

PATTERNS AND BILATERAL COORDINATION OF SCAPHOGNATHITE RHYTHMS IN THE LOBSTER *HOMARUS AMERICANUS*

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SUMMARY

The bilateral patterns of forward and reversed scaphognathite (SG) pumping are described for the American lobster. During forward pumping the two SGs usually function synchronously, but may also function independently. The nine muscles of one SG are arranged into four functional groups which are sequentially active during forward pumping. During reversed beats, motor neurones to one group of muscles are inactive while bursts to another are either delayed or missing. Reversal beats do not appear to alter the phasing of the central oscillators that generate the basic SG rhythm.

Phase analysis of bilateral SG beating demonstrates two types of relationship: phase coupling or phase drifting with a tendency to couple. An animal may remain in one state for long periods of time or may alternate between states. Coupling can occur at more than one phase indicating phase multistability. The coupled state may remain constant at markedly different frequencies of beating, indicating phase rather than latency coupling between SGs. During the drifting state each SG tends to assume its 'intrinsic' rate of oscillation. The drift state reflects the inherent asymmetry of the two SG systems. The influence of several parameters of sensory stimulation on phase and frequency of SG beating are analyzed.

INTRODUCTION

The scaphognathites (SGs) of decapod crustaceans are modified portions of the second maxillae which by their pumping movements ventilate the gill chambers. The movements of the SGs and the hydrodynamics of branchial ventilation have been studied in the lobster (Wilkins & McMahon, 1972) and a crab (Pilkington & Simmers, 1973), and information is available on the neuronal and muscular components of these appendages (Pasztor, 1968). In the lobster, 11 muscles are organized into 2 antagonistic groups, each group being innervated by a separate motor root arising from the suboesophageal ganglion. Rhythmic movements are generated by a pair of oscillator neurones, one localized in each half of the suboesophageal ganglion (Mendelson, 1971). Mendelson examined some of the characteristics of these oscillators and their control by higher order command interneurones. A separate

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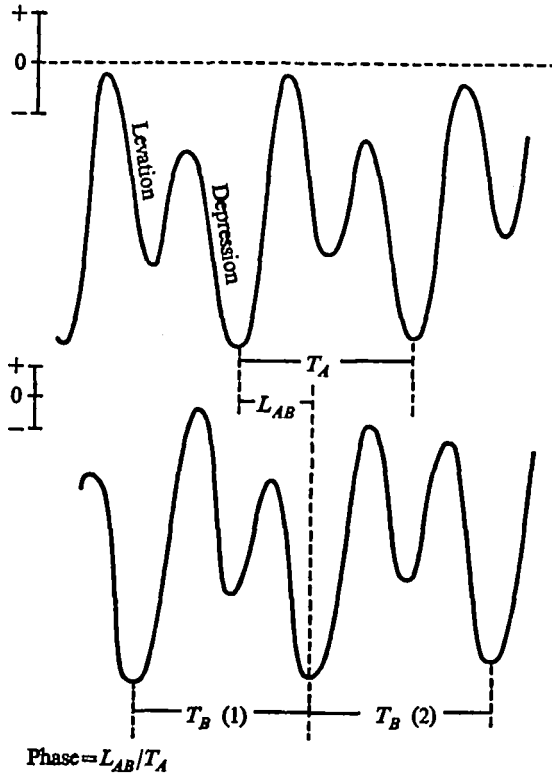


Fig. 1. Terminology for bilateral beat analysis. A complete SG beat includes both leviation and depression of the appendage. The beat period, T_A and T_B , is measured from successive minima on the biphasic pressure waveform from each SG. L_{AB} is the latency between beats on the reference and test sides. $\text{Phase} = L_{AB}/T_A$, and can vary from 0 to 1.

analysis of the command fibres which make connections with the SG motor system and the heart has also been made in the crab *Cancer magister* (Wilkins, Wilkins & McMahon, 1974).

Although the two SGs are not mechanically coupled and are thus capable of independent action (Segaar, 1934), they often show simultaneous changes in rhythms (McMahon & Wilkins, 1972). To determine to what extent these paired appendages are interrelated we here examine two aspects of bilateral beating behaviour. First, the patterns of beating, as they relate to gill ventilation, have been recorded from lobsters held at standard conditions. Particular attention was paid to the relative extents of synchronized and independent activity in the two SGs. Secondly, the interrelationships of the two appendages with respect to time, temperature and mechanical stimulation have been examined with particular attention to bilateral coordination. From these two types of analyses we feel we can define the limits of scaphognathite behaviour.

METHODS

Lobsters, *Homarus americanus*, were obtained from commercial sources and held in running sea water at 8–10 °C and P_{O_2} 120–130 mmHg for at least 3 days before use. After an initial acclimation, the animals did not appear to be under undue

stress either in the holding tanks or in the experimental chamber. Lobsters were fed frozen shrimp and have been maintained in a healthy condition for as long as 6 months. During experiments, the animals were held in a plexiglass chamber similar to that described by Larimer (1961).

Scaphognathite movements were monitored by water pressure transducers as previously described (Wilkins & McMahon, 1972). By these techniques, the forces (amplitude of pressure waves), rates and modes (forward, reverse, or pause) of SG beat were examined. Pressures were recorded simultaneously from both gill chambers in all cases.

A complete SG beat includes both levation and depression of the appendage. These movements produce a biphasic pressure waveform of two unequal negative pressures with depression of the SG generating the greatest negativity (Fig. 1). In analysing the bilateral pressure records for interrelatedness of SG movement, the instants of minimum pressure were taken as reference points. The relative timing between the successive minima in the trains for the two appendages was expressed as sequential points of relative phase. Relative phase was defined as the absolute time between a beat in the test train and the time of the preceding beat in the reference train (L_{AB}), divided by the period length (T_A) of the corresponding reference cycle. Coincident beats were assigned to phase one rather than to phase zero. Phase varies between zero and one. Beginning at the time when the two appendages were beating perfectly together the phase is taken as zero. If the reference SG beats more rapidly than the other, the phase progresses from zero until the time at which the two once again beat in the same phase which is designated one, and the phase plot will progress with a positive slope. When the slower SG has lagged a whole cycle after the faster, the phase will appear to jump from zero to one and the progression will begin again.

The analogue data were digitized, the digitized points punched onto computer cards, and the analyses done by appropriate computer programs. Some data have also been analysed by hand and by direct digitizing of the analogue pressure wave from the oscilloscope output.

Electromyograms were recorded from several of the SG muscles by means of fine silver wires (0.127 mm) insulated except for the tip. To gain access to a SG the overlying branchiostegite was cut away. Wires were positioned and glued in place by Eastman 910 adhesive. Moulded cellulose windows were glued over the opening to restore normal pumping hydrodynamics. Signals were amplified, stored on magnetic tape and later photographed.

RESULTS

I. Patterns of ventilation

The lobster can control independently the mode (forward, reverse and pause), rate and force of beating for each SG (Fig. 2). Fig. 2A shows symmetrical bilateral responses both in the occurrence of reversal beats and in changes in wave-form during forward beating. The reversals were delayed approximately a half to one beat between slides, as would be expected, since reversal beats arise only during levation (Wilkins & McMahon, 1972), and at the time of this record the two SGs

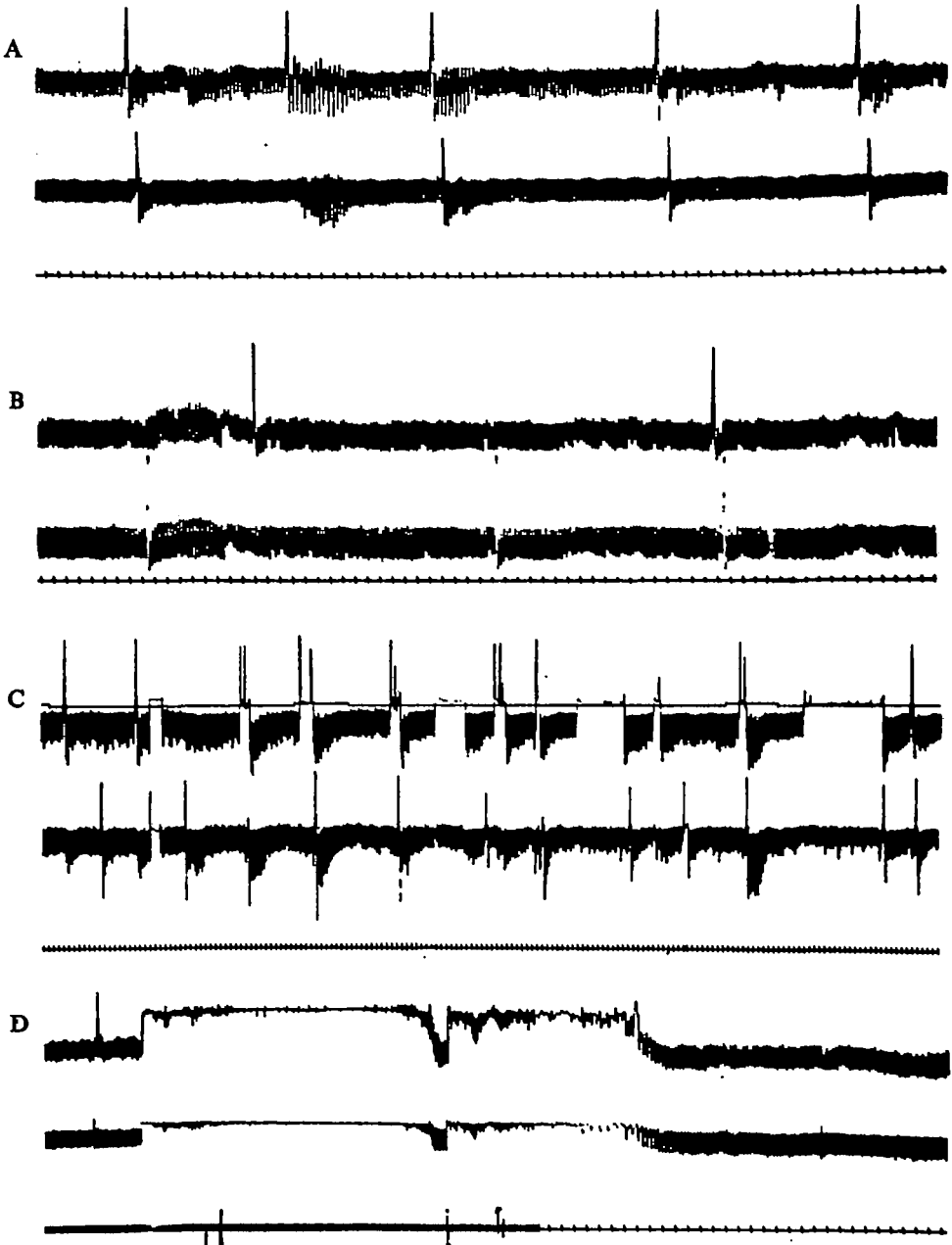


Fig. 2. Hydrostatic pressures recorded from both gill cavities of the lobster. The right pressure is always displayed above the left. Each pair is from a different animal. Negative pressure is downward. See text for detailed explanations.

were beating slightly out of phase. In Fig. 2B three reversals occurred unilaterally while the contralateral pattern remained unaffected (see also Fig. 3A). Also seen in this trace was an abrupt and unilateral change in frequency and amplitude of forward beat not accompanied by a reverse beat. Often such changes in frequency and amplitude begin with a reversal beat. The records in Fig. 2C and 2D illustrate

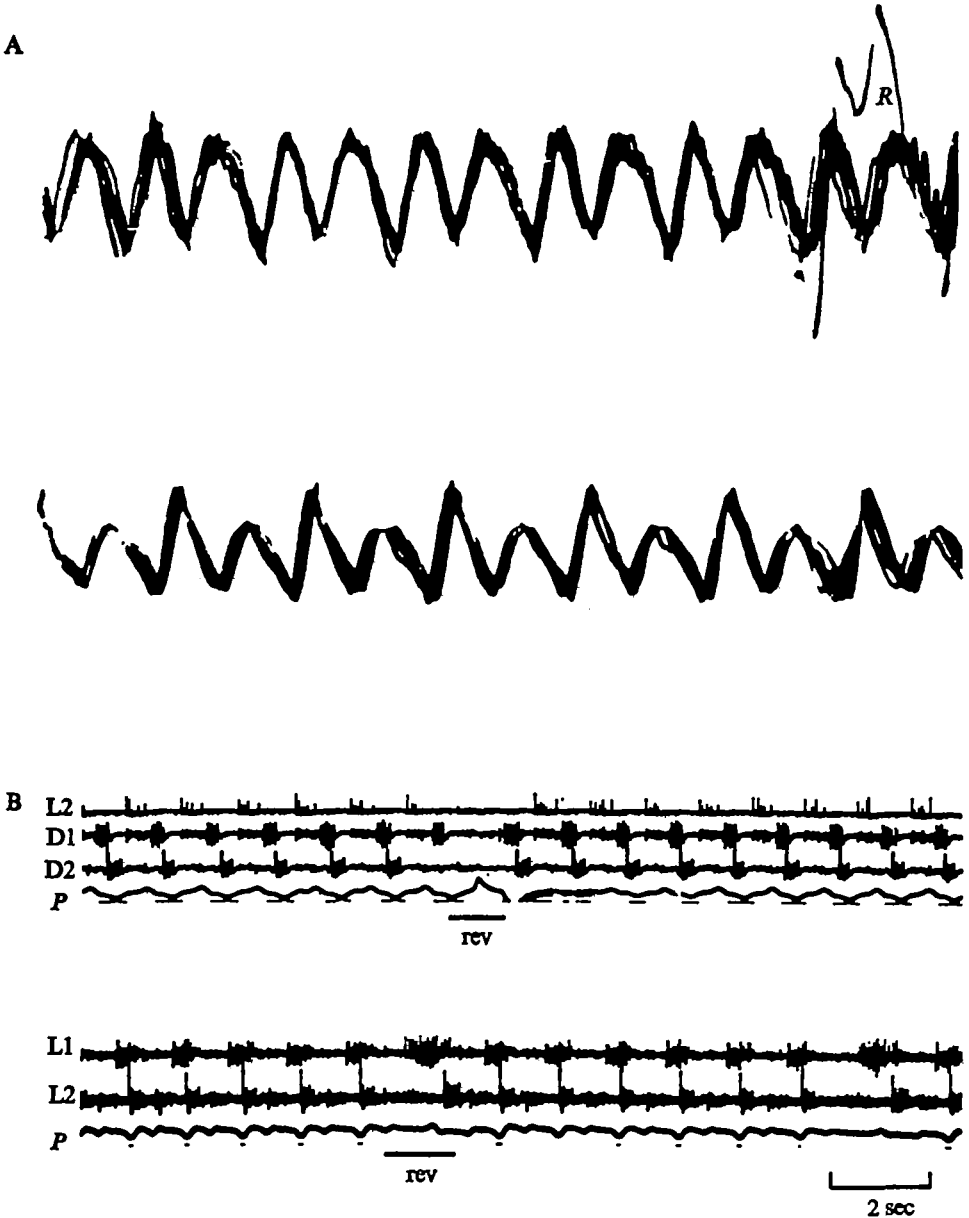


Fig. 3. (A) Storage oscilloscope display of 5 consecutive sweeps of bilateral SG activity. A reversed beat (*R*) occurred on the right side near the end of the second sweep. This altered beat did not alter the rhythms of either side beyond a minor perturbation for the beat following on the ipsilateral side. (B) Electromyograms from muscles in each of the four groups (*L*₁, *L*₂, *D*₁ and *D*₂) which produce forward and reversed pumping. The reversed beats are underlined. *P* = hydrostatic pressure.

unilateral as well as simultaneous bilateral pauses in pumping. Pausing in both SG and cardiac rhythms has been reported previously (McMahon & Wilkens, 1972) and is only observed in animals in aerated water. In Fig. 2C numerous pauses of short duration occurred, sometimes unilaterally and sometimes bilaterally, whereas the pause in Fig. 2D was bilateral and lasted for $5\frac{1}{2}$ min. At the termination of the long pause several beats were required before the original rates and amplitudes were re-established. The general impression gained is that both SGs beat at approximately the same rate and amplitude and show many altered responses in parallel; however, they may also behave independently as seen here and as will be examined more closely in the following section.

Reversal beats of the SGs result in a backward movement of water over the gills, and are produced by a negative angle of attack of the SG only during levation. The overall rates of reversals are similar for both SGs, do not show a positive correlation with oxygen tension, and may merely reflect symmetrical responses to external and internal stimuli that affect both sides of the animal simultaneously. Unilateral reversals also occur, and can be induced by stimulating ipsilateral branchiostegite hairs. Whatever the cause, it is significant that the occurrence of a reversal beat does not alter the normal rhythm of beating. In the example presented in Fig. 3A a reversal occurred on the right side during the second of five consecutive sweeps as displayed on a storage oscilloscope. The event did not break the rhythm on that side and had no effect on the contralateral side. More rarely the beat following a reversal would be delayed slightly, but re-entrainment to the standard intervals would again occur within one or two beats.

The 11 muscles of each SG (Pasztor, 1968) are arranged into four functional groups (Young, in preparation) which account for the 4 components of the pressure waveforms generated during forward and reversed beating. *Forward beating*: beginning with the SG completely depressed the 4 components of a beat cycle and the muscle group causing that movement are: (i) levation of the posterior end of the blade (L_1), (ii) levation of the remainder of the blade (L_2), (iii) depression of the posterior end of the blade (D_1), and (iv) depression of the anterior end of the blade (D_2). The sequence in which these muscle groups are active during a complete forward pumping cycle would thus be $L_1, L_2, D_1, D_2, \dots$ (Fig. 3B). The muscles included in each group are described in the discussion. *Reversed beats*: contrary to the impression left in a previous paper (Wilkens & McMahon, 1972) a re-examination of the video analysis of SG movements and preliminary recordings from the 4 muscle groups indicates that a reversal beat, which in the lobster only occurs singly during levation, is generated by the altered sequence $L_1, L_2, D_1, -, L_1, (L_2), D_1, D_2, \dots$. The D_2 muscles are completely inhibited during a reversal while the L_2 group appears to receive weaker inhibition causing either delayed onset of activity on some occasions or silence on others.

The presence of the surplus negativity following some, but not all, reversal beats appears to be more related to the action of the epimera attractor muscle (Wilkens & McMahon, 1972) than to SG beating. Thus, the reversal when present, is followed by epimera attraction, which causes the re-establishment of a level of branchial negativity greater than the mean negativity maintained by the SG beat. This surplus negativity never persists for more than a few beats.

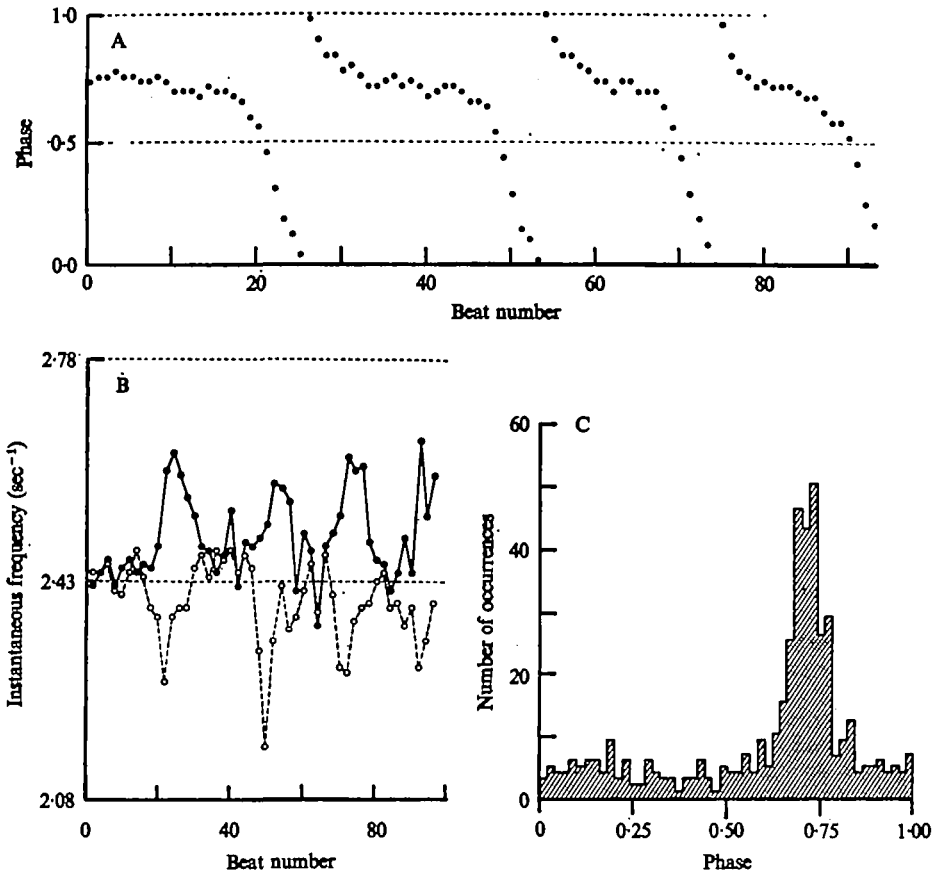


Fig. 4. Temporal analysis of bilateral coordination between SGs. (A) Phase *vs.* beat number relationship with the right period (T_A) taken as reference. Periods of phase coupling are interrupted by phase drift sequences. (B) Instantaneous frequency of each SG from the same data as used in (A). Each point is derived by the sliding average of two consecutive beats. ○ = right SG, ● = left SG. (C) Phase histogram of the cumulative number of occurrences of beats in B at a fixed phase in T_A .

II. Bilateral coordination

The foregoing section has demonstrated that the two SGs generally respond as though they receive common input from higher centres in the CNS, and also that they are each capable of independent action. Both of these responses may arise as a consequence of sensory induced perturbations of normal rhythms.

Since each SG is driven by a separate central oscillator, the question remains whether there exists transganglionic coordination between the two systems in addition to the higher levels of control. When the two SG rhythms are temporally analysed it is seen that bilateral coordination does occur. The data from one of the 20 animals studied have been selected since they contain examples of all observed coordination patterns. In Fig. 4A the phase *vs.* beat number relationship for beats on the two sides has been plotted. There were periods of 'coupled' beating (complete entrainment) during which the two sides maintained a stable, but jittery, phase relationship, which was periodically broken when the two appendages began to

'drift' apart for a number of cycles only to stabilize again at the same phase at which stable coupling occurred. The left SG of this animal beat more rapidly than the right (taken as reference side) during drift, giving a negative slope to the drift curve. The phase at which coupling occurred generally remained fixed for any set of experimental conditions on a given day. Comparing all determinations at standard conditions (10° – 11° , air-equilibrated sea water) some animals were found which maintained coupled beating throughout, others which drifted continuously although a 'coupling notch' (incomplete entrainment) was seen, and some which showed alternate coupling and drift as in the illustration presented.

Fig. 4C is a phase histogram of this experiment which clearly shows the preferred phase that occurs during coupling. Such a histogram for an animal that continuously drifts may still show a slight tendency for a preferred phase, but not as dramatic as in this case.

It is significant in the phase relationship analyses that the slope of the drift lines throughout any determination is very consistent for all drift sequences. This means that the difference between the beat frequencies of the two SGs during each drift is similar and may represent the inherent frequency of each oscillator when not coupled. The direction of the slope, positive or negative, changed only once during more than 50 determinations. More rarely, however, the slope did change from day to day and after temperature changes; i.e. the SG which beat the most rapidly during drift had changed.

When the instantaneous frequency of beating is plotted it is possible to analyse the performance of each SG separately (Fig. 4B). The frequencies of the two appendages are necessarily the same when in the coupled mode. During drift both frequencies diverged from the preferred coupling frequency with one SG slowing and the other speeding up.

The performance of two other lobsters is worthy of note. One animal showed uniform coupling throughout a long determination of over 400 beats. In the middle of this run both SGs slowed down simultaneously and significantly (also see Fig. 6B), but the phase relationship was not broken. The other case is an animal which beat in the coupled mode throughout the period of observation, but abruptly shifted from one phase to another on two occasions, thus showing three preferred phases of coupling during one observation. The phase histogram accordingly was trimodal, but the instantaneous frequency was uniform throughout. These two observations are of some interest, and indicate that the preferred phase of coupling may be independent of the mean beat frequency. It has long been known to lobster fishermen that the two chelae of lobsters are morphologically different. One bears sharp dentition and numerous hairs presumably of a sensory nature, and appears specialized for tearing and cutting. The other has blunt dentition, is more massive and is specialized for crushing. We have found that the SG on the side of the lobster with the sharp chela almost always beats more rapidly during drift than the other. The significance of this observation is uncertain.

An anatomical basis for bilateral coordination

When the two nerve trunks which innervate each SG were back-filled with cobalt chloride (Iles & Mulloney, 1971; Pitman, Tweedle & Cohen, 1972) it was possible

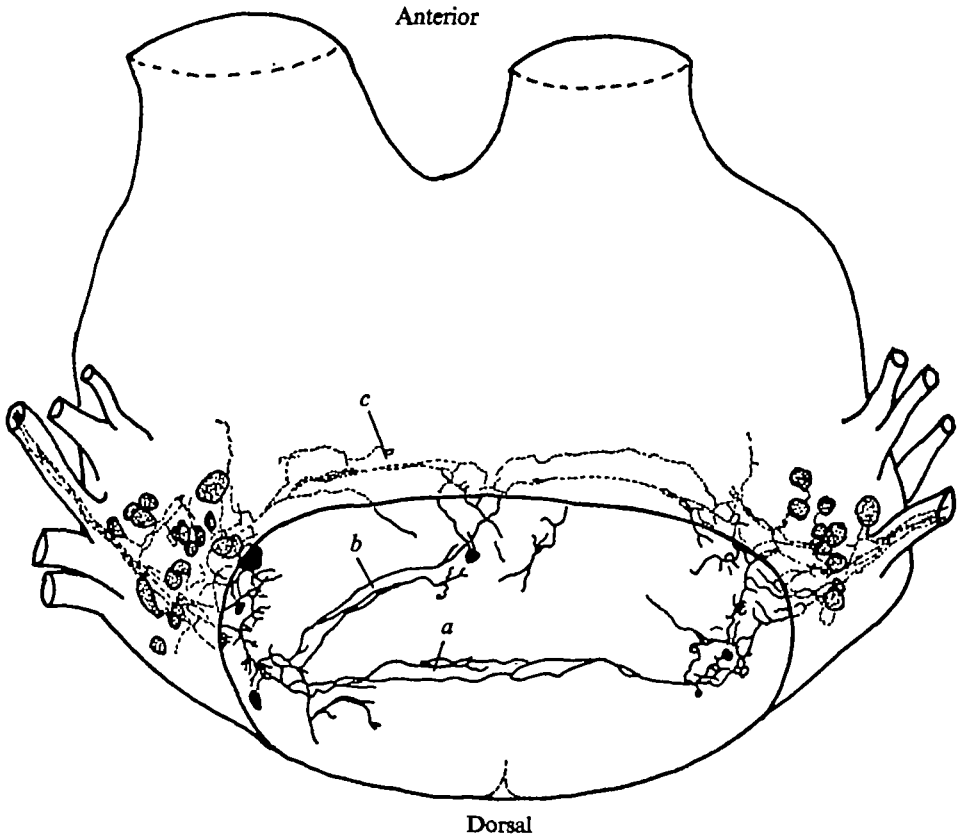


Fig. 5. Drawing derived from camera lucida tracings of the suboesophageal ganglion showing cobalt-stained motor neurones of the depressor nerve trunks. This ventral view shows the superficial clusters of somata and an underlying neuropile. From this neuropile three sets of collaterals arise and traverse the ganglion toward the midline. The three tracts are the ventral collaterals (*c*) which cross and join near the midline, descending collaterals (*b*) derived from the dorsal portion of the neuropile which join the ventral collaterals, and collaterals (*a*) which arise and cross the ganglion dorsally and appear to intermingle at the midline. One or two somata are located near the ventral midline.

to trace the motor neurones to their cell bodies which lie as clusters around the points of departure of these trunks from the suboesophageal ganglion. A dense neuropile is formed in this region with contributions from both the levator and depressor motor neurones. When the depressor nerve trunks alone were filled, several nerve collaterals were seen to cross the ganglion (Fig. 5). At least two collaterals cross on the ventral surface from each side and appear to meet in the midline. Two or more collaterals leave the neuropile dorsally, but descend as they cross the ganglion and then join the ventral collaterals. The ventral and descending collaterals form a loose circle at the ventral midline with those from the opposite side. Also arising from the dorsal neuropile are a few bilaterally symmetrical collaterals which cross on the dorsal aspect and appear to intertwine at the midline. One midventral cell body was filled on most occasions. In order to have stained, all of these processes must be collaterals of motor neurones. They could not

Table 1. Listing of the phase angles at which coupling occurred at different temperatures and at intervals up to one month – also indicated is the mode of coordination during each analysis (each analysis lasted from 2–5 min (200–500 beats))

Lobster number	Interval after 1st analysis	Preferred phase angle and dominant mode at different temperatures		
		4 °C	9–11 °C	18–20 °C
I	—	0.84 C	0.83 C	0.74 C/D (81/19)
	4 weeks		0.20 C	
II	—	0.78 C/D (84/16)	0.78 C/D (48/52)	0.12 C/D (83/17)
	2½ weeks		0.69 D*	
	3½ weeks		0.60 C	
III	—	0.94 C	0.30 C	0.82 C
	2½ weeks		0.40 D*	
IV	—	0.44 C/D (83/17)	0.94 C/D (89/11)	0.96 D*
	4 weeks		0.82 C	
V	—	0.80 C	0.64 C/D (88/12)	0.04 C/D (96/4)

C or D indicates coupling or drifting for duration of the analysis.

C/D indicates coordination characterized by periods of coupling alternating with periods of phase drifting. Numbers in parentheses () are the percentage times spent in each mode.

* Continuous drifting with only a slight break in the drift curve which was taken to represent a coupling tendency. The phase histograms for these analyses also showed a preferred phase which corresponded to this break.

originate from oscillators or other cells wholly contained within the ganglion. Whether these commissural collaterals make synaptic or electrotonic contact with one another is not yet known, but it seems likely that some contact does occur. No commissural collaterals were found after filling the levator nerve trunk.

III. Responses to sensory stimulation

We have examined bilateral coordination with respect to time and temperature, and as a function of mechanical stimulation of the cephalothorax and SGs (Table 1). Lobsters I, II and IV maintained the same preferred phase and same mode, i.e. coupled or drifting, at two of the three temperatures analysed; however, in each case there was a dramatic shift in the preferred phase at the third temperature. It is not possible to predict over which temperature range the animals will change in this manner, but it is apparent that the underlying mechanism which regulates phase is stable over a fairly broad range of temperature. Frequency varies linearly with temperature over this range. Equally apparent is the abrupt change in coupling which often accompanies a change in temperature.

Turning to the stability of bilateral coordination, it is seen that the preferred phase for coupling and the mode of coordination is not constant with respect to time. Four lobsters were analysed at intervals over one month (Table 1). All observations were made at 9–11 °C on unrestrained animals. No consistent pattern emerges. Only one animal kept the same mode of coordination and all showed changes in the preferred phase angle for coupling. Thus, one must assume that the neurophysiological basis for coupling is a labile system whose states of interaction change with time.

The respiratory system of the lobster, as of the crab (Wilkins *et al.* 1974), is sensitive to a wide variety of chemical, mechanical and light stimuli. We have

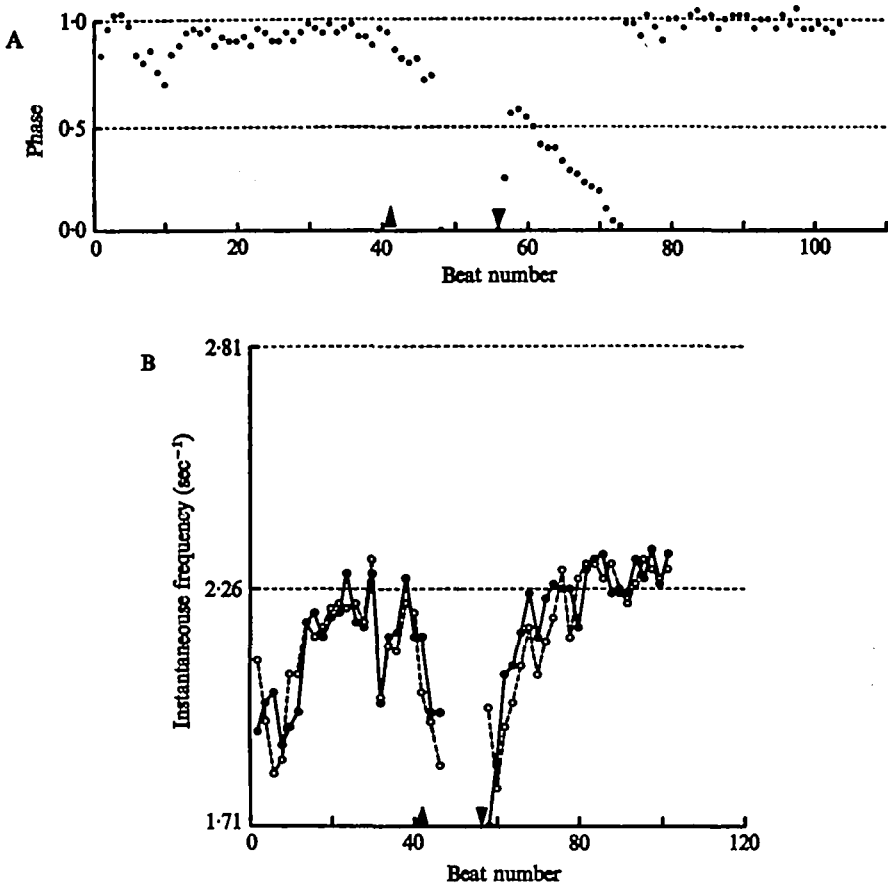


Fig. 6. (A) Phase analysis and (B) instantaneous frequency of beating through bilateral pause induced by trapping the rostrum. Period of stimulation indicated by arrows. ○ = right SG, ● = left SG.

examined the effect of several parameters of mechanical stimuli on SG rhythms and coordination. Tapping the rostrum or brushing the carapace can induce an inhibition of SG beating (pause). The record in Fig. 6 illustrates one such pause. After stimulation the animal began to drift for approximately 8 beats, paused for the equivalent of a further 10 beats, resumed pumping at the point one would have predicted had the pause not occurred, and finally re-established the coupled mode of beating. That the drift pattern was not interrupted by the pause is interpreted to mean that the oscillators were not affected by the pause-inducing commands. Rather it seems that the inhibitory commands were effective at the level of the motor neurones and that the oscillators continued to cycle throughout the pause.

In Fig. 7 the anterior branchiostegite on the right side was brushed with a camel-hair brush. This region of the carapace is innervated by the branchiostegite nerve, which enters the suboesophageal ganglion dorsal and adjacent to the SG nerve trunks. The response to brushing was an immediate reduction in the beat frequency of both SGs but there was no break in the phase relationship. There was a small

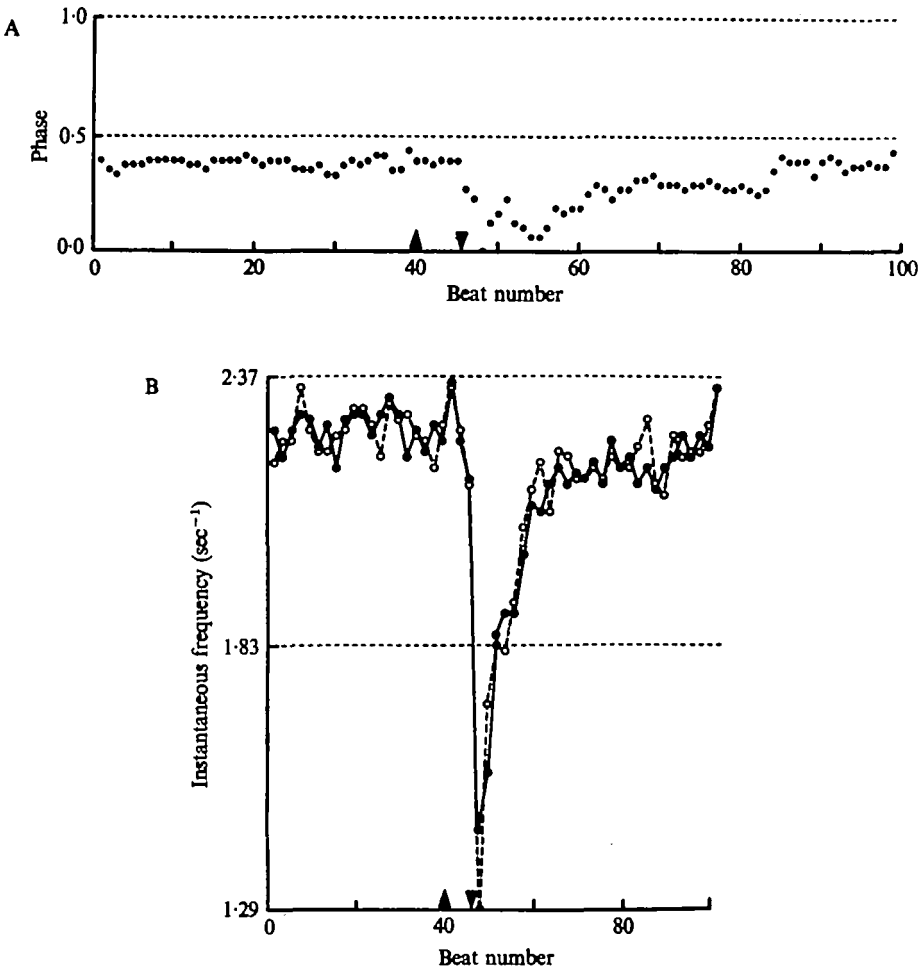


Fig. 7. Phase and frequency changes induced by brushing one branchiostegite. Period of stimulation indicated. \circ = right SG, \bullet = left SG.

change in the preferred phase following stimulation, but drifting did not occur. It appeared as if the unilateral stimulation had bilateral and symmetrical influences at the level of the SG oscillators.

When one SG was restrained, either by holding it with forceps or by packing the appendage with cotton, the manipulated SG continued to beat (or struggle), but at a reduced frequency (Fig. 8). The SGs of this lobster were beating in the coupled mode until one SG was grasped by forceps, but drifted wildly while restrained. The SGs continued to drift for two further cycles when released before resuming the coupled mode of beating. The instantaneous frequency plot shows that during the last two drift cycles the two SGs were progressively re-entrained toward the same frequency. The latency of re-entrainment was 18 beats. The contralateral SG accelerated slightly during stimulation, while on other occasions the contralateral SG slowed in response to restraint of the opposite SG, but never to the same extent. It would appear as if the sensory afference from the manipulated appendage had a greater effect on the ipsilateral than on the contralateral pacemaker.

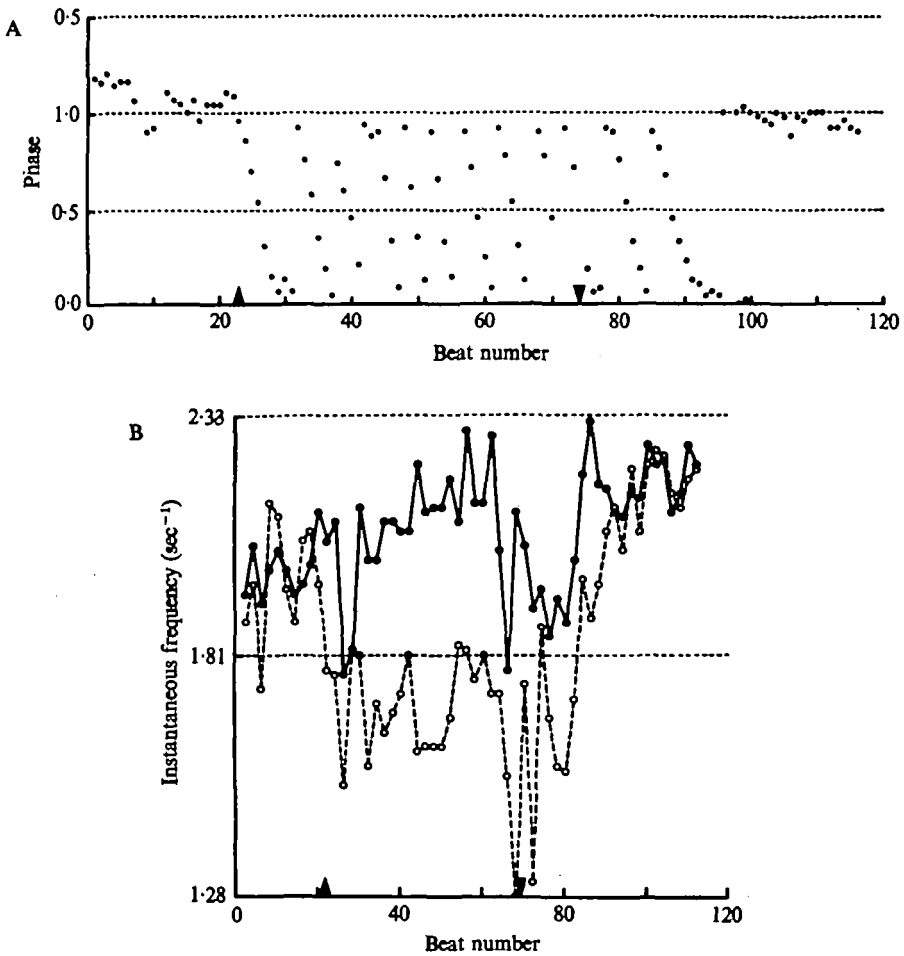


Fig. 8. The effect of restraining one SG on bilateral co-ordination and frequency of beating. Open circles and arrows on the phase plot indicate the period when the right SG was restrained. ○ = right SG, ● = left SG.

The overall impression from this series of experiments is that sensory afference, whether arising from the carapace or directly from the SGs, has bilateral ramifications. However, the afference from the carapace and other regions remote from the respiratory system appears to have roughly equal input to the two SG systems, while the re-afference from the SGs themselves demonstrates a predominantly ipsilateral influence.

DISCUSSION

All the observations in this paper assume that the nervous and muscular components controlling the SGs are as described by Pasztor (1968) and Mendelson (1971) for *Homarus americanus*. The components of the nervous system consist of a pacemaker oscillator in each half of the suboesophageal ganglion which programmes the reciprocal activity of two nerve trunks to the SGs. One trunk primarily innervates levator muscles with one motor neurone to a depressor (Young, 1973). The other trunk

innervates the depressor muscle pool. Eleven muscles and the sequence in which they are active were identified during the forward beating rhythm by Pasztor (1968). We have chosen here to use a simpler nomenclature to describe these muscles, one which focuses attention on function rather than anatomical origin and insertion. The 4 functional groups (Young, in preparation) which generate the 4 components of the pressure waveforms (and the muscles identified by Pasztor, 1968, abbreviations in parenthesis) are:

L_1 (CEM, ACEM), L_2 (PSDM, BEM), D_1 (CFM, BFM, BTM),
and D_2 (ASDM, SFM).

Muscles ABEM and ECM (Pasztor) are not classified as SG muscles as they are located so as to move the endites of the maxillae rather than the SG. The sequential activity of these muscles during forward beating and the altered sequence during reversal beats is:

$D_1, D_2, L_1, L_2, D_1, \text{--}, L_1, \underbrace{(L_2), D_1, D_2, L_1}_{\text{reversal}}$

The D_2 group is always absent during a reversal, while the L_2 group is active later than normal or also deleted. Studies on the crab *Carcinus maenas* (Young, 1973) indicate that the muscles responsible for depressing the anterior tip of the SG (D_2) also lose a burst at the onset of reversed beating. In the crab, however, reversed beating continues for many cycles and involves complete reversal of the sequence, the transition from forward to reversed beating being:

$D_1, D_2, L_1, L_2, D_1, \text{--}, L_2, L_1, D_2, D_1, \dots$
 fwd ←————— ↑ —————→ rev'd

As noted, neither reversals nor pauses necessarily affect the ongoing rhythm of beating. The obvious interpretation of this is that the 'commands' leading to these phenomena may bypass the oscillators to exert their effects upon the pattern generator (motor neurones?). With reversals this seems quite clear and logical. In the case of pauses however the evidence is more tenuous. Pilkington & Simmers (1973) maintain that, in *Cancer*, pausing may represent a prolongation of the retracted (depressed) phase. This would imply that pausing is associated with prolonged depolarization or hyperpolarization of the oscillator so that rhythmic beating is abolished and one set of motor units, the depressor set, discharges tonically. Our data on the lobster, however, differ from this. We found that beats interpolated at the pre-pause rhythm during bilateral pauses fall into step with the relative rhythm established on the resumption of bilateral activity (Fig. 6A). This would imply that the oscillators are unaffected by the pause-inducing commands. In addition, the SGs pause in both levated and depressed positions, and during such periods tonic activity was not seen in EMGs from any of the muscles. A click mechanism (Pilkington & Simmers, 1973), rather than tonic motor activity to any muscle group, could account for the position of the appendage during a pause, but such a mechanism has not been demonstrated in the lobster. Further information will probably be required before these observations can be reconciled.

The essential independence of the SGs on both sides is now well established by our observations on independent frequency changes, independent reversals and independent pausing, as well as by Mendelson's (1971) observations. Under severe hypoxic stress also (McMahon & Wilkens, 1975), the right and left SGs frequently beat alternately. As in many rhythmic systems in animals, however (Hoyle, 1964; Wendler, 1966, 1974), the activities in the two appendages are very clearly inter-related. Co-ordinated pauses, reversals and rate changes are commonplace. The respective neural sub-systems therefore must have provision both for unilateral modulation and for bilateral modulation from common sources. As noted previously, sensory input from the individual appendages may make largely ipsilateral connexions whilst that from other surface receptors may frequently have equal bilateral effects. The combination of such inputs would explain most of the present observations of unilateral and bilateral responses in SG beating.

The anatomical observations provide insight into the neuronal basis of bilateral coordination, as well as into the properties of the oscillators themselves. The tendency for coupling demonstrates the need for some form of cross-ganglionic connection. This cross-connexion appears to be bilaterally symmetrical and may be effected by the nerve collaterals identified by cobalt chloride back-filling of the depressor nerve trunks. Pasztor (personal communication) has also seen trans-ganglionic collaterals in lobsters, crayfish and *Callinectes*. The symmetrical nature of the coupling influence is apparent in the instantaneous frequency plots (Fig. 4), where it is often seen that during coupling both SGs reside at a fixed frequency, but both diverge in opposite directions upon breakaway into the drift mode. Both SGs appear to be entrained to each other at a relatively constant frequency, although coupling may also continue during major fluctuations in frequency (Fig. 7).

Less easy to explain is the cycle to cycle phase coupling between right and left SGs. The pattern of the coordination is strongly reminiscent of the relative or 'gliding' coordination described first for fin beating in teleosts by von Holst (1939) and later between contralateral walking legs in cockroaches (Hughes, 1957) and the stick insect (Wendler, 1966). Closer examination, however, reveals some, perhaps superficial, differences. Although at times there appears to be a dominant side whose frequency influences, but is not influenced by, that of the other side, it seems to be more general that both rhythms are influenced. The first condition would correspond to instances where the frequency of one side consistently remains uninfluenced during either relative drifting or relative coupling, while the frequency of the other side approaches that of the uninfluenced side during coupling.

The data are now being analysed in greater detail, but a possible mechanistic basis for the observed coupling patterns is not yet fully clear. Any mechanistic model for the system must be able to explain the following features. First, it seems that within limits, the phase of locking is independent of beat frequency. Both SGs can show simultaneous, transient frequency changes without corresponding changes in the phase of locking. Also, the coupling phase may remain the same at different temperatures although the beat frequencies change markedly. As shown in Table 1 the SGs of animal I remained coupled at a phase of 0.83–0.84 at temperatures of 4 °C and 11 °C, although the mean period lengths of these temperatures were 710 and 414 ms respectively. Similarly in animal II the SGs remained coupled at a

phase of 0.78 at 4 °C and 10.5 °C although the period lengths changed from 764 and 403 ms over this range. It should be emphasized that phase is defined as L_{AB}/T_A where L_{AB} is the latency from a beat in train *B* to the previous beat in train *A*, and T_A is the period length of the corresponding cycle in train *A*. Coupling therefore does not involve simple entrainment of trains of equal frequency so that one set lags at a fixed latency behind the other, the particular latency being determined by chance (Perkel, Gerstein & Moore, 1967). The phenomenon represents phase locking rather than latency locking. This is borne out by the fact that periods of phase coupling are frequently interrupted by periods of phase 'sliding' (Wyman, 1969) but the trains become locked again as they enter the preferred phase conformation. It seems, in addition, that the particular latency adopted during coupling is a function of the period length of the reference train, so that as the period length increases or decreases, so does the latency.

Secondly, the preferred phase may remain constant for long periods but also may change over a period of days. Changes in temperature in experiments conducted over a total period of 5–6 h may also result in a change in the preferred phase or mode of coupling, or both.

Thirdly, analyses of experiments showing the coupled/drift type coordination indicate that as the relative phasing approaches the extant preferred phase, the period lengths of both trains are usually affected. The interaction between the SGs no doubt persists irrespective of relative phasing, but the effectiveness of the interaction in causing coupling is clearly strongest at the preferred phasing. Under certain circumstances the effectiveness of this interaction is insufficient to lead to entrainment, but causes marked slowing of the drift through the preferred phase; under other circumstances the interaction leads to prolonged entrainment.

Perhaps the most plausible system for explaining the observed interactions is a phase exclusive system as described by Wyman (1969) and by Levine (1973) for coupling between insect flight motor units. The interactions between the two SGs in fact resembles those between reciprocally inhibiting synergistic insect flight motor neurones (Levine, 1973). First, when the appendages are in the coupled mode, a fixed phase is not rigidly held, but many sequences show gradual 'phase progression' (Wyman, 1969). In the coupled mode, however, this progression does not persist until all possible phases are encountered, but the relationship is continuously restored to a value within the preferred phase band. Secondly, in the drift mode, the rate of drift generally slows as the relative phase passes through the preferred band. Thirdly, the preferred phase band may shift over a period of days or, less frequently observed, abruptly to a completely different value. In the latter case, the shift from the first to the second preferred band may be achieved by a 'phase slide' (Levine, 1973).

One important difference is that there is no particularly obvious exclusion of closely synchronous cycling in the SGs. This, however, could be more apparent than real. Our analyses were performed on the essentially biphasic pressure waves produced by the activity of the SGs. It should be noted that Hughes, Knights & Scammel (1969) point out that there are no data on how the phasing of the underlying neuromuscular activity is related to the form of the branchial chamber pressure wave. Further analyses of the interactions on the assumption of a reciprocally inhibiting

interaction between the two SG systems promises to be very rewarding. If possible, exploitation of our knowledge of the anatomical location of the putative cross-linkage might also provide further understanding of the nature of the bilateral coordination.

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