J. exp. Biol. **120**, 173–188 (1986)
Printed in Great Britain © *The Company of Biologists Limited 1986*

ACTIVITY OF CRAYFISH ABDOMINAL-POSITIONING INTERNEURONES DURING SPONTANEOUS AND SENSORY-EVOKED MOVEMENTS

BY JOHN JELLIES* AND JAMES L. LARIMER

Department of Zoology, The University of Texas, Austin, Texas, 78712, U.S.A.

Accepted 17 July 1985

SUMMARY

1. The premotor interneurones that produce coordinated abdominal movements in crayfish (*Procambarus*) when stimulated directly, are also 'sensorimotor'. Sets of these interneurones respond in predictable ways to touching the body surface. One set of interneurones (type I) is activated to spiking by touch, while another (type II) receives only subthreshold influences.

2. Several of these interneurones have overlapping receptive fields on the body surface. Touching areas of overlap activates groups of interneurones which discharge at low to moderate frequencies, rather than producing a high-frequency discharge of a single cell.

3. No single positioning interneurone has been identified which is solely responsible for a 'voluntary' (spontaneous) motor programme. When active, the positioning interneurones contribute to the production of the behaviour as a member of a constellation of such cells.

4. The results show that this motor system comprises interneurones with sensory as well as motor properties. Although single cells can produce coordinated movements when stimulated at high frequencies, these positioning interneurones appear to function as 'command elements' within a large 'command system' and not as individual units.

INTRODUCTION

It has been known for several years that behaviour of some invertebrates can be altered by stimulation of certain single interneurones termed 'command neurones' (Wiersma & Ikeda, 1964). A large number of these neurones are known in crayfish (see reviews by Bowerman & Larimer, 1976; Larimer, 1976; Page, 1982; Wine & Krasne, 1982) and many of these produce coordinated postural adjustments of the crayfish abdomen.

The crayfish abdomen is a jointed structure capable of a broad range of tonic movements. In each segment there are two discrete sets of muscles and motoneurones to provide tension across the joints. Stimulating specific, single interneurones was found to cause the abdomen to assume particular positions (Kennedy, Evoy, Dane & Hanawalt, 1967). Interneurones which initiated an abdominal flexion

* Present address: Department of Biology, U.C.S.D., La Jolla, California, 92093, U.S.A.

Key words: command elements, sensorimotor systems, *Procambarus*.

were termed flexion command neurones while those that produced the opposite action were termed extension command neurones (Evoy & Kennedy, 1967).

We have conducted an intracellular study of the interneuronal organization of this positioning system in an effort to discover how simple behaviour and movements are controlled by the central nervous system. A large (but presently unspecified) number of position-producing interneurones, many of which have axonal processes that course from the 'brain' to the caudal-most abdominal ganglion, are repeatably identifiable (Larimer & Jellies, 1983; Larimer & Moore, 1984; Miall & Larimer, 1982*b*). While their somata have been identified in many ganglia, their axon terminations have been found only in the caudal-most abdominal ganglion (Larimer & Jellies, 1983). Their morphological organization gives the appearance of a system of largely parallel interneuronal elements. However, recent work suggests that there is a hierarchical arrangement in addition to a parallel organization. There are extensive unidirectional synaptic interactions among these positioning interneurones involving at least two tiers (Jellies & Larimer, 1983, 1985; Miall & Larimer, 1982*b*). One set of these interneurones (type I) was found to be generally presynaptic to another set (type II).

There are several important consequences of this functional organization. First, because of the high probability that any single positioning interneurone would recruit others, these cells are not 'command neurones' in the strictest sense. In other words, these cells do not appear to operate singly, rather they appear to be elements in a 'command system' (Kupfermann & Weiss, 1978). Second, these interneurones, which are identified on the basis of their motor effects, may also be 'sensory interneurones' in the sense that many of them might serve as conduits for input into the premotor circuitry. The apparent hierarchical arrangement of these interneurones might then be a reflection of the interneurones' roles in integrating and transmitting sensory information as well as in organizing motor output.

The present study addressed both of the above hypotheses in an effort to gain insight into how the very simple patterns of behaviour involved in abdominal posture might be initiated, modulated and coordinated. Our results show that the position-producing interneurones do respond to tactile input and that their combined activity is probably responsible for fictive abdominal-positioning. In addition, we show here that these interneurones form a hierarchy of at least two tiers based on how they respond to sensory input. Finally, we present evidence consistent with the view that constellations of interneurones, rather than single interneurones, are used by the animal to produce positional adjustments, even though one can experimentally produce such movements by high-frequency stimulation of single interneurones.

Portions of this work have appeared in abstract (Jellies & Larimer, 1984) and thesis (Jellies, 1984) form.

MATERIALS AND METHODS

These experiments were carried out on adult crayfish (*Procambarus clarkii*) obtained from Louisiana Procambarus, St. Martinville, Louisiana. The animals

were maintained in a laboratory culture prior to use. The preparation was a largely intact animal that was partially restrained.

Chilled crayfish were first perfused with about 150 ml of cold, oxygen-saturated saline (van Harreveld, 1936). This proved essential for subsequent viability. After perfusion was completed, the animal was again chilled in ice prior to dissection. Although we wanted the animal as intact as possible, it was necessary to remove the exopodite and endopodite of each swimmeret. The propodite segments were free to move, and often showed spontaneous, coordinated metachronal beating. All other appendages were left intact. A portion of the nerve cord was exposed in the ventral aspect by removing the central 5–7 mm of two adjacent sternal ribs and the associated soft cuticle just rostral and caudal to them (Fig. 1). The ventral midline blood vessel was pulled carefully to the side of the ganglion to be explored with a microelectrode, but was otherwise left intact. The ganglion was desheathed (Miall & Larimer, 1982*a*,*b*). The animal was then partially restrained in the recording chamber (Fig. 1), allowing complete freedom of movement for the rostral appendages while preventing abdominal movement. Ten preparations were examined in which the last segment of the abdomen and the tail-fan were allowed freedom of movement. Since no obvious differences were found in the response properties of the ten interneurones examined in this 'unrestrained' preparation *versus* the more restrained ones, the majority of the experiments reported here were conducted on the more restrained (and stable) preparation.

Glass suction electrodes monitored slow flexor motoneurones (SFMNs) in a superficial third root and slow extensor motoneurones (SEMNs) in a second root on opposite sides of a ganglion other than that being probed with the microelectrode. To stabilize the ganglion for recording intracellularly from interneurones, the first roots were cut and pinned to a waxed metal platform inserted beneath the ganglion of interest. The third roots containing the axons of phasic motoneurones were cut, allowing insertion of the platform. Neuropilar recordings were made using glass microelectrodes (100–400 MΩ) filled with 3 % aqueous Lucifer Yellow CH (Sigma) in the tips and 1 mol l^{-1} lithium chloride in the shanks (Stewart, 1978). The microelectrodes were coupled to a Dagan 8001-1 preamplifier operated in the balanced-bridge mode. Each impaled cell was depolarized through the microelectrode to determine whether it excited either an abdominal flexion or extension motor output, or had no noticeable effect. Interneurones which produced a flexion motor output, in which the excitatory SFMNs and the peripheral inhibitor motoneurone to the extensor muscles were excited and the excitatory SEMNs were inhibited, were called flexion-producing interneurones (FPIs) while those which produced the opposite effects when stimulated were termed extension-producing interneurones (EPIs). Most experiments were conducted by impaling interneurones in the third abdominal ganglion (G_3), but G_4, G_5 and G_6 were also examined. When cells were impaled in G_6, the motoneurone activity was monitored from G_5.

A major goal of using this preparation was to examine the effects of tactile stimulation on abdominal-positioning interneurones. Tactile stimuli consisted of a fine brush manually applied to the body surfaces. The ventral and lateral aspects of

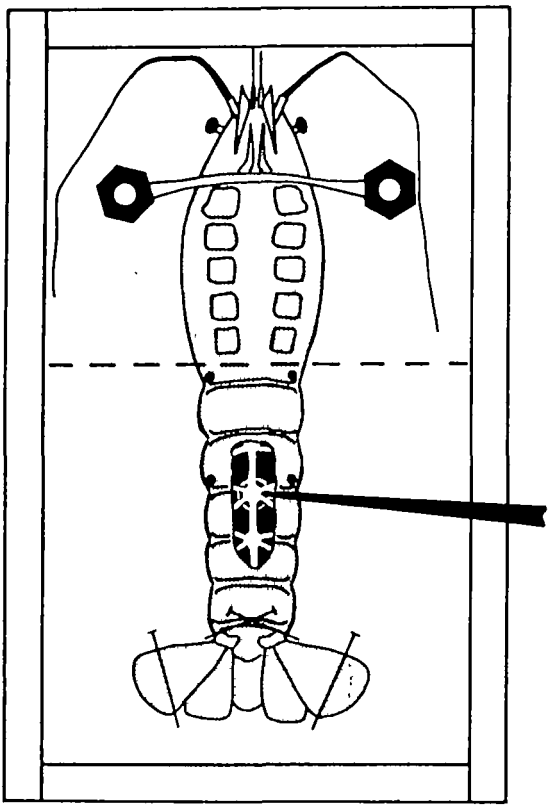

Fig. 1. A diagrammatic drawing of a largely intact crayfish restrained in the recording chamber. The legs are not shown here but all were left intact during the experiments. The crayfish was fixed to a sculptured wax base in a Plexiglas box. Two bolts secured a thin Lexan strip over the cephalothorax to clamp the rostral portion of the animal. Four pins (black dots) secured the rostral abdomen (see text), while sets of pins crossed over the last joint and the exopodites of the tail-fan to stabilize the caudal portions of the tail. Here, the third ganglion is shown being supported on a platform for intracellular penetrations. A vertical plastic partition (dashed line) prevented the animal from grasping or rubbing the electrodes and platform. The propodite segments of the swimmerets are not illustrated.

the animal and all but about a 1-cm strip of the dorsal carapace could be stimulated. A portion of the dorsal exopodites of the tail-fan was also available. To simplify the presentation of receptive fields, they are shown to extend to the dorsal midline although this most dorsal aspect could not be stimulated. This is probably a valid assumption since crayfish interneurones, in general, have tactile receptive fields over continuous segmental regions (Calabrese, 1976*a*; Sigvardt, Hagiwara & Wine, 1982). Although all receptive fields were explored using a fine brush, a blunt glass probe fixed to a tension transducer was used for tactile stimulation during filming of responses, so that an artifact could be displayed to show the timing of the stimulus relative to the interneuronal response. Stimulation with the glass probe did not change the responses.

Following electrophysiological examination, Lucifer Yellow was injected into the neurones by passing 2–12 nA pulses (750 ms duration, 1 Hz) of hyperpolarizing current for 10 min to 1 h. Filled neurones were examined briefly before fixation. If the site of electrode penetration was axonal the tissues were kept in the dark for an additional 1–4 h to facilitate dye movement into adjacent ganglia (Larimer & Jellies, 1983). The nerve cord was fixed overnight in 4 % paraformaldehyde (Stewart, 1978), rinsed for 1 h in Sorenson's buffer, dehydrated, and cleared in methyl salicylate. Whole mounts were examined with a fluorescence microscope and cells were drawn using a Zeiss drawing tube. The tissues were embedded in Spurr's medium and the positions of filled axons were located in thick cross-sections of the connectives to aid further in identification.

RESULTS

Abdominal motoneurone activity was very sensitive to touching the body surface. For example, when the exopodite of the tail-fan was touched (Fig. 2) several tonic flexor and extensor motoneurones were usually activated. This motor activity produced an abdominal posture intermediate between complete extension and flexion (Page, 1975*a*,*b*). Only occasionally was a 'pure' flexion or extension produced (evidenced by complete reciprocity), indicating that it is unusual to evoke an exceptionally strong movement in either direction in this manner. This is consistent

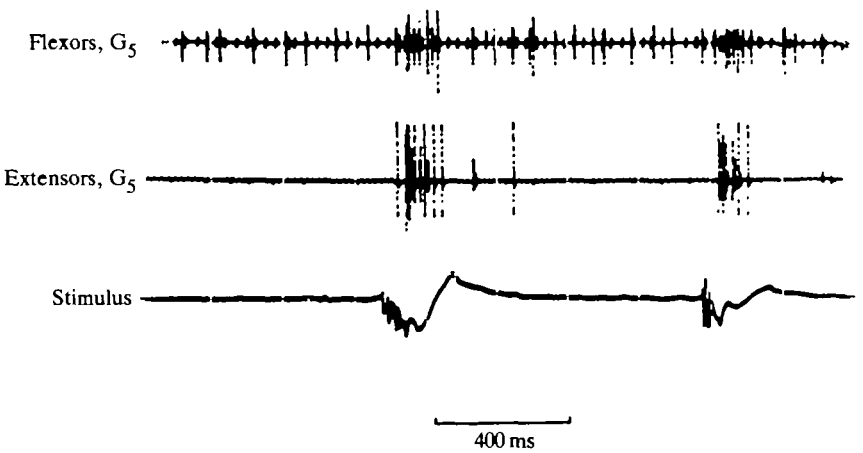

Fig. 2. The effect of tactile stimulation on tonic motoneurones. Tonic motoneurones were monitored extracellularly from opposite sides of a single ganglion as described in the text. Their activity is shown in the upper two traces. The tail-fan was touched with a probe to evoke motoneurone activity. The tactile stimulus was indicated by mounting a fine, blunt probe on a tension transducer and recording the transducer's output on the oscilloscope face (stimulus). Touching the intact animal usually evoked an increase in motoneurone activity, indicating an attempt to change abdominal position. Touching the largely intact animal produced an increase in tonic discharge of both the SFMNs and the SEMNs. Only very rarely did touch stimulation produce a strong, reciprocal output in the tonic motoneurones.

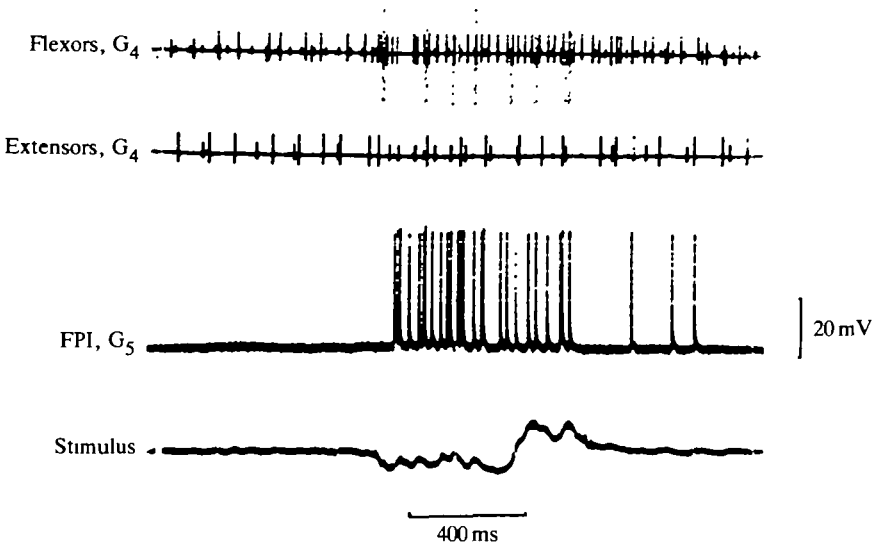

Fig. 3. The tactile activation of an abdominal-positioning interneurone (FPI). The electrophysiological records here are labelled as before with the upper traces being extracellular recordings. Touching the tail-fan increased the activity in tonic motoneurones (upper traces) and also evoked action potentials in the impaled FPI. Activation such as that shown here always occurred with relatively short latency (< 50 ms) depending on the distance between the recording site and the site of stimulation. These latencies are consistent with the presence of only one or a very few synapses between the input and the interneurone.

with observations on the unrestrained animal (Larimer & Eggleston, 1971; Page, 1975*a*,*b*; Sokolove, 1973).

The electrophysiological results presented in this paper encompassed 134 interneuronal impalements. The proportion of different kinds of interneurones (based upon their motor outputs) was similar to that seen when recording from the isolated abdominal nerve cord (Jellies & Larimer, 1985; Larimer & Moore, 1984): 111 were FPIs; 21 were EPIs; and two were inhibitory interneurones which suppressed tonic motoneurone activity when stimulated. Almost all of the interneurones could be placed into two groups based on the general way that they responded to the touch stimulus. They either responded with a short latency discharge of action potentials, or they were more weakly influenced, in a subthreshold manner, by the stimulus (a minor fraction of the interneurones showed no response).

The responses of interneurones in which the touch stimulus gave rise to a train of impulses after a short latency were coincident with the evoked motoneurone activity (Fig. 3). The interneuronal responses ranged from about 20–70 Hz depending on the cell and the stimulus strength (this was not quantified). While a few responses were more or less phasic, most were tonic and thus maintained during touch stimulation.

Two observations indicated that touch-evoked activity in these positioning interneurones was at least partially responsible for the activation of the tonic motoneurones. First, the frequencies at which the interneurones were activated were consistently above those required to produce a motor output when the interneurone

was stimulated by injecting current through the microelectrode. Second, in two instances it was possible to silence the active interneurone and observe a decrease (but not an elimination) of the touch-evoked motor output. In the great majority of cases, perhaps because of their relatively large size, these interneurones could be neither silenced nor slowed by injecting hyperpolarizing current.

Many quiescent interneurones were not activated to spiking by touch. To determine whether the sensory influence was isolated from the interneurone or had a subthreshold influence upon it, the quiescent interneurone was stimulated with a small amount of depolarizing current to excite it weakly. After 1 s the interneurone was again stimulated with the same amount of current (repeated stimulation never had effects on the impulse discharge as long as the interstimulus interval was 200 ms or greater) and at the same time some region of the body was touched lightly. The results of this procedure showed that touch had an excitatory influence on the frequency of action potential firing of some FPIs (Fig. 4A) while a similar stimulus had an inhibitory influence on other FPIs (Fig. 4B). These influences were often accompanied by a barrage of postsynaptic potentials.

By filling the interneurones with dye it was possible to correlate their morphologies with physiological identification of them as either type I or type II interneurones (Jellies, 1984; Jellies & Larimer, 1985). Soma positions of type I interneurones were either unknown (most axonal penetrations, Fig. 5G) or in the terminal ganglion, G_6. Type II interneurones known so far have their somata in the more rostral ganglia, G_2–G_5 (Jellies, 1984). The morphologies of most of these interneurones have been published in the literature (Larimer & Jellies, 1983; Larimer & Moore, 1984; Miall & Larimer, 1982*b*). Type I interneurones can be subdivided into categories represented by identified cells F, H and J (Jellies & Larimer, 1985) with morphologies as shown in Fig. 5. Each cell was identified by comparing its major features among each other as well as with a growing library of such interneurones. These comparisons focused on the size and position of somata and major processes in relation to the ganglionic outline. The position of the axons in nerve cord cross-sections support these identifications.

The sensory responses given by the neurones examined are given in Table 1. Interneurones with their somata in G_6 (type I; F, H and J) were always activated to spiking by touching the body surface (Fig. 3). Type II interneurones only exhibited subthreshold sensory influences. Only a few interneurones were unaffected by touch. Unidentified interneurones were those for which no repeatably identifiable morphology could be obtained. Axonal penetrations (Fig. 5G), while morphologically unidentified, usually responded as type I interneurones.

The receptive fields of 90 of the 134 interneurones examined in this study were systematically mapped: 52 were type I interneurones and 38 were type II. For type I interneurones the receptive fields were well-defined (Fig. 6; Table 2). There were at least four different receptive fields (probably more) which overlapped in the tail-fan; each of the identifiable type I interneurones had the same receptive field each time it was examined; and they were excited rather than inhibited. Thirty interneurones had a receptive field (Fig. 6A) that encompassed one-half of the tail-fan. Three

additional receptive fields (Fig. 6B,C,D) complete the majority of receptive fields that were associated with type I interneurones. Of the remaining five interneurones that were activated (not shown), one had a receptive field that encompassed the entire abdomen (less the tail-fan), one receptive field covered the whole abdomen, one cell was activated only by touching the walking legs, one only by touching the endopodites of the uropods and one only by touching the soft cuticle around the anus. The responses of these interneurones to stimulation were excitatory. In two cases (classified as receptive field A, Fig. 6) axons (soma position unknown) were excited by touching one-half of the tail-fan and inhibited by touching the contralateral half.

The responses to touch stimulation of type II interneurones were much more variable. Generally, a touch anywhere on the body surface (that evoked motoneurone

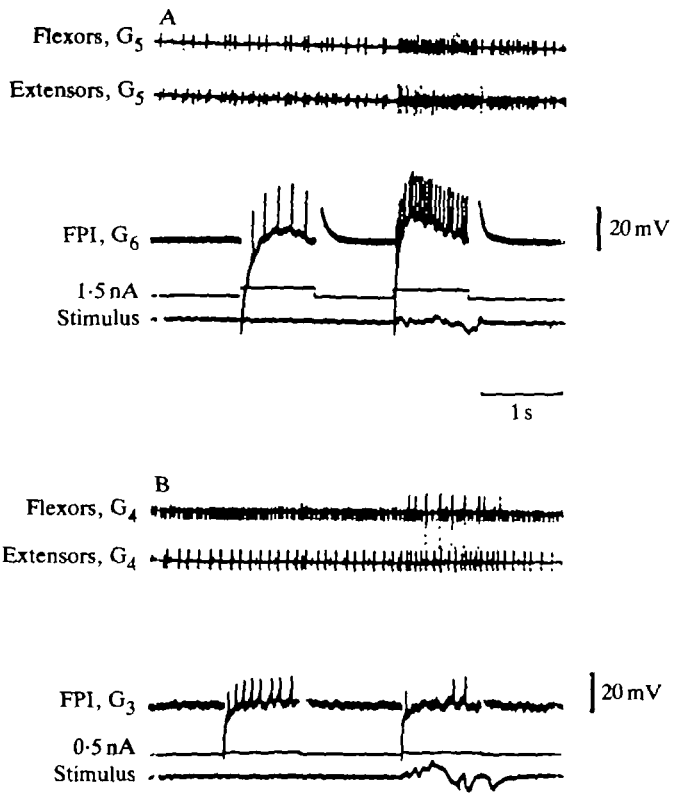

Fig. 4. Tactile influences on abdominal-positioning interneurones (FPIs). This figure demonstrates that interneurones which could not be activated to spiking by touch were in fact affected by touch although those influences were apparently subthreshold. The procedure was first to stimulate the interneurone with a small amount of depolarizing current to determine its response, and then after a suitable delay (see text) to stimulate it with current injection again, but in conjunction with touching some area of the body. A subthreshold influence was indicated by a change in the firing frequency over that obtained with current injection alone. The results shown here are from two different interneurones to demonstrate that both excitatory (A) and inhibitory (B) influences were found. The fourth trace in each panel is a record of the current monitor.

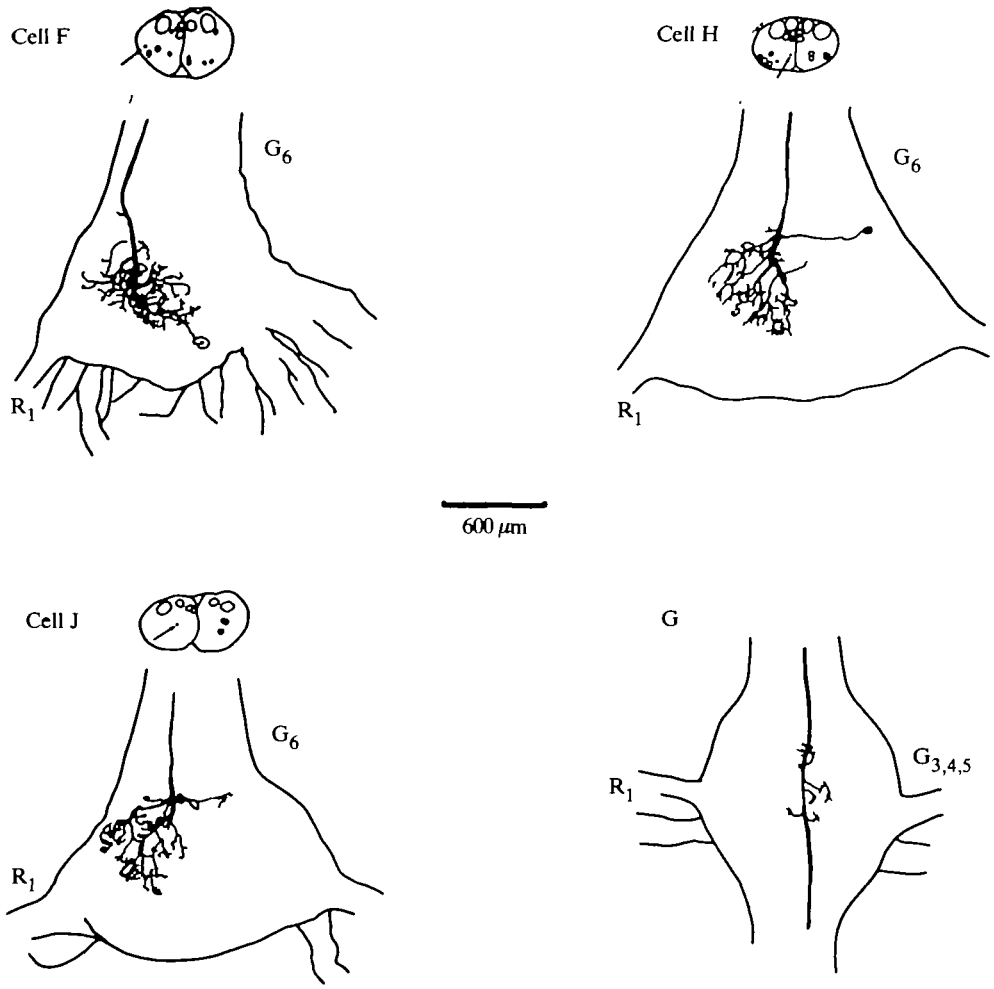

Fig. 5. The morphology of several repeatedly identifiable type I interneurones. Drawings made from Lucifer Yellow-filled interneurones from G_6 are shown here with the position of their axons in cross-sections of the nerve cord. Cell F was an FPI encountered eight times in this study, cell H was also an FPI encountered three times and cell J was an EPI encountered three times in this study. Each of these cells has been encountered numerous times prior to this work (see text). The axonal segment shown here (G) represents axonal penetrations in G_3, G_4 and G_5 for which the soma position remains unknown, although most such axons are type I cells (see text, Table 1) based on their physiological properties. All cells were drawn in whole-mount in their ventral aspects. R_1, first nerve root.

discharges) was effective in influencing the type II interneurone's activity. This influence was usually excitatory, but exclusively inhibitory as well as mixed excitatory and inhibitory influences were also seen. For example, touching the tail-fan might excite an interneurone at one time and inhibit it at a later time. Additionally, several examples were found in which touching one area of the body surface inhibited the interneurone while touching another area excited it.

Table 1. *The number of interneurones in each of several classes and their responses to tactile stimulation*

Interneurone identity	Touch-activated	Touch-influenced	No effect	Undetermined
Type I, F	8	0	0	0
Type I, H	3	0	0	0
Type I, J	3	0	0	0
Axons, G	33	2	3	5
Type II, all	0	28	0	9
Unidentified	19	6	6	8

Interneurones are designated as in Fig. 5. Unidentified interneurones are those which could not be filled with dye.

An undetermined effect was the result of a clogged electrode such that subthreshold influences could not be adequately tested for.

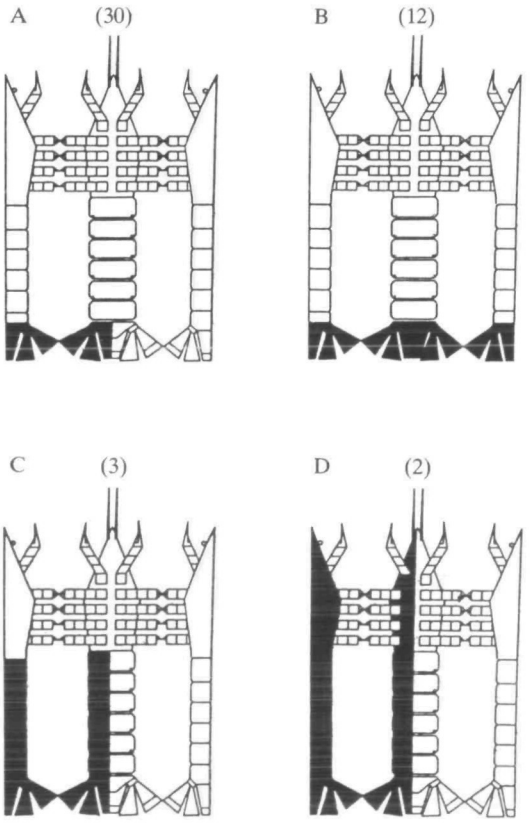

Fig. 6. The common tactile receptive fields of type I abdominal-positioning interneurones. The four most common receptive fields are shown here on the diagrammatic crayfish body surface. The central portion of these diagrams represents the ventral surface of the animal with the lateral and dorsal surfaces split down the midline and shown at the sides. The receptive fields are represented by black areas. The number of times an interneurone with that particular receptive field was impaled is indicated in parentheses above each diagram. These four (A–D) accounted for over 90% of all mapped, type I receptive fields.

Data from the activity of abdominal-positioning interneurones during 'voluntary' (spontaneous) attempts by the animal to change abdominal position support the hypothesis that such movements are mediated by sets of coactive interneurones, rather than by coding of particular positions by single interneurones. Although all preparations were responsive to tactile stimulation only 48% (64) of the animals produced spontaneous movements. These fictive abdominal movements associated with spontaneous activity were similar to those produced by touching the animal.

Table 2. *The interneurones having particular tactile receptive fields*

	Tactile receptive field				
Interneurone	A	B	C	D	Other
F	0	7	0	0	0
H	3	0	0	0	0
J	3	0	0	0	0
G	18	2	2	0	2
Unidentified	6	3	1	2	5

The receptive fields are as designated in Fig. 6. Interneurones are as designated in Table 1.

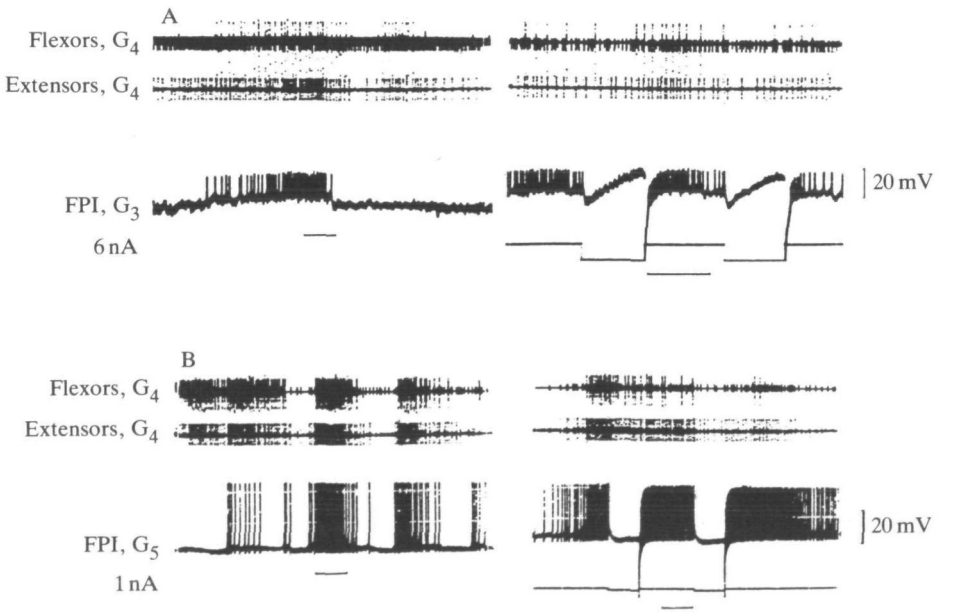

Fig. 7. Activation of positioning interneurones during spontaneous motoneurone activity. Two different FPIs from different animals are shown here. In (A) the left panel shows that this FPI was activated in conjunction with the motor activity. The right panel demonstrates that silencing the interneurone by hyperpolarizing it resulted in only a minor decrease in motoneurone activity. The results shown in (B) are similar, but the interneuronal activity was more closely correlated with the initiation of some motoneurone activity. The left panel shows that the interneurone was activated just before and during some, but not all, of the motoneurone bursts. When this interneurone was hyperpolarized during a more sustained bout of activity, a decrement of motor activity was again observed. All horizontal calibration marks are equal to 1 s.

The motoneurone activity involved both flexors and extensors with little obvious reciprocity. Only very occasionally could a motor output be described as predominantly flexion or extension. Nonetheless, in these experiments, as in the previous ones, it was possible, from the motoneurone discharges, to determine when the animal was attempting to change abdominal position.

Of the 64 animals that evidenced spontaneous motor discharges, the majority (46) showed no correlated firing in the particular interneurone impaled. Considering the large number of positioning interneurones in this system (Larimer & Jellies, 1983; Larimer & Moore, 1984; Miall & Larimer, 1982*b*) such a result is not unexpected. Thus, a given interneurone would often remain quiescent during spontaneous changes in abdominal position.

In the minority of cases (18), positioning interneurones were active during fictive spontaneous changes in abdominal position (Fig. 7). In one example (Fig. 7A) an FPI was activated in conjunction with an increase in spontaneous motoneurone output, to a peak frequency of about 30 Hz. When this interneurone was stimulated directly (through the microelectrode) at 30–40 Hz, a barely discernible motor output was produced (not shown). This implies that the interneurone was not solely responsible for the output. This conclusion was supported by the finding that hyperpolarizing this interneurone reduced, but did not eliminate the motor output (Fig. 7A, right panel). A second FPI in a different animal showed similar behaviour (Fig. 7B). The activity in this interneurone was correlated with the initiation of some of the motoneurone bursts, but not all of them (Fig. 7B, left panel). However, the interneurone was not active during the entire period of spontaneous motoneurone activity. Such a rough correlation is again consistent with the interpretation that interneurones other than this one were participating in the production of the motor output. Hyperpolarizing the active interneurone decreased but did not eliminate the motor output (Fig. 7B, right panel). In no case did we find evidence to support the notion that single positioning interneurones were responsible for producing the entire motor output.

DISCUSSION

We have demonstrated that many of the premotor positioning interneurones involved in producing changes in abdominal posture can also be considered as 'sensory interneurones'. Based on their responses to touch, there appear to be at least two tiers of interneuronal organization. One set of interneurones was activated to spiking by light touch. The relatively short latency of this activation is consistent with one or only a few interposed synapses. These corresponded to the type I interneurones which are generally presynaptic to the remaining (type II) interneurones (Jellies, 1984; Jellies & Larimer, 1985). The second type of response to touch was a subthreshold influence, routinely seen in type II interneurones.

The effects of touch on type II interneurones were much more variable than those on type I interneurones. It is beyond the scope of this report to determine the causes of variability in type II responses: it did not appear to be due to inconsistencies in

stimulation since these did not result in any obvious variability of type I responses. A second possible source of type II response variability might be an inconsistency in neuronal interconnections. While this possibility cannot be entirely eliminated, our work on the synaptic interactions among these interneurones (Jellies & Larimer, 1985) indicates that their interconnections are largely invariant.

Both type I and II interneurones appeared to be responsible for the production of spontaneous fictive movements. During spontaneous movements the animals might well have provided some self-stimulation by moving the tail-fan slightly against the pins, which activated the type I interneurones. Certainly the legs were free to move, and did. At least, such self-stimulation might have increased the excitability of some interneurones and decreased that of others. The spontaneous activation of type II interneurones is not as easily explained in terms of self-stimulation since these were the very same preparations in which touching the animal influenced, but did not activate type II interneurones. Spontaneous movements, when present, were vigorous. Yet in almost three-quarters of those instances, no correlated activity was observed in the impaled cell. If self-stimulation was a major source of activation in these experiments, one might expect considerably fewer silent interneurones than were observed.

Our data support the role of positioning interneurones as 'command elements' in a 'command system' (Kupfermann & Weiss, 1978) for crayfish abdominal positioning. Groups of interneurones would be activated by touching much of the body surface since many of their receptive fields overlap (Fig. 6). It is unlikely that touch stimuli would activate single interneurones, and even if they did so, there is a high probability that they would recruit additional command elements. The behaviour of individual positioning interneurones during spontaneous tonic motor output also supports their role as command elements in this system. Although we cannot eliminate the possibility that single 'command neurones' produce movements, our data support a more distributed functional role for these positioning interneurones.

Command elements as sensorimotor interneurones

When positioning interneurones were examined in the largely intact preparation, it became clear that many of the properties generally ascribed to crayfish mechanosensory interneurones were manifested by these 'motor' interneurones. Most crayfish sensory interneurones have receptive fields that gather input from adjacent, homologous segments; many of them have overlapping receptive fields and some have very restricted receptive fields (Hughes & Wiersma, 1960; Kennedy & Mellon, 1964; Wiersma & Bush, 1963; Wiersma & Hughes, 1961). In addition, Wiersma & Mill (1965) showed that some interneurones receive a more direct afferent input than others. This conclusion was supported by intracellular studies (Calabrese, 1976*a*,*b*; Preston & Kennedy, 1960). Finally, Calabrese (1976*b*) demonstrated that a few ($< 5\%$) of those interneurones have bilateral, tactile receptive fields and show contralateral inhibition.

These 'sensory interneurone' properties were seen in the abdominal positioning interneurones examined in this study. The interneurones had receptive fields which

collected input from contiguous, homologous segments. A minor fraction of them had very restricted receptive fields and two examples were found where contralateral inhibition was seen. Additionally, most of the positioning interneurones were sensitive to tactile input, but one group was apparently more directly activated than the other (Table 1). Thus, some sensory input has relatively straightforward access to the premotor circuitry. It remains to be seen how such input is organized by the command elements.

Spontaneously activated command elements

During spontaneous fictive abdominal movement there was a high probability that any given positioning interneurone would remain quiescent. However, on occasion both type I and type II interneurones were activated at moderate frequencies. While this activity in single interneurones could not account entirely for the robust output seen, the frequencies at which the interneurones were activated were consistent with their being responsible for some fraction of the motor output. Although it was not possible simultaneously to hyperpolarize groups of interneurones in the largely intact animal to silence all members of the active group, one might predict that doing so would completely eliminate the spontaneous movement. In agreement with this model, silencing active, individual interneurones reduced the output, thus supporting a causal role for groups of these positioning interneurones functioning as command elements in a command system.

Although stimulating single crayfish interneurones might easily lead one to conclude that particular postures are produced by the activation of single 'command neurones', the results described in this paper do not support that conclusion. Other workers have implicated groups of neurones (rather than single neurones) in the initiation of behaviour, such as leech swimming (Kristan & Weeks, 1983), mollusc swimming (Getting, 1975), lobster swimmeret beating (Davis & Kennedy, 1972*a*,*b*,*c*), *Aplysia* locomotion (Fredman & Jahan-Parwar, 1983), *Pleurobranchaea* feeding (Gillette, Kovac & Davis, 1982; Gillette & Gillette, 1983), rhythmic abdominal movements in crayfish (D. Moore & J. L. Larimer, in preparation), cockroach running and flight (Ritzmann, Tobias & Fourtner, 1980; Ritzmann, Pollack & Tobias, 1983) and locust walking (Kien, 1983).

The collection of interneurones involved in controlling abdominal posture in the crayfish offers an approachable system in which to examine the control mechanisms that may operate to some degree in many motor systems. Our findings that command elements in crayfish might themselves be considered mechanosensory interneurones allows for modulating influences to enter the premotor system *via* many conduits at early stages of processing. This set of premotor interneurones appears to be a more complex system than was previously thought, involving a large number of elements, both parallel and serial processing, and a variety of afferent and interneuronal inputs.

We thank Drs D. Moore and W. Thompson for commenting on an earlier version of this manuscript, and J. Hsieh and M. Brot for technical assistance. We also thank two anonymous reviewers for their constructive comments. Supported by NIH

Research Grant NS-05423 and BRSG261694-1401 to JLL and by a University of Texas predoctoral fellowship to JJ.

REFERENCES

BOWERMAN, R. F. & LARIMER, J. L. (1976). Command neurons in crustaceans. *Comp. Biochem. Physiol.* **54**, 1–5.

CALABRESE, R. L. (1976*a*). Crayfish mechanoreceptive interneurons. I. The nature of ipsilateral excitatory inputs. *J. comp. Physiol.* **105**, 83–102.

CALABRESE, R. L. (1976*b*). Crayfish mechanoreceptive interneurons. II. Bilateral interactions and inhibition. *J. comp. Physiol.* **105**, 103–114.

DAVIS, W. J. & KENNEDY, D. (1972*a*). Command interneurons controlling swimmeret movements in the lobster. I. Types of effects on motoneurons. *J. Neurophysiol.* **35**, 1–12.

DAVIS, W. J. & KENNEDY, D. (1972*b*). Command interneurons controlling swimmeret movements in the lobster. II. Interactions of effects on motoneurons. *J. Neurophysiol.* **35**, 13–19.

DAVIS, W. J. & KENNEDY, D. (1972c). Command interneurons controlling swimmeret movements in the lobster. III. Temporal relationships among bursts in different motoneurons. *J. Neurophysiol.* **35**, 20–29.

EVOY, W. H. & KENNEDY, D. (1967). The central nervous organization underlying control of antagonistic muscles in the crayfish. I. Types of command fibers. *J. exp. Zool.* **165**, 223–238.

FREDMAN, S. L. & JAHAN-PARWAR, B. (1983). Command neurons for locomotion in *Aplysia*. *J. Neurophysiol.* **49**, 1092–1117.

GETTING, P. A. (1975). *Tritonia* swimming: triggering of a fixed action pattern. *Brain Res.* **96**, 128–133.

GILLETTE, M. & GILLETTE, R. (1983). Bursting neurons command consummatory feeding behavior and coordinated visceral receptivity in the predatory mollusk *Pleurobranchaea*. *J. Neurosci.* **3**, 1791–1806.

GILLETTE, R., KOVAC, M. P. & DAVIS, W. J. (1982). Control of feeding motor output by paracerebral neurons in brain of *Pleurobranchaea californica*. *J. Neurophysiol.* **47**, 885–908.

HUGHES, G. M. & WIERSMA, C. A. G. (1960). Neuronal pathways and synaptic connexions in the abdominal cord of the crayfish. *J. exp. Biol.* **37**, 291–307.

JELLIES, J. A. (1984). Premotor interneurons involved in abdominal positioning in crayfish: synaptic interactions, sensory receptive fields and activity during spontaneous movements. Ph. D. dissertation, The University of Texas, Austin, Texas.

JELLIES, J. & LARIMER, J. L. (1983). Synaptic interactions between flexion-producing interneurons in crayfish. *Soc. Neurosci. Abstr.* **9**, 382.

JELLIES, J. & LARIMER, J. L. (1984). Tactile activation of interneurons which produce abdominal movements in crayfish. *Soc. Neurosci. Abstr.* **10**, 626.

JELLIES, J. & LARIMER, J. L. (1985). Synaptic interactions between neurons involved in the production of abdominal posture in crayfish. *J. comp. Physiol.* (in press).

KENNEDY, D., EVOY, W. H., DANE, B. & HANAWALT, J. T. (1967). The central nervous organization underlying control of antagonistic muscles in crayfish. II. Coding of position by command fibers. *J. exp. Zool.* **165**, 239–248.

KENNEDY, D. & MELLON, D., JR. (1964). Synaptic activation and receptive fields in crayfish interneurons. *Comp. Biochem. Physiol.* **13**, 275–300.

KIEN, J. (1983). The initiation and maintenance of walking in the locust: an alternative to the command concept. *Proc. R. Soc.* B **219**, 137–174.

KRISTAN, W. B., JR. & WEEKS, J. C. (1983). Neurons controlling the initiation, generation and modulation of leech swimming. In *Neural Origin of Rhythmic Movements*, (eds A. Roberts & B. Roberts), pp. 293–260. *Symp. Soc. exp. Biol.* **xxxvii**.

KUPFERMANN, I. & WEISS, K. R. (1978). The command neuron concept. *Behav. Brain Sci.* **1**, 3–39.

LARIMER, J. L. (1976). Command interneurons and locomotor behavior in crustaceans. In *Neural Control of Locomotion*, (eds R. M. Herman, S. Grillner, P. S. G. Stein & D. G. Stuart), pp. 293–326. New York: Plenum Press.

LARIMER, J. L. & EGGLESTON, A. C. (1971). Motor programs for abdominal positioning in crayfish. *Z. vergl. Physiol.* **74**, 388–402.

LARIMER, J. L. & JELLIES, J. (1983). The organization of flexion-evoking interneurons in the abdominal nerve cord of the crayfish, *Procambarus clarkii*. *J. exp. Zool.* **226**, 341–351.

LARIMER, J. L. & MOORE, D. (1984). Abdominal positioning interneurons in crayfish: projections to and synaptic activation by higher CNS centers. *J. exp. Zool.* **230**, 1–10.

MIALL, R. C. & LARIMER, J. L. (1982*a*). Central organization of crustacean abdominal posture motoneurons: connectivity and command fiber inputs. *J. exp. Zool.* **224**, 45–56.

MIALL, R. C. & LARIMER, J. L. (1982*b*). Interneurons involved in abdominal posture in crayfish: structure, function and command fiber responses. *J. comp. Physiol.* **148**, 159–173.

PAGE, C. H. (1975*a*). Command fiber control of crayfish abdominal movement. I. MRO and extensor motoneuron activities in *Orconectes* and *Procambarus*. *J. comp. Physiol.* **102**, 65–76.

PAGE, C. H. (1975*b*). Command fiber control of crayfish abdominal movement. II. Generic differences in the extension reflexes of *Orconectes* and *Procambarus*. *J. comp. Physiol.* **102**, 77–84.

PAGE, C. H. (1982). Control of posture. In *The Biology of Crustacea*, Vol. 4, (eds D. C. Sandeman & H. L. Atwood), pp. 33–59. New York: Academic Press.

PRESTON, J. B. & KENNEDY, D. (1960). Integrative synaptic mechanisms in the caudal ganglion of the crayfish. *J. gen. Physiol.* **43**, 671–681.

RITZMANN, R. E., POLLACK, A. J. & TOBIAS, M. L. (1983). Flight activity mediated by intracellular stimulation of dorsal giant interneurons of the cockroach *Periplaneta americana*. *J. comp. Physiol.* **147**, 313–322.

RITZMANN, R. E., TOBIAS, M. L. & FOURTNER, C. R. (1980). Flight activity initiated via giant interneurons of the cockroach: evidence for bifunctional trigger interneurons. *Science, N. Y.* **210**, 443–445.

SIGVARDT, K. A., HAGIWARA, G. & WINE, J. J. (1982). Mechanosensory integration in the crayfish abdominal nervous system: structural and physiological differences between interneurons with single and multiple spike initiation sites. *J. comp. Physiol.* **148**, 143–157.

SOKOLOVE, P. G. (1973). Crayfish stretch receptor and motor unit behavior during abdominal extensions. *J. comp. Physiol.* **84**, 251–266.

STEWART, W. W. (1978). Functional connections between cells as revealed by dye-coupling with a highly fluorescent naphthalamide tracer. *Cell* **14**, 741–759.

VAN HARREVELD, A. (1936). A physiological solution for freshwater crustaceans. *Proc. Soc. exp. Biol.* **34**, 428–432.

WIERSMA, C. A. G. (1958). On the functional connections of single units in the central nervous system of the crayfish, *Procambarus clarkii* Girard. *J. comp. Neurol.* **110**, 421–471.

WIERSMA, C. A. G. & BUSH, B. M. H. (1963). Functional neuronal connections between the thoracic and abdominal cords of the crayfish, *Procambarus clarkii* (Girard). *J. comp. Neurol.* **121**, 207–235.

WIERSMA, C. A. G. & HUGHES, G. M. (1961). On the functional anatomy of neuronal units in the abdominal cord of the crayfish, *Procambarus clarkii* (Girard). *J. comp. Neurol.* **116**, 209–228.

WIERSMA, C. A. G. & IKEDA, K. (1964). Interneurons commanding swimmeret movements in the crayfish, *Procambarus clarkii*, (Girard). *Comp. Biochem. Physiol.* **12**, 509–525.

WIERSMA, C. A. G. & MILL, P. J. (1965). "Descending" neuronal units in the commissure of the crayfish central nervous system; and their integration of visual, tactile and proprioceptive stimuli. *J. comp. Neurol.* **125**, 67–94.

WINE, J. J. & KRASNE, F. B. (1982). The cellular organization of crayfish escape behavior. In *The Biology of Crustacea*. Vol. 4, (eds D. C. Sandeman & H. L. Atwood), pp. 241–292. New York: Academic Press.