

NONSPIKING STRETCH RECEPTORS OF THE CRAYFISH SWIMMERET RECEIVE AN EFFERENCE COPY OF THE CENTRAL MOTOR PATTERN FOR THE SWIMMERET

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Summary

In crayfish, the movement of each swimmeret is monitored by a pair of nonspiking stretch receptors (NSR) with central somata and dendrites that are embedded in an elastic strand at the base of the appendage. I provide evidence that the neuropile segments of these primary sensory neurones receive synaptic input from the hemiganglionic central pattern generator for the swimmeret.

In nonbursting isolated abdominal nerve cords of *Pacifastacus leniusculus* Dana, the membrane potentials of the NSRs (recorded in the neuropile) are stable; whenever the central pattern generator is active, they oscillate in phase with the motor output. Every perturbation of the central pattern generator's activity is precisely reflected in analogous changes (in phase and/or amplitude) of the NSRs' oscillations. This activity must arise *via* central, synaptic input to the NSRs, because it occurs when all ganglia except the sixth are deafferented.

Lucifer Yellow dye-fills show that the neurites of the NSRs are confined to the ipsilateral lateral neuropile, which is the region of the hemiganglion where swimmeret functions are integrated.

These results imply that during rhythmic beating of the swimmerets, the NSRs receive an efference copy of the motor output to the limb whose movements they monitor. *In vivo*, therefore, the incoming sensory signal must be subject to modulation (gating) by the limb's central pattern generator.

Introduction

In their seminal paper on crayfish swimmerets, Hughes & Wiersma (1960) concluded that 'both the inflow from peripheral proprioceptors and intrinsic properties of the central ganglia play essential parts in the coordination of the metachronal movements of the swimmerets' (Hughes & Wiersma, 1960, p. 669). Confirmation of the autogenic production of swimmeret motor patterns by the isolated nerve cord (Ikeda & Wiersma, 1964) was followed by investigations that emphasized the properties of the intrinsic neuronal circuits. (1) Each hemiganglion contains a centre of coordination for the swimmeret it innervates (Ikeda & Wiersma, 1964), in which nonspiking interneurones are principal elements

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(Heitler & Pearson, 1980; Paul & Mulloney, 1985*a,b*). (2) Interconnections between the different centres mediate interganglionic coordination (Hughes & Wiersma, 1960; Paul & Mulloney, 1986; Stein, 1971). (3) There are neuronal (Wiersma & Ikeda, 1964; Davis & Kennedy, 1972) and neurotransmitter (Mulloney *et al.* 1987) mechanisms 'which can trigger the centres of the swimmerets to rhythmic firing' (Hughes & Wiersma, 1960). But, without proprioceptive feedback, the proper metachronal movements of the swimmerets do not occur (Hughes & Wiersma, 1960).

In other types of locomotory behaviour, sensory feedback is needed to sculpture the output of central rhythm generators to achieve fully coordinated movements (Altman, 1982; Grillner & Wallén, 1984; Sillar *et al.* 1986; Wolf & Pearson, 1987).

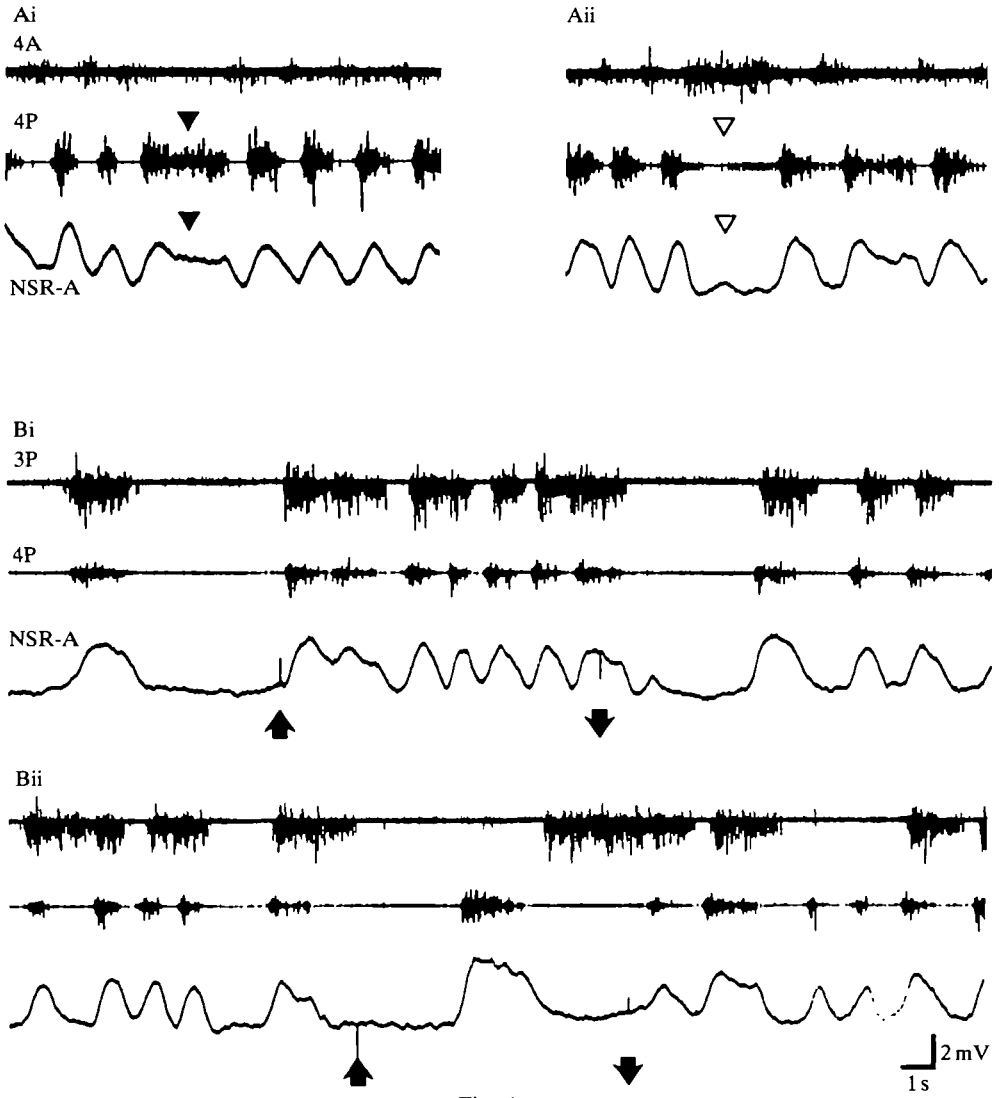


Fig. 1

Sensory influences on swimmeret motor activity have been investigated in various contexts in crayfish (Deller & MacMillan, 1985; Heitler, 1986; West *et al.* 1979) and also in the lobsters *Homarus americanus* (Davis, 1968, 1969), *Homarus gammarus* and *Nephrops norvegicus* (Miyan & Neil, 1986). Nevertheless, the neuronal interface between proprioceptive input and the intrinsic pattern-generating circuits remains obscure. I provide evidence here that sensory neurones signalling limb retraction receive rhythmic input directly from the hemiganglionic central pattern generator (CPG). This suggests that during rhythmic beating of the swimmerets the central nervous system could actively control, presynaptically, the effect of sensory feedback from the moving appendage.

In crayfish, movement of each swimmeret with respect to the abdomen is monitored by a pair of nonspiking stretch receptors (NSR) whose distal, dendritic terminals insert on an elastic strand at the base of the swimmeret (Heitler, 1982). While recording from the central processes of the NSRs in isolated nerve cords, I have discovered that these primary sensory neurones receive input from the hemiganglionic CPG for the swimmeret such that their membrane potentials oscillate in synchrony with the efferent bursting of that swimmeret's motoneurones. In this respect the swimmeret NSRs resemble their segmental homologues, the TCMRO, at the base of the walking legs (Sillar & Skorupski, 1986).

Materials and methods

All my experiments were on excised abdominal nerve cords, with tailfan attached, of *Pacifastacus leniusculus* (see Paul & Mulloney, 1985*a,b*, for details of methods). I used microelectrodes filled with Lucifer Yellow dye to impale the central processes of the NSRs in the lateral part of the lateral neuropile (LN, Skinner, 1985). In nerve cords in which all connections with the swimmerets are cut, the NSRs can be recognized physiologically by the large-amplitude, slowly decaying depolarizations with which they respond to brief electric shocks delivered

Fig. 1. Oscillations of the membrane potential of an NSR-A in ganglion (G4) (for structure, see Fig. 2F) in an isolated nerve cord are precisely coupled to activity of swimmeret motoneurones (MNs). (A) The amplitude and shape of each depolarizing wave are correlated, respectively, with the strength and duration of the ipsilateral N1P burst (trace 4P) with which it is synchronized. (Ai) NSR-A's oscillations cease at a depolarized plateau during prolonged firing of intermediate-sized 4P MNs (▼), whereas during failure of 4P bursting and the concomitant, prolonged 4A activity (Aii) the NSR-A is hyperpolarized (▽). Note the two small oscillations, suggesting that, although the MNs are not bursting, the CPG is active at a low level. (B) An identified G3 interneurone (IN) with axon to G4 (trace not shown; see fig. 5 in Paul & Mulloney, 1986) controls the frequency of the CPG in G4 (as well as in G3) and thereby the membrane potential of the G4 NSR-A. (Bi) Depolarizing current injected into the IN in G3 accelerates N1P bursting (both ganglia) and NSR-A oscillations, whereas injection of hyperpolarizing current (Bii) transiently inhibits N1P bursting and NSR-A oscillations. Arrows mark onset and offset of current injections in Bi, Bii. Note that the G4 CPG escapes before the G3 CPG and drives a 4N1P burst and synchronous NSR-A oscillation.

to the swimmeret nerve containing their peripheral dendrites, as well as by the complete absence of any spiking activity (the latter feature is shared with many local interneurons). I confirmed their identity morphologically by ionophoretically injecting the Lucifer Yellow dye (2–4 nA, 500 ms hyperpolarizing current pulses, 1 Hz, for 20–60 min) and observing the filled cells in fixed and cleared whole mounts of the ganglia, using a fluorescence microscope. The neurites of NSRs are confined to LN (see Fig. 2E–G), which is where swimmeret functions are integrated (Paul & Mulloney, 1985*a,b*). This report is based on recordings and dye-injections of 12 NSRs in as many experiments; nine were NSR-A [soma in anterior quadrant of ganglion (see Fig. 2F,G)] and three were NSR-P [soma in

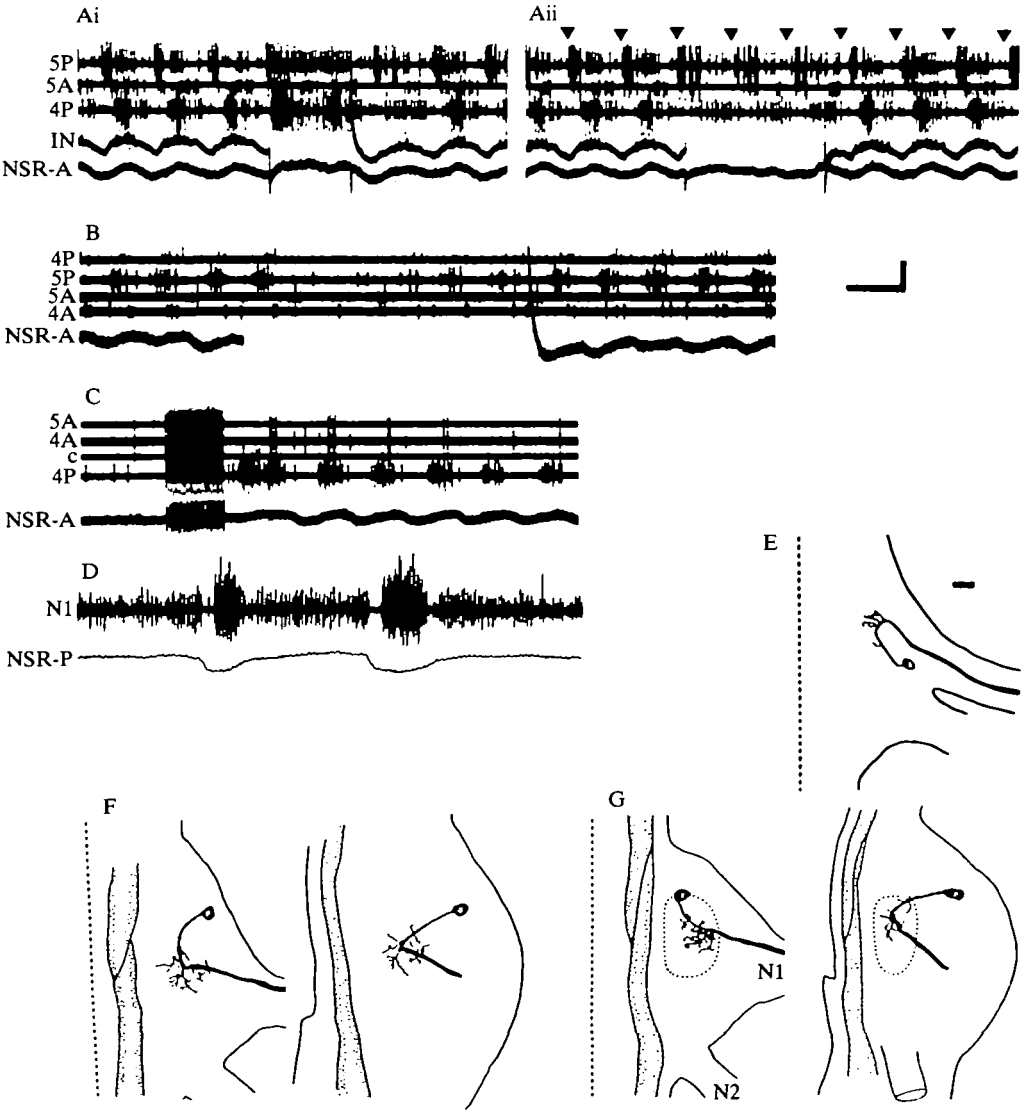


Fig. 2

posterior quadrant of ganglion (Fig. 2E); the terminology of Heitler (1982) is NSSR-A and NSSR-B].

Results

The NSRs in abdominal ganglia 2–5 show rhythmic oscillations of their membrane potentials recorded in the LN during autogenic production of swimmeret motoneurone bursts in abdominal nerve cords which are deafferented except for input to the terminal ganglion from the tailfan [Fig. 1; extracellular recordings made from the posterior, P (=remotor), branch and the anterior, A (=promotor), branch of segmental nerve 1, which innervates the swimmeret]. NSR-A depolarizes during N1P (power-stroke motoneurone) bursts (Figs 1, 2A–C) and NSR-P hyperpolarizes but with a different waveform (Fig. 2D). In each case, the oscillations of the NSR's membrane potential are precisely correlated with the moment-to-moment motor pattern of the hemiganglion in which the receptor is located and, because all ganglia except the sixth are deafferented, they must be driven by central input from the hemiganglionic CPG for the swimmeret whose movement the sensory neurone would have monitored *in vivo*. During periods of inactivity or during tonic motor output, there were occasional postsynaptic potentials but no rhythmic fluctuations in NSR membrane

Fig. 2. Simultaneous intracellular records from NSR-A in ganglion 5 (G5) and a spiking IN impaled in G4 during vigorous bursting. Both cells depolarize in phase with similar phases of N1P bursts in their respective ganglia. (Ai) 2 nA depolarizing current injected into the G4 IN drove N1P activity in both ganglia and sustained concomitant depolarization of the NSR-A for the duration of the current pulse. (Aii) 2 nA hyperpolarizing current injected into this G4 cell greatly reduced 4P bursting, weakened 5A and 5P bursts and slowed (reset) the rhythm (arrowheads mark anticipated onset of large-amplitude 5P bursts had resetting not occurred). Note the small depolarizations of the NSR-A that continue in phase with the weak firing of intermediate-sized units in 5P during the current pulse (compare with Fig. 1Aii). Current injections into this NSR-A during such vigorous bursting had undiscernible effects on motor output. (B) 2 nA depolarizing current injected into a G5 NSR-A (different preparation) during very weak bursting transiently inhibited motor output without, in this case, resetting the rhythm. (C) In a quiescent nerve cord, approx. 40 Hz stimulation of a command fibre (stimulus artefacts on all traces), contained in a lateral-ventral bundle of axons stripped from G1–G2 connective, initiated bursting and concomitant oscillations of a G4 NSR-A (trace c is from the contralateral 4A branch). (D) Abrupt hyperpolarizations of NSR-P accompany cessation of putative N1A activity preceding onset of N1P bursts (compact bursts in whole N1 recording, see traces 4P in Figs 1, 2A). (E–G) Frontal (right half of ganglion shown, anterior towards top of page; midline is dashed vertical line) and sagittal (right-hand drawing, F, G, dorsal to left) views of whole mounts of dye-filled NSRs, drawn with the aid of a *camera lucida*. (E) NSR-P in G2 (of *Procambarus clarkii*); (F) NSR-A in G4; (G) NSR-A in G5. The stippled structure is the lateral giant axon used as a landmark; the area enclosed by the dotted line (in G) is the lateral neuropil. N1, N2, segmental nerves 1 and 2. Scale bars (in B): A–C, 2 mV (10 mV for IN), 1 s; D, 7 mV, 1.2 s; E–G (bar in E), 100 μ m.

potentials (-55 to -80 mV, mean -66 mV, $N = 12$). But, whenever the hemiganglionic CPG was spontaneously active, whether weakly or strongly (Figs 1, 2A,B,D), or was driven by command fibre stimulation (Fig. 2C), the membrane potential of the NSR fluctuated by 2–5 mV in phase with the swimmeret motor output. In each experiment, the amplitude of the oscillations varied directly with the strength of the efferent bursts. Since the recordings were very similar in different experiments, the cells were probably penetrated in approximately similar locations each time. Neuropile depolarizations of these magnitudes in response to stretch of the peripheral dendrites of homologues of the swimmeret NSRs in the telson effect transmitter release, eliciting postsynaptic responses in local interneurons (Paul, 1988), although much larger depolarizations probably occur *in vivo* (Heitler, 1982; D. H. Paul, unpublished observations). Changes in phase or amplitude of the CPG's output were mirrored by similar changes in NSR membrane potential, whether the altered CPG activity occurred spontaneously (Fig. 1A) or was elicited by passing current into an identified, interganglionic coordinating neurone (fig. 5 in Paul & Mulloney, 1986) in an adjacent ganglion, or into any other neurone that altered swimmeret motor output in the ganglion of the impaled NSR (Figs 1B, 2A).

The dye-filled microelectrodes' high resistances and propensity to block precluded systematic examination of the effects of artificially depolarizing or hyperpolarizing NSRs during motor pattern generation. Small depolarizing currents (<3 nA) had overt motor effects only in quiescent nerve cords [when one or a few intermediate-sized N1P units were driven tonically for the duration of the current pulse (not shown)], and in weakly bursting nerve cords (Fig. 2B). In the latter, transient interruption of the weak bursting occurred in two ganglia (Fig. 2B) only when the impaled NSR-A was in the caudal ganglion, presumably because intersegmental coupling is stronger in the rostral than in the caudal direction (Ikeda & Wiersma, 1964; Stein, 1971). When the depolarizing current was increased to 3 nA in the preparation shown in Fig. 2B, intermediate-sized motoneurons were driven in 5N1P; these were probably the neurons that fire tonically in this branch of N1 concurrent with the depolarizing plateaus in NSR-A (Fig. 1Ai).

Discussion

In vivo, the NSRs of the swimmeret are depolarized by stretching their peripheral dendrites during remotion (power stroke) of their appendage (Heitler, 1982, 1986). Since these primary sensory neurones are also 'informed' of the intended movement whose actual execution they monitor (Figs 1, 2), the potential exists for central gating of reafference from the swimmeret. In the TCMROs, rhythmic central input to the sensory neurones that is coupled with motor output from the thoracic ganglia modulates the receptor potentials elicited by stretching the peripheral dendrites (Sillar & Skorupski, 1986) and accounts for the phase-dependence of the reflexes mediated by the TCMROs (Skorupski & Sillar, 1986).

Analogous central modulation of input from the serially homologous, uropod NSRs in the sand crab *Emerita* may explain the absence of double power-stroke bursts during slow swimming (when sensory feedback and central drive of power strokes are out of phase). At high frequencies of uropod beating, the reflex appears to act as a positive feedback, reinforcing central drive of power strokes, whereas in the absence of centrally driven power strokes during 'treading water', the uropod NSRs reflexly excite power strokes cycle by cycle (Paul, 1976, 1988).

Details of the reflex connections and roles in their respective forms of locomotion are likely to be different for the TCMROs, swimmeret NSRs and uropod NSRs. But, in addition to their similar morphologies, physiologies and peripheral function (sensing appendage retraction), the sensory neurones of these stretch receptors also appear to have in common the continuous modulation of their input by the central rhythm-generating circuits for their respective appendages. Perhaps, apart from its uninterrupted signalling capacity (Bush, 1981), the nonspiking physiology of these members of the larger set of basal joint receptors may have evolved to provide smoother efferent control of whole-limb proprioception because synthesis of the fundamental motor rhythm, at least for the swimmerets, is accomplished by interactions among nonspiking interneurons (Heitler & Pearson, 1980; Paul & Mulloney, 1985*a,b*). There may be an adaptive relationship between the analogue mode of conduction and a need for the premotor circuitry continuously to integrate the reafferent signal. If this is so, one would expect that the nonspiking cells of the squat lobster's uropod stretch receptor (Maitland *et al.* 1982) [which presumably are the larger cells whose terminations are embedded in vacuolated strings, a structural feature of all other NSRs (Wilson, 1988)] might also be presynaptically modulated by the CPG (for tailflipping). In contrast, the spiking cells of this receptor and of the swimmeret stretch receptors of lobsters (Miyan & Neil, 1986) would not be rhythmically modulated.

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References

- ALTMAN, J. (1982). Sensory inputs and the generation of the locust flight motor pattern: from the past to the future. In *Insect Flight. Biona Report 2* (ed. W. Nachtigall), pp. 127–136. Stuttgart: G. Fisher.
- BUSH, B. M. H. (1981). Non-impulsive stretch receptors in crustaceans. In *Neurones without Impulses* (ed. A. Roberts & M. M. H. Bush), pp. 147–176. Cambridge: Cambridge University Press.
- DAVIS, W. J. (1968). Quantitative analysis of swimmeret beating in the lobster. *J. exp. Biol.* **48**, 643–662.
- DAVIS, W. J. (1969). Reflex organization in the swimmeret system of the lobster. I. Intrasegmental reflexes. *J. exp. Biol.* **51**, 547–563.
- DAVIS, W. J. & KENNEDY, D. (1972). Command interneurons controlling swimmeret movements in the lobster. I. Types of effects on motoneurons. *J. Neurophysiol.* **35**, 1–12.

- DELLER, S. R. T. & MACMILLAN, D. L. (1985). Proprioceptive entrainment of the swimmeret rhythm in the crayfish, *Cherax destructor*. *Proc. Austr. physiol. pharmac. Soc.* **16**, 162P.
- GRILLNER, S. & WALLÉN, P. (1984). How does the lamprey central nervous system make the lamprey swim? *J. exp. Biol.* **112**, 337–357.
- HEITLER, W. J. (1982). Non-spiking stretch-receptors in the crayfish swimmeret system. *J. exp. Biol.* **96**, 335–366.
- HEITLER, W. J. (1986). Aspects of sensory integration in the crayfish swimmeret system. *J. exp. Biol.* **120**, 387–402.
- HEITLER, W. J. & PEARSON, K. G. (1980). Non-spiking interactions and local interneurons in the central pattern generator of the crayfish swimmeret system. *Brain Res.* **187**, 206–211.
- HUGHES, G. M. & WIERSMA, C. A. G. (1960). The coordination of swimmeret movements in the crayfish, *Procambarus clarkii* (Girard). *J. exp. Biol.* **37**, 657–670.
- IKEDA, K. & WIERSMA, C. A. G. (1964). Autogenetic rhythmicity in the abdominal ganglia of the crayfish: the control of swimmeret movements. *Comp. Biochem. Physiol.* **12**, 107–115.
- MAITLAND, D. P., LAVERACK, M. S. & HEITLER, W. J. (1982). A spiking stretch receptor with central cell bodies in the uropod of the squat lobster *Galathea strigosa* (Crustacea, Anomura). *J. exp. Biol.* **101**, 221–231.
- MIYAN, J. A. & NEIL, D. M. (1986). Swimmeret proprioceptors in the lobsters *Nephrops norvegicus* L. and *Homarus gammarus* L. *J. exp. Biol.* **126**, 181–204.
- MULLONEY, B., ACEVEDO, L. D. & BRADBURY, A. G. (1987). Modulation of the crayfish swimmeret rhythm by octopamine and the neuropeptide proctolin. *J. Neurophysiol.* **58**, 584–597.
- PAUL, D. H. (1976). Role of proprioceptive feedback from nonspiking mechanosensory cells in the sand crab, *Emerita analoga*. *J. exp. Biol.* **65**, 243–258.
- PAUL, D. H. (1988). A neurophylogenist's view of decapod Crustacea. *Bull. Mar. Sci.* (in press).
- PAUL, D. H. & MULLONEY, B. (1985a). Nonspiking local interneuron in the motor pattern generator for the crayfish swimmeret. *J. Neurophysiol.* **54**, 28–39.
- PAUL, D. H. & MULLONEY, B. (1985b). Local interneurons in the swimmeret system of crayfish. *J. comp. Physiol. A* **156**, 489–502.
- PAUL, D. H. & MULLONEY, B. (1986). Intersegmental coordination of swimmeret rhythms in isolated nerve cords of crayfish. *J. comp. Physiol. A* **158**, 215–224.
- SILLAR, K. T. & SKORUPSKI, P. (1986). Central input to primary afferent neurons in crayfish, *Pacifastacus leniusculus*, is correlated with rhythmic motor output of thoracic ganglia. *J. Neurophysiol.* **55**, 678–688.
- SILLAR, K. T., SKORUPSKI, P., ELSON, R. S. & BUSH, B. M. H. (1986). Two identified afferent neurones entrain a central locomotor rhythm generator. *Nature, Lond.* **323**, 440–443.
- SKINNER, K. (1985). The structure of the fourth abdominal ganglion of the crayfish, *Procambarus clarkii* (Girard). II. Synaptic neuropils. *J. comp. Neurol.* **234**, 182–191.
- SKORUPSKI, P. & 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.
- STEIN, P. S. G. (1971). Intersegmental coordination of swimmeret motoneuron activity in crayfish. *J. Neurophysiol.* **34**, 310–318.
- WEST, L., JACOBS, G. & MULLONEY, B. (1979). Intrasegmental proprioceptive influences on the period of the swimmeret rhythm in crayfish. *J. exp. Biol.* **82**, 289–301.
- WIERSMA, C. A. G. & IKEDA, K. (1964). Interneurons commanding swimmeret movements in the crayfish, *Procambarus clarkii* (Girard). *Comp. Biochem. Physiol.* **12**, 509–525.
- WILSON, L. J. (1988). The functional morphology of the uropod stretch receptor of the sand crab *Emerita analoga* including a comparison with the uropod stretch receptor of the squat lobster *Munida quadrispina*. MSc thesis, University of Victoria, BC.
- WOLF, H. & PEARSON, K. G. (1987). Flight motor patterns recorded in surgically isolated sections of the ventral nerve cord of *Locusta migratoria*. *J. comp. Physiol. A* **161**, 103–114.