J. exp. Biol. **157**, 205–226 (1991)
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THE SWIMMERET RHYTHM AND ITS RELATIONSHIPS WITH POSTURAL AND LOCOMOTOR ACTIVITY IN THE ISOLATED NERVOUS SYSTEM OF THE CRAYFISH *PROCAMBARUS CLARKII*

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Accepted 30 November 1990

Summary

An *in vitro* preparation was developed consisting of the five thoracic and six abdominal ganglia of the crayfish nerve cord, isolated from anterior nervous structures and from peripheral sensory inputs.

The central activities of the thoracic leg, swimmeret and abdominal positioning motor systems and their relationships were studied. When motor outputs were tonic in the thoracic leg nerves (90 % of the preparations), continuous rhythmic activity occurred and persisted for several hours in the swimmeret nerves. Interruptions of the swimmeret rhythm were associated with rhythmic motor outputs in the leg nerves (10 % of the preparations). Motor activity in the abdominal positioning system was mainly tonic.

Swimmeret rhythm reversibly disappeared during application of a sucrose block between the thoracic and abdominal parts of the nerve cord. Electrical stimulation of the connectives posterior to the block induced bouts of rhythmic swimmeret activity.

Comparisons of the swimmeret rhythm (period) and the metachronal wave (duration, phase) showed that sectioning of the connectives between the thoracic and abdominal ganglia modified the period but did not affect the properties of the metachronal wave.

We conclude that the presence of descending inputs from thoracic ganglia is necessary for persistent swimmeret activity.

Introduction

In the last few decades, isolated nervous systems of invertebrates have been used to study the neuronal mechanisms generating rhythmic motor behaviour. The concept of a central pattern generator (CPG) has emerged from these studies and is defined as a network of neurones, located within the central nervous system,

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Key words: *in vitro* preparation, *Procambarus clarkii*, swimmeret rhythm, posture, locomotion, sucrose block.

that is able to generate rhythmic motor outputs in the absence of sensory inputs. Such networks have been analysed in detail in swimming leeches (Friesen *et al.* 1978; Kristan and Weeks, 1983), in flying and walking insects (Sombati and Hoyle, 1984; Reichert and Rowell, 1986; Robertson, 1986; Ramirez and Pearson, 1988) and in the stomatogastric system of crustaceans (see review by Selverston and Moulins, 1987).

In crustacean locomotion, three networks have been described and studied both *in vitro* and *in vivo*. Two of these are located in the abdomen: one controls the swimmeret system and the other the abdominal positioning system; the third network is located in the thorax and controls the leg movements.

The swimmeret motor system has been extensively studied since the pioneering work of Hughes and Wiersma (1960), Ikeda and Wiersma (1964) and Wiersma and Ikeda (1964), who described a centrally generated rhythmic activity responsible for swimmeret beating in a semi-isolated preparation of the abdominal nerve cord. The swimmeret motor system has been shown to consist of a series of CPGs (one for each swimmeret), organized as serial bilateral pairs in the abdominal ganglia (Ikeda and Wiersma, 1964; Davis, 1968*a*; Mulloney *et al.* 1990). Each CPG produces cyclic alternating bursts in the powerstroke (PS) and returnstroke (RS) swimmeret motoneurones it controls (Heitler, 1978). Within the abdominal nerve cord, all the CPGs are interconnected by intra- and intersegmental interneurones which control the overall swimmeret motor pattern (e.g. the sequential activation of the CPGs from rear to front producing a metachronal wave) or which can trigger or modify the swimmeret rhythm (Stein, 1971; Heitler and Pearson, 1980; Paul and Mulloney, 1985, 1986). Numerous studies following those of Ikeda and Wiersma (1964) were performed on descending neurones affecting the abdominal swimmeret motor outputs (Davis and Kennedy, 1972*a*,*b*,*c*; Bowerman and Larimer, 1974*a*,*b*; Williams and Larimer, 1981; Murchison and Larimer, 1990), which were called 'command fibres'. Since 1964, the concept of 'command fibres' or 'command neurones' has evolved greatly (Kupfermann and Weiss, 1978; Larimer, 1988); they are now considered as premotor interneurones able to turn on or to maintain a given motor pattern. The evolution of this concept was mainly due to studies on the abdominal positioning system (Evoy and Kennedy, 1967; Bowerman and Larimer, 1974*a*,*b*; Jellies and Larimer, 1985), which can be rhythmically activated during backward walking.

In the crayfish, the recent development of an *in vitro* preparation of thoracic ganglia (Sillar and Skorupski, 1986) allowed an intracellular approach to the walking CPG that, spontaneously or using pharmacological compounds, is able to express rhythmic patterned motor outputs defined as fictive locomotion (Chrachri and Clarac, 1990). The sensory-motor integration (Sillar and Skorupski, 1986; Skorupski and Sillar, 1986; Cattaert *et al.* 1990) and the neuronal wiring underlying intraleg coordination have also been studied (Chrachri and Clarac, 1989).

These three motor systems have been shown to interact in several different behaviour patterns in intact animals. The swimmeret system is rhythmically active while the abdomen is extended during walking (Williams and Larimer, 1981;

Kovac, 1974; Cattaert and Clarac, 1983), during postural righting reactions (Davis, 1968*b*; Neil and Miyan, 1986) or during antagonistic behaviour (Atema and Cobb, 1980; Barthe *et al.* 1989). However, in most studies using isolated or semi-isolated preparations, each of these three systems was considered as a separate entity.

In this paper, we present a new totally isolated *in vitro* preparation consisting of all the thoracic and abdominal ganglia. We have focused our study on the spontaneous activity of the three motor systems it contains in order to investigate the relationships between them and to try to define how central neuronal connections may be involved in producing complex coordinated behaviour patterns.

Materials and methods

Male and female crayfish *Procambarus clarkii* (Girard) were obtained from local suppliers and kept in tanks of circulating oxygenated fresh water.

Before dissection, the dorsal cuticle of the animal was removed and the sternal artery perfused with oxygenated saline (NaCl 195 mmol l^{-1}, KCl 5.5 mmol l^{-1}, $CaCl_2$ 13.5 mmol l^{-1}, $MgCl_2$ 2.5 mmol l^{-1}, Tris 10 mmol l^{-1} at pH 7.6) for 10 min to flush out the blood from the ganglia.

The isolated thoraco-abdominal preparation (Fig. 1) consisted of the nerve cord with the five thoracic ganglia (T1–T5) and the six abdominal ganglia (A1–A6) with the nerve roots of one side kept long enough to allow extracellular recordings. No sensory receptors were kept. The preparation was pinned down, dorsal side

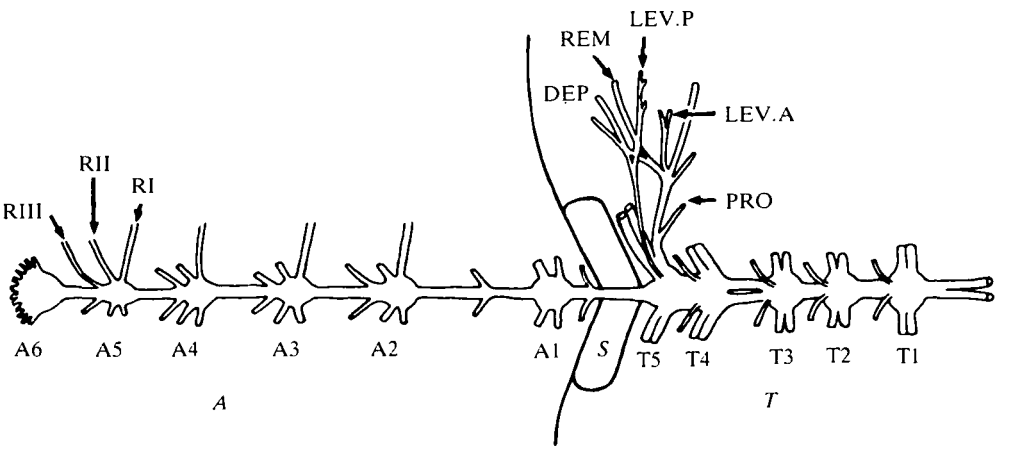

Fig. 1. The *in vitro* preparation consists of the five thoracic ganglia (T1–T5) and the six abdominal ganglia (A1–A6). Motor nerves to proximal leg muscles (promotor, PRO; remotor, REM; anterior levator, LEV.A; posterior levator, LEV.P; depressor, DEP) are labelled as well as the motor roots in the abdomen, the swimmeret nerve (RI), the second root to extensor muscle (RII) and the third root to flexor muscle (RIII). Solid lines correspond to Vaseline walls built to isolate the thoracic ganglia (*T*) from the abdominal ganglia (*A*) by a perfusion chamber (*S*) for sucrose application.

up, in a Petri dish coated with Sylgard (Dow Corning) and superfused with oxygenated saline maintained between 13 and 16°C by means of a Peltier cooling cell. To allow good perfusion of the tissue, most of the ganglia were desheathed. The connectives between ganglia T5 and A1 were also desheathed before Vaseline walls were built to form three chambers: *T*, *S* and *A* (Fig. 1). The *S* chamber could be perfused with an isotonic solution of sucrose (450 mmol l^{-1}, pH adjusted to 7.6 with 0.1 mol l^{-1} NH_4OH) to produce a conduction block.

Extracellular recordings were made with platinum wire electrodes (200 μm in diameter) from the proximal motor nerves of the fifth leg, chosen because their activity is crucial in characterizing the walking pattern, from the swimmeret nerve of each abdominal ganglion (first root, RI of A2–A5) and from the nerves to the extensor (second root, RII) and to the flexor (third root, RIII) muscles of the abdomen.

Intracellular recordings were made in the neuropiles of the desheathed ganglia using glass microelectrodes filled with 3 mmol l^{-1} KCl (resistance 10–25 MΩ). Current was injected into the neurones through the recording electrode *via* a bridge circuit of the amplifier (WPI, model 767).

The experiments considered for further analysis were selected according to several criteria which suggested that the central nervous system had survived the dissection: (1) regular sustained tonic activity or patterned rhythmic activity in the fifth thoracic leg nerves; (2) a modification of the motor activity of the three systems after stimulation of the ventral nerve cord, (3) rhythmic activity in the swimmeret nerves. If one or more of these criteria was not fulfilled, the experiment was not considered for analysis.

The results obtained from 30 isolated preparations were stored on a digital tape recorder (Biologics) and/or recorded on an electrostatic paper recorder (Gould ES 1000). Data analysis was performed using Sigmaplot software (version 3.1, Jandel Scientific).

Results

In the isolated thoraco-abdominal preparation, the outputs of the central networks of the three locomotor systems were recorded extracellularly; these were (1) the five proximal leg motor nerves of T5 for the thoracic leg system, (2) the first roots (RI) of A2–A5 for the swimmeret system and (3) the second and third roots (RII and RIII) of A5 for the abdominal positioning system.

The spontaneous outputs of all these motor systems were recorded and the swimmeret activities were analysed in greater detail.

Spontaneous activity in the thoraco-abdominal preparation

Thoracic leg and swimmeret activities

The activity recorded in the leg nerves was tonic in 90% of the preparations (e.g. PRO in Fig. 2). In motor nerves to the antagonist muscles of the two proximal joints (e.g. PRO–REM, LEV–DEP) it was consistently observed that

when strong firing occurred in one of the motor nerves (3–5 units firing at 4–10 Hz), weaker activity (1–3 units firing at 1–3 Hz) was present in the nerve to the antagonist muscle (not shown).

Tonic activity in the thoracic motor nerves was always associated with continuous rhythmic activity in the swimmeret motor nerves and both could last for 12–24 h. The rhythmic motor activity in the swimmeret system is organized by a set of eight hemiganglionic CPGs generating cyclic bursting activity in the swimmeret motoneurones. In each swimmeret nerve, two distinct bursts were recorded; they were characterized by the amplitude of the action potentials of the motor units as the returnstroke and powerstroke bursts (respectively RS and PS in Fig. 2). In our preparations, the swimmeret periods (measured as the time interval between the onset of two successive PS bursts) were between 1 and 2 s (Table 1). In the abdominal ganglion, interneurones of the CPG can be impaled whose membrane potential oscillates in phase with the swimmeret rhythm. The activity of each of the CPGs in the abdominal nerve cord is tightly correlated with that of the others, producing a sequential activation of the swimmerets in a metachronal wave travelling from A5 to A2.

In 10 % of the experiments, spontaneous rhythmic bursts were displayed in the leg motor nerves (Fig. 3). The temporal relationships between the motor bursts always corresponded to a very slow backward walking pattern, i.e. synchronous bursts in promotor and depressor alternating with synchronous bursts in remotor and levator (Chrachri and Clarac, 1990). The period of this fictive locomotion was very long and irregular (45–60 s). During these sequences of fictive backward walking the bursting activity observed in the swimmeret system became slower and there were prolonged interruptions. The period of the swimmeret rhythm increased by 50–80 % compared to that when the thoracic motor outputs were tonic. Moreover, the range of values of the swimmeret periods was comparable (1.8–2.5 s) with those observed in preparations in which abdominal structures were disconnected from the anterior structures. Fig. 3 shows such a preparation in which the interruption of the swimmeret rhythmic activity is associated with the beginning of the burst in the depressor nerve. In our experiments, no pharmacological compounds, such as muscarinic agonists, were used to maintain the spontaneous rhythmic activity in the thoracic nerves. This activity, therefore, disappeared progressively while the interruptions of the swimmeret rhythm became less frequent. When the rhythmic bursts in the leg nerves changed to tonic activity, the swimmeret activity became rhythmic, similar to the activity shown in Fig. 2.

Activity in the abdominal positioning system

Tonic activity was usually recorded in the abdominal motor nerves to postural muscles (Figs 2, 3). However, rhythmic activity in some of the motor units to these muscles occurred in one-third of our experiments (Fig. 4A). In these it was possible to observe, in both the flexor and the extensor nerves, motoneurones whose firing frequency was modulated within the cycle of the rhythmic swimmeret

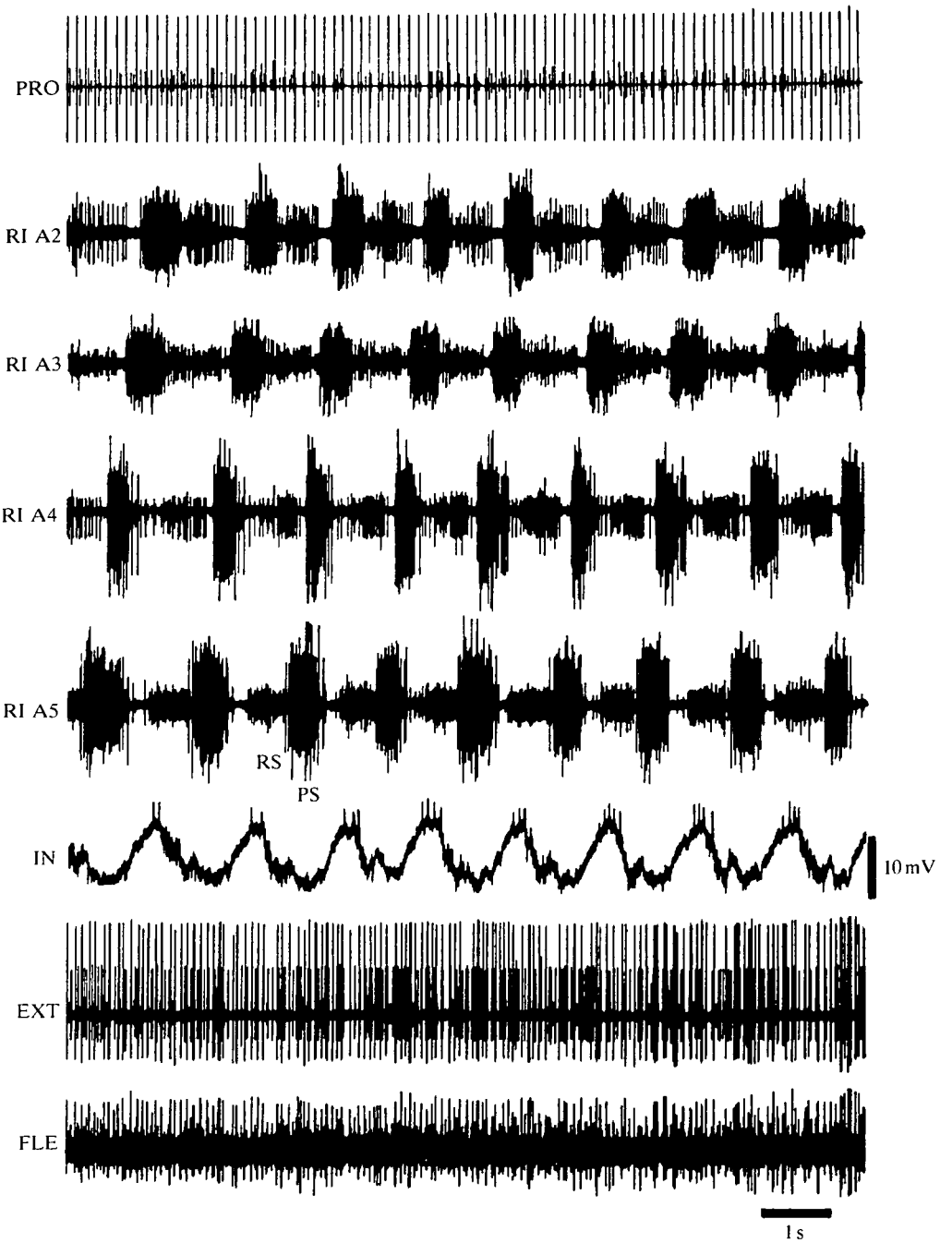

Fig. 2

activity (Fig. 4A). Moreover, in some experiments (less than 25%), a succession of long-lasting bursts (10–20 s) in the flexor and/or the extensor nerves was observed (Fig. 4B). This bursting activity could last up to 10 min and was

Fig. 2. Relationships between tonic thoracic activity and abdominal motor activity. Tonic motor activity is recorded in the thoracic leg nerves (e.g. PRO) as well as in the nerves to abdominal extensors (EXT) and flexors (FLE), while rhythmic motor activity is displayed in the swimmeret nerves (RI A2 to RI A5). The returnstroke (RS) and powerstroke (PS) bursts in the swimmeret nerves are indicated in the RI A5 trace. An intracellular recording from a swimmeret interneurone (IN) impaled in A5 shows membrane potential oscillations with depolarizations in phase with the returnstroke of the swimmeret activity in the same ganglion.

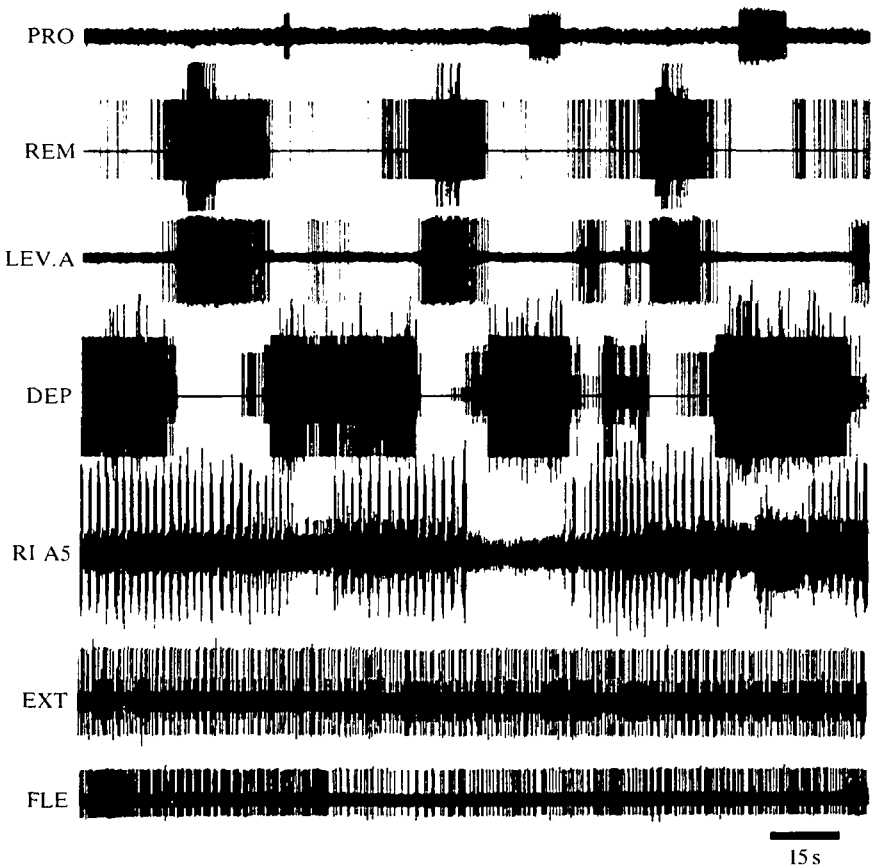

Fig. 3. Relationships between rhythmic thoracic activity and abdominal motor activity. Spontaneous rhythmic activity in the T5 motor nerves is recorded, and has a backward walking pattern: REM and LEV.A display synchronous bursts which alternate with the synchronous PRO and DEP bursts. Motor activity in the nerves to EXT and FLE is tonic. The swimmeret rhythmic activity in RI A5 is periodically interrupted at the onset of the DEP burst in this experiment. The swimmeret rhythm is slower when rhythmic thoracic outputs are recorded than when tonic activity is displayed (compare Figs 2 and 3; note the difference in the time scale).

Table 1. *Analysis of the rhythmic swimmeret motor activity in 19 experiments in which thoracic motor outputs were tonic*

	Before section			After section			
Experiment	Mean period±S.E.	Shorter period	Longer period	Mean period±S.E.	Shorter period	Longer period	*t*
*1	978.05±14.77	650	1900	–	–	–	–
*2	1024.00±6.11	800	1200	–	–	–	–
*3	1099.50±2.89	1000	1200	–	–	–	–
4	1122.50±7.50	900	1300	1917.50±16.19	1550	2500	44.54
*5	1123.50±6.53	1000	1350	–	–	–	–
*6	1141.76±15.07	950	2100	–	–	–	–
7	1165.70±5.24	1010	1280	2215.70±23.60	1840	3540	43.43
*8	1190.20±6.98	1000	1350	–	–	–	–
*9	1252.00±5.36	1150	1450	–	–	–	–
*10	1252.50±22.91	1050	2450	–	–	–	–
11	1261.00±8.92	1000	1500	2776.00±23.60	2200	3800	44.17
12	1367.00±6.60	1250	1600	1970.00±38.79	1600	4200	15.34
13	1384.25±10.90	1200	1800	1876.50±12.72	1650	2400	29.39
*14	1423.40±3.23	1360	1480	–	–	–	–
15	1451.25±13.88	1225	1900	2244.00±29.17	1650	3150	24.54
*16	1610.25±7.28	1300	1800	–	–	–	–
*17	1662.50±9.60	1000	1800	–	–	–	–
†18	–	–	–	1937.00±15.58	1200	2350	–
†19	–	–	–	1967.00±43.38	1100	4300	–

Period values are given in ms, for each sample $N=100$.

* The rhythmic swimmeret activity did not reappear after section of the connectives.

† Only the abdominal nerve cord was dissected.

The far right column gives values for Student's *t*-test calculated to compare the difference in the period before and after section.

For each experiment: degrees of freedom=198, for $P<0.001$ $t=3.291$. Student's *t*-test shows highly significant values of *t* for a nearly 100 % confidence interval.

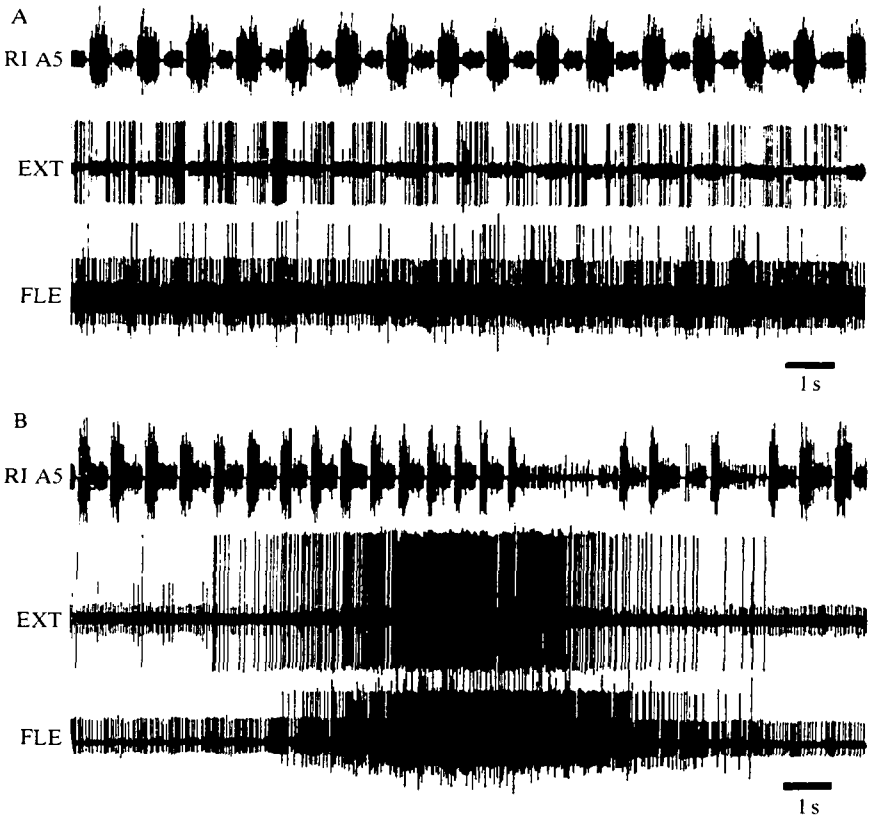

Fig. 4. Relationships between swimmeret activity and abdominal positioning motor activity. In some experiments the motor activity in the nerves of the abdominal positioning system (EXT and FLE) shows burst-like activity that is strongly correlated with the swimmeret rhythm (RI A5) (A) or long-lasting bursts that seem to be associated with a perturbation of the swimmeret rhythm (B).

correlated with a perturbation of the rhythmic swimmeret activity, which slowed down or even stopped.

These observations suggest the presence of central mechanisms that modulate the balance of activity between the abdominal positioning and swimmeret motor systems.

Relationships between thoracic and swimmeret activities

To characterize the action of thoracic inputs onto the abdominal motor systems, two preparations were utilized: (1) a thoraco-abdominal preparation with all 11 (thoracic and abdominal) ganglia and (2) an abdominal preparation with only the six abdominal ganglia. This was obtained either by applying a sucrose block or by sectioning the connectives between T5 and A1. In both types of preparation, the motor activity of the swimmeret system was recorded and three variables were

analysed: the period of the swimmeret rhythm, and the duration and phase of the metachronal wave.

The period of the swimmeret rhythm

Sucrose block on the T5–A1 connective. A reversible way of experimentally isolating the thoracic and abdominal ganglia was the application of an isotonic solution of sucrose to the desheathed connectives between T5 and A1. It is known that the propagation of action potentials is stopped by the superfusion of isotonic sucrose, but recovers after washing with normal saline (Russell, 1979). The efficacy of the sucrose block was tested by recording the swimmeret motor activity after electrical stimulation of the connectives between T5 and A1, either rostral (Fig. 5A) or caudal (Fig. 5B) to the sucrose block. During sucrose application, rostral stimulation of the connectives should not affect motor outputs caudal to the block. In control conditions (Fig. 5Ai,Bi), the stimulation elicited two modifications of the swimmeret motor pattern: it reset the swimmeret rhythm (see arrowheads in Fig. 5Ai) with a short latency and, after 3–5 s, it induced an increase in firing of some of the motor units in the fifth swimmeret nerve. Five to fifteen minutes after the superfusion of sucrose, the bursts in the swimmeret nerves stopped and tonic activity was recorded, while the thoracic motor outputs were not significantly modified. The number of swimmeret motoneurones spiking when sucrose was applied was also reduced; in Fig. 5Aii only three sizes of spikes can be distinguished. The units spiking during sucrose block were mainly small units, suggesting RS rather than PS activity. When trains of stimuli with the same characteristics as those in control conditions were applied to the connectives rostral to the sucrose block (Fig. 5Aii), no modification of the activity in the swimmeret nerves could be evoked, whereas stimulation of the connectives caudal to the sucrose block (Fig. 5Bii) elicited a short bout of bursting activity in the swimmeret nerves. The delay between the stimulation and the sequence of bursting activity varied in different experiments (compare Fig. 5Bii and Fig. 6B) and may well have depended on the position of the stimulating electrodes. When sucrose was washed off, the bursting activity in the swimmeret nerves recovered and the effects of the connective stimulations at the two sites were similar to those in the controls (Fig. 5Aiii,Biii).

A quantitative analysis of the effects of sucrose block on the swimmeret rhythm was performed in three experimental cases and results for one experiment are presented in Fig. 6. Each histogram represents the variations of the swimmeret

Fig. 5. Extracellular recordings from two swimmeret nerves (RI A4 and RI A5) during a sucrose block experiment. (A) Stimulation of the connectives between ganglia T5 and A1, rostral to the sucrose block, before (Ai) and during (Aii) sucrose application and after recovery (Aiii). (B) Stimulation caudal to the sucrose block, before (Bi) and during (Bii) sucrose application and after recovery (Biii). Stimulation train characteristics were identical: 400 ms at 30 Hz with 0.5 ms pulse duration at 6 V. Arrowheads in Ai indicate the onset of each PS burst in RI A5 if no stimulation of the connectives had been applied.

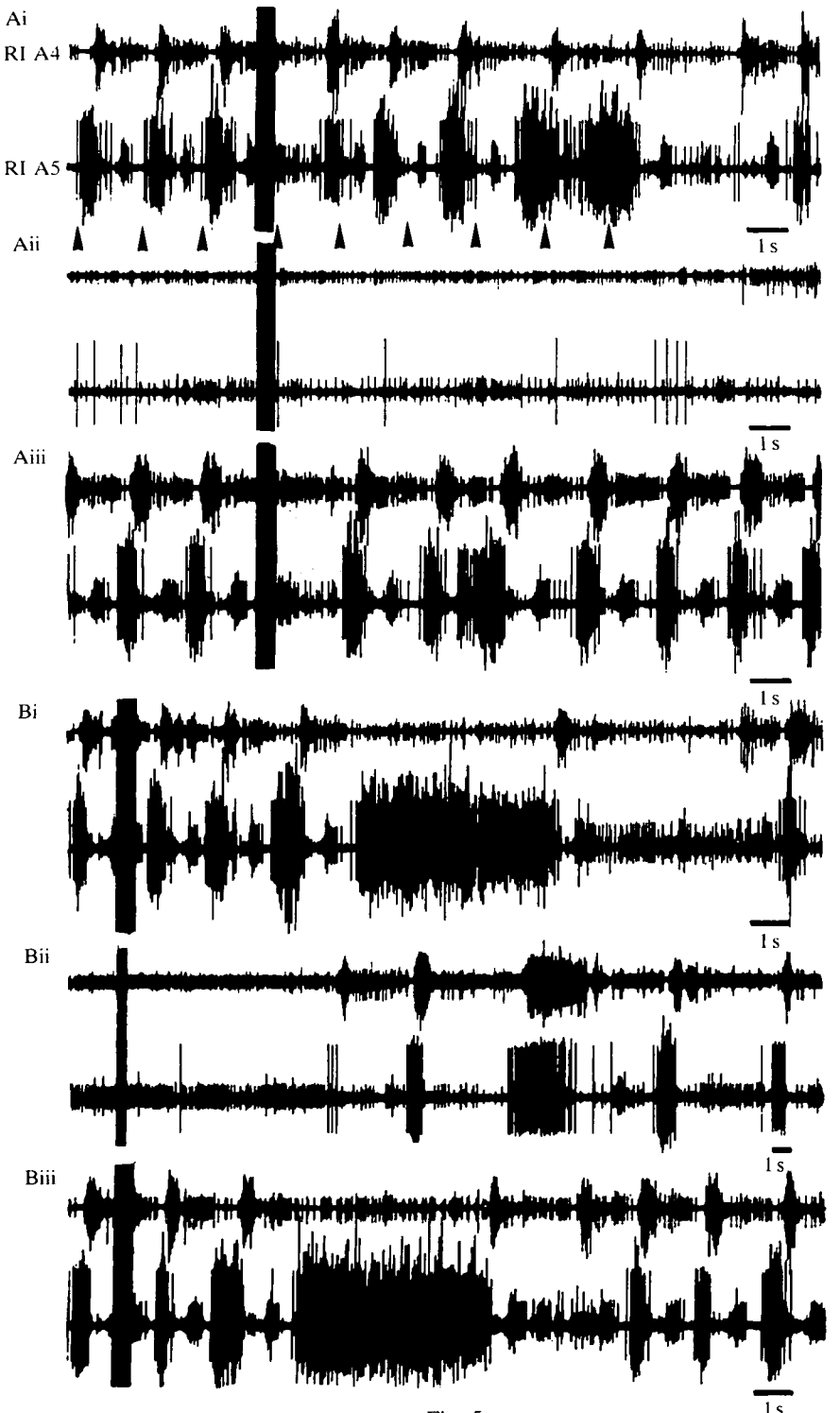

Fig. 5

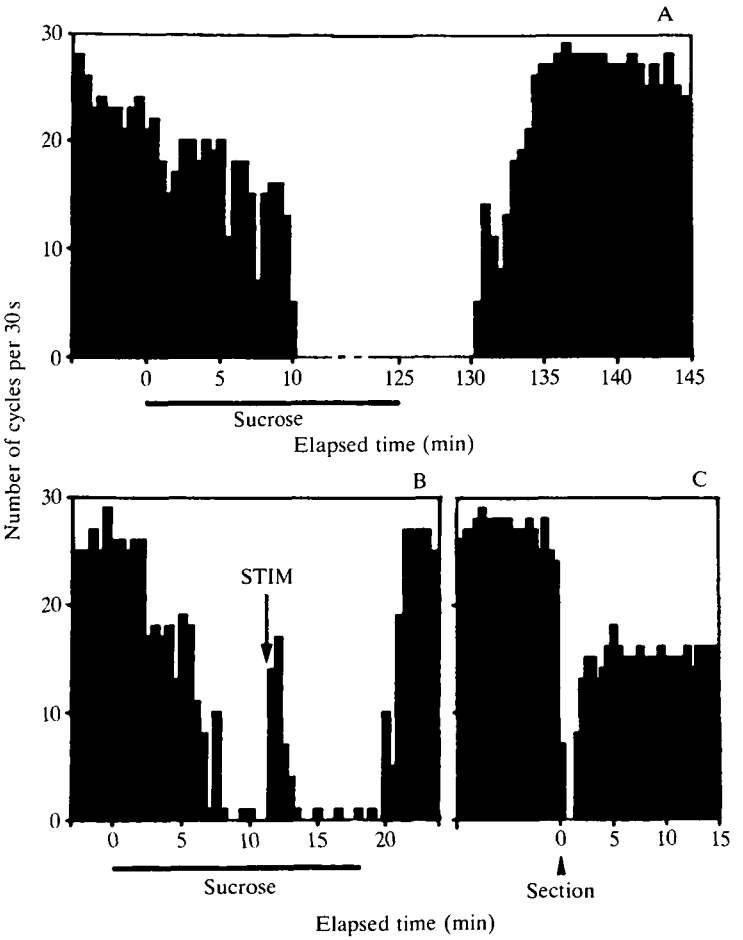

Fig. 6. Quantitative analyses of swimmeret activity in a single experiment. Each histogram shows the number of swimmeret cycles occurring during 30 s intervals as time elapses: (A) during application of sucrose (solid bar), (B) during a stimulation (STIM is one pulse: 0.5 ms duration at 6 V) of the connectives posterior to the sucrose block and (C) after section of the connectives caudal to the ganglion T5 (at time zero).

rhythm, as time elapsed, expressed as the number of cyclic bursts per 30 s interval. When the sucrose block was applied (Fig. 6A,B), the bursting activity in the swimmeret nerves decreased and then stopped. Washing off the sucrose led to progressive recovery to the control values. Application of the sucrose did not affect the ability of the swimmeret motor system to produce rhythmic activity; as shown in Fig. 6B, an electrical stimulation of the connectives caudal to the sucrose block (here a single shock) elicited some bursting activity in the swimmeret system. However, the number of bursts was smaller than before the sucrose application and the bursting activity was similar to that recorded in the isolated abdominal preparation after section of the connectives (Fig. 6C).

Section of the connectives. Rhythmic swimmeret activity can be recorded in the isolated abdominal nerve cord. When the connectives were cut between T5 and A1 in a thoraco-abdominal preparation, a large injury discharge was seen in the abdominal nerves followed by a disappearence of the swimmeret rhythm. In nearly half the experiments, swimmeret rhythm recovered within 10 min, but it had a longer cycle period than before section (Fig. 7A) and was frequently interrupted by tonic activity. However, if such a section was performed within the sucrose block, no injury discharge was seen and, from our observations, the swimmeret rhythm failed to reappeared within an hour.

When the swimmeret rhythm reappeared after section of the unblocked connectives, the period of rhythmic activity increased and the distribution of the period values widened, as shown in Fig. 7B and in Table 1. In the histograms (Fig. 7B), periods of more than 5 s were considered as interruptions of the rhythmic activity and were not included in the statistical analyses. In Table 1, the mean swimmeret periods for all experiments are given for a sample of 100 periods. The period was always shorter in the thoraco-abdominal preparation than in the corresponding abdominal preparation. Moreover, the standard error of the mean period was smaller for the former than for the latter, which explains the larger distribution of the periods in Fig. 7B.

A Student's *t*-test was performed on the periods for each experiment of Table 1 in which rhythmic swimmeret activity reappeared after section of the connectives. The *t* values (Table 1) gave a probability ($P \ll 0.001$), with a nearly 100% confidence interval, that the difference in the swimmeret periods resulted from the experimental condition, i.e. the section of the connectives. We can therefore conclude that there are significant differences in swimmeret activity in the two types of preparation: (1) the swimmeret period increases after section of the connectives and fluctuates more than in the thoraco-abdominal preparation, and (2) section of the connectives interrupts descending information from the thoracic ganglia that maintains a continuous rhythm in the swimmeret system.

The metachronal organization of the swimmeret activity

The other characteristic of the swimmeret rhythm that can be studied is the intersegmental coordination of the rhythmic activity in the four (A2–A5) abdominal ganglia. As the swimmeret period differed depending on whether the thoracic ganglia were present, we wanted to know if the metachronal organization was modified when the connectives were cut.

Two variables were studied: (1) the duration of the metachronal wave, which corresponds to the time interval between the consecutive onset of the PS burst in RI A5 and in RI A2 (*D* in Fig. 7A) and, (2) the phase of the metachronal wave, calculated as the duration over the RI A5 cycle period (*D*/*P* in Fig. 7A).

The duration of the metachronal wave was calculated under three experimental conditions: before section of the connectives, after section of the connectives, and during sucrose application when electrical stimuli applied caudal to the block were able to induce swimmeret rhythmic activity. In Fig. 8, the duration of the

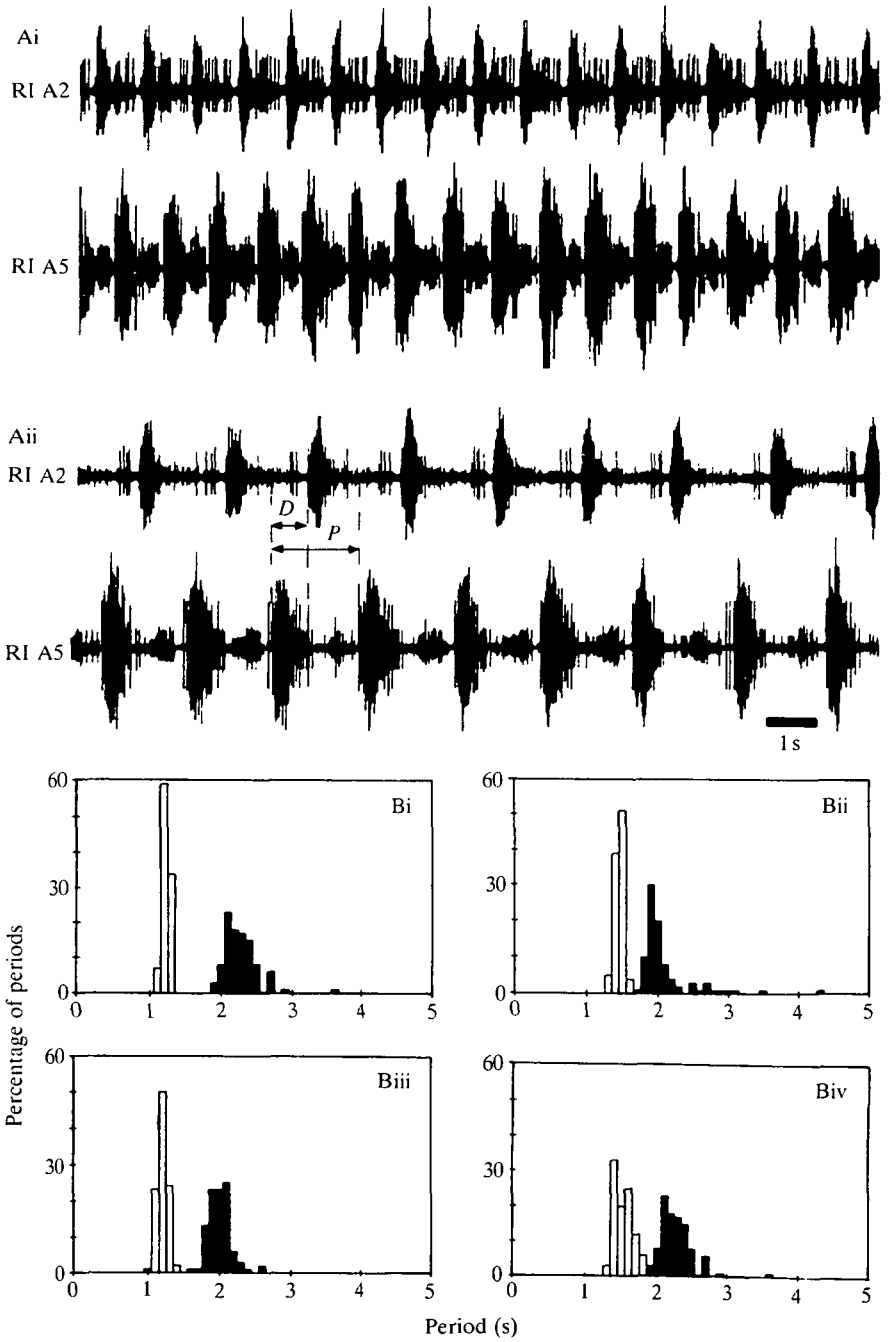

Fig. 7

metachronal wave is plotted against the swimmeret period measured in RI A5. In Table 2, the mean periods and standard errors are given as well as the coefficient of correlation for a first-order regression for each plot ($r1$). These last values indicate that, under each experimental condition, the duration of the metachronal

Fig. 7. Comparison of rhythmic activity before and after section of the abdominal connectives between T5 and A1. Extracellular recordings were obtained from the swimmeret nerves RI A2 and RI A5 in the same experiment before (Ai) and after (Aii) section. The duration of the metachronal wave (*D*) and the swimmeret period (*P*) are labelled. Four histograms (Bi–Biv) represent the distribution of the period of the swimmeret activity before (open bars) and after (filled bars) section of the T5–A1 connectives in four different experiments. The periods (between 0 and 5 s) are distributed in 50 bins of 100 ms.

wave is a linear function of the RI A5 period. Moreover, when a first-order regression is performed for all the data in each experiment, the coefficient of correlation ($r2$) indicates that they all correspond to the same linear function. Thus, we can conclude that the suppression of the thoracic information did not modify the intersegmental organization of the swimmeret system. This was confirmed when the phase of the metachronal wave was calculated (Table 3) and Student's *t*-tests were performed on paired series for experiments in which swimmeret activity was recorded before and after section of the connectives. The *t* value calculated ($t=0.1877$) does not show a predictable or significant difference of the phase in the two experimental conditions.

Discussion

The thoraco-abdominal preparation described in this paper allows the investigation of the central connections between three different motor systems whose CPGs control swimmeret beating, abdominal positioning and walking (Ikeda and Wiersma, 1964; Moore and Larimer, 1987; Chrachri and Clarac, 1990).

The novelty of this preparation is the total isolation of the ventral ganglia and nerve roots from the superior ganglia (brain and suboesophageal ganglia) and from all the sensory receptors except the sensory cells in the abdominal cord (Pabst and Kennedy, 1967). The main difficulty was to maintain sustained motor activity as the dissection took around 3 h. Preparations were obtained that remained fairly active for 12–24 h if (1) the blood was flushed out of the structures at the beginning of the dissection, (2) oxygenated saline was continuously superfused and (3) ganglia were desheathed.

Without the use of pharmacological compounds in this preparation the swimmeret system displayed rhythmic activity in 90 % of the dissections, whereas fictive locomotion (Chrachri and Clarac, 1990) occurred in only 10 % of the cases. In the abdominal positioning system, tonic activity was always recorded in the nerves to the abdominal extensor and flexor muscles, even though phasic bursts of spikes did occasionally occur.

The striking feature of the thoraco-abdominal preparation was the continuous stable rhythmic activity in the swimmeret system which persisted for hours, whereas in the abdominal preparation interruptions to the rhythm were frequent. In both types of preparations, the overall motor pattern of the swimmeret system was organized as described by several authors (Wiersma and Ikeda, 1964; Davis,

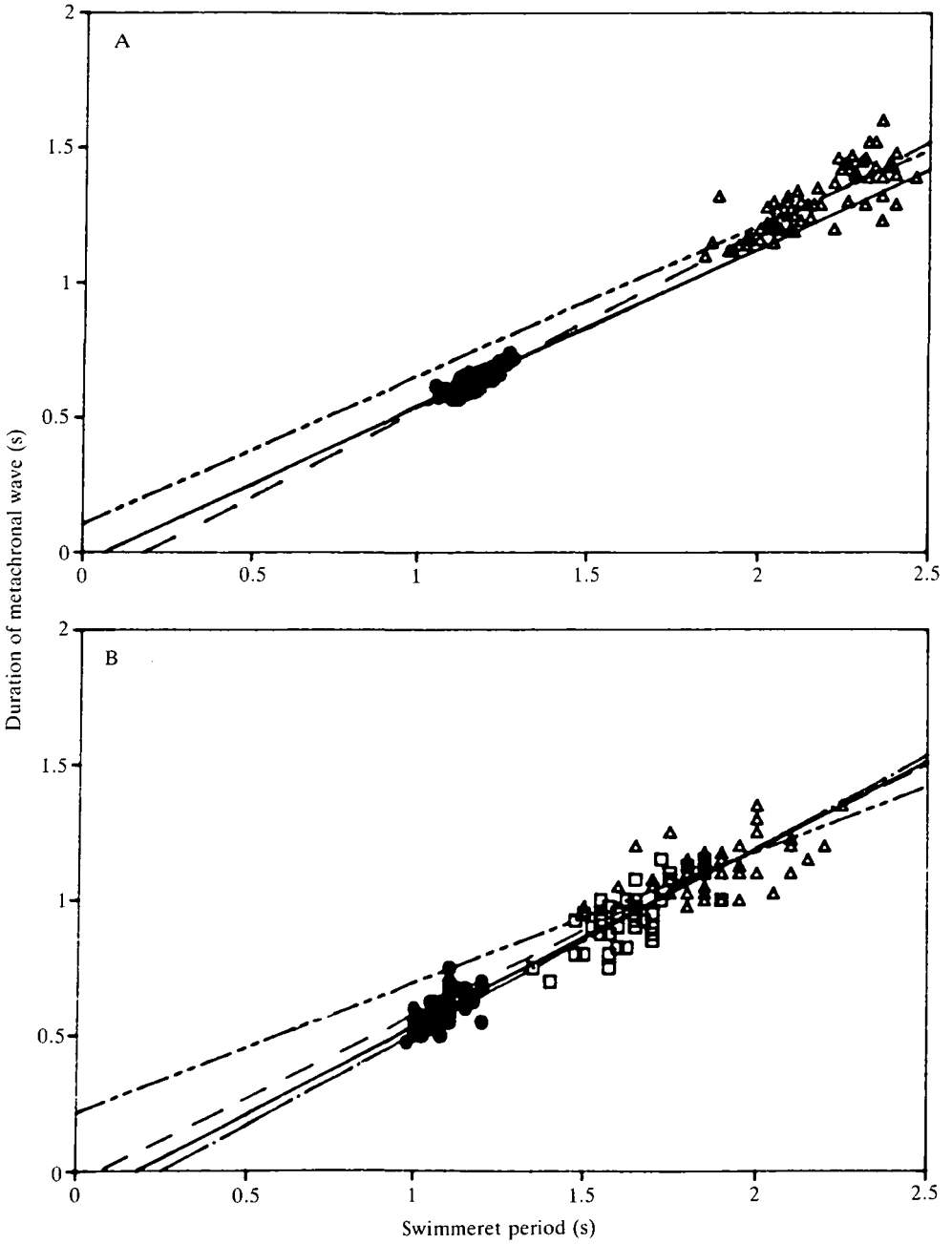

Fig. 8

1968*a*; Heitler, 1978; Mulloney *et al.* 1990). However, the mean period of the swimmeret rhythm was longer (980–1660 ms in thoraco-abdominal preparations and 1880–2800 ms in abdominal preparations) for *Procambarus clarkii* than in the crayfish *Pacifastacus leniusculus* [600–1000 ms in the thoraco-abdominal preparation (J.-Y. Barthe, personal observation) and 800–2000 ms in the abdominal

Fig. 8. The graphs show the duration of the metachronal wave as a function of the swimmeret period in A5 (see Fig. 7). The data are taken from two experiments. In A, two experimental conditions have been studied: before (●) and after (△) section of the connectives. In B three experimental conditions have been studied: before (●) and after (△) section of the connectives and when rhythmic activity is induced by electrical stimulation of the connectives during a sucrose block (□). Linear regression lines for each group of values are drawn (—— before section; –-- after section; –·– after stimulation of the connective during sucrose application) as well as the regression line (———) for all the values in each experiment. The coefficients of correlation of the regressions are given in Table 2.

preparation (Paul and Mulloney, 1985; Mulloney *et al.* 1990)]. This difference may be due to the species and/or to the methods: in most of the former studies parts of the animal (e.g. telson, rami,) or sensory receptors have been kept (Paul and Mulloney, 1986) or neuroactive compounds (e.g. proctolin) have been used to induce or enhance the swimmeret activity (Mulloney *et al.* 1987, 1990).

In the thoraco-abdominal preparation, the swimmeret rhythmic activity was spontaneously altered in several cases. When fictive locomotion was recorded, its pattern was always that of backward walking (Chrachri and Clarac, 1990) and the swimmeret rhythm slowed down and was interrupted by bouts of tonic activity that were locked with a particular phase of the walking pattern. This is not surprising since, under natural conditions during backward walking, the abdomen rhythmically flexes and extends while the swimmeret activity is inhibited during abdominal flexions (Kovac, 1974; Williams and Larimer, 1981). Furthermore, when phasic bursts occurred in the motor nerves to the abdominal flexor and extensor muscles, they seemed to be correlated with a slowing of the swimmeret rhythm. These relationships between the spontaneous activity in the three different motor systems are in agreement with previous observations: fibres able to inhibit the swimmeret rhythm during backward walking have been described by Kovac (1974) and interneurones acting on both the abdominal positioning system and the swimmeret system have been identified in abdominal ganglia (Williams and Larimer, 1981; Barthe *et al.* 1988; Murchison and Larimer, 1990). Such interneurones could be involved in driving and/or coordinating the different motor systems to produce complex motor interactions.

In thoraco-abdominal preparations in which the swimmeret rhythm was persistent, we were able to suppress it by application of a sucrose block between the thoracic and the abdominal part of the nerve cord. This effect lasted as long as the sucrose was present and the rhythmic activity always recovered after the sucrose had been washed out. Moreover, the ability of the swimmeret motor system to produce rhythmic motor patterns was not altered: stimulation of the connectives posterior to the block triggered a bout of rhythmic swimmeret activity with a similar pattern, but with a longer cycle period, than before application of the sucrose block. However, when the connectives were cut, two results were obtained. First, when the section was performed within the sucrose block while the rhythm in the swimmeret motor system was absent, the rhythm did not reappear.

Table 2. *Mean period of swimmeret activity and mean duration of the metachronal wave for two experiments for different experimental conditions*

	Experiment 1				Experiment 2					
	Before section		After section		Before section		*Sucrose block		After section	
	Period	Duration	Period	Duration	Period	Duration	Period	Duration	Period	Duration
Mean	1170.86	642.00	2189.71	1319.29	1088.00	591.00	1635.00	944.00	1897.96	1130.60
S.E.	48.57	34.54	195.26	131.59	53.51	58.85	112.48	105.41	179.41	126.99
$r1$	0.818		0.823		0.592		0.729		0.683	
$r2$	0.987						0.952			

Period and duration are given in ms.
*Swimmeret rhythmic activity was elicited by electrical stimulation of the nerve cord caudal to the sucrose block.
The coefficient of correlation of the linear regression is given for each condition ($r1$) and for each experiment ($r2$).
Note that thoracic motor outputs were tonic in these two experiments.

Table 3. *Mean phase values and standard deviation (S.D.) of the metachronal wave before and after section of the connectives in six experiments*

Before section		After section	
Phase	S.D.	Phase	S.D.
0.54	0.04	0.60	0.05
0.55	0.02	0.60	0.03
0.49	0.03	0.48	0.02
0.61	0.05	0.56	0.06
0.55	0.04	0.58	0.04
0.58	0.04	0.52	0.06

A Student's *t*-test on paired series [*t*(calculated)=0.187] does not show any significant difference between the phases for the two experimental conditions [degree of freedom=5, for $P<0.001$ *t*(table)=6.869].

Second, when the section was made posterior to the block or in the thoraco-abdominal preparation, a strong discharge from motor units in all the abdominal nerves was recorded and, after several minutes, in nearly half the preparations, the swimmeret rhythm reappeared, even though the cycle periods were longer and the rhythm was frequently interrupted. The analogies with the results obtained in the crustacean stomatogastric (STG) system are striking. In the STG preparation (see Selverston and Moulins, 1987), the continuous rhythmic activity in the pyloric and gastric CPGs is suppressed as long as a sucrose block is held on the only nerve (stomatogastric nerve) linking the superior ganglia and the STG ganglion in which the CPGs are located (Russell, 1979; Robertson and Moulins, 1981; Nagy and Miller, 1987). Moreover, when the stomatogastric nerve is cut, a stable but significantly slower motor pattern can be recorded (see review by Nagy and Miller, 1987). In these motor systems, it was concluded that inputs from interneurones located within the superior ganglia were necessary for the generation of the rhythmic motor patterns. These interneurones were described (see review by Nagy and Moulins, 1987) either as having modulatory effects that unmasked the cellular properties of the CPG neurones or as premotor oscillators that appeared to be necessary to sustain the rhythmic activity.

In the thoraco-abdominal preparation, similar interneurones may well be present, as the persistent expression of the swimmeret rhythm needs descending inputs from the thoracic ganglia. They may correspond to command neurones, as redefined by Larimer (1988), given that fibres found at different locations in the nerve cord are able to affect the swimmeret motor system (Wiersma and Ikeda, 1964; Davis and Kennedy, 1972*a*,*b*,*c*; Bowerman and Larimer, 1974*a*,*b*; Williams and Larimer, 1981). However, such interneurones in the thoracic ganglia need to be characterized, their pathways and types of action need to be described, and their modulation by biogenic compounds (e.g. amines, peptides; J.-Y. Barthe, M. Bévengut and F. Clarac, in preparation) needs to be clarified.

We are grateful to Dr R. A. DiCaprio for critical reading of the manuscript and for correcting the English. We thank Dr C. Chasserat for providing statistical analysis programs.

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