J. exp. Biol. 169, 181–206 (1992)
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REMOTE CONTROL OF THE SWIMMERET CENTRAL PATTERN GENERATOR IN CRAYFISH (*PROCAMBARUS CLARKII* AND *PACIFASTACUS LENIUSCULUS*): EFFECT OF A WALKING LEG PROPRIOCEPTOR

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Accepted 7 April 1992

Summary

1. An isolated preparation of the crayfish nervous system, comprising both the thoracic and the abdominal ganglia together with their nerve roots, has been used to study the influence of a single leg proprioceptor, the coxo-basal chordotonal organ (CBCO), on the fictive swimmeret beating consistently expressed in this preparation. Both mechanical stimulation of the CBCO and electrical stimulation of its nerve were used.

2. In preparations not displaying rhythmic activity, electrical or mechanical stimulations evoked excitatory postsynaptic potentials (EPSPs) in about 30% of the studied motor neurones with a fairly short and regular delay, suggesting an oligosynaptic pathway. Such stimulation could evoke rhythmic activity in swimmeret motor nerves. The evoked swimmeret rhythm often continued for several seconds after the stimulus period.

3. When the swimmeret rhythm was well established, electrical and mechanical stimuli modified it in a number of ways. Limited mechanical or weak electrical stimuli produced a small increase in swimmeret beat frequency, while more extreme movements of the CBCO or strong electrical stimuli had a disruptive effect on the rhythm.

4. The effect of low-intensity stimulation on existing swimmeret beating was phase-dependent: it shortened the beat cycle when applied during the power-stroke phase and lengthened it when applied during the returnstroke phase.

5. Rhythmic mechanical stimulation of CBCO or electrical stimulation of the CBCO nerve entrained the swimmeret rhythm within a limited range in relative or absolute coordination.

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Key words: *in vitro* preparation, swimmeret rhythm, leg proprioceptor, *Procambarus clarkii*, *Pacifastacus leniusculus*.

6. Electrical stimuli also produced systematic effects on the whole metachronal pattern of the swimmeret rhythm, perturbing the interganglionic coordination.

Introduction

One of the main questions that arise in studying the organisation of motor acts is to define how central neuronal networks and sensory inputs interact in the elaboration of motor patterns. From extensive studies in both vertebrates and invertebrates an answer to this question is beginning to emerge. Motor acts are under the control of central pattern generators (CPGs) able to produce the basic motor pattern, but themselves modulated, controlled or even rebuilt by peripheral sensory afferences (for reviews, see Roberts and Roberts, 1983; Bush and Clarac, 1985; Cohen *et al.* 1988).

The importance of sensory information depends to a great extent upon the characteristics of the motor systems involved. Thus, regular swimming movements performed in the uniform, supportive medium of water are less dependent upon continuous, accurate proprioceptive information than are walking or climbing, which demand continuous adaptations to changing terrain (Clarac, 1991).

In studies of the effect of sensory inputs on motor patterns controlling limb movements in arthropods, most attention has been paid to intrajoint reflexes evoked by proprioceptors. Thus, the thoraco-coxal muscle receptor organ (TCMRO) has been studied with regard to its control over protraction and retraction of the leg around the thoraco-coxal joint (Skorupski and Sillar, 1986), as has the coxo-basal chordotonal organ (CBCO) with regard to levation and depression of the leg at the coxo-basal joint (Clarac *et al.* 1978; El Manira *et al.* 1991*a*). However, individual proprioceptors can also have more widespread effects on other motor systems. The CBCO elicits interjoint reflexes in all segments of the leg (Bush *et al.* 1978), but also produces interleg reflexes (Clarac, 1985) and indeed influences motor activity in almost every appendage of the body, from the antennae (Clarac *et al.* 1976; Neil *et al.* 1982, 1984; Neil and Miyan, 1986) to the uropods (Schöne *et al.* 1976).

Moreover, these sensory–motor interactions result in phase-dependent reflexes during the existing movement. This has been demonstrated both for intrajoint reflexes (Sillar and Skorupski, 1986) and for interjoint reflexes (El Manira *et al.* 1991*b*). Such phase dependence of sensory integration is obviously also important during walking to coordinate the different CPGs controlling each leg (Müller and Clarac, 1990). These observations raise the question of whether there are more widespread interactions between proprioceptors and distinct CPGs involved in the same behavioural task.

The simultaneous activation of different motor systems during locomotion is a commonly observed phenomenon. In the lobster *Homarus gammarus* swimmeret beating accompanies forward walking, and there is evidence for phase coordination between the thoracic and abdominal rhythms (Cattaert and Clarac, 1983). Central interneuronal connections between the thoracic and abdominal oscillators

may play a role in this coordination, in much the same way as coordinating neurones between the individual swimmeret CPGs act to promote metachronal coupling (Stein, 1971; Paul and Mulloney, 1986). However, an indirect coupling arising from the activity of leg proprioceptors projecting onto the swimmeret CPGs might also exist. Such movement-related sensory feedback could provide timing signals that draw the abdominal oscillatory network into coordinated activity.

A number of *in vitro* studies on the isolated crayfish nervous system have shown that descending influences from the thorax can modulate the fictive swimmeret rhythm (Chrachri, 1990; Barthe *et al.* 1991). In this study, we have focused on the effects produced by a single leg proprioceptor, the CBCO, on the pattern of metachronal rhythmicity expressed by the abdominal ganglia controlling swimmeret beating. Our results provide evidence that these proprioceptive signals have a powerful influence on the whole swimmeret system, depending upon the state of the preparation, the strength of the stimulus and its timing within the metachronal cycle. A short communication of some of these results has been presented by Cattaert and Neil (1989).

Materials and methods

Experiments were performed on two species of crayfish, *Procambarus clarkii* (Girard) and *Pacifastacus leniusculus* (Dana) with similar results. In both species, an *in vitro* preparation of the nervous system was developed in which the thoracic and abdominal chains were isolated in continuity with each other and with their normal connections to particular thoracic and abdominal nerve roots retained (Fig. 1). On one side of the fifth thoracic ganglion the roots containing the motor axons to the major coxal leg muscles (i.e. the promotor, remotor, levator and depressor) were exposed. The CBCO and its sensory nerve supply were also dissected out intact. In abdominal segments 2–5, the ganglionic first roots supplying the swimmeret were retained on both sides, and in some cases were split into anterior (1a) and posterior (1b) branches (Fig. 1).

The isolated preparation was pinned out in a Sylgard dish and perfused with oxygenated saline (195 mmol l^{-1} NaCl, 5.5 mmol l^{-1} KCl, 13.5 mmol l^{-1} $CaCl_2$, 2.5 mmol l^{-1} $MgCl_2$, 10 mmol l^{-1} Tris at pH 7.6) at 10–12 °C. Extracellular recordings were made from chosen nerve roots using platinum pin electrodes, and the signals were amplified differentially using conventional techniques.

Following removal of the ganglionic sheaths, intracellular recordings were made from cells in the abdominal ganglia (the third or the fifth) using glass microelectrodes filled with either 3 mol l^{-1} KCl or Lucifer Yellow (3 %). Impaled neurones were identified as motor neurones when a one-for-one correlation could be established between spikes recorded intracellularly and either orthodromic spikes recorded extracellularly in motor nerves or antidromic spikes elicited by electrical stimulation of motor nerves. If no correlation existed they were classified as

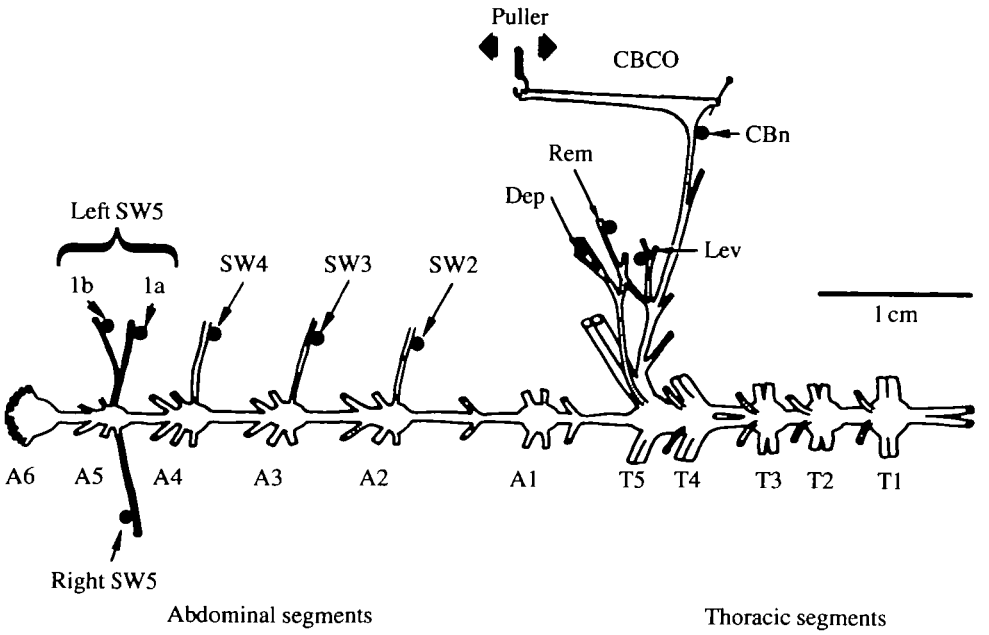


Fig. 1. Schematic drawing of the preparation. The ventral nerve cord was dissected out from the first thoracic to the last abdominal segments (T1–A6). The proximal innervation of one of the fifth legs (here the left) was retained, with the coxo-basal chordotonal organ (CBCO) and its nerve (CBn) attached. The motor activity to the basal leg muscles was monitored in the levator (Lev), depressor (Dep) and remotor (Rem) nerves (dots). The proximal end of the CBCO was pinned down in the Sylgard dish while its distal end was attached to the probe of an electromechanical puller (Puller). Activity in the swimmeret (SW) system was monitored by recording from the first roots of abdominal ganglia A2–A5 (dots). The SW root was sometimes split into its two main branches (1a and 1b) containing axons of the main returnstroke and powerstroke motor neurones, respectively.

interneurones. In some experiments, these electrophysiological identifications were confirmed by Lucifer Yellow staining of the intracellularly recorded neurone.

In order to activate selectively the different populations of fibres known to be present in the CBCO (Bush, 1965; Whitear, 1962), and to stimulate them in a natural way, stretch and release movements of the receptor strand were imposed by clamping the proximal end of the receptor strand and attaching the distal end to the probe of an electromechanical puller. To monitor the induced sensory activity, *en passant* recordings were made from the CBCO sensory nerve. It was found that excessive stretch of the strand produced irreversible damage to the sensory structures, with consequent loss of afferent activity. Therefore, length increases of 10–15% above the resting value (2.4–2.8 mm) were routinely used (limited movements) and in no case were length changes of greater than 30% imparted (extreme movements), although *in vivo* stretches may reach 40%. Mechanical

stimulation of the CBCO by the electromechanical puller was driven by the output voltage signal from a sine/ramp generator. Electrical stimulation of the CBCO nerve, and of other chosen nerves, was produced by an isolated stimulator with variable-voltage output (Grass) and achieved by delivering trains of electrical stimuli through bipolar pin electrodes.

For detailed investigation of the relationships between CBCO stimulation and the rhythmic activity in swimmeret motor roots, electrical and mechanical stimuli were used. Mechanical stimulation has the advantage of separately activating stretch- and release-sensitive sensory inputs. For precise timing, step movement were used, with the disadvantage that, during steady states, position-coding fibres fire continuously. Electrical stimulation also allows precise timing, with the advantage of recruiting the largest axons (velocity coding) at low intensity; when higher intensities are used, small fibres (position coding) are also recruited. One disadvantage of this approach, however, was that electrical stimuli recruited the afferent fibres from all the different receptor types of CBCO indiscriminately, a situation that would never occur naturally. Allowance has to be made for this in the interpretation of experiments involving electrical stimulation.

The recorded signals were fed to a chart recorder (Gould 1000S) or to an FM (Racal) or digital (DTR 800, Biologic) tape recorder. For data analysis, individual nerve spikes or bursts of spikes were converted to events by an A/D interface (Cambridge Electronic Design 1401) linked to a microcomputer (Tandon PCA20). The relationships between these events were then determined by a number of programs written within the environment of the software package SPIKE2 (Cambridge Electronic Design). The time of initiation of each swimmeret powerstroke burst was extracted by the program (see Figs 4 and 5) and the swimmeret period was calculated from these values. To highlight the effect of a CBCO stimulation on the swimmeret rhythm, a mean period over a number of cycles before stimulation was calculated (3–10 depending on the stimulus regime). A relative period (the real period as a percentage of the mean value) was then calculated for equal numbers of cycles before and after stimulation. An average over all the stimulations was then calculated and plotted to show how stimulation of the CBCO induced modification of the swimmeret rhythm. Modifications of the membrane potentials in intracellularly recorded neurones resulting from CBCO stimulation were analysed using the SIGAV software package (Cambridge Electronic Design).

Results

Triggering of swimmeret beating by CBCO stimulation

The isolated nervous system preparation (Fig. 1), which included both the thoracic and abdominal chains, expressed a strong and regular pattern of fictive swimmeret beating (see Figs 4, 5, 6, 7) in 90% of the experiments. Under the conditions we employed, this would continue for periods of at least 12 h without

significant alteration. This contrasts with the behaviour of the isolated abdominal chain alone, in which rhythmic activity is irregular (Barthe *et al.* 1991).

In recordings from the complete first motor roots of abdominal ganglia A2–A5, bursting activity occurred at frequencies of 0.2–2.0 Hz. In recordings from the separated 1a and 1b branches of the fifth swimmeret motor roots, the main returnstroke and powerstroke bursts could be clearly identified, firing in a reciprocal relationship (see Figs 5, 6, 7). The rhythmicity of the swimmeret has been described by several authors in the past and has recently been re-examined by Barthe *et al.* (1991). Only in a few preparations (10%) was the abdominal swimmeret system non-rhythmic, and in these cases there was a predominant discharge in the 1a branches of the swimmeret roots. Preparations displaying rhythmic activity in the thoracic motor nerves were not considered since it has been shown that such activity strongly altered the swimmeret rhythm (Barthe *et al.* 1991). Nevertheless, the thoracic motor roots displayed characteristic reflex responses to CBCO stimulation, and their activities provided convenient monitors of the integrity of central synaptic connections of this proprioceptor (see Fig. 6).

To test whether the CBCO could evoke responses in the swimmeret motor neurones independently of abdominal and thoracic CPGs, we have analyzed the effects of CBCO stimulation on swimmeret motor neurones in tonic preparations, i.e. those showing no tendency to produce rhythmic patterns in the thoracic and abdominal motor nerves. From intracellular recordings, it appeared that a large population of motor neurones (30% of the motor neurones recorded in the third abdominal ganglion, A3) received depolarizing events evoked by CBCO stimulation, with a fairly constant delay of about 30 ms (Fig. 2). If the distance between the recording and the stimulation sites (15–18 mm) is taken in account, it seems most likely that such events are due to oligosynaptic connections between CBCO terminals and swimmeret motor neurones. Both mechanical stimulation of a CBCO strand and electrical stimulation of the CBCO nerve have been used. Stretch of a CBCO strand had a very powerful effect, giving large EPSPs in the swimmeret motor neurones (Fig. 2A) as did electrical stimulation of the CBCO nerve (Fig. 2C). In contrast, release of the strand was ineffective (Fig. 2B). All the responses obtained in the motor neurone were excitatory (i.e. no inhibitory postsynaptic potentials occurred), and in some cases they led to the expression of the swimmeret rhythm. Nevertheless, the short latency of excitatory response could be followed by a delayed hyperpolarisation of the motor neurone when a large intensity of stimulus was used (see Fig. 7).

In the preparations displaying tonic activity in the swimmeret nerves, the CBCO was systematically stimulated, and the evoked response of the swimmeret system was observed through both extracellular and intracellular recordings. Imposed sinusoidal movements of the CBCO strand at 0.75 Hz (a frequency that approximates to the rate of movement in the walking leg) could elicit strong rhythmic activity (Fig. 3). This continued for as long as the stimulus was maintained, and for tens of seconds thereafter (10 s in Fig. 3), before subsiding to tonic activity. Intracellular recordings from interneuronal members of the swimmeret oscillator

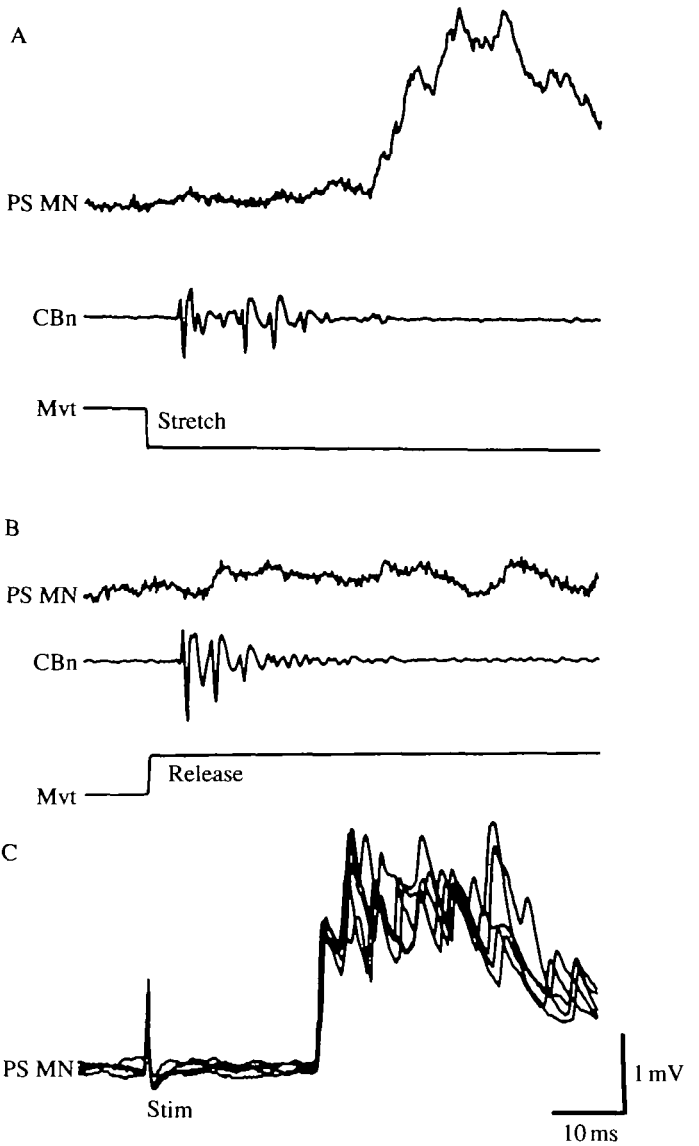


Fig. 2. In a quiescent preparation, mechanical stimulation of the CBCO strand and electrical stimulation of the CBCO nerve (CBn) evoke depolarizing events in swimmeret motor neurones, recorded intracellularly. (A,B) Responses to mechanical stimulation of the CBCO strand. Stretching the strand by 15% of rest length (A) induces depolarisation of a powerstroke swimmeret motor neurone (PS MN) while releasing it is not effective (B). (C) The same motor neurone shows depolarizing events evoked by electrical stimulation which are similar to the stretch-evoked depolarizing events. A and B are averaged traces over 20–30 strand movements. In C, the responses to five successive stimulations are superimposed. Mvt, movement.

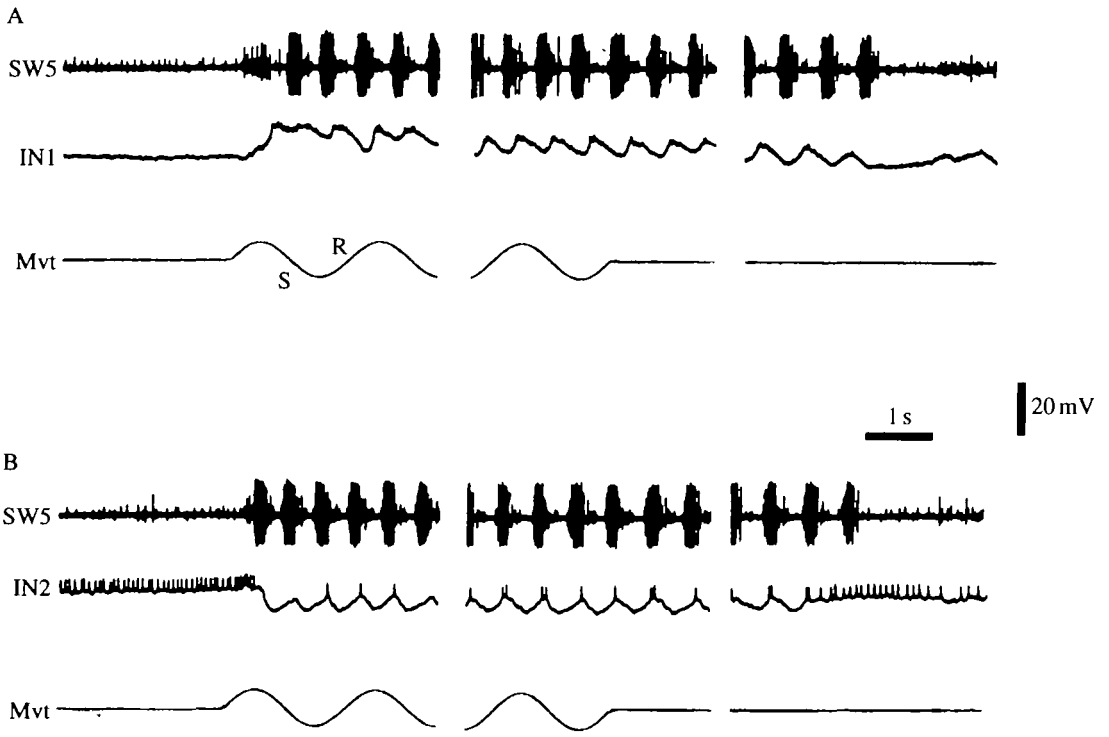


Fig. 3. In a quiescent preparation, an imposed sinewave movement of the CBCO strand (excursion 15% of rest length) induces rhythmical activity of the swimmeret (SW) system, monitored extracellularly in the first root of SW5. Two sequences are presented in A and B with two antagonistic interneurones (IN1 and IN2, recorded intracellularly in the SW5 ganglion), which start oscillating after depolarization (A) and after hyperpolarization (B). Left part: control and onset of the imposed movement. Middle part: during the imposed movement. Right part: after CBCO movement. S, stretch; R, release; Mvt, movement.

in A5 conveniently demonstrate the transition between these two states of activity (Fig. 3). The swimmeret interneurones that were silent during tonic activity started oscillating through an initial depolarization, while those that were spontaneously spiking became oscillatory through an initial hyperpolarisation (although a slight transient depolarisation could be observed in some cases).

Electrical stimuli were also able to trigger swimmeret rhythmic activity. In a non-rhythmic preparation, a single shock (0.3 ms) or a short train (0.3 ms pulses at 20 Hz for 0.2 s) of electrical stimuli to the CBCO nerve influenced the activity in the whole series of ganglia, A5–A2, which control the four pairs of swimmerets. In these cases, CBCO nerve stimulation evoked at least one metachronal wave of powerstroke bursts (see Fig. 12B). However, using a single mechanical stretch or release of the CBCO strand, such metachronal waves were more difficult to elicit.

Changes in the existing swimmeret rhythm induced by CBCO stimulation

Weak stimulation of the CBCO

Continuous weak mechanical stimulation of the CBCO strand. When limited oscillatory movements were imparted to the CBCO strand in preparations displaying regular rhythmic activity, small (1.5%), transient (5s) but consistent decreases occurred in the burst period of swimmeret 5 (Fig. 4). The period of the swimmeret rhythm was measured before and after the beginning of a stimulus bout (i.e. a 1 Hz sinewave movement of the CBCO strand for 20 s with 1 min between each bout) and expressed as a percentage of the mean period before stimulation. The average over 75 bouts showed a consistent decrease in period during mechanical stimulation of the CBCO strand (control mean 100±0.332; CBCO stimulation mean 98.76±0.293; ±S.D., N=810, P<0.01).

Weak electrical stimulation of the CBCO sensory nerve. Short trains of low-voltage stimuli (pulses of less than 4 V at 20 Hz for 0.2 s, repeated at intervals of

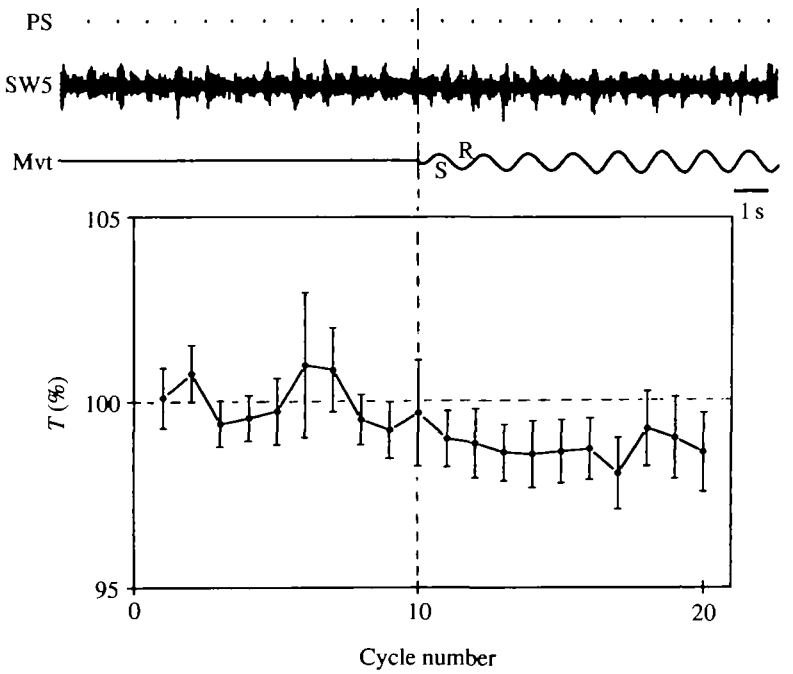


Fig. 4. Activation of the swimmeret system by imposed sinewave movement of the CBCO strand in a preparation displaying rhythmic activity. Each stimulus bout consisted of a 1 Hz sinewave movement of the CBCO strand (excursion 15% of rest length) applied for 20 s. Data were collected from 75 bouts with 1 min between each bout. The onset of powerstroke (PS) bursts has been derived from the extracellular record of activity in root 1b of SW5. The plot represents swimmeret beat periods before and during CBCO movement, expressed as a percentage of the mean period calculated over the 10 cycles immediately before the stimulation (T). Data represent mean values (±S.D.) (see text for statistical data). S, stretch movement; R, release; Mvt, movement.

30 s) were delivered to the CBCO nerve during periods of stable rhythmic activity in swimmeret 5. This voltage level represented the threshold for stimulation and would, therefore, have recruited only the large CBCO fibres. In most cases these stimuli caused distinct accelerations of the swimmeret rhythm persisting for at least 10 cycles, as seen both in the recorded traces and in the averaged data (Fig. 5A,B).

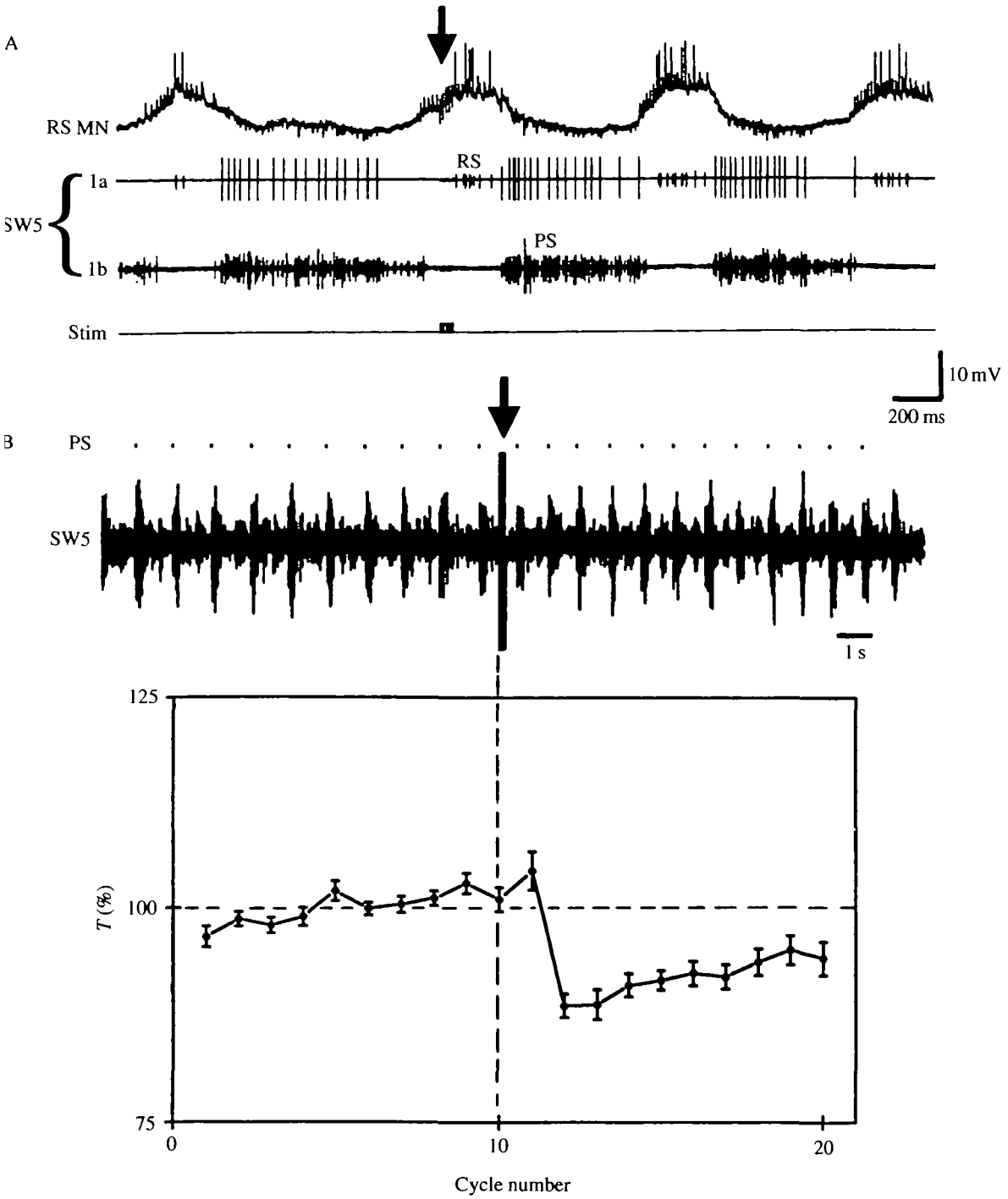
When the swimmeret rhythm was very slow (period greater than 1 s), CBCO nerve stimulation induced a shortening of the following cycles, the firing frequency being higher in both powerstroke and returnstroke bursts, as shown by the extracellular recordings (Fig. 5A). A statistical analysis of 80 such low-voltage stimulus trains to the CBCO nerve (Fig. 5B) confirms the impression given by the raw data shown in Fig. 5A; the swimmeret periods are shortened after electrical stimulation of the CBCO nerve. In fact, the main response was found to occur in the second cycle following the stimulation and to persist for a further 10 cycles (control mean 100±0.344; CBCO stimulation mean 93.35±0.538; ±S.D., N=680, P<0.001).

Strong stimulation of the CBCO

Extreme mechanical stimulations. When the amplitude of imposed CBCO movements reached 30 % of the resting length, their effect on swimmeret motor activity was found to change. When ramps of either stretch or release were imposed from a mid-position a classical resistance reflex onto the non-rhythmic thoracic motor roots was consistently observed: the firing rate of depressor motor neurones decreased during stretch (Fig. 6A) and increased during release (Fig. 6B), whereas the levator motor neurones behaved reciprocally.

In preparations displaying rhythmic swimmeret activity, an acceleration of bursting in swimmeret 5 was found to occur in response to ramps of extreme stretch (Fig. 6A), while bursting decelerated when the strand was released to the original mid-position. The effect of CBCO strand release on the swimmeret rhythm was more obvious when it was applied from the mid-position (Fig. 6B). The rhythm then nearly stopped for as long as the strand was released. CBCO

Fig. 5. Activation of the swimmeret system by weak electrical stimulation (Stim) of the CBCO nerve (trains of 3 V pulses at 20 Hz for 0.2 s). (A) Intracellular recording of a returnstroke motor neurone (RS MN) and extracellular monitors of activity in the 1a and 1b roots of the SW5 ganglion. Note that the returnstroke (RS) burst in 1a alternates with the powerstroke (PS) burst in 1b. The large-amplitude unit in 1a that fires simultaneously with the PS burst probably represents a ramus MN (see Cattaert and Clarac, 1987). The arrow on the intracellular trace indicates a change in the slope of depolarisation induced by electrical stimulation (Stim) of the CBCO nerve. (B) Data collected from 80 repetitions of the stimulus train, delivered at intervals of 30 s. The plot shows the swimmeret beat periods before and after electrical stimulation of the CBCO nerve, expressed as a percentage of the mean period calculated over the 10 cycles immediately before stimulation (T, same tests as Fig. 4). Data represent mean values (±S.D.) (see text for statistical data). The arrow marks the onset of stimulation.


A
RS MN
RS
1a
SW5
1b
PS
Stim
10 mV
200 ms
B
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SW5
1 s
125
100
75
T (%)
0
10
20
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Fig. 5

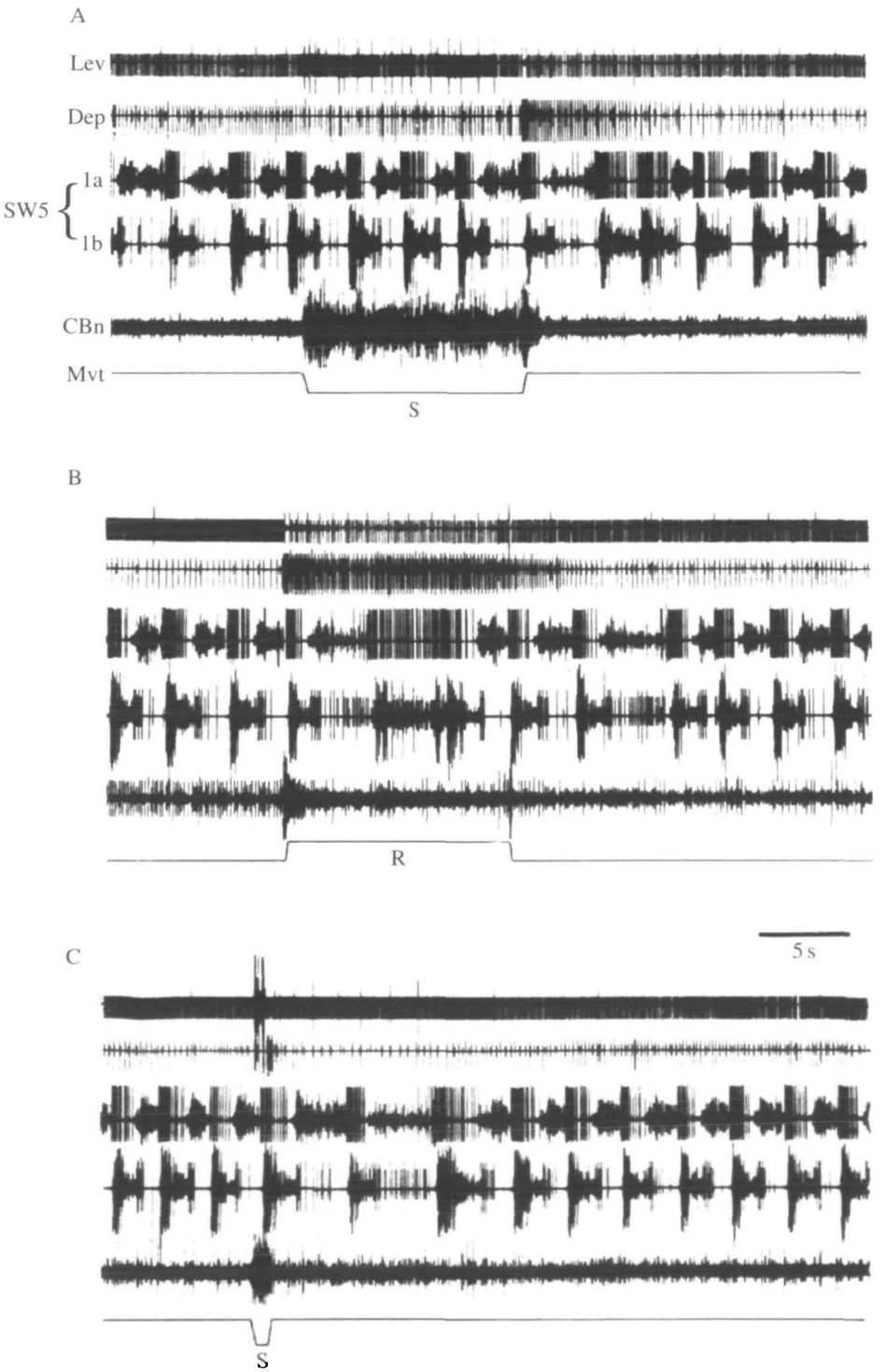

Fig. 6

Fig. 6. Simultaneous extracellular recordings from the CBCO nerve (CBn), levator nerve (Lev) and depressor nerve (Dep) on the left side of the fifth thoracic ganglion and of roots 1a and 1b on the left side of the fifth abdominal ganglion. (A) CBCO stretch (S) (excursion 30% of rest length) induces an acceleration of the rhythm. (B) Release (R) of the CBCO (from its mid-position) induces a slowing of the rhythm. (C) A rapid stretch (starting from the mid-position) and release of the CBCO induces a slowing of the rhythm that persists for 20s after the end of the movement. Mvt, movement.

stretch from this released position towards the mid-point then initiated a new sequence of bursting activity in the swimmeret roots.

A rapid stretch and release of the CBCO strand was also found to elicit a strong disruption of the swimmeret rhythm (Fig. 6C), suggesting a long-lasting effect on the system elicited by a strong but brief stimulation of the CBCO. Therefore, extreme movements of the CBCO strand evoke clear-cut direction-dependent responses (Fig. 6A,B), in contrast to the effect of limited sinewave movements imposed on the CBCO strand, which only accelerate the swimmeret rhythm (Fig. 4).

Strong electrical stimulation. In an 'active' preparation (Fig. 7) strong electrical stimuli (voltages ranging from 5 to 8 V) of the CBCO nerve stopped the rhythm. Since both the frequency and duration of the pulses were the same as in Fig. 5A and since the stimulation was delivered in the same phase of the swimmeret cycle, it can be assumed that the opposite effects obtained are related to the intensity of the stimulation. A short stimulation resulted in a long-lasting powerstroke burst (Fig. 7A) following an inhibition of the returnstroke activity, as shown by the intracellular recording of a returnstroke motor neurone. However, the rhythm recovered spontaneously after a few seconds. If, during rhythmic activity, the stimulation was prolonged over more than one cycle (Fig. 7B,C), this caused a slight increase in powerstroke activity, but no new returnstroke activity appeared. The rhythm was then stopped in a powerstroke phase for up to several minutes (data not shown). This occurred on both the ipsilateral and contralateral sides of the preparation. To confirm such actions, we have repeatly applied the same stimulation as that shown in Fig. 7A. This stopped the rhythmic activity (although transient excitation of a returnstroke motor neurone was observed during some of the CBCO stimulations) and facilitated powerstroke firing for as long as the stimulus train was applied (Fig. 7B). When the stimulation was terminated, the rhythm started again with a variable delay (cf. Fig. 7B and 7C).

Temporal interactions between CBCO stimulations and the swimmeret central pattern generator

To determine whether changes in the swimmeret rhythm evoked by weak stimulation of the CBCO involved either a general increase of motor activity or more specific effects on the swimmeret CPG, we have analyzed the temporal relationship between sensory inputs and the swimmeret rhythm.

Phase response curve

We first studied the effect of the phase of the CBCO stimulation within the swimmeret cycle in order to establish a phase response curve (Fig. 8). These

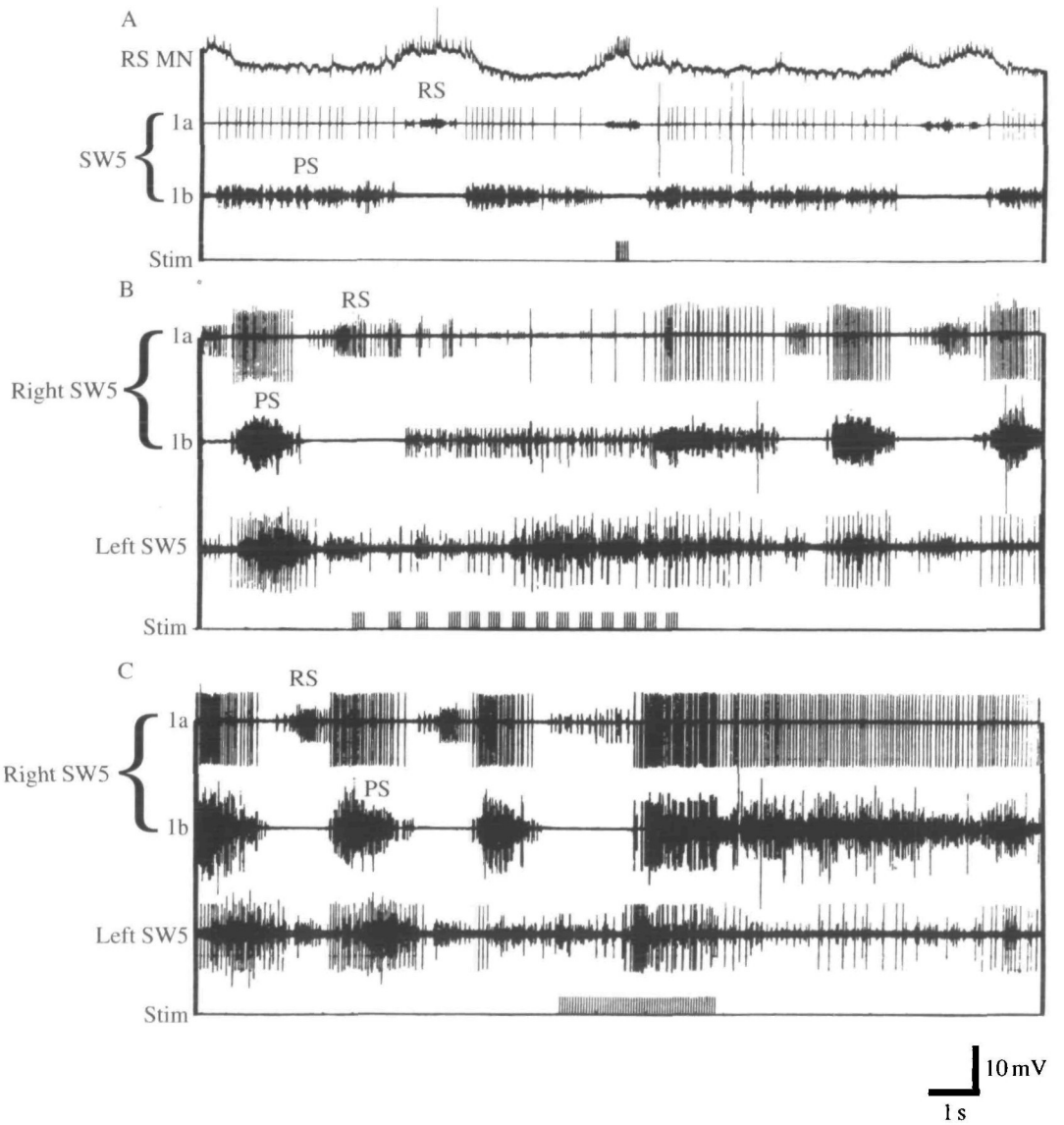

Fig. 7. Suppression of swimmeret rhythm by strong electrical stimulation (Stim) of the CBCO nerve (6 V pulses at 20 Hz). A single short train (0.2 s) modifies the firing (A), while repeated trains (B) or a longer train (for 3 s in C) block the rhythm. (A) Intracellular recording of a returnstroke motor neurone (RS MN) and extracellular recording of roots 1a and 1b of the fifth abdominal ganglion. (B,C) Extracellular recordings from roots 1a and 1b on the right side of the fifth abdominal ganglion (SW5) and from the undivided root 1 on the left side. RS, returnstroke; PS, powerstroke.

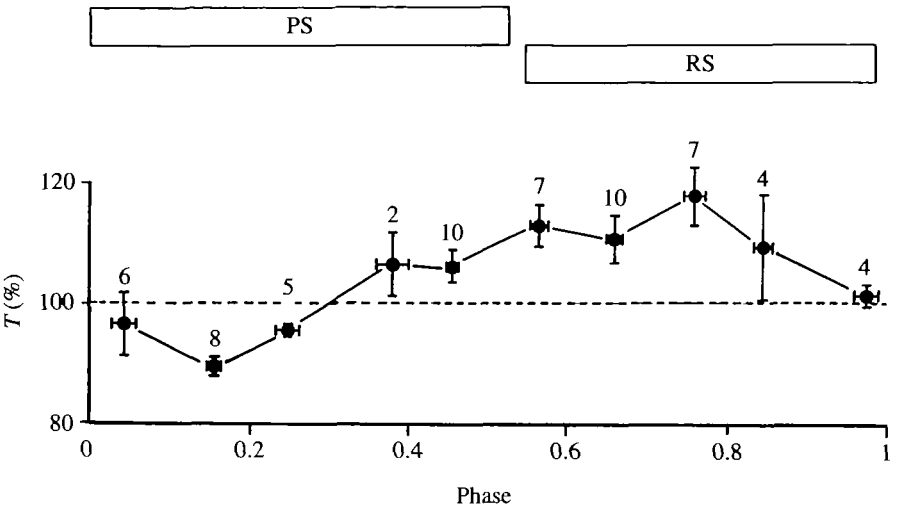

Fig. 8. The phase-dependent effect of weak electrical stimulation (3 V pulses at 20 Hz for 0.2 s) of the CBCO nerve on the swimmeret rhythm. The time of occurrence of stimuli within the swimmeret beat cycle (defined between the onsets of successive powerstroke bursts) is represented on the abscissa, and the changes in the swimmeret period relative to control values, determined over the three cycles before each stimulation (100 %), are shown on the ordinate (T). The mean phase values of powerstroke (PS) and returnstroke (RS) are indicated above the group. N=63. Vertical bars show standard error.

experiments were performed on preparations displaying a stable swimmeret rhythm. Considering the mean phase value (measured in relation to the mean of the three cycle periods before each stimulation), it appeared that when the stimulus was applied during the powerstroke phase it reduced the period, whereas when it was applied during the returnstroke phase it increased the period. This can also be seen in Fig. 5A, where the stimulation was applied just before the returnstroke burst: the course of depolarization of the recorded returnstroke motor neurone is interrupted by the stimulation (arrow in Fig. 5A) and its burst of spiking is subsequently delayed.

This result explains why averaged data indicated that the effect of electrical stimulation on the period of swimmeret beating appeared only during the second cycle following the stimulation (Fig. 5B). Since the stimulation was applied randomly with regard to the swimmeret cycles, and since the first powerstroke burst following electrical stimulation of the CBCO nerve was either delayed or advanced according to the stimulus phase within the swimmeret beat cycle, these effects would cancel out during the averaging process. The average value for the first period would, therefore, be expected to be close to the control value.

These experiments confirmed the twofold action of the CBCO in facilitating a very slow rhythm and in regulating the two phases of swimmeret movement. Stronger stimulation could even stop the rhythm in the powerstroke phase.

Entrainment of the swimmeret rhythm by CBCO stimulation

This problem has been studied using both mechanical and electrical stimulations.

Mechanical stimulation. When mechanical stimuli, similar to those used in Figs 2 and 3, were applied to the CBCO strand the result was a triggering of the swimmeret rhythm or, if it was already present, an acceleration of the rhythm. On occasion, the temporal relationships between the stimulus and the swimmeret rhythm also displayed relative coordination. Such coordination is shown in Fig. 9A, where the phase of the swimmeret 5 powerstroke burst onset was calculated in the cycle of the sinusoidal mechanical stimulus to the CBCO. The upper part of the graph shows a plot of the phase values analysed over 1 min. The

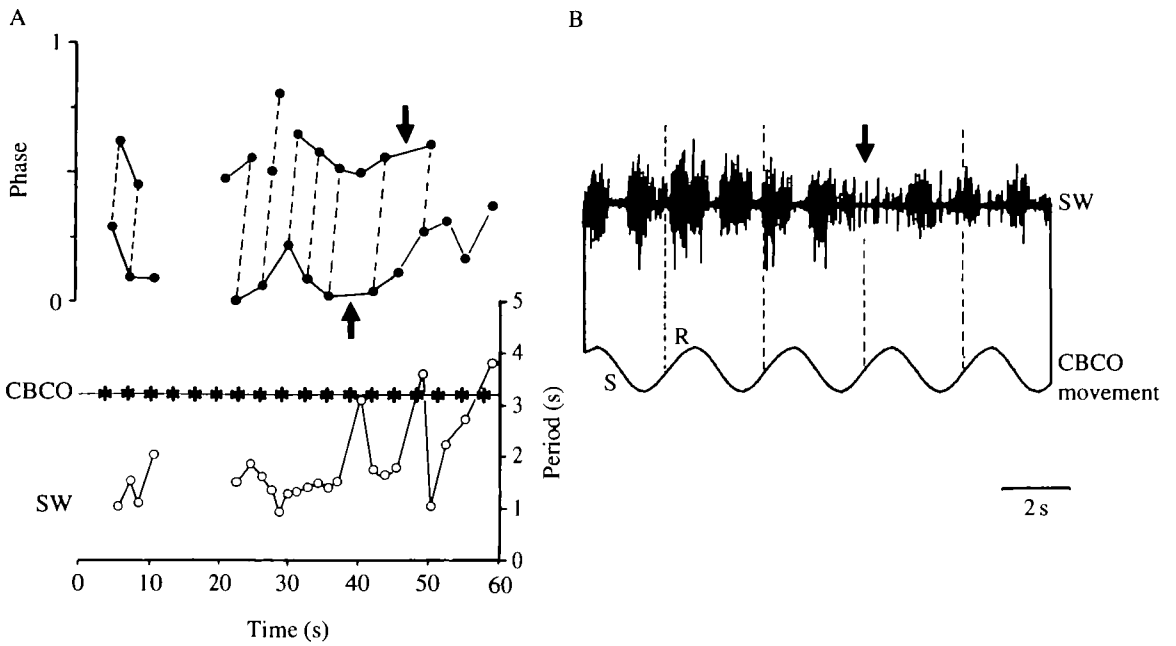

Fig. 9. Mechanical entrainment of the swimmeret rhythm by sinusoidal movement of the CBCO strand (15% excursions). (A) The lower plot shows the periods of the swimmeret rhythm (SW) (defined as the duration between the onset of successive powerstroke bursts) and of the imposed cycles of sinusoidal movement of CBCO strand. The period of the swimmeret rhythm is approximately half that of the mechanical stimulation. The upper plot shows the phase positions of the swimmeret beat cycles (measured from the start of the powerstroke bursts) within the cycles of imposed movement of the CBCO strand (defined between successive points of mid-release; see successive vertical broken lines in B). In the record shown, the swimmeret beat period is phase-locked to the stimulus with a 2:1 relationship, even after a transient arrest of the rhythm (arrows). (B) An example of an extracellular recording from the swimmeret nerve (SW), showing the 2:1 relationship and the occasional missing burst (arrow). S, stretch movement; R, release movement.

CBCO stimulation period was about twice the period of the swimmeret rhythm (Fig. 9A, lower part). In this experiment the phase values were preferentially locked at 0.1 and 0.6 over the period analysed. This is also true when the swimmeret rhythm stops transiently (see arrow in Fig. 9A,B). In these cases the period is twice as long as the previous ones and corresponds to the stimulus period. However, the next phase value is unaffected. In this experiment, the swimmeret rhythm was irregular and stopped for 10 s (break between 10 and 20 s).

This kind of relative coordination between the swimmeret motor output pattern and the cyclical stimulus to the CBCO was found only occasionally, depending upon the state of the preparation. When the swimmeret motor output displayed a strong and regular rhythm, entrainment was difficult to observe. But when the rhythm was more irregular, in every preparation in which the CBCO was able to elicit EPSPs in motor neurones some coordination could be observed. The coordinated sequences represented 5–25 % of the recording time. Sequences that displayed relative or absolute coordination were generally short (10 cycles) and were separated by sequences without any coordination.

Electrical stimulation. The same kind of analysis has also been carried out using electrical stimulations of the CBCO nerve. The voltage level of the stimulus trains was low (below 4 V), similar to that used in Fig. 5. Fig. 10A shows a preparation in which repetitive stimuli (6 s period) were able to induce a relative 2:1 coordination between the CBCO nerve stimulation and the swimmeret powerstroke bursts. More surprisingly, these repetitive stimuli, when applied with a period 80 % longer than the swimmeret period, were able to entrain the swimmeret rhythm, so that the swimmeret period extended to become equal to the stimulus period (Fig. 10B). Such relationships are shown in Fig. 11, where the electrical stimulation induced both a 2:1 and a 1:1 coordination. If during the experiment the periodicity of the stimulation was changed (here from 6 to 9 s), there was a subsequent modification of the swimmeret period, which at first followed the stimulus period (1:1 coordination, see arrow) and then decreased (2:1 coordination again).

One of the main features of the 2:1 coordination was the fact that there was a series of alternating short and long periods. This was a common finding when the period of the stimulus was less than twice the period of the entrained biological rhythm: whereas the first swimmeret burst was directly linked to the occurrence of the stimulus, the second one corresponded to a 'free-run' cycle (see star in Fig. 11A). In such instances, the first period was always shorter than the second one. This is shown in Fig. 11C: in this graph, periods of the swimmeret rhythm are plotted against the phase values. The shorter periods are associated with the shorter phases (first burst following the stimulus; filled symbols in Fig. 11C).

Effect of CBCO stimulation on intersegmental swimmeret coordination

Entrainment of the swimmeret rhythm by CBCO stimulation raises the possibility that this leg proprioceptor has multiple sites of action at different levels in the abdominal chain. We have investigated this possibility by studying the effect

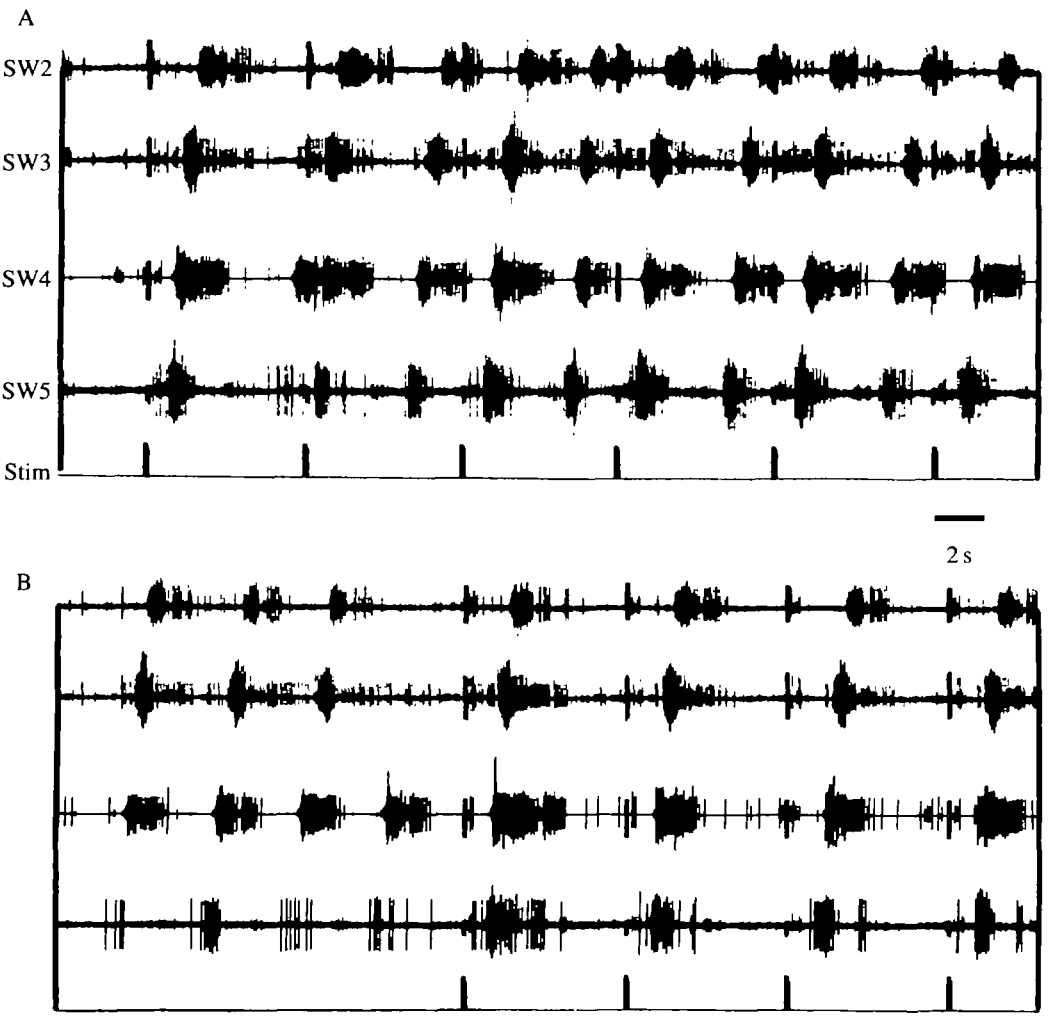

Fig. 10. Entrainment of swimmeret metachronal rhythm by weak electrical stimulation of the CBCO nerve (trains of 3 V pulses at 20 Hz for 0.2 s, delivered at intervals of 1 min). Extracellular recordings from roots 1b to SW2–SW5. (A) Relative coordination of the swimmeret rhythm to the electrical stimuli with a 2:1 relationship. (B) A free-running rhythm is slowed down by the same trains of electrical stimuli (Stim).

of CBCO stimulation on the interganglionic coordination of swimmeret activity. For this we measured metachronal wave delays, i.e. delays between powerstroke bursts from one abdominal ganglion ($d1$) to the more anterior one ($d2$). In Fig. 12A the delays between powerstroke bursts of adjacent ganglia are all identical (see the dotted line at burst onsets). Nevertheless, in some instances, when the swimmeret rhythm stopped the metachronal wave was incomplete since the swimmeret bursts in the anterior ganglia were missing (see arrows showing the times of expected bursts). From the data shown in Fig. 12B it can be inferred that

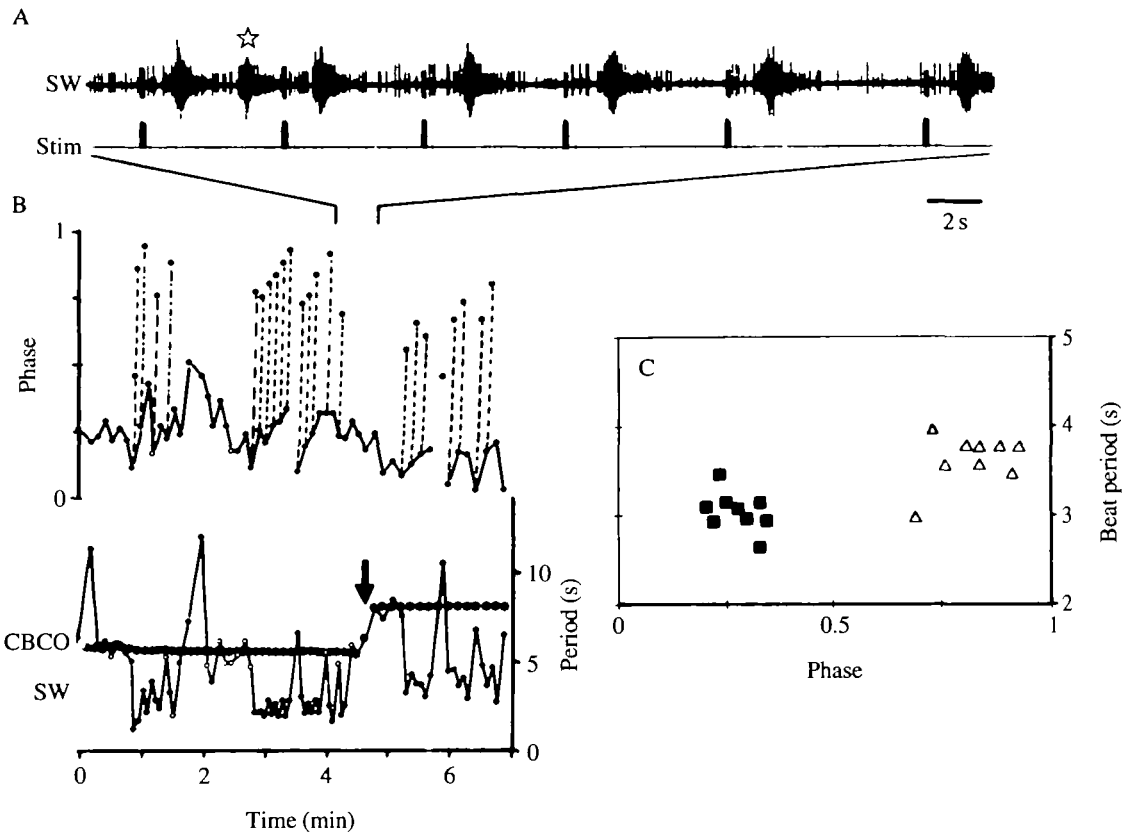

Fig. 11. Entrainment of the swimmeret rhythm to a changing frequency of weak electrical stimulation of the CBCO nerve (trains of 3 V pulses at 20 Hz for 0.2 s). (A) Extracellular recording from root 1 to SW5 (SW) during the transition in the period of the stimulation (Stim) from 6 to 9 s. Note the extra 'free-run' powerstroke (PS) burst (star) between the first two entrained bursts (see text for further description). (B) The lower plot shows the periods of the swimmeret rhythm (defined as the duration between the onset of successive powerstroke bursts) and of the imposed trains of electrical stimuli to the CBCO nerve. The upper plot shows the phase positions of the swimmeret beat cycles (measured from the start of the PS bursts) within the cycles of imposed electrical stimuli. Note the period of 1:1 coordination during the transition in stimulus period (arrow). (C) Plot of swimmeret beat period against the phase of powerstroke bursts within the cycles of electrical stimuli. Filled squares, first powerstroke burst after a stimulus; open triangles, second ('free-run') powerstroke burst after a stimulus.

the CBCO stimulations act on the swimmeret CPGs in the fifth ganglion since the stimulation elicited a single metachronal wave starting from swimmeret 5. However, when applied just after an incomplete metachronal wave, the stimulation of CBCO elicited a burst in swimmeret 3 that was no longer related to the metachronal wave (see asterisk in Fig. 12C). The swimmeret rhythm appeared to

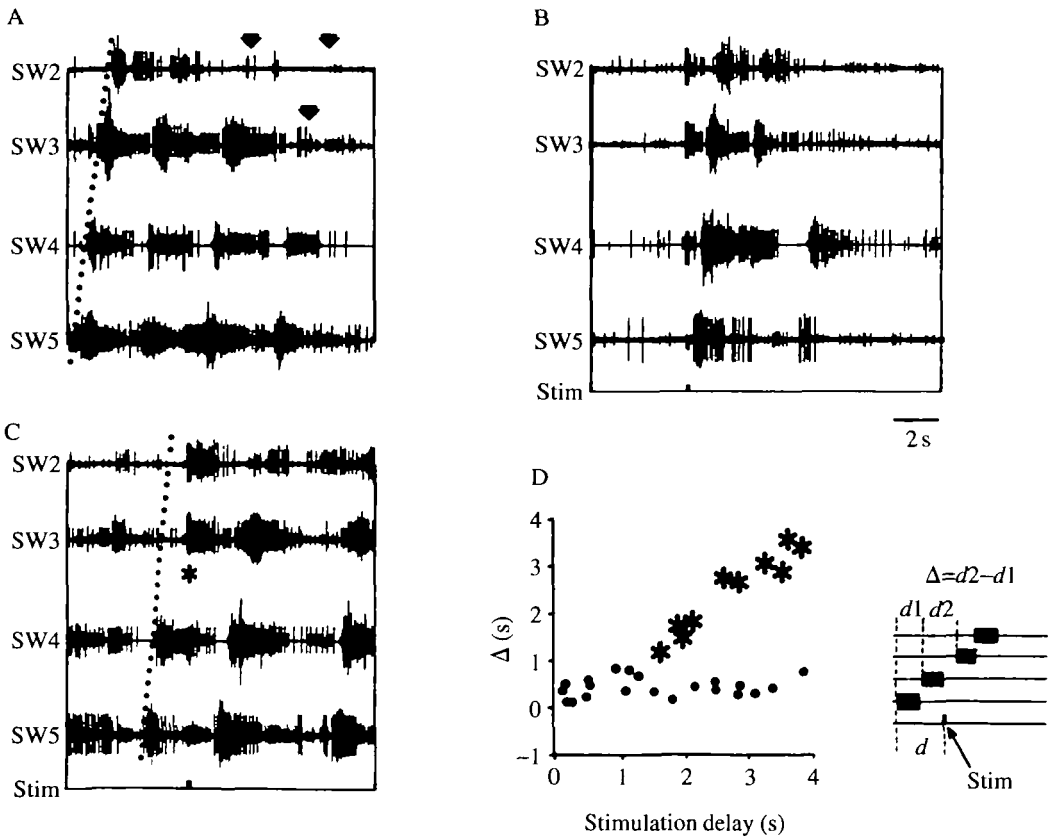

Fig. 12. CBCO nerve stimulation may affect intersegmental coordination mechanisms. (A) Extracellular recordings of spontaneous activity in roots 1b of SW2–SW5. The dotted line joining the onsets of the powerstroke bursts indicates the metachronal delay. At the end of a powerstroke burst, activity is sometimes absent in SW3 and SW2 (arrows). (B) A single electrical stimulus (Stim) to the CBCO nerve (train of 3 V pulses at 20 Hz, 0.2 s) induces a metachronal cycle of powerstroke bursts in all four ganglia. (C) When the metachronal cycle is incomplete, an electrical stimulus induces a series of powerstroke bursts which starts with SW2 and SW3 (asterisk). (D) The difference between the delay in the powerstroke bursts in two adjacent ganglia ($\Delta = d2 - d1$) is plotted as a function of the delay (d) between the SW5 powerstroke burst and the CBCO nerve stimulation (see inset). The rhythm is usually regular (filled circles: the delays are with in the same range), but when the rhythm stops between A4 and A3 (asterisks) the powerstroke burst in A3 is apparently delayed. However, this corresponds to a restarting of the rhythmic activity in A3 after it has stopped, and not to a metachronal delay.

restart from where it had stopped: the missing bursts occurred and then another metachronal wave started from swimmeret 5.

The 're-activation' of the swimmeret system after it had stopped bursting reveals a dependence of the swimmeret CBCO response on the central state of the preparation. When the metachronal wave was complete, the swimmeret 4 to

swimmeret 3 delay was unaffected by the timing of the stimulus in the metachronal cycle (i.e. it was about the same as the swimmeret 5 to swimmeret 4 delays; filled circles in Fig. 12D), whereas it was strongly related to the time of the CBCO stimulation when the latter occurred after a missing burst. The swimmeret 4 to swimmeret 3 delay then varied directly with the swimmeret 4 to stimulus delay (asterisks in Fig. 12D), suggesting a strong activation of the swimmeret 3 CPG by the CBCO stimulation.

The occurrence of these anomalous metachronal waves, which restart from their point of interruption following CBCO stimulation, suggests that control can be exerted by the CBCO on the intersegmental coordinating elements of the swimmeret system.

Discussion

The present report demonstrates that a single leg proprioceptor in the crayfish is able to exert control over the centrally generated rhythmical activity of an abdominal motor system. This finding is of great interest for our understanding of both the plasticity of the swimmeret rhythm and the role of a distant chordotonal organ. Moreover, it indicates the multiplicity of linkages that could exist between different CPGs. In a previous article (Barthe *et al.* 1991) it was established that central pathways exist between the locomotor thoracic CPG and the swimmeret CPG. Here we have demonstrated that proprioceptive inputs coding for leg movements (CBCO) control the swimmeret CPG.

Functional significance of CBCO stimulation

The CBCO has been described in detail both morphologically and electrophysiologically. The organ is composed of about 40 sensory bipolar cells embedded in connective tissue. These code the angular position and movements of the coxobasal joint, which is mainly involved in levation and depression of the leg. Most of the cells are activated at the most extreme angular positions, while only a few respond in the mid-range of angular position (Whitear, 1962; Bush, 1965).

A striking feature of our results is that the intensity of CBCO stimulation, whether mechanical or electrical, is crucial in determining the type of effect elicited in the swimmeret system. Limited movements of the CBCO strand, which approximate to the CBCO joint movements that occur during normal behaviour, produced only weak reflex effects. However, stretch or release of the strand to an extreme position, which activated sensory fibres coding position as well as those coding movement, was much more effective in eliciting reflex effects. This must be related to the number and the properties of the sensory cells recruited at the different stimulus strengths. In the case of electrical stimulation of the CBCO nerve, the opposite responses observed with weak and strong stimuli might relate to the fact that the larger sensory fibres are stimulated by low voltages while the smaller fibres are recruited only when strong stimulations are applied. This can be

correlated with the observations of Whitear (1962) describing the larger receptor cells as coding for movement while the smaller ones coded for position.

In interpreting the effects of different stimulus strengths, the experimental situation must also be considered. *In vitro* only a single proprioceptor is retained for stimulation, although under natural conditions several legs are moving simultaneously and a summation of sensory inputs from all CBCOs would be expected to occur.

Expression and control of swimmeret central pattern generators

In intact crayfishes and lobsters, and in isolated nerve cord preparations, the motor activity of the swimmeret demonstrates a characteristic rhythmical pattern of alternating powerstroke and returnstroke motor neurone activities (Hughes and Wiersma, 1960; Davis, 1969; Cattaert and Clarac, 1987). This pattern is a property of the neuronal network that constitutes the hemiganglionic oscillator of the swimmeret, and the synaptic relationships of the neurones involved are now fairly well understood (Heitler, 1978, 1983; Paul and Mulloney, 1985*a*; Mulloney *et al.* 1990). Additionally, strong bilateral coupling synchronises each pair of swimmerets (Paul and Mulloney, 1985*b*), and a forward-running metachronal wave is established by the action of coordinating interneurones (Stein, 1971; Paul and Mulloney, 1986).

Although the centrally generated pattern of swimmeret beating is rather stereotyped, variations in beat frequency and phase relationships can occur in different behavioural contexts (Cattaert and Clarac, 1983). It is possible that such effects are due, at least in part, to the action of neuromodulators on the swimmeret CPG. It has been found in isolated preparations that proctolin induces an acceleration of the swimmeret rhythm, while octopamine reduces its frequency (Mulloney *et al.* 1987). Sensory feedback from proprioceptors within the swimmeret represents another potential influence for modifying the swimmeret motor pattern. A number of studies have demonstrated that such phase-related proprioceptive feedback can indeed influence the timing and strength of the beat (Davis, 1969; Heitler, 1986) and also its periodicity (Miyan and Neil, 1986; Cattaert and Clarac, 1987; Macmillan and Deller, 1989). Furthermore, Paul (1989) has shown that the incoming sensory signals from one receptor, the non-spiking stretch receptor (NSSR), are modulated by presynaptic inputs from the CPG itself. A picture thus emerges of a central gating of re-afferent signals, which could form the basis for phase-dependent reflex effects.

Other powerful sensory effects can also arise from outside the swimmeret. Descending interneurones from the statocyst, which convey tonic signals coding body position (Takahata and Hisada, 1982; Knox *et al.* 1987), cause the swimmeret powerstroke to be redirected and uncoupled from that of its bilateral partner (Davis, 1968; Neil and Miyan, 1986; Knox and Neil, 1991). The present study reveals yet another source of extrinsic sensory modulation, the CBCO proprioceptor. Its signals are potentially re-afferent to the rhythmic activity of the thoracic motor system, but also have a distinct effect on the timing and periodicity of the

swimmeret system. The results we have obtained indicate that these effects of the CBCO depend on the state of the preparation, and on the strength and precise timing of the stimulus. In general, our findings also emphasise the extent to which the central networks controlling swimmeret beating are susceptible to modification by other neuronal elements.

The CBCO acts on the swimmeret at two levels: on the motor neurones but also on the CPG. The motor neurones can be excited separately when the level of activity is low. When excitability is high enough, the swimmeret CPG can be turned on (Fig. 2). When the swimmeret CPG is active, two opposite responses are obtained depending upon the intensity of the CBCO stimulus. This problem of the level of activity is important since we know that within a thoracic ganglion the CBCO can induce a resistance reflex in a 'resting' preparation and an assistance reflex when the thoracic CPGs are active (El Manira *et al.* 1991*b*).

The interneurones connecting the thorax and the swimmeret abdominal system are largely unknown. In the past Wiersma (1958) has characterized a great number of sensory interneurones whose activity is induced or enhanced by leg manipulation. It is probable that a large population of fibres receives CBCO input and sends it to the abdominal region. Davis and Kennedy (1972) have found command fibres that are able, depending on their discharge frequency, to turn on swimmeret beating. Work is in progress to see how such interneurones could integrate leg sensory inputs and so modulate swimmeret beating.

Entrainment of swimmeret beating by CBCO stimulation

In a free-walking decapod such as a lobster or crayfish, the thoracic locomotor system operates at a much slower frequency than that of swimmeret beating (around 0.5 Hz and 2 Hz, respectively). In such cases the rhythms are independent for most of the time. However, in certain behavioural sequences the frequencies of both rhythms can be very similar (Cattaert and Clarac, 1983, 1987), and in these situations they can interact, with one entraining the other.

Entrainment of a given CPG by a rhythmical sensory activation is a very widespread phenomenon within the arthropods, and has been studied both in insects (Wendler, 1974; Pearson *et al.* 1983) and crustaceans (Cattaert and Clarac, 1987; Sillar *et al.* 1986; Müller and Clarac, 1990). Such entrainment involves phase-dependent relationships and was only found to occur in our experiments with low-intensity stimulations. Figs 10 and 11 demonstrate an important aspect of this effect: in the absence of the swimmeret rhythm, a single stimulus is enough to induce a single cycle of activity. If such stimulation is repeated regularly, the rhythm can be entrained up to a frequency limited by the intrinsic properties of the swimmeret oscillators. However, using strong electrical stimuli, the effect was not phase-dependent: the swimmeret rhythm was always arrested in a powerstroke burst that persisted for as long as the rhythm was blocked.

The CBCO action is not limited to modulation of the rhythmic properties of a single swimmeret. It also seems to be able to control the interganglionic coordination of the swimmeret system. Fig. 12 demonstrates that the swimmeret

metachrony can be disturbed. The delay in the 5–4–3–2 sequence may be changed when the CBCO stimulus arrives within such an existing wave. As shown by Davis (1968), and confirmed by Cattaert and Clarac (1983), the duration of the wave is directly related to the period of swimmeret 5 beating. However, when incomplete metachronal waves are elicited (Fig. 12B,C), the CBCO stimulus is able to produce abnormal metachronal waves (Fig. 12D). It is therefore probable that the CBCO can exert control over each segmental oscillator. This is the first demonstration, to our knowledge, that sensory information can act on both the elementary, segmental rhythm and on the global wave.

The data we have obtained also highlight the redundancy of the central nervous system in regulating a rhythm. As demonstrated recently by Barthe *et al.* (1991), in a preparation consisting of completely isolated thoracic and abdominal nerve chains, a thoracic locomotor rhythm greatly influences the swimmeret pattern. At the same time, several sensory afferents such as the CBCO can control the swimmeret system; this means that such an abdominal rhythm is under both central and peripheral influences. It is now necessary to characterise the pathways between the thorax and the abdomen and to determine the relationship between the drives from the thoracic locomotor CPG and from CBCO sensory afferents.

The data presented here must be referred to the behavioural observations made by Cattaert and Clarac (1983) on the relationship between swimmeret beating and thoracic locomotion in intact lobsters. In their work they distinguished two patterns of swimmeret activities during forward walking: (i) high-frequency swimmeret beating, and (ii) steady swimmeret beating. In the first case, both systems are at different frequencies and if any correlation exists it has a complex ratio (sometimes 5:2). Under these conditions, the locomotor rhythm seems to stabilize swimmeret beating. In contrast, during slow swimmeret beating, locomotion appears to have an excitatory action and induces a metachronal swimmeret wave for each leg step (1:1 correlation). Cattaert and Clarac (1983) hypothesized the existence of the two types of walking leg effect on the swimmeret which we have found here *in vitro*: (i) a modulation of the swimmeret rhythm and (ii) a phase-locked response demonstrating a direct effect on the swimmeret CPG.

It seems to be important for the stability of locomotion that the two locomotor systems can be linked. The swimmerets add a propulsive force that can reinforce forward progression or can adjust the direction of movement. This linkage involves both central connections and sensory pathways, of which the one driven by the CBCO seems to be particularly powerful. Our results demonstrate how the linkage between the CBCO of the fifth leg and the swimmeret could assist in the propulsion of the body at each fifth leg levation. The combined effects of the CBCOs of all the thoracic legs acting in this way during locomotion would be expected to have a significant effect on the timing relationships between the walking legs and swimmeret.

This work was supported by a 'Twinning' Grant ETP/TW/651 from the European Science Foundation, which is gratefully acknowledged.

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