FURTHER STUDIES OF CRAYFISH ESCAPE BEHAVIOUR

II. GIANT AXON-MEDIATED NEURAL ACTIVITY IN THE APPENDAGES

By IAN R. C. COOKE

Department of Zoology, University of Melbourne, Parkville 3052, Victoria, Australia

SUMMARY

Stereotyped responses were evoked in a number of motoneurones in the appendages of semi-intact crayfish when the command neurones for escape behaviour were activated. The medial giant neurones mediated short latency responses in pereiopod common inhibitor, promotor and extensor motoneurones, several abdominal first root neurones and one uropod exopodite promotor motoneurone. The lateral giant neurones mediated short latency responses in the pereiopod common inhibitor neurones, the same abdominal first root neurones and one uropod protopodite promotor motoneurone. These responses can be correlated with stereotyped movements of the appendages which occur in the normal escape behaviour of crayfish.

INTRODUCTION

In the preceding paper (Cooke & Macmillan, 1985), it was found that the escape behaviour of crayfish involves stereotyped movements of the thoracic and abdominal appendages, in addition to the characteristic abdominal movements described previously (Larimer, Eggleston, Masukawa & Kennedy, 1971; Wine & Krasne, 1972). This report describes giant axon-mediated responses of motoneurones in these appendages which are likely to generate the stereotyped movements in normal escape behaviour.

MATERIALS AND METHODS

All recordings were made from semi-intact, Australian freshwater crayfish (*Cherax destructor*). Animals were chilled in crushed ice and then transferred to cold (3–4 °C), aerated crayfish saline [van Harreveld's (1936) solution, buffered with $10 \, \text{mmol} \, \text{l}^{-1}$ Tris to pH 7·6] for preparation. They were restrained firmly in a

ventral side up position using plasticine and were able to ventilate normally. Nerves were exposed for stimulation and recording by removing the overlying cuticle and deflecting muscles and blood vessels where necessary. The bath temperature was raised gradually to 12 °C for experimentation.

The medial giant (MG) and lateral giant (LG) axons were stimulated at various levels of the nerve cord. In the head, they were stimulated with a pair of stainless steel wire electrodes, insulated except at the tips, which were placed through holes in the epistome to span the circumoesophageal connectives. At other levels in the thorax and abdomen, the giant axons were stimulated with silver hook electrodes which were positioned against the dorsal surface of the nerve cord. Fine (50 μm diameter) silver hook electrodes were used to make extracellular recordings of activity in the ventral nerve cord, in major nerve roots and in motor nerves innervating single muscles in the appendages. The latter recordings were made by placing the electrode under the motor nerve where it branched across or through the muscle. The exposed tips of the hook electrodes were insulated from the bath using Vaseline. Signals were amplified conventionally and photographed from an oscilloscope. Ganglionic delays in the activation of appendage motoneurones by spikes in the MG or LG axons were estimated using axonal conduction velocities and distances measured for each preparation.

<div align="center">RESULTS</div>

Stereotyped responses were elicited at short latencies (<10 ms) from a small number of motoneurones in each of the thoracic and abdominal appendages when either the MG or LG axons fired a spike. Motoneurones not involved in these responses always remained silent for at least 30 ms after a spike in one of the pairs of giant axons. The MG and LG axons mediated responses from different sets of appendage motoneurones. These are described below for each group of appendages in turn.

<div align="center">*Pereiopods*</div>

Similar motor responses were evoked in each of the three anterior pairs of pereiopods when the MG axons were stimulated to fire a spike. In each leg, the response consisted of short burst of spikes in two promotor motoneurones, the fast and slow extensor motoneurones and the common inhibitor neurone (Figs 1, 2, 3). The two promotor motoneurones fired one or two spikes each in the response (Fig. 2). The ganglionic delay to the first of these was about 1·0 ms, that to the second about 2·5 ms. The fast and slow extensor motoneurones each fired bursts of 2–5 spikes at 6- to 10-ms intervals (Figs 1, 3). These units were distinguished easily in recordings from the extensor nerve since the fast extensor axon had the faster conduction velocity and produced the larger spike. In addition, the two axons ran in different roots of the pedal nerve at their origin at the thoracic ganglion (Fig. 1). The ganglionic delay in the firing of the first fast extensor spike in the response was about 1·0 ms while that to the first slow
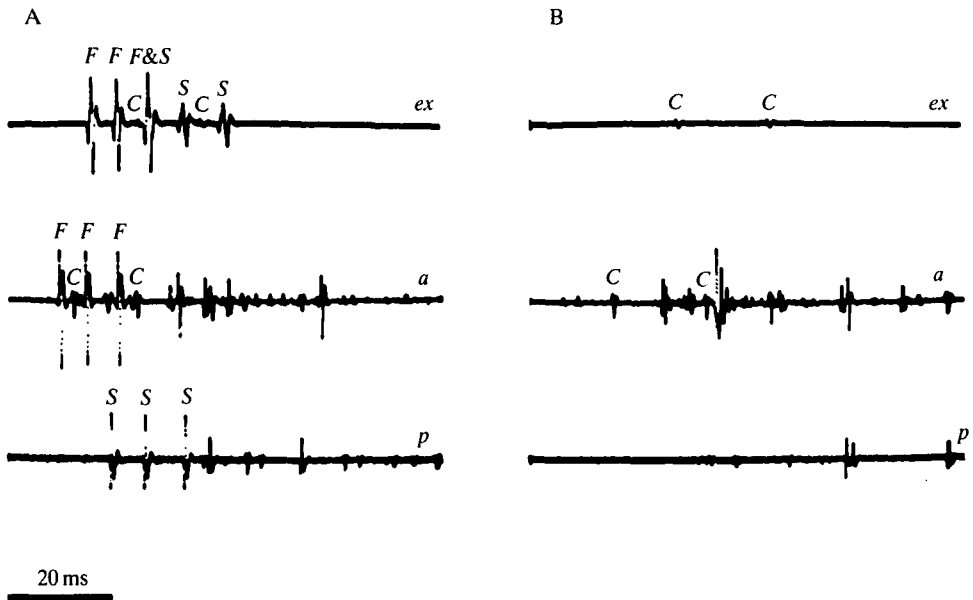
A

B

20 ms

Fig. 1. Giant axon-mediated activity recorded from the extensor nerve (*ex*) in the merus of a second pereiopod and from the anterior (*a*) and posterior (*p*) roots of the pedal nerve, adjacent to the thoracic ganglion. The promotor motor nerve branched from the pedal nerve just proximal to the recording site. (A) Response elicited by stimulation of the MG axons in the circumoesophageal connectives with a single pulse. Short bursts of spikes were produced in the common inhibitor (*C*) and the fast (*F*) and slow (*S*) extensor motoneurones. (B) Response elicited by stimulation of the LG axons in the abdomen with a single pulse. Only the common inhibitor responded at a short latency. All sweeps were triggered by the stimulus pulse.

extensor spike was much longer, ranging from 6–9 ms between preparations. The common inhibitor fired 2–4 spikes at 8- to 10-ms intervals with the ganglionic delay to the first spike ranging from 2–3 ms between preparations (Figs 1, 2, 3).

The order and timing at which spikes in these neurones first reached their target muscles after the MG axons fired in the circumoesophageal connectives was as follows: promotor motoneurones, 6–8 ms; common inhibitor branch to the promotor muscle, 10–12 ms; fast extensor motoneurone, 10–12 ms; slow extensor motoneurone, 15–19 ms; common inhibitor branch to the extensor muscle, 14–16 ms. Thus, the common inhibitor neurone fired in the promotor and extensor muscles well after the promotor and fast extensor motoneurones. However, spike activity in the common inhibitor and slow extensor axons at the extensor muscle was largely coincident.

The motor responses evoked in the posterior pair of pereiopods when the MG axons fired differed from those in the anterior legs in two respects. First, the fast extensor motoneurone was not activated but remained silent for at least 30 ms after the MG axons fired (Fig. 4). Second, the ganglionic delay to the first slow extensor spike was about 15 ms, much longer than that in the three anterior pereiopods. The responses of promotor motoneurones and the common inhibitor neurone were similar to those in the anterior pereiopods. Thus, in the posterior

20 ms

Fig. 2. Activity evoked in the promotor nerve of a third pereiopod by stimulation of the MG axons in the circumoesophageal connectives. All traces were triggered by the stimulus pulse. These recordings were made at the proximal end of the promotor nerve, close to the thoracic ganglion and the small potential (arrowed) that precedes each response is a field potential from the descending MG axon spike. Upper trace: response to a single stimulus pulse. The large units A and B are probably spikes in excitatory motoneurones. C is a spike in the common inhibitor. The second B spike masks an earlier spike in the common inhibitor. Lower trace: superimposed responses to five successive stimuli at 1 Hz. The latency of the A spike remained constant, that of the B spike increased through the series.

pair of pereiopods, the promotor motoneurones fired at the promotor muscle well before the common inhibitor but at the extensor muscle the common inhibitor fired first, several milliseconds before the slow extensor motoneurone (Fig. 4).

Activation of the LG axons elicited a burst of 2–4 spikes at 8- to 10-ms intervals in the common inhibitor neurone in each of the pereiopods (Fig. 1). The ganglionic delay to the activation of the first spike in the response varied from 2 to 3 ms between preparations. All other pereiopod motoneurones remained silent for at least 30 ms after the LG axons fired.

Responses similar to those described above were produced when the giant axons were stimulated to fire a short burst of spikes (2–3 spikes at 4- to 8-ms intervals), as are seen in the normal escape behaviour of crayfish (Wine & Krasne, 1972; Cooke & Macmillan, 1985). These stereotyped responses failed to be elicited when the giant axons fired repeatedly at 1 Hz. The various pereiopod
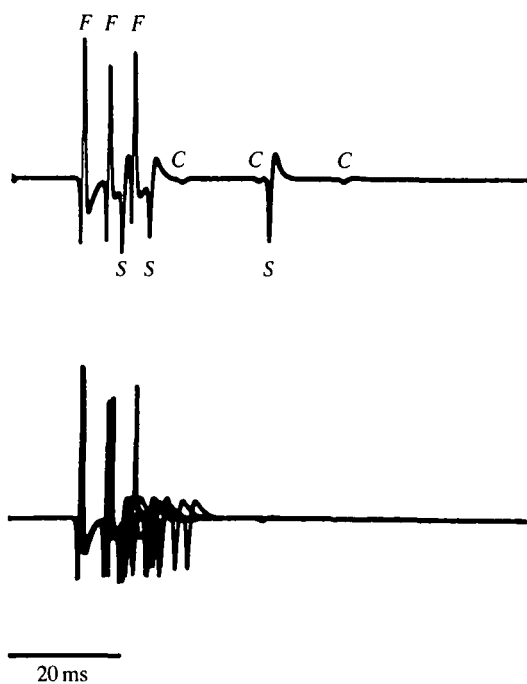
Fig. 3. Activity evoked in the extensor nerve in the merus of a second pereiopod by stimulation of the MG axons in the circumoesophageal connectives. All sweeps were triggered by the stimulus pulse. Upper trace: response to a single stimulus pulse. Bursts of spikes were produced in the fast (F) and slow (S) extensor motoneurones and in the common inhibitor neurone (C). Lower trace: superimposed responses to five successive stimuli at 1 Hz. The latency of the first fast extensor spike remained constant, those of other spikes in the response increased throughout the series.
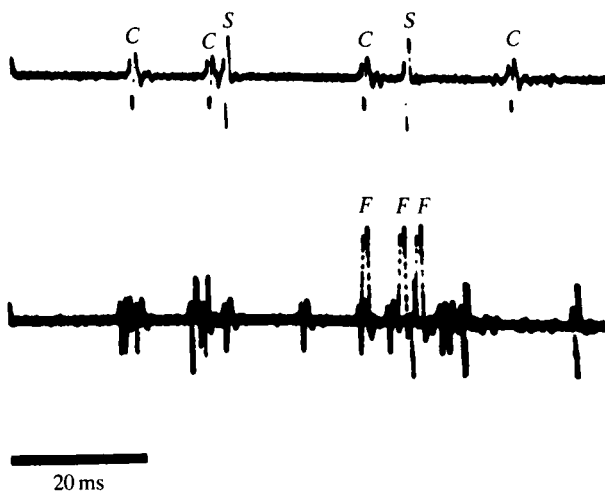

Fig. 4. Activity evoked in the extensor nerve of a fourth pereiopod by stimulation of the MG axons in the circumoesophageal connectives. All sweeps were triggered by the stimulus pulse. Upper trace: response to a single pulse. Spikes were elicited in the common inhibitor (C) and the slow extensor motoneurone (S). Lower trace: superimposed responses to three successive stimuli at 15-s intervals. The fast extensor motoneurone (F) fired at a long latency in these responses.

motoneurones differed in the persistence of their responsiveness during these trains of giant axon spikes. When the MG axons fired at 1 Hz, the slow extensor motoneurone was the most labile, usually failing to respond after about five stimuli. The latency of the initial slow extensor spike in each response increased by several milliseconds over this period (Figs 3, 4). The common inhibitor neurone was more persistent but also dropped out after 5–10 stimuli, with the latency of the initial spike in its response increasing by up to 2 ms. A similar trend in the common inhibitor response also occurred when the LG axons fired repeatedly at 1 Hz. The fast extensor and promotor motoneurones showed the most persistent responses during repeated firing of the MG axons, and the latency of the initial spike of each unit in the response increased little over 10–15 cycles at 1 Hz (Figs 2, 3). All units eventually failed to respond if the giant axons continued to fire repeatedly.

Swimmerets

In *Cherax destructor*, segments 2–5 of the abdomen each bear a pair of swimmerets which are innervated by the first root of the abdominal ganglion. Identical responses were recorded from each of these first roots when either pair of giant axons fired a spike (Fig. 5). The first unit in the volley of spikes recorded at the proximal end of a first root when the giant axons fired showed many of the characteristics of the segmental giant (SG) neurones found in *Procambarus clarkii*
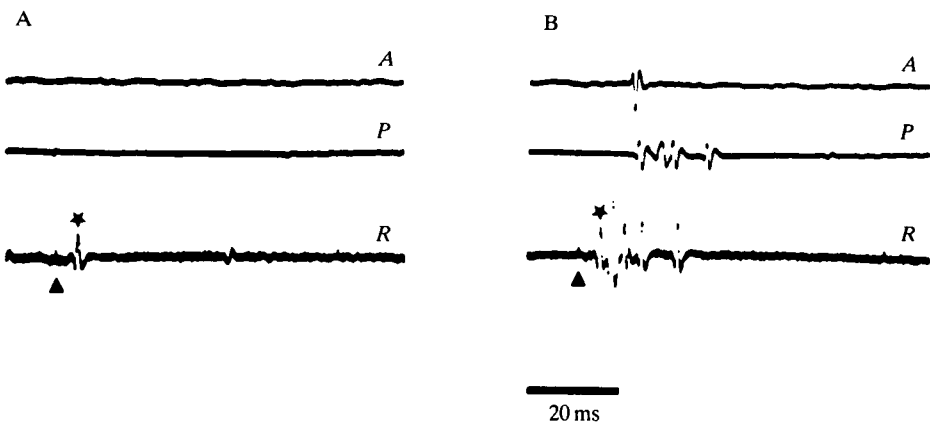

Fig. 5. Responses of abdominal first root neurones to spikes in the LG axons. Recordings were made from the first root of the fifth abdominal ganglion at its base adjacent to the ganglion (R), and from the anterior (A) and posterior (P) distal branches which innervate the swimmeret returnstroke and powerstroke muscles respectively. The LG axons were stimulated in the fifth segment of the abdomen. The time of stimulation is marked ▲. (A). Response to a spike in the LG axons, recorded during a train of high frequency (50 Hz) spikes in the LG axons. Only one first root neurone (★) responded at a short latency. This cell was unique in that a corresponding axon spike was not seen in either of the distal branches A or B, nor in the proximal branch of the root which contains sensory axons from the sternal area. The cell is probably the segmental giant (SG) neurone. (B) Response to a single LG axon spike after the preparation had been rested for several minutes. In addition to the SG cell, several swimmeret motoneurones also fired at short latencies.

(Kramer, Krasne & Wine, 1981; Roberts *et al.* 1982). This spike was never correlated with a synchronous spike in any of the distal branches in the first root, yet was shown to be efferent in nature by its persistence when the root was transected immediately distal to the recording electrode (Fig. 5). The ganglionic delay in the activation of this unit was less than 1·0 ms and the unit followed giant axon spikes on a one-to-one basis at frequencies of up to 100 Hz. A single spike in one MG axon excited the unit bilaterally.

In addition to the SG neurone, several swimmeret motoneurones also fired at short latencies when the giant axons were stimulated (Fig. 5). The ganglionic delay in the activation of the first of these was about 1·5 ms. The responses of these motoneurones were much less persistent than the response of the SG neurone during trains of giant axon spikes and they usually failed within 5–10 cycles when the giant axons fired at 1 Hz. All other motoneurones in the swimmerets remained silent for 30 ms or more after the giant axons fired.

Uropods

Stimulation of the MG axons to fire a spike elicited a burst of 2–3 spikes at 8- to 12-ms intervals in a motoneurone innervating the intrinsic lateral promotor muscle (Fig. 6). This muscle promotes the uropod exopodite relative to the endopodite and protopodite (Larimer & Kennedy, 1969). The ganglionic delay in the activation of the first spike in this response was about 1·5 ms. The burst of spikes reached the lateral promotor muscle about 16–18 ms after the MG axons fired in the circumoesophageal connectives. No other uropod motoneurones fired at a short latency after the MG axons were stimulated. However, a group of remotor motoneurones with axons in the second root of the terminal ganglion characteristically fired a long burst of spikes, beginning at least 40 ms after the MG axons fired (Fig. 6). In contrast to the lateral promotor motoneurone, which continued to respond for at least 10 cycles when the MG axons fired repeatedly at 1 Hz, the responses of these remotor motoneurones failed after several cycles. Also, the lateral promotor motoneurone fired only when there was a spike in the ipsilateral MG axon whereas the late responses of the remotor motoneurones were evoked bilaterally.

Only one uropod motoneurone responded at a short latency when the LG axons fired. This unit has its axon in the second root of the terminal ganglion and innervated the extrinsic uropod muscle termed the lateral remotor by Larimer & Kennedy (1969) (Fig. 6). In *C. destructor*, this muscle inserts dorsal and lateral to the point of articulation between the uropod protopodite and the carapace, so that its action is to extend and slightly promote the protopodite. The muscle will be termed the protopodite promotor muscle here. The ganglionic delay in the activation of the protopodite promotor motoneurone was about 1·5 ms and the spike reached the muscle 5–6 ms after the LG axons fired in the abdomen. The response of the neurone persisted for 5–10 cycles of repeated activation of the LG axons at 1 Hz before failing. There was also a late burst of spikes in the second root remotor motoneurones after the LG axons fired, similar to that which followed a spike in the MG axons.
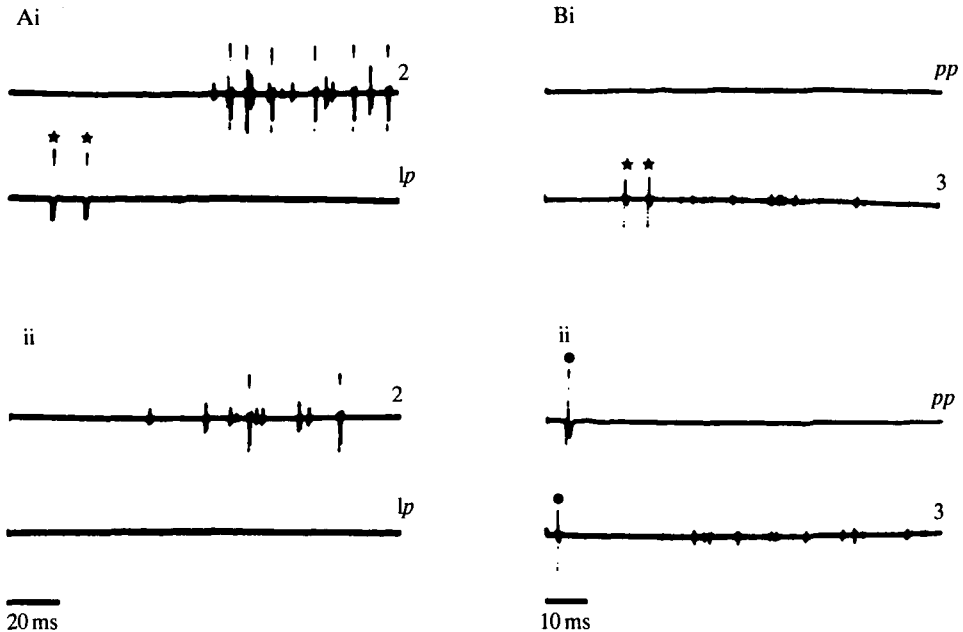
Fig. 6. Giant axon-mediated responses of uropod motoneurones. (A) Responses recorded from the second root of the terminal abdominal ganglion (2) and the motor nerve to the intrinsic lateral promotor muscle (1p). (i) Stimulation of the MG axons in the head elicited a pair of spikes at short latency in one lateral promotor motoneurone (★) and a later burst of activity in several remotor motoneurones in the second root. (ii) Stimulation of the LG axons in the abdomen elicited only a long latency burst of activity in second root motoneurones. (B) Responses recorded from the motor nerve to the protopodite promotor muscle (pp) and the third root of the terminal ganglion (3). (i) Stimulation of the MG axons in the head elicited the pair of spikes in the lateral promotor axon in the third root (★). (ii) Stimulation of the LG axons in the abdomen elicited a single spike (●) in a motoneurone to the protopodite promotor muscle. All traces were triggered by the stimulus pulse.

DISCUSSION

The patterns of activity recorded from appendage motoneurones in these experiments correlate well with the stereotyped movements of the appendages which occur in giant axon-mediated escape behaviour (Cooke & Macmillan, 1985). The burst of activity in the common inhibitor in each pereiopod after the MG or LG axons fire a spike should promote passive streamlining of these limbs by causing relaxation of tonic muscle fibres (Cooke & Macmillan, 1983). In MG flips, this action is supplemented by the activity of promotor and extensor motoneurones. The promotor and extensor muscles both contain populations of phasic muscle fibres which contract rapidly in response to one or a few spikes in fast motoneurones (Wiersma, 1961; Crabtree & Tse, 1982; Govind & Atwood, 1982). The delayed firing of the slow extensor motoneurones in these responses should also contribute to the rapid contraction of the extensor muscles in the pereiopods. The slow extensor motoneurone mainly innervates tonic fibres which normally contract slowly (Govind & Atwood, 1982). However, coincident activity at the terminals of the common inhibitor and slow extensor axons in the extensor

muscles should prevent these fibres from contracting but will not affect facilitation of transmitter release at the terminals of the slow extensor axon. Continued firing in the slow extensor motoneurone after the common inhibitor burst ceases should produce large facilitated junction potentials, resulting in more rapid contraction of the tonic muscle fibres (Marmont & Wiersma, 1938; Atwood & Walcott, 1965; Florey, 1977).

The extreme delay in the firing of the slow extensor motoneurones in the most posterior pair of pereiopods is consistent with the delayed extension of these limbs in MG flips (Cooke & Macmillan, 1985). It is not clear why the fast extensor motoneurone is not recruited in this response.

Roberts *el al.* (1982) have demonstrated that the SG neurones in abdominal ganglia serve as local spiking interneurones which drive abdominal fast flexor motoneurones and other interneurones and motoneurones in the giant axon-mediated tailflip circuitry. The other first root motoneurones which respond when the giant axons fire probably cause the twitches of the swimmerets observed in these experiments but the significance of these actions is not clear.

Larimer & Kennedy (1969) found that the intrinsic lateral promotor muscles and extrinsic lateral remotor muscles in the uropods of *P. clarkii* both contained only phasic fibres. If the uropod muscles of *C. destructor* have similar properties, then the brief responses of the exopodite lateral promotor and protopodite promotor motoneurones would cause the observed promotion of the uropod exopodites and protopodites in MG and LG flips respectively. The delayed burst of activity in second root remotor motoneurones could account for the remotion of the uropods at the end of giant axon-mediated tailflips. In normal escape behaviour, uropod remotion during the abdominal extension phase of swimming is probably also driven by elements of the non-giant tailflip circuitry.

Although the techniques used to estimate ganglionic latencies in this study only yielded approximate values, the responses of the appendage motoneurones to giant axon spikes can still be separated into three categories. First, there were the extremely short latency and reliable responses of the SG neurones which are known to be driven directly by the giant axons themselves (Roberts *et al.* 1982). Second, there were the short latency responses of the pereiopod promotor, fast extensor and common inhibitor neurones and those of the uropod lateral promotor and protopodite promotor neurones. These motoneurones all responded at longer latencies than the SG neurones and were less persistent during repeated firing of the giant axons. This would be consistent with the presence of one or more driving interneurones being interposed between the giant axons and these motoneurones. Finally, there were the much longer latency responses of the pereiopod slow extensor motoneurones which suggest the involvement of some form of delay-line interneuronal circuitry activated by the MG axons.

Command-derived inhibition of motoneurones to the abdominal tonic flexor, tonic extensor and phasic extensor muscles is important in ensuring that contraction of the phasic flexor muscles is not impeded in LG flips (Wine, 1977; Kuwada & Wine, 1979; Kuwada, Hagiwara & Wine, 1980). Similar processes

might be expected to act on appendage motoneurones to ensure the integrity of movements of the appendages in MG and LG flips. Circumstantial evidence pointing to this includes the consistent observation in the present experiments that all appendage motoneurones not involved in the short-latency, stereotyped responses remained silent for 30 ms or more after the giant axons fired. Intracellular recordings from these motoneurones will be necessary to confirm this hypothesis.

Much is now known about the organization of the neural pathways which coordinate the activity of the axial muscles of the crayfish in escape swimming (Wine & Krasne, 1982; Kramer & Krasne, 1984). The axial muscles of crayfish show a clear division into phasic and tonic components: the phasic flexor and extensor muscles are used exclusively for swimming while the tonic muscles are reserved for postural control and are inhibited during escape (Wine, 1977; Kuwada & Wine, 1979; Kuwada et al. 1980). By contrast, the muscles of the appendages, particularly those of the pereiopods, are used in a variety of behavioural roles. It will be interesting to see if the central neural pathways which coordinate the activity of the appendage motoneurones during escape differ substantially from those which control abdominal movements, or whether they utilize the same basic principles and share common elements.

REFERENCES

ATWOOD, H. L. & WALCOTT, B. (1965). Recording of electrical activity and movement from legs of walking crabs. *Can. J. Zool.* **43**, 657–665.

COOKE, I. R. C. & MACMILLAN, D. L. (1983). The common inhibitory innervation of the pereiopods of the crayfish *Cherax destructor*. *J. exp. Zool.* **225**, 513–517.

COOKE, I. R. C. & MACMILLAN, D. L. (1985). Further studies of crayfish escape behaviour. I. The role of the appendages and the stereotyped nature of non-giant escape swimming. *J. exp. Biol.* **118**, 351–365.

CRABTREE, R. L. & TSE, F. (1982). An ultrastructural-histochemical dichotomy in crayfish muscle. *J. exp. Zool.* **221**, 259–262.

FLOREY, E. (1977). Role of peripheral inhibition in the control of arthropod muscle. In *Identified Neurons and Behaviour of Arthropods*, (ed. G. Hoyle), pp. 117–130. New York, London: Plenum Press.

GOVIND, C. K. & ATWOOD, H. L. (1982). Organization of neuromuscular systems. In *Biology of Crustacea*, Vol. 3, (ed. D. E. Bliss), pp. 63–104. New York, London: Academic Press, Inc.

KRAMER, A. P. & KRASNE, F. B. (1984). Crayfish escape behaviour: production of tailflips without giant fiber activity. *J. Neurophysiol.* **52**, 182–211.

KRAMER, A. P., KRASNE, F. B. & WINE, J. J. (1981). Interneurons between giant axons and motoneurons in crayfish escape circuitry. *J. Neurophysiol.* **45**, 550–573.

KUWADA, J. Y., HAGIWARA, G. & WINE, J. J. (1980). Postsynaptic inhibition of crayfish tonic flexor motoneurones by escape commands. *J. exp. Biol.* **85**, 343–347.

KUWADA, J. W. & WINE, J. J. (1979). Crayfish escape behaviour: commands for fast movements inhibit postural tone and reflexes and prevent habituation of slow reflexes. *J. exp. Biol.* **79**, 205–224.

LARIMER, J. L., EGGLESTON, A. C., MASUKAWA, L. M. & KENNEDY, D. (1971). The different connections and motor outputs of lateral and medial giant fibres in the crayfish. *J. exp. Biol.* **54**, 391–402.

LARIMER, J. L. & KENNEDY, D. (1969). Innervation patterns of fast and slow muscle in the uropods of crayfish. *J. exp. Biol.* **51**, 119–133.

MARMONT, G. & WIERSMA, C. A. G. (1938). On the mechanism of inhibition and excitation of crayfish muscle. *J. Physiol., Lond.* **93**, 173–193.

ROBERTS, A., KRASNE, F. B., HAGIWARA, G., WINE, J. J. & KRAMER, A. P. (1982). The segmental giant: evidence for a driver neuron interposed between command and motor neurons in the crayfish escape system. *J. Neurophysiol.* **47**, 761–781.

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

WIERSMA, C. A. G. (1961). The neuromuscular system. In *The Physiology of Crustacea*, Vol. 2, (ed. T. H. Waterman), pp. 191–240. New York, London: Academic Pres, Inc.

WINE, J. J. (1977). Crayfish escape behaviour. II. Command-derived inhibition of abdominal extension. *J. comp. Physiol.* **121**, 173–186.

WINE, J. J. & KRASNE F. B. (1972). The organization of escape behaviour in the crayfish. *J. exp. Biol.* **56**, 1–18.