PHASE CO-ORDINATION IN THE CARDIAC AND VENTILATORY RHYTHMS OF THE LOBSTER *HOMARUS AMERICANUS*

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SUMMARY

1. Relative co-ordination is demonstrated between the rhythms of the right and left scaphognathites (SG) and between the heart and either right or left SG or both simultaneously.

2. The co-ordination between these rhythms results from mutual phase-dependent alteration of the period lengths as demonstrated by the phase-response curves for the interactions.

3. The standard deviation of the SG period lengths is an inverse linear function of the percentage coupling between the two SGs ($r = 0.76$; $P < 0.01$).

4. The right SG motor output may show relative co-ordination with respect to a sinusoidal, dorso-ventral movement applied along the radial axis of that appendage.

5. This is taken to indicate that sensory reafference resulting from the interplay between the applied and intended movements can alter the SG rhythm depending upon the relative phasing of the two movements. The effects of sensory reafference are therefore not merely tonic as has been believed until now.

6. Cautery of the right oval organ, a major phasic mechanoreceptor of the SG, has no significant influence on any of the above relationships. The significance of this and of the widespread occurrence of relative co-ordination between rhythmic activities is discussed.

INTRODUCTION

The neuromuscular control of ventilation in the decapod Crustacea has now been studied by several authors (Segaar, 1934; Pasztor, 1968; Mendelson, 1971; Pilkington & Simmers, 1973; Wilkens, Wilkens & McMahon, 1974; Wilkens & Young, 1975; Young, 1975) and reviewed recently by Wilkens (1976). It seems clear from these studies that the rhythmic beating of each of the two ventilatory appendages, the scaphognathites (SG), is controlled by a separate oscillator neurone in each half of

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the sub-oesophageal ganglion. Sinusoidal variation of the membrane potential of this oscillator neurone is associated with reciprocal activation of the levator and depressor muscles of the appendage (Mendelson, 1971). The levator muscles fall into two groups (L1 and L2) as do the depressor muscles (D1 and D2). These four groups of muscles are activated in the sequence D1, D2, L1, L2, D1...during forward pumping, so that the latencies between the starts of bursts in the respective groups, and the durations of the bursts are proportional to the period length of the cycle (Pilkington & Simmers, 1973; Wilkens & Young, 1975; Young, 1975). Young (1973, 1975) observed that although the latencies between the bursts seemed unaffected by immobilization of the appendage, the durations of the bursts were somewhat altered. Even the detailed pattern of motor activity therefore seems largely independent of proprioceptive feedback which appears to have primarily a tonic effect – the rhythm being slower in its absence (Pasztor, 1968; Mendelson, 1971).

In spite of the apparent insensitivity of the phasing of the SG output pattern to sensory reafference, the SG is provided with numerous mechanosensory structures (Pasztor, 1969) which must feed back into the sub-oesophageal ganglion information of both dynamic and positional content. Most prominent among these is the dorsally located oval organ (Pasztor, 1969) which generates large phasic spikes in response to movements of the appendage. It therefore seemed possible that such phasic information might be averaged over several cycles as proposed by Wilson (1961) and Wilson & Gettrup (1963) for similar tonic effects on wingbeat frequency in the locust, due to phasic reafference from stretch receptors in the wing. These authors found that stimulation of the wing sensory nerves which carry the stretch receptor axons did not re-set the wing-beat cycle depending upon timing in the cycle, but merely led to increased beat frequency. However, Wendler (1974) has now shown that in spite of this, all phasic reafference from the wing is not lost but can in fact lead to phase-dependent alteration of the wing-beat rhythm, giving rise to gliding or relative co-ordination (von Holst, 1939; Wendler, 1966) between the periodic motor output to the wing muscles and sinusoidal movements imposed on the wing by an external driver. Burrows (1975) moreover shows that stimulation of the stretch receptor nerve only can affect the time of firing of flight motoneurones.

In view of these observations, closer examination of the phasic responses of the SG motor output to sensory reafference seemed to be indicated. Also, since relative co-ordination between the right and left SGs has already been demonstrated (Wilkens & Young, 1975) it is clear that the SG system itself must be responsive to at least some phasic input. In the present study, therefore, we examine four different aspects of phase co-ordination involving the ventilatory rhythm in the lobster. We investigate (1) the co-ordination between right and left SGs, (2) the co-ordination of the SGs with the heart, and (3) the effects upon these relationships and upon the timing of the cycles in the right SG, of sinusoidal movements imposed upon this appendage. And finally we examine (4) the influence on the above relationships of destruction of the oval organ. We demonstrate that relative co-ordination can occur not only between the two SGs, but between the heart and either SG, as well as between the motor output to the SG and the imposed sinusoidal movements. We analyse some of the detailed features of these relationships and their interactions, and show that oval organ cautery has no significant effect on the coupling strength in any of the above cases.
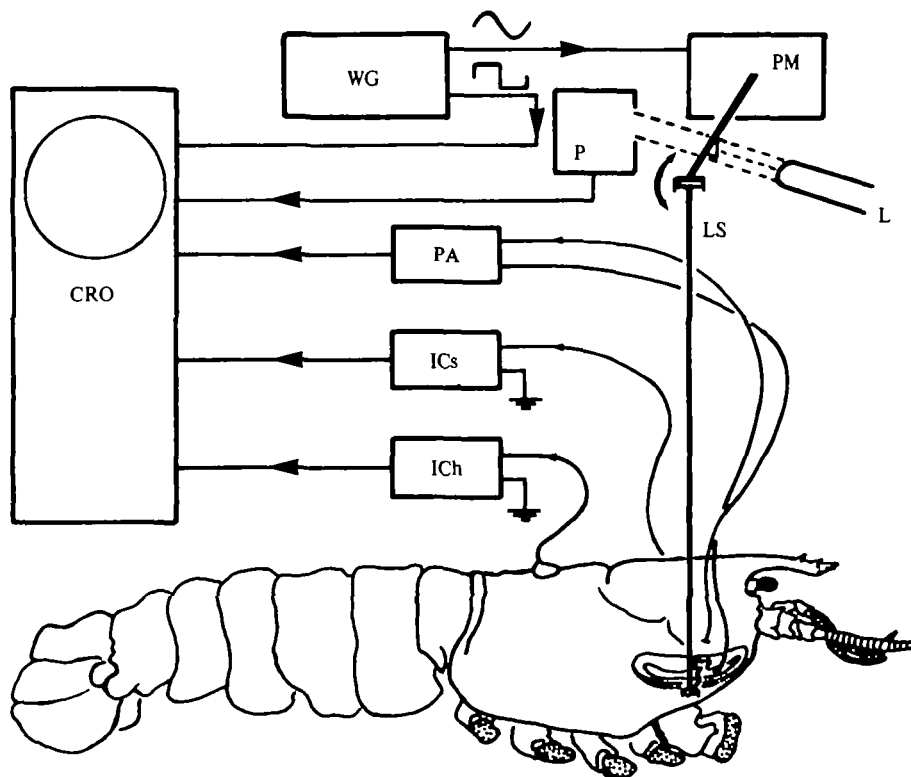
Fig. 1. Schematic diagram of the preparation and associated apparatus. All the signals going to the oscilloscope were simultaneously recorded on magnetic tape but this is not shown here. The legs of the animal have been omitted for clarity, but in reality only the right chela was removed by eliciting autotomy. The pen-motor could be uncoupled from the SG at the point of attachment of the lever system to the 'wand' on the SG (see text). ICh, Impedance converter for heart beat; ICs, impedance converter for left SG; L, light source; LS, lever system; P, photocell; PA, AC pre-amplifier for right scaphognathite EMG; PM, pen-motor; WG, waveform generator. (Apologies to J. L. Wilkens and B. R. McMahon for the lobster.)

MATERIALS AND METHODS

(a) The preparation

Animals were bought from local sea food markets and held in aquaria in the laboratory for short periods (about 1 week) before and during use. Records of the motor output to the right SG were taken by inserting fine insulated copper wires with bared tips into the depressor muscle D2a (Young, 1975). Access to the muscle was gained by removing a portion of the branchiostegites from over the exhalant canal where the SG is located (Fig. 1).

In a few cases, EMGs were recorded from the left SG as well, but in these cases the disruption of ventilation was severe and the branchial chambers had to be cannulated and perfused with aerated sea water. In most cases the left SG and branchiostegites were left intact and the pumping activity monitored using an impedance converter.

Cardiac activity also was recorded by means of an impedance converter which provides excellent, non-disruptive monitoring of mechano-electric activities. For both the heart and the left SG, the active lead of the impedance converter consisted

of a length of insulated wire ending in a disc of silver (*ca.* 2–3 mm diam.). This disc was placed in a depression drilled in the cuticle over the SG or heart and completely covered with Epoxy resin to hold it in place and insulate it from the bath. Correspondence between the actual movements of the SG and the output from the impedance converter was verified.

The movements of the SG in *Homarus* consist of dorso-ventral levation/depression about a longitudinal axis, combined with a rocking movement (pronation/supination) about a radial axis, roughly at right angles with the first (Wilkens & McMahon, 1972). By analogy with the situation in the crab it might be assumed that the dorso-ventral movement along the pronation/supination (P/S) axis will be approximately sinusoidal (Pilkington & Simmers, 1973; Young, 1975). Dorso-ventral sinusoidal movements, therefore, were applied to the right SG via an appropriately contoured length of wire (usually a portion of a long entomological pin) cemented along the P/S axis using cyanoacrylate glue. Part of this 'wand' protruded about ½ in beyond the border of the SG and could be attached via a lever system to the arm of a Harvard pen-motor (Fig. 1). To avoid any undue stress on the appendage the lever system was pivoted on longitudinal axes at the points of attachment to the wand and to the arm of the pen-motor (see Fig. 1). Hence, only the dorso-ventral component of the applied movements could be effectively transmitted to the appendage.

Sinusoidally modulated input to the pen-motor from a waveform generator resulted in corresponding movements of the arm, which could be varied independently in frequency, amplitude and offset. Amplitude and offset were adjusted so that nearly the full normal sweep of the SG was effected, without causing the wand to abut against the roof or floor of the exhalant canal. The actual movements of the pen-motor arm were monitored with a photocell device, and simultaneously, a square wave output from the waveform generator, in step with the sinusoidal output, was recorded in order to facilitate analysis (Figs. 1, 3). The animal was visually screened off from the peripheral devices.

(b) *Experimental protocol*

Having prepared the experimental animal and allowed a period of 1–2 h for recovery, a series of recordings were taken of activity in the heart and the two SGs under the following conditions:

(1) With both SGs freely moving and the oval organ intact.

(2) With the right SG being 'driven' (moved via the pen-motor/lever system) at various frequencies, usually in the range of its own natural frequency.

The right oval organ was then cauterized using a soldering iron with a fine, specially contoured tip. Care was taken to keep the cautery superficial or else the nerve to the D2a muscle would also be damaged. The animal was allowed to recover overnight or for about 2–3 h in the case of the bilaterally dissected animals whose branchial chambers had to be perfused. After this, further recordings were taken of heart and SG activity.

(3) With both appendages freely moving and the right oval organ cauterized and,

(4) With the right SG being driven at various rates as above.

In order to ensure that the cautery was effective, the right SG was then dissected and recordings made of activity in nerve *b* (Pasztor, 1969) while applying stimuli that normally would excite oval organ discharge. Fig. 2 shows the responses in one
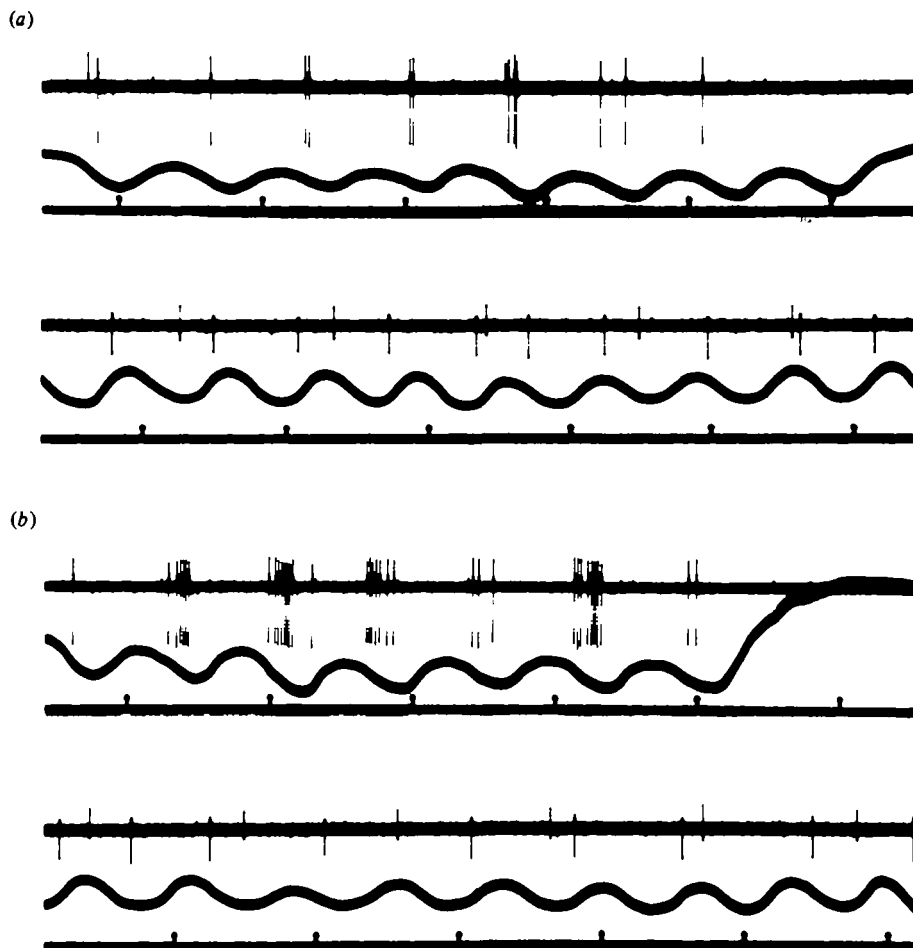
(a)

(b)

Fig. 2. Afferent activity in nerve *b* (Pasztor, 1969) during manipulation of the SG with the oval organ intact (upper record in *a* and *b*) and with the oval organ cauterized (lower record in *a* and *b*). In each record: top trace, activity in nerve *b*; middle trace, movement monitor; bottom trace, time pulse at 1/s. In (*a*) the SG is held at the distal border along the radial axis and moved dorso-ventrally as when driving via the pen-motor. The upper record shows strong, phasic discharge in the left SG in which the oval organ was intact. The lower record shows low amplitude, tonic activity which persisted even when movements were stopped. The lower record is from the right SG of the same animal after cautery of the oval organ (see text). The arrangement in (*b*) is similar but here the stimulus is pressure applied ventrally to the basipodal sclerite near the origin of the D2a muscle. Note here also the strong phasic activity in the intact appendage (upper record) and the weaker, tonic activity in the cauterized appendage (lower). In (*a*) upward movement of the middle trace = levation. In (*b*) downward movement = increased pressure.

such preparation in which records were also taken from the left (uncauterized) SG for comparison.

(c) Analysis of results

The essence of the present investigation lies in the analysis of the phase relation-ships between pairs of periodic processes. To permit analysis, heart beat, left SG movements, motor outout to the right SG and imposed movement signals were dis-played together and filmed over periods of 300–400 SG beats. Fig. 3 shows segments
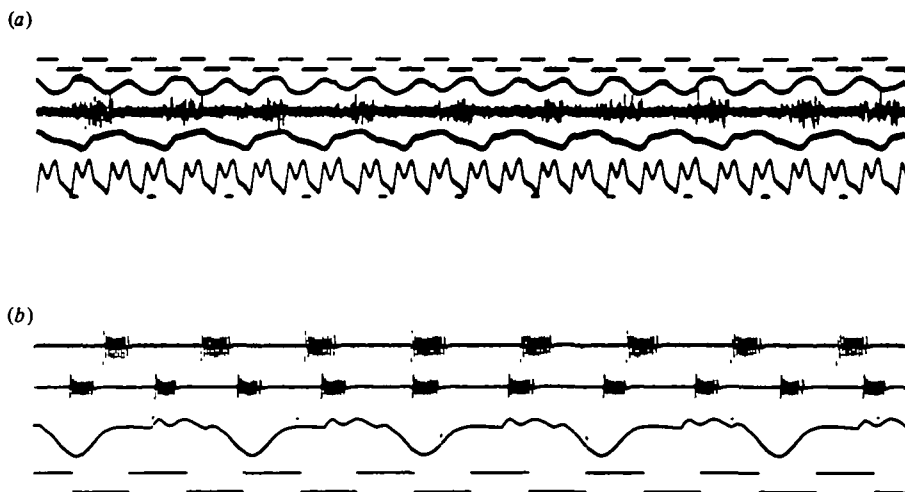
(a)

(b)

Fig. 3. Representative records filmed while driving the right SG via the pen-motor. (a) Trace 1 (top), square wave output from the waveform generator. Trace 2, photocell monitor of pen-motor arm movement; upward deflexion = levation. Trace 3, EMG from D2a muscle of right SG. Trace 4, impedance converter signal of heart beat. Trace 5, impedance converter signal of left SG beat. Trace 6, time pulses at 1/sec. Notice that the right SG here is beating much more slowly than the left, and has in fact adopted a rate about equal to the heart rate. Quite strong coupling between the heart and right SG was observed during this run. (b) Here the photocell trace has been omitted and EMGs are recorded from the D2a muscle in both the right and the left SG. Trace 1, EMG right SG. Trace 2, EMG left SG. Trace 3, impedance converter record of heart beat, with super-imposed time pulses at 1/sec. Trace 4, square wave output from the waveform generator; rise = point of maximal levation.

of typical records obtained while driving the right SG. The times of fixed identifiable points in each set of repeating cycles were digitized onto computer cards. Henceforth the sets of continuously varying cycles were treated as sets of repeating point processes or 'events' and analysed as such using appropriate digital computer programmes.

Sequential phase plots were used to display the phasing of consecutive events in one train (the 'test' train) in the cycles of another (the 'reference' train). Frequency distribution histograms for the phasing of the test events in the reference cycles were derived from these plots. And concurrently, the data were subjected to polar co-ordinate analysis as described in detail by Hughes (1972). This analysis treats events occurring at different phase angles in the reference cycle as equally weighted points at appropriate angular displacements on the rim of a circle of radius 1. The angular displacement of the centre of mass of the resulting distribution and its distance from the centre give respectively the mean angular displacement between the two sets of cycles, and the strength of coupling (percentage coupling) between the trains. From the latter a t-distributed parameter T may be derived whose value gives an index of the statistical significance. This method is suitable only for uni-modal distributions since symmetrically placed peaks in bi- and tri-modal distributions tend to negate each other, giving weak coupling percentages and low T values. For such distributions the χ^2 test of significance on the frequency distribution histogram is more appropriate.

In order to reveal systematic interactions depending upon phasing between pairs of event trains, phase-response curves (Wendler, 1966; Pavlidis, 1973; Pittendrigh

973) also were constructed. These were used in two forms. For the most part they were expressed as conditional phase drift plots as defined by Wendler (1974). In this plot, which is derived directly from the sequential phase plot, the mean phase advance $\Delta\phi$ from one cycle (n) to the next $(n+1)$ is computed for each phase category (ϕ_i) so that

$$\Delta\phi_i = [\Sigma(\phi_{n+1} - \phi_{i,n})]/N_i.$$

A plot of $\Delta\phi_i$ against ϕ_i should then reveal any systematic dependence of the rate of phase drift upon the relative phasing between the two trains.

Alternatively the phase-response curve may take the form of a plot of the deviation of mean period lengths for different relative phasing, from the overall average period length. This phase-dependent deviation in period length (ΔP_i) is plotted as a function of the associated phase category (ϕ_i). This type of plot was used for one set of analyses (Fig. 9) because it was particularly illustrative of the way in which phase co-ordination between trains may be effected. Indeed since rate of phase drift (phase advance) depends upon the fractional difference between the period lengths of the two trains, this latter type of plot seems more direct and hence may be more generally useful for the present type of analysis. One limitation of these phase-response analyses is that the measurements are taken usually while relative phasing is changing rather than under steady-state conditions. The resulting plots therefore will not be true phase-response curves if there is a delay of one or more cycles in response to the influence of a given phase relationship, or if the influence persists for more than one cycle.

RESULTS

1. *Coupling between the right and left scaphognathites*

In almost all cases coupling between the ventilatory cycles of the two SGs was significant $(P < 0.05)$ following the pattern of lock and drift or gliding co-ordination (Fig. 4a), or phase locked with no drift (absolute co-ordination) as described previously by Wilkens & Young (1975). Frequently the lock/drift type of co-ordination was accompanied by equalization of the period lengths during the locked periods, with the beat frequencies in one SG increasing while the other simultaneously fell during the drift periods. This pattern of activity also was described previously by Wilkens & Young (1975). Of 44 different measurements on seven different animals only seven showed no preferred phasing between the two SGs. All these were from the same animal in which the left SG tended to beat considerably faster than the right. In most cases, however, the two appendages maintained similar average frequencies with period length ratios close to 1·0. Fig. 5 shows the coupling percentages between the two SGs as a function of the period length ratios. Even with ratios close to 1·0 widely varying though significant coupling strengths occur. Outside of this region however only weak coupling is found.

Fig. 4(b) shows the frequency distribution histogram for the whole run from which Fig. 4(a) was taken, and Fig. 4(c) shows the conditional phase drift plot. There is a strong tendency in this case for the SGs to beat in synchrony, but other preferred phase relationships may also be found. Clearly (Fig. 4c) this preference results from systematic variations in the rate of phase progression depending upon the relative
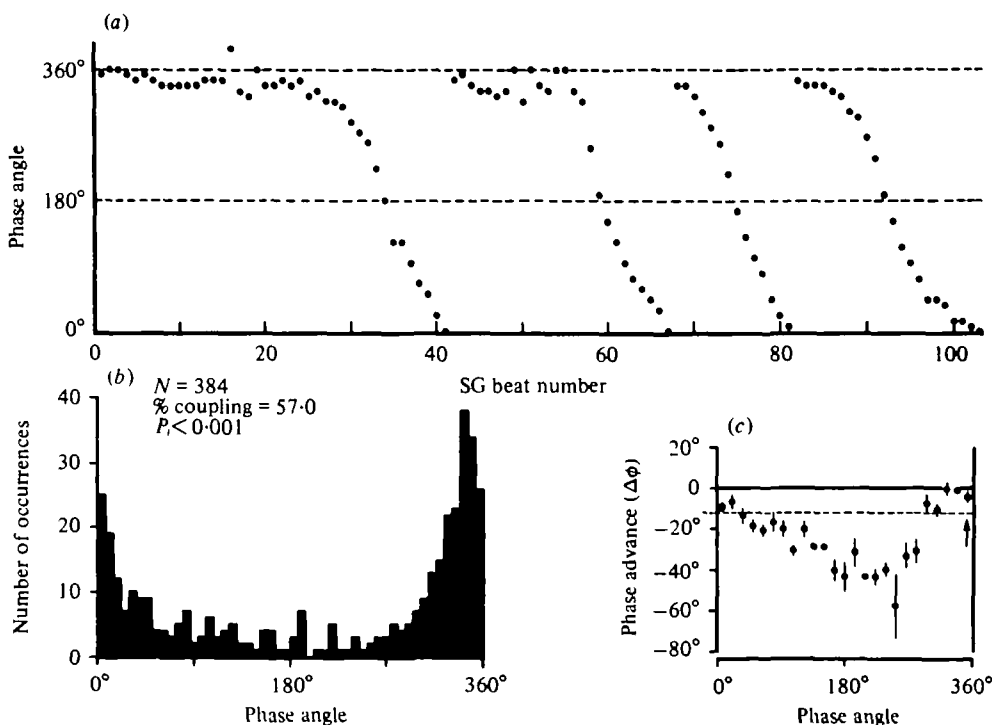
Fig. 4. Relative co-ordination between the right and left SGs after cautery of the right oval organ. (a) Sequential phase plot for the phasing of the left SG (mean period length = 604 ms) beats in the cycle of the right SG (mean period length = 625 ms) for consecutive beats of the left SG. In this and in all subsequent plots of this sort 360° = 0°. (b) Frequency distribution histogram for the phasing of the left SG beats in the cycle of the right. The mean preferred phase angle was 354°. (c) Phase-response curve for the mean phase advance ($\Delta\phi_i$) for different relative phasings (ϕ_i) of the left SG in the right. The mean overall phase advance (broken line) was $-12°$. The vertical lines indicate ± 1 s.e. about the mean $\Delta\phi$ values. The arrow indicates the preferred phase position of the left SG beat in the cycle of the right. The reciprocal plot for the phasing of the right SG beats in the cycle of the left is similar but positive-going due to the longer average period length of the right SG cycles.

phasing of the two trains. This in turn must result from systematically varying perturbation of the period lengths of the cycles in each train by the other depending upon their relative phasing.

In view of this phase-dependent variation in period length of the cycles it might be expected that the overall variability of the period lengths should increase as the strength of coupling decreases – that is, as the amount of random drift through all possible phases increases. Accordingly it was found that for a total of 11 separate runs on one animal, the standard deviations of the period lengths of the right SG were strongly and negatively correlated with percent coupling between the two SGs ($b = -0.56$; $a = 107$; $r = 0.76$; $P < 0.01$). This could imply that even when the SGs are not strongly coupled, phase-dependent perturbation of the period lengths persists, leading to greater irregularity of beating in the uncoupled than in the coupled, stabilized mode. However, it may also be taken simply to indicate that one of the advantages arising from the coupling of the two rhythms is increased stability and regularity of pumping.
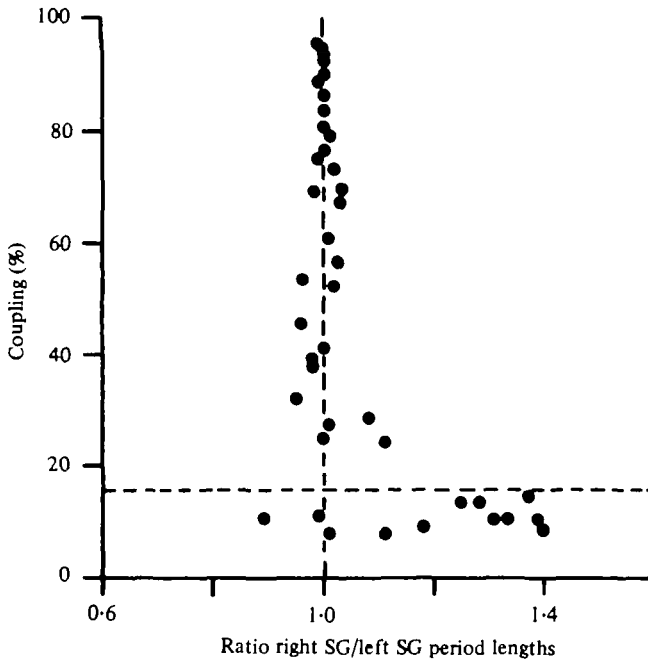
Fig. 5. Percentage coupling between the right and left SGs as a function of the ratios of the mean period lengths of the right to the left SG beats. The horizontal broken line indicates that coupling percentage below which $P > 0.01$. For runs with period length ratios close to 1·0 a wide range of coupling percentages occurs, but most are significant at the 0·01 level. Outside of this region few significant values are seen.

Table 1. *The influence on percentage coupling between the two scaphognathites (SGs) of movements applied to the right SG and of cautery of the right oval organ*

(The numbers in the body of the table are the coupling percentages from different runs.)

Animal	SG free, no cautery	SG driven, no cautery	SG free, oval organ cauterized	SG driven, oval organ cauterized
II	92·4	75·2, 76·6	95·5	28·5, 57·0, 69·7
IV	79·4	27·5, 53·3	88·9	67·2, 72·9, 86·4, 90·4

Cautery of the oval organ of the right SG seemed to have little influence on the coupling between the SGs. The percentage coupling after cautery was on the average slightly higher than before, but not significantly so (see Table 2). Similarly, forced movement of the right SG by means of the external driver may have a slight disruptive effect on the inter-scaphognathite coupling, but this is certainly not statistically significant. Table 1 shows an illustrative sample of some typical data from two animals.

2. *Heart–scaphognathite coupling*

The heart and SG rhythms may at times become either relatively or absolutely co-ordinated. However, the incidence of significant coupling is less frequent than between the two SGs and the strength of coupling is generally considerably weaker. The period length of the heart beat ranged between 1·4 and 3·9 times that of the SGs,
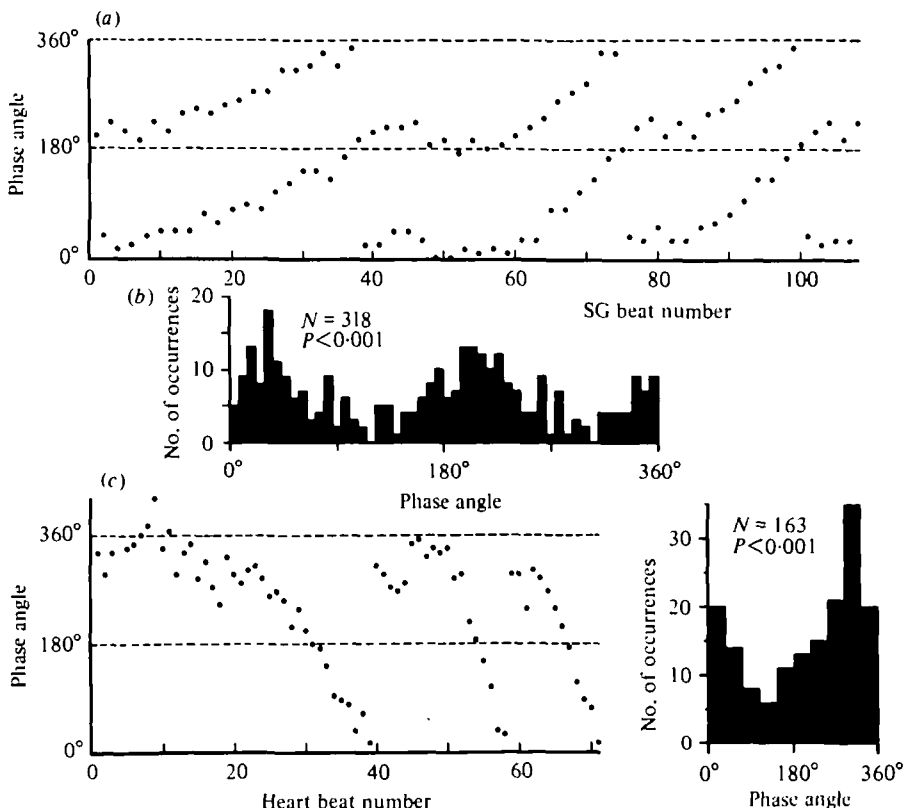
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Fig. 6. Relative co-ordination between cardiac and ventilatory rhythms showing a di-harmonic relationship. (a) Phasing of sequential beats of the left SG in the cardiac cycle. Notice the typical lock and drift pattern of relative co-ordination with two preferred phases. (b) Phase histogram showing two highly significant peaks in spite of the low coupling percentage (0·33 %) returned by polar co-ordinate analysis. (c) The reciprocal plot for the phasing of sequential heart beats in the left SG cycles. Here also the typical pattern of relative co-ordination is seen but the relationship is monotonic. (d) Frequency distribution histogram for the phasing of the heart beats in the left SG cycle. The percentage coupling for this uni-modal relationship was 32·6 %. The simultaneous coupling between the heart and right SG was very weak (0·81 %).

with the strongest coupling occurring at period length ratios between 1·7–2·0 and 2·8–3·1 (see Fig. 8). Thus, where significant phase coupling occurred between the two rhythms the SG beats were either bi-modally (Fig. 6 a, b) or tri-modally (Fig. 7 a, b) distributed in the cycle of the heart beat. In both Figs. 6 and 7 the characteristic lock and drift pattern of gliding co-ordination can be discerned, as it can be also for the reciprocal, uni-modal distribution of the heart beats in the SG cycle (Fig. 6 c, d) Often, however, the pattern for the distribution of the heart beats in the SG cycle was less organized, with the heart beats showing a less systematic tendency to occur at a given region of the SG cycle (Fig. 7 c, d).

In all cases, the calculated percentage coupling for the multimodal relationships was misleadingly low for reasons already indicated (see Materials and Methods). Hence significance levels had to be determined using the χ^2 test on the phase histograms or via coupling percentages calculated for the reciprocal uni-modal distributions of the heart beats in the SG cycle. Fig. 8 shows these latter coupling percentages a
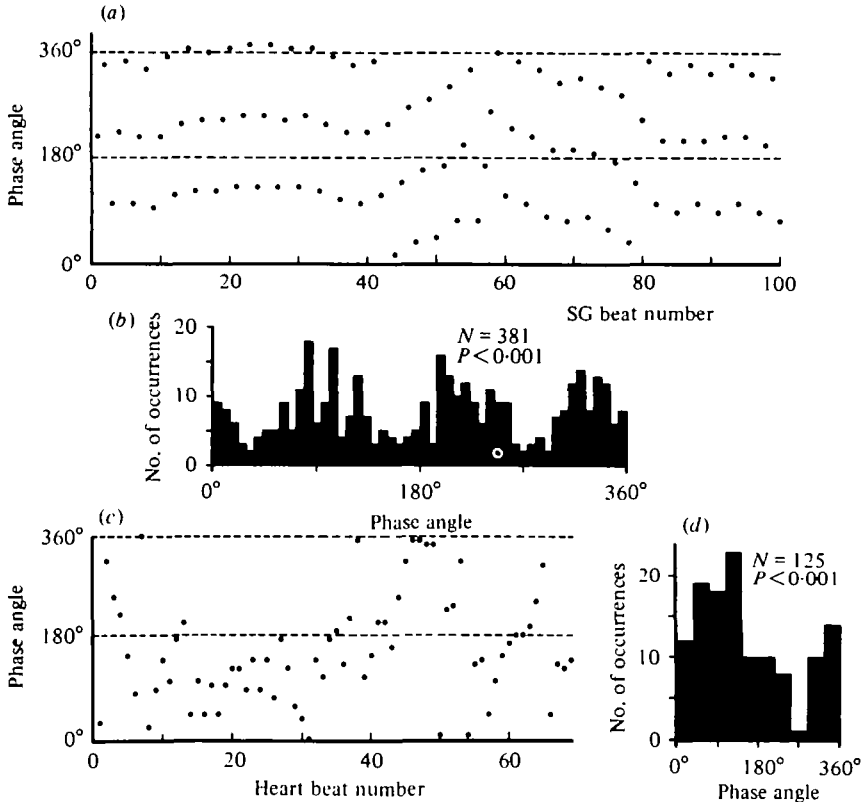
Fig. 7. Tri-harmonic coupling between the heart and the left SG. (a) Phase position of sequential SG beats in the heart cycle. Here we see at first a period of stable entrainment followed by a period of drift, then re-stabilization at the same phases as before. (b) Phase histogram with three highly significant peaks, in spite of the low indicated coupling percentage (0·70 %). (c) Reciprocal plot of the phasing of sequential heart beats in the left SG cycles. Here, a less organized relationship is seen that in Fig. 6c. Nevertheless (d), the phase histogram, shows a strong tendency for the heart 'beats' to occur in the first half of the SG cycle. The percentage coupling between the heart and left SG was 26·4 %. Coupling of the heart to the right SG also was significant but weaker (19·3 %).

function of the heart/SG period length ratios. Comparison of this plot with Fig. 5 for two SGs reveals the relative weakness of the heart–SG coupling and the comparatively wide spread of the period length ratios.

It is clear from Figs. 6 and 7 that phasing between the heart and SG cycles may be non-random and that this might result from systematically varying perturbations of the period lengths, contingent upon the relative phasing between the two cycles. This is shown more clearly by the phase-response curves in Fig. 9 in which differences between the 'conditional mean period lengths' and the overall mean period length (ΔP_i) are plotted as a function of relative phasing (ϕ_i). Fig. 9 (a, top) shows the phase-response curve (PRC) for the left SG, and Fig. 9 (a, bottom) for the heart beat period lengths. The data were taken from the run illustrated in Fig. 6. There seems to be no systematic relationship between ΔP_i and ϕ_i for the left SG. However, there clearly is systematic alteration of the period lengths of the heart cycles depending on the phasing of the SG beats. Moreover, the peaks in the phase histogram for the
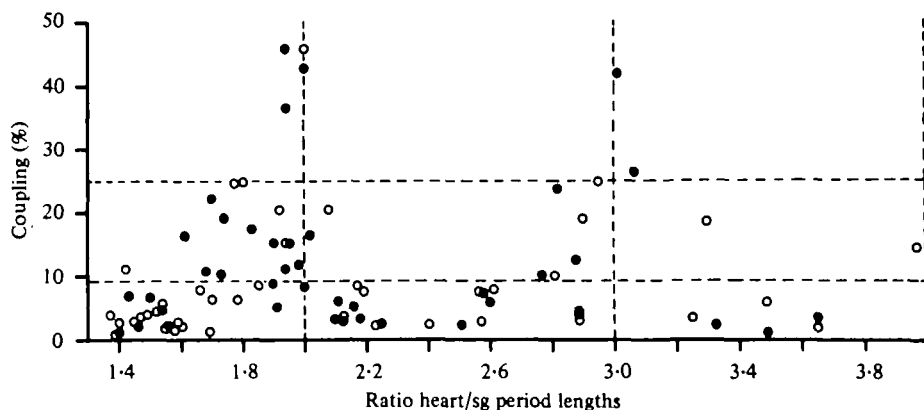
Fig. 8. Coupling percentages for heart beats in SG cycles as a function of the ratio of the mean period length of the heart to SG cycles. The vertical broken lines indicate whole number ratios. The horizontal broken lines enclose a region in which some coupling percentages are significant at the 0·05 level, and others not, depending on the number of cycles in the run. All points above the lines are significant; all points below are not. Circles, right SG; dots, left SG. There is no significant difference between the two. Notice that compared to Fig. 5 the coupling percentages are low and widely scattered. Relatively high values seem to occur at ratios not so close to whole numbers.

distribution of the SG beat in the heart cycle (Fig. 6b) correspond with those points where the mean period length of the heart cycles become maximally increased above the overall average. This seems reasonable since the ratio of the average period lengths of heart/SG for this run was 1·96 or less than 2·00. One therefore might expect that phase stabilization would involve increasing the period length of the heart beat, bringing the ratio closer to the integral multiple 2·00.

Fig. 9(b), derived from data for a different animal, shows that the situation is not always so simple. Here the ratio of the heart/SG period lengths is 2·02 so that the period length of the heart beat is slightly greater than a whole number multiple of the SG period lengths. One might therefore deduce that the preferred phasing now should lie in a region of the PRC in which the period length of the heart beat is reduced below average. However, the preferred phases once more correspond to the maxima in Fig. 9 (b, bottom) where the period lengths of the heart beats are *increased* with respect to the overall average.

Fig. 9(b, top) reminds us that we are dealing here with two variable rhythms. In Fig. 9(a) the SG rhythm appears to be independent of the heart rhythm, while the heart rhythm depends upon the relative phasing with the SG. In Fig. 9 (b, top) it is seen that the SG rhythm also may show systematic alterations in period length depending on the relative phasing of the heart beat. At the preferred relative phasing, the period length of the SG cycles is increased above average to 605 ms, with the corresponding average period lengths of the heart beats being 1213 ms, giving a ratio of 2·00. Interestingly, in spite of the fact that the two rhythms are brought to, and not just close to an integral multiple at the preferred phasing, absolute entrainment does not result. Evidently it is not merely the period length ratios which serve to determine the extent of co-ordination between rhythms.

Earlier work (Coyer, 1977, 1978) indicates that the strength of coupling between the heart and SG might also be related to the strength of coupling between the
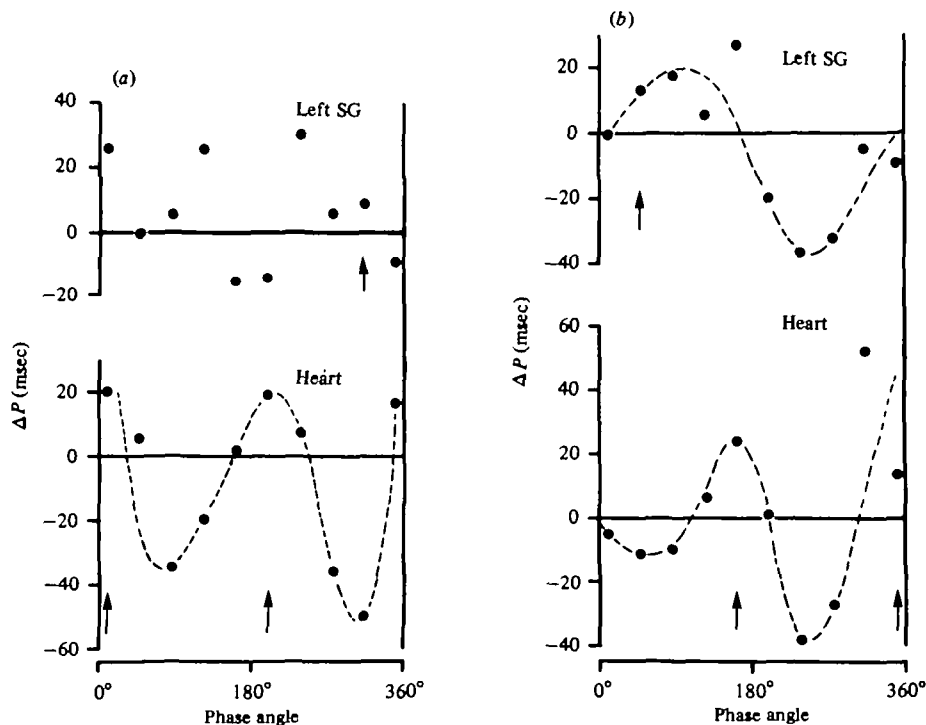
Fig. 9. Phase-response curves (PRCs) for the left SG and heart rhythms during di-harmonic coupling in two different preparations (*a*, *b*). Taking a run of 300–400 consecutive SG cycles, each SG cycle was divided into 10 equal parts or 'phases' (ϕ_i, *i* = 1–10). The mean SG period length was found for all cycles in which the heart 'beat' occurred in phase 1, in phase 2 and so on. The deviations (ΔP_i) of these means from the overall mean period length for the run were then plotted as a function of the corresponding phases categories (ϕ_i) in the upper graphs. The reciprocal relationship between ΔP for the heart cycles and ϕ, the relative phasing of the SG beat is plotted in the lower graphs. The broken trend lines were fitted by inspection. (*a*) The period lengths of the left SG show no systematic variation depending on the phasing of the heart beats. The cardiac cycles however do vary systematically depending on the phasing of the left SG beats. Arrows indicate the preferred phases. Note the correspondence of the preferred phases with the maxima in the cardiac PRC. (*b*) Similar plots for a different preparation. Here both the SG and heart show reasonably systematic PRCs indicating that each rhythm may systematically perturb the other. Notice that here as in (*a*) the preferred phases for the SG beats in the heart cycle correspond with the maximum mean period lengths of the heart cycles. The coupling of the heart to both right and left SG was about equal (44 %) and that between the two SGs also was strong (38 %).

SGs. However, the present data reveal no significant correlation between SG–SG percentage coupling and heart–SG coupling. Nor are perturbations of the heart rhythm, as measured by the standard deviations of the period lengths, related to the strength of SG–SG coupling ($b = -1.00$; $a = 163$; $r = -0.41$; $P < 0.05$; 9 degrees of freedom). Similarly, heart–SG coupling seemed to be completely unaffected by cautery of the right oval organ (Table 2).

3. *Heart–driver co-ordination*

No correlation could be found between heart beat frequency and the frequency of movements imposed on the right SG, either between preparations or within a given preparation. Nor were any systematic differences apparent in the heart rates when the
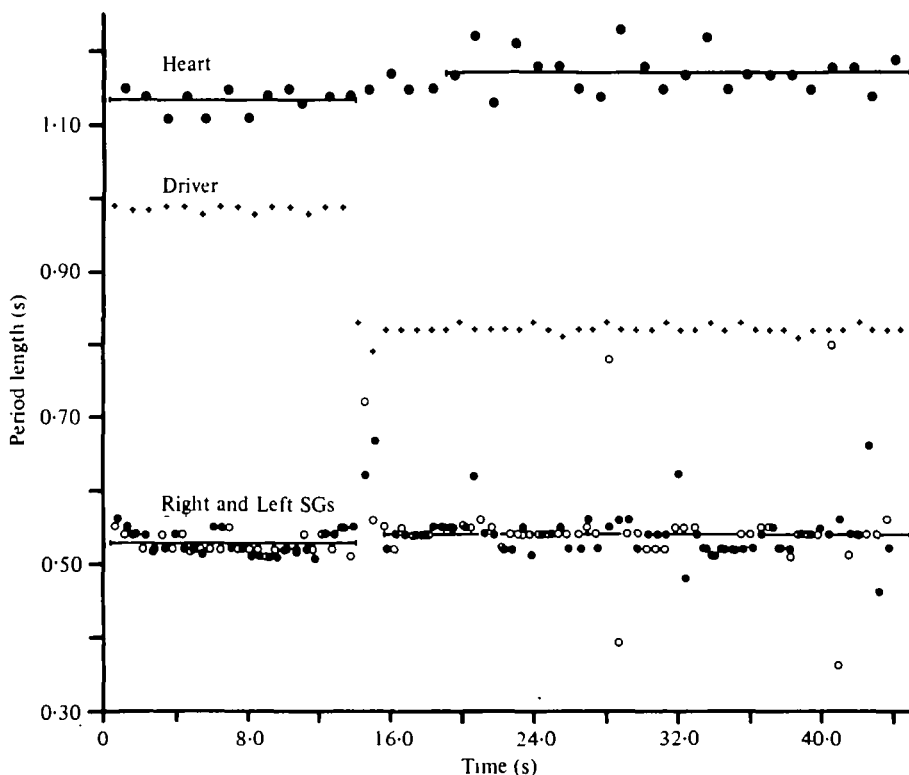
Fig. 10. Alterations in the heart and SG rhythms accompanying abrupt alteration in the rate of the rhythmic movements applied to the right SG. The mean period length of the heart rhythm (large dots) was 1·14 s before and 1·17 s after the driving rate was increased. The period length of the driver was altered from 0·99 to 0·82 s. Notice the transient increase in scatter of the heart cycles after the switch. The mean period lengths of the right SG (small dots) and the left SG (circles) were identical throughout. Before the switch the mean values were 0·53 s and after, 0·54 s. The horizontal lines drawn through the points indicate the mean values for the period lengths. Notice the increased incidence of divergent cycles in both the right and the left SG after the switch. This type of change was not a constant feature of the experiments of this type.

appendages were freely moving as compared with when the right SG was being driven. In at least three separate individual cases however, abrupt alteration in the rate of driving was accompanied by increased scatter in the period lengths of the heart beat (Fig. 10). This seemed to be a transient effect and probably is just another example of the extreme sensitivity of the heart rhythm to diverse stimuli (Larimer, 1964), without any special significance.

'Significant' preferred phasing between the heart and driver rhythms were obtained in 2 out of 22 cases in normal animals and in none out of 14 cases after cautery of the right oval organ. Since significance was being judged at the 0·05 level, there seems to be no cogent reason for supposing that these two significant cases out of 36 were anything but fortuitous. In any event there was no sign of systematic relationships between relative phasing of the heart and driver rhythms, and the period length of the heart beat. In short there was no evidence either of persistent tonic or of phasic relationships between the heart rhythm and the rhythm of movements imposed upon one of the ventilatory appendages.
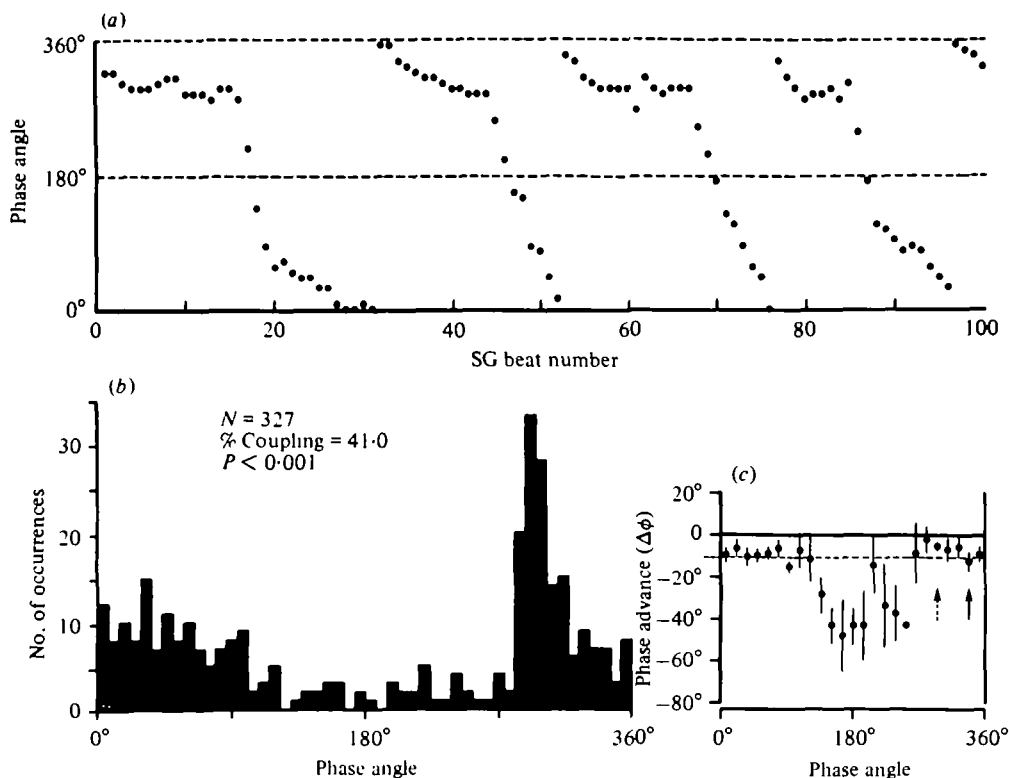
Fig. 11. Relative co-ordination between a forced rhythmic movement applied to the right SG and the motor output to the right SG, with the oval organ intact. (*a*) Phasing of sequential beats of the right SG in the cycle of the imposed movement. There is regular, quite typical lock and drift behaviour. (*b*) Phase histogram. The highly significantly peak is asymmetrical, with a sharp rise at about 290°, then a slow fall-off. The modal phase angle (broken arrow) is thus different from the mean phase angle (solid arrow). (*c*) Phase-response curve for mean conditional phase advance (Δφ) as a function phase. The overall mean phase advance was −11° (dotted line). Notice the systematic variation in the PRC.

4. *Scaphognathite–driver coupling*

The rate of movements applied to the right SG had very little if any obvious relationship with the rate of beating of the SGs (Fig. 10). Nonetheless, there was at times distinct and significant non-random phasing between the centrally generated motor output to the right SG and timing of the imposed movements, confirming earlier preliminary findings (Coyer, 1977; Coyer, Young & Wyse, in preparation). The typical pattern of relative co-ordination between the two rhythms is seen quite clearly in Figs. 11(*a*) and 12(*a*) in which the rates of phase drift slow markedly as the cycles pass through a given, preferred phase. The respective phase histograms (Figs. 11*b* and 12*b*) and conditional phase-drift plots (Figs. 11*c* and 12*c*) demonstrate that over the whole run systematic changes in the rate of phase progression with relative phasing give rise to significant phase coupling between the two rhythms. As noted already, this must result from systematic changes in the fractional difference between the two period lengths depending upon their relative phasing. There was no con-ent indication, however, that the standard deviation of the SG period lengths
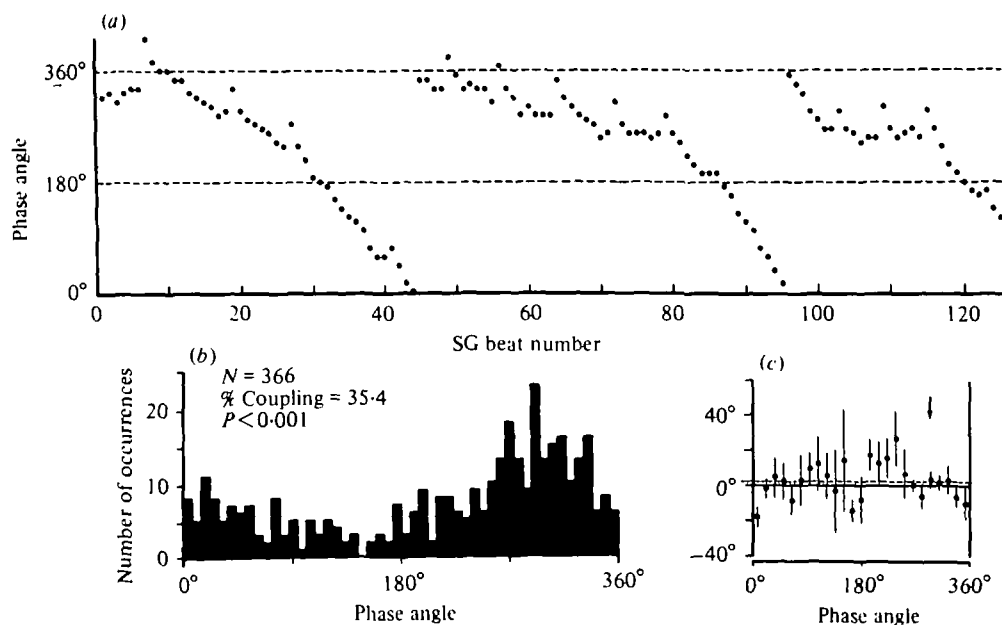
Fig. 12. Relative co-ordination between imposed rhythmic movements and the motor output to the right SG after oval organ cautery. (a) Phasing of sequential beats of the right SG in the cycle of the imposed rhythm. In this particular run there was a regular, recurrent perturbation of the relative phasing, the source of which is unknown. Nevertheless the general pattern of relative co-ordination is apparent. (b) Phase histogram showing that over the whole run there was a highly significant preferred phasing. (c) Conditional phase drift PRC. This is considerably less clear than that in Fig. 10 (c) partly due to the anomalous perturbation, but a systematic pattern can still be discerned. Note also that in spite of the generally negative phase drift seen in (a) the mean phase advance for the whole run was $+2\cdot2°$, the mean period lengths of the SG and driver being 529 and 523 ms respectively (ratio = 1·01). The arrow indicates the preferred phasing.

varied with the strength of coupling with the imposed rhythm, or with the rate of driving.

Fig. 13 shows the overall distribution of the coupling percentages between the right SG and driver rhythms, as a function of the ratio of SG/driver period lengths. The strength of co-ordination is very weak, more comparable to that between the heart and SG than to that between the two SGs. This figure includes pooled data, values obtained before and after oval organ cautery not being separated since no significant change in coupling strength resulted from destruction of the oval organ.

In order to ascertain the effect of oval organ cautery on the SG–driver co-ordination it is necessary to remember that the ratio of the period lengths is an important determinant of coupling strength. Differences between indiscriminately pooled means could simply reflect differences in the overall ratios of the SG beat rates to driving speeds, which were rather arbitrarily selected. To avoid this problem, Table 2 compares, for SG–driver coupling before and after cautery of the oval organ, the means ($\pm$ s.e.) of the coupling percentages obtained with period length ratios between integral values plus or minus 0·10. Interestingly, the mean coupling percentage of SG–driver increases slightly though not significantly, after oval organ cautery. The oval organ activity therefore if anything, might have a tendency to disrupt rather than to generate phase coupling of the SG rhythm to an imposed movement.
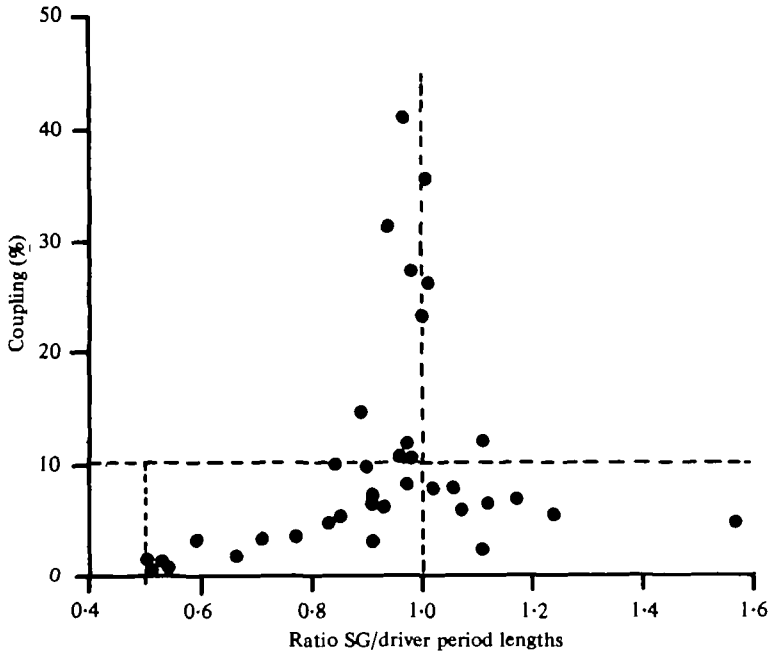
Fig. 13. Percentage coupling between the SG and driver rhythms as a function of the ratio of the mean period lengths of the right SG to driver. The vertical broken lines indicate harmonic ratios. Horizontal broken line indicates the percentage coupling level below which $P > 0.05$. Notice that the strength of coupling is very weak compared with the inter-SG coupling strength, but is very similar to the heart-SG coupling.

Table 2. *The influence of cautery of the right oval organ on the coupling between the right scaphognathite (SG) and driver, SG and heart and between the two SGs*

Entries with the associated parenthetic numbers represent mean coupling percentages for runs in which the mean period length ratios fell between integral values plus or minus 0·10. The numbers in parenthesis are the standard errors of the means. In no case was there a significant change after cautery, but in all cases there was a slight rise in percentage coupling. The pooled means afford a good comparison of the relative strengths of the three relationships.

	SG–driver		SG–heart		Right SG–left SG	
	Before cautery	After cautery	Before cautery	After cautery	Before cautery	After cautery
N % coupling	13·2 ($\pm$4·1)	19·3 ($\pm$4·0)	20·7 ($\pm$3·9)	24·7 ($\pm$3·8)	57·4 ($\pm$6·4)	66·0 ($\pm$7·4)
	9	8	15	6	17	15
Pooled means	16·1 ($\pm$2·9)		21·7 ($\pm$2·9)		61·5 ($\pm$4·6)	
N (total)	17		21		32	

Table 2 also shows for comparison similarly derived mean coupling percentages before and after oval organ cautery, for the SG – heart and the SG–SG relationships. In no case does oval organ cautery result in a significant change in coupling strength although in all cases there is a slight increase on the average after cautery. The pooled means included in the Table also afford a good comparison of the coupling strengths of the three relationships. There is no significant difference between the SG–driver and SG–heart coupling strengths and the SG–SG relationship is about three times as strong as either of these.

DISCUSSION

I. *The basis of co-ordination of the ventilatory and cardiac rhythms*

In the actively ventilating lobster there are at least three coupled oscillators operating, associated with the two scaphognathites and the heart. Wilkens & Young (1975) have already described cross-ganglionic motoneuronal processes which conceivably could mediate co-ordination between the two SGs in the lobster. Pilkinton & MacFarlane (1978) have demonstrated similar processes in the crab. But the possibility remains that the required phasic information could be transferred either from premotoneuronal levels, or via sensory reafference finding its way across the ganglion. Oval organ sensory reafference at least is apparently not a primary contributor to the coupling of the oscillators since cautery of the right oval organ has no significant influence on either interscaphognathite coupling or coupling between heart and SG.

Young (1973, 1978) provides evidence that in the Norwegian lobster *Nephrops* the phasic cues which serve to entrain the heart and SG rhythms may reach the heart via the cardioregulatory nerves. These nerves may carry spikes, particularly in the cardioinhibitory units, which have non-random phasing in the cycle of the ipsilateral SG. This may well be of significance in view of the fact that in both cases analysed (Fig. 9) the period lengths of the heart cycles are maximally increased (inhibited?) at the preferred phasing with the SG. Also of interest is the fact that the SG rhythm (Fig. 9*b*, top) may be modulated depending on the phasing of the heart cycles. This conceivably could mean that in addition to receiving phasic information concerning the SG cycle, the heart may feed back to the SG, phasic information concerning its own cycle. Indeed, sensory reafference from the heart to the sub-oesophageal ganglion has been described (Taylor, 1970; Field & Larimer, 1975) but its possible functional role has not been investigated. Similar phase co-ordination between cardiac and ventilatory activity has been observed in the dogfish (Hughes, 1972) and in *Limulus* (Watson & Wyse, 1978). The latter study also shows that rate covariation of the cardiac and ventilatory rhythms is mediated via the cardioregulatory nerves, as it is in the decapod crustaceans (Young, 1978). Presumably the phase-related information necessary for phase co-ordination also is transferred via this same route as it is in the decapods.

It still remains unclear how the relative phasing of the cycles in the two SGs will influence the entrainment of the heart. If the phasic information in both the right and left cardioregulatory nerves is similarly phase locked to activity in the ipsi-lateral SG, then there should be reinforcement of the phasic information transmitted to the heart when the two SGs are locked in phase as in Fig. 4. Likewise, there might be mutual obliteration if the two SGs are coupled 180° out of phase, of if they drift randomly. There is, however, no indication here of any relationship between SG–SG coupling and SG–heart coupling. In fact, there is contrary evidence (Coyer, 1977, 1978) that the tendency for coupling between the heart and SG is strongest when the SGs are uncoupled, when one of the SGs may become strongly coupled to the heart. From Coyer's (1977, 1978) study it appears that the three oscillators might be mutually attracting but tend to become co-ordinated only in pairs, to the exclusion of the third. However, several cases were observed here of relatively strong coupling between both SGs and between heart and SG rhythms simultaneously (e.g. Fig. 9

It seems therefore that some important determinants of the strength of coupling between the cardio-ventilatory rhythms are still being overlooked.

One possible source of variability might simply be individual variation, due to low selective pressures operating on the specification of the strength of coupling between the rhythms. For example, Hughes (1972) also observed that there was a great deal of individual variation in the cardio-ventilatory coupling parameters in the dogfish, variations within individuals being much more limited. This suggests that while coupling between these rhythms might be of some consequence to organisms, the actual strength of the relationships is not important. However, it is not an adequate explanation, since the amount of unexplained variation within the individuals both for the dogfish and the lobster is still very great.

II. *Sensory reafference and the entrainment of rhythms to an external Zeitgeber*

The phasic entrainment of the SG rhythm to an imposed movement demonstrates that sensory reafference must play some role, albeit a weak and variable one, in regulating the timing of the SG cycles. Sensory input arising from the manipulation of the appendage may influence period length of the SG cycle depending upon the phasing of the stimulus in the SG cycle. In the thinly cuticularized, flexible SG the sensory responses to an applied movement will not be simple, but will reflect complex interactions between the effects of the intended and applied movements. None-the-less the net effect is a resetting of the SG rhythm depending on the relative phasing of the two movements (Fig. 11c). This is a necessary feature of relative co-ordination. If the difference between the period lengths of the two rhythms is sufficiently small, and the influence of the interactions sufficiently large, then the two frequencies may become equal at a certain relative phasing. Absolute co-ordination might then result, particularly if the form of the phase-response curve is such that any phase advance or retardation from the preferred phase will lead to alterations in period lengths, which will tend to restore the original preferred phasing. In most cases studied here, absolute co-ordination did not persist for long periods, perhaps due partly to the weakness of the interactions and partly to the strength of random, unrelated perturbations.

Gliding entrainment of a centrally generated rhythm to an imposed movement has been seen in the locust flight system (Wendler, 1974), in the ventilatory system in *Limulus* (Wyse, 1975; Wyse & Page, 1976) and now in the ventilatory system in the lobster. A common feature of these three systems is the absence of obvious phase-related responses to proprioceptive feedback. The locust flight system showed no phasic responses to stimulation of the sensory wing nerves in the hands of Wilson (1961) and Wilson & Gettrup (1963). In *Limulus* immobilization or forced movements of the gill plates over short periods, exert only very weak and largely tonic effects on the patterning of the motor output (Wyse, 1975), and movement of the first gill plate has no discernible effect (Wyse & Page, 1976). In the crab SG, immobilization does not apparently affect the phasing of the bursts of activity in the muscles (Young, 1975) and destruction of a major phasically active sense organ does not affect significantly the coupling of the SG rhythm to an imposed movement. Clearly only very subtle phasic sensory cues and very subtle changes in the motor output are required

in order to produce significant relative co-ordination to an applied movement. We might well take note of Wendler's (1974) suggestion for the locust flight system, that no one sensory organ need be fully responsible for the phasic effects. Individual sense organs studied separately, such as the wing stretch receptors in the locust or the oval organ in the lobster, may produce no obvious phasic effects. But the total response profile in the sense organs to more 'natural' stimulus configurations may be phasically effective due to additive, multiplicative or compensatory interactions. Interestingly, Burrows (1975) showed that whereas high frequency stretch receptor firing generally does not induce spikes in the ipsilateral flight motoneurones in the locust, it does produce sub-threshold depolarization which may sum with other inputs to induce spiking. Indeed the elegant experiments of Burrows (1975) provide us with a unique insight into the possible neural basis for weak and variable coupling to sensory inputs in the locust flight system.

III. *The importance of relative co-ordination*

Relative co-ordination between centrally generated, oscillatory motor patterns clearly is a ubiquitous phenomenon among both vertebrates and invertebrates. A moment's reflexion will reveal many examples, such as the difficulty of tapping out different rhythms which are not harmonically related, with either hand, or with a hand and a foot. The rhythms tend to become coupled. Entrainment of a centrally generated pattern to an external Zeitgeber forms another subset of commonly occurring phenomena which is probably related in underlying mechanism to the first. In the crustacean SG, sensory mechanisms may even be by-passed and the central oscillator entrained to a sinusoidally modulated AC current, applied directly to the ganglion (Pilkington, 1976).

Why should this tendency towards phasic co-ordination between rhythms be so ubiquitous? Clearly, the answer must lie either in some important property of oscillatory phenomena *per se*, or in some basic, universal feature of the organization of neural oscillators. Oscillatory activity forms such a large and important component of an animal's behavioural repertory (Craig, 1918) that the persistence of such interactions can hardly be accidental, and an understanding of the principles and mechanisms involved may not be at all so trivial as it might at first appear to be. In some instances the advantages of co-ordination are obvious. For example, relative co-ordination may contribute to organizing the stepping pattern in insects (Hughes, 1957; Wendler, 1966). Or on a longer time scale, it may contribute to the entrainment of internal circadian oscillators to the extant day-length (Pavlidis, 1973; Pittendrigh, 1973). But in many if not most instances, the advantages are not so clear. Perhaps the co-ordination between intrinsic oscillators may aid in stabilizing their rhythms, allowing them to reinforce each other, and perhaps thereby serving as an energy conserving device. The decreased scatter of the SG period lengths with increased percentage coupling between the two appendages does support the argument for stabilization, although the correlation is open to other interpretations. Co-ordination of rhythmic motor output to extrinsic, rhythmic stimuli might result from a need during normal activity to phasically alter the motor output depending upon aberrant sensory reaff°rence resulting from activity produced by the output. This could aid both in regularizing the rhythm and in adjusting the motor output to compensate fo

loss, fatigue or other malfunction of some of the motor elements. Whatever the advantages, the widespread occurrence of relative co-ordination between rhythms and the importance of rhythmic activities to the organism, indicate that this is an important neurophysiological phenomenon whose neural basis is as yet inadequately understood.

REFERENCES

BURROWS, M. (1975). Monosynaptic connexions between wing stretch receptors and flight moto-neurones of the locust. *J. exp. Biol.* **62**, 189–219.

COYER, P. E. (1977). Neuronal mechanisms underlying the co-ordination of heart and gill-bailer rhythms in decapod Crustacea. Ph.D. thesis, University of Massachusetts, Amherst.

COYER, P. E. (1978). Heart and one gill-bailer rhythm phase couple in *Cancer borealis* and *Cancer irroratus*. *Comp. Biochem. Physiol.* (In the Press).

CRAIG, W. (1918). Appetites and aversions as constituents of instincts. *Biol. Bull.* **34**, 91–107.

FIELD, L. H. & LARIMER, J. L. (1975). The cardioregulatory system of the crayfish: neuroanatomy and physiology. *J. exp. Biol.* **62**, 519–530.

HOLST, E. von (1939). Die relative Koordination als Phänomen und als Methode zentralnervöser Funktionsanalyse. *Ergebn. Physiol.* **42**, 228–306.

HUGHES, G. M. (1957). The co-ordination of insect movements. II. The effect of limb amputation and cutting of commissures in the cockroach, *Blatta orientalis*. *J. exp. Biol.* **34**, 306–333.

HUGHES, G. M. (1972). The relationship between cardiac and respiratory rhythms in the dogfish, *Scyliorhinus canicula* L. *J. exp. Biol.* **57**, 415–434.

LARIMER, J. L. (1964). Sensory induced modifications of ventilation and heart rate in crayfish. *Comp. Biochem. Physiol.* **12**, 23–36.

MENDELSON, M. (1971). Oscillator neurones in crustacean ganglia. *Science, N.Y.* **171**, 1170–1173.

PASZTOR, V. M. The neurophysiology of respiration in decapod Crustacea. I. The motor system. *Can. J. Zool.* **46**, 585–696.

PASZTOR, V. M. (1969). The neurophysiology of respiration in decapod Crustacea. II. The sensory system. *Can. J. Zool.* **47**, 435–441.

PAVLIDIS, T. (1973). *Biological Oscillators. Their Mathematical Analysis.* New York and London: Academic Press.

PILKINGTON, J. B. S. (1976). Experimental coupling of crab (*Carcinus maenas*) second maxilla to an alternating current. *Experientia.* **32**, 1435–1437.

PILKINGTON, J. B. S. & MACFARLANE, D. W. (1978). Numbers and central projections of crab second maxilla motor neurones. *J. mar. biol. Ass.* **58**, 571–584.

PILKINGTON, J. B. S. & SIMMERS, A. J. (1973). An analysis of bailer movements responsible for gill ventilation in the crab, *Cancer novae-zelandiae*. *Mar. Behav. Physiol.* **2**, 73–95.

PITTENDRIGH, C. S. (1974). Circadian oscillations in cells and circadian organization of multicellular systems. In *The Neurosciences. Third Study Program* (ed. F. O. Schmitt and F. G. Worden), pp. 437–458. MIT Press.

SEGAAR, J. (1934). Die Atmungsbewegungen von *Astacus fulviatilis*. *Z. vergl. Physiol.* **21**, 492–512.

TAYLOR, E. W. (1970). Spontaneous activity in the cardio-accelerator nerves of the crayfish *Astacus pallipes* Lereboullet. *Comp. Biochem. Physiol.* **33**, 859–869.

WATSON, W. H. & WYSE, G. A. (1978). Coordination of heart and gill rhythms in *Limulus*. *J. comp. Physiol.* **124**, 267–275.

WENDLER, G. (1966). The co-ordination of walking movements in arthropods. *Symp. Soc. exp. Biol.*
  20, 229–250.
WENDLER, G. (1974). The influence of proprioceptive feedback on locust flight co-ordination *J. comp.
  Physiol.* **88**, 173–200.
WILKENS, J. L. (1976). Neuronal control of respiration in decapod Crustacea. *Fed. Proc.* **35**, 2000–2006.
WILKENS, J. L. & MCMAHON, B. R. (1972). Aspects of branchial irrigation in the lobster *Homarus
  americanus.* I. Functional analysis of scaphognathite beat, water pressures and currents. *J. exp. Biol.*
  56, 469–479.
WILKENS, J. L., WILKENS, L. A. & MCMAHON, B. R. (1974). Central control of cardiac and scapho-
  gnathite pacemakers in the crab, *Cancer magister. J. comp. Physiol.* **90**, 89–104.
WILKENS, J. L. & YOUNG, R. E. (1975). Patterns and bilateral co-ordination of scaphognathite rhythms
  in the lobster *Homarus americanus. J. exp. Biol.* **63**, 219–235.
WILSON, D. M. (1961). The central nervous control of flight in a locust. *J. exp. Biol.* **38**, 471–490.
WILSON, D. M. & GETTRUP, E. (1963). A stretch reflex controlling wingbeat frequency in grasshoppers.
  J. exp. Biol. **40**, 171–285.
WYSE, G. A. (1975). Neural control of arthropod gill ventilation. In *Respiration of Marine Organisms*
  (ed. J. J. Cech Jr., D. W. Bridges and D. B. Horton). The Research Institute of the Gulf of Maine,
  South Portland.
WYSE, G. A. & PAGE, C. H. (1976). Sensory and central nervous control of gill ventilation in *Limulus.
  Fed. Proc.* **35**, 2007–2012.
YOUNG, R. E. (1973). Nervous control of ventilation in the shore crab *Carcinus maenas.* Ph.D. thesis,
  University of St Andrews, Scotland.
YOUNG, R. E. (1975). Neuromuscular control of ventilation in the crab *Carcinus maenas. J. comp.
  Physiol.* **101**, 1–37.
YOUNG, R. E. (1978). Correlated activities in the cardioregulator nerves and ventilatory system in the
  Norwegian Lobster, *Nephrops norvegicus* (L.) *Comp. Biochem. Physiol.* **61A**, 387–394.