J. exp. Biol. **120**, 387–402 (1986)
Printed in Great Britain © *The Company of Biologists Limited 1986*

ASPECTS OF SENSORY INTEGRATION IN THE CRAYFISH SWIMMERET SYSTEM

BY W. J. HEITLER

The Gatty Marine Laboratory, University of St Andrews, St Andrews, Fife, KY16 8LB, Scotland

Accepted 6 September 1985

SUMMARY

The chief sensory effects observed in response to movement and position of a single swimmeret are ipsilateral reflexes such that the amplitude of spontaneous rhythmic activity is augmented when the swimmeret is held protracted, and diminished when the swimmeret is held retracted. A major source of these reflexes appears to be the non-spiking stretch receptors (NSSRs) at the base of the swimmeret. Sinusoidally-varying current injected into a single NSSR produces a beat-frequency modulation of spontaneously-generated rhythm very similar to that produced by applying sinusoidal movement to the whole swimmeret. The spontaneous rhythm does not entrain to the applied movement. Spiking receptors responding to movement and cuticle distortion may be largely responsible for a dynamic component of the reflex, and also for inconsistently-observed arousal effects and changes in frequency of spontaneously-generated rhythms.

INTRODUCTION

The swimmerets of decapod Crustacea are bilaterally paired appendages on the ventral surface of the abdomen. They beat back (retraction or power stroke) and forth (protraction, or return stroke) in a metachronal rhythm in behaviour such as locomotion, burrow ventilation, and the ventilation of eggs on a gravid female. Like many rhythmic systems, the oscillatory motor programme is the result of interactions between a centrally generated rhythmic component and feedback from peripheral sense organs. The central pattern generator (CPG) has been extensively studied, and although it is by no means fully understood, there is considerable information about it (Hughes & Wiersma, 1960; Ikeda & Wiersma, 1964; Davis, 1969*a*; Heitler, 1978, 1981, 1985; Heitler & Pearson, 1980). Comparatively little attention has been paid, however, to the peripheral component.

Previous work on the crayfish has shown that sensory input from the swimmerets is capable of modulating the period of the swimmeret rhythm, but with a high degree of variability (West, Jacobs & Mulloney, 1979). In these experiments the swimmeret rhythm was induced by stimulating command fibres, and it was suggested that the

Key words: sensory, swimmerets, crayfish.

variability reflected variations in the specific command fibres activated. No attempt was made to study reflexes onto specific motor neurones or classes of motor neurones.

In this study the aim has been to investigate more fully the reflexes mediated by sensory input from the swimmerets of crayfish. Microelectrodes have been used to record from and stimulate various identified and unidentified neurones while perturbing the activity of a single swimmeret in a semi-isolated preparation. First intra- and intersegmental reflex modulation of the swimmeret rhythm is described. Next a rapid review of the main proprioceptive systems present in the swimmeret is given. Finally, evidence is presented as to the function of a specific proprioceptive system, the non-spiking stretch receptors (NSSRs) (Heitler, 1982).

MATERIALS AND METHODS

The search for reflex input to the swimmeret system was undertaken using a preparation in which the chain of abdominal ganglia from the second (G2) to the fifth (G5) were isolated from the periphery, except for a single swimmeret left attached to G3 or G4 to provide the sensory input. This swimmeret was dissected free from the carapace, but left attached to its base which included a short section of the sternal rib and part of the lateral pleural plate. The chain of ganglia was mounted dorsal surface upwards on a Sylgard platform, and submerged in Van Harreveld's crayfish saline. A twist was put in the first root (R1) of the attached swimmeret, so that this could be positioned ventral surface upwards for ease of manipulation. R1 contains the entire innervation of the swimmeret. The saline level in the bath was kept low, and the swimmeret was thus normally held by surface tension either in the protracted or retracted position. It could be moved from these positions either manually, or with an electromechanical transducer.

Extracellular recordings were made from the appropriate R1s, using hook electrodes for the R1 to which the swimmeret was attached and pin electrodes for other R1s. Intracellular recordings were made from various neurones within the central nervous system, using microelectrodes which were either filled with 3 mol l^{-1} potassium acetate, or a 5% solution of Lucifer Yellow in 1 mol l^{-1} lithium chloride. Neurones recorded intracellularly were identified by various characteristics. The NSSRs could be easily recognized by their highly specialized physiology and anatomy. Motor neurones were classified as neurones which were not NSSRs, but which had an axon in R1. This was determined by physiological correlation of orthodromic intracellular and extracellular spikes, or by the presence of an antidromic spike on stimulating R1, or anatomically. Motor neurones could also sometimes be functionally identified according to the swimmeret structures which moved when the neurones were induced to spike by injecting depolarizing current. If no movement was induced, functional identification could not be achieved, since the motor neurones could be inhibitors, or very slow, or innervating a muscle damaged in dissection. All neurones in this study identified as motor neurones received a background barrage of postsynaptic potentials. In no case out of numerous

experiments has subsequent anatomical investigation shown that a neurone with these characteristics was a spiking primary afferent.

RESULTS

Three classes of stimulus may occur during swimmeret movements. The first results from the position and change of position of the swimmeret itself (static and dynamic components of position). The second results from the reactive forces of water on the swimmeret, produced by the movement and detected by sensory hairs and cuticle stress detectors. The third results from mechanical stimuli such as touching the swimmeret to prevent its movement, or squeezing it and distorting its cuticle. The first two classes are the important ones so far as the natural functioning of the swimmerets is concerned, but the latter stimulus class is usually an unavoidable concomitant of experimental manipulation.

Sensory effects on the swimmeret system

In several experiments, movements applied to a single swimmeret attached to an otherwise isolated ventral nerve cord (VNC) induced no obvious reflex motor output whatever. Preparations of this sort tended to show little spontaneous activity, and although definitely alive, were regarded as not being 'aroused'. In contrast, preparations showing a high level of spontaneous activity frequently demonstrated quite powerful responses to sensory input. These typically had a form which in many respects resembled resistance reflexes. In one such preparation (Fig. 1) an intracellular recording was made from a power-stroke motor neurone of G3 to which a swimmeret was left attached, while extracellular recordings were made from the R1 of G3 and G4. Continuous and relatively constant rhythmic motor output was expressed by G4 irrespective of the position of the G3 swimmeret, indicating that in this preparation there was no static interganglionic effect (although a dynamic effect was apparent when the swimmeret was moved). In contrast, the output of G3 was strongly dependent on the position of its swimmeret. If the swimmeret was held retracted, very little extracellular activity was recorded, and there was only a slight indication that this activity was rhythmic. If the swimmeret was held protracted, obvious rhythmic bursts with the correct metachronal relationship to G4 were recorded from several units. The intracellular recording showed that the G3 motor neurone membrane potential was undergoing rhythmic oscillations in *both* the swimmeret positions, but that the amplitude of oscillation was much less in the retracted than in the protracted position. The oscillations could not be abolished even in an extremely retracted position, probably indicating that it was the output of the CPG, rather than the CPG itself, which was being gated by sensory input. There was no change in the *frequency* of the oscillations recorded in the motor neurone in response to maintained swimmeret position, merely in the *amplitude*. There was, however, a brief increase in frequency during the actual movement, both in protraction and retraction. This had the effect of resetting the period of the rhythm.

In these experiments the swimmeret was held in the two static positions by surface tension (see Materials and Methods). Mechanical forces acting on the swimmeret to maintain its position must have been varying with the spontaneous rhythm, but they would have been distributed evenly over the entire surface, and since the spontaneous movement was not strong it is unlikely that significant cuticular distortion was induced. The static component of the resistance reflex is thus probably largely due to swimmeret *position* detectors, rather than cuticular stress detectors activated by mechanical resistance to the spontaneous movement. In contrast, when the swimmeret was experimentally moved from one position to the other this involved breaking the surface tension, and considerable cuticular distortion resulted. The large 'dynamic' reflex response probably resulted in part from this distortion, as well as including some velocity component from the position detectors.

It is not possible from these results to determine whether sensory input was affecting the motor neurones directly, or indirectly through some other set of neurones. A factor complicating interpretation is that some crayfish motor neurones are coupled in an excitatory manner to their homoganglionic agonists, and in an inhibitory manner to their antagonists (Heitler, 1978, 1981; Nagayama, Takahata & Hisada, 1983). In the motor neurone described above the spontaneous oscillations were subthreshold, but spiking could be induced with low levels of depolarizing

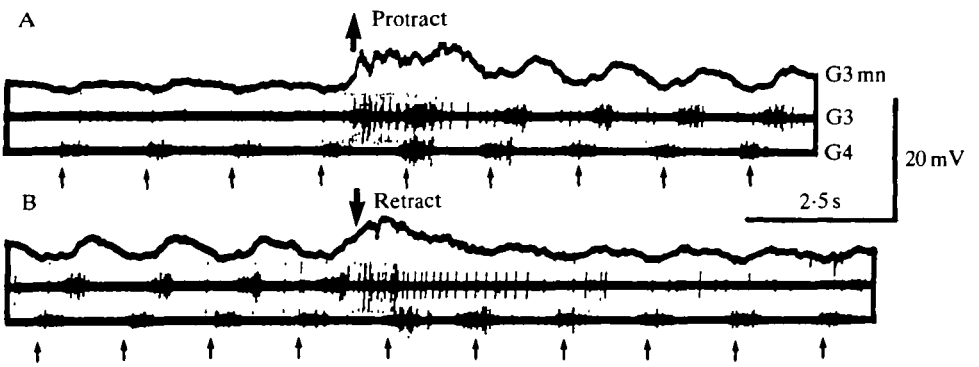

Fig. 1. Reflex modulation of power stroke swimmeret motor neurones. Intracellular recording from functionally-identified G3 fast protopodite retractor and lateral ramus curler motor neurone of G3 (first trace, G3 mn), extracellular recordings from G3-R1 (2nd trace, G3) and G4-R1 (third trace, G4). Power stroke activity is clearly apparent in the extracellular records, but return stroke activity is minimal. This is a common feature of recordings from the intact R1. A swimmeret was attached to G3-R1, but G4-R1 was isolated from the periphery. (A) The swimmeret was moved (large up arrow) from retracted to protracted position and kept there. (B) The swimmeret was moved from protracted to retracted position (large down arrow). Note that G3 and G4 motor neurones are transiently excited by movement in either direction, but only G3 motor neurones (i.e. homoganglionic to the moving swimmeret) are continuously excited by maintained protraction. The phase of the rhythm is reset by movement in both directions (small arrows under traces), but the period is unaffected by steady state position.

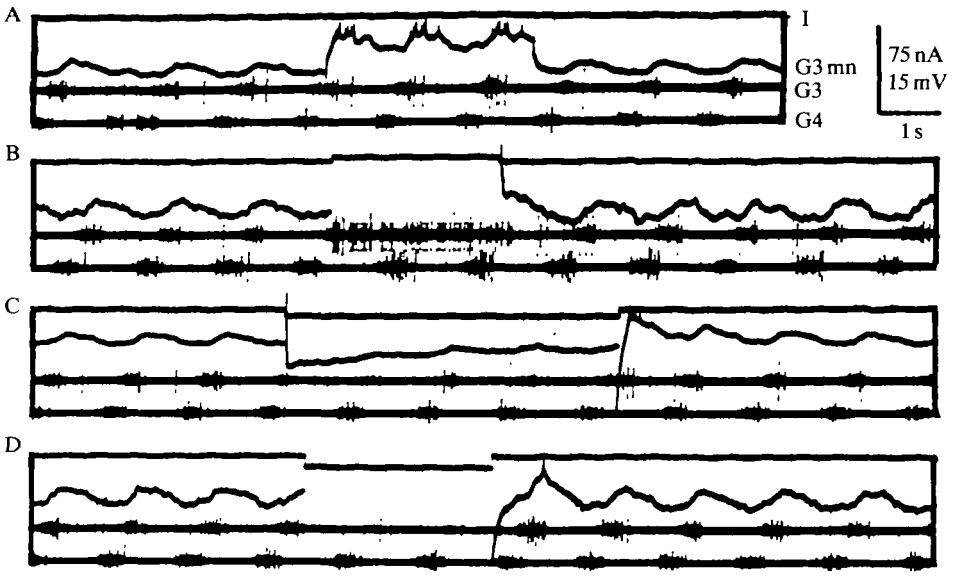

Fig. 2. Central coupling between motor neurones revealed by current injection after isolating R1 from the periphery (current in first trace, I, other traces as in Fig. 1). (A), (B) The motor neurone was injected with depolarizing current. (C),(D) The motor neurone was injected with hyperpolarizing current. The activity of several neurones recorded extracellularly in G3-R1 was modulated by the current, as well as that of the motor neurone recorded intracellularly.

current (Fig. 2A). The associated extracellular spike was very small. With larger amounts of depolarization, a second, larger extracellular unit was recruited (Fig. 2B). Similarly, with hyperpolarizing current the spike activity of several motor neurones, other than that into which current was injected, was abolished (Fig. 2C,D). With low levels of current the amplifier bridge could be balanced, and it was apparent that depolarizing current increased the amplitude of spontaneous oscillations, while hyperpolarizing current decreased it. Neither polarity affected the period of the rhythm. These results are compatible with three hypotheses. First, since hyperpolarizing current decreases the amplitude of oscillation, the input from the CPG to this motor neurone may consist largely of periodic inhibition (cf. motor neurones in the scaphognathite system; Simmers & Bush, 1983, but note that this is not a universal feature of swimmeret motor neurones). Second, since hyperpolarizing current mimics the steady state effects of swimmeret retraction, the input to the system from position-detecting sensory components may consist partly of inhibition in response to swimmeret retraction. Third, since depolarizing or hyperpolarizing current injected into a single motor neurone can alter the output of several neurones, sensory input which was targeted to only a few specific sites could spread throughout a population of coupled motor neurones.

Recordings have also revealed pathways which are, in principle, capable of mediating interganglionic effects and modulating the CPG. Thus simultaneous

recordings were made from two neurones in G4 in a preparation which was spontaneously rhythmic, and in which a swimmeret was attached to G3. These neurones did not fulfil the criteria for motor neurones, and are tentatively identified as interneurones. One of these neurones oscillated with depolarizations in phase with power stroke (Fig. 3, n1), and appeared to be a non-spiker of a type described previously in both the scaphognathite and swimmeret systems (Mendelson, 1971; Simmers & Bush, 1983; Heitler & Pearson, 1980; Heitler, 1985). This neurone excited homoganglionic power-stroke motor neurones when injected with depolarizing current, inhibited them when injected with hyperpolarizing current, and reset the rhythm in both G4 and G3 when injected with 10 nA pulses of the appropriate (antagonistic) polarity (Fig. 3A,B). However, lower levels of sustained current injection only modulated the amplitude of the rhythm and not its period. The second neurone did not oscillate, but received a continuous barrage of apparently unitary EPSPs which was not significantly modulated with the rhythm (Fig. 3, n2). This second neurone had no effect when injected with hyperpolarizing current, but had very powerful homoganglionic inhibitory effects when injected with depolarizing current. It virtually abolished G4 motor output, and reduced the amplitude of the oscillations in the other neurone, but did not alter the period of the rhythm (Fig. 3C,D). Thus this neurone did not have direct access to the CPG, but was a powerful modulator of its output. The preparation showed interganglionic resistance reflexes similar to the homoganglionic reflexes described above. If the G3 swimmeret was moved from the retracted to the protracted position, the amplitude of G4 motor output increased, the oscillating neurone depolarized and the amplitude of its oscillations increased, and the neurone receiving the tonic barrage of PSPs hyperpolarized (Fig. 3E). Thus these neurones, which had powerful effects on motor output, were themselves modulated by sensory input impinging on an adjacent segment. This constitutes a potential pathway for interganglionic reflexes. Furthermore, one of the neurones had access, albeit weak access, to the CPG.

No consistent effects of steady state sensory input on the period of the swimmeret rhythm have been observed. However, sensory input has occasionally been seen to 'switch on' the rhythm. Recordings were made from a G3 slow power-stroke motor neurone in a preparation which showed lengthy bouts of spontaneous rhythmic activity. During this activity, homoganglionic reflexes from the G3 swimmeret were observed, with little interganglionic effect (Fig. 4A). However, if the swimmeret was held in the retracted position for an extended period the rhythmic output from both G3 and G4 sometimes stopped, and no subthreshold oscillations were visible in the intracellular recording. It seems likely that in these circumstances the CPG itself had stopped oscillating. Protraction of the swimmeret in this state could initiate strong rhythmic activity from both G3 and G4 (Fig. 4B). However, after initiating rhythmic activity by protracting the swimmeret in this manner, subsequent retraction usually failed immediately to return the preparation to its previous quiescent state (Fig. 4C). Rather, the homoganglionic reflexes were observed. In such cases it may well have been the arousal stimulus of touching the swimmeret that initiated rhythmic activity, rather than the positional information itself.

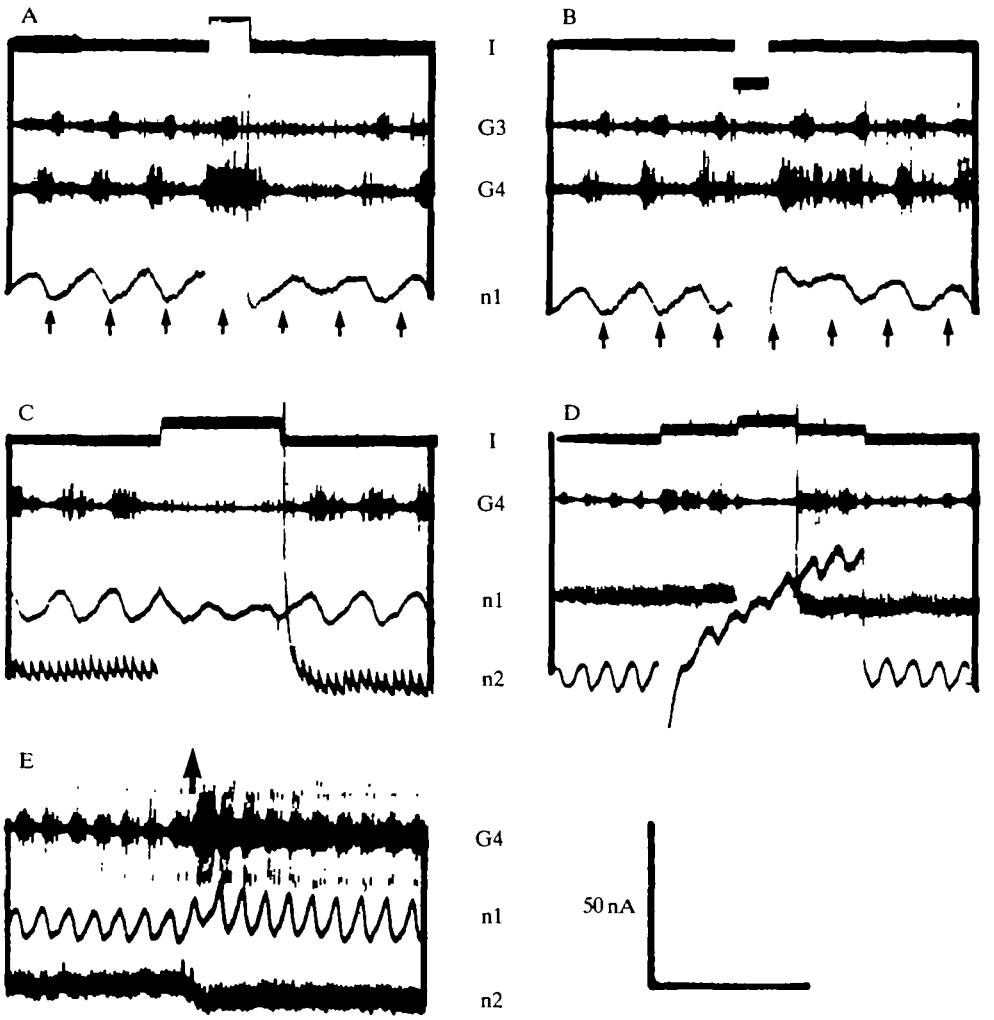

Fig. 3. Interganglionic sensory effects upon presumed interneurones. (A),(B) Depolarizing or hyperpolarizing current (first trace, I) injected into an oscillating G4 non-spiking interneurone (fourth trace, n1) reset the rhythm recorded extracellularly in G3-R1 (second trace, G3) and G4-R1 (third trace, G4). Arrows under intracellular recording indicate phase-resetting. (C) Depolarizing current (first trace) injected into a non-oscillating G4 interneurone (fourth trace, n2) inhibited rhythmic activity recorded extracellularly in G4-R1 (second trace) and intracellularly in the oscillating non-spiking interneurone (third trace). (D) Depolarizing current (first trace) injected into the oscillating interneurone (fourth trace) increased G4-R1 motor output recorded extracellularly (second trace), but this effect was swamped by inhibition resulting from a pulse of depolarizing current (middle of first trace) injected into the non-oscillating interneurone (third trace). (E) Protracting the swimmeret attached to G3-R1 (large arrow) increased activity recorded extracellularly in G4-R1 (first trace), depolarized and increased the amplitude of oscillations in the G4 oscillating non-spiking interneurone (second trace), and hyperpolarized the G4 non-oscillating interneurone (third trace). Scale: vertical A–C, 50 mV; D,E, 60 mV; horizontal A–E, 1 s; D,E, 2 s.

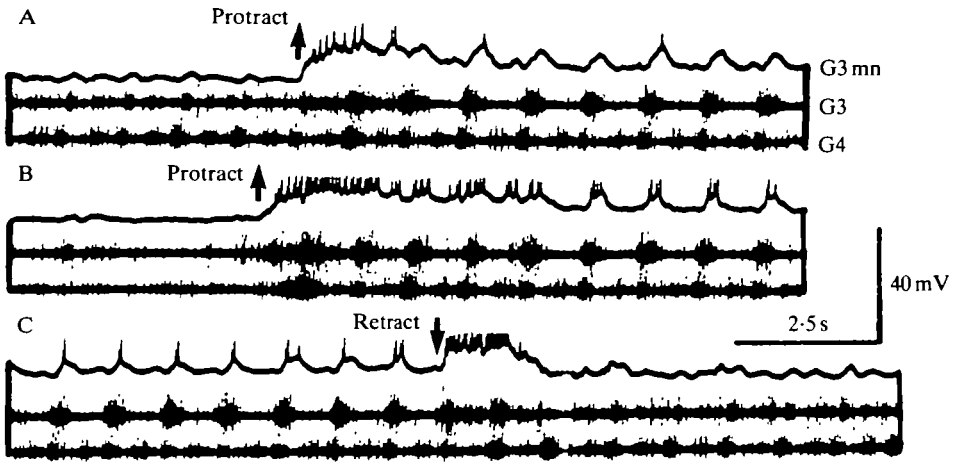

Fig. 4. Rhythm-initiating function of sensory input. Slow power stroke motor neurone recorded intracellularly from G3 (first trace, G3 mn), extracellular recordings from G3-R1 (second trace, G3, swimmeret attached) and G4-R1 (third trace, G4, isolated root). (A) During bouts of spontaneous rhythmic activity normal reflexes were observed (protraction at large up arrow). (B) If the swimmeret was maintained in the retracted position for 20–30 s, spontaneous rhythmic activity often ceased, but could be reinitiated by protraction (large up arrow). (C) Subsequent retraction (large down arrow) reduced the amplitude of rhythmic activity (normal reflex), but did not inhibit it completely.

Sensory components of the swimmerets

There are two types of sensory receptors innervating the swimmerets: non-spiking and spiking. The non-spiking system is composed of two large axons whose peripheral terminals innervate an elastic strand (S1) spanning the base of the swimmeret. These neurones, called non-spiking stretch receptors (NSSRs), transmit a graded depolarization to the CNS in response to swimmeret retraction. One has a cell body in the anterior quadrant of the ganglion (NSSR-A), while the other has one in the posterior quadrant (NSSR-P). They appear to have essentially similar responses, and neither adapts much to maintained stimuli. The response characteristics and anatomy of these neurones has been described in detail previously (Heitler, 1982).

The spiking sensory component of the swimmeret system is more complex, and a variety of units are excited by both protraction and retraction (Fig. 5A). The origin of this activity has been investigated by peripherally-directed cobalt staining through R1, and by extracellular recording from R1 while selectively stimulating and ablating parts of the swimmeret. A group of small diameter axons innervate a strand (S2) adjacent to that innervated by the NSSRs in the base of the swimmeret (see Fig. 9A, Heitler, 1982). No cell bodies appear to be associated with these axons in the periphery, and they are thus assumed to have cell bodies located centrally. These two receptor strands are the only sensory systems that cobalt stains have revealed located in the base of the swimmeret. If the swimmeret was amputated distal to its base, and the remaining stump moved back and forth, a burst of spikes could be recorded in the

anterior branch of R1 in response to retraction or lateral movement of the swimmeret, but not protraction or medial movement (Fig. 5B). Since all other swimmeret sensory organs had been removed, these spikes must have originated in the small diameter axons innervating the strand S2. This strand thus comprises a spiking stretch receptor, excited by swimmeret retraction and lateral movement, which adapts moderately rapidly to maintained stimuli.

Distal to the base of the swimmeret the nerves break up into several fine branches. Cobalt stain has never been successfully traced into these branches, but methylene blue stains have revealed no specific proprioceptors such as chordotonal organs in this distal region. However, there are numerous hair cells on the surface of the cuticle, and a ramifying plexus of nerve branches is visible below the cuticle, especially below the arthrodial membrane on the posterior surface of the basipodite and rami. A jet of water directed at the posterior surface of the swimmeret elicits spiking sensory activity, as does any mechanical stimulus such as squeezing or stroking (Fig. 6). The response elicited by the water jet does not come from the fringing setae of the rami, since specific mechanical stimulation of these structures elicited little response. As well as sensory axons innervating the swimmeret there are also axons within R1 which innervate hair cells on the medial and lateral surfaces of the pleural plates. Some of these are large, and their spikes are amongst the largest in R1. These hair cells are very sensitive to water-borne vibration, and care is needed when stimulating the swimmeret left attached to its base and part of the pleural plate to distinguish between the response of these receptors and those of the swimmeret itself.

Non-spiking stretch receptors

Reflexes could be demonstrated by injecting current specifically into an NSSR through a microelectrode. Extracellular recordings showed that depolarizing current injected into a single NSSR (which mimics retraction) inhibited a number of power stroke motor neurones, while hyperpolarizing current (which mimics protraction) excited them. This was confirmed by simultaneous intracellular recording from a

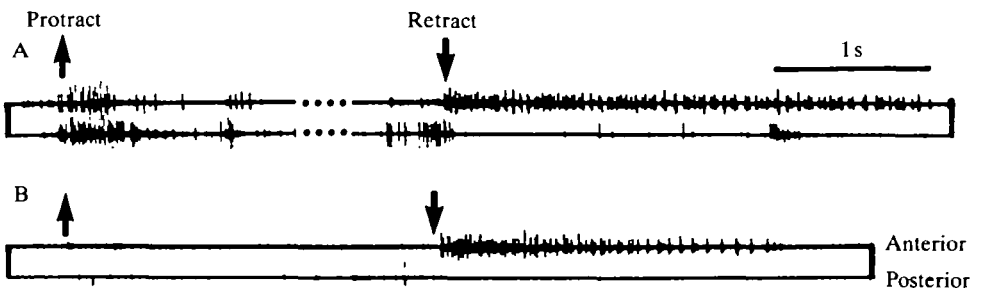

Fig. 5. Spiking sensory responses to swimmeret protraction (up arrow) and retraction (down arrow). Extracellular recordings from anterior (first trace) and posterior (second trace) branches of R1, with the swimmeret isolated from the CNS. (A) A burst of spikes is recorded in both branches upon swimmeret movement in either direction. (B) After amputating the swimmeret just distal to the base of the protopodite, spikes are recorded in the anterior branch alone, and only in response to retraction.

power stroke motor neurone (Fig. 7). Only the amplitude of motor output was modulated, there was no change in frequency of spontaneous rhythmic activity.

The reflex effects of current injected into a single NSSR could be compared with those caused by movement of the whole swimmeret. A G4 swimmeret was held stationary in the mid position in a preparation displaying spontaneous rhythmic activity at 1·25 Hz. Rhythmic activity was recorded extracellularly from R1 and intracellularly from a power stroke motor neurone, which showed membrane potential oscillations. Small oscillations were also apparent in the NSSR, even though no overall movement of the swimmeret was possible (Fig. 8A). This may have been due to slight movements of the base of the swimmeret resulting from

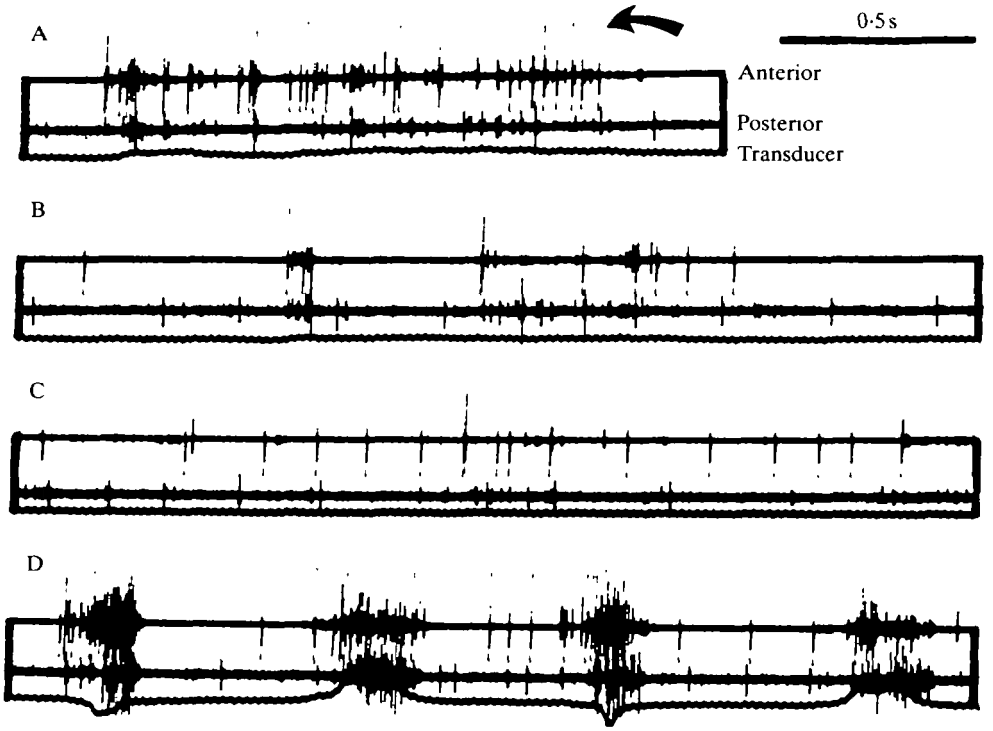

Fig. 6. Spiking response of receptors other than the strand receptors in the swimmeret base. The swimmeret protopodite was held stationary relative to the sternal socket. Extracellular recordings from the anterior (first trace) and posterior (second trace) branches of R1 isolated from the CNS. The pleural plate and sternal rib were dissected away to a minimum, to try to ensure that most of the response actually comes from the swimmeret receptors, but the largest spike (curved arrow) in each trace still comes from a hair cell on the remaining sternal socket. (A) A strong jet of water was directed at the posterior surface of the swimmeret (a force transducer placed on the opposite side of the swimmeret acted as a semi-quantitative monitor; third trace). (B) A weaker jet was directed to the same position. (C) The transducer was used to stroke the fringing setae of the rami, eliciting little sensory response. The transducer fails to register the low force of this stimulus. (D) The transducer was used to flex (monitor deflects downwards) and extend (monitor deflects upwards) the rami, producing considerable cuticular distortion and a powerful sensory response.

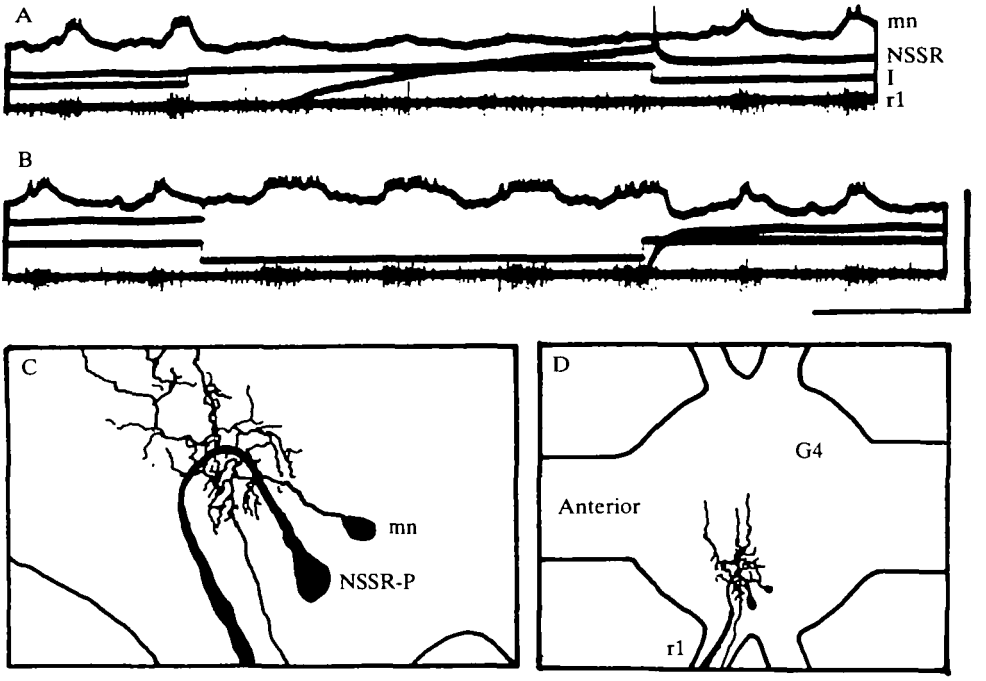

Fig. 7. Reflexes are induced by injecting current into an NSSR in a preparation exhibiting spontaneous rhythmic activity. Dual microelectrode penetrations of a power stroke motor neurone (first trace, mn) and NSSR-P (second trace, NSSR), extracellular recordings from R1 (fourth trace, r1). (A) Depolarizing current (third trace, I) reduced the amplitude of oscillations recorded intracellularly in the motor neurone, and inhibited power-stroke activity recorded extracellularly. (B) Hyperpolarizing current injected into the NSSR had the opposite effect. (C) Both neurones were subsequently stained with Lucifer Yellow, and their anatomy determined. Their dendritic fields overlap in an area of complex branching, but specific points of contact could not be determined. (D) Diagram showing the location of the neurones within the ganglion (not to scale). Scale: vertical 30 mV, 30 nA; horizontal 1 s, 150 μm.

contractions of the main power and return stroke muscles (the swimmeret was clamped relatively distally, at the base of the rami), or it may have been due to central input to the NSSR. The swimmeret was then moved experimentally in a sinusoidal arc at 1 Hz frequency. This caused much larger 1 Hz oscillations in the NSSR, but disrupted the oscillatory activity of the motor neurones (Fig. 8B). The intracellular recording clearly showed beating with a frequency of about 0·25 Hz. (Beating is used here in the sense of the modulation of amplitude which occurs when two independent oscillators with slightly different frequencies sum.) During the applied movements in this experiment the distal part of the swimmeret was not allowed to touch the surface of the saline, but continually protruded above it. Thus there was no cuticular distortion resulting from breaking the surface tension, and the main proprioceptive systems activated would have been those at the base of the swimmeret. Very similar beating was produced by injecting sinusoidally-varying current into the NSSR at the same frequency as the applied movement, but with the swimmeret held stationary (Fig. 8C). The beating was more pronounced with movement stimulation than with

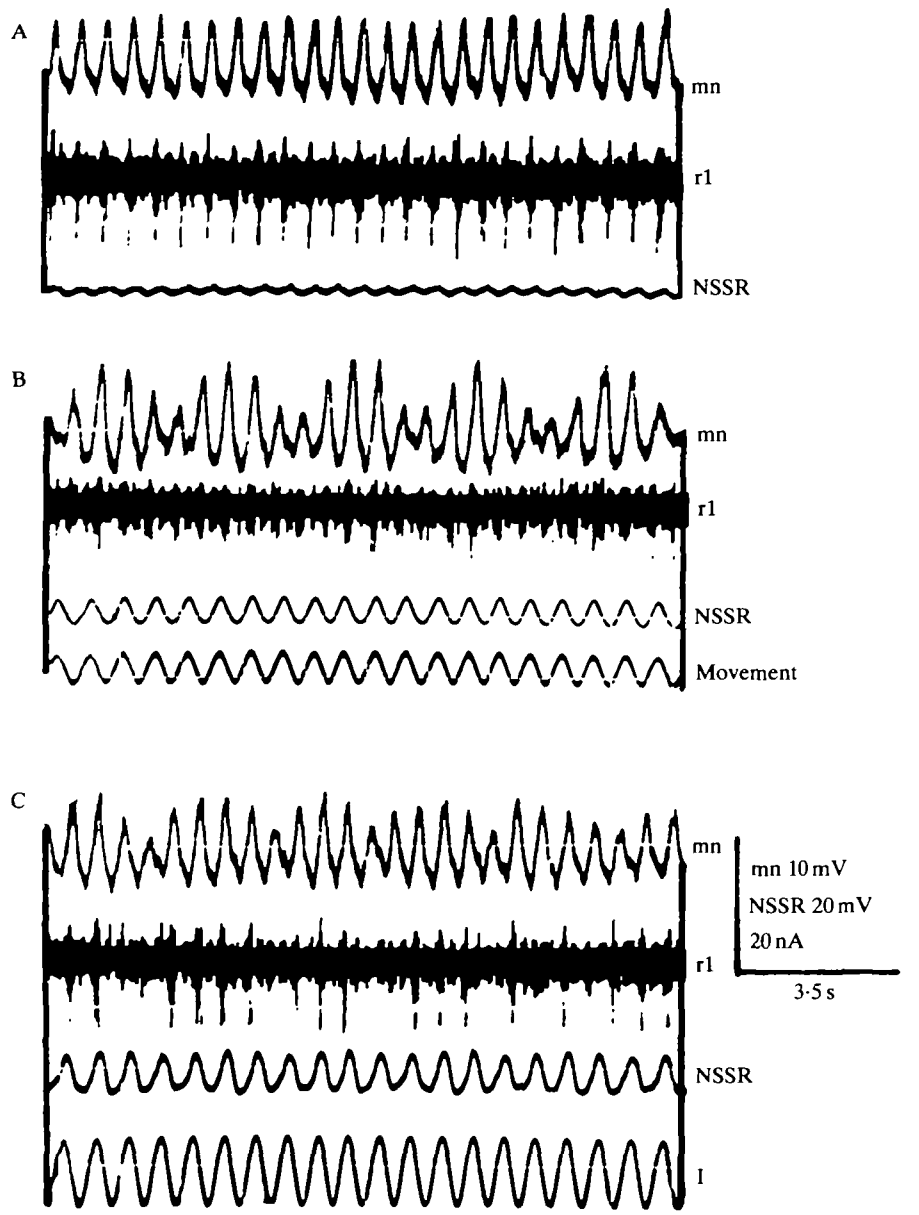

Fig. 8. NSSR input is a major sensory input mediating reflexes in a spontaneously active preparation. Intracellular recordings from a power stroke motor neurone (first trace, mn) and an NSSR (third trace, NSSR), extracellular recording from R1 (second trace, r1). (A) The swimmeret was held stationary by a clamp at the base of the rami, and uniform oscillations are apparent in the motor neurone. (B) The swimmeret is moved (monitor; fourth trace, movement) at a frequency slightly different to that of the spontaneous rhythm, and beat-frequency modulation of the motor neurone membrane potential oscillations occurs. (C) Similar modulation is caused by injecting sinusoidal current (monitor; fourth trace, I) into the NSSR (bridge circuit slightly unbalanced).

current injection, possibly because the current was injected into only one NSSR, while the movement would have stimulated the entire complement of sensory receptors at the swimmeret base. However, the qualitative similarity suggests that the NSSR plays a major role in mediating the beat modulation. There was no change in the fundamental frequency of oscillation expressed by the motor neurone.

DISCUSSION

In these experiments significant reflex modulation of motor activity was only observed in preparations showing a relatively high level of spontaneous, usually rhythmic, activity. Because of the large number of motor neurones present in the swimmeret system, and the difficulty of distinguishing between individuals within a class, it is not certain that exactly the same motor neurones have been penetrated in rhythmic and non-rhythmic preparations. However, numerous intracellular and extracellular recordings have been made from motor neurones in non-rhythmic preparations, and few have shown consistent significant reflex modulation. In contrast, the reflexes observed in the rhythmic preparations described in this report were obvious. This suggests (although it does not prove) that the expression of these reflexes is dependent on concurrent expression of the motor programme. Such gating of reflex effects is increasingly becoming recognized as an important element in motor control (e.g. Zill & Forman, 1983).

Two types of reflex effects can be distinguished deriving from a single swimmeret. There is a static component apparent when the swimmeret is held in a fixed position, and a dynamic component apparent when the swimmeret is moved (Figs 1, 4). The static component has a form similar to that of a resistance reflex. Protraction increases the amplitude of the depolarization phase of the oscillations in power stroke motor neurones, thus tending to increase their spike activity and drive the swimmeret into retraction. This steady state reflex is maintained as long as the swimmeret is held protracted. The NSSRs are the only proprioceptors which do not eventually adapt to a maintained position, and so they must be responsible for the steady state reflex. This is supported by the evidence from current injected into a single NSSR (Fig. 7). Unfortunately, only one of the two NSSRs has been specifically identified in these experiments, and so it is possible that the other NSSR may have different effects. However, the similarity in the response characteristics of the two NSSRs, coupled with the qualitative similarity in the effects of current injection into one NSSR and movement of the whole swimmeret (Fig. 8), suggests that any such differences are likely to be subtle. Intracellular recordings have only been made from power stroke motor neurones in this study, and so it is not known whether return stroke motor neurones are also modulated. However, both the intracellular and extracellular recordings confirm that the steady state reflexes from a single swimmeret only modulate the amplitude of the motor programme, there is no effect on its frequency.

In contrast to the steady state effects, modulation of amplitude *and* frequency has been observed in the dynamic phase of stimulation (Fig. 1). In some preparations

both protraction and retraction briefly increase the frequency of the rhythm, indicating that some sensory systems have access to the CPG. These effects are most marked when the swimmeret has previously been maintained stationary for a time, but they have not been consistently observed. The source of such modulation could be the dynamic response of the NSSRs (which is small but definite), the dynamic response of the spiking stretch receptors at the base of the swimmerets, or the spiking response of receptors responding to non-specific stimulation resulting from the experimenter manually picking up the swimmeret and moving it. Continuous rhythmic movements applied mechanically to the swimmeret do not entrain the spontaneous rhythm (Fig. 8), nor do they alter its frequency from that which is expressed spontaneously. This suggests that an element of novelty may be important for sensory access to the CPG, rather than the dynamic component *per se*.

What is the role of sensory feedback in the unperturbed preparation? NSSR-P mediates negative feedback which modulates amplitude but not frequency. In theory such feedback could oppose all central drive, and maintain the swimmeret stationary in the mid position. Obviously this is not the case. It seems more likely that the feedback stabilizes the oscillation, helping both to initiate and terminate the power stroke. Understanding the precise role of the feedback requires knowledge of the dynamics of the reflex, which is not yet available. The functional role of the spiking feedback is even less clear. All such feedback adapts quickly, and only the receptors at the swimmeret base are specific for direction. These receptors cannot be stimulated without also stimulating the NSSRs, and the latter have not been ablated, so their effects in isolation are not known. Continuous sinusoidal movement applied to the whole swimmeret (with the rami protruding above the saline surface) produce qualitatively similar effects to current injection into a single NSSR, suggesting that the spiking and non-spiking receptors at the swimmeret base may act essentially synergistically. The more distal spiking receptors are not strongly activated by water jets directed at the swimmeret, suggesting that they are not stimulated by the relatively weak water currents produced in reaction to normal swimmeret movements, and that they may have no function at all in such movements. However, these receptors are powerfully activated by mechanical stimuli such as touch, and such stimuli may increase both amplitude and frequency of the rhythm. Perhaps this helps the crayfish overcome any obstacle encountered in locomotion, or increases ventilation of the burrow if it becomes obstructed by debris.

The absence of steady state modulation of CPG period found in this study is in contrast to the results of West *et al.* (1979), who found variable, but definite, frequency modulation. Three possible explanations exist for this difference. First, the latter authors stimulated 'command fibres' to initiate rhythmic activity, whereas all rhythmic activity described in this study was spontaneous. It is possible that command-driven activity may involve different central pathways from those utilized in spontaneous activity. Second, the previous study used a much more intact preparation, in which several swimmerets were left attached to the CNS. The present study has shown that pathways exist by which neurones involved in the CPG may be influenced by sensory activity, but that these effects are relatively small when

resulting from a single swimmeret. They may be much larger when several swimmerets act in concert. Thirdly, it is possible that the previously observed effects on CPG period were not produced by sensory input monitoring swimmeret position or movement itself, but rather from non-specific arousal caused by cuticular distortion resulting from blocking the normal movement of the swimmeret. Similar possible effects were seen in this study, when moving the swimmeret initiated rhythmic activity during the non-rhythmic periods of a preparation which exhibited frequent bouts of spontaneous rhythmic activity. In the steady state experiments of this study, stable swimmeret position was maintained by surface tension, which distributed the load fairly evenly over a large area, and thus minimized cuticular distortion.

The absence of maintained modulation of frequency fits with results from the lobster *Homarus* (Davis, 1969*b*). However, it is difficult to compare these data in detail, because NSSRs do not appear to exist in the lobster. This has been confirmed in the Norwegian lobster *Nephrops* (Miyan, 1982), in which the author was aware of the presence of NSSRs in crayfish, and so is unlikely to have missed them. In the lobster, spiking proprioceptors at the base of the swimmeret are activated by retraction and excite power stroke motor neurones in a positive feedback, in contrast to the negative feedback from NSSRs found here. The same lobster proprioceptors also excite return stroke motor neurones, but this input can be swamped by inhibition resulting from mechanically stimulating the rami. It was suggested that this latter stimulation mimics that produced naturally by water currents during the power stroke. In crayfish, such mechanical stimulation is definitely not an adequate mimic of stimulation produced by water currents, since water jets directed at the rami have to be very fierce before they elicit significant sensory activity (Fig. 6). Although spiking input has been found onto various motor neurones as a result of squeezing the rami, the effects observed have been extremely variable. The reasons for such considerable differences in the proprioceptive complement and reflexes between closely related animals is not known.

This work was supported by grants from the SERC and the Royal Society. I thank K. Fraser for technical assistance and for critically reading the manuscript.

REFERENCES

DAVIS, W. J. (1969*a*). The neural control of swimmeret beating in the lobster. *J. exp. Biol.* **50**, 99–117.

DAVIS, W. J. (1969*b*). Reflex organization in the swimmeret system of the lobster. I. Intrasegmental reflexes. *J. exp. Biol.* **51**, 547–563.

HEITLER, W. J. (1978). Coupled motorneurones are part of the crayfish swimmeret central oscillator. *Nature, Lond.* **275**, 231–234.

HEITLER, W. J. (1981). Neural mechanisms of central pattern generation in the crayfish swimmeret system. In *Adv. Physiol. Sci.*, Vol. 23, *Neurobiology of Invertebrates,* (ed. J. Salanki), pp. 369–383. Oxford: Pergamon Press.

HEITLER, W. J. (1982). Non-spiking stretch-receptors in the crayfish swimmeret system. *J. exp. Biol.* **96**, 355–366.

HEITLER, W. J. (1985). Motor programme switching in the crayfish swimmeret system. *J. exp. Biol.* **114**, 521–549.

HEITLER, W. J. & PEARSON, K. G. (1980). Non-spiking interactions and local interneurones in the central pattern generator of the crayfish swimmeret system. *Brain Res.* **187**, 206–211.

HUGHES, G. M. & WIERSMA, C. A. G. (1960). The co-ordination of swimmeret movements in the crayfish, *Procambarus clarkii* (Girard). *J. exp. Biol.* **37**, 657–670.

IKEDA, K. & WIERSMA, C. A. G. (1964). Autogenic rhythmicity in the abdominal ganglia of the crayfish: the control of swimmeret movements. *Comp. Biochem. Physiol.* **12**, 107–115.

MENDELSON, M. (1971). Oscillator neurons in crustacean ganglia. *Science, N.Y.* **171**, 1171–1173.

MIYAN, J. A. (1982). The neuromuscular basis of the swimmeret equilibrium reaction in the lobster, *Nephrops norvegicus* (L.). Ph.D thesis, University of Glasgow.

NAGAYAMA, T., TAKAHATA, M. & HISADA, M. (1983). Local spikeless interaction of motoneuron dendrites in the crayfish *Procambarus clarkii* Girard. *J. comp. Physiol.* **152**, 335–345.

SIMMERS, A. J. & BUSH, B. M. H. (1983). Central nervous mechanisms controlling rhythmic burst generation in the ventilatory motoneurones of *Carcinus maenas*. *J. comp. Physiol.* **150**, 1–20.

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.

ZILL, S. N. & FORMAN, R. R. (1983). Proprioceptive reflexes change when an insect assumes an active, learned posture. *J. exp. Biol.* **107**, 385–390.