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SENSORY SYSTEMS IN THE SWIMMERETS OF THE CRAYFISH *CHERAX DESTRUCTOR* AND THEIR EFFECTIVENESS IN ENTRAINING THE SWIMMERET RHYTHM

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Summary

The branching patterns and profiles of the main sensory nerves of the swimmerets of *Cherax destructor* are described. The innervation is generally similar to that in *Pacifastacus* but there are important differences that may be due to species variation or that may explain apparent differences in the physiology of the two species. The two large axons from the long strand of the stretch-receptor complex in *Cherax* give rise to non-spiking signals whereas those from the short strand are not active during passive protraction and retraction of the swimmeret.

The rhythmic movements of one swimmeret are sufficient to entrain the fictive swimmeret rhythm of an otherwise deafferented ventral nerve cord. The parameters of this entrainment match those of the known reflexes associated with swimmeret movement. Ablation experiments show that the stretch-receptor complex is necessary and sufficient for this entrainment and that input from the pleural plate hairs and swimmeret hairs is not required. Sinusoidal current injection into the non-spiking stretch receptors can also entrain the fictive rhythm of the deafferented cord.

From these results it is argued that the non-spiking stretch receptors play an important part in stabilizing and adjusting the frequency of the swimmeret rhythm. The swimmeret system is therefore another example of patterned motor output generated by a distributed, multicomponent network and this may be a general feature of cyclically rhythmic systems.

Introduction

It has recently been shown that the frequency of beating in the swimmeret system of the freshwater crayfish *Cherax destructor* can be entrained by controlling some of the sensory input if its timing and phasing approximates that produced during normal swimmeret beating (Deller & Macmillan, 1985, 1989). Prior to that

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report it was considered that in the swimmeret system sensory input could only alter intraburst structure (Davis, 1969, 1973; Heitler, 1986).

There are four known sensory systems in the swimmerets of crayfish and lobsters which could be responsible for the observed entrainment of the swimmeret rhythm. The setae fringing the two rami of the swimmeret and the setae on the pleural plates along the lateral margin of the abdomen both respond to water currents, water-borne vibrations and contact with the substratum (Heitler, 1986; Killian & Page, 1987, 1988). The swimmeret setae are involved in a reflex that occurs during the powerstroke movement in the lobster *Homarus americanus* (Davis, 1969) and crayfish *Cherax* (Oakley, 1982). In *Cherax*, the setal receptors of the swimmeret that are activated during the powerstroke excite the powerstroke motor neurones and inhibit the returnstroke motor neurones, whereas those activated during the returnstroke inhibit the returnstroke motor neurones and activate returnstroke inhibitors and some powerstroke excitors (Oakley, 1982).

Located in the base of each swimmeret of *Pacifastacus leniusculus*, is a two-stranded stretch-receptor complex that is maximally stretched at the end of the powerstroke. The largest of the strands is innervated by two large-diameter neurones which produce graded depolarizations. The other strand appears to produce spiking activity (Heitler, 1982, 1986). This receptor complex mediates powerstroke and returnstroke reflexes in both *Pacifastacus* and *Cherax* (Heitler, 1982, 1986; Oakley, 1982) but it is not clear whether both spiking and non-spiking receptors are involved.

Towards the end of the powerstroke, feedback from the stretch receptors excites the returnstroke motor neurones. Because the inhibition from the setae diminishes as the end of the cycle approaches, the returnstroke cycle could be reflexly initiated by the powerstroke cycle. Davis (1969) found no such reflex initiation of the powerstroke by the returnstroke movement in *Homarus* but Oakley (1982) found evidence for it in *Cherax*. Davis (1969) concluded that the reflexes in *Homarus* would not be sufficient to initiate, maintain or regulate swimmeret beating without a central pacemaker and results to date suggest that the situation is similar in *Pacifastacus* (Heitler, 1986). Oakley (1982), however, concluded that the reflexes present in *Cherax* were in the right direction to support a rhythm once it had started, although he did not argue, or produce evidence, for the proposition that the rhythm is maintained by reflexes.

Static swimmeret manipulations and imposed swimmeret movements have been shown to affect the amplitude of motor neurone activity in all three species (Davis, 1973; Heitler, 1986; Oakley, 1982) but only in *Cherax* has it been shown that the frequency can be altered by phasic sensory manipulations (Deller & Macmillan, 1989). This could be due to species variability such as the apparent differences in reflexes discussed above. A more likely explanation is that it is only in *Cherax* that the search has been undertaken in a way that reveals the effect.

The aim of the experiments described here was to describe the responses of the four receptor systems and to determine which of the elements are capable of producing entrainment of the swimmeret rhythm in *Cherax*.

Materials and methods

The specimens of *Cherax destructor* used in the experiments were collected in north-central Victoria, housed in polystyrene boxes in the open and fed on commercial cat food. Large specimens (55–75 mm cephalothorax length) were used for the behavioural experiments as they were easier to handle – only males fell into this size range in our samples. Smaller animals of both sexes were used in the physiological and anatomical experiments.

Anatomy

Peripheral innervation and sensory structures

Preparations of the fourth abdominal ganglion, a first root and the base of the swimmeret were fixed in alcoholic Bouin's solution, embedded in soft resin and stained. Sections were cut at intervals between the ganglion and the receptors of the swimmeret so that axon profiles could be examined.

To examine the sensory structures in the swimmeret the entire first root or its two main branches separately (1A or 1B, after Oakley, 1982) were filled towards the periphery with 1·5 % cobalt chloride for 3–5 h at 20–25 °C. This was then precipitated with 0·1 % ammonium sulphide. The preparation was fixed in alcoholic Bouin's solution, dehydrated, cleared in methyl salicylate (Altman, 1981; Altman & Tyrer, 1980) and subsequently silver-intensified (Bacon & Altman, 1977). A microscope and drawing tube were used to draw the preparations.

Central projections

The central projections of the stretch receptor in the base of the swimmeret (Heitler, 1982; Oakley, 1982) were examined by tracing nerve 1A to the point where it divides into three, just dorsal to the orbit of the swimmeret. The ventral nerve cord, together with the nerve innervating the stretch-receptor complex ($1A_3$) was carefully removed from the abdomen, secured in a Sylgard dish and covered with cold saline. The stretch receptor was left attached to its nerve to allow positive identification. Nerve $1A_3$ was carefully teased away from the first root and traced back towards the nerve cord (see also Lupone & Macmillan, 1988). Once the nerve had been separated close enough to the ganglion, it was filled with cobalt and processed as described previously.

Behavioural experiments

A rhythm was imposed upon selected swimmerets by attaching them to a mechanical system that produced movements closely matching the natural ones in position and phase (Deller & Macmillan, 1989). The movements of the attached swimmerets were synchronized with those of the unattached swimmerets for 20–30 cycles. The movement system was then accelerated by approximately 0·1 Hz. After 1 min or 40 cycles the movement rhythm was returned to its original level

and the frequency monitored for a further 20–30 cycles. The behavioural results were analysed with a video–computer system (Deller & Macmillan, 1989).

Sensory isolation

The isolation experiments were performed only on animals which showed entrainment responses when intact, so that direct comparisons could be made before and after removal of a particular sensory system.

Pleural plate hairs. The hairs or setae which fringe the lateral–ventral cuticular pleural plates were removed from the same side as the controlled swimmerets. It is possible that the rim of the excised area around the base of hairs could still produce some postoperative injury input or aberrant mechanical afference, but this would not be substantial and would not code the movement in the normal way.

Rami removal. The exopodite and endopodite of the swimmeret were removed from animals which had already had their pleural plate hairs excised. This was done by cutting the rami of all swimmerets attached to the movement system at their point of articulation with the basipodite, which completely removed the input from the setae which fringe the rami.

Stretch receptor ablation. The stretch receptors were ablated from animals with their rami and pleural plate setae intact. To ablate the stretch receptors, a small incision was made in the articulating membrane of the basipodite. The receptor complex can be seen clearly in this region and a mounted minutien pin with a hooked point was used to sever the posterior insertion of the complex in the posterior-medial region of the coxopodite–basipodite joint. The rotator muscle 6 was also cut because the receptor complex is closely associated with it. The receptor ablation was performed on all attached swimmerets. A post-experimental autopsy was done to confirm the success of the ablation.

Limited swimmeret movement.

To move the swimmerets through only limited fractions of the normal range, smaller cams were used on the movement machine (Deller & Macmillan, 1989). These cams produced only a 20–30° arc of movement.

Physiological experiments

Deafferented nerve cord

In these experiments, the fourth swimmeret, which was the only swimmeret with an intact neural connection with the ventral nerve cord, was attached to the movement system. The loose connective tissue was removed from under the nerve cord and all other peripheral nerve roots cut. The abdominal cavity was perfused with cold saline so that the preparation was effectively an isolated nerve cord *in situ* with one swimmeret still attached in a natural way. The motor rhythms were recorded by inserting bipolar platinum electrodes into Sylgard next to the nerves and insulating them from contact with the surrounding physiological saline using Vaseline.

The frequency of the movement system and the cyclic motor activity wer

synchronized or compared by displaying an analogue of the movement together with the electrical recording from the nerve cord on an oscilloscope screen. Once the basic frequency of the motor rhythm had been established the frequency of the movement system was adjusted to match it. It was not possible to be sure of correct matching during the experiment and all experiments were analysed from photographed records.

Intracellular recording and current stimulation of receptors

Intracellular recording and stimulation were carried out using glass microelectrodes filled with $3\,mol\,l^{-1}$ potassium chloride or potassium acetate. For recording the response of the receptors to movement, the animal was prepared as described in the preceding section. A waxed platform attached to a micromanipulator was manoeuvred beneath nerve 1A where it emerges from the orbit of the swimmeret. The receptor fibres were visualized and penetrated where they passed over the platform. All signals were direct-current amplified for tape recording or photographing.

Entrainment experiments using single receptor fibres were carried out on isolated abdominal nerve cords pinned out on Sylgard (Dow Corning) where the sensory fibres were readily visualized by transillumination so that intracellular current could be injected.

Entrainment experiments using two receptor fibres were carried out by isolating the cut ends of the two receptor fibres within a small saline-filled, Vaseline cup. Small silver/silver chloride pellet electrodes were positioned close to the nerve immediately adjacent to either side of the Vaseline wall and sinusoidally varying current was passed along the axons in the Vaseline gap between the two.

Results

Anatomy

Stretch receptor

The first root innervating the swimmeret bifurcates into two main branches, 1A and 1B. Nerve 1B, the posterior branch, supplies the powerstroke muscles, the rami opener and closer muscles and the hairs fringing each ramus. The more anterior nerve 1A innervates the returnstroke and rotator muscles, the pleural plate hairs and the stretch-receptor complex (Fig. 1).

Cobalt filling of nerve 1A showed that it divides into three branches: $1A_1$, which breaks up into smaller branches in the connective tissue near the integument, $1A_2$, which separates from $1A_3$ close to the receptor complex and innervates the pleural plate hairs, and $1A_3$, which runs to the receptor complex itself (Figs 1, 2A).

The stretch-receptor complex is made up of two innervated elastic strands, one longer (*lsr*) than the other (*ssr*) (nomenclature after Oakley, 1982). The *lsr* has the dendrites of two very large axons (Fig. 2C) embedded in it (Fig. 2D) and these run into nerve $1A_{3b}$ (Fig. 2A).

The shorter strand joins the *lsr* strand close to its anterior insertion but runs to a

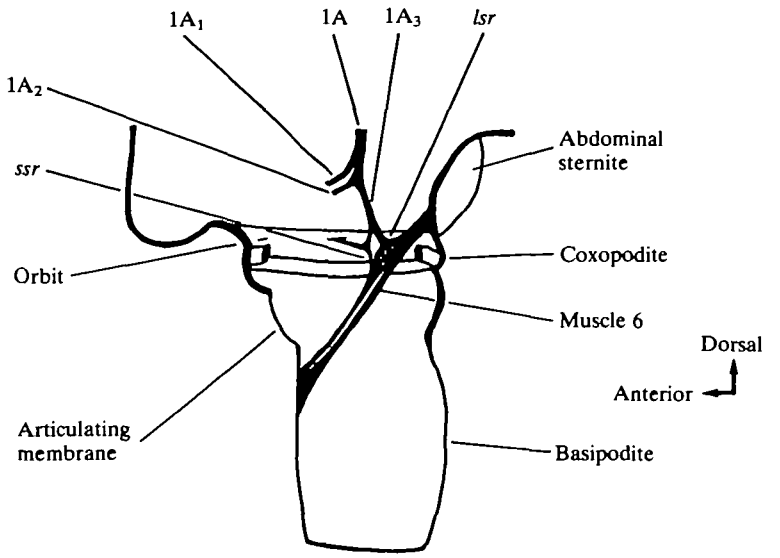


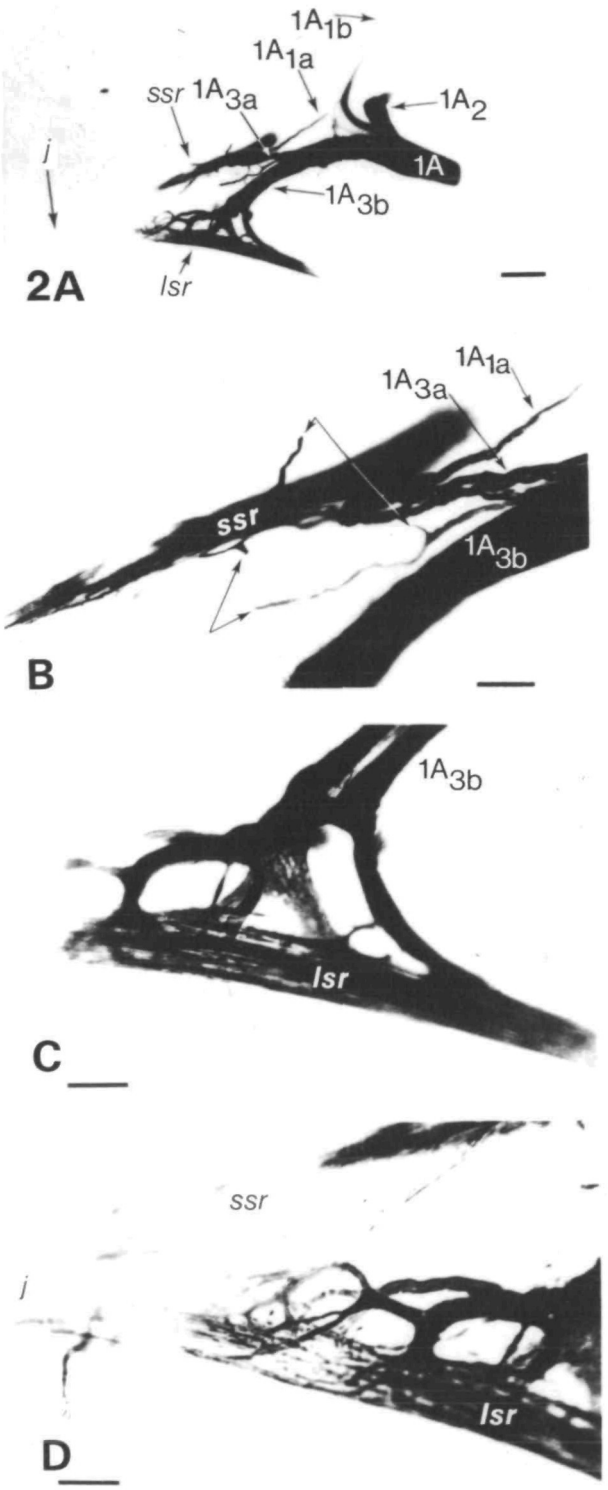
Fig. 1. Medial view of a swimmeret showing the position and orientation of the stretch-receptor complex within the coxopodite–basipodite joint of the swimmeret. This was drawn from cobalt fills of nerve 1A. *lsr*, long strand receptor; *ssr*, short strand receptor.

separate insertion on the anteromedial edge of the swimmeret orbit (Fig. 1). It is innervated by 7–12 small-diameter axons in nerve $1A_{3a}$ (Fig. 2A,B) but we found no evidence of cell bodies associated with the strand.

Sections at different distances from the ganglion and through successively finer branches leading to the swimmeret receptors confirmed this analysis of the cobalt preparations. Nerve $1A_{3b}$ to the *lsr* carries the two large axons from the two large sensory cells and also two smaller previously undescribed axons of unknown destination and function (Fig. 3E). Nerve $1A_{3b}$ combines with the smaller fibres of $1A_{3a}$ from the *ssr* (Fig. 3D) and with $1A_2$ from the pleural plate hairs (Fig. 3C). This group of fibres runs as an identifiable bundle in the anterior border of nerve 1A and of the first root (Fig. 3A,B).

Centrally directed cobalt fills of the two large axons showed them entering the ganglion together dorsally and then running medially towards the midline, before separating (Fig. 4A). One axon travels in a medial and slightly anterior direction

Fig. 2. Cobalt fills of nerve 1A towards the periphery. (A) Branching pattern of nerve 1A showing $1A_{3a}$ to the short strand of the receptor complex (*ssr*) and $1A_{3b}$ to the long strand (*lsr*). Note the close proximity to the receptor of the $1A_2$ branch. (B) Higher power view of the innervation of the *ssr*. Unlabelled arrows indicate some broken ends of fibres in nerve $1A_{3a}$. (C) Higher power view of the innervation of the *lsr*. Nerve $1A_{3b}$ divides into two branches that run to opposite sides of the receptor strand. (D) Higher power view of the relationship between the dendrites of the large receptor cells and the elastic strand. Note the junction (*j*) of the *lsr* and the *ssr*. Scale bars, A, 100 μm; B–D, 50 μm.


1A1b
1A1a
ssr
1A3a
1A2
j
1A
1A3b
2A
lsr
1A1a
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ssr
1A3b
B
1A3b
lsr
C
ssr
j
lsr
D

before it dives ventrally and medially to its ventral cell body (Fig. 4B). The second branch runs medially and posteriorly before turning back on itself, also diving ventrally and laterally to its cell body located in the ventral neuropile (Fig. 4C). At the point where the axons separate, there is a region of dense dendritic arborization in the lateral and dorsal neuropile of the ganglion (Fig. 4B,C). Closer

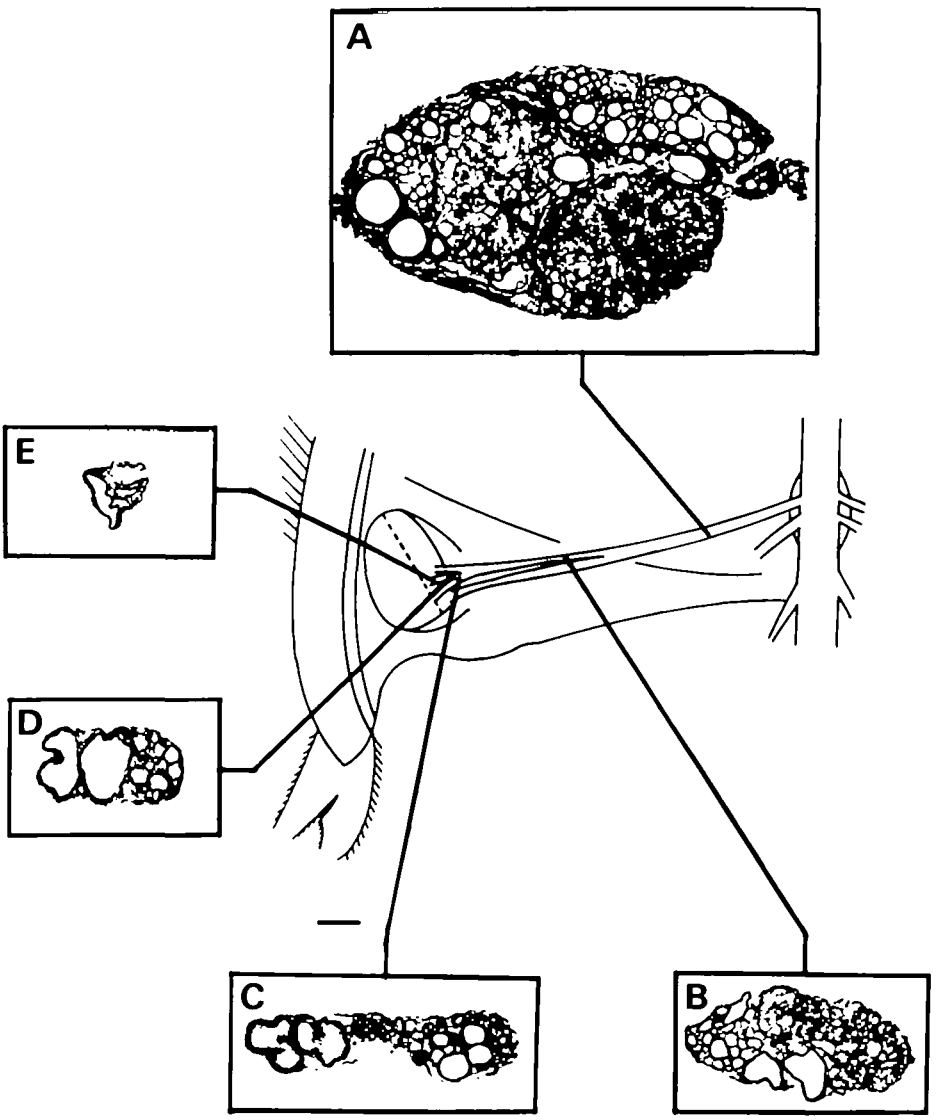

Fig. 3. Transverse sections through the abdominal first root and its branches that are associated with the swimmeret stretch receptor complex. Central figure shows a scale drawing of the first root on the left side of the fourth abdominal ganglion and its relationship to the swimmeret orbit and receptor complex (dotted line). (A) Transverse section of the first root; (B) nerve 1A; (C) nerves $1A_3$ and $1A_2$; (D) nerves $1A_{3a}$ and $1A_{3b}$; (E) nerve $1A_{3b}$. Scale bar, central diagram, 1 mm; B–E, 25 μm.

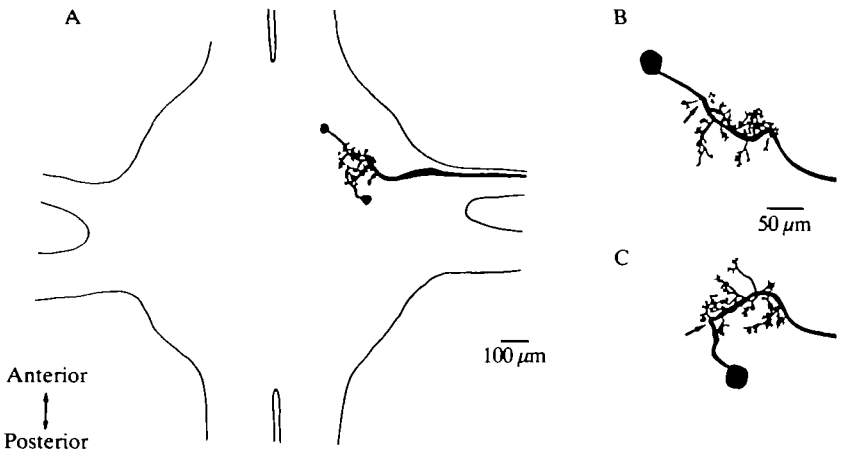

Fig. 4. (A) Cobalt fill of the central projections of nerve $1A_3$ in the fourth abdominal ganglion of the crayfish (ventral view). This shows the position of the nerve $1A_3$ within the first root, the division of this nerve into two axons and the location of the two central cell bodies. There is a region of dense dendritic branching at the point of bifurcation. (B,C) Higher power view of a cobalt fill of the central projections of the swimmeret stretch receptors (ventral view). The two neurones have been drawn separately to show the dendritic branching pattern of each in more detail. (B) Anterior medial neurone, (C) posterior lateral neurone. The arrow in each drawing indicates the approximate position along each neurone where the axons dive ventrally into the ganglion to their ventrally located cell bodies.

to the cell bodies both neurones show a small amount of dendritic branching which again is restricted to the lateral and dorsal neuropile.

Behavioural experiments

To isolate the afference necessary and sufficient for the entrainment the various sources of sensory input were progressively eliminated.

Are the hairs of the pleural plate and rami involved in entrainment?

The pleural plate hairs and subsequently the rami were removed from animals which had shown either full or partial entrainment to the externally controlled movements of three or four swimmerets.

The pleural plate hairs were removed first. Fig. 5A,B shows an example of an entrainment response after pleural plate hair removal. Full or partial entrainment still occurred in all cases following this ablation (Table 1). The finding that removal of the pleural plate hairs does not interfere with entrainment was not unexpected. The hairs may be indirectly stimulated by water currents set up by swimmeret movements, but they are more likely to be involved in signalling movement of the body relative to the surrounding water and *vice versa*. If this surmise is correct, they would not provide the type of information necessary for changing the timing of the rhythm.

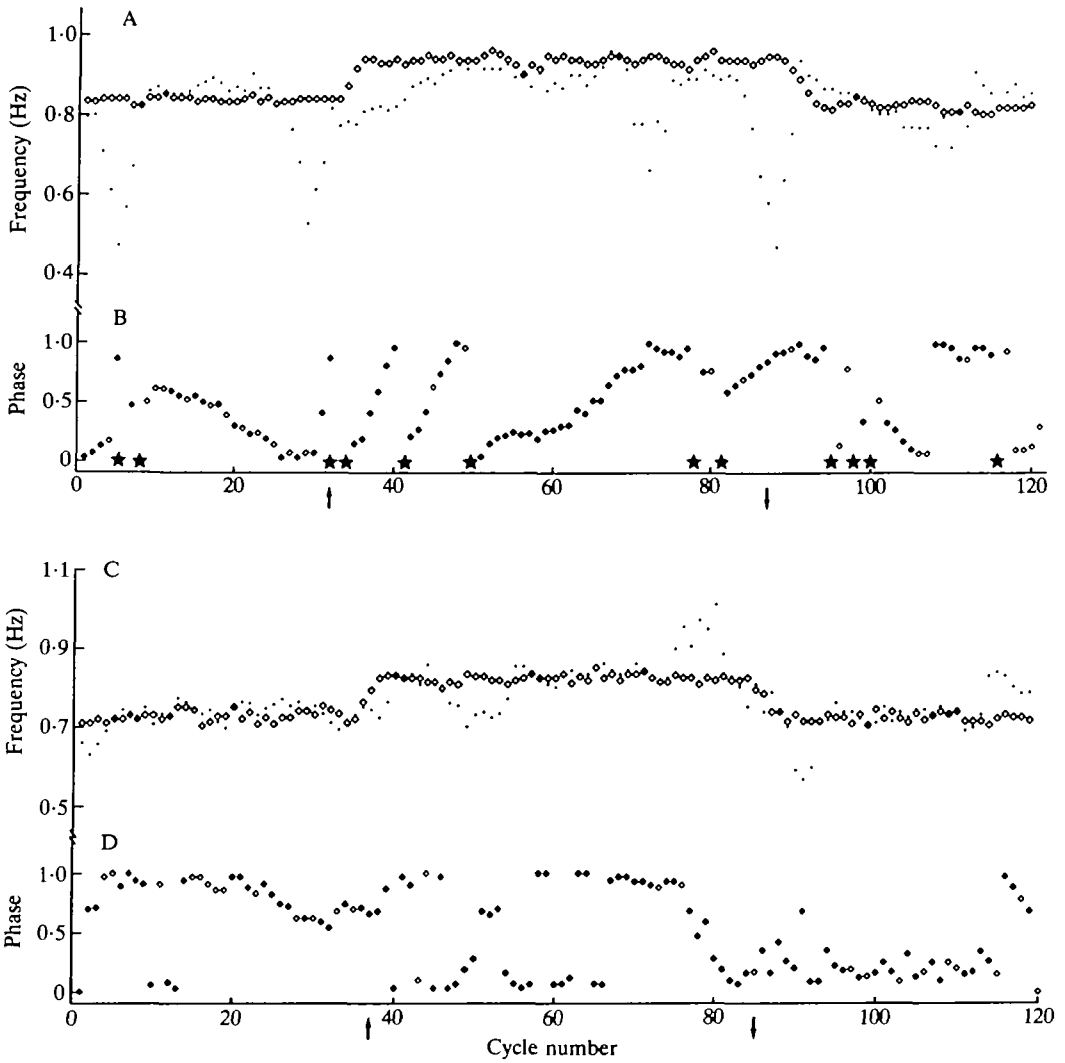

Fig. 5. (A) Sequential frequency (three-cycle average) graph of the fourth attached (◇) and unattached (●) swimmeret when four swimmerets were moved after removal of the pleural plate hairs from the same side. Note the delay of 12 cycles before the unattached swimmerets followed the imposed frequency. There were also two transient deviations of about five cycles each during the initial synchronization and the entrainment period. (B) Sequential phase position of the fourth unattached swimmeret movement within the cycle of the fourth attached swimmeret during the sequence shown in A. Stars indicate missing values. (C) Sequential frequency (three-cycle average) graph of the fourth attached (◇) and unattached (●) swimmeret when the movements of the bases of four swimmerets with their rami removed were controlled. The pleural plate hairs on the same side as the controlled swimmerets were also removed. (D) Sequential phase position of the fourth unattached swimmeret within the cycle of the fourth attached swimmeret during the sequence shown in C. The arrows on the abscissa indicate the time of the imposed frequency changes.

Table 1. *Occurrence of different types of behavioural responses following removal of the different receptor systems from the controlled swimmerets*

Sensory system removed	No. of animals	No. of trials	Entrainment Full	Partial	No entrainment
Pleural plate	4	9	3	6	0
Hairs on rami	3	7	4	1	2
Stretch-receptor complex	4	18	0	0	18

Entrainment still occurred in most cases following removal of those parts of the rami distal to the end of the basipodite. This operation removes the sensory hairs and any other receptors associated with the ramus (Table 1). In a small number of cases there was no entrainment response following this treatment, but the proportion of full entrainment or partial entrainment responses seen was the same as that encountered in the intact animal when four swimmerets were moved (Deller & Macmillan, 1989). Fig. 5C,D shows a full entrainment response in a preparation with pleural plate hairs and rami removed from the controlled side.

These results indicate that neither the hairs on the rami nor the hairs on the pleural plate are necessary for the entrainment of the swimmeret rhythm.

Is the stretch-receptor complex necessary for entrainment?

Since the rami and pleural plate hairs are not necessary for the entrainment of the swimmeret rhythm, the morphological evidence points to the only known sensory system remaining: the stretch-receptor complex.

The animals used in these experiments showed full or partial entrainment to controlled movements of four swimmerets. With the stretch-receptor complexes of the attached swimmerets ablated and the rami and pleural plate hairs intact, it was not possible to entrain any of the preparations to the imposed rhythm (Table 1). In some cases the rhythm of the unattached swimmerets showed some frequency variation around the original level, or another non-specific response (Fig. 6), but this was neither directional nor sustained. The results show that the stretch-receptor complex located at the base of the swimmeret is necessary and sufficient for entrainment of the non-attached swimmerets.

How does the entraining capacity of the stretch-receptor complex relate to its known static reflexes?

The stretch-receptor complex produces stronger reflex effects at some swimmeret positions that at others (Heitler, 1982, 1986; Oakley, 1982; Deller & Macmillan, 1985) so the question of whether some parts of the arc swept out by a swimmeret are more likely to produce entrainment than others is pertinent to any consideration of receptor influence. To examine this question, all four attached swimmerets were moved through a limited arc in either the anterior or the posterior part of the normal range of movement. When the attached swimmerets

were moved in the anterior part of the range, most preparations failed to show entrainment (Table 2), although some showed non-specific oscillation (Fig. 7A,B).

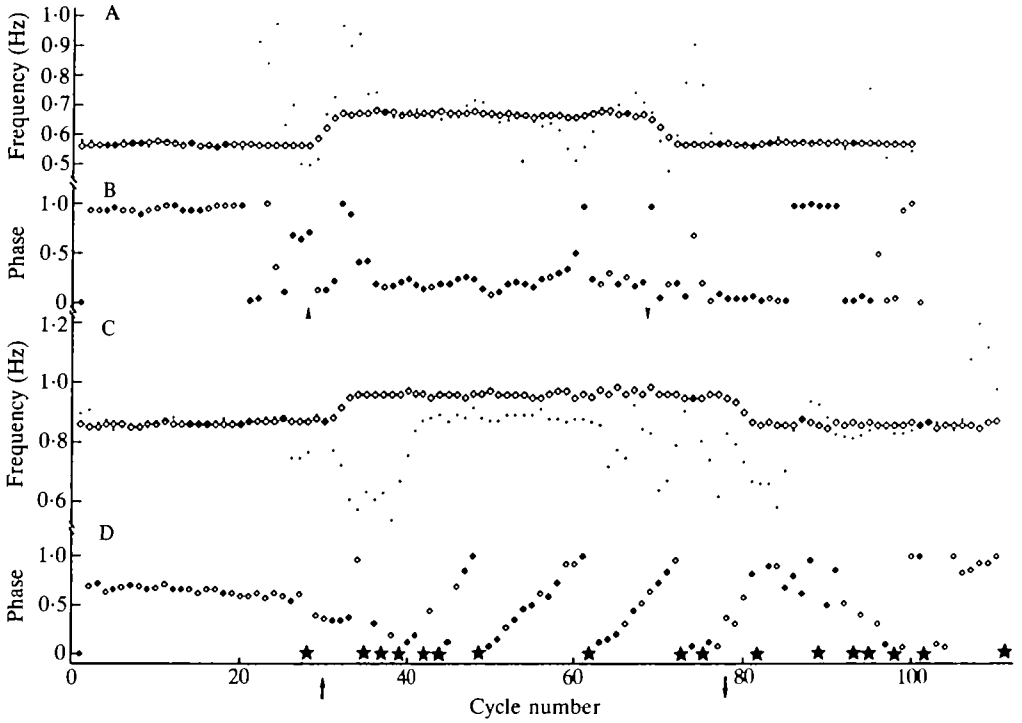

Fig. 6. (A) Sequential frequency (three-cycle average) graph of the fourth attached (◇) and unattached (●) swimmeret before ablation of the stretch receptors from the four attached swimmerets. Apart from two short transient deviations during the frequency increase and following the subsequent decrease, the unattached swimmerets followed the imposed frequency changes closely. (B) Sequential phase position of the fourth unattached swimmeret movement within the cycle of the fourth attached swimmeret during the same sequence. The two arrowheads indicate the time of the imposed frequency changes. (C,D) Sequential frequency (three-cycle average) graph and phase position graphs from the same preparation after ablation of the stretch receptors from the four attached swimmerets. All symbols as in A with the two arrows on the abscissa indicating the time of the frequency changes for the post-ablation trials. Stars indicate missing values.

Table 2. *Occurrence of different types of behavioural responses following movement of the attached swimmerets in the anterior and posterior part of their range*

Part of range used	No. of animals	No. of trials	Entrainment		No entrainment
			Full	Partial	
Anterior	3	10	0	2	8
Posterior	2	11	3	8	0

When the four attached swimmerets were moved in the posterior part of the arc, full or partial entrainment was seen in all cases (Table 2). A full entrainment response under these conditions is illustrated in Fig. 7C,D. The results indicate

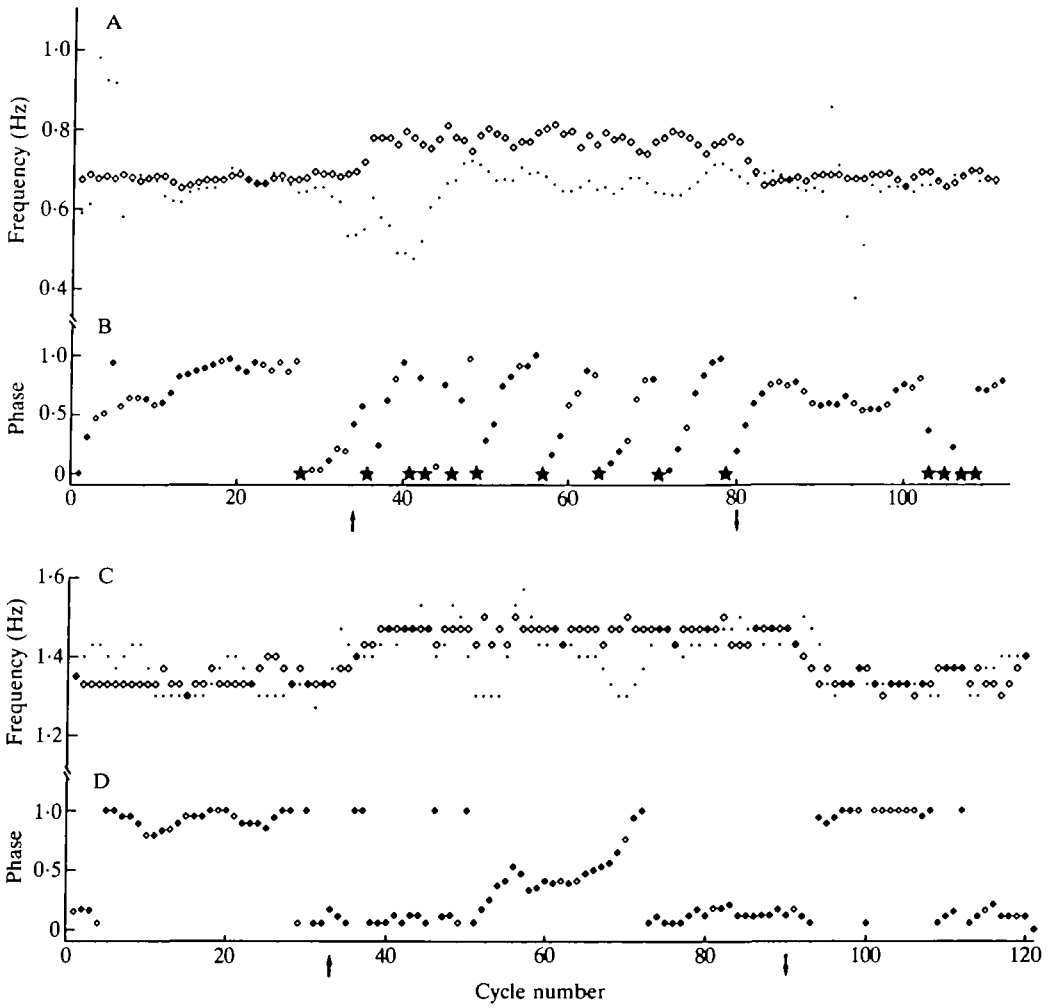

Fig. 7. (A) Sequential frequency (three-cycle average) graph of the fourth attached (◇) and unattached (●) swimmeret when four swimmerets were moved in the anterior part of the normal arc of movement. The unattached swimmerets showed some oscillation about the pre-treatment level but did not follow the controlled frequency. (B) Sequential phase graph of the fourth unattached swimmeret movement within the cycle of the fourth attached swimmeret for the sequence shown in A. (C) Sequential (three-cycle average) frequency graph of the fourth attached (◇) and unattached (●) swimmeret when four swimmerets were moved in the posterior part of the normal arc of movement. (D) Sequential phase plots of the fourth unattached swimmeret movement within the cycle of the fourth attached swimmeret during the sequence shown in C. The two arrows on the abscissa indicate the time of the imposed frequency changes. Stars indicate missing values.

that entrainment only occurs if the attached swimmerets are moved in the range where static reflexes from the stretch-receptor complex are active.

Physiological experiments

Entrainment of fictive swimmeret rhythm by appendage movement

In the preceding paper (Deller & Macmillan, 1989), we showed that the efficacy of an entraining influence on the rhythm of the intact abdominal preparation depends on the number of swimmerets controlled. Control of four swimmerets ensures a high level of entrainment, one swimmeret alone does not entrain the rhythm. One interpretation of these results is that the strength of the entraining influence is a function of the proportion of controlled to uncontrolled afference. This interpretation is supported by the finding that cutting the contralateral first roots increased the efficacy of the entraining movements on the fictive rhythm (Deller & Macmillan, 1989). To examine this proposition further we investigated the effect of controlling one swimmeret on the fictive rhythm produced by an otherwise isolated ventral nerve cord. In this case, afference from this one swimmeret was the only sensory input in conflict with the motor pattern produced by the central nervous system.

The difference between the burst structure from intact and isolated nerve cords was described in the earliest reports on the swimmeret rhythm (Hughes & Wiersma, 1960; Ikeda & Wiersma, 1964) and noted in a number of subsequent studies. Two changes are seen: occasional sudden discontinuities in rhythm, and raggedness and variability in the internal structure of the bursts. We eliminated the first by only using sequences where no pauses or discontinuities occurred. The second arises mainly because the normally tight coupling between powerstroke and returnstroke is much looser in the isolated preparation (see Fig. 9). This was not a problem for the synchronization experiments because the frequency of the whole cycles produced by the isolated system varies only slightly around some given frequency over periods of time much longer than our trials. The frequency of the imposed movement was achieved by matching a sinusoidal analogue of the movement to the overall cyclic rhythmicity of the motor output over many cycles. Verification of correct synchrony prior to a step change and of the frequency adopted afterwards was by subsequent analysis of photographed records. The slight variation in frequency of the swimmeret rhythm coupled with the loose intracycle structure meant that specific cyclic events varied somewhat in phase position within the cycle but this occurred without loss of overall synchrony between the cycles. The element of the cycle chosen as a reference point for comparing the two cycles was not critical for the result. Although all elements moved around somewhat within the cycle, the use of a three-cycle running average (as used for the movement correlations – see Deller & Macmillan, 1989) provided a stable measure of overall cycle frequency.

Using this type of frequency analysis we found that the rhythm in the contralateral first root of most deafferented preparations was readily entrained t

Table 3. *Occurrence of the response of fictive swimmeret activity when one swimmeret was moved on an otherwise isolated central nerve cord*

	No. of animals	No. of trials	Entrainment Full	Partial	No entrainment
Movement of one swimmeret	2	20	9	7	4

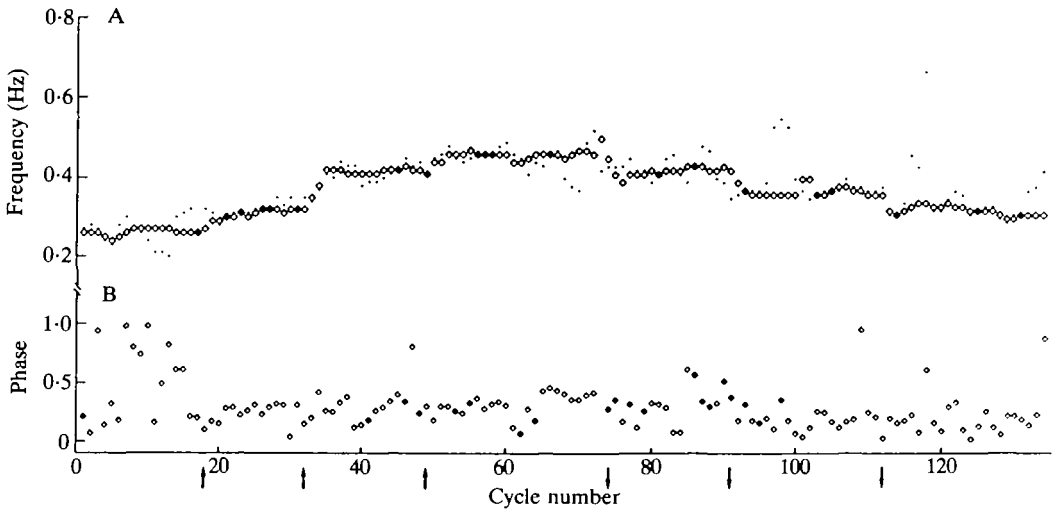

Fig. 8. (A) Graph of frequency (three-cycle average) of the fourth attached swimmeret (◇) and of the cyclic activity (●) recorded from the first root of the fourth contralateral swimmeret. All first roots other than the attached one have been cut. In this case, the controlled frequency was changed twice. (B) Phase of the motor neurone bursting activity within the cycle of the fourth attached swimmeret during the same sequence. The first appearance of motor activity within each cycle was used as the reference point for the phase calculation for this figure. The two arrows on the abscissa indicate the time of the imposed frequency changes.

the movements of one swimmeret (Table 3). Fig. 8 shows the result from a typical experiment in which the fictive rhythm followed the imposed rhythm closely as the frequency increased and decreased and examples of recordings from these experiments are shown in Fig. 9. In some cases the fictive rhythm entrained to the imposed rhythm for small frequency steps but the coupling was lost when the imposed frequency was moved too far from the natural rhythm (Fig. 10).

What is the nature of the sensory system responsible for entrainment?

Having demonstrated that the stretch-receptor complex is necessary and sufficient for entrainment and that one complex can entrain the deafferented ventral nerve cord, we were in a position to determine the nature of the entraining sensory signal. Earlier studies on both *Pacifastacus* (Heitler, 1982, 1986) and

Cherax (Oakley, 1982) indicated that the nerve from the stretch-receptor complex gives rise to non-spiking, decrementally conducted movement signals in the two large-diameter fibres and to movement-sensitive spiking activity in some of its smaller axons.

We recorded intracellularly from both the large axons and confirmed Heitler's observation that they are non-spiking. In *Cherax* the resting potential is typically

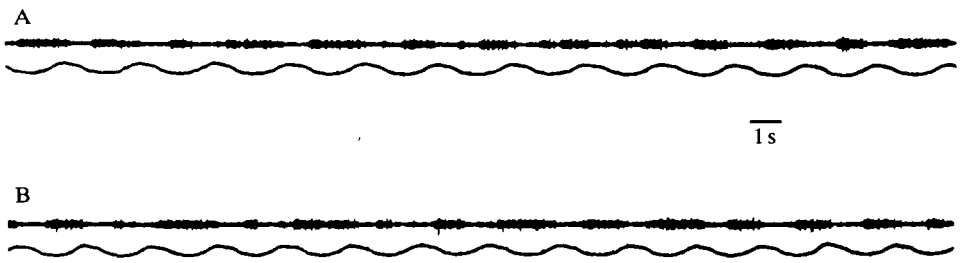

Fig. 9. Sample of data used to construct the frequency and phase plots such as those used in the previous figure. Cyclic motor activity recorded from the first root of the fourth swimmeret (upper trace) and movement of the fourth contralateral (attached) swimmeret. The figure shows the attached swimmeret being moved at two frequencies: (A) 0·42 Hz (B) 0·47 Hz. Upward deflection of the movement trace corresponds to a powerstroke.

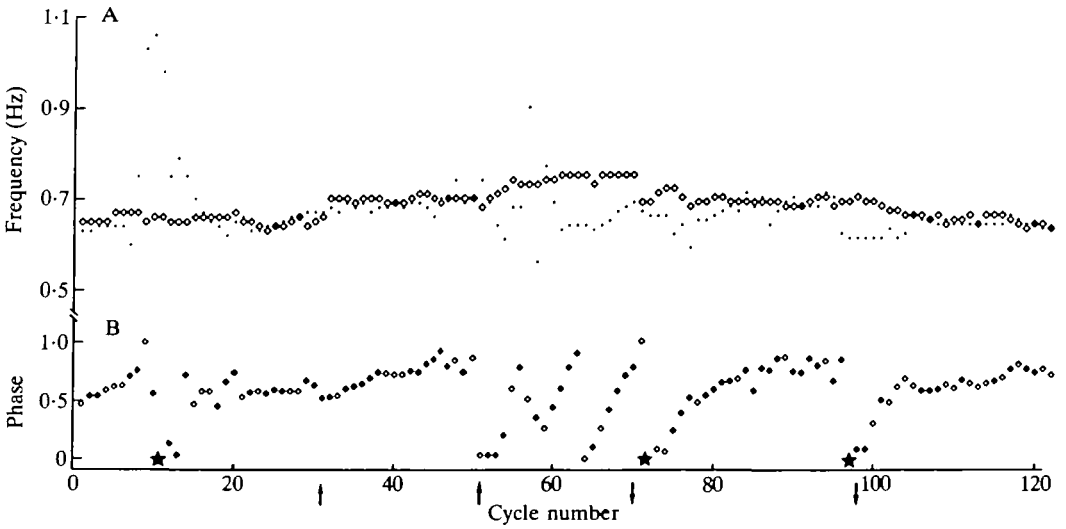

Fig. 10. (A) Frequency plots (three-cycle average) of the fourth attached (◇) swimmeret and of the motor neurone bursting activity (●) recorded from the contralateral first root of the fourth swimmeret. This shows that for the first two changes, the motor neurone activity followed the imposed frequency but on the third, it did not. (B) Phase plot of the motor neurone bursting activity within the period of the fourth attached swimmeret during the sequence shown in A. Stars indicate missing values. The arrows on the abscissa show step increases and decreases in applied frequency.

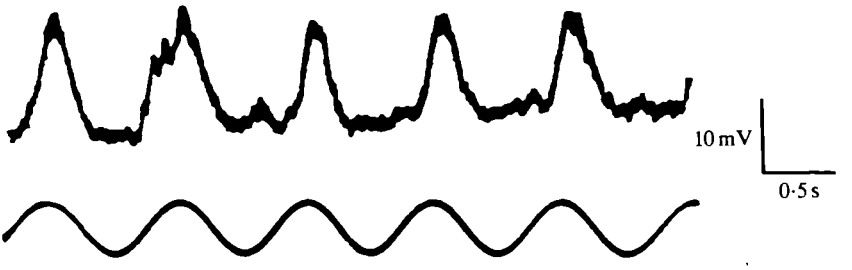

Fig. 11. Intracellular recording from a non-spiking stretch-receptor axon close to the receptor. Upper trace shows intracellular depolarization of 18 mV below the resting potential of 70 mV in response to a 30° arc of movement in the posterior part of the swimmeret range. Retraction movement produces upwards deflection of trace.

60–70 mV close to the receptor. Retraction of the swimmeret through an arc of approximately 30° at the posterior end of its range of movement evoked a depolarization of some 15–20 mV from resting potential (Fig. 11). Occasionally, larger depolarizations have been observed in preparations where the swimmeret orbit has been extensively dissected (V. M. Pasztor & D. L. Macmillan, unpublished observations) but whether such potentials could occur in the intact animal is not known. Although we did not record from the two receptors simultaneously, we could see no apparent difference between their responses in either range sensitivity or dynamic response. The range of movement over which they showed depolarization corresponds to the range that is effective for entrainment.

Recordings from the peripheral cut end of the first root showed a number of units that responded cyclically to sinusoidal movements of the swimmeret (Fig. 12A). Recordings in the same position after nerve 1B had been cut (Fig. 12B) showed that most of this response to movement arose in the receptors associated with the fringe of the ramus (Killian & Page, 1988). It was more difficult to determine the origins of the remainder of the signal. Nerves $1A_3$ and $1A_2$ normally separate so close to the receptor complex that it is not possible to cut nerve $1A_2$ without damaging the receptor or its suspension. In preparations where it was not possible to cut the nerve we found that carefully cutting away the ipsilateral pleural plate hairs on that segment abolished the remaining spiking response to passive swimmeret movement (Fig. 12C). This result surprised us because previous reports (Heitler, 1982, 1986; Oakley, 1982) together with our nerve cross-sections led us to predict a residual spiking component from the small axons in nerve $1A_{3a}$ innervating the short receptor strand. In all cases, however, where we thought initially that we might have a residual spiking signal we were able to eliminate it by locating and removing hairs we had overlooked. Furthermore, very occasionally we found animals in which nerve $1A_2$ separated from $1A_3$ further away from the receptor than usual and so we were able to record from $1A_3$ exclusively. These fortuitous anatomical variations allowed us to confirm that in *Cherax* there is no spiking signal from the short stretch receptor in response to

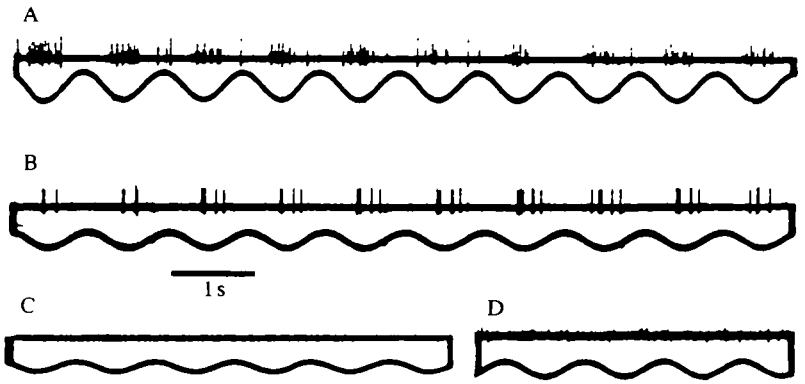

Fig. 12. (A) Recording of the response in the first root of the left, fourth swimmeret in response to rhythmic movement of the appendage over approximately a 20° arc at the posterior end of its range. Retraction causes an upwards deflection of the movement trace. (B) Recordings from the same preparation after nerve 1B has been cut. (C) Recording from the same preparation after cutting nerve 1B and removing all ipsilateral pleural plate hairs. (D) Recording from an anatomically unusual animal where it was possible to place electrodes on nerve $1A_3$ from the receptor complex alone.

passive swimmeret protraction and retraction throughout the normal range and velocity of movement (Fig. 12D). Great care was required with these recordings because the spiking activity from nerve $1A_2$ travelling back from the junction with $1A_3$ could, in the confined space of the swimmeret orbit, create the illusion of small spikes in nerve $1A_{3a}$.

Can the non-spiking stretch receptor entrain the swimmeret rhythm?

Since the stretch receptor complex can entrain the rhythm of the deafferented ventral nerve cord and, at least in *Cherax*, the *ssr* does not contribute a sensory signal during passive swimmeret movement, it should be possible to entrain the rhythm using appropriate rhythmic electrical stimulation of the stretch receptors. We found that intracellular injection of current into one of the stretch receptor axons, to mimic the voltage changes evoked by swimmeret movement, successfully entrained the fictive rhythm in two out of seven trials. Simultaneous stimulation of both non-spiking stretch receptors using the Vaseline-gap method produced full entrainment in three out of five trials (Fig. 13), partial entrainment in one trial and no entrainment in the other.

Discussion

The swimmeret system of *Cherax destructor* appears to have a similar complement of receptors to that in *Pacifastacus leniusculus* (Heitler, 1982). Now that we have more details of the morphology and physiology of the receptors in *Cherax* it remains to be seen whether species differences will emerge at that level or wheth

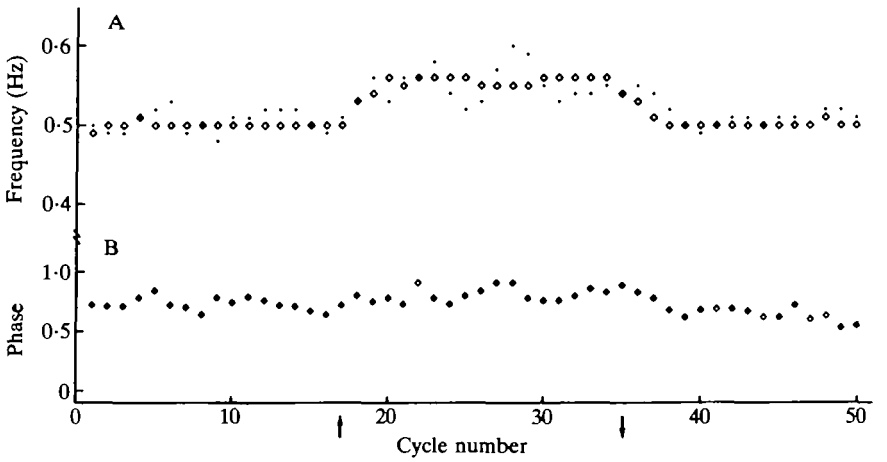

Fig. 13. (A) Sequential (three-cycle average) frequency graph of the fictive (●) swimmeret rhythm recorded from the fourth right root of an otherwise deafferented ventral nerve cord. The frequency of a sinusoidally varying current applied to the non-spiking stretch receptor axons of the contralateral fourth root is shown by the diamonds. The arrows on the abscissa show a step increase and decrease in the applied frequency. (B) Phase of the fictive rhythm within the cycle of the applied rhythm during the sequence shown in A.

some of the earlier assumptions concerning *Pacifastacus* (Heitler, 1982, 1986) will require modification as they did in *Cherax* (Oakley, 1982).

The non-spiking stretch receptors in the two species appear to be very similar in structure although the sensory signal generated may be somewhat smaller in *Cherax*. Although we subjected the receptors to a wider range of stimulus parameters than was used in testing *Pacifastacus*, we too detected no signs of range fractionation or separation of dynamic response between the two receptors. As in *Pacifastacus*, the main difference appears to be their central destinations. The disposition of nerves to the stretch-receptor complex is also closely similar in the two species and our cross-sections of specific small branches broadly confirm Heitler's (1982) analysis of the innervation. It was surprising, therefore, to find clear physiological differences from the earlier work. Our investigations revealed no evidence that the axons from the *ssr* carry a spiking sensory signal like that described in *Pacifastacus* (Heitler, 1982). A similar signal is carried, however, by closely associated nerves from adjacent hair receptors. There are a number of possibilities that would explain the differing findings. It could simply be due to species differences, although the close anatomical correspondence makes this unlikely. Separating the sources of the various signals is probably as difficult in *Pacifastacus* as it was in *Cherax* and, as the result is counter-intuitive, it is possible that Heitler misinterpreted his results as Oakley (1982) did, and as we did initially. Another possibility is that the fibres from the *ssr* only respond if rotational or other components are present in the swimmeret movement; such components may

normally require a dynamic rather than a passive movement. The absence of peripheral cell bodies is often associated with non-spiking, decremental conduction of the sensory signal but the small diameter of the axons makes it unlikely in this case. The somewhat unusual arrangement of the *ssr* and its innervation as described for *Pacifastacus* is perhaps even more enigmatic in the light of our results for *Cherax*.

Davis (1969) first suggested that the reflexes seen in the lobster swimmerets could be partly due to the stretch receptors located in the base of the limb. He found that they excite the returnstroke excitor motor neurones and contribute to the reflex initiation of the returnstroke. West *et al.* (1979) found further evidence that the stretch receptors could modulate the motor neurone activity in *Procambarus clarkii*, but individual sensory systems were not identified. Oakley (1982) proposed a model for *Cherax* where the stretch-receptor reflexes could not only initiate the returnstroke, as Davis (1969) suggested, but could also signal the end of the returnstroke and initiate the powerstroke. Oakley, however, based his model on sensory signals recorded from nerve 1A which he believed originated from the spiking stretch receptors. This nerve innervates the stretch receptors, swimmeret muscles and sensory setae on the pleural plate and so it is likely that the recordings included other afferent inputs, especially from the pleural plate hairs which are very sensitive to water currents or water-borne vibrations. These unresolved questions concerning clear identification of receptor inputs make it difficult to reconcile the findings of the earlier reflex investigations with the present entrainment results.

Heitler (1982, 1986) provided the first clear evidence that the stretch receptors could influence the swimmeret motor pattern in the crayfish. Depolarizing current injected into a non-spiking stretch receptor neurone inhibited powerstroke motor neurones and hyperpolarizing current excited them. This suggests a negative feedback resistance reflex, whereas in the lobster there is an assistance reflex (Davis, 1969). When Heitler (1986) moved one swimmeret on an effectively isolated nerve cord, the amplitude of the motor neurone bursting activity was modulated although it seemed unlikely that the kind of response he found to be evoked by the stretch receptors of one swimmeret would be strong enough to produce entrainment.

Our results show that the non-spiking stretch receptors are necessary and sufficient for the entraining influence of controlled swimmeret movements on the swimmeret rhythm. It is again possible that apparent differences may be due to species variation. We feel that this is not likely because the non-spiking stretch-receptor organs and their responses are so similar and because other studies have shown the animals to be very close in other aspects of their behaviour and nervous system function (Cooke, 1985; Cooke & Macmillan, 1985; Macmillan *et al.* 1983). A more likely explanation for the difference is that our behavioural experiments were performed on relatively intact animals and that relatively natural swimmeret manipulations were used. Heitler used a more artificial arrangement when he moved the swimmeret in an inverted position which required putting a twist in th

first root. Another complication with this method is that only the basipodite was covered in saline so that the sensory input from the hairs on the rami, which are water-activated, would be absent or disrupted. This could affect the normal structure of the bursts although, as we have shown, it would not be likely to affect entrainment experiments. We also found that it is important to commence entrainment attempts close to the natural frequency and that there appear to be limits to the difference in frequency that can be sustained.

The finding that the deafferented ventral nerve cord of the crayfish can produce a relatively stable cyclic motor output has generally been taken as an indication that the pattern-generating mechanism is dominated by central oscillators. One of our most interesting results, therefore, is that the rhythm of the deafferented cord was readily entrained by the non-spiking receptors of one swimmeret whereas the intact system never was. This suggests that although the central connections are sufficient to produce patterned output in the absence of any sensory input they require constant reinforcement by correctly phased, rhythmic afference for stability during normal operation. This model is supported by our previous finding that only the lower frequencies of the possible range are produced by deafferented cords (Deller & Macmillan, 1989) and by the raggedness of the intracyclic structure in deafferented preparations. If this interpretation is correct it could be argued that the central element appeared dominant in the earlier work on this system precisely because the afference was significantly reduced or otherwise affected (Hughes & Wiersma, 1960; Ikeda & Wiersma, 1964). The parts of the afferent input that stabilize and can alter the periodicity of the system could be considered a constituent part of the temporal control mechanism so that the distributed control network model for rhythmic patterning of insect flight proposed by Altman (1983), Delcomyn (1985) and Wendler (1985) could also be applied to the swimmeret system.

Our experiments show that a sinusoidal input in one of the pair of stretch-receptor fibres can entrain the rhythm of the deafferented cord. Although there is some suggestion that the entrainment effect is stronger or more reliable when both fibres are used, the significance of this apparent duplication in the swimmeret control system remains to be determined.

Proprioceptors that respond to limb movement about a joint or to body movement have now been shown to produce similar effects on the periodicity of motor output in other systems. The stretch receptors located in the lateral edge of the spinal cord are thought to be the sensory receptors responsible for the very strong entrainment response seen in lampreys and dogfish (Grillner & Wallén, 1982, 1984). The wing-hinge stretch receptors in the locust entrain the flight rhythm (Wendler, 1974; Pearson *et al.* 1983) but only if the frequency of the proprioceptive input is close to that of the spontaneous flight rhythm. The swimmeret system appears to be similar in this respect. Many different sensory systems, proprioceptive and exteroceptive, are integrated components of the normal stable flight rhythm (Horsman *et al.* 1983; Horsman & Wendler, 1985; Neumann, 1985; Wendler, 1978, 1983). By comparison, there are fewer relevant

sensory inputs for regulating the swimmeret rhythm. This is perhaps not surprising considering the obvious difference in complexity of the behavioural output generated. The setae and the stretch receptors are the only systems identified so far and only the input from the stretch receptors has been shown to affect the period of beating. Although this does not preclude others, the morphological investigations of several species have been quite extensive and none has been found. Heitler (1986) suggests that the spiking and non-spiking inputs from the stretch receptors may act synergistically so that fewer receptor inputs are required to ensure that the proprioceptor input is adequate in all parts of the frequency range.

The results presented here indicate that the swimmeret system of the crayfish can no longer be considered a classic example of a central network that determines frequency on its own. It appears instead to be another example where control is distributed over several components of the system. As the swimmeret system provided perhaps the strongest evidence for the existence of patterning determined by a central oscillator, the possibility that distributed control is a general feature of rhythmic systems must now be seriously entertained.

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