ENTRAINMENT OF THE SWIMMERET RHYTHM OF THE CRAYFISH TO CONTROLLED MOVEMENTS OF SOME OF THE APPENDAGES

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Summary

A machine was used to impose controlled movements, closely resembling natural movements, on some of the swimmerets of crayfish with their ventral nerve cords cut between thorax and abdomen. The rhythm of the unrestrained swimmerets could be entrained to the imposed frequency. Full entrainment occurred most readily when three or four swimmerets were controlled and was uncommon with two. When one was controlled, only partial entrainment was seen. A small proportion of preparations could not be entrained irrespective of the number of swimmerets controlled. Entrainment of the neural rhythm also occurred when movement was imposed on one or more swimmerets attached to an otherwise isolated nerve cord.

This is the first demonstration that sensory input affects the periodicity of the swimmeret rhythm. In the light of this result, the hypothesis that swimmeret rhythm is largely controlled by a central pattern generator should be viewed with caution. It now appears that there is also an influential sensory component responsible for stabilizing and adjusting the timing of the swimmeret rhythm.

Introduction

The swimmeret system of the freshwater crayfish Cherax destructor comprises four pairs of biramous, paddle-like appendages on the ventral surface of the abdomen. These beat metachronously, with each limb showing alternating powerstroke and returnstroke movements. Contralateral swimmerets are in phase whereas those on adjacent segments show a constant phase-lag (Hughes & Wiersma, 1960). The swimmerets are used in righting responses, forward swimming, egg transport, egg aeration and burrow ventilation.

Hughes & Wiersma (1960) and Ikeda & Wiersma (1964) have shown that the abdominal nerve cord can produce relatively normal motor rhythms in the absence of sensory input. This finding has stimulated investigation into the nature of the sensory input.

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central control system. In each abdominal hemiganglion there is an oscillator or central pattern generator (CPG) (Hughes & Wiersma, 1960; Ikeda & Wiersma, 1964), and the CPGs are coupled to form a distributed pattern-generating network. Although a great deal is now known about various components of the CPGs, their function is still not understood (Davis, 1969a; Heitler, 1978, 1980; Heitler & Pearson, 1980; Paul, 1981; Paul & Mulloney, 1985a,b). The level of excitation of CPG elements is controlled by tonic input from descending interneurones (Wiersma & Ikeda, 1964; Davis & Kennedy, 1972a,b) and intersegmental coordinating neurones are involved in regulating the phase relationship between segments (Macmillan et al. 1983; Paul & Mulloney, 1986; Stein, 1971; Wiersma & Hughes, 1961). It is possible that the motor neurones themselves may be part of the CPG (Heitler, 1980; Macmillan et al. 1983) but there is to date little evidence for the involvement of the sensory system in coordination (Deller & Macmillan, 1985; Macmillan et al. 1983).

Although sensory input has not been ignored, its role has not attracted as much interest as that of the CPG. The effects of some non-cyclic sensory signals on the rhythmicity of the system have been examined by holding one swimmeret in the retracted position. In Homarus americanus, reflexes produced in this way have been found to amplify and stabilize the swimmeret rhythm but not to influence periodicity (Davis, 1969b). West et al. (1979) have demonstrated that static swimmeret manipulations can modulate the period of the rhythm in the crayfish Procambarus clarkii but were not able to determine the conditions necessary for this because of the variability they encountered. Similar procedures have been shown to produce transient frequency changes in Cherax (Oakley, 1982), but only in the amplitude of motor neurone output in Pacifastacus leniusculus (Heitler, 1986). The effects of some simple dynamic manipulations on the frequency of the rhythm have also been looked at. Heitler (1986) found that moving a swimmeret from one stationary position to another results in a brief, transitory increase in frequency but sustained movement affects only the amplitude of the motor neurone activity with no frequency responses being observed. Each of these studies has concluded that sensory input does not have a specific role in the periodicity of the swimmeret rhythm and when they are considered together, the variability and range of reported results indicates that this is a sound judgement.

A re-examination of these reports suggests that elements in the experimental designs could have reduced the role of sensory input and overemphasized that played by the CPG. For example, static manipulations are difficult to interpret because the situation is unlikely during normal swimmeret beating. Many of the results also come from isolated abdomens or significantly reduced ventral nerve cord preparations so that the balance of sensory input may have been diminished prior to the trial.

This interpretation was supported by our preliminary work on Cherax which showed that clear changes in the period of the rhythm could be evoked by cyclic control of a few of the swimmerets of an intact animal (Deller & Macmillan, 1985). The aim of the present study was to examine the proposition that the experimenta
methods used to date have masked the role of sensory input in the coordination of the swimmeret rhythm.

Materials and methods
Specimens of *Cherax destructor* were collected in north-central Victoria, housed in polystyrene boxes and fed on commercially prepared cat food. Males were used for behavioural experiments because of their large size (55–75 mm in cephalothorax length). Both sexes were used in physiological experiments.

Behavioural experiments

Swimmeret movement system
The proportions of the swimmeret cycle devoted to powerstroke, returnstroke and interstroke delay were measured from video recordings. Using these measurements, a swimmeret movement system was constructed to produce movements closely mimicking the natural swimmeret movement. The machine consisted of a single drive shaft with four cams on it. Upon each cam rested a weighted lever hinged at the other end. Each lever was connected to the swimmeret by a system of thread and eyelets. The shape of the cams was designed to produce a movement which matched natural swimmeret movement (Fig. 1). The constant phase-lag between the four ipsilateral swimmerets was obtained by adjusting the radial position of the four cams relative to each other. The machine was driven by a motor with a variable speed control connected to the drive shaft by a pulley and non-slip belt. Each attached swimmeret could be moved through an arc of approximately 60–70° which compares well with the 90–110° natural arc. The output of the system provided an accurate copy of metachronal beating that could be imposed on the swimmerets of one side at any chosen frequency. When the frequency of the attached swimmerets was increased, the duration of the powerstroke, returnstroke and intercycle delay decreased and the powerstroke-returnstroke ratio remained constant (Fig. 2), thus mimicking the natural cycle structure in both crayfish (Oakley, 1982) and lobster (Davis, 1968).

Experimental procedure
Animals were anaesthetized by immersion in ice for 15–30 min. They were induced to autotomize the last two pairs of pereiopods and one cheliped because these interfered with the movement system. The animal was secured in a harness, placed ventral surface up in a large Petri dish and covered with ice. The abdomen was exposed and an eyelet baseplate attached to the animal with a mixture of α-cyanoacrylate glue and talcum powder (Fig. 3A). The eyelets were placed such that each swimmeret had a pair bracketing it anteriorly and posteriorly at about the height of the distal rim of the basipodite (Fig. 3B). A fine thread was attached to the basipodite with α-cyanoacrylate glue and threaded through the eyelets ready for connection to the arm of the machine. Depending on the experiment, one to four swimmerets were attached to threads in this way.
Fig. 1. (A) Diagram of the movement system (SMS) used to mimic natural swimmeret movement (see Materials and methods for description). The mirror on one of the levers was used in conjunction with a photoelectric sensor and light beam to monitor movement during physiological experiments. (B) Schematic diagram of the apparatus used in the behavioural experiments. The animal's rostral end is placed in an isolation chamber to stop it interfering with the movement machine. Both the harness and isolation chamber are firmly clamped such that the abdomen is positioned directly above the SMS. This shows the connection of one thread to the machine. The experiment is filmed by video camera and stored on a video cassette recorder (VCR).

The ventral nerve cord was exposed by making a small incision in the fused thoracic sternum between the last pair of pereiopods and removing the underlying connective tissue, care being taken not to damage the posterior ventral abdominal
Fig. 2. Four parameters of the movement cycle of the fourth attached swimmeret during a typical experimental trial. The arrows on the abscissa indicate when the controlled frequency was changed. (A) Interval between powerstroke and return-stroke movements. (B) Ratio of the powerstroke–returnstroke durations. (C) Return-stroke duration. (D) Powerstroke duration. Variables A, C and D decrease as SMS frequency increases whereas B remains constant.

artery. The cord was cut between the last thoracic and first abdominal ganglia and the wound sealed.

The animal was secured to a holding bracket in an aquarium which contained water at 12-17°C. The thread attached to each swimmeret was secured to the appropriate lever underneath which were another two eyelets through which the thread was passed before being re-secured to itself by means of a soft Perspex cleat (Figs 1, 3B). The threads were made of siliconized silk except where they passed through the eyelets, where short sections of small-gauge nylon were substituted to resist fraying.

After a wait of 20 min for the animal to acclimatize to the aquarium, the experiment was commenced. Once the animal began to beat its free swimmerets, the attached swimmerets were synchronized with them. The machine frequency was then either accelerated or retarded by approximately 0.1 Hz. After 1 min or 40 cycles the driving frequency was returned to its original level. A typical experiment consisted of approximately 100 cycles of swimmeret beating. This sequence was made up of 30 cycles of synchronization, 40 cycles of frequency change and a further 30 cycles at the original synchronization frequency. This was
repeated three or four times. The experiment was filmed at 25 frames s\(^{-1}\) with a video camera equipped with a stopwatch function for time reference.

**Video and computer analysis**

The experiment was analysed frame by frame with a variable, slow-speed video cassette recorder. Owing to the constant phase-lag between swimmerets (crayfish: this study; Ikeda & Wiersma, 1964; lobster: Davis, 1968), the frequency of the whole system can be monitored by following the movements of one appendage. The powerstroke and returnstroke start-stop times were recorded for the fourth swimmerets, one attached and one unattached, and the details entered directly into a microcomputer. Data were transferred to a Hewlett-Packard series 200 computer for analysis and display.

For our analysis we calculated the instantaneous frequency, powerstroke and returnstroke durations, the powerstroke-returnstroke ratio, intercycle delays of the attached and unattached swimmerets and the phase of the fourth unattached swimmeret relative to the fourth attached one. The term ‘phase’ is used in accordance with Wilson’s (1966) definition: ‘the phase of an event is the fraction of some cycle which has elapsed at the time of the event’s occurrence’. The phase values could range from 0 to 1 phase units. Sequential phase plots and frequenc
plots were generated from the data. The individual frequency values used for plotting were the average of three points: the point before, the point after and the point itself. This three-cycle averaging was necessary to smooth systematic variation caused by jitter in the video display.

**Behavioural controls**

The ventral nerve cord was cut because this operation markedly increased the proportion of time the swimmerets spent beating steadily. The validity of this procedure was checked by using the methods described previously to compare the swimmeret beating patterns from operated animals with those from intact animals.

**Physiological experiments**

*Experimental procedure*

Animals were anaesthetized in ice for 30 min. The eyelet baseplate was attached to the abdomen and the control threads to four swimmerets as for the behavioural experiments. The head, thorax and abdominal musculature were removed and the preparation was then pinned dorsal surface up in a Sylgard dish, and immersed in aerated crayfish saline (Van Harreveld, 1936) buffered to pH 7.4 with 10 mmol l⁻¹ Tris instead of bicarbonate. The cord was cut by a dorsal approach at the same point as in the behavioural experiments. Then the preparation was secured to a Perspex holding rod and placed so that the swimmerets and ventral abdominal surface were immersed in an aquarium filled with cold water (5°C), and the abdominal cavity was filled with cold saline. The four ipsilateral swimmeret threads were attached to the machine. *En passant* recordings were made from the first root of the fourth unattached swimmeret with fine silver hook electrodes. The movements of the attached swimmerets were recorded with a photoelectric cell which monitored a light beam reflected from a small mirror attached to the most posterior lever. The neural signals were amplified, filtered and stored on FM tape for subsequent display and analysis. The experimental design was the same as that used in the behavioural experiments although the length of the experimental sequences was somewhat shorter.

*Analysis*

The period of the controlled cycle and of the powerstroke bursting activity were measured. Phase and three-cycle average frequency plots were drawn as for the behavioural experiments.

*Results*

**Behavioural controls**

To ensure that cutting the ventral nerve cord is an acceptable experimental manipulation for increasing the amount of swimmeret beating without altering the structure of the beating, activity from intact animals was compared with that from animals with their ventral nerve cords cut.
Intact animals had fewer periods of beating under the experimental conditions. Some did not beat at all and only a few produced long sequences of steady beating. Of 19 animals prepared for behavioural experiments with cord sections, 13 exhibited long sequences of activity suitable for analysis, confirming the finding of Wiersma & Ikeda (1964) that this operation removes rostral inhibitions. There were no differences in the basic pattern of swimmeret movement exhibited by intact and operated animals. The operated animals had a normal phase-lag between ipsilateral swimmerets and normal contralateral synchrony. The proportions of each swimmeret cycle devoted to powerstroke, returnstroke and pauses were also the same.

The frequency of beating did not vary much during a continuous sequence. One effect of cutting the nerve cord was a change in the frequency range of different sequences. The mean frequency of sequences from intact animals showed a range of 1–3 Hz whereas operated animals produced sequences with mean frequencies in the 0.5–1.5 Hz range. A comparison of beating activity from operated animals and from intact animals, using the narrower band characteristic of the operated animals, showed that the variability of the two groups was similar. The variation in this frequency range was due either to deviations of short duration or to slow, long-term frequency drifts. It is unlikely that these changes would be confused with responses to imposed swimmeret movement. Intact animals showed greater variability when using higher frequencies (1.5–3 Hz) and occasional dramatic fluctuations.

**Imposed swimmeret movement**

**Possible responses**

In some preparations, the frequency of the unattached swimmerets was too erratic to allow synchronization of the attached ones for periods long enough for acceptable controls. Only sequences with clear initial synchronization were used in the analysis. Once synchrony had been established, the response of the unattached swimmerets was scored for both changes of machine frequency: the initial increase and the return to synchronization. Following a frequency change in the attached appendages, the unattached swimmerets could follow the change, ignore the change or respond to the change in a way that was not frequency-linked. If one considers the attached and unattached swimmerets as separate oscillating systems, then entrainment is the ability of one oscillator to phase-lock the output of the other. A number of responses short of full entrainment were observed, and were classified in the following way.

**Specific responses**

We classified a response as specific if it had a frequency-specific element. We found five categories within this group (Fig. 4B).

*Full entrainment:* the unattached swimmerets followed the imposed frequency change in both directions.
Swimmeret entrainment

Fig. 4. Summary of the categories used to classify observed frequency and phase responses in freely moving swimmerets following frequency changes imposed upon those attached. (A) The typical imposed frequency change to which the summarized responses are related. The dotted vertical lines in each graph indicate the timing of the imposed change. (B) Frequency-specific responses (entrainment or partial entrainment). (C) Nonspecific responses (no entrainment). (D) Phase responses accompanying (i) entrainment (coupling), (ii) no-entrainment (no coupling), (iii) intermittent entrainment at a preferred frequency (heterodyne synchrony).

**Partial entrainment. Delayed:** entrainment occurred but after a time delay. **Initial:** the unattached swimmerets showed initial entrainment but the frequency returned independently to its original level. **Oscillation:** the unattached swimmerets followed the imposed frequency change initially but then oscillated about the new frequency.

**General frequency response:** the frequency of the unattached swimmerets moved away from their resting level in an appropriate direction but without clear entrainment. This was an infrequently seen response.

**Nonspecific responses**

There were three types of response that were not frequency-specific and therefore had no element of entrainment (Fig. 4C).

**Sustained oscillation:** the variability of the unattached swimmeret frequency
Table 1. Occurrence and proportions of different responses to imposed frequency changes

<table>
<thead>
<tr>
<th>Number of swimmerets controlled</th>
<th>Entainment</th>
<th></th>
<th></th>
<th>No-entrainment</th>
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<tr>
<td></td>
<td>Full</td>
<td>Delayed</td>
<td>Initial</td>
<td>Oscillation</td>
<td>General response</td>
<td>Initial oscillation</td>
<td>Unpatterned</td>
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<td>Actual occurrence in each response category</td>
<td></td>
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<tr>
<td>Four</td>
<td>16</td>
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<td>2</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>2</td>
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<td>3</td>
<td>3</td>
<td>4</td>
<td></td>
<td>1</td>
<td>1</td>
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<td>Relative occurrence (%)</td>
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<tr>
<td>Four</td>
<td>45-7</td>
<td>2-9</td>
<td>5-7</td>
<td>11-4</td>
<td>11-4</td>
<td>17-2</td>
<td></td>
</tr>
<tr>
<td>Three</td>
<td>31-6</td>
<td>10-5</td>
<td>10-5</td>
<td></td>
<td></td>
<td>26-4</td>
<td>10-5</td>
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<tr>
<td>Two</td>
<td>14-3</td>
<td>14-3</td>
<td>28-6</td>
<td>28-6</td>
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<td>14-2</td>
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<tr>
<td>One</td>
<td>25-0</td>
<td>25-0</td>
<td>33-4</td>
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<td>8-3</td>
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* 13 animals were tested but some were used for a number of different swimmeret connections.

increased so that it oscillated about the initial frequency for as long as the imposed frequency was different.

Initial oscillation: initially the variability increased, as with the sustained oscillation, but then the frequency steadied to the pre-manipulation level.

Unpatterned response: there was some response by the unattached swimmerets to the imposed frequency change but it did not follow any pattern and was difficult to categorize and interpret. This was observed only infrequently.

The results of all behavioural experiments were classified according to these categories and tabulated for ease of comparison. The degree of coupling between the two oscillating systems is indicated by the phase graphs. When there was a steady frequency relationship between the systems, the phase value was constant.

If the frequencies were different, the phase decreased or increased according to which of the two oscillating systems had the higher frequency, and the phase graph had a sawtooth appearance (Fig. 4D). In some cases, the two oscillators drifted independently, except at certain preferred phase points when phase-locking occurred for only a few cycles before the drifting continued (Fig. 4D). When this heterodyne synchrony or gliding coordination occurred the phase graph had the saw-toothed appearance characteristic of independent oscillators but with the favoured phase position revealed by short periods of phase constancy.

Table 1 summarizes the results of 80 trials on 13 animals in which the movements of between one and four ipsilateral swimmerets were controlled.

Clear entrainment, in which the unattached swimmerets followed the frequency of the attached ones both up and down resulted most frequently (46 %) when four swimmerets were attached. Fig. 5 shows the results of an experiment in which full entrainment occurred and it illustrates the close synchronization achieved between
Swimmeret entrainment

Fig. 5. (A) Graph of the sequential frequency (three-cycle average) of the fourth attached (◯) and fourth unattached (●) swimmerets when the movements of four swimmerets were controlled and full entrainment resulted. Note the delay of six cycles before the unattached swimmerets followed the imposed change and the transitory undershoot in the frequency of the freely moving swimmerets after the controlled frequency was returned to its natural level. (B) The sequential phase plot of the fourth unattached swimmeret movement within the cycle of the fourth attached swimmeret during the sequence shown in A. The abscissa represents sequential cycles and the two arrows indicate the timing and direction of the imposed frequency changes.

the fourth swimmerets prior to the entrainment step and the way in which resynchronization typically occurred over about six cycles both at frequency increase and at frequency decrease. The accompanying phase plot shows that the two swimmerets maintained a relatively constant phase relationship throughout. The increased frequency of beating of the unattached swimmerets was achieved by a reduction in both powerstroke and returnstroke duration (Fig. 6C,D). As the reduction in powerstroke duration was less than that for the returnstroke and the pause between the two remained constant there was a slight increase in the powerstroke/returnstroke ratio (Fig. 6A,B). Throughout the study we used the returnstroke duration as an indicator of changes in cycle duration.

When only three swimmerets were controlled, the percentage of trials resulting in full entrainment fell (32 %) and almost half the step changes failed to produce any entraining effect (Table 1). In the trials where the unattached swimmerets were unaffected, the phase relationships of the two oscillating systems drifted independently of one another during the period of changed frequency (Fig. 7). There was a further reduction in the number of full entrainments (14 %) when the movements of two swimmerets were controlled, and no full entrainment responses
Fig. 6. Variation in basic cycle parameters in the unattached fourth swimmeret during the same experimental sequence shown in Fig. 5. (A) Interval between powerstroke and returnstroke movements. (B) Powerstroke/returnstroke ratio. (C) Returnstroke duration. (D) Powerstroke duration. The arrows on the abscissa indicate the timing of imposed step changes in the frequency of the attached swimmerets.

at all with only one swimmeret attached (Table 1). In all cases there were a number of partial entrainments involving delayed effects (Fig. 8), initial effects (Fig. 9) and oscillation effects (Fig. 10).

Physiological experiments

We examined the effect on entrainment of reducing sensory input which is phase-locked to the unattached swimmerets by severing all abdominal nerves contralateral to the attached swimmerets. While monitoring the fictive swimmeret rhythm in the ganglia of the unattached swimmerets by recording the motor activity in the cut swimmeret nerves (Fig. 11), the trials described in the behavioural experiments were repeated with four ipsilateral swimmerets attached and all contralateral nerve roots severed. The frequency measurements obtained were treated in the same way as those from intact animals to produce similar frequency and phase graphs (Fig. 12). The entrainment effect was markedly stronger under the altered experimental conditions, irrespective of the number of swimmerets controlled, and so the division into many categories of response was unnecessary. Of 15 trials on four animals 12 showed full entrainment, two showed partial entrainment and only one failed to show any entraining effect.
Swimmeret entrainment

Fig. 7. (A) Sequential frequency (three-cycle average) graph of the fourth attached (○) and fourth unattached (●) swimmeret when three swimmerets were controlled. The rhythm of the unattached swimmerets was unaffected by the imposed frequency changes. (B) The phase position of the fourth unattached swimmeret within the cycle of the fourth attached swimmeret and (C) the returnstroke duration of the fourth unattached swimmeret. Stars indicate missing values. The arrows on the abscissa indicate the timing of the frequency changes.

Discussion

The effects of sensory input on the periodicity of swimmeret movement that we have demonstrated here are quite different from those described previously. Davis (1969a,b) has shown that sensory feedback from the hairs of the lobster swimmeret evokes reflexes that are capable of initiating the returnstroke, but not the powerstroke, and has concluded that the sensory feedback is not part of the control system for swimmeret movement. Davis (1973) has also shown that when the swimmeret is restrained there is a change in the motor output to that swimmeret but no change in the period of the rhythm in the whole system. With a similar experimental approach, Heitler (1986) has examined the effect of small sinusoidal movements upon the crayfish swimmeret. With the limb in the posterior part of the range, he could demonstrate neither intra- nor intersegmental effects on motor neurones. In the anterior part of the range, small movements produced an intrasegmental reflex that increased the amplitude of motor neurone activity, evoked intersegmental reflexes and affected the activity of two unidentified interneurones, but did not affect the frequency. Sinusoidal movements of one
swimmeret were also shown to alter the amplitude of activity in its motor neurones through the activity of its nonspiking stretch receptors.

There has been some indication that sensory input may influence the timing of the rhythm but only in a limited way. West et al. (1979) found that interfering with the movement of some swimmerets sometimes affected the period of the rhythm. Oakley (1982) showed that static swimmeret manipulations may affect intercycle details in Cherax, and Heitler (1986) found that the movement of a swimmeret from one stationary position to another produced a transient increase in the frequency of the rhythm. All the reports mentioned the variability of the results and stressed the absence of long-term effects on the period of the rhythm. Although the results are not strictly comparable, the general conclusion can be made that sensory input is important for stability, for adjusting the intracycle timing details and for altering the amplitude of motor output to meet the external...
Fig. 9. (A) Initial response, sequential frequency (three-cycle average) graph of the fourth attached (◇) and unattached (●) swimmeret when two swimmerets were moved. After about a six cycle delay, the movements of the unattached swimmeret started to follow the attached swimmeret. After a short period in which the two systems showed loose coupling, the frequency of the unattached swimmeret drifted down and continued to oscillate about a level just above the original frequency until the end of the step. (B) The sequential phase position of the fourth unattached swimmeret within the cycle of the fourth attached swimmeret. Note the slowed drift in the phase relationship between the two systems during the period of partial entrainment. See Fig. 7 for an explanation of the stars. (C) The returnstroke duration of the fourth unattached swimmeret during the same sequence. The arrows on the abscissa indicate the timing of the frequency changes.

demands being made on the system; it can be considered unlikely that such inputs could affect the periodicity of the rhythm.

The very clear influence of sensory input on the frequency of rhythm shown by the present results, in contrast to the earlier studies, may be partly due to the type of preparations. Most of the previous studies were carried out on heavily dissected preparations, especially where swimmeret movements were imposed (Davis, 1973; West et al. 1979; Oakley, 1982; Heitler, 1986). Clearly a swimmeret motor pattern can be produced without any sensory feedback by an isolated nerve cord, but this does not necessarily imply that sensory input plays only a minor role in the intact animal. The earlier investigations may have altered the significance of sensory feedback because the techniques were more invasive than the present ones.

The methods used for the various swimmeret manipulations also complicate interpretation. The studies of Davis (1969a,b, 1973), West et al. (1979) and Oakley
Fig. 10. Oscillation, sequential frequency (three-cycle average) graph of the fourth attached (◇) and unattached (●) swimmerets when one swimmeret was controlled. The imposed frequency change caused the frequency of the unattached swimmeret to oscillate about a level slightly above the control level. (B) The sequential phase position of the fourth unattached swimmeret within the cycle of the fourth attached swimmeret. Note that although there was no coupling between the two systems, the phase drift was slowed at a favoured phase position which indicates interaction and transient coupling. See Fig. 7 for an explanation of the stars. (C) The returnstroke duration of the fourth unattached swimmeret during the same sequence. The arrows on the abscissa indicate the timing of the frequency changes.

Fig. 11. Recordings of motor neurone bursting activity from the first root of the fourth swimmeret (upper trace) contralateral to four attached swimmerets, moved as indicated in the lower trace. The nerves to all four ipsilateral swimmerets were cut. The figure shows the rhythm following imposed movement at 0.67 Hz (A) and 0.86 Hz (B). Upward deflection of the movement trace corresponds to swimmeret powerstroke.
Swimmeret entrainment

Fig. 12. Graph of the frequency (three-cycle average) of the fourth attached swimmeret (○) and of the motor neurone bursting activity recorded from the contralateral fourth swimmeret nerve (●) when the movements of four swimmerets were controlled. All abdominal nerves apart from those to the attached swimmerets were severed. The tendency for the rhythm to entrain to the imposed frequency changes was so strong that the rhythm often followed multiple step changes as shown here. The arrows indicate the timing and direction of the changes. (B) A plot of the phase of the motor neurone bursting activity within the cycle of the fourth attached swimmeret for the sequence shown in A. The start of burst activity was used as the reference for the phase calculations.

(1982) restrained the swimmerets so that only static interactions would be revealed. It is likely that moving the swimmerets provides a more natural situation for a comparison of peripheral and central elements. Static interactions seldom occur during normal behaviour and the results may be a special case. Interpretation of the results of West et al. (1979) may be further complicated because unidentified command neurones were stimulated to obtain a basic rhythm.

The present experiments were based on the assumption that the role of sensory feedback would be most readily revealed if the movement imposed closely mimicked natural swimmeret movement and if the animal remained as intact as possible. Attention to these two factors may explain the differences between these results and those of other investigations and lend support to Neumann's (1985) argument that rhythmic behaviour is best investigated in largely intact preparations. In view of this line of argument, some justification is required for cutting the ventral nerve cord. The cord was cut because sudden unpredictable changes in frequency make it difficult to obtain long, reproducible bouts of regular beating. Cutting the nerve cord removes input to the swimmeret system from higher centres which not only can excite and inhibit the rhythm but also can change the frequency and other aspects of the output (Davis & Kennedy, 1972a,b). Unless
this variable was controlled it would be virtually impossible to examine the effects of local sensory input on the swimmeret pattern generators. Cutting the cord not only reduces the variability but also reduces the frequency range of 1–3 Hz seen in an intact animal to 0.5–1.5 Hz. The parameters of beating over this frequency range appear to be the same before and after cutting the nerve cord, which suggests that a reduction in variability has been achieved without significant alteration to the basic structure of the swimmeret motor pattern.

What is the relative importance or strength of the sensory system relative to the central system in producing a coordinated swimmeret rhythm? The central pattern-generating mechanism could be a strong element with sensory input playing only a secondary role or it could be a weak element which is reinforced by phasic sensory input. The clearest and strongest entrainment occurred when a great deal of sensory information (three and four swimmerets) was externally controlled. This could mean that entrainment can only occur when there is sufficient sensory information to overcome a strong central component. The experimental conflict would then be not between central and peripheral information but between two conflicting sources of afference. This view is supported by our finding that the removal of conflicting sensory information, produced by severing the nerves to the contralateral swimmerets, strengthens the entraining influence of the attached swimmerets on the fictive rhythm.

The results suggest that the central network exerts weaker control over the frequency of motor output than thought previously. The fact that entrainment occurs at all indicates that the system is responding to incorrectly timed sensory input and it is reasonable to assume that correctly phased sensory input is being integrated in the same way. Thus the entraining input is overriding the combined effect of the central component and its correctly phased sensory input. The variety of classes of both entrainment and no-entrainment responses suggests that perturbation of the system by aberrant sensory information destabilizes it. It could be argued that a strong central system would not be influenced by such a conflict, and therefore not show this instability.

A small percentage of preparations failed to entrain irrespective of the number of swimmerets controlled, which suggests that our procedure did not control all factors determining the strength of the entraining influence.

The influence of both proprioceptors and exteroceptors upon motor rhythms has been shown to be phase-dependent in a number of systems (insect flight: Mohl & Nachtigall, 1978; Pearson et al. 1983; Reichert & Rowell, 1985, 1986; lower vertebrate swimming: Grillner, 1979; Grillner et al. 1976; Wallén, 1980; crayfish walking: Sillar & Skorupski, 1986; Skorupski & Sillar, 1986). These central pattern generators appear to carry out phasic gating of sensory information and effectively phase-couple it to the central rhythm. Reichert & Rowell (1986) termed this ‘phase-coupled synaptic drive’. Phase-dependent reflex modulation can occur with
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both phasic and tonic sensory inputs. Pearson et al. (1983) found that they could only entrain the insect flight rhythm if the sensory input from the wing stretch receptors arrived at the correct time within the cycle and if the frequency of stretch receptor stimulation was close to the frequency of the flight rhythm. This is a strong indication that the sensory input may be part of the central pattern generator for flight. Although the results presented here do not permit similar conclusions to be drawn, the initial and delayed entrainment responses described here could be due to a similar type of phase coupling in the swimmeret system. The drift between the oscillating systems often slowed around favoured phase positions and, furthermore, we were only successful in influencing the rhythm when the controlling frequency was adjusted so as to be close to that of the free-running rhythm.

These results question the validity of the comparison often made between the crayfish swimmeret system and other examples of rhythmic locomotion in invertebrates and vertebrates. Our view of the crayfish swimmeret system now appears to be following a path similar to the one exemplified by insect flight in many respects. Following the early investigations of Wilson (1961) and Wilson & Gettrup (1963), the insect system was considered to be an example of a strong central nervous element producing and coordinating a motor output. Wilson (1961) showed that the flight rhythm could still be expressed by a deafferented thoracic nerve cord and Wilson & Gettrup (1963) found that tonic input from the wing stretch receptors could increase the flight frequency. Subsequently, it has been shown that the flight rhythm can be influenced by phasic receptor input (Wendler, 1974; Mohl & Nachtigall, 1978; Pearson et al. 1983). The current view is that the flight motor pattern is not produced by a dominant central pattern generator but that sensory input is equally important (Altman, 1983; Delcomyn, 1985; Wendler, 1985).

It is also interesting to compare the swimmeret system and the lower vertebrate swimming system. Both systems have many features in common in terms of structural components: they both have two oscillators per segment (Cohen & Wallén, 1980; Grillner & Wallén, 1984), both show weak, local, intrasegmental coupling, both show a constant phase-lag between segments and both have a distributed central network. Dogfish swimming was originally considered to be under strong peripheral sensory control (Lissman, 1946). Once it was demonstrated that an effectively deafferented animal could still produce coordinated motor outputs (Roberts, 1969; Grillner & Kashin, 1976; Grillner et al. 1976), it was concluded that there must be a strong central influence. As in insect flight, there has been a move away from the extreme positions. In both dogfish and lamprey, the imposition of sinusoidal tail movements on an effectively deafferented animal can entrain the efferent motor activity (Grillner & Wallén, 1977, 1982; Grillner et al. 1981). In the dogfish, entrainment occurs over a range of frequencies well above and below the resting level and very small amplitudes of movements are effective, indicating that entrainment occurs more readily in these vertebrates than in the insect and the crayfish. The investigations of dogfish and lamprey swimming
suggest that rhythmic patterning in vertebrates may also be achieved by a balanced, multi-component, central–peripheral mechanism.

This is the first demonstration that sensory feedback can affect the timing of the swimmeret rhythm and not just the amplitude of its components. The results show that the observed external rhythm is a composite of central and peripheral influences. In the absence of external perturbations, the centrally determined frequency is stabilized by sensory feedback. Sensory perturbations can, however, influence the rhythm and if a sufficient proportion of the sensory input is out of phase with the centrally determined rhythm then the afferent signals can become the pacemaker. The difference between these results and the previous findings probably arises from the use of relatively intact animals and from the use of imposed swimmeret movements which accurately copy the natural ones. Our results also indicate that the entraining frequency is only effective if it falls within a limited range close to that of the free-running preparation. The emerging view that rhythmic output is produced by multi-component distributed networks, (Altman, 1983; Delcomyn, 1985; Wendler, 1985), is further supported by these results from the swimmeret system of the crayfish.

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References


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