J. exp. Biol. ***187****, 305–313 (1994)*
Printed in Great Britain © The Company of Biologists Limited 1994

SHORT COMMUNICATION

INTEGRATION OF POSITIVE AND NEGATIVE FEEDBACK LOOPS IN A CRAYFISH MUSCLE

PETER SKORUPSKI, PAUL J. VESCOVI* AND BRIAN M. H. BUSH†
Department of Physiology, University of Bristol, School of Veterinary Science, Southwell Street, Bristol BS2 8EJ, UK

Accepted 27 September 1993

It is now well established that in arthropods movement-related feedback may produce positive, as well as negative, feedback reflexes (Bässler, 1976; DiCaprio and Clarac, 1981; Skorupski and Sillar, 1986; Skorupski *et al.* 1992; Vedel, 1980; Zill, 1985). Usually the same motor neurones are involved in both negative feedback (resistance) reflex responses and positive feedback reflexes. Reflex reversal involves a shift in the pattern of central inputs to a motor neurone, for example from excitation to inhibition.

In the crayfish, central modulation of reflexes has been described in some detail for two basal limb proprioceptors, the thoracocoxal muscle receptor organ (TCMRO) and the thoracocoxal chordotonal organ (TCCO) (Skorupski *et al.* 1992; Skorupski and Bush, 1992). Leg promotor motor neurones are excited by stretch of the TCMRO (which, *in vivo*, occurs on leg remotion) in a negative feedback reflex, but when this reflex reverses they are inhibited by the same stimulus. Release of the TCCO (which corresponds to leg promotion) excites some, but not all, promotor motor neurones in a positive feedback reflex.

There are at least two ways in which the reflex control of a muscle may be modulated in this system. Firstly, inputs to motor neurones may be routed *via* alternative reflex pathways to produce different reflex outputs. Secondly, the pattern of inputs to a motor pool may be inhomogeneous, so that activation of different subgroups of the motor pool causes different outputs.

Different crayfish promotor motor neurones are involved in different reflexes. On this basis, the motor neurones may be classified into at least two subgroups: those that are excited by the TCCO in a positive feedback reflex (group 1) and those that are not (group 2). Do these motor neurone subgroups have different effects on the promotor muscle, or is the output of the two promotor subgroups summed at the neuromuscular level?

To address this question we recorded from the promotor nerve and muscle in a semi-intact preparation of the crayfish, *Pacifastacus leniusculus*. Adult male and female

*Present address: Department of Zoology, University of Melbourne, Parkville, Victoria 3052, Australia.
†To whom reprint requests should be addressed.

Key words: crayfish, motor neurone, reflex, neuromuscular junction, *Pacifastacus leniusculus.*

crayfish, 8–11cm rostrum to tail, were decapitated and the tail, carapace and viscera removed. The sternal artery was cannulated and perfused with oxygenated crayfish saline, as described previously (Sillar and Skorupski, 1986).

The promotor muscle has three heads (lateral, medial and posterior), which insert together on the anterior rim of the coxa (see Pilgrim and Wiersma, 1963). Intracellular recordings were obtained from all three heads, but simultaneous recordings from the nerve and muscle were only possible with the posterior head, because the lateral and medial heads were deflected to expose the promotor nerve.

Conventional extra- and intracellular recording techniques were used. A pair of 25 μm diameter twisted insulated Ni–Cr wires was used for electrical stimulation. The TCMRO and TCCO were stretched in parallel by a servo-controlled puller. A fine hook glued to the tip of the puller was manipulated around the two receptors about three-quarters of the way along their course between the thorax and coxa. Ramp displacements of the puller were from 0.7 to 1.5mm but, because its axis was at an angle with respect to the receptor strands, length changes imposed on the receptors would have been smaller than this. Data were recorded on FM tape and digitized, on or off line, with a CED 1401 laboratory interface and microcomputer running CED software (Sigavg and Spike2).

Promotor nerve recordings from these semi-intact preparations revealed broadly similar patterns of activity to those obtained previously in isolated preparations (Sillar and Skorupski, 1986; Skorupski *et al.* 1992). A single unit was active in the promotor nerve for much of the time, with a frequency that increased and decreased with a slow, variable period. Sometimes bursts of promotor activity alternated with periods of silence. Up to five more promotor units could be recruited during periods of increased activity, but if excitation increased still further it was impossible to distinguish single units because of signal summation in the extracellular recording.

Spontaneous activity was readily recorded from the posterior (PH) and medial (MH) promotor heads (Fig. 1). In simultaneous recordings from the promotor nerve and the PH, many muscle fibres showed excitatory junction potentials (EJPs) that were correlated 1:1 with spikes in a spontaneously active promotor unit (Fig. 2B). Simultaneous recordings from MH and PH muscle fibres revealed common inputs in all cases where both fibres showed spontaneous activity (Fig. 1B). A tonic motor neurone therefore innervates both the PH and the MH of the promotor muscle. By contrast, no spontaneous activity was recorded from the lateral head (LH) of the promotor muscle.

Tactile stimulation of the leg (particularly the anterior surfaces of the coxa and basipodite) elicited intense barrages of EJPs in MH and PH muscle fibres and a much smaller number of EJPs in the LH (Fig. 1C).

Stimulation of an ipsilateral connective in the region of the medial giant axon (MG) consistently (44/45 fibres) elicited a large (10–20mV) EJP in the LH, which exhibited rapid long-lasting depression (Fig. 1D). Its characteristics suggest that it represents activation of an MG-driven promotor motor neurone, described by Heitler and Fraser (1989). This 1:1 response to MG stimulation was also seen in the PH (3/9 fibres) and MH (3/11 fibres), although it was generally only 2–3mV in amplitude.

Different promotor motor neurones displayed different types of reflex responses to parallel stretch and release of the TCMRO and TCCO. The unit that displayed the most

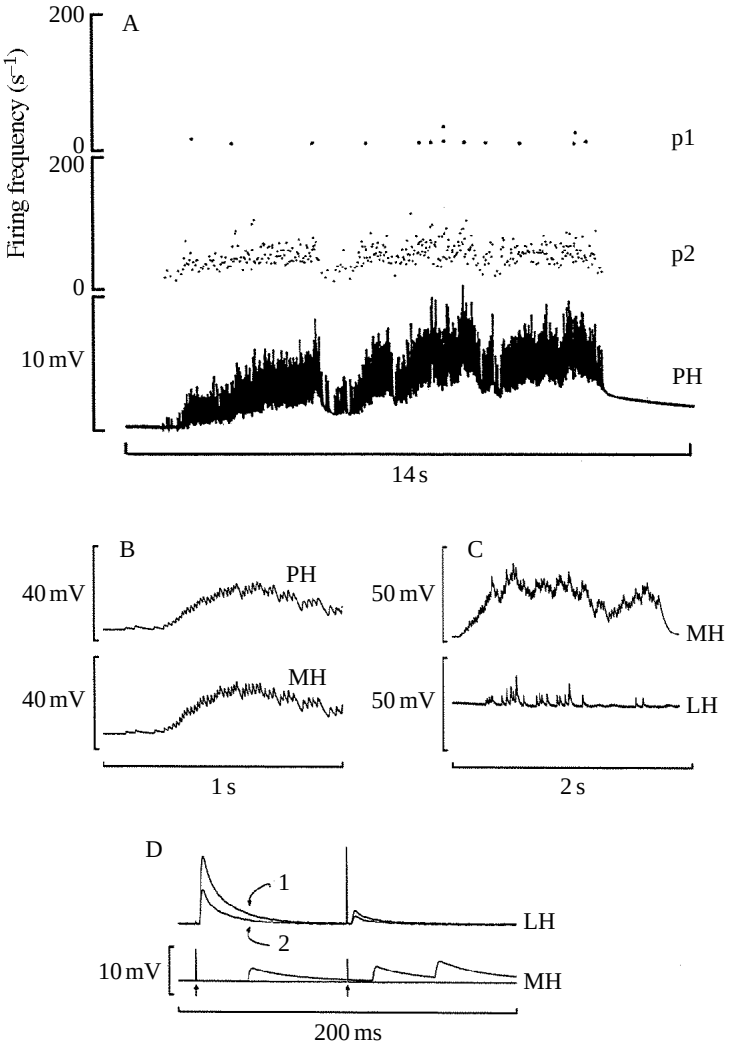

Fig. 1. Recordings from the three heads of the promotor muscle. (A) Instantaneous firing frequency of two promotor nerve units (p2, p1) and an intracellular recording from a posterior head muscle fibre (PH) during spontaneous promotor activity. (B) Spontaneous activity recorded simultaneously from posterior and medial heads, showing common excitatory junction potentials (EJPs). (C) Simultaneous recordings from lateral and medial heads. Lightly touching the anterior surface of the coxa near the beginning of the trace evoked an intense barrage of summating EJPs in the MH and weaker activity in the LH. (D) Stimulation of the anterior connective near the medial giant axon evoked large EJPs in LH (first trace), which exhibit rapid, long-lasting depression. Two pulses, 90ms apart, were given in each sweep (arrows) and there was a delay of 1min between superimposed traces 1 and 2. The response was absent in a simultaneously recorded MH fibre (second trace; the EJPs here are spontaneous).

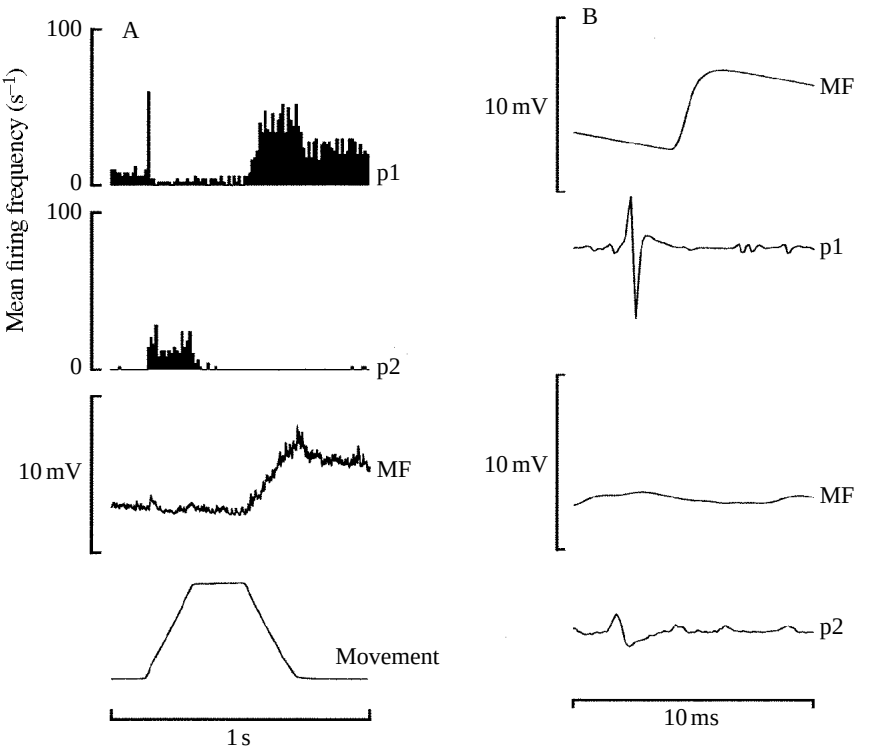

Fig. 2. Reflex responses of two promotor units and a PH muscle fibre to stretching and releasing the TCMRO and TCCO in parallel. (A) Peristimulus time histograms showing a group 1 promotor unit (p1) excited by release, a group 2 unit (p2) excited by stretch and the averaged response of a muscle fibre (MF), all recorded simultaneously during 50 cycles of stretch and release; lowest trace, movement monitor (stretch is upwards). (B) Averaged nerve (bottom traces) and muscle fibre (top traces) recordings triggered from the two classes of promotor nerve spikes. The group 1 unit (p1) elicits an EJP in the muscle fibre, but the group 2 unit (p2) does not. Data from the same experiment as Fig. 1A.

spontaneous activity (i.e. the most tonic motor unit) was either excited or inhibited by stretch. This is essentially the phase-dependent reversal of TCMRO input described by Skorupski and Sillar (1986). This unit was also excited by release, often much more markedly than by stretch (although occasionally, in less active preparations, this unit was inhibited by release). The excitation caused by release represents TCCO input and is therefore a positive feedback reflex, so the unit was classified as a group 1 promotor motor neurone (Skorupski *et al.* 1992). This response pattern is illustrated in the top trace of Fig. 2A (unit p1, also shown during spontaneous activity in Fig. 1A).

Up to three phasic promotor units could also be discriminated (Figs 1A, 2A). These units, which had smaller extracellular spikes, were excited by stretch but not by release (Fig. 2A, p2) and were therefore classified as group 2 promotor motor neurones. Intracellular recordings from this type of unit revealed no subthreshold excitatory TCCO input; instead they were hyperpolarized by release (Skorupski *et al.* 1992).

Recordings from PH muscle fibres during stretch and release stimulation of the TCMRO and TCCO showed a variety of responses that suggested innervation by either or both groups of promotor motor neurones. For example, the averaged reflex response of the muscle fibre shown in Fig. 2A suggests input from the group 1 but not the group 2 promotor unit. This is shown directly in Fig. 2B, where the muscle fibre potential is averaged with respect to each of the two units discriminated from the promotor nerve.

Similar responses to stretch and release of the TCMRO and TCCO were also recorded in MH, although simultaneous nerve–muscle recordings were only obtained with the PH. Nevertheless, results from the PH may to some extent be generalised to the MH, for common EJPs were observed in both heads. In addition, recordings from the separate nerve branches to the two heads also revealed some group 1 and group 2 units common to both. Fig. 3 shows promotor responses to stretch and release, recorded simultaneously from MH and PH nerve branches. The spike of the most tonic (group 1) promotor unit, which was excited by both stretch and release, was present in both branches. A number of smaller phasic spikes (group 2) were recruited during stretch but not release. Some of

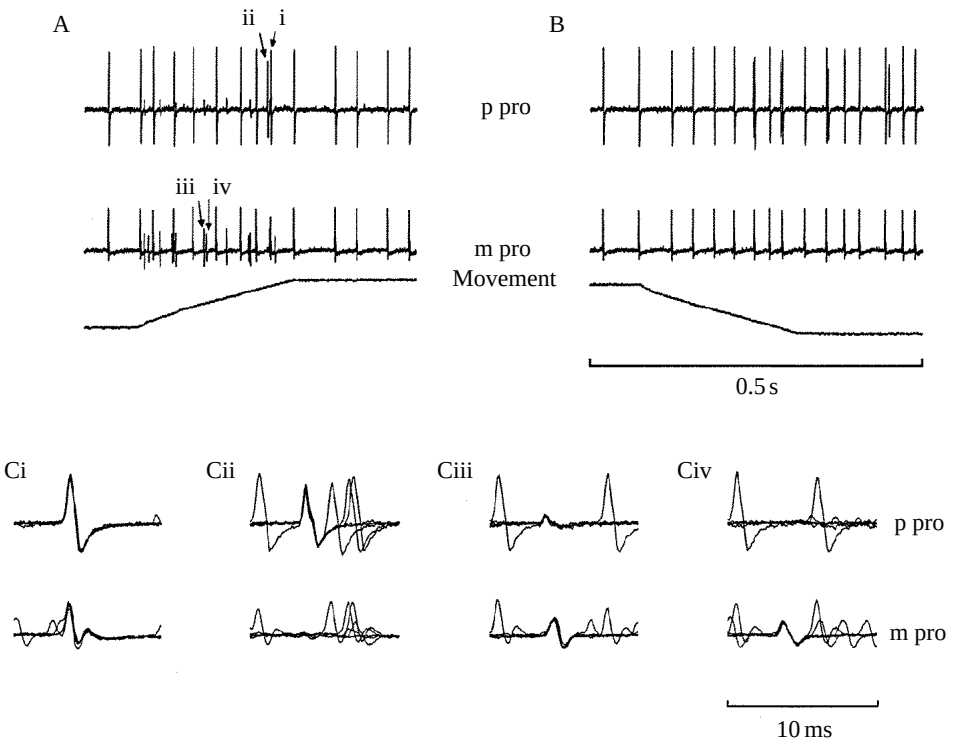

Fig. 3. Simultaneous recordings from promotor nerve branches to posterior (p pro) and medial (m pro) heads. Responses to stretch (A) and release (B): note the small (group 2) units that responded to stretch but not release. Units are shown on expanded time scale in C. Of the group 1 units, one is present in both branches (i) and another in the posterior branch only (ii). Similarly, one group 2 unit occurs in both branches (iii) and another only in the medial branch (iv).

these are also common to both branches. Additional spikes excited by either stretch or release may be recruited in more active preparations, and may be shared, or may be exclusive to one branch or the other. Thus, motor neurones of different feedback groups (as defined by responses to TCMRO and TCCO stimulation) are not segregated in grossly different regions of the promotor muscle complex. The MH and PH are both innervated by units that respond only to stretch (group 2) and by units that respond (variably) to both stretch and release (group 1).

We were also able to show that, in the PH, the same muscle fibre could be innervated by promotor motor neurones of both subgroups. Fig. 4 shows recordings from three different PH muscle fibres during reflex-evoked activity. In Fig. 4A, several group 2 promotor spikes are elicited by stretch, whereas larger group 1 units are excited by release. EJPs can be seen following both the group 2 and the smaller of the group 1 units. Averaged reflex responses of the muscle fibre and the two units indicated by the arrowheads are plotted for 16 cycles of stretch and release stimulation in Fig. 4B. Fig. 4C compares four promotor units, two classified as group 1 (i, ii) and two as group 2 (iii, iv). Three of these units, two group 1 and one group 2, innervated the muscle fibre shown in Fig. 4C. Thus, motor neurones of different subgroups do not innervate different regions of the muscle or produce different types of responses.

We did not measure the relative strengths of group 1 and group 2 motor output onto the PH. EJPs caused by either class of spike showed marked facilitation and could vary considerably in size between and within muscle fibres. Furthermore, because these preparations were spontaneously active, there was always an uncontrolled level of background facilitation. At present we can only say that the promotor motor neurones that were most readily recruited, from either subgroup, were slow facilitating excitors. Fig. 4D shows EJPs from a group 1 and a group 2 promotor motor neurone recorded in a single muscle fibre and selected from responses to receptor movement in which there was minimal background activity. They are similar in size, time course and degree of facilitation, indicating that even at this level no functional distinction between the two motor subgroups is evident.

The present analysis was necessarily restricted to promotor motor neurones that displayed suprathreshold activity, either spontaneous or evoked by receptor movement. As noted above, a single (group 1) unit was spontaneously active for much of the time, and (up to two) more group 1 units could be distinguished during occasional periods of increased spontaneous activity, before high-frequency firing and signal summation made spike discrimination unreliable. Up to three group 2 units can be recruited by stretch. Therefore, we can safely say that up to six promotor units were spontaneously active or driven by reflex at moderate levels of activity in these experiments.

We conclude that different patterns of feedback to different promotor motor neurones do not reflect different peripheral functions of these motor neurones. Instead, the output from different feedback groups (onto PH at least) seems to be summed at the muscle. This does not rule out the possibility that some form of functional subdivision exists in the promotor muscle, but the basis for any such subdivision is not the same as the subdivision of the motor pool into feedback groups on the basis of their inputs from the TCMRO and TCCO.

Non-uniform patterns of innervation have been described in both insect (Theophilidis

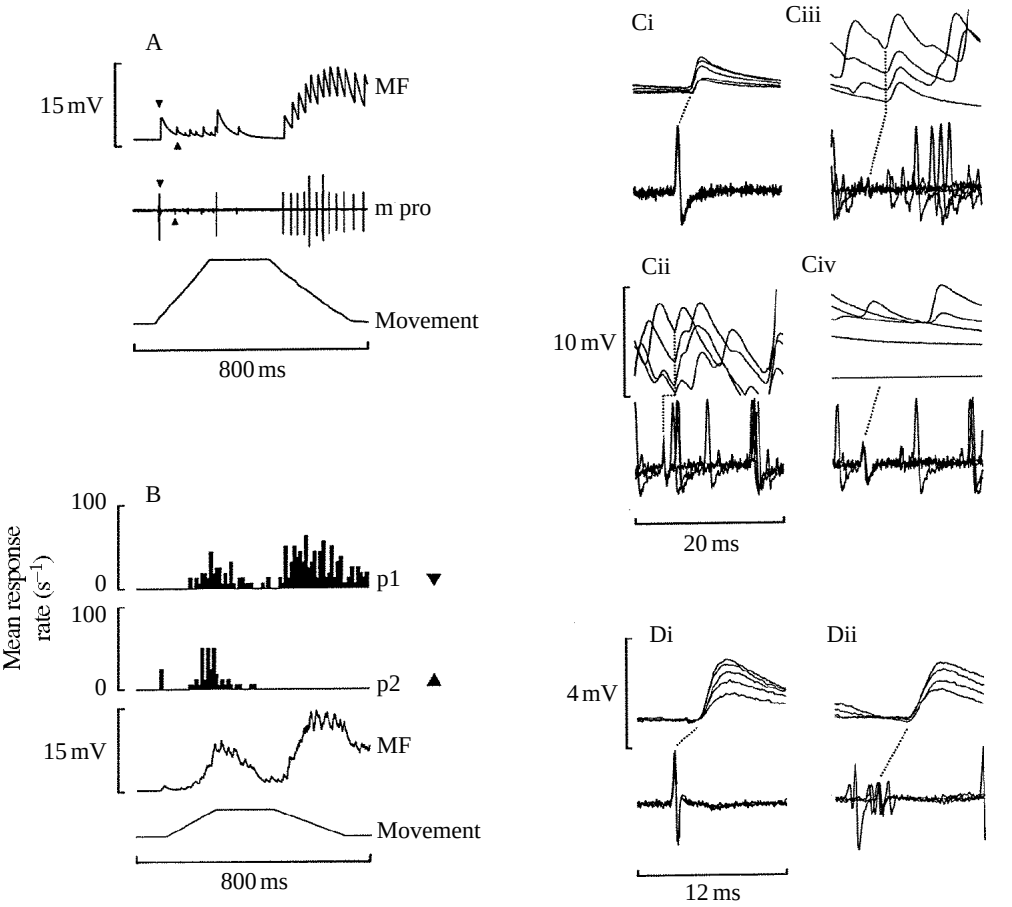

Fig. 4. Polyneuronal innervation of promotor muscle fibres by motor neurones of different feedback groups. (A) Reflex responses recorded in a PH muscle fibre (MF) and the medial branch of the promotor nerve (m pro). A group 1 promotor spike is followed 1:1 by EJPs (downward arrowheads). Smaller group 2 spikes (excited by stretch) also elicit EJPs (upward arrowheads). (B) Average reflex responses of the promotor units and muscle fibre marked in A (16 sweeps). (C) A PH muscle fibre that received EJPs from three of four promotor units discriminated from the nerve recording. On the basis of reflex responses, two units (i, ii) were classified as group 1 and two (iii, iv) as group 2. (D) Five superimposed, consecutive spikes elicited from a group 1 unit by release (i) and four from a group 2 unit by stretch (ii) cause similar facilitating EJPs in one PH muscle fibre. A–B, C, D, are from three different experiments.

and Burns, 1983) and crustacean (Hill and Govind, 1982; Parsons, 1982) muscle. In these cases, fast and slow motor neurones tend to innervate separate regions of the muscle. We analysed motor neurones that were spontaneously active, or readily recruited by receptor stimulation. In the case of the PH, where simultaneous nerve and muscle recordings could be made, all discriminable motor spikes produced slow, facilitating EJPs, regardless of whether the motor neurone belonged to group 1 or group 2.

Much less activity was recorded in the lateral head. The anatomical arrangement of this head appears to favour fast, powerful contraction. It is the longest head of the promotor muscle, originating laterally on the epimeral plate (Pilgrim and Wiersma, 1963), coursing medially and ventrally over a Y-shaped endopleural process, before proceeding laterally again to its shared insertion on the anterior rim of the coxa. This pulley-like arrangement may increase the mechanical advantage of the muscle.

In keeping with this notion, we found that stimulation of the ipsilateral connective in the region of the medial giant axon reliably produced a large EJP in LH fibres, but only a small response, or no response at all, in MH and PH fibres. Heitler and Fraser (1989) described a promotor motor neurone with direct MG input *via* a rectifying electrical synapse. It would appear that the muscle recordings in the above study were also made from the LH. Rapid promotion of the legs during MG-activated tailflips may be a function of this portion of the promotor muscle.

The possible inhomogeneity of mammalian motor pools is now an intensely debated topic (Windhorst *et al.* 1989). However, given the anatomical complexity of many mammalian muscles, it is not surprising to find a complex organization of their motor pools. The crayfish promotor muscle is relatively simple because the action of the thoracocoxal joint is restricted to one plane. Nevertheless, the motor pool innervating it is inhomogeneous in terms of feedback inputs to individual members. Different patterns of feedback, however, are not reflected in grossly different peripheral functions of the motor neurones, at least for the six or more units most readily recruited. It seems that in the crayfish the significance of feedback groups must be sought in terms of central patterning of motor output.

Supported by a SERC grant to B.M.H.B. P.J.V. was a British Council Bursar from the University of Melbourne, Australia. We thank S. A. Cayzer, G. J. Murphy and B. M. Rawat for participating in some early experiments.

References

BÄSSLER, U. (1976). Reversal of a reflex to a single motor neurone in the stick insect *Carausius morosus*. *Biol. Cybernetics* **24**, 47–49.

DICAPRIO, R. A. AND CLARAC, F. (1981). Reversal of a walking leg reflex elicited by a muscle receptor. *J. exp. Biol.* **90**, 197–203.

HEITLER, W. J. AND FRASER, K. (1989). Thoracic output of crayfish giant fibres. I. Pereiopod promotor motor neurons. *J. comp. Physiol.* A **166**, 117–124.

HILL, R. H. AND GOVIND, C. K. (1982). Functional subdivision within a lobster motor unit. *Experientia* **38**, 362–363.

PARSONS, D. W.(1982). The leg flexor muscle of *Carcinus*. I. Innervation and excitatory neuromuscular physiology. *J. exp. Zool.* **224**, 157–168.

PILGRIM, R. L. C. AND WIERSMA, C. A. G. (1963). Observations on the skeleton and somatic musculature of the abdomen and thorax of *Procambarus clarkii* (Girard), with notes on the thorax of *Panulirus interruptus* (Randall) and *Astacus*. *J. Morph.* **113**, 453–487.

SILLAR, K. T. AND SKORUPSKI, P. (1986). Central input to primary afferent neurons in the crayfish, *Pacifastacus leniusculus*, is correlated with rhythmic motor output of thoracic ganglia. *J. Neurophysiol.* **55**, 678–688.

SKORUPSKI, P. AND BUSH, B. M. H. (1992). Parallel reflex and central control of promotor and receptor motorneurons in crayfish. *Proc. R. Soc. Lond. B* **249**, 7–12.

SKORUPSKI, P., RAWAT, B. M. AND BUSH, B. M. H. (1992). Heterogeneity and central modulation of feedback reflexes in crayfish motor pool. *J. Neurophysiol.* **67**, 648–663.

SKORUPSKI, P. AND SILLAR, K. T. (1986). Phase-dependent reversal of reflexes mediated by the thoracocoxal muscle receptor organ in the crayfish, *Pacifastacus leniusculus. J. Neurophysiol.* **55**, 689–695.

THEOPHILIDIS, G. AND BURNS, M. D. (1983). The innervation of the mesothoracic flexor tibiae muscle of the locust. *J. exp. Biol.* **105**, 373–388.

VEDEL, J. P.(1980). The antennal motor system of the rock lobster: competitive occurrence of resistance and assistance reflex patterns originating from the same proprioceptor. *J. exp. Biol.* **87**, 1–22.

WINDHORST, U., HAMM, T. M. AND STUART, D. G. (1989). On the function of muscle and reflex partitioning. *Behav. Brain Sci.* **12**, 629–681.

ZILL, S. N. (1985). Plasticity and proprioception in insects. II. Modes of reflex action of the locust metathoracic femoral chordotonal organ. *J. exp. Biol.* **116**, 463–480.