. Dropping the platform caused the animal to reflexly extend its abdomen and, after a time, to flex the abdomen (Larimer and Eggleston, 1971). The intention of these experiments was to record from the appropriate flexor and extensor roots, and from a command element (either a flexion- or extension-producing type). The type was determined early in the experiment by passing depolarizing current into a cell impaled in a ganglion. Control of the command element could be imposed at will by controlling the platform (Murphy et al., 1989).

In both series of experiments, abdominal positioning command elements were impaled and the animals underwent evoked or spontaneous abdominal movements. The correlations between activity in the command element and the motor programs, however, were not always clear. In fact, it was observed occasionally that there was no correlation at all. For example, we might have a command element for abdominal flexion impaled and have the animal undergo spontaneous flexion behavior. It was not uncommon to have no observable response in the command element. An additional test was usually performed, consisting of passing hyperpolarizing current into the impaled command element to silence any activity it might have. These results have several possible explanations. First, if many command elements exist for flexion behavior, it is possible that only part of them are active at once. In the example given above, it is possible that the responsible command element was not being recorded. In other instances, however, there was a strong correlation between the firing of the impaled command element and the motor program for abdominal positioning. Hyperpolarization of the impaled command element can cause a deficit in the motor program but fail to terminate it (see Fig. 14). This might occur if several command elements were contributing to the motor output. If only one of these were impaled and removed from the circuit by hyperpolarization, one might expect to cause only a deficit rather than complete elimination of the motor program. Although strong electrical stimulation of a command element is often capable of evoking an apparently complete behavior, no single command element has been found that can account for a complete behavior under natural activation.

We have reported above that synaptic interactions are prevalent among both flexion and extension command elements. Those command elements with agonistic function tend to be associated by excitatory synapses. Those that are antagonistic in behavioral output (i.e., flexor and extensor pairs) tend to be inhibitory to one another. Agonistic command elements may recruit others of their own kind to produce a strong drive and give rise to behavior. High frequency activation of certain command elements have been shown to drive another command element to spiking (Figs. 6 and 7).

NUMERIC AND METAMERIC ASPECTS OF ABDOMINAL POSITIONING ELEMENTS IN CRAYFISH

The most recent work on the abdominal positioning system from this laboratory has dealt with estimating the total number of command elements devoted to these behaviors, the number of elements in the re-

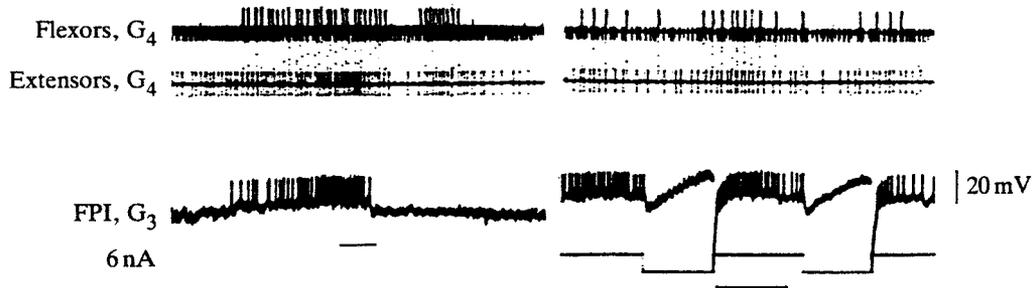


Fig. 14. Data from largely restrained, mostly intact crayfish. These animals attempt movements and the movements can be interpreted from the motor neuron and command element records. This example is from a flexion-producing interneuron (FPI) impaled in A_3 . This command element was occasionally spontaneously active. The activity in the extensor motor neurons (line 2) was composed mostly of impulses from the peripheral inhibitor, indicating reciprocity. It was

during these spontaneous bouts that hyperpolarizing current was applied through the microelectrode. Although the command element was silenced by hyperpolarization, the motor neuron firing was reduced but not silenced. It is believed that more than one command element was controlling the motor output. Reproduced from Jellies and Larimer (1986) with permission of the publisher.

recruited population, and determining how consistent these numbers may be. In other words, when a command element synaptically recruits a group, how many of the cells are shared in duplicate stimulation programs?

We wished to know as much as possible about each command element's morphology, its location in the CNS, and its physiology. With this information, we could classify each as an identified cell and determine how many times each was encountered when their ganglion of residence was sampled with the microelectrode. Over a period of more than 20 years, we have gathered a catalog of abdominal positioning command elements consisting of several hundred interneurons. These cells were recorded at random in different studies from abdominal ganglia A_3 , A_4 , A_5 , and A_6 of the crayfish, *Procambarus clarkii*. Detailed information on the interneurons in question is presented principally in the following publications: Miall and Larimer, 1982a,b; Larimer and Jellies, 1982; Jellies and Larimer, 1985, 1986; Larimer and Moore, 1984; Moore and Larimer, 1988.

We set aside those cells from each ganglion that could be classified for further analysis. The most useful ones were those impaled in the cell body or dendrites so the soma-dendrite morphology could be used as well as the location of the axon. The electrophysiology was also valuable in classifying each command element. We first counted the number of cells found in each ganglion (Larimer and Pease, 1988). For ganglia A_3 , A_4 , A_5 , and A_6 , the numbers were 29, 30, 20, and 35, respectively. We realized that these numbers did not represent all of the command elements present because with each sampling of a ganglion, we almost always found new cells. As the sampling proceeded, however, fewer new cells were found and previously discovered cells were encountered more frequently. It appeared useful to estimate the total number of abdominal positioning command elements in each ganglion. Two statistical methods were used to estimate this number, maximum likelihood, and the Lincoln index (Larimer and Pease, 1988). For A_3 , we estimated the total number to be 34 (95% confidence interval, 27–49) using the maximum likelihood method and 39 (95% confidence interval, 34–48) from the Lincoln index. With these two

methods, the estimated numbers were 60 (42–108) and 64 (48–110) for A_4 , 86 (53–186) and 91 (50–144) for A_5 , and 98 (69–181) and 101 (73–182) for A_6 . Almost all of the abdominal positioning neurons accumulated in the catalog are the projection type. In independent studies, Kondoh and Hisada (1986) and Wine (1984) estimated that the same species of crayfish used in our experiments, *Procambarus clarkii*, possesses a total of 650 neurons in each abdominal ganglion. Furthermore, these 650 neurons are evenly distributed among local (axonless) interneurons, motor neurons, and projecting interneurons, except in A_6 where the ratio is approximately 3:2:1 (Wine, 1984). For ganglia A_3 to A_5 , it is estimated that from 16 to 42% of the 217 projection interneurons per ganglion are positioning elements. Larimer and Pease (1988) estimate about 100 abdominal positioning elements in A_6 and Wine (1984) estimates 108 projection interneurons in the same ganglion. If true, virtually all of the projecting interneurons originating in A_6 are command elements for abdominal positioning! This is not easily explained. For example, there is some evidence that command elements operate at all levels of the CNS (Larimer and Moore, 1984). At each level, they may be specialized to interact with another set of interneurons (Bowerman and Larimer, 1974a). Abdominal positioning elements are known to cooperate with the interneurons involved in swimmeret movements, uropod positioning, and walking, among others (see above). This arrangement may require a number of additional neurons. In normal behavior, one can observe that the abdomen assumes an almost infinite variety of positions. We assume that each position requires at least some modification in composition of command elements from that required for another position. Even if the substitutions are partial, the total number of command elements participating in each abdominal position could be large.

We have known for some time that strong stimulation of one command element will usually cause the activated interneuron to synaptically recruit other similar command elements. Although we know that many of the recruited cells are capable of contributing to the motor output and that they are, at times, driven to impulse formation, we did not know until recently how many of the recruits comprise an active group (Brewer and Larimer, 1997). In this study, the ventral nerve

cord was isolated and suspended in van Harreveld's (1936) solution. A Lucifer-filled microelectrode was used to locate and stimulate an abdominal positioning command element. Records from ganglionic roots 2 and 3 signalled whether the evoked motor output was extension or flexion, respectively. The interganglionic connectives were teased into smaller bundles for recording any impulse activity in the CNS evoked by intracellular stimulation of the command element. Impulses moving rostrally as well as caudally were recorded. Recognition of each new CNS impulse was accomplished with a digital recorder sampling at 10 KHz, thereby spreading the impulses and revealing their shapes. The impulse profiles were copied on film and then enlarged on acetate sheets for use as templates. A comparison of templates with other impulses was accomplished simply by using an illuminated viewing box to visually match the shapes.

The average number of new interneurons recruited in a group was 18. Twenty-seven groups were examined, with the size of the group ranging from 3 to 50 interneurons. The average number of groups with ongoing activity that showed increased activity upon intracellular stimulation of the command element was 14 (range 3–32). Finally, the number of driven command elements that caused lower spontaneous activity was 10 (range 2–32). Even if they fired at low frequency, the recruited command elements contributed substantially to the overall motor output (Brewer and Larimer, 1997).

It appears that groups of 10 to 18 interneurons participate in each evoked behavior. If each synaptic group of command elements is composed of somewhat different individual elements, each unique group could produce a unique behavior. Note that this scheme allows some individual command elements to be part of several different functional groups. If we return to our early questions about crustacean command elements, we asked "how does stimulation of one neuron cause unique activity in several ganglia and even affect more than one behavior?" The answer could be that synaptic recruitment of similar command elements forms the functional group that actually produces the behaviors. Also, "how can the same neuron in two individuals (identifiable interneurons) produce recognizably similar behaviors?" The neurons involved must not only be substantially the same among different individuals of a species, but their synaptic connections must also be largely invariant (Larimer, 1988, 2000).

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