

NERVOUS CONTROL OF VENTILATION IN THE  
SHORE CRAB CARCINUS MAENAS

Ronald E. Young

A Thesis Submitted for the Degree of PhD  
at the  
University of St Andrews



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CARCINUS MAENAS

by

Ronald E. Young

Catty Marine Laboratory,  
University of St. Andrews.

1973.

A thesis submitted for the degree of  
Doctor of Philosophy.



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#### DECLARATION.

I hereby declare that the work recorded in this thesis has been carried out by myself, and that it is of my own composition. I further declare that it has not been submitted in any previous application for a higher degree.

Ronald E. Young

I certify that Ronald Young has fulfilled the conditions laid down in the regulations for a degree of Doctor of Philosophy, under Ordinance No. 16 of the University Court of the University of St. Andrews and that he has accordingly qualified to submit this thesis for the degree of Doctor of Philosophy.

#### VITAE.

I was educated at St. George's College, Kingston, Jamaica and attended the University of the West Indies where I graduated in Honors Zoology and Chemistry in 1968. I subsequently went on to complete an M.Sc. degree in Zoology at the same University in 1970. The work described in this thesis was carried out between October, 1970 and October, 1973.

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**REFERENCES.**

**SECTION I**

**GENERAL INTRODUCTION AND REVIEW OF THE SPECIFIC PROBLEM.**

## I-1. GENERAL INTRODUCTION

Perhaps one of the most outstanding characteristics of animals is their performance of the numerous short term movements and adjustments which constitute behaviour. The activities may be either apparently spontaneous or in response to obvious environmental stimuli. This net 'behaviour' can become extremely complex in even the simplest orders of animals. However, aspects of it frequently can be isolated, which seem to be whole within themselves. These usually are made up of definite, reproducible sequences of activity which can readily be elicited under given conditions. The study of these 'behavioral units' as they might be termed, constitutes one of the more fascinating aspects of Biology.

Such studies can be approached from many directions and with many goals. One approach however, is to try to push the definition to its limit - to try to get closer and closer to the nucleus of these behavioral molecules from which overt behaviour is built. One way of doing this is to attack simple systems which generate patterned activity, and attempt to untangle the underlying mechanisms involved. Perhaps if this can be achieved, the ideas and approaches evolved, and the techniques developed, may eventually allow the more complex systems to be broached.

In this approach, the study of systems which generate rhythmical stereotyped activity, offers obvious advantages. Thus, much of the progress in this line has been made on systems such as the flight mechanism in insects (Pringle, 1949; Wilson, 1961; 1964; Wyman, 1965; Waldron, 1967, and others), walking in insects (Wilson, 1965; Wendler, 1966; and others), swimmeret beat in the Crustacea (Hughes and Wiersma; 1960; Ikeda and Wiersma, 1964; Davis, 1968; 1969; and others), and the

control of respiration in arthropods (Miller, 1960; 1966; Mill and Hughes, 1966; Passtor, 1968; 1969; Mendelson, 1971; Wyse, 1972). Studies on more advanced animals undeniably have produced some interesting results and insights (Lorenz, 1950; Tinbergen, 1950; Weiss, 1950; Taub and Berman, 1963; Taub, Bacon and Berman, 1965; Burns and Salmoiraghi, 1960; Salmoiraghi and Burns, 1960), but for an understanding at the very basic, cellular level it seems clear that the simpler systems involving fewer cells, as are found in the Invertebrata offer more possibilities.

The problem at this level is that although we know a relatively large amount about the characteristics of the units comprising the system (the neurones) and even something of their connectivity, we do not understand how they generate patterned activity. The argument between the reflexogenic (Sherrington, 1895, Gray, 1950) and the purely central dogmatists (von Holst, 1939) has largely resolved itself in the frequently seen dialectical compromise. It now seems quite clear that patterned activity frequently involves both systems to varying extents, perhaps depending partly on the probability with which a given course of activity can be correctly predicted over an extended period. Thus, activity that is prone to irregular unpredictable environmental disturbances might come to rely to a great extent on sensory reafference at each stage to determine the subsequent course that should be followed. In the majority of cases, however, the basic pattern appears to be generated by endogeneous, central arrangements, sensory feedback playing only a modulating role (Wilson, 1966; Evoy and Cohen, 1971). Indeed, only one example comes to mind of simple rhythmic activity which is demonstrably patterned by sensory feedback only (Mellon, 1969).

A fruitful line of attack then, might be to try to understand the endogeneous production of patterned behaviour in its purest forms, as free as possible from dependence on sensory feedback (Wilson, 1966).

In this, the control of ventilatory activity in the Crustacea would appear in many respects, to provide a suitable system. Although highly specialized, this system is not nearly so unusual as some even more specialized examples (eg. the cardiac ganglion in the Crustacea) whose simplicity, although allowing for relative ease of study, simultaneously may prohibit a more general application of the findings.

The scaphognathite of the second maxilla in the decapod Crustacea, in effecting ventilation of the gills, repeats a roughly constant sequence of actions over extended periods. Lying enclosed under the branchiostegites as it does, it must need to make only infrequent adjustments for material changes in its environment. Not surprisingly then, sensory feedback appears to have little effect on the pattern of muscular activity generated via the innervating motoneurons, from the central nervous system (Passtor, 1968; 1969; Mendelson, 1971). The system is not completely independent of this reafference, and deafferentation does appear to cause a slowing of the rhythm (ibid). However, it represents one of the clearest examples of the purely central generation of patterned activity. But scaphognathite activity has not yet been subject to the detailed and comprehensive analyses as have been directed at flight and walking in insects for example.

This thesis therefore, intends to conduct a detailed study of the control of ventilation in some decapod Crustacea with a view to gaining a better appreciation, from the gross structural to the neuronal level, of how this unit of overt behaviour is generated, controlled and integrated into the general functioning of the animals.

In the main, the studies will concentrate on the shore crab Carcinus maenas, but in some instances Portunus depurator and Nehrons norvegicus will also be considered. The approach will be to describe the system in some detail, firstly on the gross structural level,

secondly in terms of the nature and form of the mechanical activity, and thirdly in terms of the nature and form of the nervous output. The relationship between the scaphognathite and heart rhythms and the linkage between the scaphognathites on either side shall also be investigated. From these descriptions a model will be proposed of a simple neuronal network by which such a pattern as the one described, could be generated, and linked into the total network.

It is hoped that this study will make some contribution to the understanding of how a simple 'unit of behaviour' can be effected by suitable interaction of skeletal, muscular and neuronal elements.

## I-2. THE SPECIFIC PROBLEM

This study by no means constitutes the first investigation of the control of ventilation in the decapod Crustacea. Early studies, reviewed by Wolvekamp and Waterman (1961) concentrated on the factors which influenced the frequency and amplitude of the scaphognathite beat (Johnson, 1936 etc.). More recently, Larimer and his co-workers (Larimer, 1961; Larimer and Gold, 1961; Larimer, 1964; Ashby and Larimer, 1965) and others (McMahon and Wilkens, 1971; Wilkens and McMahon, 1972) have made significant contributions in this area.

Wolvekamp and Waterman (1961) also describe work demonstrating that the respiratory control centre in Astacus is located in the sub-oesophageal ganglion, and is primarily autonomous (Kalmus, 1930; Segaar, 1934). Segaar (1934) was able to show an absence of respiratory rhythm in the cerebral ganglion, and that ventilatory rate could be increased by as much as 80% by locally warming the sub-oesophageal ganglion. These observations have now been confirmed by Mendelson (1971) who was able to record persistent organized bursts of activity in the motor axons to the second maxilla, in the isolated ganglion in the hermit crab Pagurus, for up to 48 hours. He was also able to demonstrate in neuropilar processes in this animal and in Homarus, roughly sinusoidal oscillating potentials bearing a clear relationship to the discharge of the respiratory motoneurones. One set of motoneurones fired during the upper half only of the cyclic oscillation, and the antagonists fired during the lower half only. Hyperpolarization of the impaled neuropilar process induced continued discharge of the second group, and inhibition of the first, whilst depolarization had the opposite effect. Various characteristics were cited as evidence that the oscillations were spontaneous and not simply being driven by the motoneurones. These included the absence of obvious underlying

rhythmic input during periods of polarization, and the resetting of the rhythm by injected pulses of current. One criticism of this conclusion is that the hyperpolarization illustrated in the paper was apparently not continued for more than 700 ms whilst the cycle time was of the order of 750 to 1000 ms. The duration of depolarization was not specified. In the case of the records taken during hyperpolarization and depolarization then, one may well not expect to see underlying cyclic 'membrane potential oscillation at the free running rate', since one full cycle has not elapsed. If the recorded oscillations were in fact produced by periodic inhibitory and/or excitatory input from the motoneurones, they might well be expected to disappear during periods of hyper- or depolarization when the motoneurones may no longer be firing phasically, one group having taken over completely, firing continuously. In addition to this, one wonders if the fact of resetting of the rhythm by injected pulses is really a sound basis for rejecting the possibility that the oscillator is actually being driven, whilst still playing an important, though not absolutely controlling role in the network.

Be that as it may, the oscillations were taken to be produced by a spontaneous oscillator neurone, governing the discharges in both agonist and antagonist motoneurone pools. The properties of such spontaneous oscillators have been most thoroughly studied in molluscan nervous systems (Chalazonitis, 1963; Arvanitaki and Chalazonitis, 1968). Many of their properties such as temperature sensitivity (Carpenter, 1967) and sensitivity to carbon dioxide and oxygen levels (Matheiu and Roberge, 1971) certainly are consistent with properties observed in the arthropod respiratory system. However, with our present understanding of the components, it is difficult to envisage any simple, satisfactory system whereby such a single oscillator could be made to

drive two reciprocally active motoneurone pools bursting at the normal ventilatory frequencies.

Mendelson's (1971) study, although moving directly to the area of central, overall control at the neuronal level, does leave unanswered many questions concerning the organization at the motoneurone level or above, which produces the latencies and/or phasing of the bursts, essential to the proper functioning of the ventilatory mechanism. Significant studies in this regard are those of Pasztor (1968, 1969) on the crayfish Orconectes virilis, and Wyse (1972) on the Xiphosuran Limulus. An important fact of Pasztor's (1969) paper is the discovery of phasic reafference from the oval organ in Orconectes. Phasic sensory feedback is therefore possibly, but not necessarily of some significance in the organization of the respiratory rhythm. Wilson and Wyman (1965) have shown in the control of locust flight that phasic reafference in rhythmic systems may be treated in the same way as random stimulation, simply being averaged over a number of cycles. In the case of decapod crustacean ventilatory activity, Mendelson's (1971) study has certainly shown that no sensory input is required for the generation and maintenance of the rhythm.

Pasztor's (1968) study examined the skeleton, musculature and its fine innervation and to some extent phasing of activity in the different muscles, and the structure of the bursts of activity in individual muscles. Pasztor concluded that each muscle although dually innervated was activated by one motoneurone only, that the spike frequency and burst length in the motor output to individual muscles were not correlated with the burst interval, and that there was no peripheral inhibition of muscular activity. This latter was interpreted to indicate that in the scaphognathite, fine phasing and positional control of the individual segments is not so important as control of

amplitude and frequency of beating. This was contrasted with the situation in the swimmerets of the lobster Homarus (Wiersma and Ikeda, 1964; Davis, 1969), where numbers of spikes per burst and total duration of the muscles activity per cycle, are correlated with the cycle frequency.

These studies although of obvious value, seem not to have come to grips with the problem of integration of the activity between the motoneurons governing the ventilatory muscles. Wyse's (1972) successful intracellular investigations indicate that in Limulus the integration may well occur at a pre-motor level, and that the integrating oscillator whether single or multineuronal, may influence the motoneurons at least in some cases, by inhibitory input. It therefore goes further than the previous studies in this respect, but it still allows no clear insight into the way the integration may come about.

The present work, by a relatively detailed description and analysis of the output parameters of the system, aims to clarify further the nature of the system concerned with generation and integration of the neural pattern governing ventilatory activity, in particular, in Carcinus maenas.

SECTION II  
MATERIALS AND METHODS

## II-1. ANATOMICAL

In the intact second maxilla in Carcinus the delicate and complicated skeletal arrangements tend to be obscured by the extensive musculature. Removal of the muscles in fresh specimens could not be effected without damaging the skeleton, and the use of potassium hydroxide solution tended to distort the more delicate sclerites. However, in animals left to autodigest in a cool place for a few days, the muscles were easily pulled out of the appendage with a pair of fine forceps. With this method there is little or no damage to the skeleton.

The musculature was studied in both freshly dissected material and in specimens fixed in sea water Bouin's. Methylene blue staining aided the study of the gross innervation of the appendage.

## II-2. Electrophysiological

### II.2.1. The preparation

In recording from the ventral musculature, it was usually convenient to remove the walking legs as these tended to become entangled in the recording electrodes. The legs were therefore routinely autotomized in all the experiments concerned with recording from the scaphognathite muscles. After autotomizing the legs the animals were allowed to recover for a half to one hour before proceeding. During this period ventilation appeared to be normal and the animals remained 'alert' as judged by eye responses to movement.

The ventral musculature of the second maxilla could readily be approached by removing the ventral covering of the exhalent channel, and cutting away the more anterior mouth parts. Small perforations in the thin cuticle covering the appendage allowed the recording electrodes to be placed in contact with the muscles. The electrodes were usually placed close to where the muscles took their origin so as to minimize movement artefact, and to interfere as little as possible

with the movement of the appendage. In recording from the quintus muscle (Fig.III.3) and sometimes from the sixtus (Fig.III.3) it was necessary to restrict the movement of the appendage in order to prevent excessive movement artefact, and to prevent the electrode from being thrown off from the intended recording site. For these experiments the animal was either clamped inverted in the experimental bath, or cemented with a drop of Eastman 910 adhesive on the dorsal carapace, to a metal baseplate.

The dorsal muscles were less accessible. In order to approach these muscles, the dorsal carapace on one side had to be removed, along with the hepatopancreas. These preparations were perfused with chilled, aerated and filtered sea water, via a small glass pipette inserted under the posterior border of the carapace. Perfusion was started as soon as the dorsal carapace was removed, and was continued at a relatively high rate while the hepatopancreas was being removed, and for a few minutes afterwards. This helped to wash away digestive juices spilled from the ruptured hepatopancreas. The rate of flow was then reduced to a minimum. The animals seemed to survive best under this protocol. Perfusion with Carcinus saline (Pantin, 1948) instead of sea water did not seem to improve the survivability. Pre-dissection anaesthesia by chilling also did not help significantly. The survival rate of these preparations was poor. Although in the best preparations rhythmic beating of the scaphognathite continued for up to six hours, the average survival time was of the order of one hour.

In recording from the nerve trunks to the second maxilla, an equally drastic ventral dissection was necessary. The anterior sternites and the hepatopancreas had to be removed carefully, and in order to get at the nerve trunks it was usually necessary also to cut away some major blood vessels. Perfusion technique was as in the dorsal preparation. The survival rate was similar to that in the dorsal preparation.

### II.2.2. Recording techniques

The electrical activity in the muscles, and as a rule in the nerve trunks also, was monitored using polythene suction electrodes constructed from PP55 gauge tubing drawn to a fine tip. The signals were led off by means of fine gauge silver wire threaded through the wall of the polythene tube, into the tips of the electrodes. This lowered the resistance of the electrodes and improved signal. Indifferent silver wire electrodes went to the bath, which was earthed. The signals were fed via AC differential preamplifiers filtered to pass 80 Hz to 1 KHz waveforms, into respective channels of a four channel cathode ray oscilloscope. Three recording electrodes could be used simultaneously. The fourth channel carried a time marker. Time pulses normally of 50 or 500 ms were obtained from a Tektronix 161 pulse generator triggered by a type 162 waveform generator. The timing of the pulses was calibrated against the Tektronix type 3B4 time base on the cathode ray oscilloscope. The stationary beams were photographed on Ilford NS6 oscillograph paper with a model 1428 Mk II Coeslor camera with a model 1431 drive unit. Film speeds were varied to suit the particular requirements, between 0.63 and 12.5 cm per second.

### II.2.3. Movement of the scaphognathite.

Three different methods were used to monitor the movement of the scaphognathite, depending on the type of information required. The first method allowed monitoring of the rate of beating in the intact animal without disturbing the activity of the scaphognathite. It gave no information concerning the incidence of reversals. The second was designed to allow the fine movements of specified points on the scaphognathite to be monitored accurately. And the third was devised in order to gain information concerning the linkage between the right

and left scaphognathites, not just in regard to rate and phasing, but also with respect to the incidence of reversals.

(a) The photodiode method

This method was used to monitor scaphognathite rate in the intact animal in the experiments on Nephrons norvegicus described in Section VI. The maximum dorsal excursion of the scaphognathite was located by directing a beam of light into the exhalent channel of the branchial chamber. At this point, a small depression was made in the carapace by means of a pneumatic drill. The depression was just large enough to accomodate the tip of a minature photo-duo-diode (0.8 mm diameter) powered by a 9 volt dry cell battery. The animal was held gently about the thorax by means of a clamp lined with foam rubber, in a bath of running sea water. The photodiode was sealed by slipping it into a tightly fitting length of polythene tubing, and the tip was micromanipulated up against the depression in the carapace. A beam of light from a DC powered source was then directed into the exhalent channel, from below the water level. The leads from the photodiode were taken directly to the cathode ray oscilloscope for recording as described previously. This arrangement produced a single, biphasic deflection on the monitor trace for each beat cycle. Occasionally, the movements of the first maxillipeds introduced artefacts by interrupting the light beam, but this was easily distinguished from the wave produced by the scaphognathite movement. There was normally no difficulty if the light beam was optimally adjusted.

(b) The capillary tube method

This method was used in order to determine the relative movements of the different parts of the scaphognathite in relation to the muscular activity in Carcinus. A length of glass capillary tube was held with plasticine in front of the photosensitive element of a small

photocell. The sensitive element consisted of a narrow (2 mm width) vertical strip of photoresistive material. The plasticene occluded the remaining surface of the photocell so that only light passing through the capillary tube could strike the element. A small, headless pin, about 2 mg in weight, was then inserted into the capillary tube so that the lower end rested on the scaphognathite at a predetermined point, while the upper end was free to move in the capillary tube between a DC powered light beam and the photocell. The photocell and capillary tube assembly was mounted on a micromanipulator. It therefore could easily be racked into position and adjusted, so that as the scaphognathite moved, the tip of the pin oscillated about the centre of the photosensitive element, thus affording maximum linearity of response. The photocell was powered by a 9 volt dry cell battery, and the output was fed directly into the oscilloscope and recorded in the usual manner. The most reliable recordings were from the dorsal preparations. Accurate placement of the monitor in the ventral preparation necessitated removal of the covering branchiostegites, and of the more anterior appendages. This disrupted the normal movement of the appendage. Dorsally, however, the scaphognathite abuts against a thin walled, transparent covering which allowed accurate placement of the monitor through small perforations without disturbing the normal restrictions placed on the excursion of the scaphognathite. Dissection was kept to a minimum. Just enough of the dorsal carapace and hepatopancreas were removed to allow access to the scaphognathite through the thin-walled, covering endopleurite, and for a suction electrode to be positioned on the promotor muscle (mpIIIm), the most dorsal muscle, where it enters the base of the second maxilla.

(c) The resistance method

This method was used to monitor bilateral scaphognathite activity

in experiments with Carcinus on the phasing of the beats in the right and left scaphognathites. It placed minimal restriction on the movements of the animal and disturbed only minimally, the normal activity of the scaphognathite. It utilized as a signalling device, the change in resistance to earth in a fine, insulated copper wire, as the scaphognathite moved across the bared tip. Two pairs of insulated copper wires, bared at the tips were connected via suitable resistors to a 9 volt dry cell battery, so that a small current (a few micro-amps) would flow when they were connected to earth. The two wires to each were inserted into small holes in the lateral aspect of the ventral covering of the exhalent channel, so that at the ventral extremity of its movement, the scaphognathite would come into contact with the bared tips. One of either pair was located near the anterior tip of the scaphognathite, and the other, near the posterior tip. The wires were held in place with Eastman 910 adhesive. They were then run over on to the dorsal carapace, and led off through a length of insulated sleeving attached with Eastman 910 adhesive to the centre of the animal's back. The animal was free to move about normally without disturbing the connections. It was hoped that reversal could be detected by a change in the relationship between signals in the anterior and posterior leads in each pair. This did not work as well as expected since it turned out that one of the pair frequently did not give a reliable signal. This is discussed further in Section VI.

### II-3. Analysis of data

#### II.3.1. Definitions of parameters concerning temporal relationships

##### (a) Parameters related to frequency dependence analyses

Two temporal characteristics of the bursts were investigated in terms of their dependence on period length. Firstly, the relative latencies were defined as the times of the first spikes in the test muscle with respect to the times of the first spikes in a reference

muscle in the same cycle. The start of the cycle was arbitrarily taken to be the start of the burst in the flexor muscle (Fig.III.3). Routinely, the times of the starts of the bursts in the different muscles were measured with reference to the time of the start of the burst in the flexor, since this was the only muscle that was readily accessible from both the dorsal and ventral aspects. Period length was generally defined as the time from the start of a given burst in a particular muscle, to the start of the next burst in that muscle. In analyses involving relative latencies, the period length was taken to be the mean of the corresponding period lengths for the test and reference muscles. The two estimates were usually not very different. The relative latency with respect to the flexor was designated  $L_f$  and with respect to any other muscles, simply as  $L$ .  $L_f$  was usually determined then, as the time from the first spike in the flexor, to the time of the first spike in the succeeding burst of the test muscle. These relationships are shown in Fig. II.1C. These data then, were analysed in terms of the regression of relative latency on mean period length. The data were also expressed as the phase position in the cycle of the start of the test burst with respect to the start of the reference burst. This was designated  $\phi_L$  and was computed simply as

$$\phi_L = L/\text{mean period.}$$

Analyses of the regression of  $\phi_L$  on period were also performed. The data were expressed in addition, as phase histograms.

The second parameter investigated was burst duration. This, designated  $L_D$ , was defined as the time from the first spike in the burst to the last spike in the burst in a given cycle. The period length here was taken simply as the time from the first spike in the burst to the first spike in the succeeding burst. These data also,

were expressed as phase angle of the burst duration,  $\phi_D$ , defined as

$$\phi_D = L_D / \text{period.}$$

Analyses were done on the regressions of both  $L_D$  and  $\phi_D$  on period.

#### (b) Other analyses

Most of the other analyses were done on similar lines. Most involved the time of occurrence of a recurrent event with reference to another recurrent event. In these cases, latency was defined as the time to a test event, from the occurrence of the last reference event preceding. Phase position was usually defined as the latency divided by the time from the specified reference event, to the next reference event. Coincidence between reference and test events was normally assigned to phase 0.0 rather than to phase 1.0.

#### II.3.2. Techniques of analysis

Recorded film strips were analysed with the aid of a film 'reader' (Fig.II.2) designed and built in conjunction with Mr. P.R. Balch, a fellow doctoral research student at the Gatty Marine Laboratory. The film strip to be analysed was passed over the central roller, to which it was held tightly by a pair of pinch rollers. By turning the central roller, which was covered with a rubber sleeve in order to prevent slippage, the film could be guided past a vertical, no-parallax cursor, which was graduated to facilitate sorting of spikes according to height. The central roller was so mounted as to be virtually free from backlash. As the film was moved along, the turning of the central spindle unwound a precision 10 turn linear helipot. The helipot which had a resolution of 1 degree in 3600, was powered by dry cell batteries with a stabilizing circuit to ensure absolute stability of the input voltage. The circumference of the roller was such that the lower limit

of resolution of the helipot corresponded to about a 0.2 mm advance of the film strip. This, at the usual film speed of 2.5 cm/second for recording muscle activity, would correspond to a resolution of about 8 ms. At the higher speeds used for experiments concerned with spike to spike events, this would be equivalent to a resolution of about 3 ms, and at the maximum film speeds used, of about 1.5 ms. The output of the helipot ranged from +6.9 to -6.9 volts roughly, so that the accuracy of the reading was balanced over the whole range.

As the film was turned past the cursor, the times of the succeeding events then, were converted into equivalent voltages at the output from the helipot. This output was led into a digital voltmeter/tape punch machine, designed by Drs. M. Shepherd and C. Vincent of the Physical Chemistry Department, to convert continuously varying DC voltages into digital output punched on paper tape (Shepherd and Vincent, 1973). The events of interest were sequentially aligned with the cursor and, by depressing a foot pedal triggering device, the corresponding voltages were punched sequentially with differing code numbers for the respective series of events, on to the paper tape. The voltages at given time marks were simultaneously entered to allow conversion from the voltages into milliseconds.

If the helipot reached the end of its range before the end of the strip being analysed, the final voltage was entered with a specified code number. The clutch (Fig.II.2) was released and the helipot turned back to the starting point without moving the film. The starting voltage was entered, and then the usual entries continued. In subsequent computations, the difference between the voltages before and after the turnback point was added to all succeeding entries.

Appropriate programs were written to convert the times of the events to the parameters described previously, and to plot appropriate graphs and histograms. The programs allowed the graphical data to be punched

when necessary, onto data cards as respective pairs of values, for subsequent statistical analyses. The main statistical analyses were done using the computing laboratory's 'statistical package' designed by Dr. C.P. Saksena of the computing laboratory.

#### II.2.4. Some preliminary investigations

The muscles of the second maxilla lie close together in a somewhat confined space. The likelihood that there might be some amount of cross-talk between the muscles seemed high. As will be seen (Section IV) a number of the muscles are active at the same time in the beat cycle. In the initial experiments, it was difficult to decide whether or not this coincidence was merely cross-talk. Two criteria however were used to decide whether the records were reliable. Firstly, records which showed two sessions of activity per cycle were immediately open to suspicion. Secondly, by observing the muscular activity with a pair of binocular microscopes while listening to the signal on an audio amplifier, one could usually tell whether or not the signal corresponded with the active contraction of the muscle. Once the relative positions of the different bursts in the beat cycle had been roughly determined, it became easier to distinguish between genuine activity in the muscle and cross-talk. In only two muscles, the sixtus and the tertius (Fig.III.3) was there a marked indication of cross-talk. In the tertius signals from the remotor were occasionally superimposed, and in the sixtus, some activity from the quintus was sometimes seen. Equivocal records were not analysed.

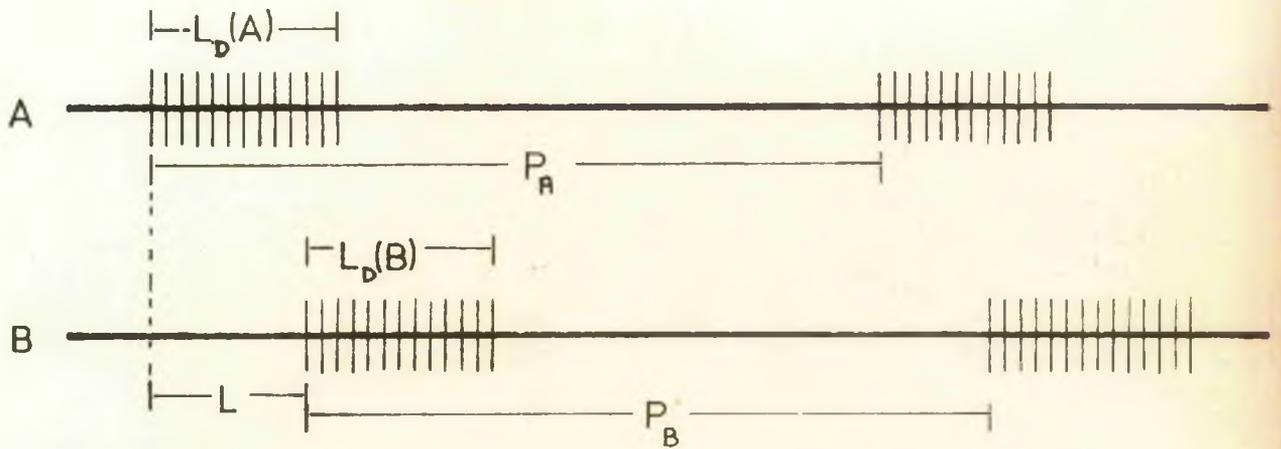
It would have been useful to immobilize the appendage when recording from two of the ventral muscles, the quintus and sixtus. It seemed necessary therefore to first see if immobilization could affect the frequency dependence of the characteristics of the muscles. To test this, the dependence on frequency of three characteristics were examined whilst the appendage was held and while it was free to

move. The three characteristics were the dependence on period of the duration of the flexor burst, and of the burst in the quartus (Fig.III.3) and the lag between the starts of these two bursts in the beat cycle. The results are summarized in Tables A - D. There were significant changes in some cases, but in no case was there absolute consistency. There may be a tendency towards increased slope in the regression of the duration of the flexor burst on period when the appendage is held. To be on the safe side, the scaphognathite was not immobilized, but the range of the ventral movement was usually restricted by means of a stop when recording from the quintus and sextus muscles. In view of the variability found in later experiments, it is probable that the changes seen in the tables were not a direct result of immobilization. Davis (1969) found that similar parameters for the muscles controlling swimmeret beat did not remain constant for more than about twenty consecutive cycles.

Figure II.1. [Faint, illegible text]

Figure II.1.

Figure II.1. Parameters used in investigating the temporal relationships for the bursts of activity in the muscles of the scaphognathite.



Plots :

$$L_D(A) \text{ vs } P_A$$

$$L_D(A)/P_A \text{ or } \phi_D \text{ vs } P_A$$

$$L_D(B) \text{ vs } P_B$$

$$L_D(B)/P_B \text{ or } \phi_D \text{ vs } P_B$$

$$L \text{ vs } (P_A + P_B)/2 \text{ or } P_{AB} \text{ (mean period)}$$

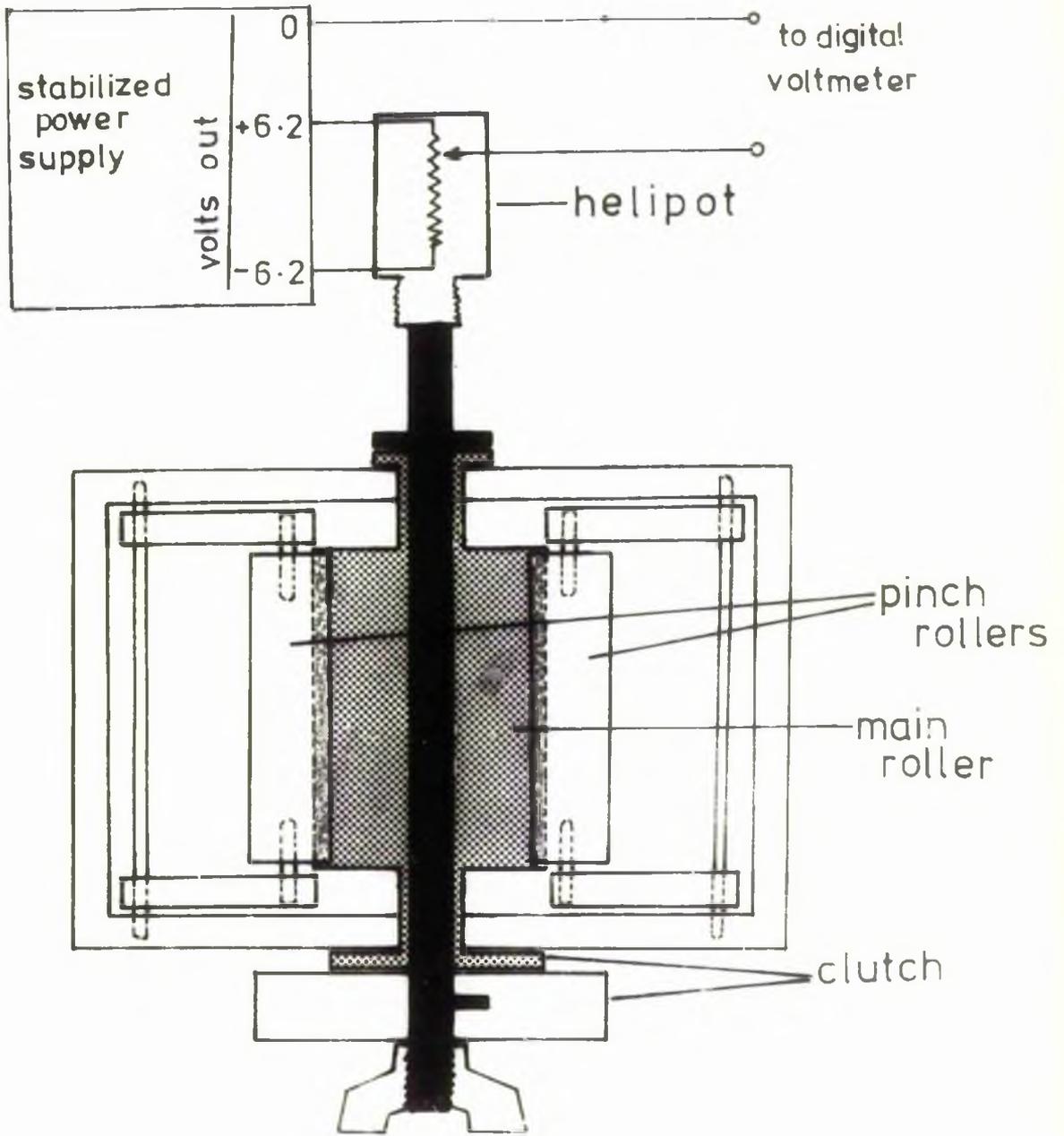
$$L/P_{AB} \text{ or } \phi_L \text{ vs } P_{AB}$$

... ..  
... ..  
... ..  
... ..

Figure II.2.

Figure II.2. Diagram of the film 'reader' used to transfer data from oscillographs to paper tape for computer analysis. The 'reader' was designed and built in conjunction with Mr. P.R. Balch, a fellow doctoral research student at the Gatty Marine Laboratory.

plan



side view

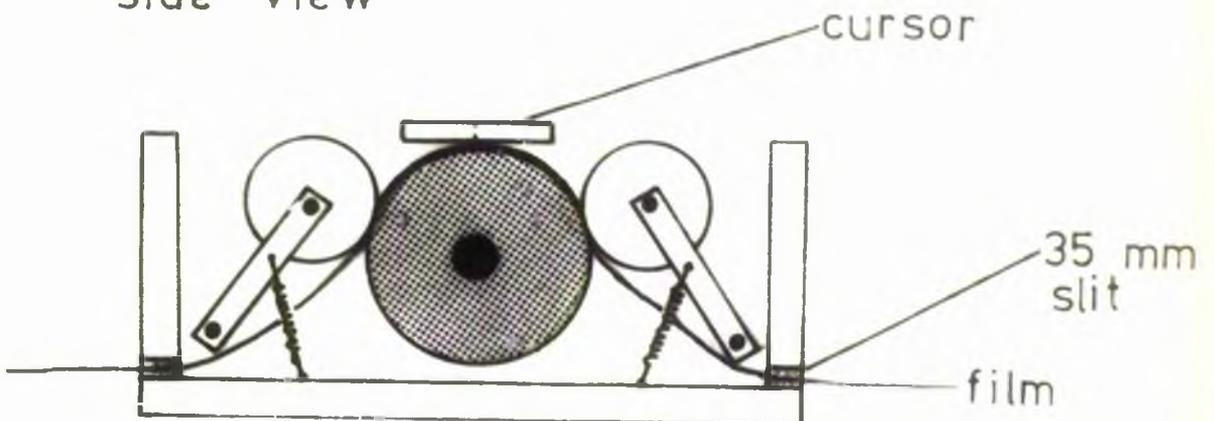


TABLE A

Comparison of some of the temporal relationships for activity in the scaphognathite musculature when the scaphognathite is first immobilized and then abruptly freed. The data pertain to analyses on plots of the parameters in the left hand column against period length.

		HELD	FREE	Significance of the difference between the b values
Duration of flexor burst	$b \pm SE$	$0.159 \pm 0.031$	$0.171 \pm 0.034$	$t = 1.906$
	r	0.609	0.717	NS
	Sy.x	26.587	14.711	
	P	0.01	$P < 0.01$	
	N	47	54	HELD = FREE
Duration of quartus burst	$b \pm SE$	$0.328 \pm 0.046$	$0.520 \pm 0.060$	$t = 2.545$
	r	0.730	0.770	$0.01 < P < 0.05$
	Sy.x	40.368	27.269	FREE > HELD
	P	$< 0.01$	$< 0.01$	
	N	47	54	
Latency ( $L_r$ ) between the starts of the flexor and quartus bursts	$b \pm SE$	$0.708 \pm 0.088$	$0.480 \pm 0.095$	$t = 1.827$
	r	0.765	0.572	NS
	Sy.x	70.302	38.894	
	P	$< 0.01$	$< 0.01$	HELD $\neq$ FREE
	N	47	54	

TABLE B

Comparison of some of the temporal relationships for activity in the scaphognathite musculature when the scaphognathite is first freely moving and then abruptly immobilized. The data pertain to analyses in plots of the parameters in the left hand column against period length.

		HELD	FREE	Significance of the difference between b values
Duration of flexor burst.	$b \pm SE$	0.190 $\pm$ 0.021	0.102 $\pm$ 0.022	t = 2.908
	r	0.776	0.554	P < 0.01
	Sy.x	11.516	28.612	HELD > FREE
	P	<0.01	<0.01	
	N	57	53	
Duration of quartus burst.	$b \pm SE$	0.273 $\pm$ 0.030	0.102 $\pm$ 0.020	t = 4.735
	r	0.771	0.600	P < 0.01
	Sy.x	20.854	32.159	HELD > FREE
	P	<0.01	<0.01	
	N	57	57	
Latency (L) of the start of the quartus burst with respect to the start of the flexor burst	$b \pm SE$	0.682 $\pm$ 0.045	0.809 $\pm$ 0.099	t = 1.166
	r	0.897	0.757	NS.
	Sy.x	27.095	112.481	HELD = FREE
	P	<0.01	<0.01	
	N	57	52	

TABLE C

Comparison of some temporal relationships for activity in the scaphognathite musculature. The scaphognathite was immobilized for about 5 mins. before the first set of data were recorded. It was then freed and the second set of data taken about 5 mins. after release.

	HELD	FREE	Significance of the difference between the slopes (b)
Duration of flexor burst	$b \pm SE$ 0.147 $\pm$ 0.021	0.003 $\pm$ 0.034	$t = 3.619$
	$r$ 0.657	0.012	$P < 0.01$
	$Sy.x$ 34.164	50.969	HELD > FREE
	$P$ < 0.01	NS	
	$N$ 67	45	
Duration of quartus burst	$b \pm SE$ 0.422 $\pm$ 0.035	0.239 $\pm$ 0.039	$t = 3.479$
	$r$ 0.833	0.679	$P < 0.01$
	$Sy.x$ 56.534	70.165	HELD > FREE
	$P$ < 0.01	< 0.01	
	$N$ 67	45	
Latency of the first spikes in the quartus with respect to first spikes in the flexor	$b \pm SE$ 0.569 $\pm$ 0.063	0.704 $\pm$ 0.228	$t = 0.571$
	$r$ 0.748	0.426	NS
	$Sy.x$ 94.543	230.528	
	$P$ < 0.01	< 0.01	HELD = FREE
	$N$ 67	45	

TABLE D

Comparison of some temporal relationships for activity in the scaphognathite musculature. The data for 'FREE' were taken initially while the appendage was freely moving. The appendage was then immobilized, and after about 5 mins., the data analysed under 'HELD' were recorded.

	HELD	FREE	Significance of the difference between the slopes
Duration of flexor burst	$b \pm SE$ 0.150 $\pm$ 0.029	0.023 $\pm$ 0.016	$t = 3.780$
	$r$ 0.721	0.210	$P < 0.01$
	$Sy.x$ 70.180	27.330	
	$P$ <0.01	NS.	HELD > FREE
	$N$ 26	47	
Duration of quartus burst	$b \pm SE$ 0.451 $\pm$ 0.034	0.422 $\pm$ 0.024	$t = 0.696$
	$r$ 0.721	0.210	NS
	$Sy.x$ 70.180	27.330	
	$P$ <0.01	NS	HELD = FREE
	$N$ 24	47	
Latency of the start of the quartus burst with respect to the start of the flexor	$b \pm SE$ 0.737	0.061	$t = 5.929$
	$r$ 0.931	0.806	$P < 0.01$
	$Sy.x$ 105.671	66.722	HELD > FREE
	$P$ <0.01	<0.01	
	$N$ 25	47	

SECTION III

FUNCTIONAL ANATOMY

### III-1. INTRODUCTION

The second maxillae in the Crustacea are highly compressed appendages, lying on either side, in the anterior exhalent channels of the branchial chambers. The appendage is antero-posteriorly compressed, but is tilted backwards on its radial axis so that the morphologically anterior surface lies on the dorsal side, and the internal angle points anteriorly. The appendage is composed of two (Passtor, 1969) or more (Borradaile, 1922; 1926) protopodal segments, two bilobed endites, an endopodite, and a broad, blade-like exopodite, the scaphognathite. The scaphognathite forms the most obvious part of the appendage. It lies across the exhalent channel of the branchial chamber, in which it fits snugly, aided by a fringe of short, simple hairs along its distal border. By its movement it pumps a current of water either forwards or in the reversed direction through the branchial chamber and over the gills.

Passtor (1969) described in detail the skeletomuscular system of the scaphognathite in the crayfish Orconectes virilis. The general plan of this organ in the Brachyura is very similar but there are a number of obvious differences. For example, the division of the brachyuran scaphognathite blade into two distinct, articulated segments, appears to contribute greatly to the seemingly more effective action of the brachyuran appendage.

Cochran (1955) gave a detailed description of the musculature of the second maxilla of the crab Callinectes, and Maynard (1961) extended this description, adding two new muscles to the thirteen described by Cochran. Neither of these studies were concerned with the skeletal system of the scaphognathite in a more than cursory fashion, and so do not permit a clear understanding of the way in which the muscles transmit their forces to the skeleton to produce the smooth,

undulating movement that can be seen in the exposed organ.

A detailed analysis of the skeletomuscular system of the scap-hognathite of Carcinus will therefore be attempted here, as a prelude to the investigation of the nature of the nervous input into the appendage. It is hoped that this will allow an appreciation of the significance of the basic pattern of the normal motor input, as well as the significance of deviations from this, for example, during reversed beating.

It was considered worthwhile to describe also the gross anatomy of the innervation as this could prove helpful in subsequent discussions of electrophysiological techniques used.

### III-2. The Skeleton

#### III.2.1. Results

Figs. III. 1a and 2a illustrate the skeletal elements of the second maxilla from the dorsal (morphologically anterior) and ventral (morphologically posterior) aspects. The portions of the head apodeme with which it articulates, and which bear the origins of some of the extrinsic muscles, are also shown. The ten discrete calcified units which are discernible are displayed more clearly in an expanded scheme in Fig. III. 2b. The diagrammatic inset (Fig. III. 2c) attempts to clarify further, their structure and linkages.

The first two sclerites (PrBS/PCS and ACS/ECS - Fig. 2c) together form an irregular annulus, proximally articulating with the head apodeme. Internally they bear two bilobed endites and a broad, flat endopodite. Externally there is an expanded, roughly conical sclerite which bears one of the proximal articulation points. Distally, the sclerites forming this annulus are linked to the proximal border of

the scaphognathite blade by four sclerites: a thin, V-shaped (RBS1) and a stout hatchet shaped (RBS2) sclerite internally, and externally by an extended three pronged sclerite (PrS) and another, heavier inverted V-shaped sclerite (DBS). This second roughly V-shaped sclerite is intercalated between the three pronged sclerite and the more proximal conical sclerite. It is hinged at the proximal tip of its ventral arm to the ventral lip of the half cone (ECS), and along the dorsal arm, is sutured to the dorsal prong of the three pronged sclerite. The apex of the cone forms an articulation point with the angle of the V. The tip of the dorsal arm is also hinged to one of the angles of a small triangular sclerite (ClS). The other two points of this sclerite are hinged between the dorsal lip of the half cone, and the distal edge of its internal prolongation (ACS).

The scaphognathite itself is composed of two segments, each having a central area of calcification (ISG; LSG). The segments are linked along the radial axis of the appendage by a flexible hinge. Distally, a narrow, sclerotized arc (HS) links the two segments across the Hinge.

### III.-2.2. Interim Discussion

#### Homologies and Nomenclature

The second maxilla might best be regarded primarily as a thin walled structure in which areas of heavier calcification have been laid down to form a system of articulated sclerites which serve as points of attachment for the muscles, and as levers and axes about which suitable movement of the appendage may be effected.

As a result of the extreme specialization of the brachyuran second maxilla, it is almost impossible to identify unequivocally the structures homologous with those in the generalized crustacean

appendage. Cochran (1955) suggested that it was useless to attempt a separation of the elements of the basipodite from those of the coxopodite. An attempt at such a characterization may be worthwhile so that the skeletal elements may be named on some logical basis rather than referred to by arbitrary letters or numbers.

Throughout the following discussion, the functional rather than the morphological terminology will be used when referring to the dorsal and ventral aspects of the maxilla. The sclerites will however be named on the basis of their morphological positions. Where confusion might arise, the morphological equivalent is noted in parenthesis after the functional usage. The functionally anterior and posterior aspects will always be referred to subsequently by their morphological positions, i.e. internal and external.

The cone shaped sclerite (ECS) capping the external edge of the maxilla, and articulating proximally with the head apodeme is arguably a part of the coxopodite (Pasztor, 1969), although Borradaile (1922, 1926) suggests that it may also contain elements of the basipodite and a pre-coxal segment. An extension (ACS) of the internal base of this sclerite curves across the dorsal (morphologically anterior) base of the appendage to form a second, internal articulation with a protrusion of the head apodeme. Across the ventral (posterior) surface, in a roughly corresponding position, another narrow, calcified band (PCS) runs from the base of the cone near its articulation with the head apodeme, to fuse with the external and proximal edges of a cup-shaped sclerite (PrBS) lying at the base of the internal edge of the maxilla. The band continues under the proximal edge of the cup-shaped sclerite to enter the base of the more proximal pair of endites (Cnd) which are associated with the coxopodite (Cochran, 1955). This whole sclerite, excluding the cup-shaped portion, will be termed

the coxopodal sclerite (CS), and the subdivisions referred to respectively, as the external coxopodal sclerite (ECS), the anterior coxopodal sclerite (ACS), and the posterior coxopodal sclerite (PCS).

If the more distal bilobed endite (Bnd) as well as the endopodite assigned to the basipodite (Brooks, 1882) then the cup-shaped sclerite (PrBS) might be regarded as a part of the basipodite. It curves round the internal edge of the appendage at the bases of the endites and endopodite, and is flexibly attached by its external edge on the dorsal (anterior) side, to the internal articulation of the CS. It will be referred to as the proximal basipodal sclerite (PrBS).

The inverted V-shaped sclerite (DBS), by virtue of its articulation with the ECS might be taken also, to be part of the basipodite, and will be termed distal basipodal sclerite (DBS). The ventral (posterior) arm will be designated the posterior distal basipodal sclerite (PDBS), and the dorsal (anterior) arm, the anterior distal basipodal sclerite (ADBS).

The juncture of the three prongs of the three pronged sclerite (PrS) coincides with the angle of the DBS, with which it is fused. The external prong runs along the free, proximal border of the external scaphognathite segment. The two internal prongs run along the proximal border of the scaphognathite blade, across the hinge between the internal and external segments. This sclerite must therefore be assigned to the scaphognathite, and will be referred to as the proximal scaphognathite sclerite (PrS). The prongs of the PrS may be identified separately as follows:

1) The anterior proximal scaphognathite sclerite (APrS) - the prong which arches along the proximal border of the scaphognathite on the dorsal (anterior) side of the blade, and crosses the hinge, to articulate with the proximal edge of the internal segment. This prong is sutured for part of its length to the anterior limb of the DBS (ADBS).

ii) The posterior proximal scaphognathite sclerite (PPrS) - which traces on the ventral side a path similar to that of the APrS on the dorsal side, but after crossing the hinge, turns proximally to form a narrow, flexible connection at the juncture of the PrBS and the PCS. This prong is in fact composed of two parts, sutured together at the angle where it turns proximally. It seems likely that the more proximal portion constitutes a part of the basipodite, whilst the more distal part only, belongs to the scaphognathite. With this in mind, the sclerite will none-the-less be treated as a single sclerite since it behaves functionally as such, and it will be regarded as part of the scaphognathite.

iii) The external proximal scaphognathite sclerite (EPrS) - which runs in an arc along the ventral (posterior), internal border of the external scaphognathite segment.

Distally, at the juncture of the three prongs, the PrS forms an articulation with the central sclerite of the external scaphognathite segment.

The figures were drawn from dried skeletons and so do not show clearly that where the PPrS runs opposite the articulation of the APrS with the ISG, it also abuts on the ISG on the ventral side. In an end on view in fresh preparations, the external border of the ISG appears to be loosely held between the two sclerites as by a pair of tongs.

Two of the three sclerites noted previously in the blade of the scaphognathite have been termed the internal scaphognathite segment (ISG) and the external segment (ESG). The third which links the two segments distally across the flexible hinge, may be referred to as the hinge sclerite (HS). The scaphognathite therefore contains four sclerites altogether, the most proximal, the PrS having three subdivisions, the APrS, PPrS and the EPrS.

Allocation is more difficult for the two internal sclerites which lie parallel to the radial axis of the scaphognathite, articulating proximally with the rim of the dorsal segment of the PrBS and distally with the proximal edge of the internal scaphognathite segment (ISG). From their position, parallel to the basipodal (?) element of the PPrS, they shall for the present, be assigned to the basipodite. They shall be referred to as:

- i) The radial basipodal sclerite 1 (RBS1) - the more internal, pronged sclerite, and,
- ii) The radial basipodal sclerite 2 (RBS2) - the stout, hatchet shaped rod articulating distally with the ISG, just internal to the articulation of the APrS.

The basipodite therefore, is represented by four sclerites, the RBS1, RBS2, PrBS and the DBS, of which the DBS has two subdivisions, the ADBS and the PDBS. The proximal portion of the PPrS in addition, possibly constitutes a basipodal element.

#### The axes for movement

From the structure of the skeleton, it is clear that the second maxilla can move about three axes. Protraction and retraction (levation and depression) can occur about a line through the internal and external articulations of the CS with the head arthropod. This has been termed the P-R axis (Pilkington and Simmers, 1973). The other two axes lie along a radial line and are more complicated. The more dominant of the two is associated with the articulations between the DBS and the ECS. It involves turning of the DBS about the apical pivot, so that the PDBS swings on its hinge with the lip of the ECS. At the same time, the triangular sclerite (CLS) turns on its hinge with the CS, and about its articulation with the ADBS. The result of this

is that the ESG and PrS effectively pivot about the apex of the ECS, along an axis at about  $45^{\circ}$  to the P-R axis. This twisting movement of the ESG is transmitted to the ISG via the Hinge, and the articulations of the APrS and PPrS with the proximal, external edge of the ISG. The ISG therefore tends to be raised and lowered as the ESG/PrS complex twists. This causes the ISG to turn about the dual axes along the RBS1 and the RBS2 which form the second radial axis. The net result is that as the hind (external) tip of the scaphognathite is lowered, the Hinge is raised, and the whole ISG simultaneously elevated, the external portion to a greater extent than the internal tip. This movement can be superimposed on the protraction-retraction movement about the CS articulations.

#### The click mechanism.

As the ADBS moves from one extreme of its range to the other rotating the CLS about its hinge with the CS as it turns (see Fig.III.1 (b)), the angle between the ADBS and the dorsal lip of the ECS increases to a maximum near the centre of the range, then falls slightly once more. At the maximum angle of separation, a strain is placed on the hinge between the ECS and ADBS. This is transmitted to the CLS so that it tends to turn about a dorsi-ventral axis (antero-posterior) through its distal articulation with the ACS. The resulting effect is an instability at the centre of the range of movement on the radial axis through the apex of the ECS. Thus as the centre is passed, the sclerites tend to snap gently into the more extreme positions. If the ACS is cut internal to its articulation with the CLS, the softer cuticle (Fig.III.1b) at the external end of the ACS does not resist the turning of the CLS about its joint with the ECS, and the instability, along with the 'click' that normally

accompanies the movement, is distinctly reduced. It does not however, disappear, even if the whole ClS is removed. It appears that the articulation of the PDBS with the ECS also plays a role.

As in the case of the ADBS it appears that the tip of the PDBS, during the rotation of the DBS through its range, is forced by virtue of its articulation with the ECS (Fig. 1c), to follow a path that it would not normally trace. Again, it appears that the stress placed on the sclerite by this linkage is greatest near the centre of the range of movement, and least at the extreme. If the tip of the PDBS is freed from the CS with the ClS intact, the 'click' is again reduced, but not abolished. If both joints are transected, the 'click' disappears. Surprisingly however, there still remains some instability, so that if the appendage is pushed dorsally just past the centre of the range, it will gradually settle into the most dorsal position. The same is true of ventral movement but to a lesser extent. It appears that this residual instability is associated with the articulation of the APrS with the ISG. It seems that near the central position when the APrS, RBS1 and RBS2 lie close to a straight line, the RBS1 and RBS2 articulations are strained, whilst this does not occur at the extremities of the movement.

### III.-2.3. Conclusions

Of the ten main sclerites found in the second maxilla, one has been assigned to the coxopodite (ACS/ECS); one has been divided between the coxopodite and the basipodite (PCS/PrBS); three others have been fully allocated to the basipodite (RBS1, RBS2 and DBS); four have been assigned to the scaphognathite (HS, ISG, ESG, PrS). One (ClS) has not been allocated, but may be a part of the coxopodite. One element of the PrS is considered to represent, in part, a residue

of the basipodite. In some cases, subdivisions to these main sclerites have been separately identified in order to facilitate later discussions, and to enable points of articulation, and later, the insertions of the muscles, to be clearly indicated.

A click mechanism associated with the pronation-supination movement of the ESG about the ECS, has been described, whereby the two extremes of the range of the movement are made more stable than the central region as a result of a peculiar skeletal arrangement. During normal beating of the scaphognathite, the expulsion of the exhalent current occurs at the extremities of the beat. The twisting movement of the ESG also is associated with the pulling of water into the branchial chamber. The 'click' mechanism is therefore so designed as to contribute to the force with which water is pulled into and expelled from the branchial chamber. In the passively moved appendage the force generated by the 'click' is minimal, and would appear to be able to contribute but little to the muscular forces. In a later section, a mechanism will be described whereby the contribution of the 'click' may be enhanced somewhat during active movement.

The existing condition of the appendage may be regarded as the result of selective sclerotization and a degree of fusion along certain lines, in a primarily thin-walled generalized limb. This produces suitable pivotal points and lines, and the necessary degree of flexibility and room for muscular activity, so as to permit the generation of an effective undulating movement of the scaphognathite. The musculature involved in the production of this movement will now be dealt with.

### III-3. The Musculature

#### III.-3.1. Results and Discussion

The muscles of the second maxilla of Carcinus are illustrated in

Fig.III.3, as they can be seen in a dorsal preparation when the more dorsal muscles are progressively removed. The muscles are divided into two groups dorsi-ventrally (morphologically anteroposteriorly) by a strong ligament (lig) running along the P-R axis between the internal and external articulations of the CS with the head apodeme. The dorsal (morphologically anterior) set is concerned with levation (promotion), and supination, and the ventral (posterior) set with depression (remotion) and pronation.

Where possible the nomenclature of the muscles follows that of Cochran (1955). Three muscles not described by Cochran are given the names assigned to them by Maynard (1961).

Two additional muscles were discovered in Carcinus in this study. They probably function as accessories and are given the names of the previously described muscles with which they are associated. The first, the accessory scaphognathite flexor muscle (fsIIIm') has not given any successful electrical recordings, but from the scarcity of motor input to this region, it might be assumed to receive the same innervation as the scaphognathite flexor muscle (fsIIIm). Only two phasic units have been recorded in the nerve to this region, and both go to the fsIIIm. The second, the accessory promotor muscle (mpIIIm') at first sight might appear to be an integral part of the main muscle (mpIIIm). There are at least two gross anatomical differences however. Firstly, the mpIIIm' has its origin more distally and dorsally as a separate bundle on the side wall of the branchial chamber, and secondly, it has a direct insertion more distally than the mpIIIm, on the distal expansion of the RBS2. Contrary to the observations of Pasztor (1969) in Orconectes and Cochran (1955) in Callinectes, as well as Pilkington and Simmers (1973) in Cancer, the mpIIIm in Carcinus has been observed in this study to insert indirectly

on the RBS2 via a number of tough, tendinous strands. This has also been observed in Portunus depurator but not in the masked crab Coxystes. The indicated studies have reported an absence of tendons in the muscles of the second maxilla. Bearing in mind the small size of the RBS2, the condition found here in Carcinus and in Portunus has the obvious advantage of allowing the massive mpII<sub>m</sub> to transmit its force to a very localized region of the appendage.

Electromyograms reveal that the activity in the mpII<sub>m</sub>' is quite different from that in the mpII<sub>m</sub>, although the two muscles may act synchronously. Phasic activity in the mpII<sub>m</sub>' is most marked during reversed beating, and tonic activity of the same sort frequently continues during pauses when the mpII<sub>m</sub> is silent. This will be discussed more fully at a later stage.

The mpII<sub>m</sub>/mpII<sub>m</sub>' complex forms the most dorsal muscle involved directly in ventilatory activity. The mpII<sub>m</sub> takes its origin on a broad, reinforced intrusion of the endopleurite which is continuous distally with the thin walled covering of the branchial chamber. It inserts after crossing the radial axis, on the distal expansion of the RBS2. Its contraction might be expected to raise the ISG, simultaneously pulling it backward, and to cause twisting about either the Hinge or the ECS/DBS articulation.

Below this, originating on a more mesal expansion of the arthro-phragm, the musculus respiratorius secundus II maxillae (mr2II<sub>m</sub>) runs forward to cross the radial axis in the opposite direction to the mpII<sub>m</sub>, and insert on the proximal lip of the ECS just external to its junction with the ACS. Its contraction would tend to raise the whole maxilla, and in particular, the ESG, about the P-R axis, pulling it slightly forward at the same time.

Alongside the mrII<sub>m</sub> and posterior (external) to it, the musculus

respiratorius primus ( $mr1IIm$ ) runs roughly parallel to the radial axis of the appendage, from an origin just mesal and ventral to that of the  $mrIIm$ , to insert on the base of the ECS just external to the insertion of the  $mr2IIm$ . Its effect would be primarily to raise the whole appendage, in particular, the ESC in an action very similar to that effected by the  $m2IIm$ .

Below and between these, lying roughly along the radial axis the musculus remotorius II maxillae ( $mrIIm$ ) originates on a more distal platform where the apodemes bearing the more dorsal muscles meet. It runs outwards and forwards to insert on the dorsal lip of the  $APrS$  where it crosses the Hinge. Since this muscle lies so close to the P-R axis, the effect of its contraction might be expected to depend upon the position of the limb when it is activated. It probably induces little movement about the P-R axis. Its major effect would probably be to swing the  $PrS$  about its articulation (through the  $DBS$ ) with the ECS. It would therefore cause the external tip of the scaphognathite to be depressed, raising the hinge, and to a smaller extent, the internal tip of the scaphognathite (see Fig. III.3).

Immediately opposite the  $mrIIm$ , the musculus respiratorius tertius ( $mr3IIm$ ) takes its origin ventrally, and more distally on the same skeletal platform as the  $mrIIm$ , from which it is separated by the ligament running between the CS articulations with the head apodeme. It inserts, like the  $mrIIm$  on the  $APrS$  where it crosses the Hinge, but on a ventrally projecting flange of the sclerite. It appears to be a natural antagonist of the  $mrIIm$ , and if it becomes active when the  $APrS$  is in the dorsal part of its range, could well produce the opposite effect to that proposed for the  $mrIIm$ . Thus, it would pull the  $PrS$  down about the ECS, depressing the hinge and the internal tip of the scaphognathite, and causing the external tip to

rise. Indeed, the arrangement of the articulation of the PPrS with the juncture of the PrBS and the PCS - deeply invaginated so that it lies on a dorsi-ventral (antero-posterior) plane through the middle of the mrIIIm - would seem to be designed to ensure this. This arrangement also places the PrBS/PCS articulation slightly dorsad (anterior) to the fsIIIm (see below) so that activity in the fsIIIm will tend to pull the ISG down about a hinge formed by the articulations of the RBS1, RBS2 and PPrS with the PrBS.

The musculus respiratorius quartus (mr4IIIm) originates on the head apodeme just below the mr3IIIm. It is a much larger muscle than the mr3IIIm, its origin extending from the external extremity of the base of the appendage for about three quarters of the total base length. It inserts on the dorsal (anterior) wall of the semi-conical ECS. Its contraction would tend to depress strongly the whole appendage, in particular the ESG.

The remaining muscles of significance in ventilation are intrinsic.

The musculus respiratorius quintus (mr5IIIm) originates on the proximal tip of the PDBS and inserts on the ventral (posterior) side of the RBS2 slightly proximal to the insertion of the mpIIIm. It would tend to oppose the 'supinating' action of the mpIIIm by depressing the internal scaphognathite segment (ISG). The Hinge would simultaneously be depressed, and the external tip of the scaphognathite raised.

Originating on the hinge of the PDBS just below the origin of the mr5IIIm are two small muscles, which insert at the angle of the PPrS. These are the musculus respiratorius sextus and septimus (mr6IIIm and mr7IIIm). No difference could be found in the electromyograms of these muscles. With their similar origins and insertions, these may therefore be treated as a single muscle. The main effect of these

muscles would be to twist the DBS downwards about its articulation with the ECS (supination).

The flexor scaphognathitis II maxillae (fsII<sub>m</sub>) is a diffuse muscle originating on the inner walls of the cup-shaped PrBS, and running outward, spreading across the ISG and inserting largely on the dorsal (anterior) side along the distal extremity of the sclerite. Its action is to curve the internal tip of the scaphognathite downwards and backwards as described previously.

The accessory flexor muscle (fsII<sub>m</sub>') first described in this study, originates at the base of the RBS2 and runs slightly distally to insert on the base of the dorsal prong of the RBS1. Its activity could conceivably reinforce the effectiveness of the fsII<sub>m</sub>, with which, as described earlier, it is probably synchronous. It could also, however, quite conceivably serve to stabilize the RBS2 during the contraction of the mr5II<sub>m</sub>, since the activity in this muscle overlaps to some extent with that of the fsII<sub>m</sub>.

There is one other muscle, the musculus depressor II maxillae (mdII<sub>m</sub>) which might play a role in ventilation. It originates ventrally on the endosternite, one head inserting at the base of the PrBS where the PCS passes below it, and a second on the proximal edge of the ventral lip of the PRBS. No electrical activity was ever recorded from this small muscle, and its removal seems to have no obvious effect on the ventilatory movements. It is well situated however, to cause depression of the appendage about the internal articulation of the CS. It is interesting too, to note that gentle pressure on the ventral side of the PrBS just distal to the insertion of the mdII<sub>m</sub> results in the discharge of a strong, slowly accommodating sensory unit. Contraction of the muscle may have similar results, but there is no actual evidence to support this.

Other muscles figured here, but thought not to play a significant role in ventilation are:

- i) The musculus proximalis coxopoditis II maxillae (pcII<sub>m</sub>)
- ii) The musculus dorsiventralis posterior (mdp)
- iii) Musculus distalis coxopoditis II maxillae (dcII<sub>m</sub>)

None of these three muscles is figures by Cochran (1955), but they are described by Maynard (1961).

- iv) The musculus adductor endopoditis II maxillae (aeII<sub>m</sub>).

With the exception of the mdp, these are very small muscles associated with the bilobed endites.

### III.-3.2. Conclusions

Twelve muscles have been described which appear to play an important role in the ventilatory movement of the second maxilla. They are so disposed as to effect movements about the axes described previously. Those on the dorsal (anterior) side of the P-R axis form two pairs. The mpII<sub>m</sub> and the mrII<sub>m</sub> appear to be largely concerned with movements about the radial axes, whilst the mr3II<sub>m</sub> and the mr4II<sub>m</sub> would be largely involved in movements about the P-R axes.

On the ventral (posterior) side of the P-R axis, most of the muscles appear to effect movements about the radial axes. Only the massive quartus (mr4II<sub>m</sub>) seems to be strictly involved with movements about the P-R axis. The remotor (mrII<sub>m</sub>) and the tertius (mr3II<sub>m</sub>) which by their positions might be expected to be antagonists in the levation and depression about the P-R axis, in fact appear to be antagonists, but not in movement about this axis. By virtue of their attachment to the PrS, they would tend to effect movements about the radial axis through the apex of the CS.

Of the other ventral muscles, the quintus (mr5II<sub>m</sub>) seems to serve as an antagonist to the mpII<sub>m</sub>/mpII<sub>m</sub>' complex, whilst the sextus

and septimus together could aid the tertius as antagonists to the *mrIIIm*. The *fsIIIm* appears to have no antagonist.

### III-4. The Cross Innervation Pattern

#### III-4.1. Results

A single nerve trunk leaves the anterior lobe of the ventral ganglionic mass ventro-laterally, ventral, and slightly anterior to the large nerve to the cheliped. It quickly bifurcates, and at a variable distance, one-half to a quarter of the way to the second maxilla, one of the branches divides again. Three roots thus arrive at the second maxilla, one large, and two smaller. Close to its entry into the maxilla, the larger trunk divides unequally, the smaller branch (*M1*, Fig. III. 4) turning dorsally between the two expanded apodemes which bear the dorsal (anterior) muscles of the second maxilla. Here, this branch divides again sending one branch externally and one internally along the *mrIIIm*, a third continuing dorsally to innervate the other dorsal muscles. This branch is apparently composed of motor axons only, so sensory discharges ever having been recorded in it in response to many varied stimuli.

The larger branch of this nerve along with the two smaller trunks, enters the second maxilla at its internal angle. The large trunk (*SM*) gives off a small branch at the base of the *PrBS*. This innervates the *mdIIIm* and the ventral muscles to the endites. The main trunk continues distally, then divides, the internal branch splitting up to innervate the *fsIIIm* and continuing on to innervate the *ISC*. The external branch runs between the *APrS* and the *PPrS* close to the insertions of the *mr6IIIm* and *mr7IIIm*, apparently without branching. It continues on to the junction of the three prongs of the *PrS* where it gives off a small branch, which splits up in this region, then turns distally into the

ESG, through which it ramifies.

Of the two small nerve trunks the more internal innervates the endites, and is apparently purely sensory (SN). The more external (MS) runs distally through the mr4IIIm along its internal edge giving off small branches to innervate it. It continues to the apex of the ECS, then turns internally along the PPrS, to the insertion of the mr6IIIm and mr7IIIm. A more dorsal branch runs proximally along the mr3IIIm, innervating it. This branch apparently carries a few sensory units in addition to the motor units to the external ventral musculature. The sensory units may be associated with a small patch of hairs lying just proximal to the origins of the quintus, sextus and septimus muscles.

#### III-4.2. Discussion and Conclusions

The second maxilla is innervated by four separate nerve trunks, one purely sensory, two sensori-motor, and one purely motor. The innervation to the dorsal musculature is carried in a single, separate trunk (M1). The ventral muscles form two groups, an internal group including the muscles of the endites and the fsIIIm, and an external group. These are innervated by separate nerve trunks. The trunk innervating the internal group (SM) carries numerous sensory units, and innervates the general cuticle of both the ISC and ESG.

It is becoming more and more clear that anatomical arrangements such as the grouping of neurons, and perhaps their peripheral processes, is not a hap-hazard process. Purely logistic considerations are undoubtedly of great importance. Thus, axons going to a common destination might be expected to be grouped together. It is interesting to speculate however, whether as with the central neurones (Kandel, 1969) functional considerations might govern peripheral axonal groupings. It is of course impossible at this stage, to say, but one

might well wonder if the innervation of the fsIIIm, independent of the other ventral muscles controlling ventilatory activity, may not be associated with a certain degree of independence in the properties of its motoneurons. This speculation may be answered by the subsequent analyses of the phasing of the muscles, their frequency dependencies, and of the fine structure of the motoneurone bursts.

### III-5. Summary

The second maxilla in Carcinus is composed of a complex system of articulated sclerites, separated by very thin walled cuticle, and which provide insertions, and in some cases origins, for ten muscles which are directly involved in ventilatory activity. Because of the small separations of the pivotal points of the sclerites, and the insertions and origins of some of the muscles; compared to the relatively large excursion of the appendage, the exact point in the movement cycle at which a muscle becomes active may be important in determining the movement produced.

The skeletal system has been described in greater detail than hitherto, and an attempt has been made to reconstruct its homologies with the plan of the generalized crustacean appendage. From a consideration of the scheme of articulations between the sclerites, three major axes for movement have been identified. One lies along a longitudinal axis, and allows levation and depression (promotion and remotion) of the whole appendage, and in particular, of the external portion. The second lies along a radial axis in the internal scaphognathite segment, and has two foci, through the RBS1 and the RBS2. This allows the internal scaphognathite segment to twist about a double radial axis composed of the RBS1 and RBS2. The third allows twisting of the external scaphognathite segment about an axis running

through the apex of the ECS at about  $45^{\circ}$  to the P-R axis.

All these movements are linked, so that activity about any one of the three axes produces associated adjustments about the others. It is thus difficult to deduce on an a priori basis from the three permitted axes of movement, what the net movement might be. In the next section therefore, the movement of various points on the scaphognathite will be monitored in relation to the cycle of activity in the muscles. The individual movements will then be superimposed in an attempt to reconstruct the total movement in relation to the activity in the participating muscles.

In the present section also, the musculature of the second maxilla has been described in detail, and has included two muscles not previously figured. The gross innervation has been described and the anatomically separate anterior (functionally dorsal) and posterior (functionally ventral) muscle groups found to receive their innervation from two separate nerve trunks, with the exception of the more internal, ventral muscles, including the fsIIIm, which are again, separately innervated.

The detailed analysis of the skeleto-muscular system has allowed a greater understanding of the movements involved in the pumping activity of the scaphognathite, and has allowed some inferences to be drawn concerning the phasing of the activity in the muscles involved.

The following table shows the results of the experiments conducted in the laboratory of the Department of Chemistry, University of Toronto, during the year 1925. The experiments were conducted by Mr. J. H. Hill and Mr. J. H. Hill, Jr. The results are given in the following table.

Figure III.1

The table contains several rows of data, likely representing different experimental runs or conditions. The columns are too faint to identify, but the structure suggests a standard data table with headers and multiple columns of numerical or descriptive values.

Figure III.1. The skeleton of the second maxilla. Dorsal (morphologically anterior) view. The inset (b) shows more clearly the dorsal (anterior) linkage of the DBS with the CS in relation to the dorsal muscles. As the DBS/PrS complex turns about the axis c'c on the pivot (ax) at the apex of the ECS, the sclerite ClS swings about the axis b'b, and the PDBS twists at its tip along the hinge ph. This peculiar skeletal arrangement gives rise to instabilities in the structure, which are greatest near the middle of the range of movement, and least at the extremes. The result is that in response to passive movement, the appendage tends to click smartly between the extreme positions with respect to turning about the axis b'b. This effect may be exaggerated during active movement (see Section IV).

KEY:      HS, hinge sclerite

ISG, ESG, internal and external sclerites in the respective segments of the scaphognathite

APrS, PPrS, EPrS, anterior, posterior and external divisions of the proximal scaphognathite sclerite

DBS, RBS1 and RBS2, PrBS, distal, radial and proximal basipodal sclerites

ECS, ACS, PCS external, anterior, and posterior coxopodal sclerites.

End, endopodite of the basipodite

Bnd, basipodal endites

Cnd, coxopodal endites

ClS, 'click' sclerite (coxopodal ?)

a

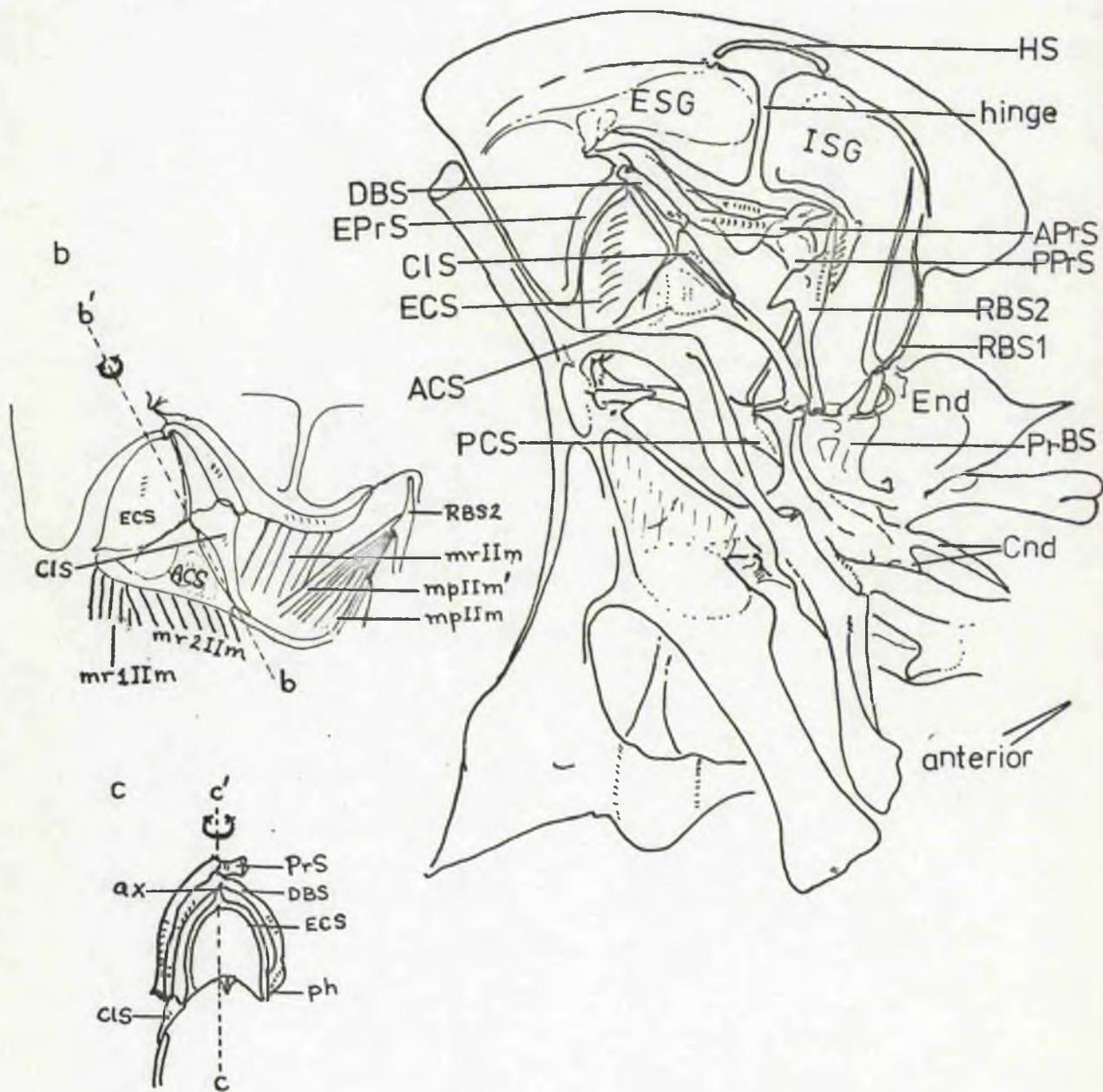
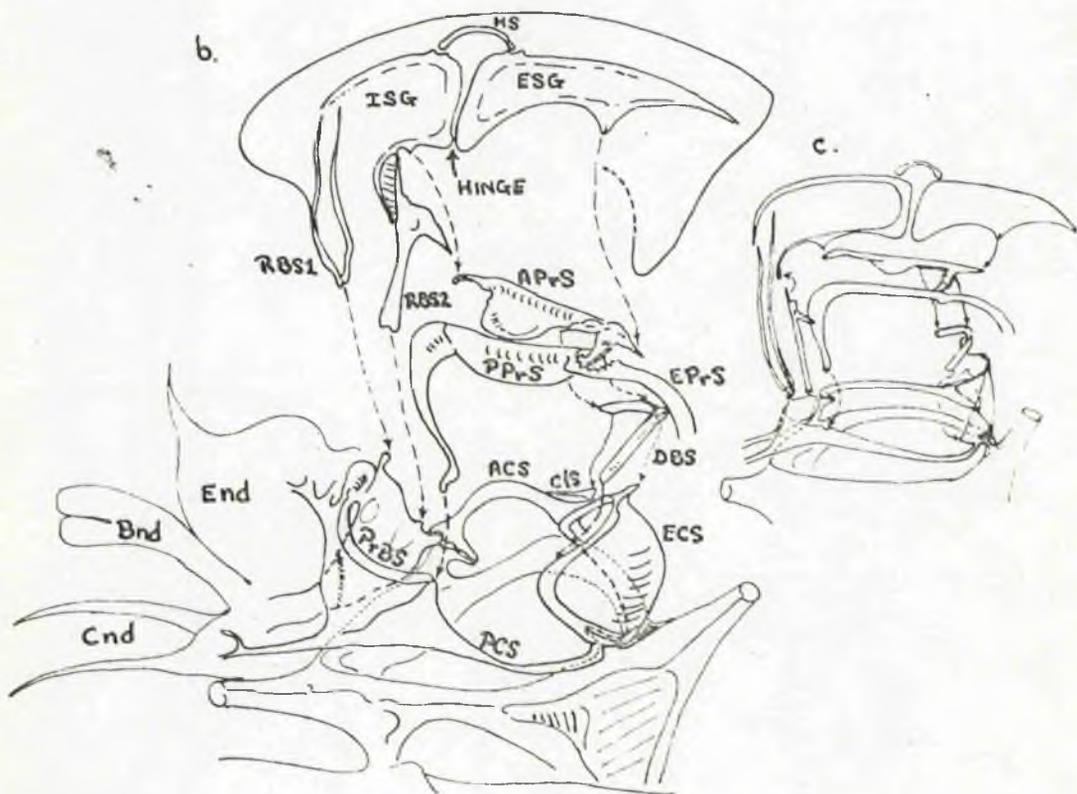
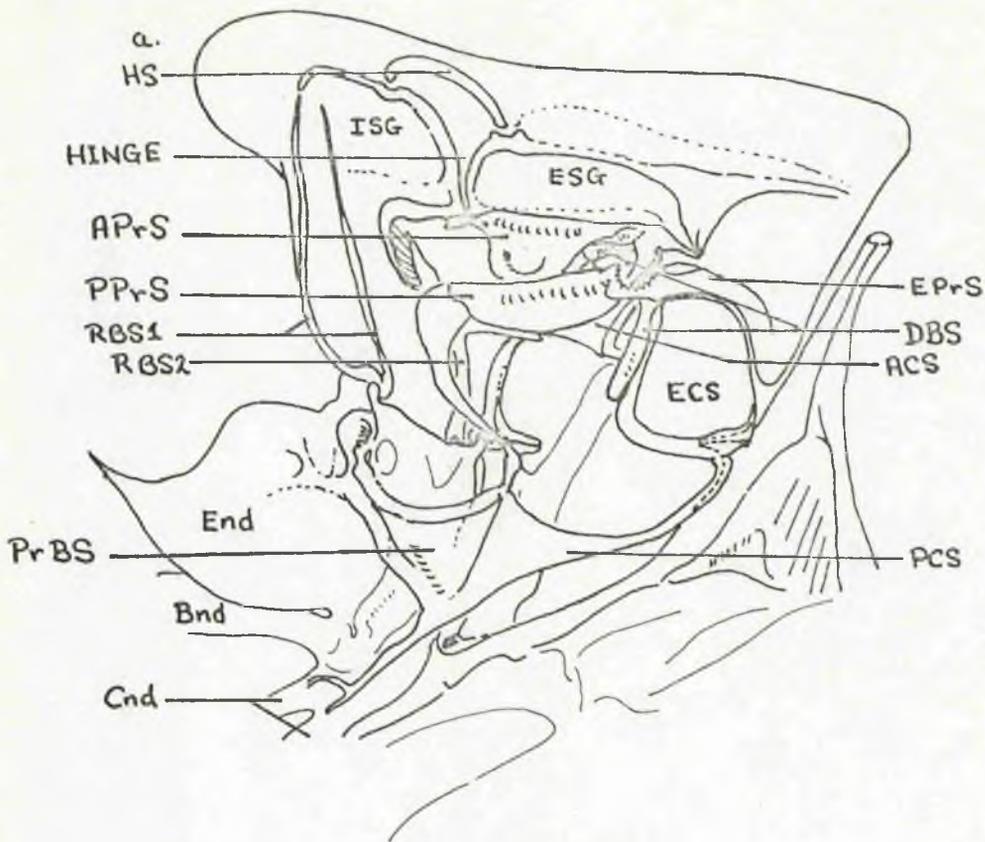


Figure III.2 shows the results of the analysis of variance for the effect of the concentration of the solution on the rate of polymerization. The rate of polymerization increases with increasing concentration of the solution. The rate of polymerization is also affected by the temperature of the reaction. The rate of polymerization increases with increasing temperature.

Figure III.2

Figure III.2. Skeletal elements of the second maxilla. In (b) the elements are disarticulated for clarification and the diagrammatic inset (c) further clarifies the interconnections.

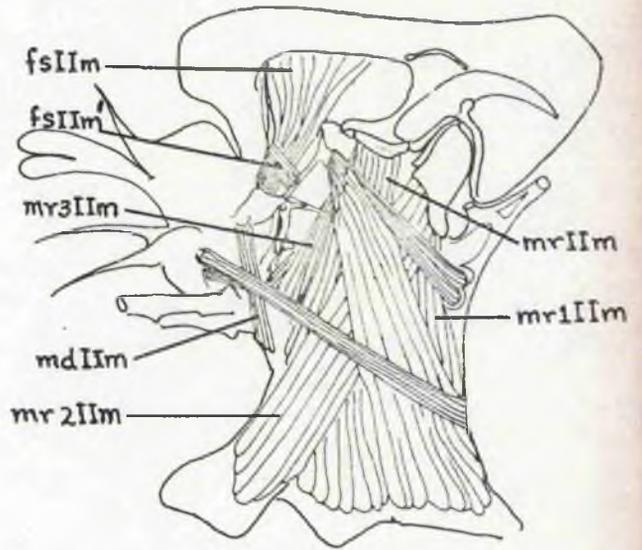
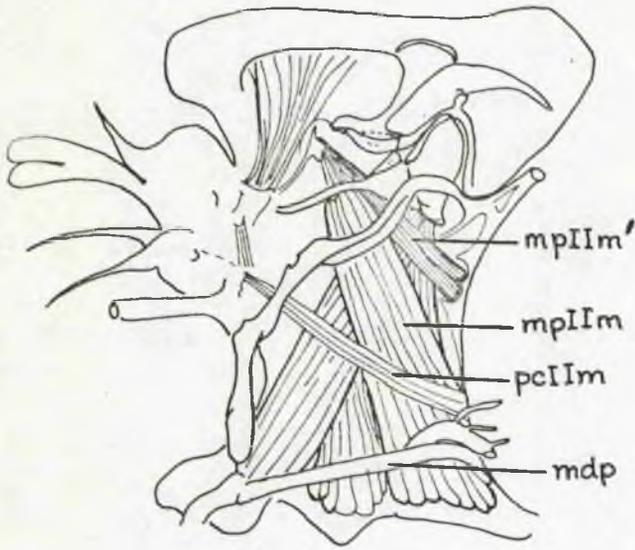


**Figure III.3**

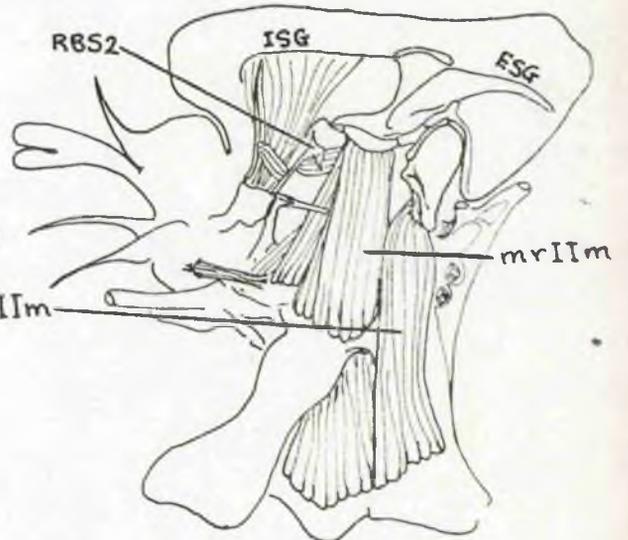
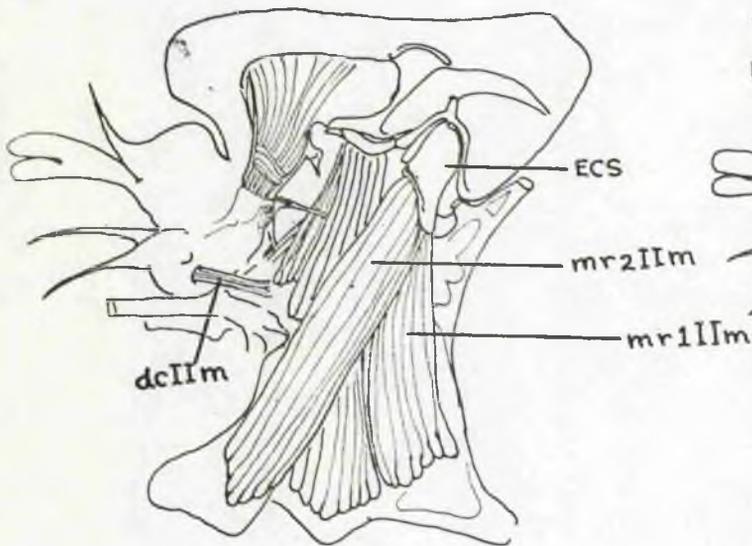
Figure III.3. Musculature of the second maxilla  
as seen from the dorsal (morphologically anterior)  
aspect (except in d, which shows the ventral view).

Figure III.3

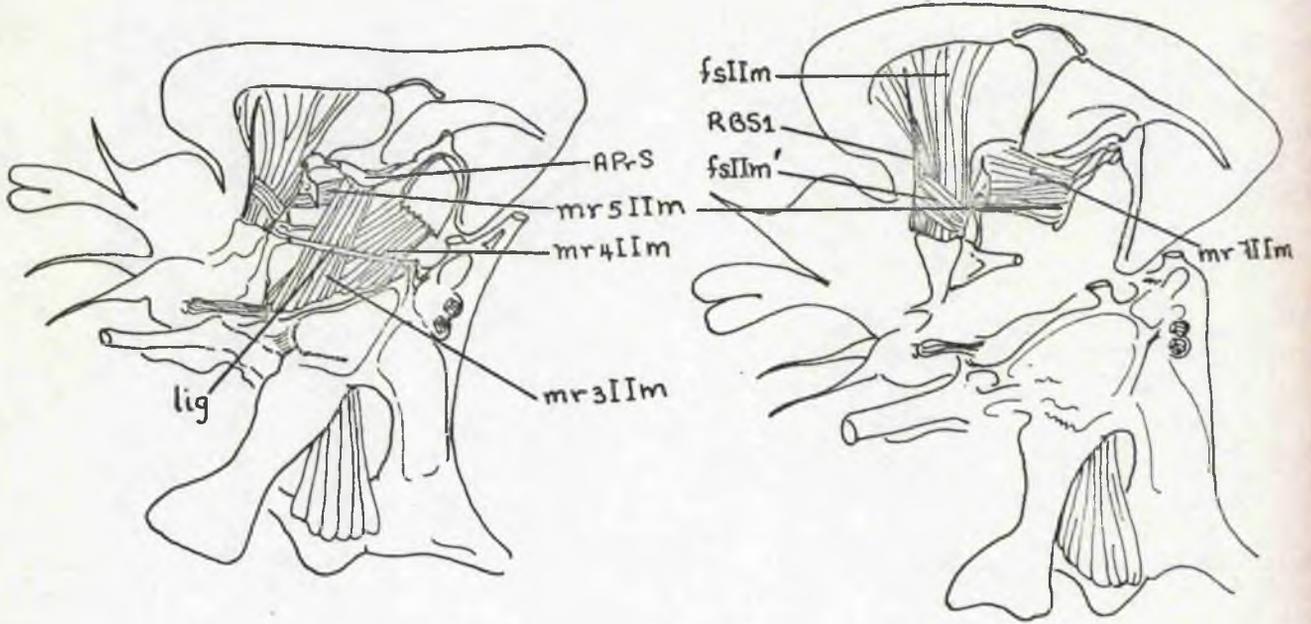
a



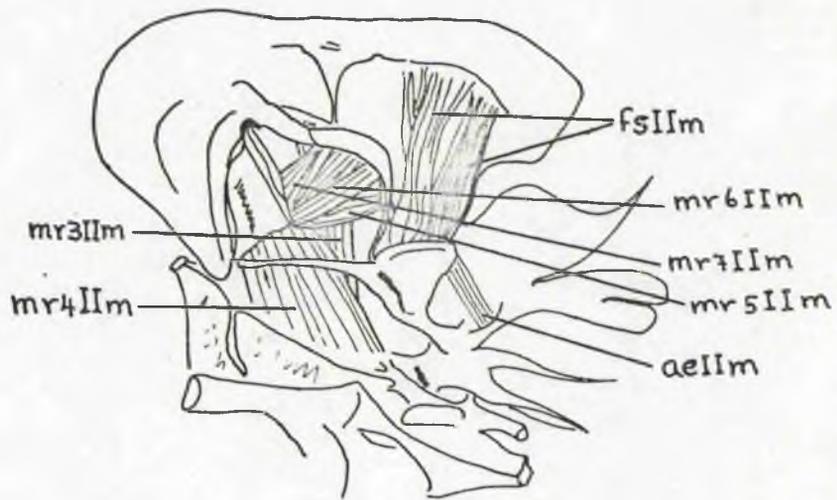
b



c



d



Key to Nomenclature for the Muscles of  
the Second Maxilla in Carcinus Maenas.

- aeIIa - musculus adductor endopoditis II maxillas  
fsIIa - flexor scaphognathitis II maxillae  
fsIIa' - accessory flexor scaphognathitis II maxillae  
mdp - musculus dorsiventralis posterior  
mdIIa - musculus depressor II maxillae  
mpIIa - musculus promotor II maxillae  
mpIIa' - accessory promotor  
mrIIa - musculus remotorius II maxillae  
mr1IIa - musculus respiratorius primus II maxillae  
mr2IIa - musculus respiratorius secundus II maxillae  
mr3IIa - " " tertius II maxillae  
mr4IIa - " " quartus II maxillae  
mr5IIa - " " quintus II maxillae  
mr6IIa - " " sextus II maxillae  
mr7IIa - " " septimus II maxillae  
pcIIa - musculus proximalis coxopoditis II maxillae

... ..

**Figure III.4**

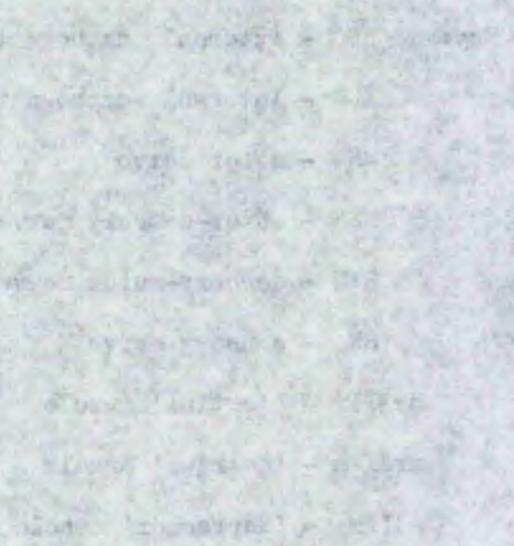


Figure III.4 The innervation pattern of the second maxilla of Carcinus. The course of the nerves is shown superimposed on the ventral side of the left scaphognathite to illustrate the relationship with the muscles and skeletal elements.

Key: ESC - external scaphognathite segment

ISC - internal scaphognathite segment

MM - motor nerve

MSN - motor-sensory nerve

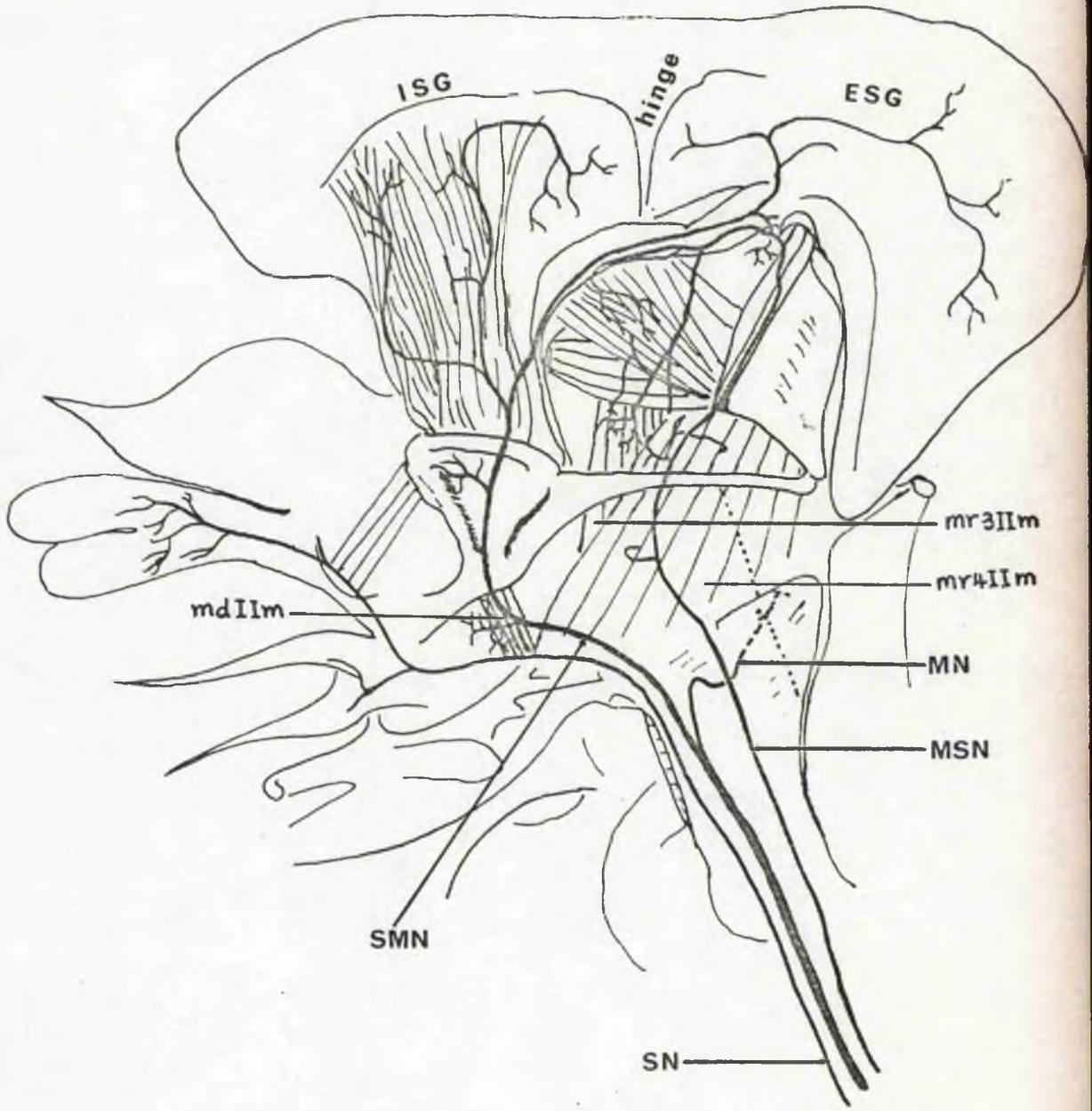
SMN - sensory-motor nerve

SN - sensory nerve

mr3IIa - 'tertius' muscle

mr4IIa - 'quartus' muscle

mdIIa - 'depressor' muscle



SECTION IV

THE TEMPORAL PATTERN OF THE BURSTS  
OF ACTIVITY IN THE VENTILATORY MUSCLES

## INTRODUCTION

In order to produce meaningful activity, and for maximum efficiency in pumping water through the branchial chamber, the muscles controlling scaphognathite movement must be brought into play in reasonably well defined sequences and for similarly well defined segments of the beat cycle. The rate of scaphognathite beating can vary widely, changes in beat frequency representing perhaps the most significant response of the scaphognathite to alterations in relevant environmental parameters such as oxygen tension ( $PO_2$ ) and carbon dioxide tension ( $PCO_2$ ) (Thomas, 1933; Segaar, 1934; Larimer, 1964), and in the concentration of various 'food' chemicals (Ashby and Larimer, 1965). Presumably therefore, one of the characteristics of the muscular phasing must be a relative fixity at differing beat frequencies, or at least, if alterations in phasing accompany changes in frequency, that the changes be consistent with effective pumping activity.

A detailed investigation of the phasing of the muscular activity and its frequency dependence can yield not only understanding of the mechanics of scaphognathite activity, but may also give some insight into the nature of the central connectivity patterns underlying the observed neural output. This section therefore presents a study of the phasing of the activity of the ventilatory muscles and the frequency dependence of this phasing. In addition, the relationship between the muscular activity and the movement of the scaphognathite will be described. Firstly however, the relationship between the motor input into the appendage and the activity in the different muscles will be described, in so far as it will aid in an appreciation of the overall pattern of the activity in the muscles.

The basic methods used for monitoring muscular activity and

scaphognathite movement have been described previously (Section II.2.3), along with the treatment of the preparations (Section II.2.1), and the techniques used in the analyses of the filmed electro-myographs (Section II.3).

IV.1. The relationship between the motor output to the second maxilla and the activity in the ventilatory muscles.

IV.1. 1. Results.

The total motor output to the second maxilla is carried to two major nerve trunks, the MSN and the combined SMN and MN. By simultaneously recording activity in the muscles and these nerve trunks, certain units in the nerve could readily be associated with certain muscles. This was especially clear for units to the flexor and the sixtus which tended to be active when the other units in the nerve trunk innervating them were silent. Fig. IV.1 A & B illustrates the activity in these nerve trunks in relation to that in the flexor, during both reversed and forward beating. The excitatory junctional potentials (ejp's) in the flexor can readily be associated with units in the combined SMN/MN. During forward beating, the unit associated with the flexor fires during a somewhat separate period from the remaining units in this nerve well inside the period when the MSN shows strong activity. In reversed beating, the unit associated with the flexor, although now overlapping with the remaining spike trains in the nerve trunk, still overlaps extensively with the spike trains in the MSN. The remaining spikes in the SMN/MN tend to alternate, with little overlap, with the bursts of spikes in the MSN. The remaining activity in the SMN/MN complex is probably associated with the activity in the levator musculature (Fig. IV.1, F,G). The figure shows that the ejp's in the secundus correspond with some of the units that are active earlier in the SMN/MN burst during forward beating (excluding the unit already assigned to the flexor), and towards the end of this burst during reversed beating (again excluding the unit assigned to the flexor). Fig. IV.1, D in a forward cycle, and Fig. IV.1, C in a reversed

cycle, show that the large units active during the period when the MSN exhibits maximal bursting are associated with the occurrence of ejp's in the quartus. The single trailing unit in this burst is associated with the sextus (Fig. IV.1, E). From the position of the burst in the sextus muscle with respect to that in the flexor (Fig. IV. 10, A) it may be inferred that the units to the sextus are the leading units in the MSN burst during reversed beating (Fig. IV.1 A & B). The tertius and quintus as well as the quartus receive their innervation via the MSN, and all are active during the same portion of the cycle (Fig. IV.2). It appears that the tertius and quartus may, to some extent, share a common innervation, whilst the quintus responds to a smaller unit which may start later in the cycle, and has no influence on either the tertius or the quartus (Figs. IV.1 D, E; IV.2). The records shown in Fig. IV.2 were taken from a preparation in relatively poor condition, but are used because they show the relationship between individual nerve spikes and their corresponding ejp's much more clearly than fully active preparations.

It was not possible to correlate the activity in the other dorsal muscles with that in the nerves, as these muscles were not easily accessible from the ventral side and the nerve trunks were not approachable from the dorsal side. However, from the positions at which these muscles become active in the cycle, with respect to those studied, the spike trains associated with their activity may to some extent, be inferred.

From the above, it appears that the depressor muscles with the exception of the flexor, receive their innervation via the MSN, whilst the levator muscles and the flexor, are innervated by the combined SMN/MN. This confirms the anatomical observations made in Section III.4.

The total motor output to the ventilatory muscles is seen to

consist of alternating bursts of overlapping spike trains to the levator and depressor muscles. During forward beating, the end of the depressor phase overlaps slightly with the start of the levator phase. There is a slight pause between the end of the levator phase and the start of the next depressor phase. During reversed beating, the pattern appears to be roughly inverted, but is not a perfect mirror image of the forward pattern. There is for example, usually no pause apparent in the cycle during reversals. Apart from the brief periods of overlap, the antagonist groups are completely silent whilst the agonists are active during both forward and reversed beating. The small, irregularly spiking unit appearing in the SMN/MN complex in Fig. IV.1, A,C, F & G during the depressor phase is probably associated with one of the smaller muscles of the appendage (see Section III, 3 & 4). Individual muscles may respond to the discharge of single units (e.g. flexor, Fig. IV.1. A,B; sixtus, Fig. IV.1. E; IV.2.D) or multiple units (e.g. secundus, Fig. IV.1. F,G; quartus, Fig. IV.1.C,D).

These then are some of the broad characteristics of the neuromuscular activity in the scaphognathite of Carcinus in particular. The question of the sensory contribution to the activity in the nerves has however, not yet been mentioned. The SMN/MN complex appears to carry the majority of the sensory information returning from the scaphognathite proper. In order to see if any phasic afferent activity contributed to the burst in this nerve, the nerve was picked up on two pairs of hook electrodes. These were separated by nearly 1 cm of nerve, and the individual hooks in each pair were separated by about 2 mm. One electrode was located close to the ganglion and the other close to the point where the nerve bifurcates before entering the maxilla (Fig. III.4). Two criteria could then be used

to distinguish afferent from efferent activity. Firstly, afferent spikes will be 'seen' by the distal before the proximal pair of electrodes. Efferent, motor output will be 'seen' first by the proximal pair. Secondly, a central wave of depolarization will come first to the more distal hook of each pair. A distad wave will come first to the more proximal hook. The biphasic depolarization due to sensory impulses will therefore be inverted with respect to that due to motor units. Fig. IV. 3A shows the overall activity in the SMN/MN complex recorded at a relatively slow film speed. In Fig. IV. 3B the activity recorded at a high speed is shown. There is no clear indication that any sensory activity contributes to the burst. Some very low amplitude activity which may constitute sensory spikes have been encircled in the records in Fig. IV. 3B. It would appear then that all the readily discernable activity in the SMN/MN complex is efferent motor output.

Subsequent chapters will attempt to describe in greater detail the phasing of the activity in the different muscles in the beat cycle, and the fine structure of the burst in at least some of the muscles.

Figure 4.1. The relationship between the amount of water...

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Figure IV.1

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Figure IV.1. The relationship between the bursts of excitatory junctional potentials in some of the ventilatory muscles, and the spike trains in the main motor nerve trunks (MSN and SMN/MN) to the second maxilla.

- Strip A Activity in the flexor (faIIa) associated with spikes in the SMN/MN complex during forward beating (fd). Note strong facilitation, the absence of any obvious response to some of the earlier spikes in the bursts, and the sharp (exponential) rise in spiking frequency through the burst.
- Strip B The same, during reversed beating (rev). Note that in A the flexor burst precedes the remaining spikes in the SMN/MN complex and that there is a gap of about 75 ms intervening. In B the flexor burst follows, and there is no gap. Also note the slow decrease in spiking frequency through the burst in B as opposed to the rapid increase in A. The arrowed spikes in A and B have been assigned to the sixtus (see strip E). Notice the close relationship between these spikes and the spikes in the flexor burst where they overlap in B.
- Strip C Spikes in the MSN associated with ejp's in the quartus (mr4IIa). The fast spikes in the MSN have not reproduced well. Close examination showed that ejp's occurred in response to different units, but this is not readily apparent in the EMGs. In the second burst in B also, note that an odd unit may suddenly replace one of the normal spikes with no corresponding change in the EMG.
- Strip D The same as above, for forward beating in different preparation. Note the tendency towards the occurrence of 'doublets' and the multi-unit innervation of the muscle.
- Strip E The trailing single unit in the MSN associated with ejp's in the sixtus (mr6IIa). Forward beating. See arrowed spikes in strip A. The arrowed spikes in B have been associated with the sixtus during reversed beating.
- Strip F Activity in the secundus (mr2IIa) associated with some of the leading spikes in the SMN/MN trunk (excluding the spikes assigned to the flexor). The trailing spikes in this burst have been assigned to the promotor and remotor. Forward beating.
- Strip G As above, reversed beating. Activity in the secundus now associated with trailing spikes in the burst (excluding the unit assigned to the flexor). Note here and in F, the multi-unit innervation of the muscle.

Note in the forward cycles, the brief pause between the end of the burst in the SMN/MN trunk and the start of the next burst in the MSN. There is no pause in the reversed cycles (B,G), but in C the overlap is minimal.

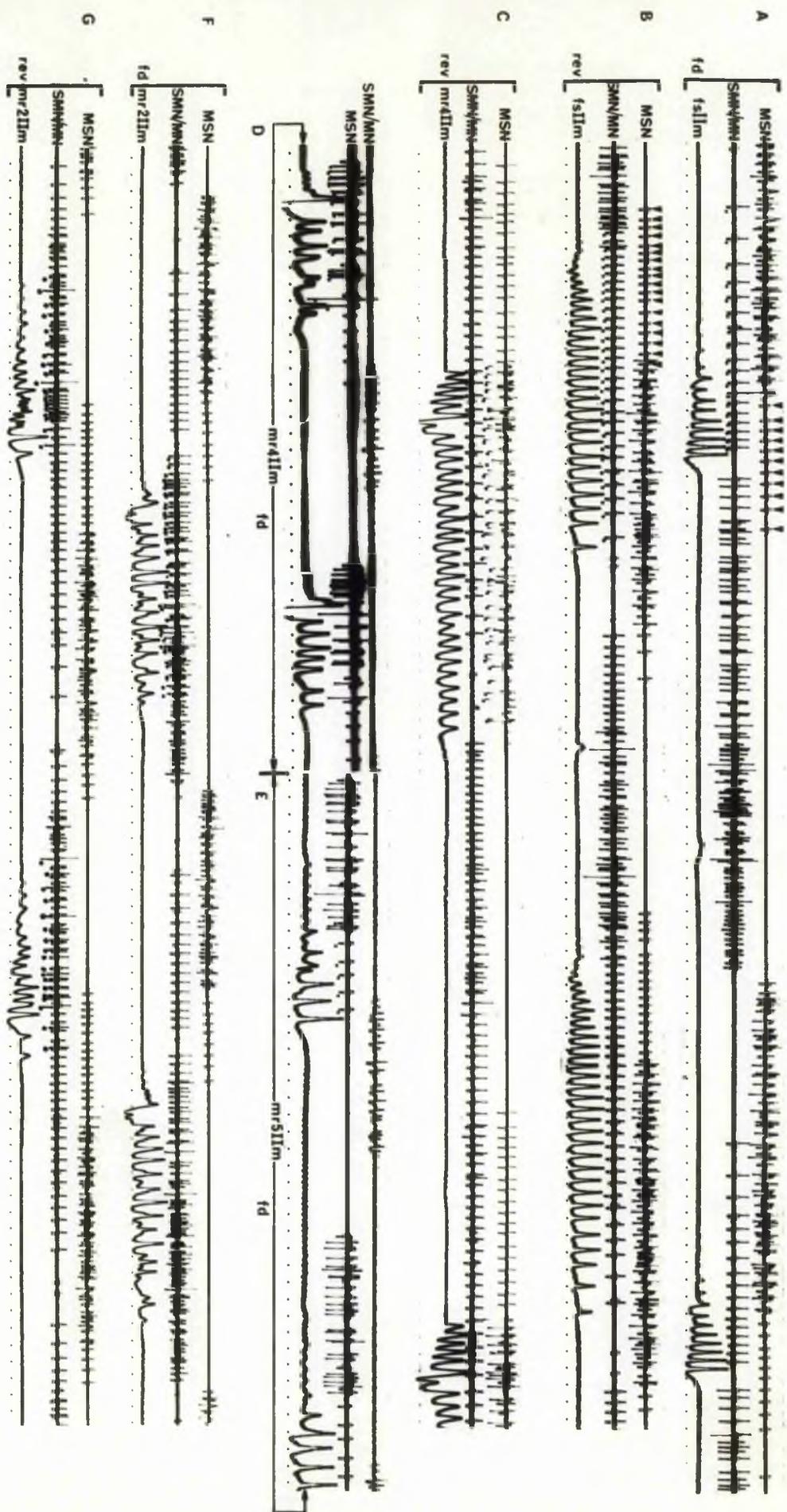
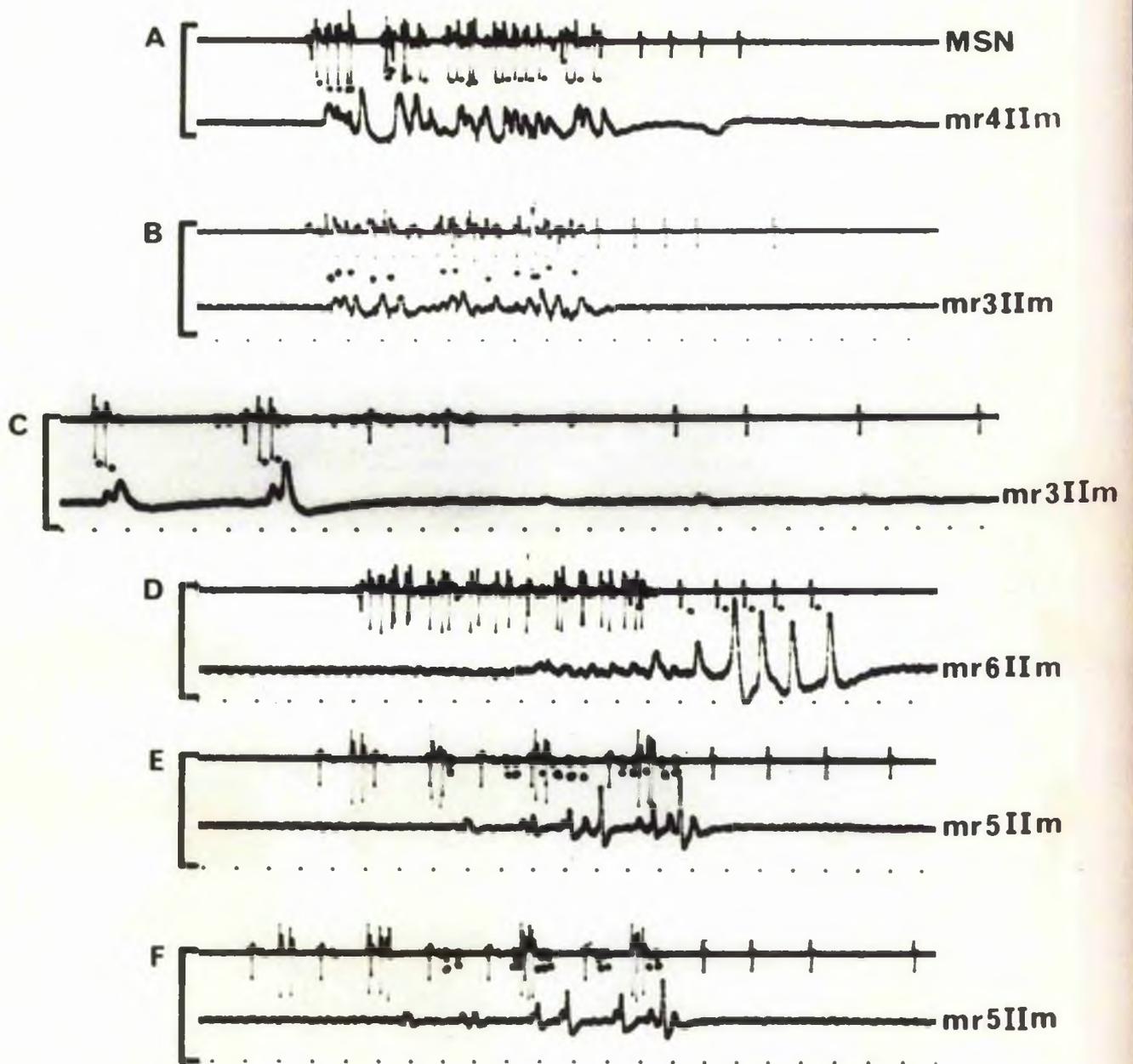




Figure IV.2. Activity in the MEN in relation to ejp's in some of the depressor muscles during forward beating. The quartus (mr4IIa, strip A) and the tertius (mr3IIa, strip B) appear to respond to common units in the MSN. The quintus appears to respond to a very small unit (or two) which may start later in the cycle than those to the quartus and tertius, but which ends at approximately the same time (strips E, F). The sextus responds to the trailing unit in the burst (strip D). Notice in strip C in a rather weak, odd burst, the clear occurrence of doublets in the input to the tertius (mr3IIa).

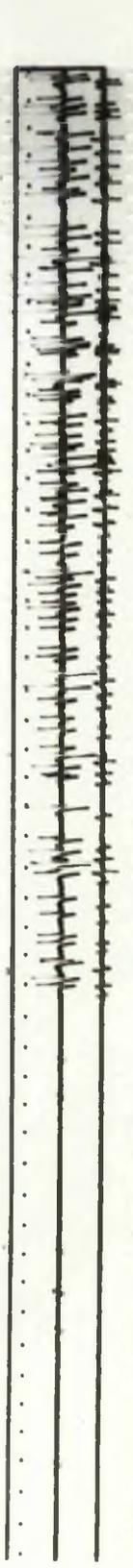
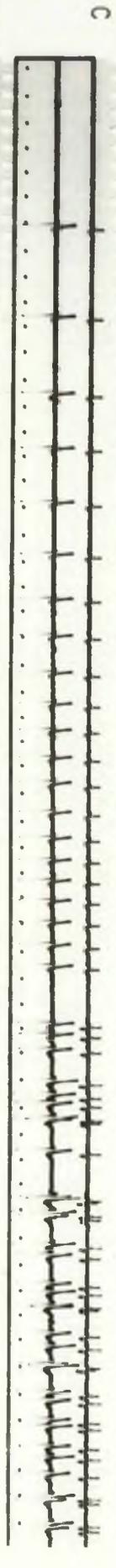
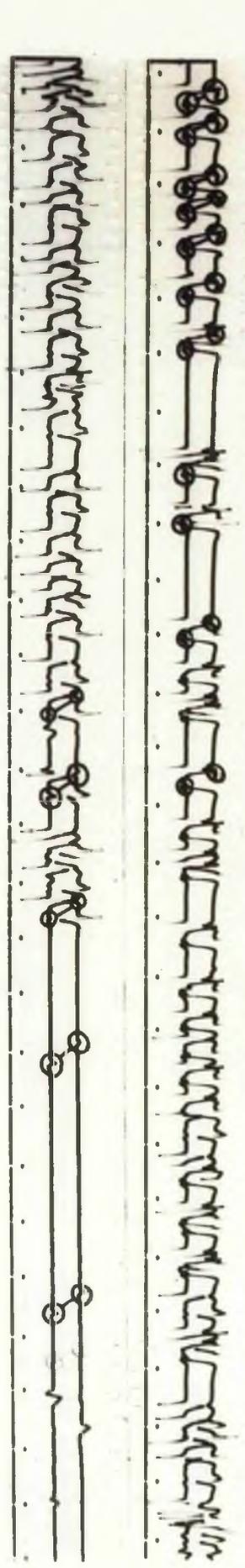
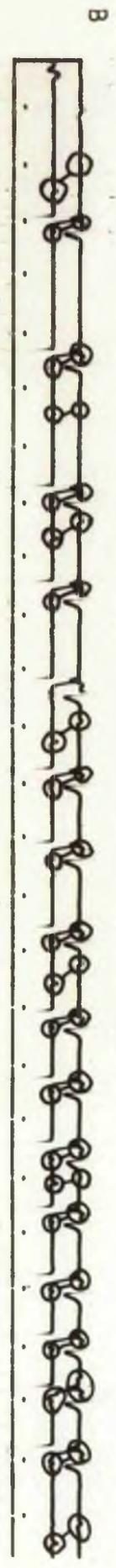


time mark = 50 ms



**Figure IV.3.** An attempt to discern the presence of phasic sensory units in the SMN/MN nerve trunk during active ventilation.

- Strip A** A single burst in the SMN/MN nerve trunk recorded at a slow speed with a single pair of hook electrodes.
- Strip B** A single burst recorded at high speed with two pairs of hook electrodes, separated by about one cm of nerve trunk. Lower trace, central electrodes; upper trace, distal electrodes. Sensory units should be distinguishable from motor by appearing firstly in the upper, and then after a short latency, in the lower trace. The biphasic action potential should also be the inverse of that for motor (efferent) impulses. No sensory spikes are readily discernable. Vague possibilities have been encircled. Most are probably artefacts. The SMN/MN nerve trunk appears to carry the main bulk of the sensory reafference from the scaphognathite.
- Strip C** As in strips B. At a lower gain and speed to show more clearly the relationship between the motor (efferent) spikes. At this gain, the presumptive sensory (afferent) units cannot be discerned.



time mark = 50 ms

## IV.2. The overall pattern of the bursts.

### IV.2.1. Results.

Figures IV.4 to 7 represent analyses of extended records from the experiment illustrated in part in Fig.IV.1. in the preceding chapter. These data show that the durations of the total levator and depressor phases of the cycle are a linear function of period length, during both forward and reversed beating. The lag between the start of activity in the depressor pool and the start in the levator pool is also linearly dependant on period (Figs.IV.4 to 6) during forward and reversed beating. During forward beating in this experiment, both levator and depressor sessions tended to occupy phase angles close to 0.5 in the cycle, and this remained essentially constant over the range of frequencies shown (Fig.IV.7). During reversed beating, the durations of the bursts remain linearly dependent on period, but whilst the levator pool still bursted for roughly half the cycle, the depressor pool came to occupy a phase angle of about 0.65. Numerous other differences can be discerned in Fig.IV.1. between the spiking patterns during forward and reversed beating, but discussion of these will be left until a later stage.

Figures IV. 8 and 10 reconstruct visually the recorded activity in the different muscles by combining film strips with the same period lengths. The pattern of the flexor (feIm) bursts in strips B to E in Fig.IV.8. are all similar in time course and in the existence of marked antifacilitation. Strip A shows an interesting anomaly. Here, not only is there facilitation as opposed to antifacilitation, but the duration of the flexor burst is extremely abbreviated. If the first spikes in these bursts are aligned with the first spikes in the remaining strips (Fig.IV.8. column II), then the position that the quartus comes to occupy in the cycle does not

correspond with the picture obtained from the records of total motor output (Fig.IV.1, preceding chapter). There is extensive overlap between the bursts in the quartus and those in both the primus and promotor sub-group muscles. If the last spikes of the different strips are aligned (Fig.IV.8, column II) a much fairer picture seems to result. Notice however, that in strip A the characteristic movement artefact associated with rapid depression of the tip of the scaphognathite (strip C) now appears relatively late in the cycle. The occurrence of this artefact will later be shown to be linked with the onset of the burst in the sixtus.

In the experiment of strip A the bursts in the tertius and quartus were exceptionally strong and extended. Taken together, the above observations seem to indicate that this potentiated activity in the quartus sub-group resulted in a delay of the onset of activity in the flexor and sixtus (See also Fig.IV.10 for interactions during reversed beating). The initiation of the levator session, however, seems to have not been equally affected. Note in the figure that whereas in strip C the movement artefact occurs during the flexor burst, in strip A it occurs after. As mentioned above, this artefact is associated with the burst in the sixtus. The sixtus is the only depressor muscle in which the burst normally overlaps with the activity in the levator phase during forward beating. It would therefore not be expected to be overly affected by a relatively early start in levator activity.

Although Column I (Fig.IV.8) presents a better picture than Column II of the interrelations between the bursts in the ventilatory muscles, it is probably not a completely true one for each of the individual strips. In Strip A for example, it seems likely that some overlap between the start of the quartus session and the end of

the levator session did in fact occur. This has been observed on occasion in recordings of the total motor output. It was not the norm however. The discontinuity seen in the rise of the ejp's (excitatory junctional potentials) at the start of the quartus burst in strip A does suggest that overlap occurred in this case. A discontinuity of this sort is frequently seen when overlapping activity occurs between certain muscles. An example of a relatively weak interaction of this sort is seen in strip E (Fig.IV.8) where the promotor overlaps with the secundus. A stronger interaction is displayed in Fig.III.28 where the primus and flexor overlap.

#### IV.2.2. Interim Discussion

Combination of recordings from different muscles in different experiments can provide a good reconstruction of the gross pattern of the activity in the ventilatory muscles during the beat cycle. Not unexpectedly, discrepancies arise as a result of variations in pattern in the different experiments. The explanations given above, provide a reasonably satisfactory interpretation of the discrepancies observed in Fig.IV.8. They imply that the activity in the flexor might not always come to an intrinsically (?) determined end. It would seem that although the burst in the flexor is terminated before any activity appears in the levator group, the mechanisms associated with the timing of the onset of the levator phase might well be responsible for its termination. It would be unwise to take these speculations any further at this point, but certainly, they seem to be points worth considering in any attempt to disentangle the circuitry of the central pattern generator.

As to the occurrence of facilitation in the flexor burst in strips A and G (Fig.IV.8), it is doubted that this has any direct

relevance to the above discussions. In records of electromyograms from the flexor muscle all ranges of response from antifacilitation (Fig.IV.8, strips B to D), no marked facilitation or antifacilitation (Fig.IV.8, strips E,F,G), to marked facilitation (Fig.IV.1, strip A; Fig.IV.8, strip A). This is in keeping with observations on the homologous muscle (ASDM - Pasztor, 1968) in the crayfish Orconectes. The remaining muscles in Carcinus show as a rule varying levels of facilitation. A plot of the height of the last ejp in the burst of the sixtus against the length of the preceding interval for example (Fig.IV.9) shows that as the interval diminishes, the amplitude of the ejp rises. The number of ejp's per burst was between 4 to 6 approximately.

Fewer records were obtained for reversed than for forward beating. However, Fig.IV.10 attempts in so far as possible, a similar reconstruction to that in Fig.IV.8, for reversed beating. It was not possible in all cases to find cycles of exactly the same period. In these cases some photographic adjustment has been made by appropriate differences in enlargement. The fit therefore assumes proportionate increase in all aspects of the cycle with increase in period length. The relationship between the different strips may therefore be taken only as a rough picture of the relative positions of the bursts in the cycle.

From examination of Figs.IV.1. and 2, IV.8 and 10, it can be seen that the muscles active during both the levator and depressor phases of the cycle can be separated each into two sub-groups consisting of muscles which show a tendency to be active together. In the levator group, the promotor (mpIIIm) and the remotor (mrIIIm) tend to show synchronous activity during both forward and reversed beating, thus forming the first levator sub-group, the promotor

sub-group. The primus (mr1IIa) and secundus (mr2IIa) together form the second levator sub-group, the primus sub-group, showing near synchrony during both forward and reversed beating. Their activity overlaps to a variable extent with that of the promotor sub-group.

In the depressor group during forward beating, the tertius (mr3IIa), quartus (mr4IIa) and the quintus (mr5IIa) tend to be active together, and form the first depressor sub-group. The relationship between the activity in these muscles has been discussed previously (Fig.IV.2). There appears to be little difference in their inter-relationship during reversed beating (Fig.IV.10.) On the basis of sheer bulk, and since it is apparently the only ventilatory muscle directly responsible for depressing the scaphognathite about its longitudinal axis, the quartus would seem to be the dominant muscle in this sub-group. The sub-group will therefore be referred to as the quartus sub-group. The second depressor sub-group, the flexor sub-group, is formed by the sextus (mr6IIa) and the flexor (fsIIa), which during forward beating show only partially overlapping activity (Fig.IV.1.A and IV.8.F). The burst in the fsIIa starts before that in the sextus, and is sharply terminated shortly before the onset of activity in the primus sub-group, whilst the sextus continues firing for some time into the levator phase of the cycle. In the experiment described previously in Figs.IV.1 to 7 the mean phase angle of this overlap was about 0.05. During reversed beating, the bursts in the flexor and the sextus become strongly linked, starting almost simultaneously in both muscles. In reversed beating however, the burst in the sextus is now frequently terminated before that in the flexor (Fig.IV.1.B and Fig.IV.10). The relationship between the bursts in these muscles will be investigated in greater detail at a later stage.

The overall pattern of activity in the ventilatory muscles then, shows two main sessions in each cycle, corresponding to bursts of activity in the levator and depressor musculature. The activity in these two sessions overlap only minimally, more during reversed than in forward beating. The activity in the levation session consists of two extensively overlapping portions. In one, the muscles of the primus sub-group, which are apparently concerned with raising the scaphognathite about the PR axis (Fig.IV.3b) are activated together. In the second, the muscles of the promotor sub-group are roughly simultaneously recruited. These muscles are perhaps concerned with supinating the scaphognathite about the PS axes (Fig.IV.3). In forward beating the activity in the levating muscles precedes that in the supinating muscles, whilst in reversed beating, the sequence is reversed.

In the depressor session also, there are two main portions, strongly overlapping each other. During forward beating, the first portion consists of roughly synchronous activation of the tertius, quartus and quintus. In the second session, the flexor and sextus are sequentially activated, and continue bursting as described previously. During reversal, the sequence of the two depressor sessions, like the levator sessions becomes inverted. The inversion of the relative phase positions of the bursts in the different sub-groups provides a good example of the overall mirror image symmetry between reversed and forward beating. On the other hand, the changes described in the relationships between the flexor and sextus also provide examples of the asymmetry which is exhibited at a finer level. These observations extend and corroborate those made at the end of the preceding chapter. The finer aspects of the phasing of the muscular activity will be developed more fully in the next and later chapters.

Figure IV.4 shows the results of the analysis of variance for the effect of the treatment on the response variable. The results are presented in the following table:

Source	SS	df	MS	F	P
Treatment	10.0	1	10.0	1.0	0.32
Error	100.0	9	11.1		
Total	110.0	10			

Figure IV.4

Source	SS	df	MS	F	P
Treatment	10.0	1	10.0	1.0	0.32
Error	100.0	9	11.1		
Total	110.0	10			

Figure IV.4. The durations of the total depressor (start sextus to end quartus) and levator bursts during reversed beating, as functions of period length. In this experiment, the levator bursts (open circles) occupied roughly 50% of the total cycle. The depressor bursts (filled circles) occupied somewhat more. The broken line passes through points corresponding to burst durations of 50% of the total period.

Depressor session:

$b = 0.548 \pm 0.019$   
 $a = 209.72$   
 $S_{yx} = 107.55$   
 $r = 0.978$   
 $n = 41$

Levator session:

$b = 0.469 \pm 0.023$   
 $a = 6.97$   
 $S_{yx} = 133.54$   
 $r = 0.955$   
 $n = 41$



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**Figure IV.5**

Figure IV.5. The dependence on period of the relative latency of the starts of the main depressor and levator bursts. Reversed beating. The broken line passes through points corresponding to a latency of 50% of the total period.

$b = 0.565 \pm 0.030$   
 $a = 77.19$   
 $S_{yx} = 170.65$   
 $r = 0.950$   
 $N = 41$

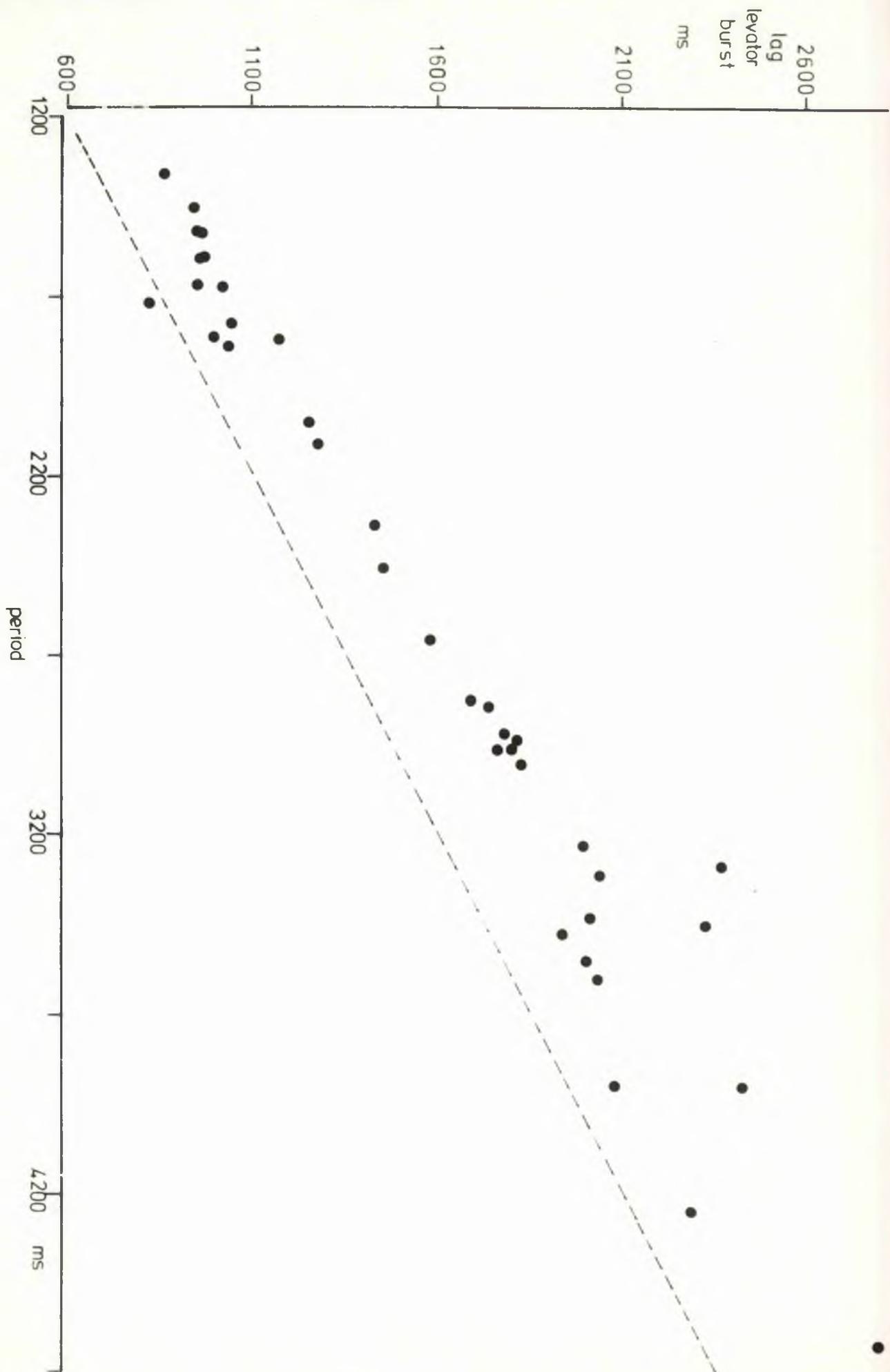


Figure 4.6. The dependence of the relative  
 intensity of the second harmonic on the  
 angle of the crystal. The solid line is  
 the theoretical curve, the dashed line is  
 the experimental curve.

100.0-0.000  
 0.000-0.000  
 0.000-0.000  
 0.000-0.000  
 0.000-0.000

**Figures IV.6 and IV.7**

The dependence of the relative intensity of the  
 second harmonic on the angle of the crystal  
 is shown in Figure 4.6. The solid line is  
 the theoretical curve, the dashed line is  
 the experimental curve. The angle of the  
 crystal is shown in degrees. The relative  
 intensity of the second harmonic is shown  
 in percent. The theoretical curve is  
 calculated from the theory of the  
 second harmonic generation. The  
 experimental curve is obtained from  
 the measurements. The agreement between  
 the theoretical and experimental curves  
 is very good. This indicates that the  
 theory of the second harmonic generation  
 is valid for the crystal used.

100.0-0.000  
 0.000-0.000  
 0.000-0.000  
 0.000-0.000  
 0.000-0.000

100.0-0.000  
 0.000-0.000  
 0.000-0.000  
 0.000-0.000  
 0.000-0.000

Figure IV.6. The dependence on period length of the relative latency between the starts of the depressor and levator bursts during forward beating. As for Fig.III.5. The slope here is not significantly different from that for reversed beating (Fig.III.5).

$$\begin{aligned}b &= 0.573 \pm 0.043 \\a &= -232.24 \\S_{yx} &= 63.67 \\r &= 0.968 \\n &= 14\end{aligned}$$

Figure IV.7. The durations of the main levator and depressor bursts as functions of period length. Forward beating. Open circles, levator bursts; filled circles, depressor bursts. Broken line as in Figs.III.4 to 6. The data in this and the previous figure were unfortunately, based on but a few irregular cycles. In spite of the marked overlap, there is a significant difference between the slopes of the regressions for the levator and depressor sessions ( $P < 0.01$ ) for the null hypothesis. There is a significant difference between the 6 values for the duration of the depressor session here and that in Fig.IV.4 for forward beating. The values for the levator sessions are not significantly different.

Depressor Session:

$$\begin{aligned}b &= 0.704 \pm 0.053 \\a &= -370.8 \\S_{yx} &= 78.90 \\r &= 0.967 \\n &= 14\end{aligned}$$

Levator Session:

$$\begin{aligned}b &= 0.407 \pm 0.044 \\a &= 236.69 \\S_{yx} &= 65.46 \\r &= 0.936 \\n &= 14.\end{aligned}$$

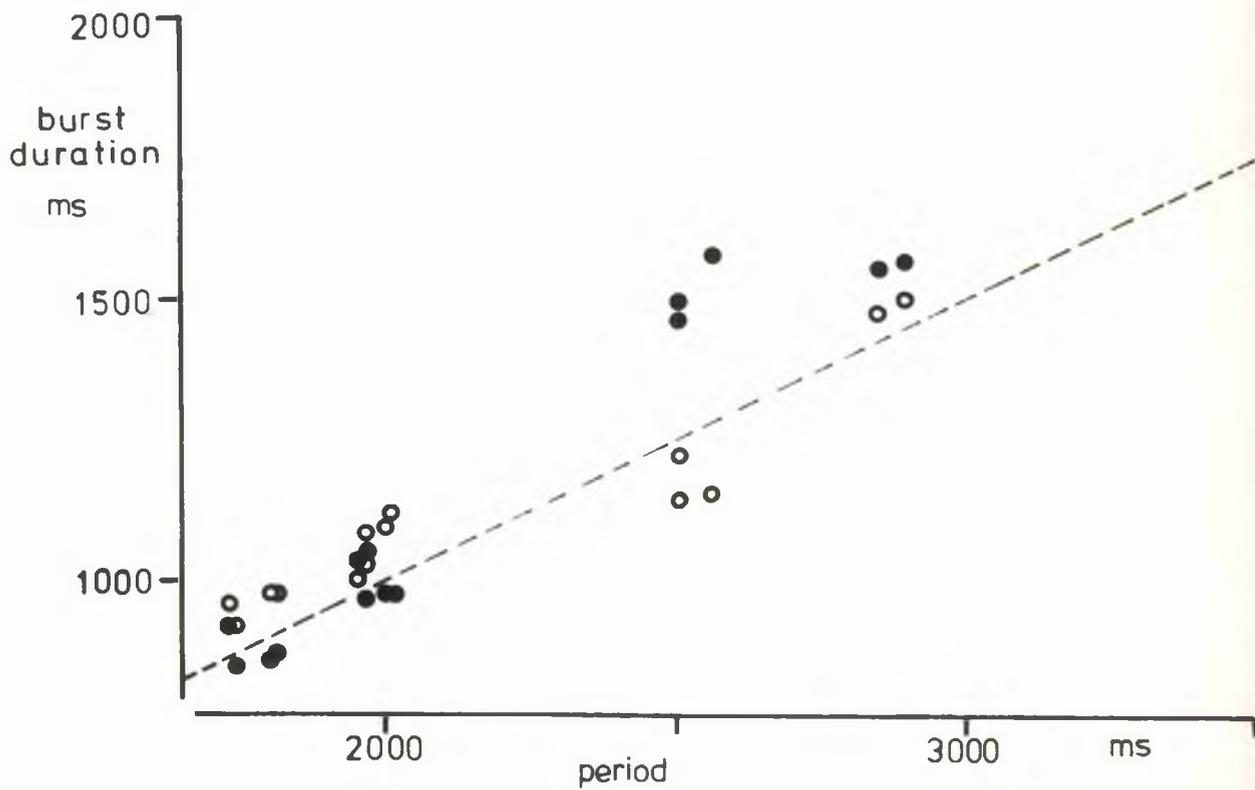
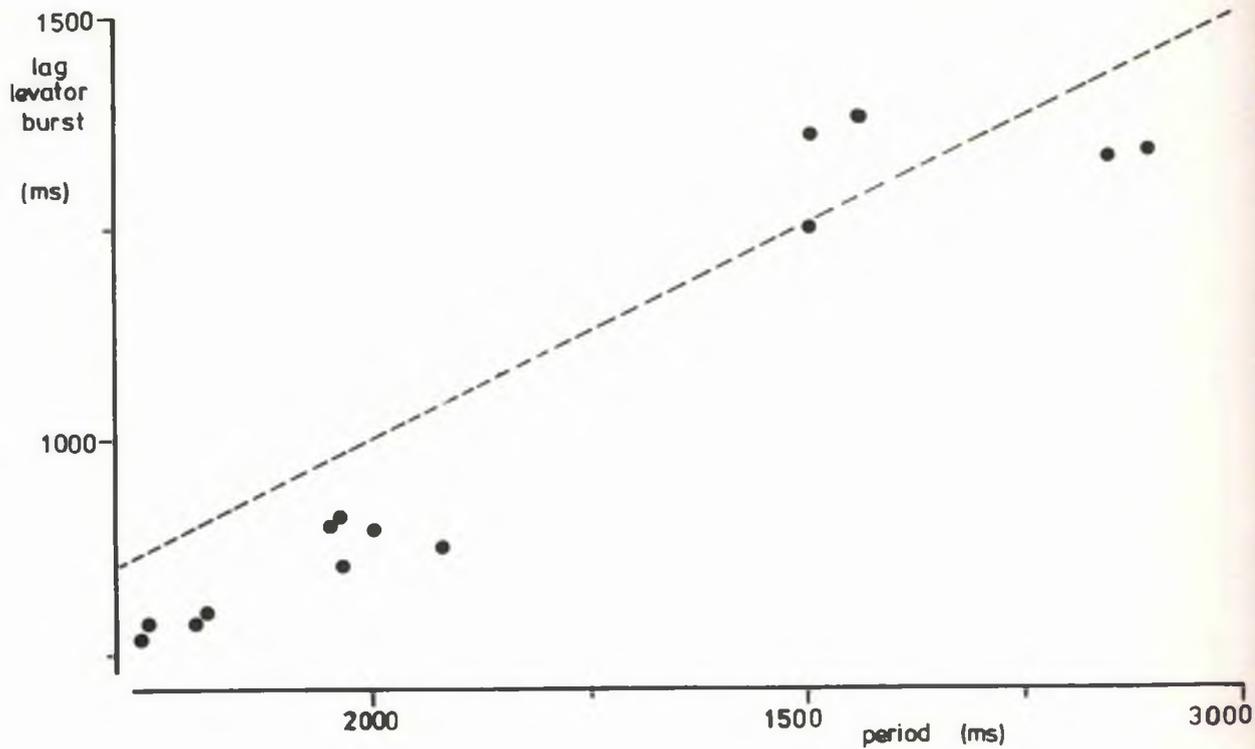
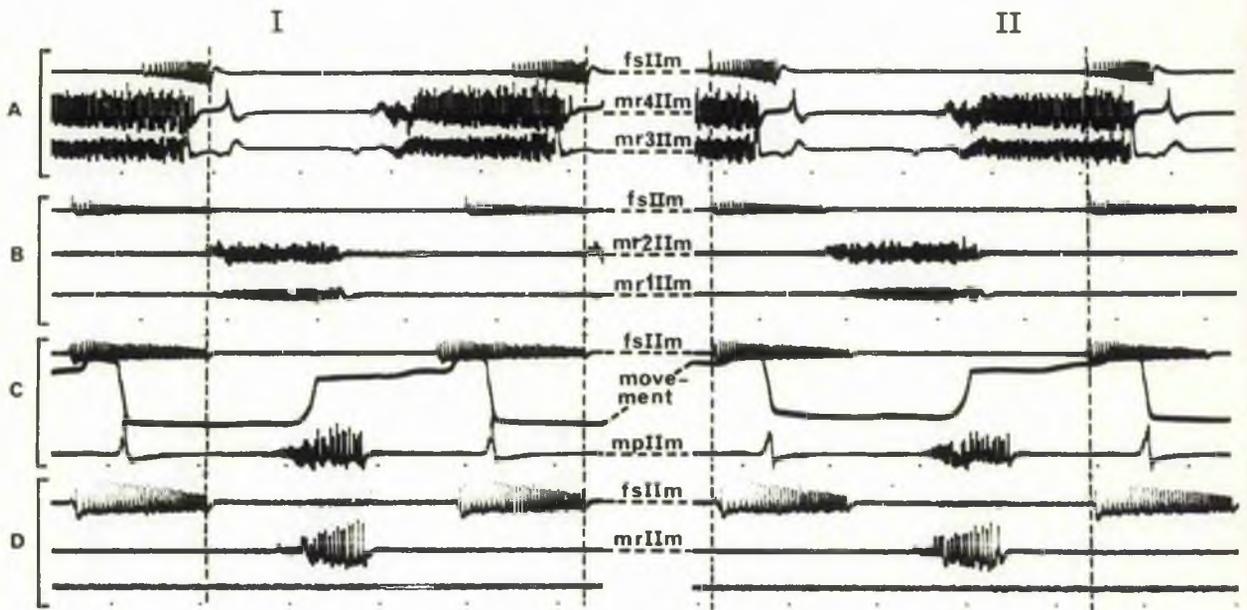




Figure IV.8. Reconstruction of the overall pattern of the bursts of activity in the ventilatory muscles during forward beating. A qualitative picture. In A to D and in F and G film strips from different experiments, but showing identical period lengths have been aligned in order to show the relative positions in the beat cycle of the bursts of activity in the different muscles. The flexor muscle (fsII<sub>m</sub>) is common to all the strips and has been used as a reference point. In column I the ends of the flexor bursts have been aligned, and in column II the starts. Column I gives the more realistic picture of the relative positions of the bursts, as compared say, to the total motor output pattern (Fig. IV.1). Note the differing types of activity in the flexor in strips A and C (facilitating e<sub>jp</sub>'s), B, C and D (marked anti-facilitation), E and F (no marked facilitation or anti-facilitation). Strip E shows in a single experiment the bursts in the flexor, promotor (mpII<sub>m</sub>) and secundus (mr2II<sub>m</sub>). This might be compared with the results of combining the filmstrips B and C, and with the total motor output pattern (Fig. IV.1, F). Note the differing types of activity in the promotor in strip C (multiunit) and in strip E (single unit). The movement monitor in strip C records the movement of the internal tip. Upward movement of the trace corresponds to upward movement of the internal tip (supination). For further explanations see text.



time mark = 500 ms

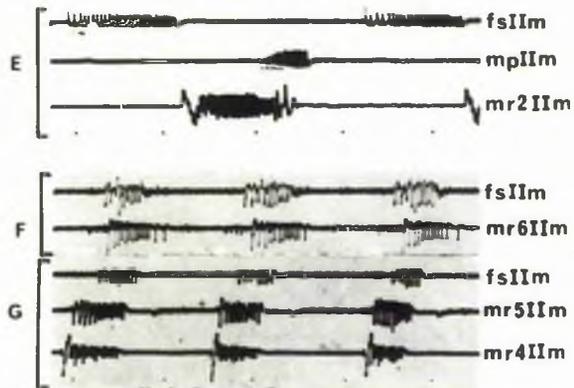


Figure IV.9 shows the results of the analysis of variance for the effect of the concentration of the solution on the rate of reaction. The results show that the rate of reaction increases with the concentration of the solution. The rate of reaction is highest at the highest concentration of the solution and lowest at the lowest concentration of the solution. The rate of reaction is also affected by the temperature of the solution. The rate of reaction increases with the temperature of the solution. The rate of reaction is highest at the highest temperature of the solution and lowest at the lowest temperature of the solution.

Figure IV.9

Figure IV.9. Height (in arbitrary units) of the last ejp in the sixtus as a function of the length of the preceding interval in milli-seconds. As the interval diminishes the ejp height increases linearly. The facilitation seen here is very pronounced. Most of the muscles displayed some degree of facilitation, perhaps to a lesser extent than seen here.

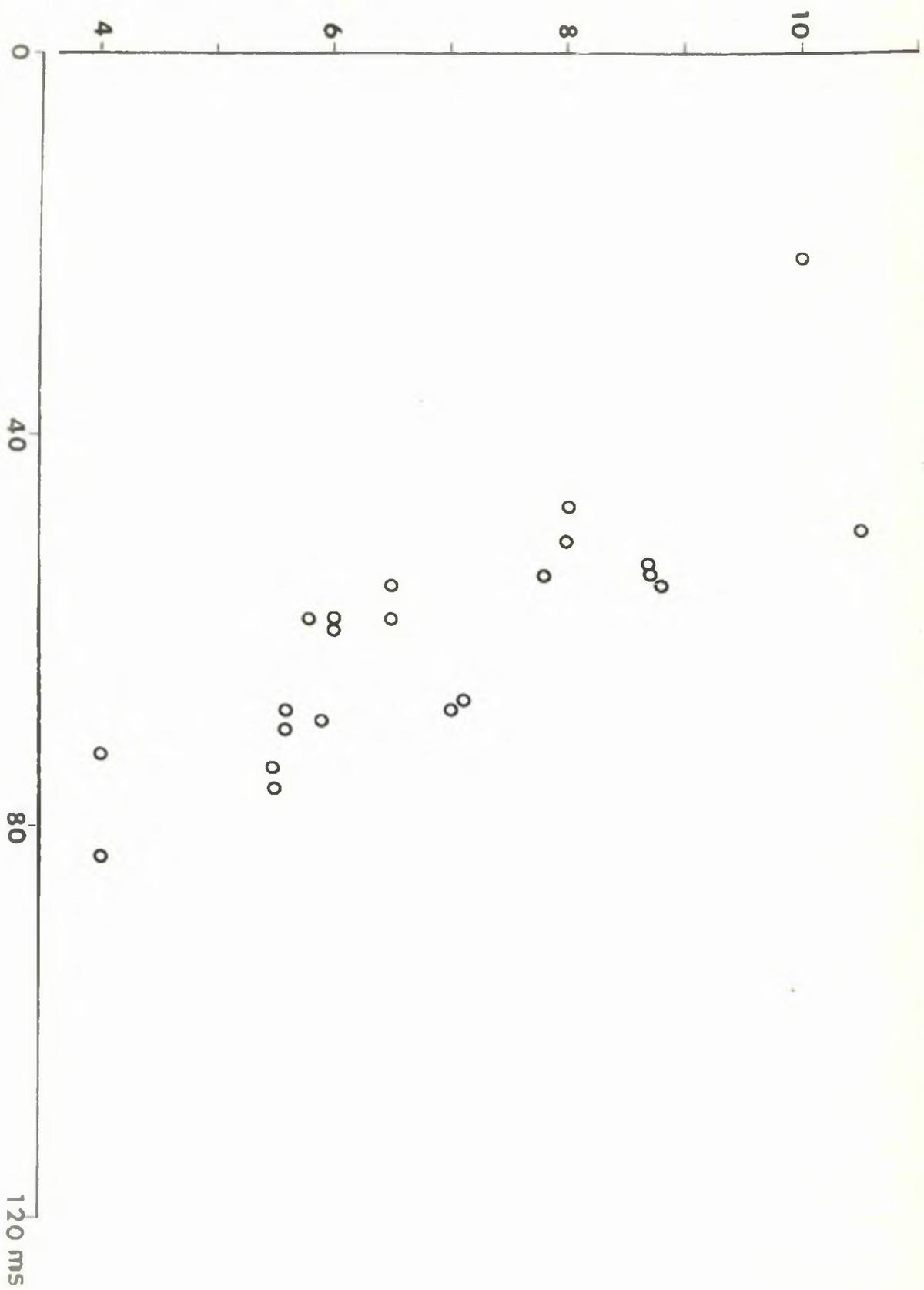


Figure IV.10. A schematic representation for a system of particles...

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...the particles are distributed in space... the particles are distributed in space...

Figure IV.10

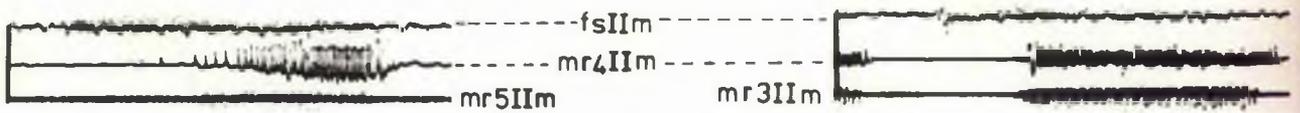
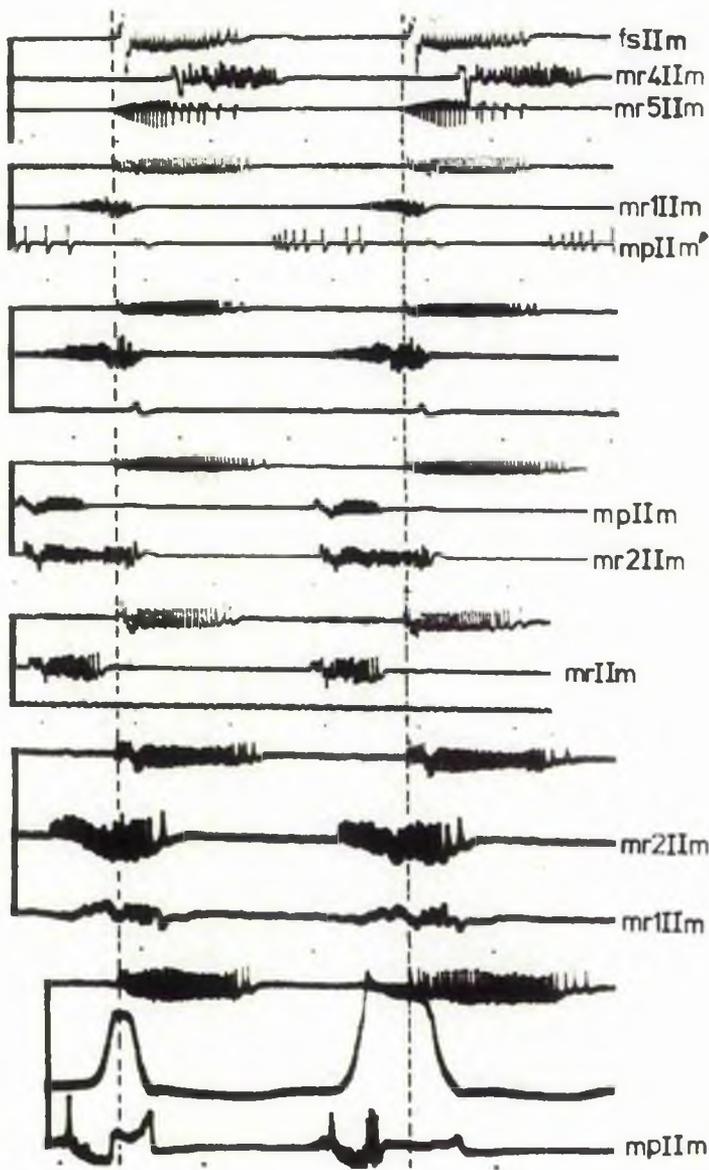
...the particles are distributed in space... the particles are distributed in space...

...the particles are distributed in space... the particles are distributed in space...

...the particles are distributed in space... the particles are distributed in space...

Figure IV.10. A similar reconstruction for reversed beating, to that in Fig.III.8 for forward beating.

- Strip A : Note the change in the structure of the bursts and the relative latencies of the burst starts in the flexor and sixtus. Also note the sharp decline in spiking frequency in the sixtus, and the more gradual decline in the flexor when the quartus starts firing. The quartus now starts after rather than before (Fig.IV.8) the flexor.
- Strips : When the accessory promotor is firing strongly (B),  
B, C the burst in the primus appears to start later in the cycle and to be slightly less intense than when the accessory is firing minimally (C). B and C are strips taken at different times in the same experiment.
- Strips : The primus and secundus and the promotor and remotor  
B - C still appear to go together during reversed beating. The bursts in the promotor sub-group now begin before those in the primus sub-group, but the relative latencies are much smaller than in forward beating (Fig.IV.8). The bursts in the promotor sub-group are terminated some time before those in the primus sub-group, but the overlap is extensive.
- Strip G : A very short reversed cycle from a transient session of reversed beating in the same experiment illustrated in Strip C (Fig.IV.8). Note the marked alteration in the burst in the promotor, as in the primus in Strip F. Some activity is apparent at the appropriate points in the cycle, but the efp's are for the most part extremely small. This type of activity is frequently associated with the transition from forward to reversed beating. It may also occur with changes from reversed to forward beating (Fig.IV.14).
- Strips : These show that the quintus (H) and tertius (I) still  
H, I tend to go with the quartus during reversed beating.



IV. 3. An analysis of the frequency dependence of the phasing  
of the onset of activity in the ventilatory muscles

IV.3.1 Results

It has already been demonstrated that the total durations of the levator and of the depressor phases of the beat cycle, and the latencies between their initiation are linear functions of period. It has also been shown that the spike trains within these respective bursts are responsible for the sequential activation of the four 'levator' and five 'depressor' muscles mainly responsible for movement of the scaphognathite. The relative timing of the initiation of activity in the different muscles during the cycle will now be described. Firstly, the relationship between the muscles recruited within the two main (levator and depressor) sessions will be dealt with. For the most part, the latencies ( $L$ ) of the starts of the bursts will be taken with respect to the start of the burst in the flexor muscle. This parameter will be designated  $L_f$ . The dependence of the latencies ( $L$ ) and the phase angle of the latencies ( $\phi_L$ ) on the mean period length for the two muscles (reference and test) will be examined for all the muscles. Wyman (1965) suggests that this is one approach towards distinguishing between primarily latency locked and primarily phase locked motor 'scores'.

A quick scan through Tables I to IV shows that the timing between the starts of the bursts in the different muscles tends to be qualitatively similar to that between the overall levator and depressor sessions. By far the predominant relationship is one in which the relative latencies tend to be longer at longer period lengths and the phase angle of the latencies, to remain roughly the same over the range of frequencies. This is particularly marked for latencies

between muscles from different, antagonist sub-groups (Tables I and II). Between muscles from the same sub-groups (Table III) the relative latencies of the burst starts tend to be largely independent of period (Figs.IV.19,21). The regression coefficients for relative latency against period tend to be very small, as a result of the small phase angle separating the starts of the bursts in the same sub-groups.

The tendency towards reduced dependence on period of the relative starting latencies in muscles of the same sub-group reaches its limit in the relationships between the tertius and quartus (Figs. III.8,10) which appear always to show near simultaneity, and between the flexor and sextus during reversed beating (Fig.IV.10). From qualitative observations, the promotor and remotor also (Fig.IV.8,10), appear to show a relationship similar to that between the tertius and quartus.

The value of  $L_F$  was routinely measured from the start of the flexor burst to the start of the next burst in the test muscle. When this is done for the quartus sub-group muscles, there appears to be a marked change during reversal in both the magnitude of  $L_F$  and the degree of its dependence on period (Table IV; Fig.IV.18). The change-over appeared to be accompanied by rotation of the regression line about a point some way above the origin. These changes are largely artefactual. They appear because in forward beating, the next quartus burst following the flexor burst lies in the consecutive depressor session, separated from the flexor burst by a relatively large phase angle. This results in a steeper slope in the latency/period plot. In reversed beating, the next quartus burst after the flexor burst occurs in the same depressor session, at a short relative latency. This results on a smaller slope to the latency/period plot, which,

with the same standard error on the regression coefficient, gives rise to the apparent fall in correlation. If the analyses for forward and reversed beating are done using the relative latencies within a single depressor session only, then there is little difference other than the shift of about 0.5 in phase angle (Figs.IV.15,16; Table III). The reversed beats in Figs. 15 and 18 represent exactly the same data plotted with a slightly contracted X axis, and an extended Y axis in Fig.IV.15. The points are widely scattered, and it is difficult to say exactly what sort of a trend they represent, even with a statistical test.

For both the remotor (Fig.IV.12) and the primus with respect to the flexor (Fig.III.13) it appears that during reversal the slope of the regression may not change significantly. In other cases, (e.g. secundus and promotor, Table I), there is obviously a significant change in regression during change from forward to reversed beating. In view of the variability of the data, it would be difficult to say whether this might be a constant feature of the respective muscle pairs. On the whole it would appear that the finer details of the timing of the onset of the bursts in the different muscles can vary quite significantly from animal to animal.

In Tables I to III it appears that there might be a slight reduction in the standard error of the regression for relative latencies on period on going from inter-antagonist to intra sub-group relationships. This is not however, very obvious. In Table V the residual mean squares (for N-2 degrees of freedom) for plots of phase angle of the relative latencies ( $\phi_L$ ) against period are compared. Four experiments are shown, which allow direct comparisons of inter-group variances (between muscles from antagonist groups), designated type A, against inter sub-group (agonist) (type B) and

1.	Promotor	rev	930±58	-74.3	67.2	0.72±0.07	0.952	S	66.1±1.4	---	---	N	140±77	14
2.	"	"	962±13	280.6	158.4	0.30±0.05	0.634	S	42.2±0.5	-0.05±0.02	-0.272	N	228±28	53

Mean: 956± 0.39 47.2 2098 67 (total)

1.	Remotor	rd	348±16	15.2	42.9	0.51±0.04	0.899	S	52.9±1.0	0.00±0.06	0.004	N	658±28	40
2.	"	"	1091±21	-38.8	77.9	0.55±0.03	0.505	S	52.9±0.4	0.01±0.01	0.061	N	2059±34	77
3.	"	"	1066±32	-181.5	87.9	0.61±0.03	0.940	S	51.8±0.6	0.07±0.01	0.553	S	2035±49	63

Mean: 917 0.56 52.5 0.03 1739 180 (total)

1.	"	"	737±22	82.2	61.2	0.59±0.03	0.932	S	66.9±0.7	-0.08±0.03	-0.395	S	1113±34	60
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**TABLE I**  
The dependence on period of the relative latencies between the starts of the bursts in muscles  
from antagonist groups.

Muscle	Node	Mean LFSB	a	S y.x	b <sub>L</sub> ±SE	r	Sig	φ <sub>L</sub> ±SE	b(φ <sub>L</sub> )±SE	r	Sig	PERIOD mean±SE	N
1. Pylorus	fd	694±16	537.8	70.1	0.81±0.07	0.835	S	45.1±0.7	0.21±0.04	0.527	S	1530±16	64
2. "	"	879±22	-263.5	95.5	0.51±0.09	0.707	S	38.9±0.7	0.05±0.04	0.315	N	2250±30	38
Mean:	fd	763			0.69			42.8	0.16			1798	120 (total)
1. Pylorus	rev	936±27	78.2	79.2	0.84±0.03	-----	S	77.0±0.6	0.03±0.02	0.176	N	1235±30	87
1. Secundus	fd	678±17	-124.3	85.6	0.39±0.03	0.811	S	32.7±0.5	0.03±-----	-----	N	2059±34	77
2. "	"	556±15	251.5	101.4	0.20±0.03	0.615	S	37.2±0.7	-0.03±0.02	-0.220	N	1503±47	69
3. "	"	763±23	-----	-----	0.72±0.09	0.747	S	34.2±0.9	0.19±0.04	0.531	S	2214±24	50
Mean:		657			0.41			34.6	0.05			1903	196 (total)
1. Secundus	rev	990±61	-78.3	72.8	0.77±0.07	0.952	S	70.6±1.4	0.05±0.05	0.248	N	1396±76	14
1. Promotor	fd	935±24	455.0	152.4	0.32±0.05	0.636	S	62.6±0.9	-0.05±0.02	-0.235	N	1504±47	68
2. "	"	1112±19	-668.6	97.9	0.86±0.10	0.738	S	54.3±0.7	-0.06±0.03	-0.219	N	2060±15	73
3. "	"	1267±37	-137.4	78.9	0.68±0.07	0.910	S	61.5±0.8	0.05±0.03	0.325	N	2052±50	25
Mean:		1063			0.61			58.8	-0.04			1831	166 (total)

**TABLE II**

The dependence on period of the relative latencies between the starts of the bursts in muscles from agonist sub-groups.

Test Muscle	Reference Muscle	Mode	Mean $I_{\pm SE}$	a	Sy.x	$b_{I \pm SE}$	r	sig	$\beta_{I \pm SE}$	$b(\beta_{I \pm SE})_{\pm SE}$	r	sig	Period mean $\pm SE$	N
1. Sixtus	Quartus	fd	423 $\pm$ 10	-27.5	63.1	0.35 $\pm$ 0.03	0.794	S	32.6 $\pm$ 4.4	0.00 $\pm$ 0.00	0.000	N	1295 $\pm$ 24	100
1. "	"	rev	459 $\pm$ 10	-136.7	92.2	0.15 $\pm$ 0.04	0.336	S	21.5 $\pm$ 0.4	0.03 $\pm$ 0.02	0.155	N	2132 $\pm$ 22	94
1. "	Quintus	fd	368 $\pm$ 5	-20.04	29.74	0.21 $\pm$ 0.03	0.694	S	22.5 $\pm$ 1.9	0.00 $\pm$ 0.02	0.026	N	1635 $\pm$ 16	70
1. Promotor Secundus	Secundus	fd	380 $\pm$ 10	-205.2	65.5	0.12 $\pm$ 0.02	0.574	S	25.5 $\pm$ 0.5	0.02 $\pm$ 0.01	0.204	N	1501 $\pm$ 47	69
1. Remotor Secundus	Secundus	fd	412 $\pm$ 7	91.8	39.3	0.16 $\pm$ 0.02	0.774	S	20.1 $\pm$ 0.3	0.03 $\pm$ 0.01	0.378	S	2059 $\pm$ 35	77
1. Flexor Quartus	Quartus	fd	360 $\pm$ 20	11.04	88.04	0.14 $\pm$ 0.02	0.755	S	13.8 $\pm$ 0.5	-0.00 $\pm$ 0.01	-0.004	N	2595 $\pm$ 114	43
2. "	"	"	759 $\pm$ 32	-206.6	116.3	0.50 $\pm$ 0.04	0.862	S	38.4 $\pm$ 0.9	0.06 $\pm$ 0.02	0.400	S	1946 $\pm$ 55	51
3. "	"	"	214 $\pm$ 12	-107.6	64.7	0.36 $\pm$ 0.04	0.745	S	23.6 $\pm$ 0.8	0.14 $\pm$ 0.04	0.399	S	885 $\pm$ 24	67
Mean:			426			0.35			25.7	0.08			1310	161 (total)
1. Flexor Tertius	Tertius	fd	704 $\pm$ 5	355.2	27.7	0.16 $\pm$ 0.05	0.494	S	32.3 $\pm$ 0.2	0.83 $\pm$ 0.02	-0.539	S	2176 $\pm$ 16	36
1. Quartus Flexor	Flexor	rev	312 $\pm$ 7	404.9	46.7	-0.04 $\pm$ 0.03	0.208	N	18.8 $\pm$ 0.7	-0.17 $\pm$ 0.02	-0.766	S	1776 $\pm$ 36	44
2. "	"	"	393 $\pm$ 9	131.8	89.3	0.12 $\pm$ 0.04	0.283	S	18.8 $\pm$ 0.4	-0.03 $\pm$ 0.02	-0.154	N	2137 $\pm$ 21	100
3. "	"	"	627 $\pm$ 40	98.0	162.3	0.23 $\pm$ 0.02	0.828	S	27.7 $\pm$ 1.0	-0.02 $\pm$ 0.01	-0.257	N	2331 $\pm$ 145	52
Mean:			437			0.11			21.2	+0.06			2107	196 (total)

TABLE III

The dependence on period of the relative latencies between the starts of the bursts in muscles from the same sub-group.

Test Muscle	Reference Muscle	Mode	Mean $I \pm SE$	$\mu$	S.y.x	$b_I \pm SE$	r	sig	$\phi_I \pm SE$	$b(\phi_I) \pm SE$	r	sig	Period mean $\pm SE$	N
1. Sixtus	Flexor	fd	35.6 $\pm$ 3.7	45.8	31.0	-0.01 $\pm$ 0.02	-0.076	N	3.4 $\pm$ 0.2	-0.02 $\pm$ 0.01	-0.215	N	881 $\pm$ 24	68
2. "	"	"	20.8 $\pm$ 1.4	9.41	7.9	0.01 $\pm$ 0.01	0.307	N	2.6 $\pm$ 1.7	-0.01 $\pm$ 0.01	-0.220	N	790 $\pm$ 29	37
3. "	"	"	222.6 $\pm$ 9.9	33.1	95.9	0.09 $\pm$ 0.04	0.251	N	10.7 $\pm$ 0.5	-0.01 $\pm$ 0.01	-0.29	N	2068 $\pm$ 27	100
4. "	"	"	373.3 $\pm$ 14.1	-93.3	95.2	0.15 $\pm$ 0.04	0.437	S	11.6 $\pm$ 0.4	0.01 $\pm$ 0.01	0.122	N	3204 $\pm$ 42	55
Mean:			177			0.065			7.8	-0.01			1816	260 (total)
1. "	"	rev	-59.3 $\pm$ 1.9	-47.4	18.5	-0.01 $\pm$ 0.01	-0.064	N	-0.6 $\pm$ 0.9	0.07 $\pm$ 0.04	0.166	N	2141 $\pm$ 21	100
2. "	"	"	25.1 $\pm$ 3.8	1.95	15.2	0.01 $\pm$ 0.01	0.376	N	1.5 $\pm$ 0.2	0.00 $\pm$ 0.01	0.012	N	1661 $\pm$ 101	18
Mean:			-46.4 $\pm$			-0.00			-0.3	0.06			2068	118 (total)
1. Quintus	Quartus	fd	109 $\pm$ 4	-18.6	24.4	0.09 $\pm$ 0.02	0.615	S	7.8 $\pm$ 0.2	0.01 $\pm$ 0.01	0.160	N	1387 $\pm$ 27	58
1. Primus	Secundus	fd	69.3 $\pm$ 5.3	1.5	32.5	0.03 $\pm$ 0.03	0.191	N	3.1 $\pm$ 0.2	-0.00 $\pm$ 0.01	0.007	N	2232 $\pm$ 33	38

intra sub-group (type C) variances, in the same sets of cycles. It is quite clear here, that there is a significant reduction in variance for the type B and type C relationships, compared to the type A relationship. In addition, there is also a significant reduction in the variance for the type C in comparison to the type B relationship. All the analyses refer to forward beating. It appears then, that the variability of the relationship might be in some way related to the magnitude of the relative latencies, being smallest for the small intra-sub-group latencies and largest for the relatively large inter-group (type A) latencies. The figure for the inter (agonist) sub-group relationship for the quartus and flexor seems inordinately large by comparison with the corresponding values. It seems therefore that without further corroboration of these figures, only tentative conclusions might be drawn from their indication.

#### IV.3.2. Interim Discussion

To summarize the findings reported in this chapter, it must first be said that obvious exceptions can be found to practically every generalization so far made. A large percentage of unexplained variability characterizes the results of most of the analyses. This would seem to imply that the models and analyses applied are perhaps inadequate to fully explain the characteristics of the system. Similar variability has been encountered by Davis (1968) in his analyses of the interrelationships between the muscles controlling swimmeret beat in the lobster Homarus. Davis' (1968) suggested explanation of this variability might well apply in the present instance: Continuous shifting of the work load between synergistic motor- and inter- neuronal units having slightly differing connectivities could indeed explain some of the anomalous regressions

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seen for example in Figs.IV.15 and 16 for the reversed beats and in the forward cycles in Figs.IV.17 and 18.

Some reasonable generalizations can none-the-less be made, if only for the sake of synthesizing and unifying the data. Except in the case of muscles from the same sub-groups, the relative latency between the starts of the bursts in the different muscles tends to be directly proportional to period length during both forward and reversed beating. In cases where a reasonable measurement is possible, the relative latencies between muscles of the same sub-group show no significant correlation with period length. It is believed that the relationship might be of the same sort as that described between the other muscles. It may however, be obscured because of a relatively high level of 'noise' in comparison to the very small latencies. A significant correlation between  $L_f$  and period for the sixtus in an experiment with very long period lengths and latencies tends to bear this out.

In plots of  $\phi_L$  against period, the scatter of the values about the predicted regression line is greatest for inter-group relationships, less for inter sub-group (agonist) and least for intra sub-group relationships. For shorter latencies between burst starts therefore, it appears that the variance is less than for longer latencies. On the basis of this observation, one might tend to rule out, in the present instance, the mechanism suggested by Wyman (1965) for the generation of timing cues which vary with period length, (specifically, constant phase cues). Fig.IV.22A shows that in such a system, at least assuming the simplest and most direct relationships, the error should be independent of L. For a sinusoidal cueing input (Davis, 1969), a more complex relationship would be expected, in which the variation between units cued close together near the ends of the range might be expected to be larger than that between muscles cued

at the end and middle respectively, of the cycle. The variation between units cued at the extremes of the range would probably be greatest. The present data cannot be said either to support or rule out this hypothesis. The indications, however, do suggest a hierarchical system of cueing. One generalized scheme which might explain the present relationships is outlined in Fig.IV.22B, but many alternative models might easily be envisaged.

In most cases, where there is a significant regression of  $\phi_L$  on period, the regression coefficient is quite small (less than 0.1). Phase histograms with a relatively high degree of resolution demonstrate that the starts of the bursts, over the normal range of frequencies, are to a great extent restricted to a given phase position in the cycle, with some scatter about a strongly peaked modal position. The system might therefore be regarded loosely as being effectively phase locked. It must be stressed that this is not strictly speaking, true. In most cases, the relationship is clearly more complicated. The marked scatter in the plots, and the fact that significant correlations may be obtained between  $\phi_L$  and period length indicate that other factors come into play. In fact, for the present purposes, the relationship might best be described by the equation,

$$L = a + b (\text{period}) + \text{error}$$

where in some cases, and at some times, a might be zero (leading to a truly phase locked relationship) whilst at other times and in other cases, it might differ significantly from zero. The error term might be dependent in some way on the magnitude of L. This would clearly add further to the complications of finding a suitable mathematical model for the system.

In the changeover from forward to reversed beating, the changes in the relative latencies of the starts of the bursts in the different

muscles may be effected in two ways. There may be a change in b, a remaining constant at 0, i.e. a rotation. Alternatively, b might remain constant whilst a changes, i.e. a displacement. In the first case, the inter-relations between the muscle pair in question will remain truly phase locked during both forward and reversed beating. In the second, the relative latencies may be phase locked in one of the modes or in neither, but not in both. In Fig.IV.12 for the relative latency between the remotor and flexor, there is a truly phase locked relationship in neither forward nor reversed mode. In Fig.IV.13 for the primus and flexor the relationship is truly phase locked during reversed beating, but is not during forward.

It might be said finally, that it seems clear that factors are operating which tend to maintain the relative phase angles in the cycle of the starts of the bursts. The relationships are, however, complex and subject to a great deal of variability. This variability is obviously restricted within reasonable limits, since the overall pattern of the onset of activity in the different muscles is not subject to undue variability. This is clearly demonstrated by the reasonably good reconstruction obtainable by combining film strips from different experiments (Figs.IV.8,10).

The data presented in this chapter give no information concerning the ongoing activity in the muscle bursts. The nature of this activity is obviously important both in terms of the organization of the central pattern generator, and in terms of the mechanical activity of the scaphognathite. An attempt will be made to characterize this aspect of the muscles' activity in the following chapters.

TABLE IV

The dependence on period of the latency ( $L_L$ ) between the start of the burst in the flexor and the start in the muscles of the quartus sub-group, in the cycle of the flexor, during forward beating. This may be compared with the data of Table II.

Muscle	Mode	Mean $L_{LS}$	a	S <sub>y.x</sub>	$b_{L \pm SE}$	r	sig	$\beta_{L \pm SE}$	$b(\beta_L) \pm SE$	r	sig	Period mean $\pm SE$	N
1. Quartus	fd	620 $\pm$ 6	9.67	27.8	0.77 $\pm$ 0.07	0.810	S	78.0 $\pm$ 0.4	0.01 $\pm$ 0.08	-0.009	N	795 $\pm$ 6	71
2. "	"	2141 $\pm$ 103	-103.0	120.2	0.88 $\pm$ 0.03	0.985	S	83.9 $\pm$ 0.7	0.02 $\pm$ 0.01	0.317	N	2533 $\pm$ 121	41
3. "	"	1179 $\pm$ 32	237.8	120.0	0.49 $\pm$ 0.04	0.853	S	61.3 $\pm$ 1.0	-0.07 $\pm$ 0.02	-0.411	S	1941 $\pm$ 56	51
4. "	"	1588 $\pm$ 38	288.2	230.5	0.70 $\pm$ 0.23	0.426	S	84.8 $\pm$ 0.7	0.00 $\pm$ 0.01	0.004	N	1846 $\pm$ 23	45
Mean:		1275			0.71			75.0	-0.02			1645	208 (total)
1. Tertius	fd	1322 $\pm$ 17	270.0	97.9	0.66 $\pm$ 0.05	0.803	S	83.1 $\pm$ 0.6	-0.08 $\pm$ 0.03	-0.267	N	1549 $\pm$ 169	91
2. "	"	1470 $\pm$ 15	-172.5	55.8	0.76 $\pm$ 0.11	0.776	S	67.5 $\pm$ 0.4	0.04 $\pm$ 0.05	0.142	N	2176 $\pm$ 15	36
3. "	"	3243 $\pm$ 38	-2010.3	119.0	1.17 $\pm$ 0.13	0.851	S	71.9 $\pm$ 0.5	0.10 $\pm$ 0.03	0.534	S	4503 $\pm$ 27	35
Mean:		1770			0.79			77.2				2352	162 (total)
1. Quintus	fd	555 $\pm$ 16	19.98	89.5	0.82 $\pm$ 0.08	0.758	S	85.5 $\pm$ 1.2	-0.07 $\pm$ 0.09	-0.082	N	649 $\pm$ 15	75
2. "	"	1442 $\pm$ 16	72.8	103.6	0.86 $\pm$ 0.09	0.741	S	90.5 $\pm$ 0.7	-0.05 $\pm$ 0.06	-0.087	N	1570 $\pm$ 14	87
Mean:		1031			0.84			88.2	-0.05			1144	162 (total)

TABLE V

Expt. Muscles (Antagonist) (Type A)	Intergroup Deviation	Muscles	Inter sub- Group Deviation (Agonist) (Type B)	Muscles	Intra sub- Group Deviation (Agonist) (Type C)	F tests		
						A/B	A/C	B/C
1.	f/r mean = 529 N = 77	2/r mean = 201 N = 77	428.2 N = 77	—	—	3.253	—	—
	f/2 Mean = 327 N = 77	1647.145 N = 77	—	—	—	3.847 F=1.72	—	—
2.	f/2 mean = 432 N = 51	2665.133 N = 51	—	2/1 mean = 31.1 N = 38	200.046 N = 38	—	13.323 F=2.13	8.920 F=2.23
	f/1 mean = 389 N = 38	1784.451 N = 38	—	—	—	—	—	—
3.	f/p mean = 626 N = 68	5421.520 N = 68	2/p mean = 255 N = 69	1489.759 N = 69	—	—	3.639 F=1.71	—
	f/2 mean = 372 N = 69	2795.539 N = 69	—	—	—	1.877 F=1.71	—	—
4.	f/4 mean = 236 N = 67	3999.947 N = 67	—	—	f/6 mean = 34.1 N = 68	—	—	16.614 F=1.79

Deviation refers to the mean squared deviations from the estimated regressions  $f$  = flexor;  $r$  = remotor;  $p$  = promotor; 1 = primus; 2 = secundus; 4 = quartus; and 6 = sextus. F values refer to 1% points. mean refers to the mean phase angle  $\times 1000$ . This was the form in which the data were expressed and the residuals determined.

TABLE VI

The interrelationships between various parameters relating to the duration of the flexor burst and its regression on period length.

(A correlation matrix)

	<u><math>L_D</math></u>	<u><math>b_{LD}</math></u>	<u><math>\phi_D</math></u>	<u><math>b\phi_D</math></u>	<u>Per.</u>	
$L_D$	1.000	<u>0.520</u>	0.345	<u>0.472</u>	<u>0.786</u>	N = 17
$b_{LD}$	<u>0.520</u>	1.000	<u>0.430</u>	<u>0.801</u>	0.291	Significant values
$\phi_D$	0.345	<u>0.430</u>	1.000	0.332	0.288	have been
$b\phi_D$	<u>0.472</u>	<u>0.801</u>	0.332	1.000	0.299	underlined.
Per.	<u>0.786</u>	0.291	0.288	0.299	1.000	

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Figure IV.11

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Figure IV.11. Starting times of the remotor (arIIa) in the cycle of the flexor (L) as a function of the mean period length of the flexor and remotor cycles. Filled circles, forward beating; open circles, reversed. There is strong correlation and a marked regression on period. This reflects the relationship between the overall levator and depressor bursts (Figs. IV.4 to 7). There is not a great change between forward and reversed beating. Note that although the remotor leads the levator burst in this mode, the values for  $L_r$  are greater during reversed beating. This is due to the increased duration of the flexor burst during reversed beating. The slopes and correlation coefficients do not differ significantly.

Forward beating:

$a = -38.8$   
 $b = 0.55 \pm 0.03$   
 $r = 0.905$   
 $N = 77$   
 $P < 0.01$   
 $S_{yx} = 77.9$

Reversed beating:

$a = 82.2$   
 $b = 0.59 \pm 0.03$   
 $r = 0.932$   
 $N = 60$   
 $P < 0.01$   
 $S_{yx} = 61.2$

( $b$  = regression coefficient  $\pm$  standard error

$r$  = correlation coefficient

$N$  = number of pairs of observations

$P$  = Probability that the computed correlation coefficient  
is not significantly different from zero.

$a$  = the intercept on the y axis.

$S_{yx}$  = standard error of the estimate.)

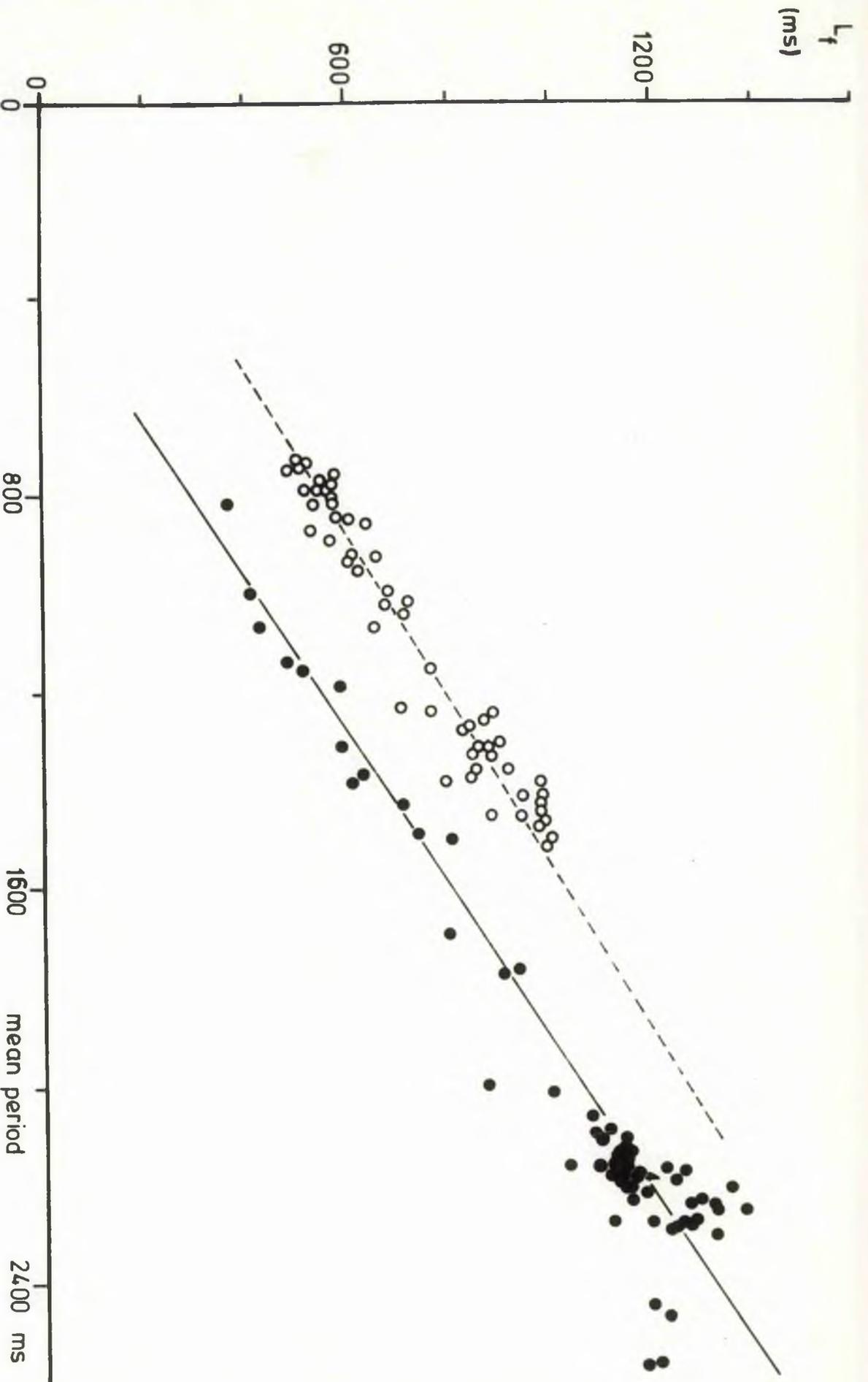




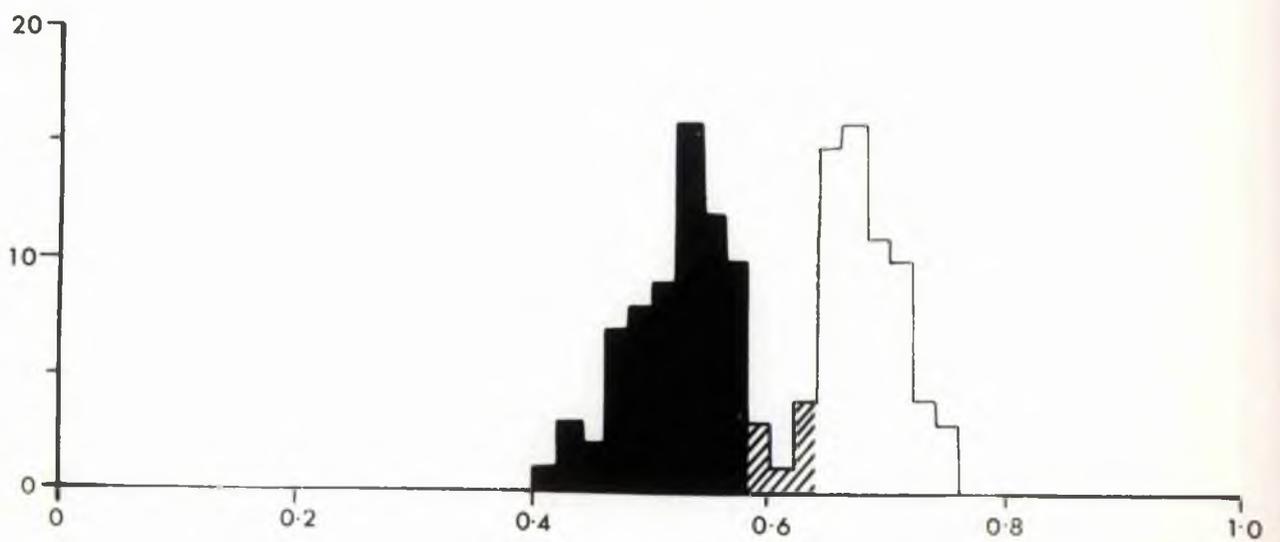
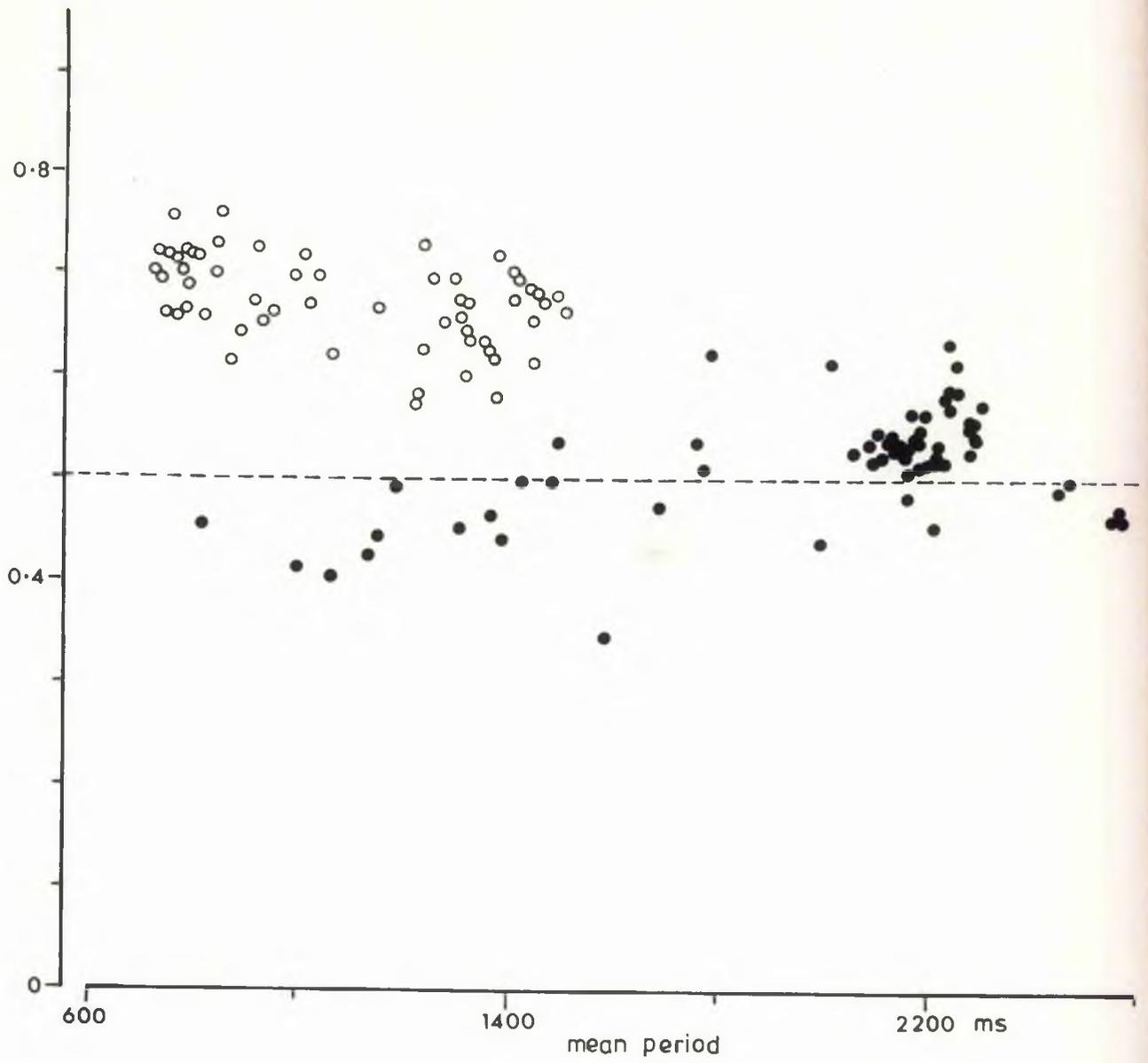
Figure IV.12. Phase angle of the start of the remotor ( $\phi_{II}$ ) burst in the cycle of the flexor ( $\phi_I$ ). Filled circles and histogram bars, forward cycles; open, reversed. Cross-hatched histogram bars, overlap between forward and reversed cycles. Broken line in plot indicates phase angle of 0.5. Note that during forward beating the lag between the start of the flexor and the start of the remotor burst is roughly 50% of the total cycle, during reversed beating. During reversed beating it is slightly greater. This might be compared with the relationship in the primus (Fig.IV.13) in which there is a relatively large shift from forward to reversed beating.

**Forward:**

$b = 0.01 \pm 0.01$   
 $r = 0.061$   
 $N = 77$   
 $P < 0.01$

**Reversed:**

$b = -0.08 \pm 0.03$   
 $r = -0.395$   
 $N = 60$   
 $P < 0.01$



The first part of the report is devoted to a description of the
 experimental apparatus and the method of measurement. The
 results of the measurements are given in the second part, and
 the discussion of the results is given in the third part.

The experimental apparatus consists of a cylindrical vessel
 of diameter  $d$  and height  $h$ , filled with a liquid of
 density  $\rho$ . The vessel is rotated about a vertical axis
 with a constant angular velocity  $\omega$ . The free surface
 of the liquid is assumed to be a paraboloid of revolution.
 The height of the liquid at the center of the vessel is
 denoted by  $h_0$ , and the height at the edge is denoted
 by  $h_1$ . The difference in height between the center and
 the edge is denoted by  $\Delta h$ .

The theoretical expression for the difference in height
  $\Delta h$  is given by

$$\Delta h = \frac{\omega^2 d^2}{8g}$$

where  $g$  is the acceleration due to gravity. The
 experimental results are compared with this theoretical
 expression in the second part of the report.

Figure IV.13

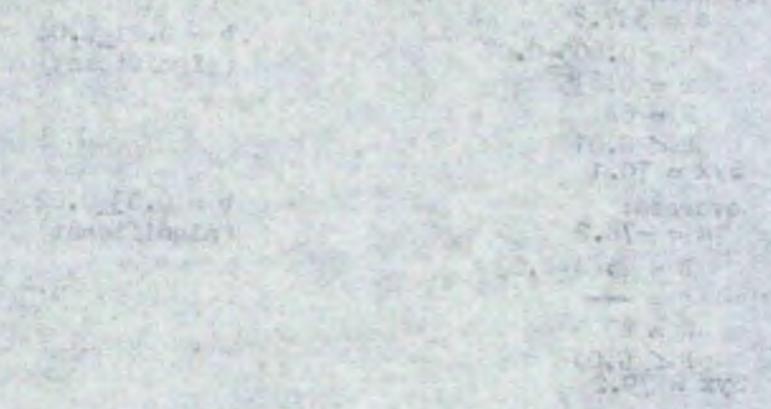


Figure IV.13. The position of the start of the primus burst in the cycle of the start of the flexor burst.

- A. Relative latency ( $L_p$ ) of the primus (mrlIIm) burst as a function of period length.
- B. Phase histogram of the same data.

Filled circles and histogram bars, forward beating; open, reversed. Histogram ordinates, number of occurrences; abscissa, phase angle. Note the relatively large shift in the starting point of the primus in the flexor cycle, as compared with that for the remotor (Figs. IV.11 and 12) between forward and reversed beating. Also note that as opposed to the change in the remotor, the primus starts earlier during forward than during reversed beating. In both cases, the regression and correlation coefficients are strong and do not change markedly between forward and reversed beating.

Forward:

$a = 537.8$   
 $b = 0.805 \pm 0.07$   
 $r = 0.835$   
 $N = 64$   
 $P < 0.01$   
 $Syx = 70.1$

$\phi_L$

$b = 0.21 \pm 0.04$   
(significant)

Reversed:

$a = -78.2$   
 $b = 0.84 \pm 0.03$   
 $r = \text{---}$   
 $N = 87$   
 $P < 0.01$   
 $Syx = 79.2$

$b = 0.03 \pm 0.02$   
(significant)

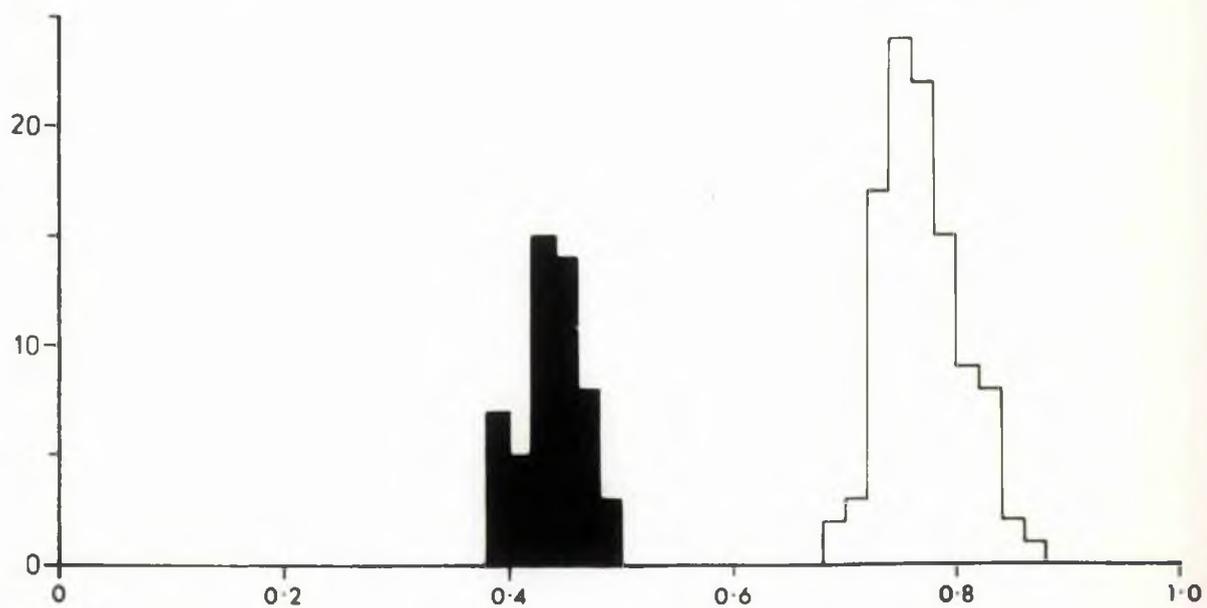
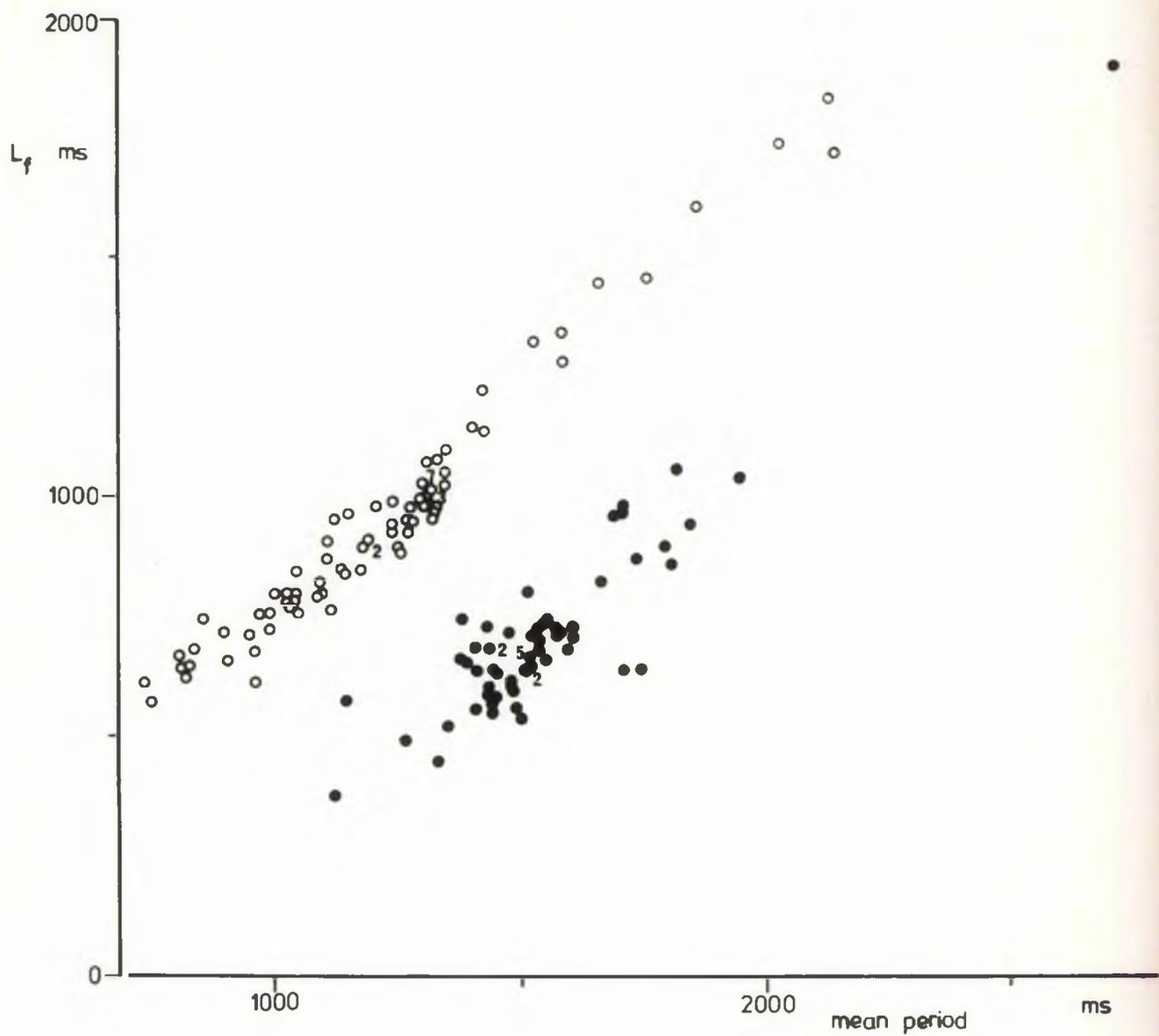


Figure IV.14. (a) Schematic diagram of a... (b) Schematic diagram of a... (c) Schematic diagram of a...

Figure IV.14.

(a) Schematic diagram of a... (b) Schematic diagram of a... (c) Schematic diagram of a... (d) Schematic diagram of a... (e) Schematic diagram of a...

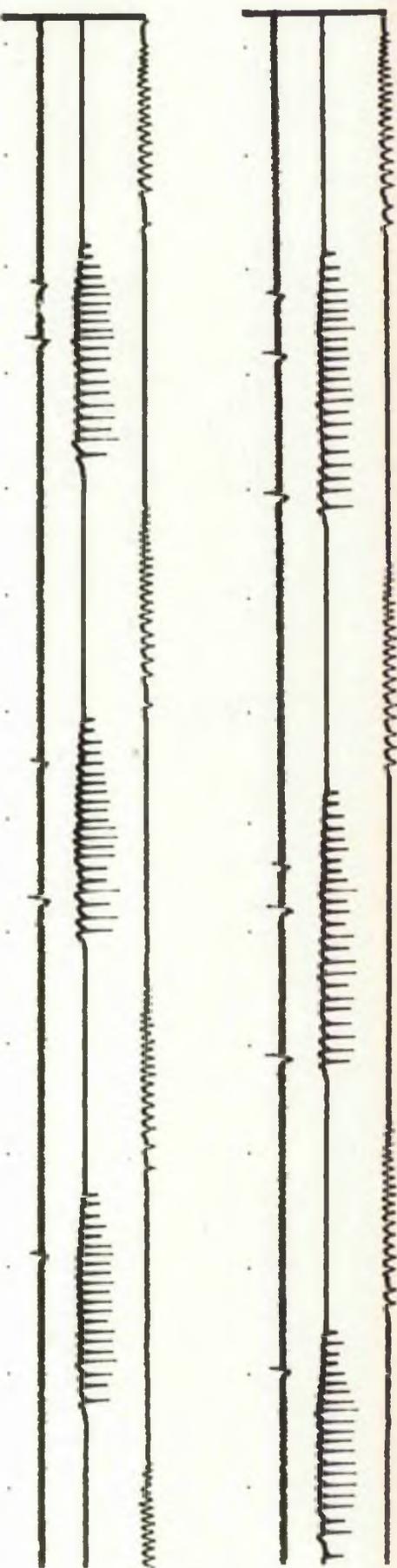
Figure IV.14. Abnormal bursting in the flexor (fsIIIm) and promotor (mpiIIIm) muscles.

(A) Notice that the bursts in these two muscles only occupy almost the whole cycle. This is contrary to the normal pattern so far established. Presumably the quartus bursts must terminate at roughly the same time as the flexor, since the overlap between the quartus and levator bursts is never very great. It would have been interesting to see how the bursts in the primus sub-group were affected by this type of activity in the promotor.

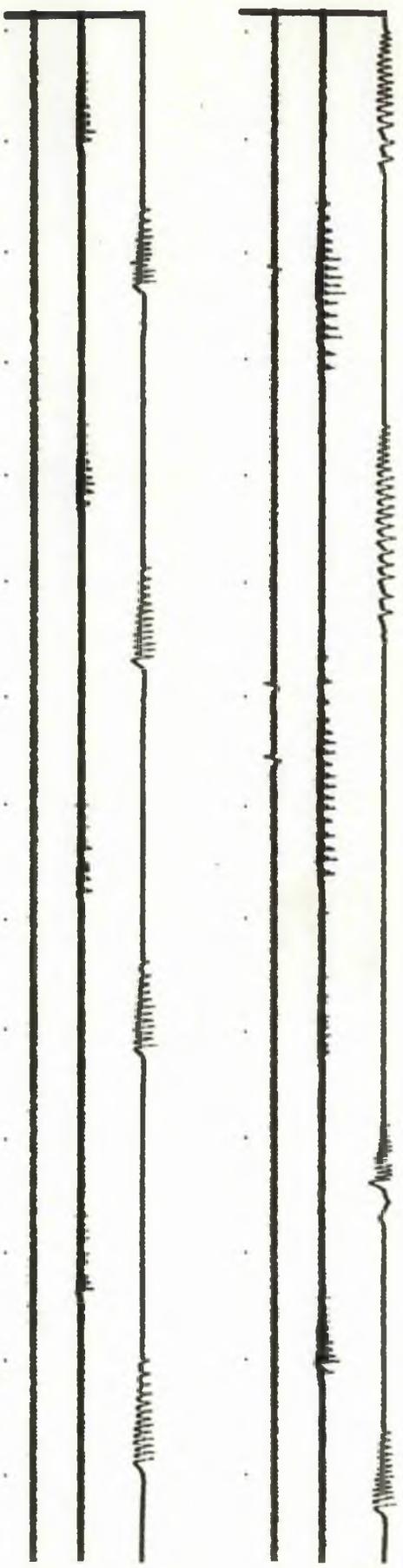
(B) A number of cycles later. Changeover from reversed to forward beating. Notice the drastic reduction in the amplitude of the ejp's although the spiking frequency in the burst is apparently not greatly diminished. This is a common feature of changes in beat direction, either from reversed to forward or vice versa. In this preparation, the attenuation was very marked, and sustained. A few cycles later, the promotor burst had all but disappeared. Note the change in the burst structure in the flexor, and the disappearance of all activity in the accessory promotor during forward beating.

Top trace : Flexor  
Middle : Promotor  
Bottom : Accessory promotor.

A



B



time mark = 500 ms

Figure IV.15A shows the results of the analysis of variance for the dependent variable of time taken to complete the task. The results are shown in Table IV.15A. The analysis shows that there is a significant difference between the two conditions,  $F(1, 18) = 10.5, p < .01$ . The results also show that there is a significant difference between the two conditions for the dependent variable of time taken to complete the task,  $F(1, 18) = 10.5, p < .01$ . The results also show that there is a significant difference between the two conditions for the dependent variable of time taken to complete the task,  $F(1, 18) = 10.5, p < .01$ .

Figure IV.15A

Source	SS	df	MS	F	p
Between	10.5	1	10.5	10.5	< .01
Within	180	18	10		
Total	190.5	19			

The results of the analysis of variance for the dependent variable of time taken to complete the task are shown in Table IV.15A. The analysis shows that there is a significant difference between the two conditions,  $F(1, 18) = 10.5, p < .01$ . The results also show that there is a significant difference between the two conditions for the dependent variable of time taken to complete the task,  $F(1, 18) = 10.5, p < .01$ . The results also show that there is a significant difference between the two conditions for the dependent variable of time taken to complete the task,  $F(1, 18) = 10.5, p < .01$ .

Figure IV.15A The regression on period of the absolute (irrespective of sign) relative latency between the starts of the bursts in the flexor and quartus (mr4IIm) muscles, for bursts from the same depressor session.

Conventions as in previous figures. Note the relatively weak regression. This was an extreme case\* but was chosen since it showed both forward and reversed beats in the same experiment. This pattern might be compared with that in Fig. IV.18 in which the same data were analysed in terms of the lag between the start of the flexor burst and the start of the next burst in the quartus. A very different and perhaps misleading picture results.

Forward:

a = 11.04  
b = 0.14 ± 0.02  
r = 0.755  
N = 43  
P < 0.01  
Syx = 88.04

Reversed\* :

a = 404.9  
b = 0.04 ± 0.03  
r = 0.208  
N = 44  
P < 0.10  
Syx = 46.7

---

\* these results for reversed beating were in fact the odd one out in three analyses. This by itself would perhaps not be adequate grounds for being skeptical about the normalcy of the picture. Bearing in mind the strong clumping of the greater proportion of the points and the large deviation from these and the wide scatter, in the remaining few, it does seem that not much reliance should be placed on the results of regression analyses, and the indicated level of significance of their results. It might be more reasonable to give greater weight to the mutually corresponding results of the other two analyses in which the points were more evenly distributed (Fig. IV. 15B).

$L_f$

ms

600

300

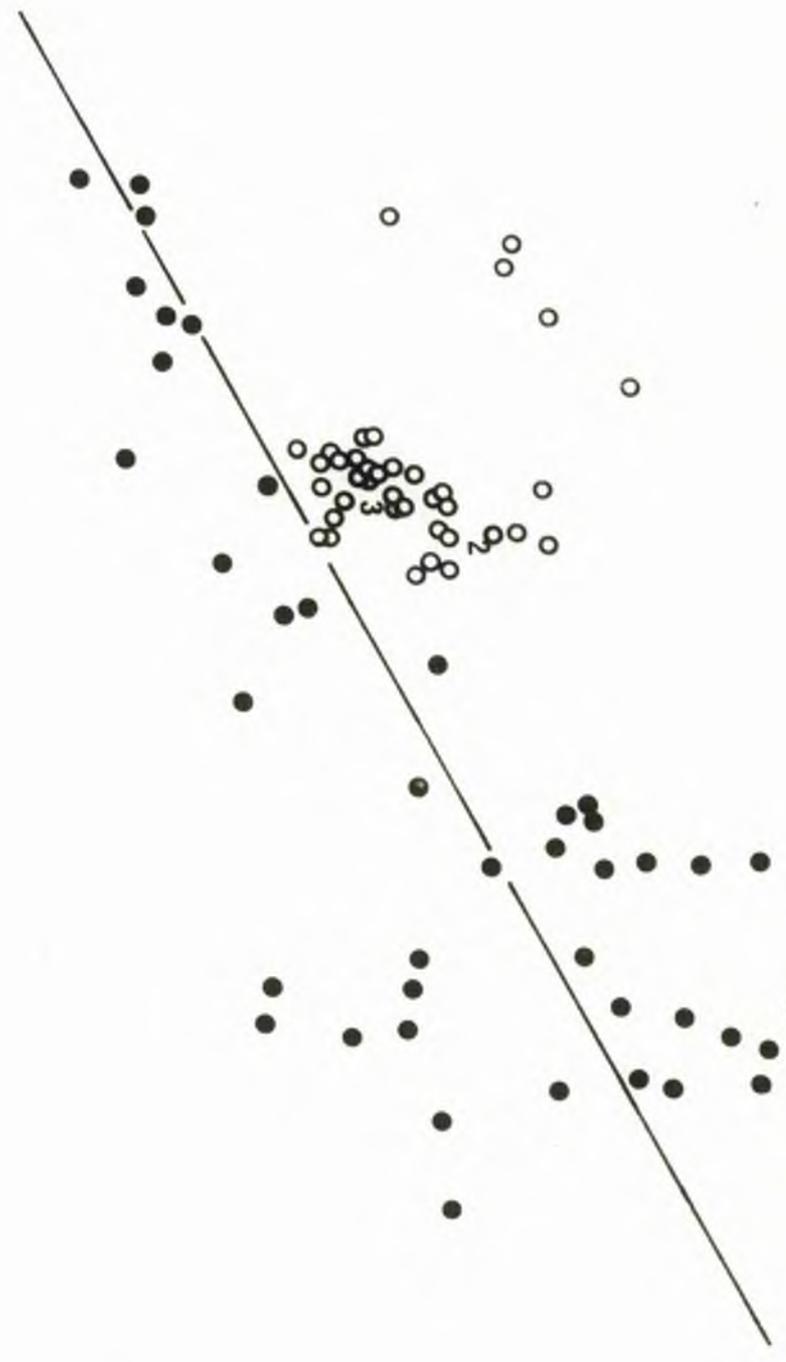
0

0

2000

mean period

4000 ms



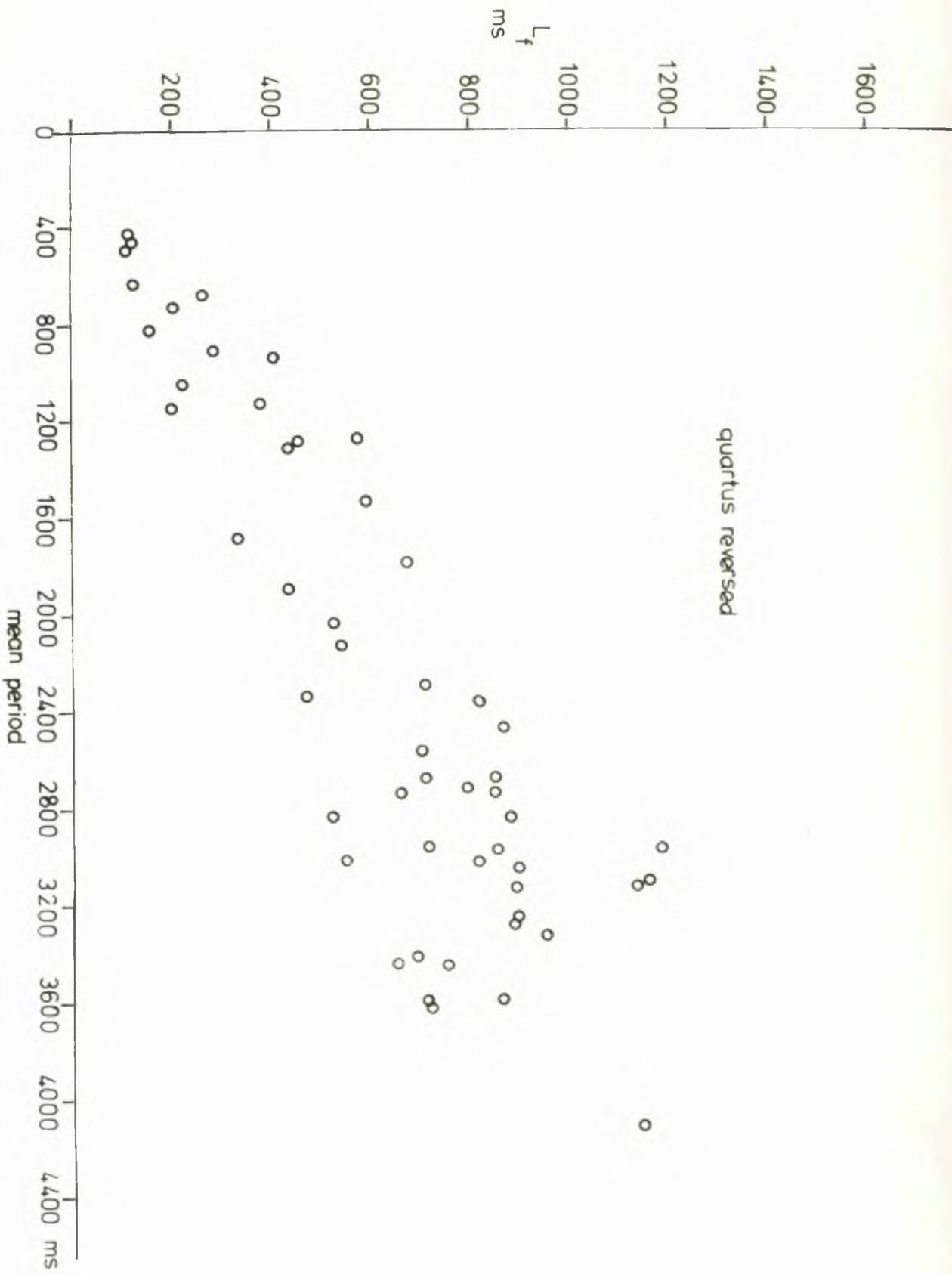


Figure IV.16 shows the results of the analysis of variance for the effect of the concentration of the solution on the rate of the reaction. The results show that the rate of the reaction increases with the concentration of the solution. The rate of the reaction is directly proportional to the concentration of the solution. The rate of the reaction is also affected by the temperature of the solution. The rate of the reaction increases with the temperature of the solution. The rate of the reaction is also affected by the surface area of the reactants. The rate of the reaction increases with the surface area of the reactants. The rate of the reaction is also affected by the nature of the reactants. The rate of the reaction increases with the nature of the reactants.

Figure IV.16

Rate of reaction  
vs. concentration of solution

Rate of reaction  
vs. temperature of solution

The results of the analysis of variance for the effect of the concentration of the solution on the rate of the reaction are shown in Figure IV.16. The results show that the rate of the reaction increases with the concentration of the solution. The rate of the reaction is directly proportional to the concentration of the solution. The rate of the reaction is also affected by the temperature of the solution. The rate of the reaction increases with the temperature of the solution. The rate of the reaction is also affected by the surface area of the reactants. The rate of the reaction increases with the surface area of the reactants. The rate of the reaction is also affected by the nature of the reactants. The rate of the reaction increases with the nature of the reactants.

Figure IV.16. Phase position of the start of the quartus burst in the cycle of the start of the flexor bursts ( $\phi_1$ ) expressed as a function of mean period, and as a frequency distribution histogram. Conventions as in the preceding figures. Note the large change in the phase position of the start of the quartus burst in forward and reversed beating. This compares with the change in the position of the start of the burst in the primus (Fig.IV.13) with respect to the flexor.

Forward:

$$b = 0.02 \pm 0.01$$

$$r = 0.317$$

$$N = 41$$

$$0.02 < P < 0.05$$

Reversed:

$$b = -0.17 \pm 0.02$$

$$r = -0.766$$

$$N = 43$$

$$P < 0.01$$

Notice that the phase angle of the start ( $\phi_1$ ) is independent of period during forward beating. The significant negative regression of  $\phi_1$  on period during reversed beating is due largely to a few trailing points. These appear as a separate peak on the phase histogram.

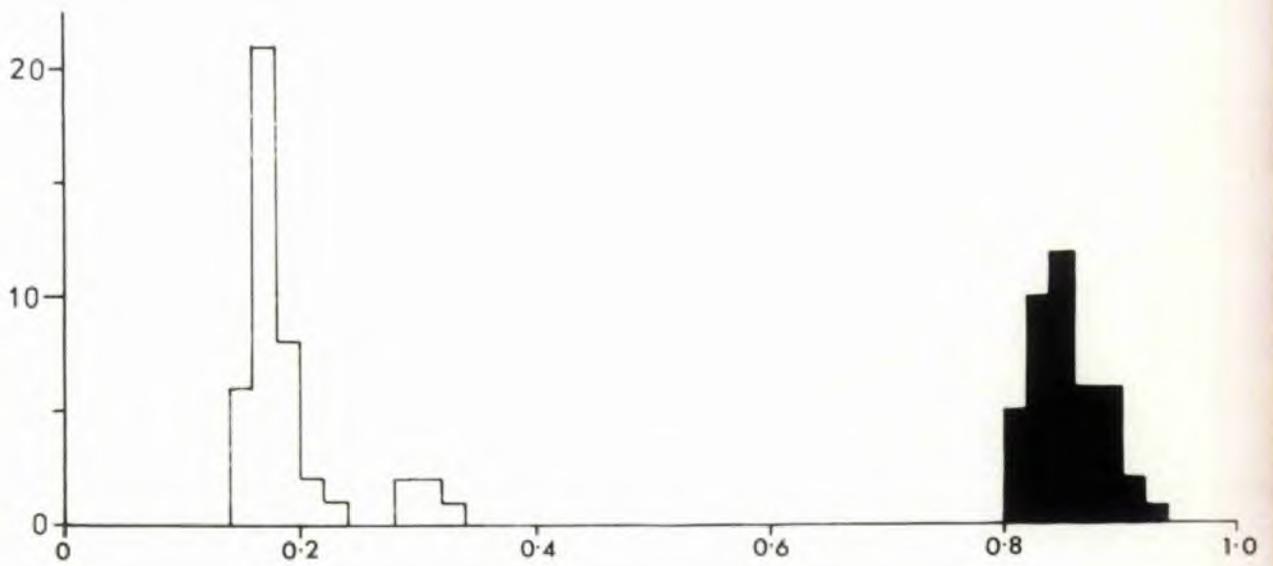
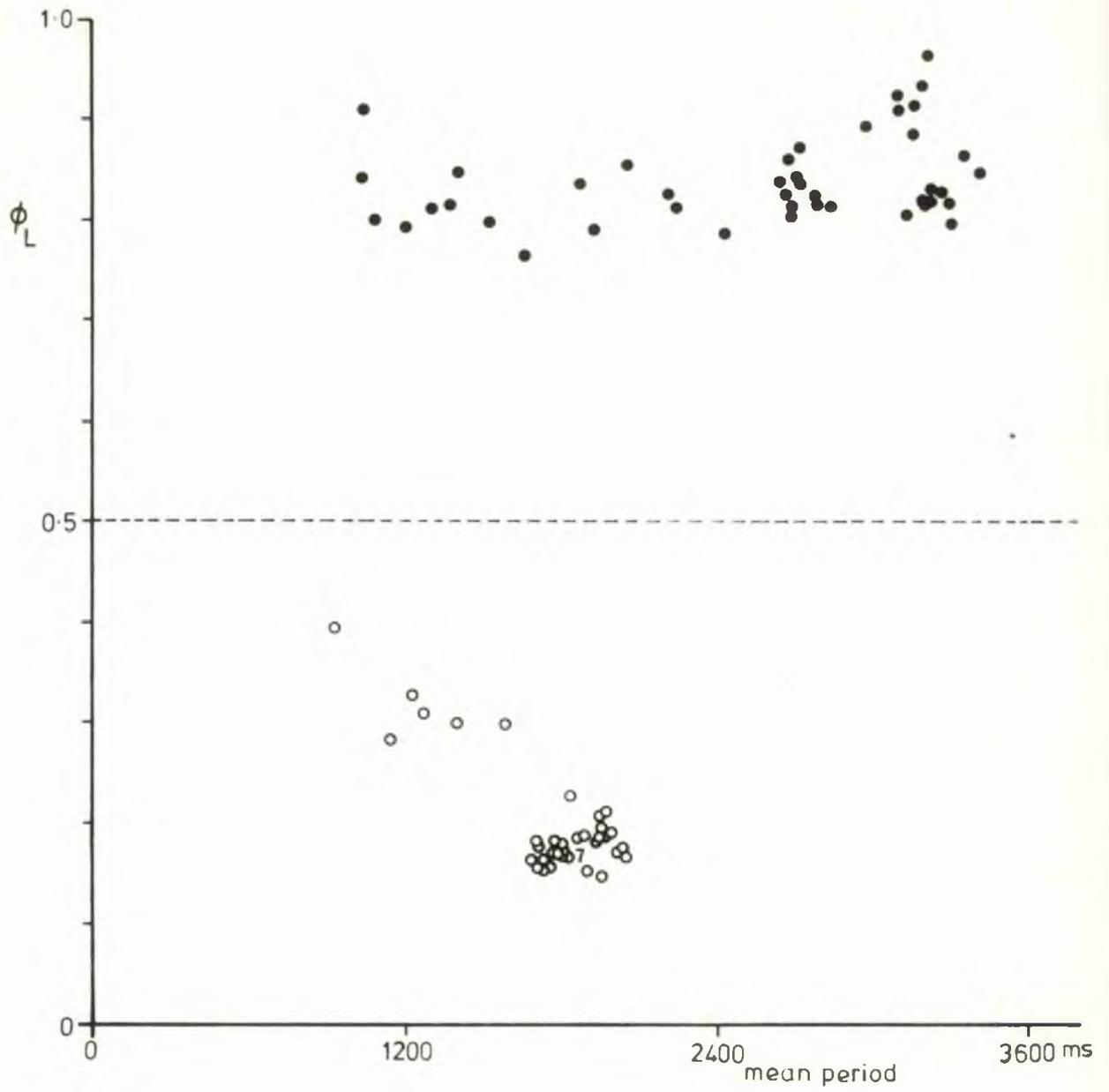




Figure IV.17. The relationships between the relative latency between the promotor and secundus, and period length. Conventions as in the preceding figures. The relationships between muscles from the different levator sub-groups may be compared with those between the different depressor sub-groups (Fig.IV.16) and contrasted with those between muscles from the levator and depressor groups (Figs. IV.11,12,13). Note the sudden aberrant regressions seen for a few cycles in many of these plots. It is interesting to compare this plot with that in Fig. IV.29 for the duration of the promotor against period length. Davis (1969) noted a similar variability in the muscles of the swimmerets of the lobster.

A. Relative latency (L) as a function of period length.

Forward:

$a = -205.2$   
 $b = 0.12 \pm 0.02$   
 $r = 0.524$   
 $N = 69$   
 $P < 0.01$   
 $S_{yx} = 65.5$

Reversed:

$b = 0.05 \pm 0.01$   
 $r = 0.850$   
 $N = 15$   
 $P < 0.01$

B. Phase angle of lag as a function of periods with phase histogram as inset.

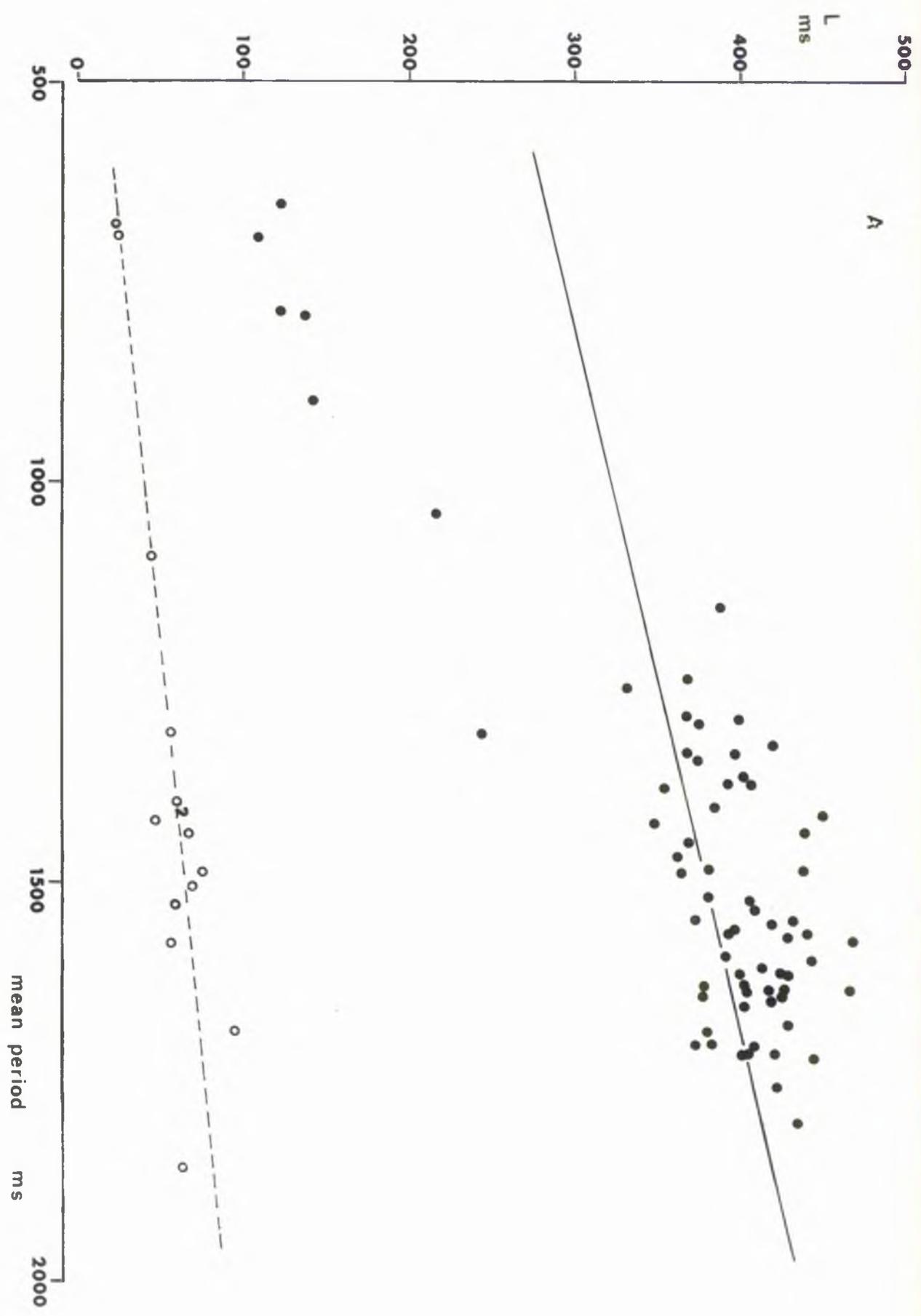
Forward:

$b = 0.02 \pm 0.01$   
 $r = 0.204$   
 $N = 69$   
 $P < 0.10$

Reversed:

$b = 0.005 \pm 0.006$   
 $r = 0.206$   
 $N = 15$   
 $P < 0.10$

A



B

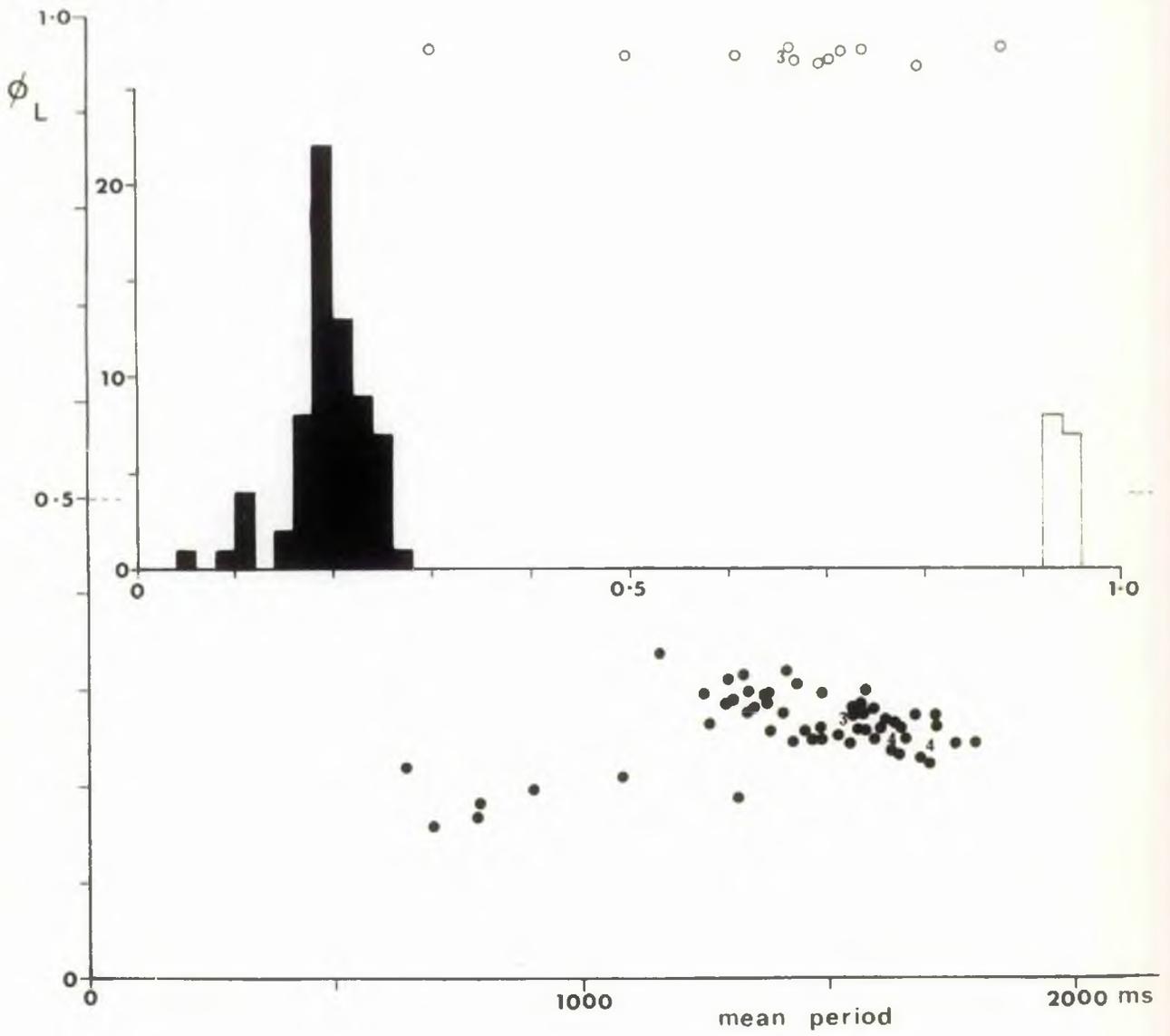




Figure IV.18. The dependence of  $L_c$  for the quartus (mr4II<sub>m</sub>) on the mean period length of the corresponding cycles in the flexor and quartus. Same experiment as illustrated in Figs. IV.15A and 16. Here,  $L_c$ , as defined, refers to the time from the start of the flexor burst to the start of the next burst in the quartus. During forward beating this next burst lies in the subsequent depressor burst, and the duration of the levator burst intervenes (see Fig. IV.8). This relatively long latency accounts for the marked regression and strong correlation seen in this figure, as compared with the results in Fig. IV.15A. The latency in Fig. IV.15A is the absolute latency (irrespective of sign) between the starts of the flexor and quartus bursts in the same depressor burst. In forward beating, the quartus burst occurs before the flexor burst in the same depressor session; in reversed beating, it occurs after (see Fig. IV.1).

**Forward:**

$a = -103.0$   
 $b = 0.88 \pm 0.03$   
 $r = 0.985$   
 $N = 41$   
 $P < 0.01$   
 $S_{yx} = 120.2$

**Reversed:**

$a = 405.9$   
 $b = -0.04 \pm 0.03$   
 $r = -0.208$   
 $N = 43$   
 $P < 0.10$   
 $S_{yx} = 27.7$

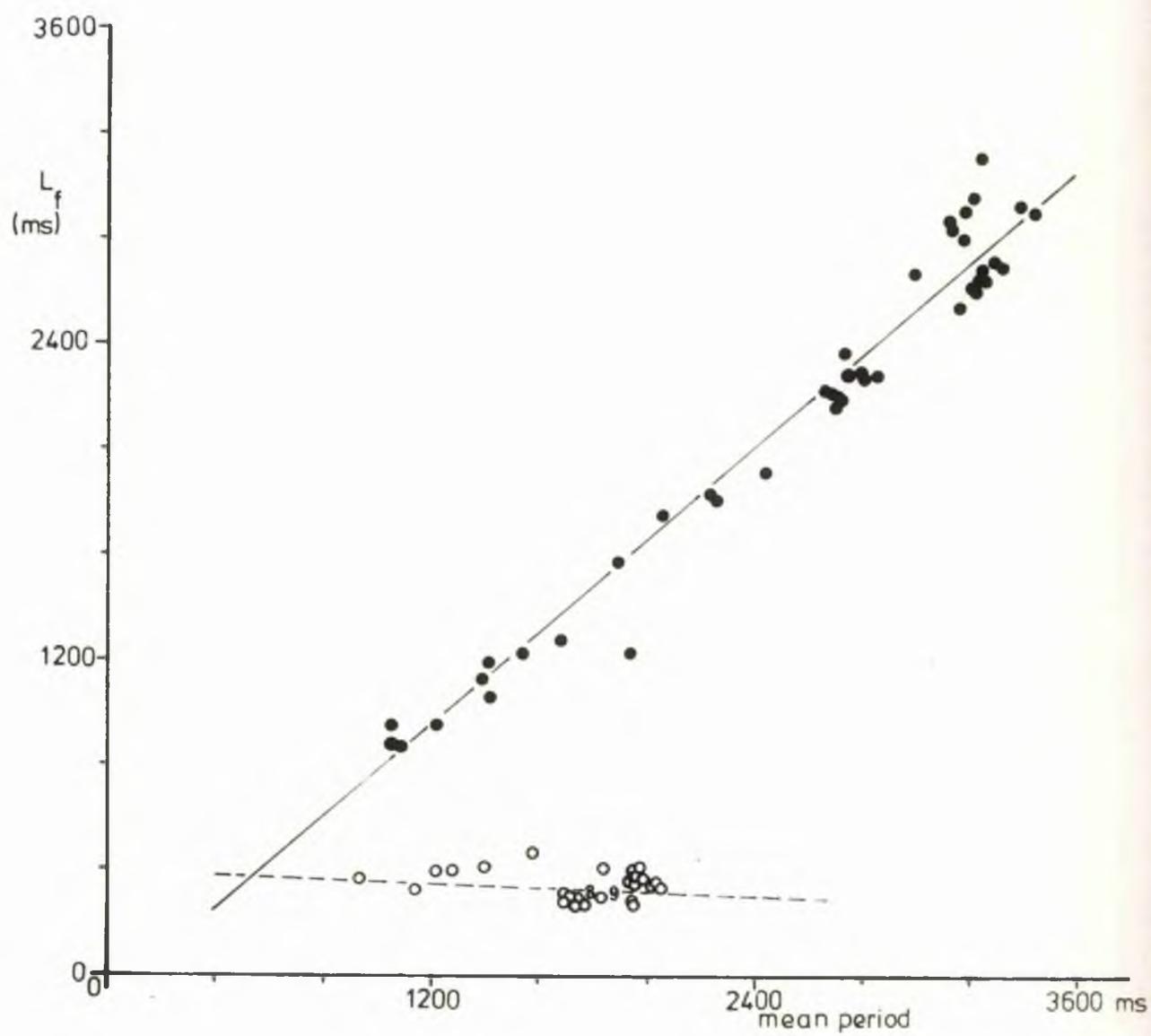




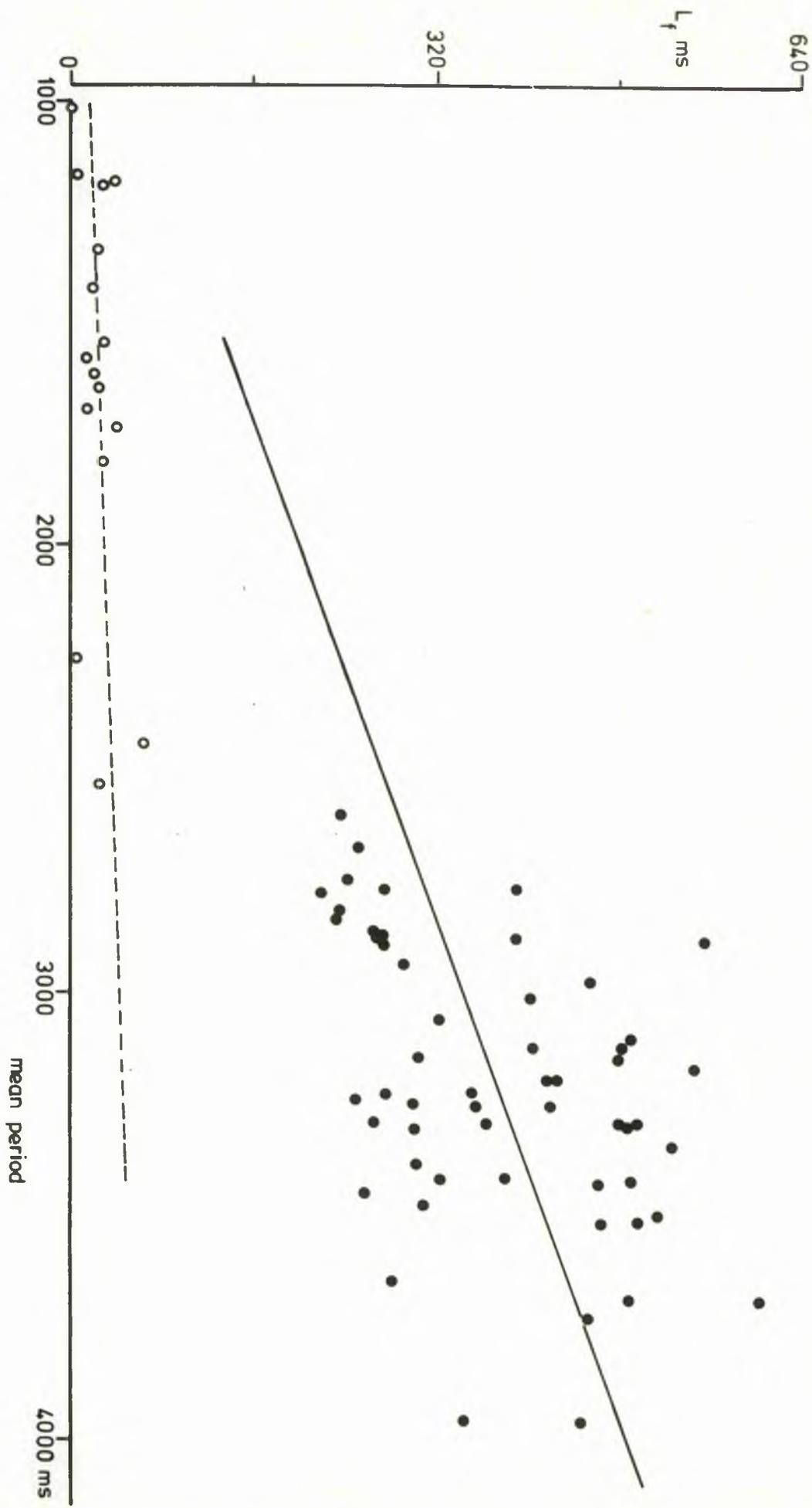
Figure IV.19. Regression on period of  $L_1$  for the sixtus during forward and reversed beating. Note the large relative scatter during forward beating (filled circles) compared with the weak regression. This represents the 'typical' relationship within the flexor sub-group. This may be contrasted with the pattern seen between sub-groups from agonist and antagonist groupings (preceding figures).

**Forward:**

$a = -93.3$   
 $b = 0.15 \pm 0.04$   
 $r = 0.437$   
 $N = 55$   
 $P < 0.01$   
 $S_{yx} = 95.9$

**Reversed:**

$a = 1.95$   
 $b = 0.01 \pm 0.01$   
 $r = 0.376$   
 $N = 18$   
 $P < 0.10$   
 $S_{yx} = 15.2$



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Figure IV.20

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Figure IV.20 Same data as in Fig.IV.19 plotted as a phase histogram (inset) and as  $\phi_L$  against period. Note the negligible slope of the regression, and that in reversed beating the muscles (flexor and sixtus) start roughly synchronously.

**Forward:**

$b = 0.01 \pm 0.01$   
 $r = 0.122$   
 $N = 55$   
 $P < 0.10$

**Reversed:**

$b = -0.00 \pm 0.01$   
 $r = -0.012$   
 $N = 18$   
 $P < 0.10$

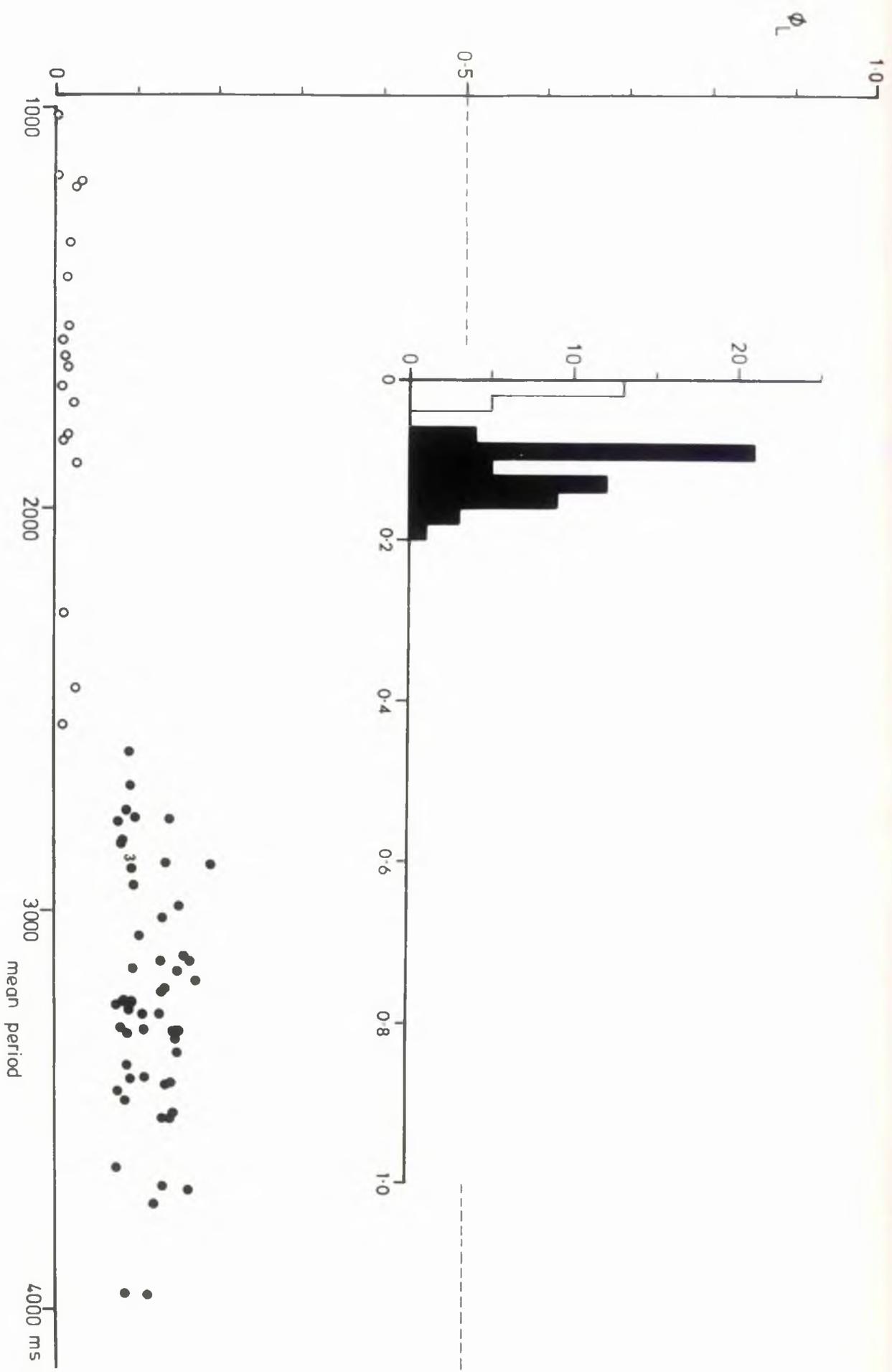


Figure IV.21 shows the results of the analysis of variance for the effect of the concentration of the solution on the rate of reaction. The results show that the rate of reaction increases with increasing concentration of the solution.

The rate of reaction is directly proportional to the concentration of the solution. This is shown by the linear relationship between the rate of reaction and the concentration of the solution.

Rate of reaction

Concentration

The results of the analysis of variance for the effect of the concentration of the solution on the rate of reaction are shown in Figure IV.21.

**Figure IV.21**

Rate of reaction  
Concentration

The results of the analysis of variance for the effect of the concentration of the solution on the rate of reaction are shown in Figure IV.21. The results show that the rate of reaction increases with increasing concentration of the solution. This is shown by the linear relationship between the rate of reaction and the concentration of the solution.

Figure IV.21. Relative timing of the starts of the bursts in the primus and secundus (same sub-group) as a function of period length.

A. Relative latency (ordinates) against period (abscissae).  
Forward beats only.

$$a = 1.5$$

$$b = 0.03 \pm 0.03$$

$$r = 0.191$$

$$N = 45$$

$$P < 0.10$$

$$S_{yx} = 32.5$$

B. Phase angle of the lag against period. Same data transformed.

$$b = 0.03 \pm 0.01$$

$$r = 0.424$$

$$N = 45$$

$$P < 0.01$$

Note as in Fig. IV.20 the negligible slope of the regression. This is a reflection of the fact that the intercept of the latency against period plot is not significantly different from zero.

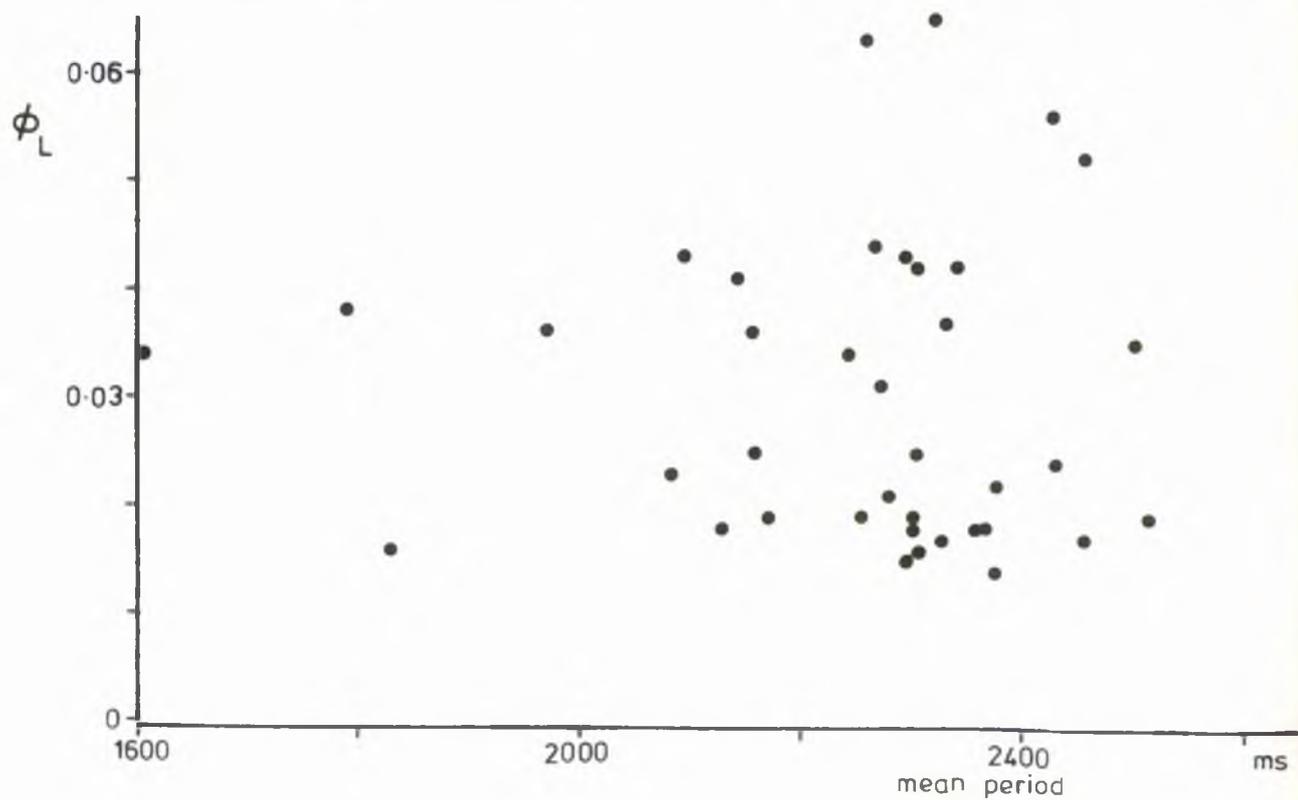
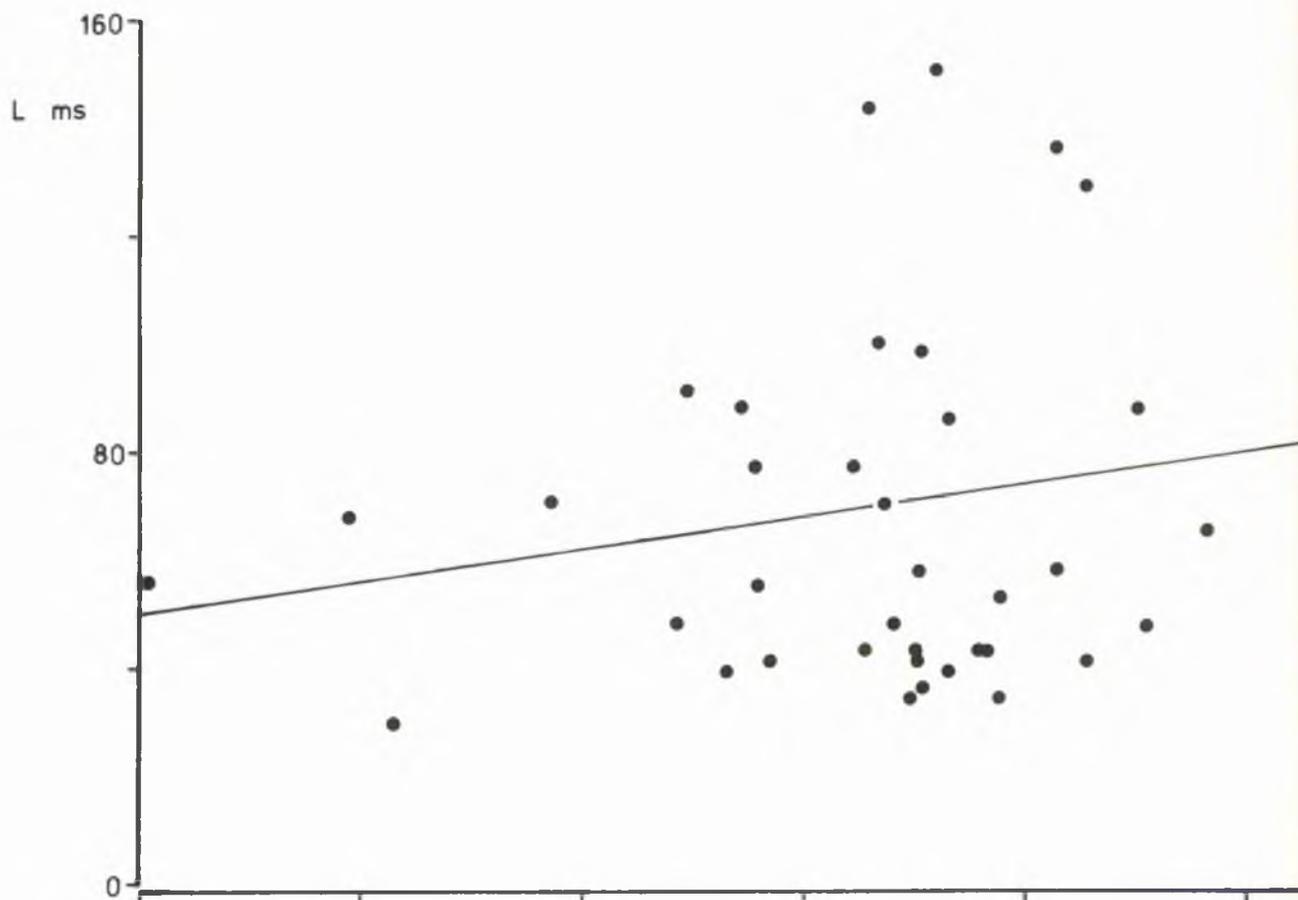
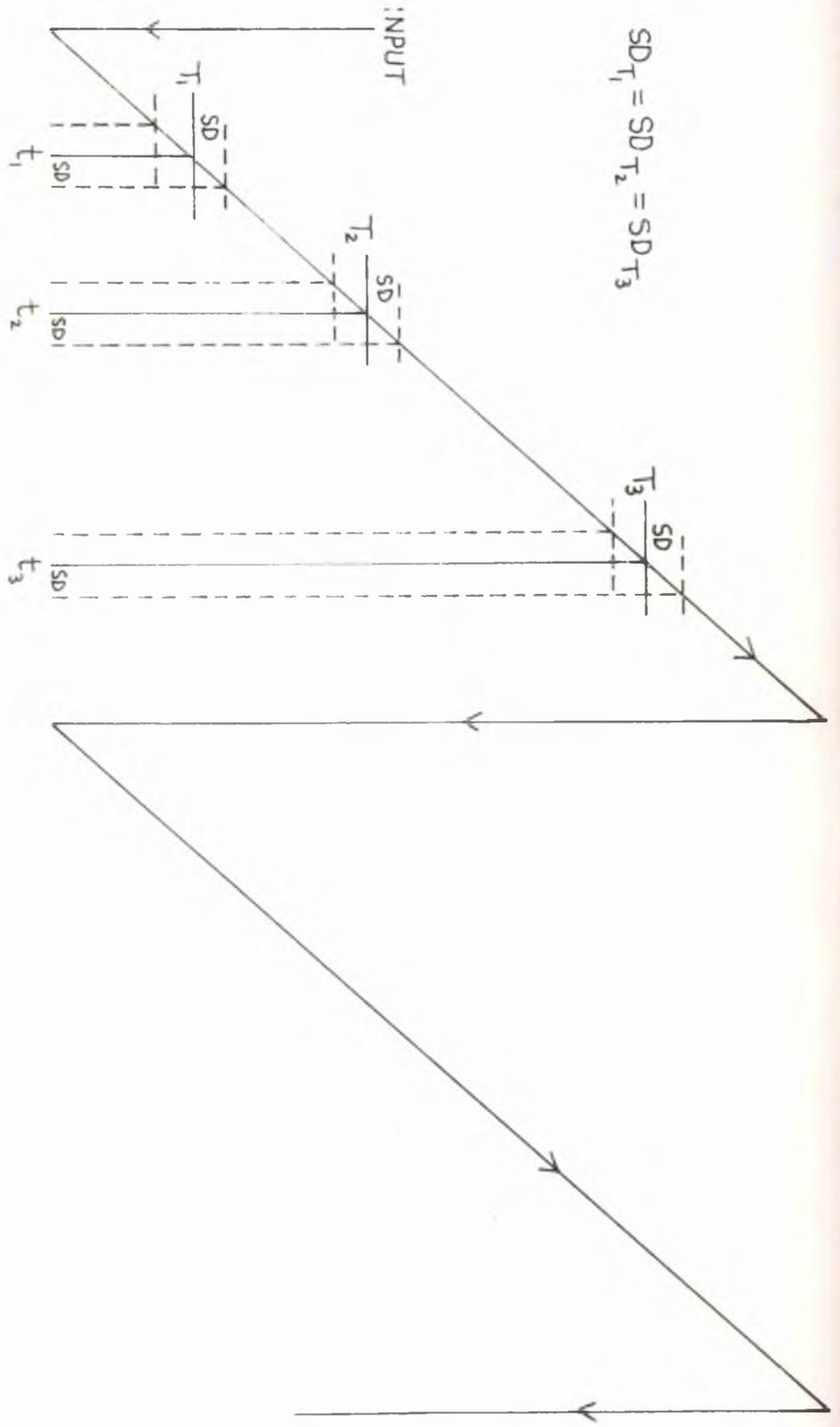


Figure IV.22A shows the results of the experiment. The data points are plotted against the concentration of the reactants. The curve shows a decrease in the rate of reaction as the concentration of the reactants increases. This is characteristic of a reaction that is first order with respect to the reactants.

Figure IV.22A.

Figure IV.22A. Adapted from Wyman (1968).  $T_1$ ,  $T_2$ ,  $T_3$  represent threshold levels which when exceeded by the input, result in activation of the respective units. If it is assumed that the error in each of these thresholds is the same, then the error in the relative latencies of the cued events will be independent of the magnitude of  $\Delta t$ .

$$SD_{t_1} = SD_{t_2} = SD_{t_3}$$



$$\Delta t_{1,2}$$

$$SD_{1,2} = SD_1 + SD_2$$

$$SD_1 = SD_2 = SD_3$$

$$\Delta t_{1,3}$$

$$SD_{1,3} = SD_1 + SD_3$$

$$SD_{1,2} = SD_{1,3}$$



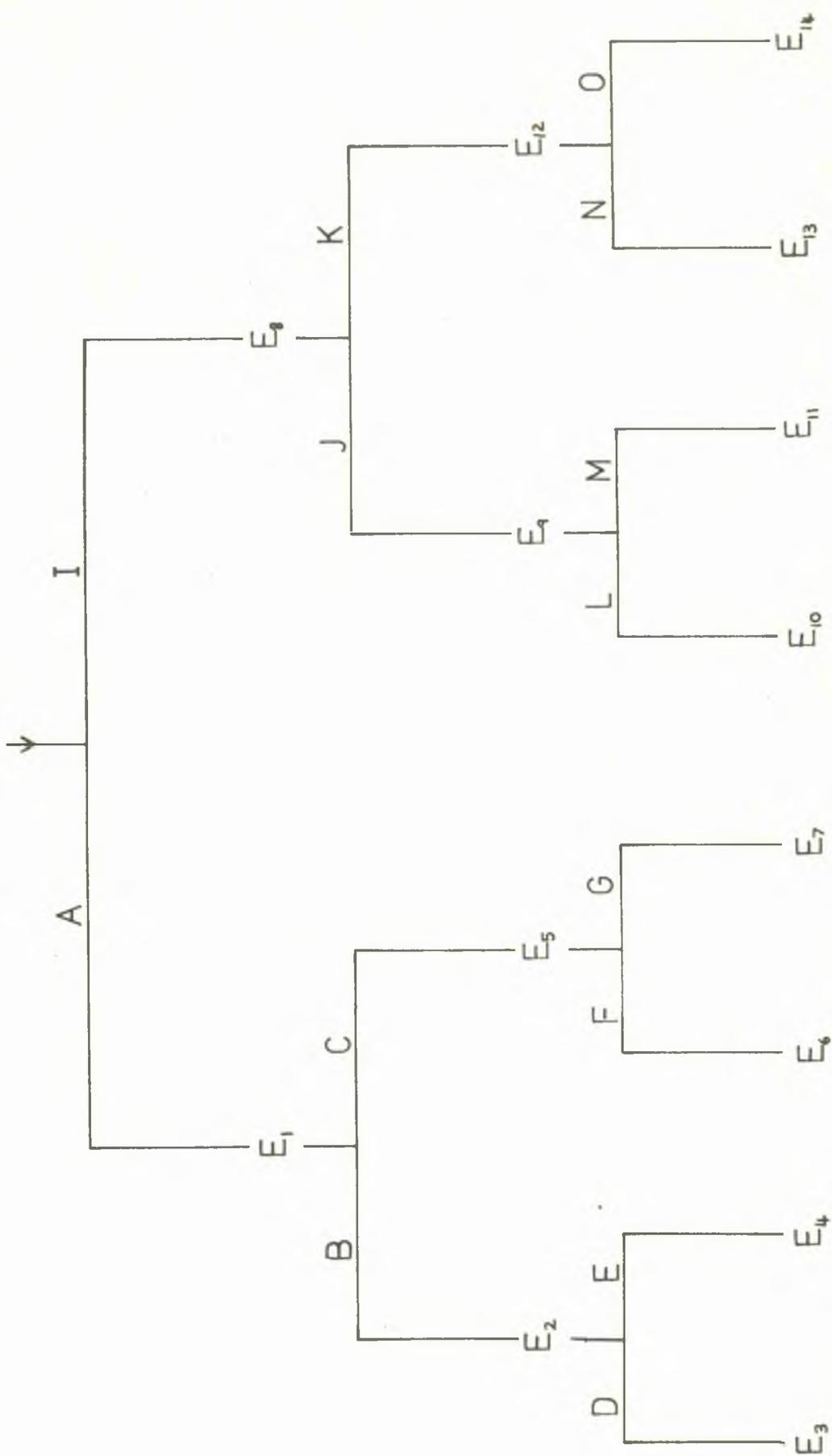
Figure IV.22B. A generalized scheme which might be consistent with some of the observations so far made in this study on the characteristics of the relative latencies of the bursts in the different muscles. The input first triggers A which in turn triggers B then, after a delay, C. B triggers D and E then C triggers F and G. The triggering of I is then 'enabled' or effected as the input rises above some given level. The triggering in this limb then proceeds as in the A limb, which must now be regarded as being refractory or fatigued, or actively subdued, as the triggering in the I limb proceeds.

Opportunities for error are envisaged at each branch point, or at the point of input (or output) of each subunit.

Note that the error in the timing between D and E would be only  $E_3 + E_4$ . That between D and F would be  $E_3 + E_4 + E_5 + E_6$ , between D and L,  $E_3$  and  $E_2 + E_1 + E_8 + E_9 + 3E_{10}$  and so on. These errors assuming all the errors to be equal would be for D/E, 2E; D/F, 4E and D/L, 6E. This would explain the increasing variance in relative latencies going from intra-sub-group (D/E type), via inter agonist sub-group, (D/F type) to inter group (D/L type) relationships. Clearly this is a gross simplification since the relative variances here do not follow such a simple pattern.

Undoubtedly many other explanatory systems could be devised, and it would require much more extensive and detailed analyses than those done here in order to decide with any certainty between the different alternatives.

INPUT



IV. 4 The relationship between burst duration and  
period length in the ventilatory muscles.

IV.4.1. Results.

Tables VII to XII summarize the relationships between the durations of the bursts in the different muscles and period length, for both forward and reversed beating. There is perhaps a greater degree of variability, but the pattern is much the same as that for the relative latencies. By far the predominant relationship is a significant correlation between burst duration and period length. In addition, at least among the depressor muscles, the phase angle of the duration ( $\phi_D$ ) appears to be predominantly, independent of period length. However, there are significant differences between the slope and intercepts of the regressions both between muscles and in a given muscle in different animals. Amongst the levator muscles, especially during forward beating, there appears, in general, a significant regression of  $\phi_D$  on period reflecting significant deviations of the intercepts in the latency plots, from zero.

The phase angle during which the different depressor muscles are active in forward beating varies between 0.29 and 0.42. The flexor appears to be active for a shorter segment of the cycle than the other depressor muscles. The mean values for  $\phi_D$  in different experiments varied between 0.19 to 0.38 as compared with a range of 0.28 to 0.53 for the muscles of the quartus sub-group. The duration of the sixtus is also somewhat brief. During reversed beating there appears to be little change in  $\phi_D$  in the quartus sub-group (Fig.IV.25), whilst in the flexor sub-group, the mean values change from 0.29 (forward) to 0.42 (reversed). This increase in the phase angle occupied by the flexor burst during reversed beating can readily be seen in Fig.IV.23. As there are statistically significant differences between the different

analyses in each group, it is not strictly legitimate to pool the values in order to find an overall mean for each group. In each of three analyses including both forward and reversed beats in the same strip however, (Table VII 1,5,8 (Reversed) corresponding respectively with Table VIII,2,3,4 (Forward)) a marked increase in the fraction of the cycle occupied by the flexor burst is seen. The change can therefore be confirmed without the need for comparison between results from different animals. Like  $\phi_L$  then,  $\phi_D$  here, may be taken to be effectively governed by some mechanism which might tend to maintain the phase angle of the burst duration, but which tolerates some degree of variability, systematic as well as random.

An extreme case of this variability is seen in the analyses on the flexor burst during forward beating (Table VII) where both slopes and intercepts for the regression of  $L_D$  on period vary widely. In fact, in the eighteen separate analyses (Table VII), seven indicated that there was no significant correlation between period length and the phase angle occupied by the muscles activity. Six showed significant negative, and five, significant positive correlations, all at the 0.01 level of significance. No relationship could be discerned between these three groups of results and other factors such as mean period length or the nature of the burst in associated muscles. Nor did they appear to be associated with variations in the fine structure or form of the flexor burst itself (parameters such as spike frequency, magnitude and speed of ejp's, presence of facilitation or anti-facilitation, and the number and intensity of units discernable in associated muscles were used as indices in the comparisons). If a plot is erected of the regression coefficient for  $\phi_D$  on period ( $b(\phi_D)$ ) as a function of the mean period length in the different experiments (Fig. IV.24), it becomes apparent that with respect to period length the

values are randomly distributed about zero, the mean regression coefficient being 0.006 or virtually nil. The question still arises though, as to why three out of every six samples differ significantly when only one percent should. To some extent, the discrepancies might be explained by the marked clumping in the distribution of the period lengths in some of the samples (e.g. Fig.IV.16), along with the occurrence of a few widely aberrant values. Unwarranted indications of significance might well result in such cases. It is doubted however, that this provides a complete explanation.

The relationships in the levator muscles show limited similarity to those in the depressor group. The two levator sub-groups show marked differences between themselves. In the primus sub-group there is a positive correlation between  $L_D$  and period during both forward and reversed beating (Fig.IV.26) but the slope of the regression is weak compared with that seen in the depressor group. The intercept and hence phase angle  $\phi_D$  occupied by the primus and secundus bursts show a significant, negative regression on period. The slope of the regression however, is very gentle (less than 0.1).  $\phi_D$  therefore will probably change only slightly over the normal range of frequencies. The mean phase angle occupied by the secundus burst is 0.35, roughly the same as that taken by the muscles of the quartus sub-group. That occupied by the primus is 0.29, virtually identical to the value for the flexor. Neither the slopes of the regression nor the values of  $\phi_D$  appear to change significantly on the average during reversed beating (Figs. IV.26,27). In this, both the muscles of this sub-group resemble more closely the quartus than the flexor sub-group. It should be noted that in view of the variability of the data, similarities between results from different animals may be taken as being meaningful, but differences except when quite clear cut, must be interpreted with

caution. However, it might be taken that significant differences associated with observed functional changes within single runs on one animal, are meaningful.

Fig.IV.27 shows the relationship between  $L_D$  and period for the primus during forward and reversed beating. The data were obtained from a single experiment in which both modes of beating were displayed. Together, the points appear to form a single population with a non-linear regression on period. The points plotted in Fig.IV.27 as split circles correspond to cycles in which the accessory promotor ( $mpIIIm'$ ) showed strong, phasic activity. The other points follow the established convention. This type of activity in the  $mpIIIm'$  occurred only during reversed beating. When the points are considered separately, the regression appears to be composed of three separate, largely linear relationships (Fig.IV.28). It would appear that the occurrence of strong activity in the accessory promotor directly or indirectly, might exert some influence on the duration of the burst in the primus. There was however, no marked influence on the characteristics for the flexor bursts or in the phasing of the onset of activity in the primus in the beat cycle.

The relationships in the second levator sub-group, the promotor sub-group, are unlike those in any of the other sub-groups described previously. The regression of  $L_D$  on period during forward beating is very small in both the promotor and remotor (means:  $0.01 \pm 0.03$  in promotor;  $0.05 \pm 0.01$  in remotor. Different animals). The values for the two muscles are not significantly different, and the combined mean is  $0.03 \pm 0.02$  which is scarcely different from zero.

Conversely,  $\phi_D$  shows a more pronounced, negative regression on period, so that the duration of the burst of activity in these muscles will tend to become relatively shorter as period increases (Fig.IV.29).

During reversed beating, the pattern conforms more closely to that established previously for the muscles of the other sub-groups. The regressions of  $L_D$  on period are not significantly different in the promotor and remotor (Table XII), and the combined mean regression coefficient is 0.31. There is no significant correlation between  $\phi_D$  and period, the combined mean regression coefficient being  $-0.02 \pm 0.06$ .

There is some reason to disregard the data from experiment 2 for the promotor during reversed beating, since the activity in the muscle seemed somewhat abnormal (Fig.IV.14). This abnormality shows up as an exceptionally large value for  $\phi_D$  (Table XII). This is not unexpected as in Fig.IV.14 it can clearly be seen that the promotor remained active for almost all that portion of the cycle when the flexor was silent. This might be compared with the records in Fig.IV.10. If these data are disregarded, then it appears that the phase angles occupied by the bursts in the promotor and remotor are very similar. This would be expected from the data in Fig.IV.10. The values of  $\phi_D$  found were 0.22 and 0.25 respectively.

#### IV.4.2. Interim Discussion

It might now be useful to outline the more important characteristics of the bursts as described above. In both depressor sub-groups during forward and reversed beating, the burst duration in the muscles tended to last for a constant fraction of the beat cycle. During forward beating, the actual magnitude of this fraction was less in the muscles of the flexor sub-group than in those of the quartus sub-group. During reversed beating, this fraction remained the same for the quartus sub-group. In the flexor sub-group, it increased markedly, becoming larger than the fraction occupied by the quartus sub-group muscles. Among the levators, in the primus sub-group during forward beating, the

fraction of the cycle occupied by the bursts tended to decrease with increasing period length. The change was very small. When mean period length varied between 1.5 to 2.2 seconds (47%), the fraction of the cycle occupied by the burst in the secundus decreased by 12 percent. Over the same range the fraction in the primus actually increased by 4 percent, in contradiction to what would be expected on the basis of the qualitative results. During forward beating the fraction of the cycle occupied by the primus burst is less than that occupied by the burst in the secundus. The fraction occupied by the secundus is roughly equal to that for the muscles of the quartus sub-group; that taken by the primus is roughly equal to the fraction taken by the flexor during forward beating. During reversal, these values do not change significantly. They may, however, become less dependent on period length. In all cases during both forward and reversed beating, the absolute burst durations in the muscles of the primus sub-group showed significant regression on period.

In the muscles of the promotor sub-group, the dependence of the burst duration on period is apparently less marked, the fraction of the cycle occupied by the bursts in these muscles being very small. It is smaller than that for any of the other ventilatory muscles, and does not appear to increase very markedly during reversal. There is, however, an enhanced regression of burst duration on period length during reversed beating. The fraction of the cycle occupied by the remotor appears to be slightly larger than that for the promotor, but it is doubted that this difference is truly significant.

The small change in the regression of  $L_D$  on period between forward and reversed beating in the flexor, along with the apparent change in phase angle of the duration, means that the change during reversal must arise by displacement of the regression rather than rotation (Fig.IV.26).

Presumably then, similar considerations to those for the relative starting latencies must hold in the present instance. Here, and for the other muscles in general, the relationship between duration of the bursts and period length might best be described by an equation of identical form to that for the starting latencies. Thus,

$$L_D = a + b(\text{period}) + \text{error} \quad (2)$$

As with the starting latencies, there appears to be considerable variability in the values that may be assumed by a and b in the equation. There is consequently parallel variation in the degree to which  $\phi_D$  varies with changing period length. This, as for  $\phi_L$ , is frequently not significant, and usually small. The obvious exception in the case of  $\phi_D$  is in the relationships for the muscles of the promotor sub-group during forward beating.

In the case of the flexor muscle during forward beating, a might be regarded as fluctuating widely about zero, the values assumed being quite independent of period length. In fact, as shown in the, perhaps somewhat unorthodox, correlation table (Table VI) there is a significant correlation between  $b(\phi_D)$  and but one other of the parameters considered here. This parameter is  $b(L_D)$  the slope of the plot of duration against period, and the relationship is only to be expected. There is no significant correlation between  $b(\phi_D)$  and  $\phi_D$ . From the plot in Fig.IV.24B however, in comparison with that in Fig.IV.24A, there might just be a case for the following argument: that there is an optimum phase angle for the duration of the flexor burst during forward beating, and that deviations about this optimum value may occur, reflecting, or being reflected in, alteration of the constant a (the intercept) in a relationship of the type described by equation 2. This then results in variation in the dependence of  $\phi_D$  on period.

Intuitively, it appears likely that the earlier speculations on

the truncation of the flexor burst by the scheduling of the start of the levator session (discussion, previous chapter), might provide some basis for rationalizing the above abstractions. No entirely satisfactory connection has been observed. It might however, be imagined that for relatively long burst lengths, the prematurely truncated fraction of the duration of the flexor burst becomes increasingly small, resulting in a decrease in the relative size of  $\underline{a}$ , the fraction of the burst (equation 2) which is independent of period length. At the optimum phase angle,  $\underline{a}$  would be zero. A tendency towards runaway activity in the flexor might conceivably result in increasing burst durations and negative intercepts, giving rise to positive regression coefficients for  $\phi_D$  on period. Excessive truncation of the burst could at the other extreme, limit the rate of increase of  $\phi_D$  with increasing period length, and so result in positive intercepts and negative regression of  $\phi_D$  on period for shorter burst durations. No doubt, other, perhaps more plausible explanations could be devised.

The duration of the flexor burst in all but one case, showed significant regression on period length during forward beating. The muscles of the promotor sub-group, described on the basis of fewer samples, show virtually no correlation between burst duration and period length. The situations in the flexor and in the promotor sub-group nonetheless show marked similarity. The bursts in both instances are curtailed shortly before the onset of activity in the antagonist group. In both cases also, the burst duration is relatively short. It seems likely then that the relationships in the promotor sub-group may represent a more drastic example of the same type of activity as that proposed in the case of the flexor. In the promotor sub-group it would appear that the extent of truncation is greater than in the flexor. This would explain the lack of correlation between

$L_D$  and period, as well as the marked negative regressions of  $\phi_D$  on period. Unlike the case in the flexor sub-group, where the flexor but not the sixtus is truncated, it appears that both the promotor and remotor are halted by the terminating influence. The situation in the remotor is well shown in Fig.IV.29. For longer periods (above 2000 ms) and burst durations, the points for forward beating correspond roughly with those for reversed beating, both sets of points lying on a line passing roughly, through the origin. Below a critical period length however, there appears to be a change in the relationship for forward beating, so that the intercept of the regression no longer passes through the origin. This gives some support to the hypothesis, but certainly does not clarify the mechanisms by which the relationship arises. It must be remembered too, that these speculations are no more than mere speculations, since taken in the light of the total variability in the whole data, the significance of any variations at all, might well become questionable.

The activity in the ventilatory muscles has now therefore been described in terms of the points of initiation of the bursts in the beat cycle, and the duration of the ongoing activity. In both cases, it appears that the relationships might be summarized by the equation

$$L = a + b(\text{period}) + \text{error}$$

where the value of  $a$ , the intercept might vary widely about zero; from time to time in a given muscle, as well as from muscle to muscle. This gives rise to a situation in which truly phase locked relationships are found in some cases at some times, whilst a weak (or even quite marked) dependence of  $\phi$  on period is obtained in other cases, or at other times. In addition to the variability in  $a$ ,  $b$  might also vary considerably from experiment to experiment. There remains in all the relationships, a large amount of residual, unexplained variability,

which might, perhaps without sufficient grounds, be ascribed, for the present purposes, to 'biological noise'.

It should now be possible to reconstruct from the data presented a rough, average picture of the relative time courses of the bursts in the different muscles during the beat cycle. This will be done in the next chapter, and an attempt will be made to relate the resulting pattern to the movements produced in the scaphognathite during the beat cycle.

TABLE VII

Muscle	Mode	Mean $I_{D \pm SE}$	a	S y.x	$b_{\pm SE}$	r	sig	Mean $\phi_{D \pm SE}$	$b(\phi_{D})_{\pm SE}$	r	sig	Mean $\pm SE$ period	N
1. Flexor	fd	549 $\pm$ 22	115.4	55.0	0.170 $\pm$ 0.010	0.929	S	22.2 $\pm$ 0.4	-0.023 $\pm$ 0.004	-0.681	S	2527 $\pm$ 121	43
2. "	"	150 $\pm$ 3	92.6	21.5	0.089 $\pm$ 0.017	0.526	S	23.5 $\pm$ 0.4	-0.116 $\pm$ 0.023	-0.514	S	645 $\pm$ 17	75
3. "	"	160 $\pm$ 7	65.0	36.9	0.121 $\pm$ 0.034	0.507	S	20.8 $\pm$ 0.7	-0.100 $\pm$ 0.034	-0.439	S	785 $\pm$ 29	38
4. "	"	198 $\pm$ 3	-4.9	16.0	0.256 $\pm$ 0.035	0.663	S	24.9 $\pm$ 0.2	0.006 $\pm$ 0.043	0.017	NS	793 $\pm$ 7	73
5. "	"	58 $\pm$ 16	-541.9	51.7	0.742 $\pm$ 0.040	0.920	S	38.1 $\pm$ 0.6	0.222 $\pm$ 0.025	0.745	S	1519 $\pm$ 20	64
6. "	"	680 $\pm$ 24	-27.9	110.5	0.340 $\pm$ 0.031	0.825	S	31.9 $\pm$ 0.8	0.146 $\pm$ 0.025	0.641	S	2200 $\pm$ 28	55
7. "	"	639 $\pm$ 16	-201.0	69.9	0.408 $\pm$ 0.026	0.875	S	30.8 $\pm$ 0.4	0.053 $\pm$ 0.013	0.043	S	2064 $\pm$ 35	76
8. "	"	704 $\pm$ 21	-13.7	86.7	0.356 $\pm$ 0.026	0.894	S	34.8 $\pm$ 0.5	0.005 $\pm$ 0.013	0.044	NS	2016 $\pm$ 51	65
9. "	"	677 $\pm$ 17	-978.6	66.3	0.803 $\pm$ 0.050	0.889	S	32.6 $\pm$ 0.6	0.066 $\pm$ 0.023	0.325	S	2041 $\pm$ 30	73
10. "	"	552 $\pm$ 8	139.7	49.2	0.258 $\pm$ 0.025	0.740	S	34.6 $\pm$ 0.3	-0.040 $\pm$ 0.014	-0.287	S	1597 $\pm$ 3	91
11. "	"	382 $\pm$ 14	58.0	71.0	0.167 $\pm$ 0.025	0.689	S	19.9 $\pm$ 0.5	-0.028 $\pm$ 0.011	-0.335	NS	1939 $\pm$ 57	51
12. "	"	131 $\pm$ 3	12.9	18.7	0.211 $\pm$ 0.021	0.763	S	19.1 $\pm$ 0.3	0.033 $\pm$ 0.031	0.124	NS	683 $\pm$ 12	76
13. "	"	478 $\pm$ 19	-124.6	54.2	0.295 $\pm$ 0.042	0.828	S	23.2 $\pm$ 2.6	0.040 $\pm$ 0.019	0.400	NS	2043 $\pm$ 53	25
14. "	"	498 $\pm$ 8	493.2	51.0	0.003 $\pm$ 0.034	0.012	NS	27.6 $\pm$ 1.9	-0.263 $\pm$ 0.043	-0.694	S	1830 $\pm$ 40	45
15. "	"	549 $\pm$ 7	14.8	44.2	0.340 $\pm$ 0.031	0.767	S	34.9 $\pm$ 0.3	-0.005 $\pm$ 0.027	-0.027	NS	1573 $\pm$ 17	87
16. "	"	650 $\pm$ 12	37.6	192.9	0.296 $\pm$ 0.034	0.661	S	31.4 $\pm$ 0.4	-0.005 $\pm$ 0.017	-0.029	NS	2068 $\pm$ 26	100
17. "	"	804 $\pm$ 21	-2103.6	67.2	0.646 $\pm$ 0.069	0.852	S	17.8 $\pm$ 0.4	0.108 $\pm$ 0.015	0.775	S	4500 $\pm$ 28	35
18. "	"	685 $\pm$ 7			0.076 $\pm$ 0.022	0.434	S	21.4 $\pm$ 0.3	-0.041 $\pm$ 0.007	-0.650	S	3208 $\pm$ 42	35

Means:

499

0.329

28.4

0.006

1755

1107  
(total)

TABEL VIII

Muscle	Kode	Mean $L_D \pm SE$	a	S y.x	b( $L_D$ ) $\pm SE$	r	sig	Mean $\beta_D$	b( $\beta_D$ ) $\pm SE$	r	sig	Mean period $\pm SE$	N
1. Flexor	rev	839 $\pm$ 14	100.1	74.2	0.323 $\pm$ 0.049	0.680	S	36.7 $\pm$ 0.4	-0.008 $\pm$ 0.020	0.052	NS	2281 $\pm$ 29	53
2. "	"	776 $\pm$ 15	217.8	50.5	0.310 $\pm$ 0.029	0.859	S	43.4 $\pm$ 0.5	-0.089 $\pm$ 0.020	-0.571	S	1795 $\pm$ 32	43
3. "	"	539 $\pm$ 13	40.3	36.4	0.401 $\pm$ 0.014	0.953	S	43.5 $\pm$ 0.3	-0.021 $\pm$ 0.011	-0.194	NS	1242 $\pm$ 30	89
4. "	"	452 $\pm$ 12	105.9	35.1	0.313 $\pm$ 0.017	0.927	S	41.5 $\pm$ 0.5	-0.094 $\pm$ 0.015	-0.633	S	1105 $\pm$ 35	61
5. "	"	1697 $\pm$ 71	-9.0	309.1	0.525 $\pm$ 0.055	0.804	S	52.0 $\pm$ 1.1	-0.007 $\pm$ 0.014	-0.072	NS	3252 $\pm$ 108	53
6. "	"	1140 $\pm$ 73	-2.8	100.0	0.491 $\pm$ 0.013	0.982	S	48.4 $\pm$ 0.7	0.012 $\pm$ 0.007	0.235	NS	2325 $\pm$ 146	52
Mean:		861			0.395			44.1	-0.033			1907	351

TABLE IX

Muscle	Mode	Mean $L_D$	$s$	$S$ y.x	$b(L_D)$	$r$	sig	Mean $\beta_D$	$b(\beta_D)$	$r$	sig	Mean period	$n$
		$\pm$ SE			$\pm$ SE			$\pm$ SE	$\pm$ SE			$\pm$ SE	
1. Sirtus	fd	306 $\pm$ 13	-114.9	46.7	0.477 $\pm$ 0.030	0.888	S	34.2 $\pm$ 0.6	---	---	NS	881 $\pm$ 24	68
2. "	"	262 $\pm$ 15	-89.6	53.2	0.447 $\pm$ 0.050	0.829	S	32.8 $\pm$ 1.1	0.121 $\pm$ 0.059	0.324	NS	786 $\pm$ 28	38
3. "	"	250 $\pm$ 9.1	129.2	88.8	0.093 $\pm$ 0.037	0.247	NS	19.6 $\pm$ 7.8	0.078 $\pm$ 0.032	-0.241	NS	1296 $\pm$ 24	100
4. "	"	572 $\pm$ 9	-106.6	53.9	0.416 $\pm$ 0.050	0.711	S	35.0 $\pm$ 0.4	-0.028 $\pm$ 0.025	-0.135	NS	1641 $\pm$ 19	70
5. "	"	782 $\pm$ 11	206.0	194.8	0.278 $\pm$ 0.025	0.749	S	38.0 $\pm$ 2.9	-0.060 $\pm$ 0.013	-0.420	S	2068 $\pm$ 29	100
6. "	"	816 $\pm$ 16	487.1	113.0	0.103 $\pm$ 0.047	0.288	NS	25.6 $\pm$ 0.5	-0.052 $\pm$ 0.014	-0.446	S	3201 $\pm$ 44	55
7. "	"	1686 $\pm$ 27	-1221.7	89.6	0.646 $\pm$ 0.074	0.835	S	37.5 $\pm$ 0.4	0.072 $\pm$ 0.017	0.614	S	4503 $\pm$ 36	35
Mean:		596			0.309			31.1				1877	466
1. "	rev	758 $\pm$ 10	211.9	88.3	0.257 $\pm$ 0.042	0.537	S	36.1 $\pm$ 0.6	0.027 $\pm$ 0.026	0.106	NS	2130 $\pm$ 22	93
2. "	"	579 $\pm$ 30	117.4	44.1	0.278 $\pm$ 0.025	0.941	S	35.2 $\pm$ 0.8	-0.043 $\pm$ 0.015	-0.584	NS	1662 $\pm$ 101	18
Mean:		731			0.260			36.0		0.016		2059	111

TABLE X

Muscle	Mode	Mean $L_D \pm SE$	a	S y.x	$b(L_D) \pm SE$	r	sig	Mean $\phi_D \pm SE$	$b(\phi_D) \pm SE$	r	sig	Mean period $\pm SE$	N
1. Tertius	fd	648.4(12.8)	-65.5	76.0	0.66(0.052)	0.802	S	40.6(0.48)	0.015(0.023)	0.070	NS	1592(22)	91
2. "	"	1038(9.31)	2.3	26.8	0.476(0.044)	0.881	S	47.6(0.02)	0.004(0.021)	-0.035	NS	2177(17)	36
3. "	"	1748.0(13.9)	587.8	68.5	0.258(0.065)	0.571	S	38.8(0.27)	-0.029(0.014)	-0.335	NS	4507(31)	35
Mean:		972.5			0.532			41.8	0.001			2352	162
1. Quartus	fd	300.5(3.94)	-10.4	25.4	0.391(0.054)	0.656	S	37.7(0.37)	0.005(0.066)	0.009	NS	795(6.6)	71
2. "	"	719.7(35.0)	9.1	103.4	0.276(0.021)	0.895	S	27.8(0.56)	0.004(0.008)	0.072	NS	2559(115)	43
3. "	"	1046.3(38.3)	202.3	87.4	0.643(0.031)	0.948	S	53.4(0.63)	0.048(0.014)	0.432	NS	1943(57)	51
4. "	"	605.2(14.1)	163.5	70.2	0.239(0.039)	0.679	S	33.1(0.45)	-0.133(0.039)	0.469	S	1831(16)	44
5. "	"	425.2(10.3)	-9.5	34.3	0.336(0.015)	0.920	S	32.8(0.26)	-0.005(0.011)	-0.042	NS	1295(24)	100
6. "	"	406.2(7.53)	74.6	30.8	0.239(0.020)	0.847	S	29.3(0.31)	-0.045(0.014)	-0.391	S	1388(27)	58
Mean:		540.5			0.355			34.5	-0.016			1515	367
1. Quintus	rev	781.8(21.3)	-289.0	60.1	0.601(0.043)	0.908	S	43.7(0.64)	0.123(0.027)	0.584	S	1796(30)	43
2. "	"	731.8(12.0)	151.9	103.8	0.271(0.046)	0.511	S	33.9(0.46)	-0.053(0.020)	-0.260	NS	2127(23)	100
3. "	"	800.4(60.9)	-47.1	223.3	0.363(0.030)	0.864	S	33.7(1.15)	0.011(0.011)	0.142	NS	2337(145)	100
Mean:		768.9			0.367			35.6	0.005			2155	243
1. Quintus	fd	181.1(4.46)	80.0	30.9	0.155(0.024)	0.609	S	28.0(0.44)	-0.077(0.028)	-0.312	S	653(18)	75
2. "	"	534.3(6.47)	-42.6	30.0	0.354(0.028)	0.835	S	32.7(0.22)	0.016(0.017)	0.109	NS	1628(15)	70
3. "	"	603.5(13.5)	-204.1	94.2	0.515(0.063)	0.666	S	38.3(0.65)	0.085(0.039)	0.230	NS	1569(17)	87
Mean:		446.1			0.350			33.3	0.012			1291	232

TABLE XI

Muscle	Node	Mean L ±SE	b	S <sub>y,x</sub>	b(L <sub>D</sub> ) ±SE	r	sig	Mean ϕ <sub>D</sub> ±SE	b(ϕ <sub>D</sub> ) ±SE	r	sig	Mean Period ±SE	N
1. Secundus	fd	72±9	---	---	0.195±0.037	0.609	S	32.8±0.4	-0.068±0.018	-0.471	S	2208±28	50
	"	700±8	338.4	43.2	0.176±0.016	0.785	S	34.4±0.4	-0.100±0.084	-0.807	S	2059±34	77
	"	548±10	359.6	70.7	0.125±0.022	0.570	S	37.2±0.5	-0.080±0.008	-0.761	S	1505±47	69
Mean:		652		0.163			35.0	-0.085				1902	196
1.	rev	50±31	-76.4	31.4	0.411±0.032	0.965	S	35.7±0.7	0.050±0.040	0.349	NS	1465±52	13
	fd	429±4	441.3	21.0	0.128±0.023	0.565	S	28.4±0.3	-0.104±0.016	-0.624	S	1518±20	67
2.	"	665±11	246.5	56.3	0.186±0.048	0.545	S	29.7±0.5	-0.058±0.023	-0.392	S	2247±31	38
Mean:		514		0.149			28.9	-0.087				1782	105
1.	rev	366±8	131.3	45.1	0.187±0.015	0.795	S	29.5±0.4	-0.056±0.012	-0.438	S	1256±33	91

**TABLE XII**

Muscle	Mode	Mean $L_D$ ± SE			r	sig	Mean $\phi_D$ ± SE	b( $\phi_D$ ) ± SE			r	sig	Mean Period ± SE	N
		$\bar{x}$	S <sub>y,x</sub>	b( $L_D$ ) ± SE				b( $\phi_D$ ) ± SE	r	sig				
1. Promotor	fd	269 ± 2	244.0	19.3	0.017 ± 0.006	0.326	S	18.8 ± 0.6	-0.093 ± 0.009	-0.785	S	1509 ± 48	68	
2. "	"	484 ± 4	503.3	35.1	-0.009 ± 0.029	-0.039	NS	23.9 ± 0.5	-0.167 ± 0.011	-0.869	S	2051 ± 25	73	
3. "	"	375 ± 11	248.2	54.5	0.062 ± 0.047	0.265	NS	18.3 ± 0.6	-0.064 ± 0.022	-0.519	S	2063 ± 48	25	
Mean:		380			0.012		21.0	-0.121				1831	166	
1.	rev	300 ± 24	-70.4	31.1	0.270 ± 0.025	0.947	S	21.7 ± 0.8	0.066 ± 0.024	0.620	NS	1398 ± 79	14	
2.	"	1042 ± 15	263.9	84.8	0.341 ± 0.056	0.647	S	36.7 ± 0.8	-0.008 ± 0.020	-0.052	NS	2283 ± 29	53	
Mean:		887			0.326		33.6	0.007				2098	67	
1. Remotor	fd	483 ± 4	389.6	27.7	0.045 ± 0.011	0.443	S	24.0 ± 0.4	-0.114 ± 0.005	-0.929	S	2058 ± 35	76	
2. "	"	473 ± 4	335.8	26.4	0.067 ± 0.010	0.663	S	23.6 ± 0.6	-0.117 ± 0.006	-0.921	S	2056 ± 43	64	
3. "	"	129 ± 5	98.4	27.7	0.046 ± 0.022	0.338	NS	21.1 ± 0.7	-0.112 ± 0.042	-0.418	S	654 ± 26	37	
Mean:		405			0.053		23.2	-0.115				2057	177	
1.	rev	280 ± 12	-40.1	56.8	0.288 ± 0.028	0.806	S	24.9 ± 0.6	-0.042 ± 0.024	-0.288	NS	1112 ± 35	59	

The following table shows the results of the tests conducted on the specimens of the material under investigation. The specimens were tested in accordance with the requirements of the standard. The results are given in the table in the form of the average values and the standard deviations.

**Figure IV.23**

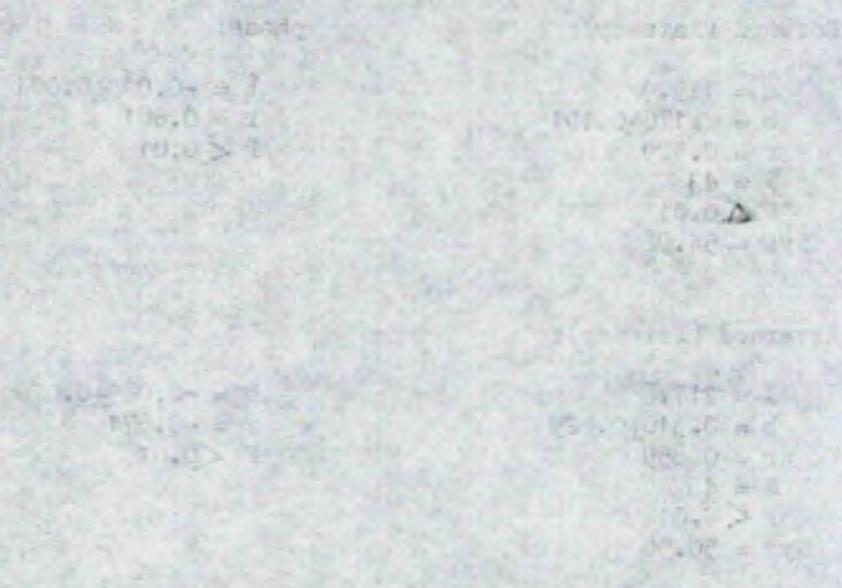


Figure IV.23 The regression on period of  $L_D$  (top) and  $\phi_D$  (bottom) for the flexor muscle.

Note that the slope (top) changes but little during reversed beating. The change in phase angle (bottom) is therefore effected by 'displacement' of the regression (top) rather than 'rotation' about the origin. The results of this is that there is a truly phase locked relationship in forward beating, but in reversed beating, with the intercept now different from zero, the relationship is no longer really phase locked (open circle, bottom).

Forward (latency):

$a = 115.4$   
 $b = 0.170 \pm 0.101$   
 $r = 0.929$   
 $N = 43$   
 $P < 0.01$   
 $S_{yx} = 54.98$

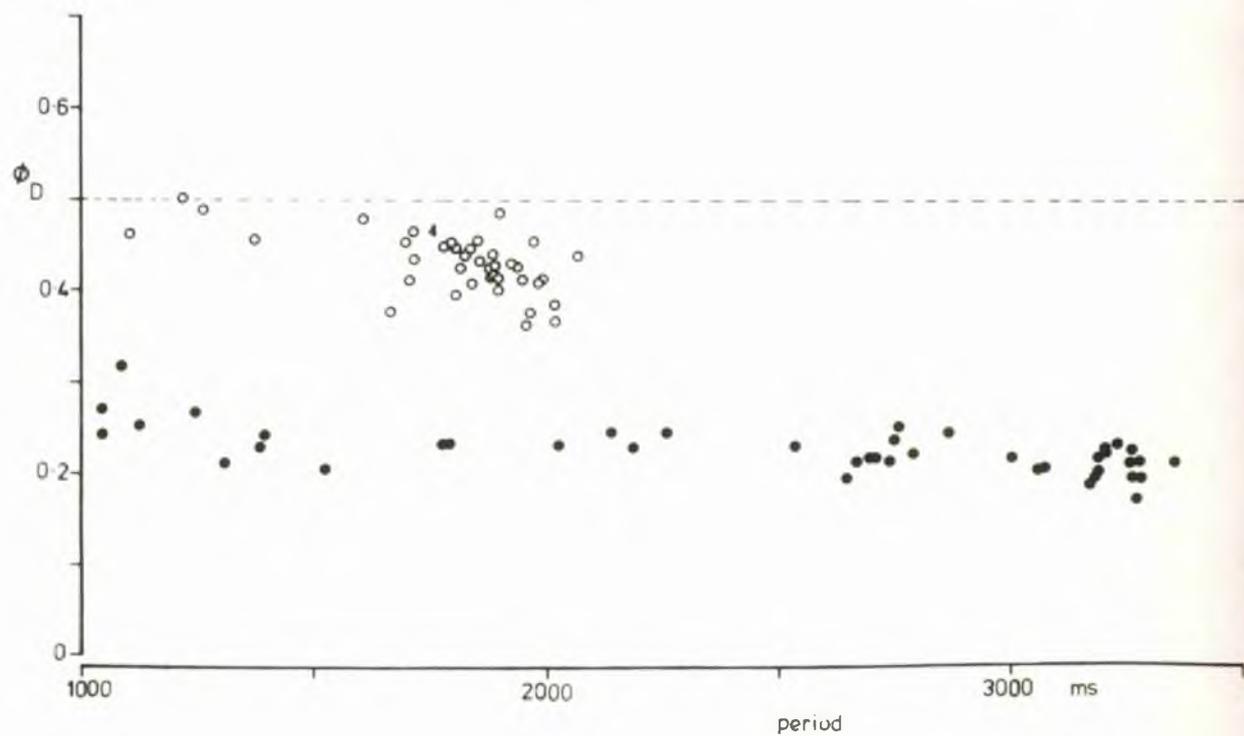
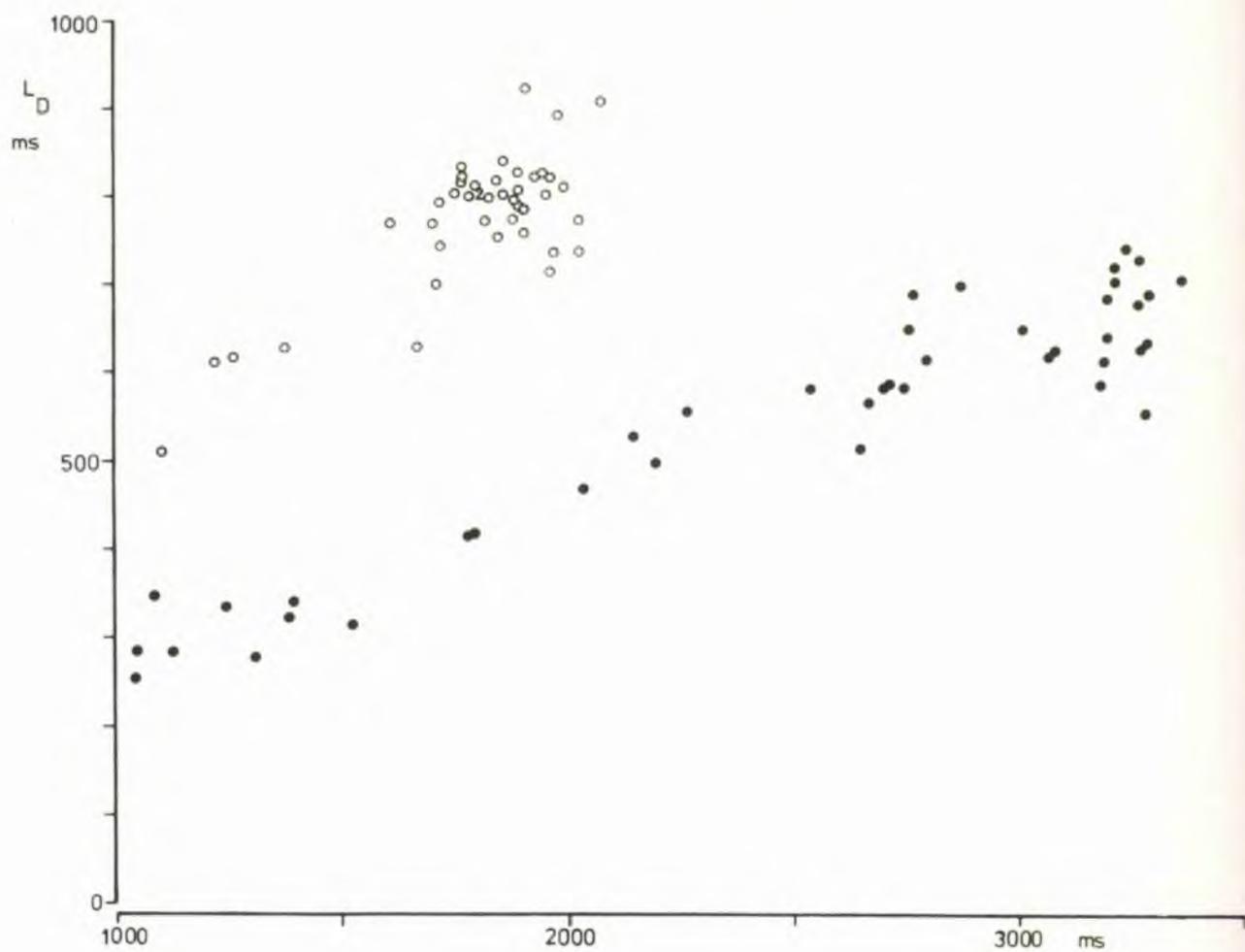
phase:

$b = -0.023 \pm 0.004$   
 $r = 0.681$   
 $P < 0.01$

Reversed (Latency):

$a = 217.8$   
 $b = 0.310 \pm 0.029$   
 $r = 0.859$   
 $N = 43$   
 $P < 0.01$   
 $S_{yx} = 50.50$

$b = -0.089 \pm 0.020$   
 $r = -0.571$   
 $P < 0.01$



Faint, illegible text, possibly bleed-through from the reverse side of the page.

Figure IV.24

Figure IV.24A. The relationship between the regression coefficient of  $\phi_D$  on period as a function of the overall mean period length in the different experiments. Note that with respect to period length the values of the regression coefficient are randomly distributed about zero. The discrepancies in their values must have some explanation which is independent of period length. This is discussed more fully in the text. The most likely explanation would in fact, seem to require that some relationship should exist between  $\phi_D$  and period length.

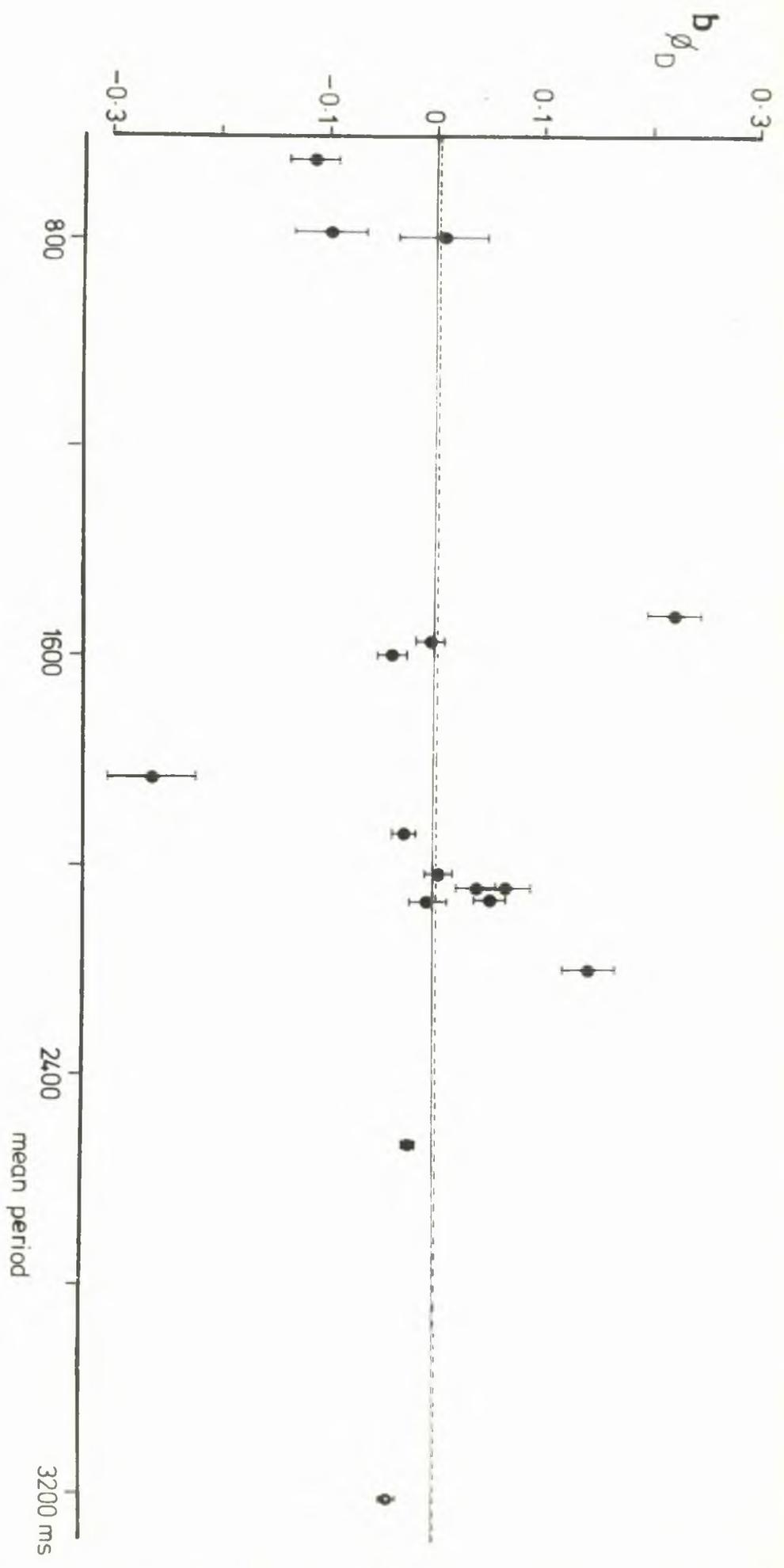


Figure IV.24B shows the relationship between  $\log V$  and  $\log \tau$  for the liquid state during the cooling process. The data points are plotted on a log-log scale. The relationship is linear, indicating a power-law dependence between  $V$  and  $\tau$ . The slope of the line is approximately 1.5, which is consistent with the theoretical prediction for a liquid state. The data points are scattered around the line, suggesting some uncertainty in the measurements. The overall trend shows that the volume  $V$  increases as the relaxation time  $\tau$  increases, which is characteristic of a liquid state.

**Figure IV.24B.**

Figure IV.24B. The relationship between  $b(\phi_D)$  and mean  $\phi_D$  for the flexor muscle during forward beating. There is not a significant correlation between these two variables. Comparison with Fig. IV.24, A however, does suggest that there is a case for arguing that there is an optimum mean value of  $\phi_D$  for the flexor burst. At this optimum,  $b(\phi_D)$  (the regression coefficient of  $\phi_D$  on period), is zero. At phase angles greater or less than the optimum, the values of  $b(\phi_D)$  show increasingly great tendencies to deviate from zero. This might mean that such 'random' variations away from the optimum  $\phi_D$  are probably associated with displacement of the duration period curve, rather than rotation about the origin: i.e. a change from zero in  $a$  in the equation  $L_D = a + b(\text{period})$ .

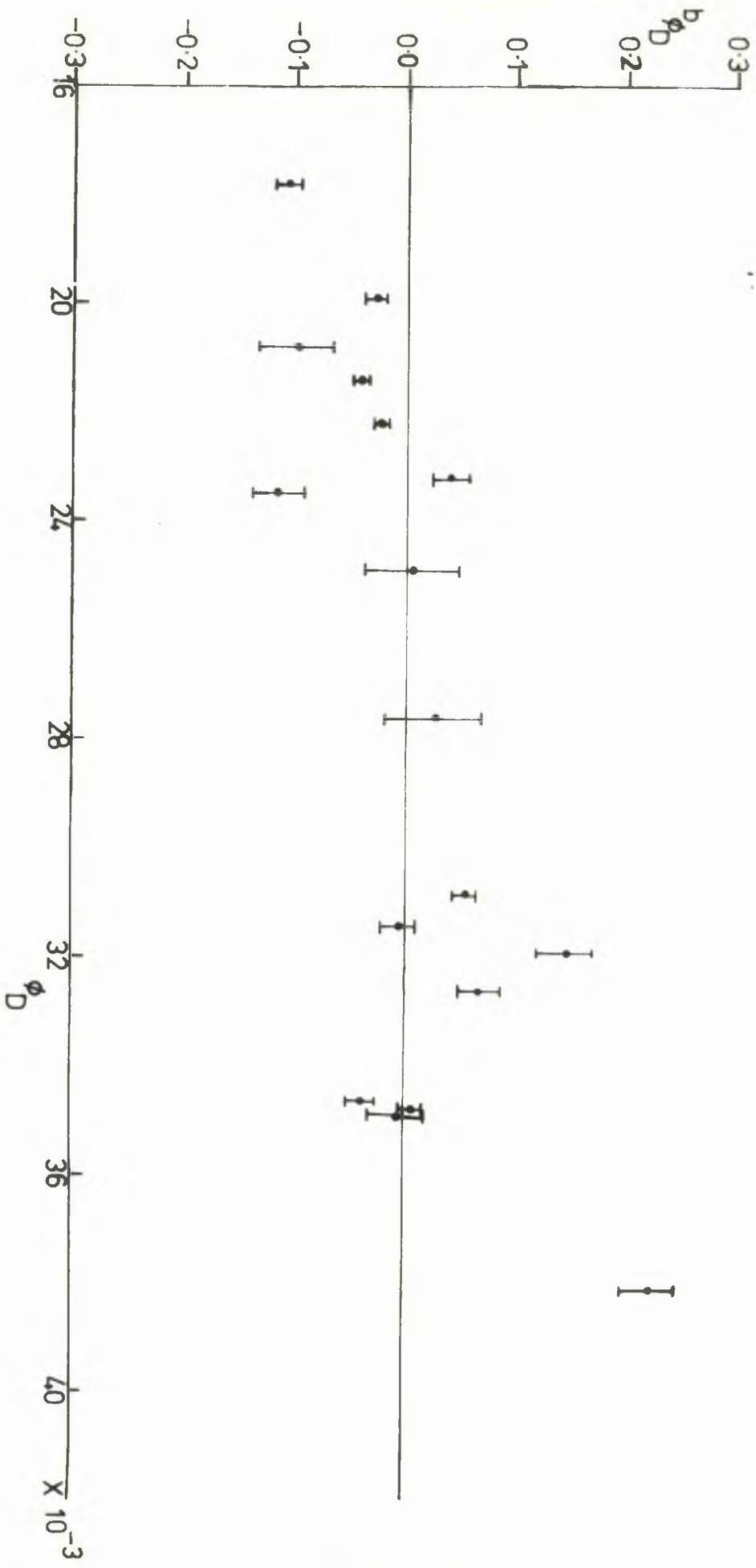


Figure IV.25 shows the results of the analysis of variance for the data presented in Figure IV.24. The analysis of variance is presented in Table IV.10. The results of the analysis of variance are as follows:

Table IV.10  
Analysis of Variance

Figure IV.25

Table IV.11  
Analysis of Variance

Figure IV.25. The regression of  $\phi_D$  on period for the quartus muscle. Note the small change in the mean phase angle occupied by the burst during forward and reversed beating. This change shown here is perhaps greater than the norm, but is still small with respect to that in the flexor (Fig.IV.23).

**Forward:**

$$\begin{aligned} b &= 0.00 \pm 0.01 \\ r &= 0.072 \\ N &= 42 \end{aligned}$$

**Reversed:**

$$\begin{aligned} b &= 0.12 \pm 0.03 \\ r &= 0.584 \\ N &= 43 \end{aligned}$$

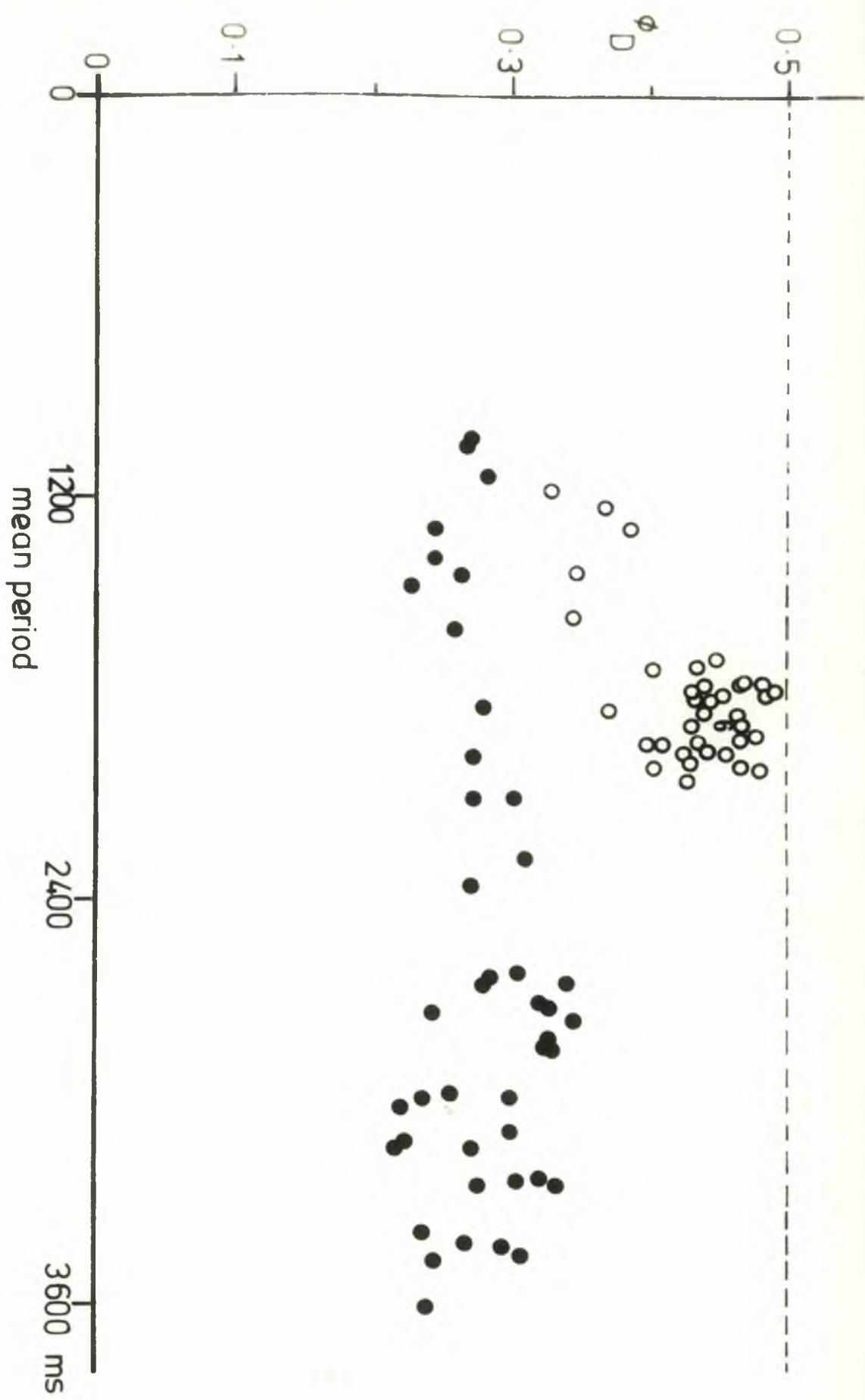


Figure IV.26 shows the results of the analysis of variance for the dependent variable of the level of the dependent variable. The results are presented in Table IV.26.

Table IV.26

Source of Variation	Sum of Squares	df	Mean Square	F	p
Between Groups	10.00	2	5.00	1.00	.37
Within Groups	10.00	18	.56		
Total	20.00	20			

Figure IV.26

Source of Variation	Sum of Squares	df	Mean Square	F	p
Between Groups	10.00	2	5.00	1.00	.37
Within Groups	10.00	18	.56		
Total	20.00	20			

The results of the analysis of variance are presented in Table IV.26. The results show that there is no significant difference between the groups ( $F = 1.00, p = .37$ ).

Figure IV.26. The relationship between period length and the duration of the burst in the secundus during forward (o) and reversed (o) beating.

Top: Duration ( $L_D$ ) against period.

Forward:

$a = 359.6$   
 $b = 0.125 \pm 0.022$   
 $r = 0.570$   
 $N = 69$   
 $P < 0.01$   
 $Syx = 52.87$

Reversed:

$a = -78.28$   
 $b = 0.765 \pm 0.05$   
 $r = 0.965$   
 $N = 14$   
 $P < 0.01$   
 $Syx = 31.39$

Bottom: Phase angle of duration ( $\phi_D$ ) against period.

Forward:

$b = -0.03 \pm 0.02$   
 $r = -0.220$   
 $N = 69$   
0.10  $P < 0.05$

Reversed:

$b = 0.05 \pm 0.05$   
 $r = 0.248$   
 $N = 14$   
 $P < 0.10$

Note: One point at  $\phi = 165$  period = 3958 is omitted from the plot but was included in the analyses for forward beating.

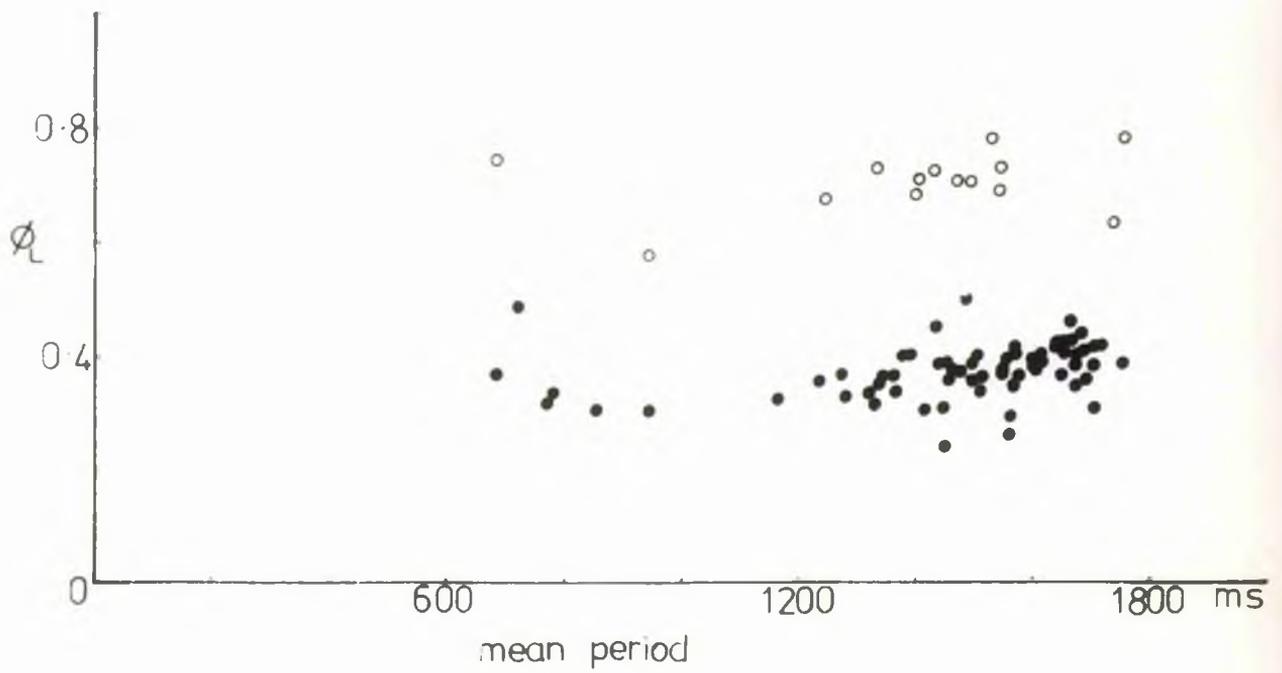
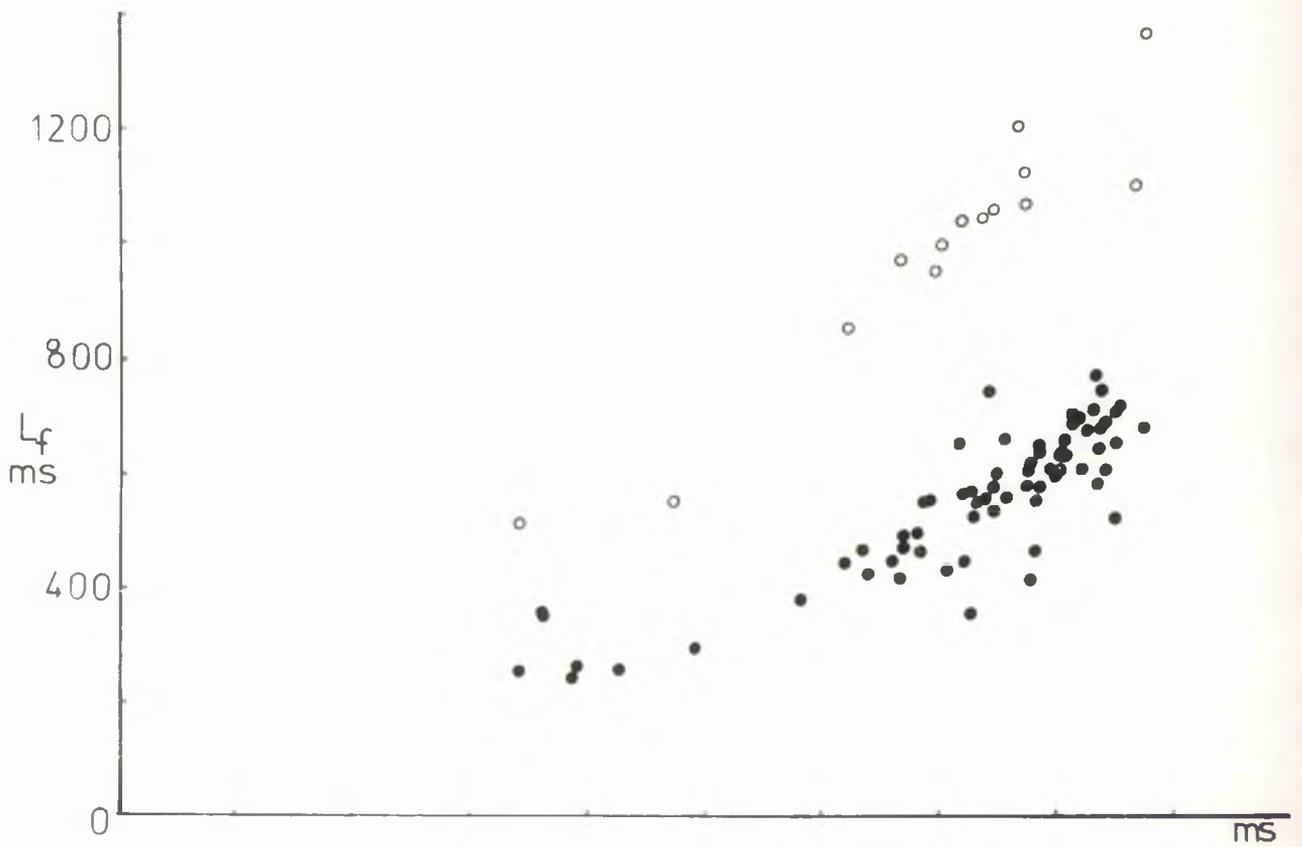


Figure IV.27. The relationship between the number of...  
the curves. Note that the overall relationship appears to  
be... in fact the second order relationship  
... as indicated by the slight...  
...-on

Figure IV.27

Figure IV.27. The relationship between  $L_p$  and period for the primus. Note that the overall relationship appears to be curvi-linear. In fact the second order regression coefficient appeared to be significant. See Fig.IV.28 however.

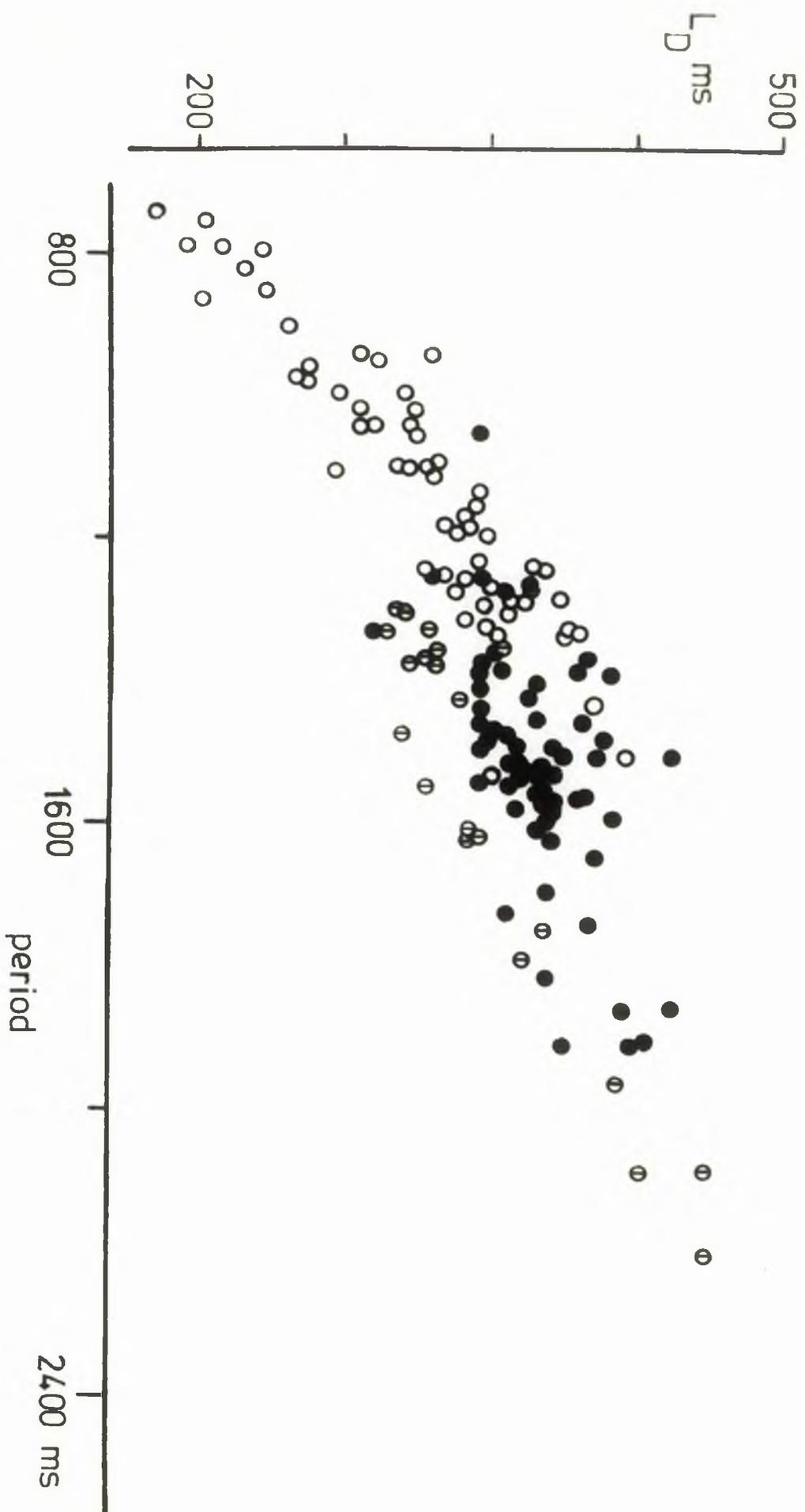




Figure IV.28. Comparison of the regressions of  $L_D$  on period for the primus muscle during three different types of activity, as shown in the insets.

Top - reversed beating, low level of activity in the accessory promotor. The last 'spike' coinciding with the start of the flexor burst in this and the middle strip, is probably a movement artefact associated with the operation of the 'click' mechanism.

Middle - Same, but with strong activity in the accessory promotor. Note the changes in the intensity and the starting position of the primus burst.

Bottom - Forward beating in the same experiment. The electrodes have not been moved. There is no activity in the accessory promotor. Note the change in the position in the cycle of the primus relative to the flexor.

Particularly clear in the top strip is the discontinuity in the burst structure of the primus where it overlaps (perhaps starting just before the start of the flexor burst) with the flexor burst. It may be that a different motoneurone or motoneurones take over at this point.

In all the film strips, the top trace refers to the flexor, the middle to the primus and the bottom to the accessory promotor. The time mark in each case is 500 ms.

The respective plots illustrate the relationship between burst duration and period length in each of the modes of activity. Fig.IV.27 shows these points plotted together on the same grid.

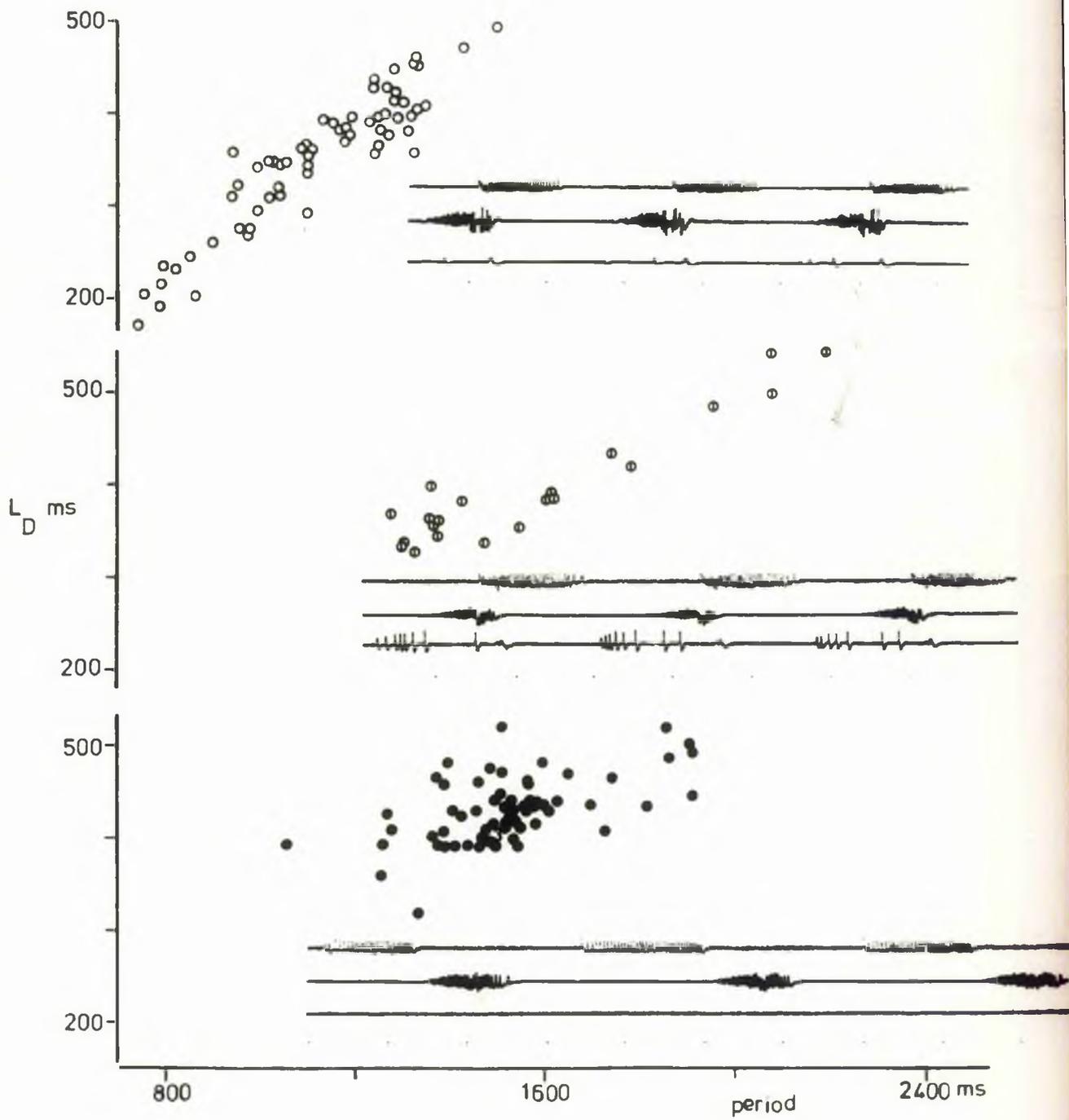




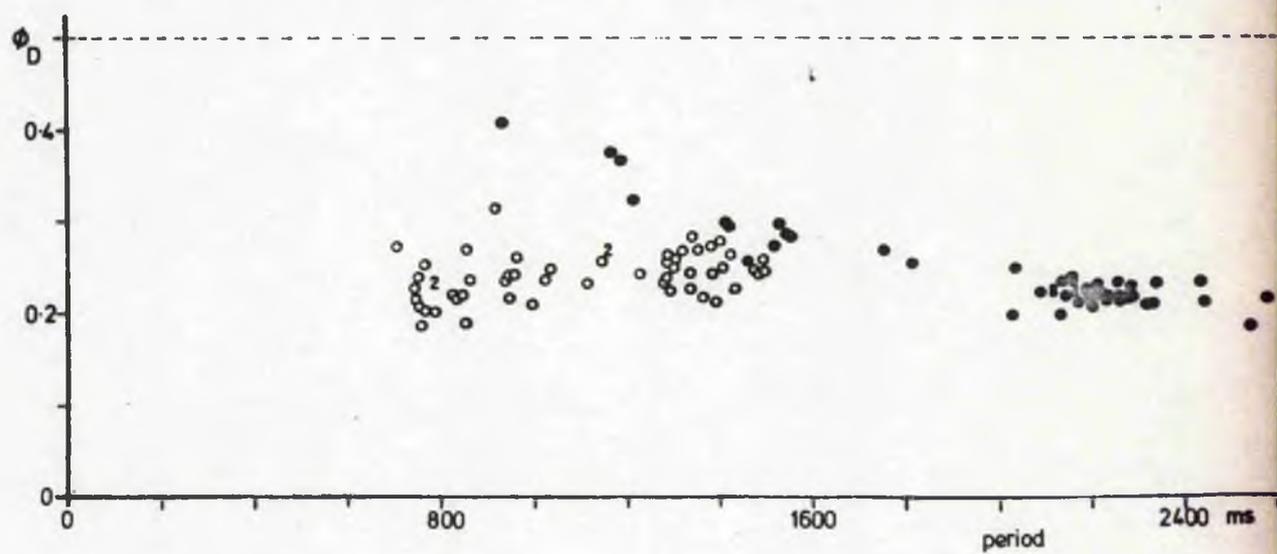
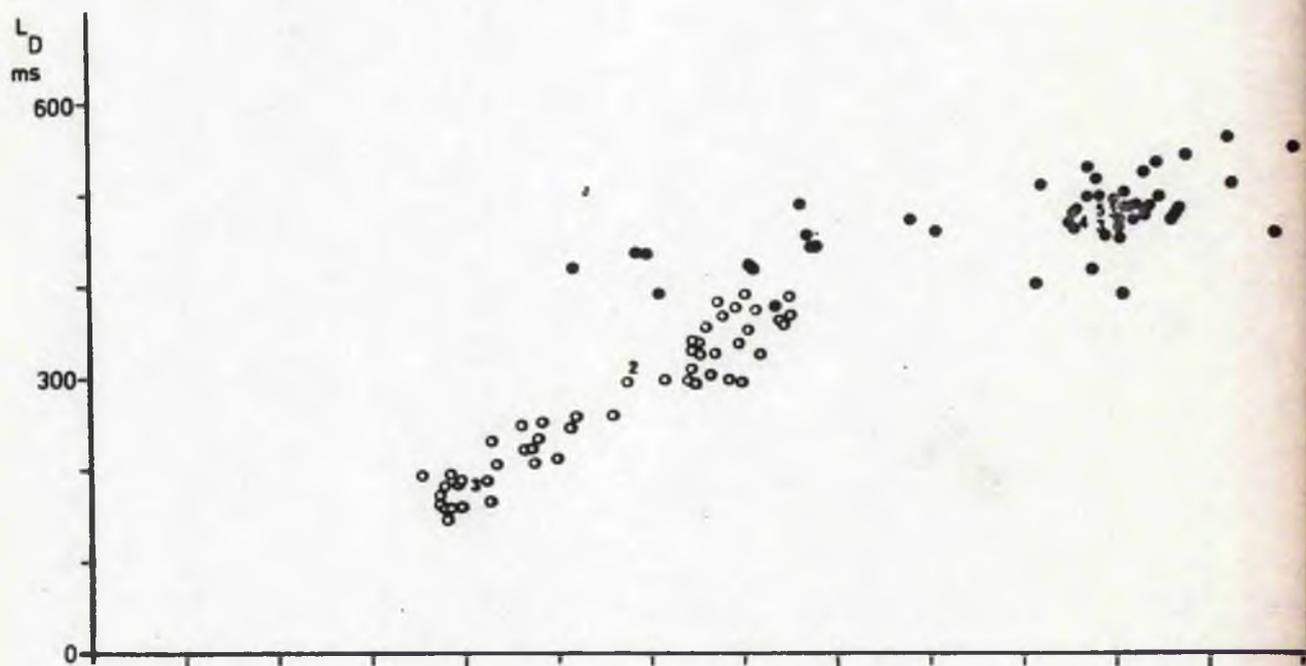
Figure IV.29. The relationship between burst duration and period length in the remotor muscle. Note that in forward beating, above a critical period length, the relationships appear to be the same as during reversed beating. At shorter period lengths however, the nature of the relationship apparently changes, and the duration of the burst becomes somewhat independent of period length.

Forward:

$a = 335.8$   
 $b = 0.067 \pm 0.010$   
 $r = 0.663$   
 $N = 64$   
 $P < 0.01$   
 $S_{yx} = 17.24$

Reversed:

$a = -40.11$   
 $b = 0.288 \pm 0.028$   
 $r = 0.806$   
 $N = 59$   
 $P < 0.01$   
 $S_{yx} = 56.75$



IV.5. The movement of the scaphognathite in relation  
to the pattern of activity in the muscles.

IV.5.1. Results

The complicated skeletal arrangements, the anatomical organization of the musculature, and the temporal patterning of the activity in the muscles, together subserve one ultimate goal. This is the generation of an effective pumping action in the scaphognathite. The present chapter describes the movements of the scaphognathite in relation to the overall, mean pattern of muscular activity as reconstructed using the data presented in the preceding chapters.

Fig.IV.30A combines records from a single experiment in which the movement monitor (Section II.2.3) was located at different points on the scaphognathite blade. The preparation was dorsally dissected; the reference muscle was the promotor; and the pre-branchial chamber was left undisturbed, except for small perforations in the thin, transparent dorsal wall, allowing placement of the movement monitor.

Fig.IV.30C combines similar records from a ventral preparation. Of necessity, the ventral extremity of the movement was disturbed by removal of the mouthparts anterior to the scaphognathite, and of portions of the branchiostegites overlying the scaphognathite in its ventral aspect. This was to permit access to the reference muscle, the flexor, and to allow placement of the movement monitor. Dissection was kept to a minimum so that ventral movement of the scaphognathite was still restricted by portions of the branchiostegites which were left intact. Note that the beautiful symmetry of the movement of the internal tip is all but lost. There are nonetheless more elements of similarity in the movement of the tip in Figs.IV.30 and IV.32 than between these figures and Fig.IV.8C in which the scaphognathite was free to move to its fullest extent dorsally. The

movement of the hind end was apparently less drastically altered by dissection, and compares reasonably well in Figs.IV.30 and IV.32.

In one minimally dissected, ventral preparation the dissecting technique seems to have been particularly effective. The movement of the internal tip (Fig.IV.31B) was almost identical to that in Fig.IV.30A. This record serves to highlight the strong symmetry which can be manifested in the movement of the internal tip of the scaphognathite during forward beating. It shows too, that the promotor and flexor sub-groups probably fulfill equivalent functions during the upstroke and downstroke respectively, of the scaphognathite. The rising phase of the movement trace in Fig.IV.31A is associated with dorsal movement of the scaphognathite, and in Fig.IV.31B with ventral movement. The shapes of the curves can scarcely be distinguished. Moreover, the sharp movement artefact seen at about the mid-point of the promotor cycle associated with the sharp depression of the tip in Fig.IV.31A now appears in Fig.IV.31B at a similar point in the flexor cycle in association with the sharp elevation of the tip. As mentioned previously, this artefact is probably related to the passing of the 'click point'. In some experiments, the movement artefact was most prominent during ventral movement of the tip; in others it was most marked during dorsal movement; in some, it occurred twice in each cycle, during both dorsal and ventral sweep of the internal tip. In Fig.IV.33 the artefact can clearly be seen to occur during pronation - that is, rotation about the radial (PS) axes bringing the front end down, and the hind end upwards.

In spite of the anatomical asymmetries in the depressor and levator musculature then, it appears that the activity in the muscles is so structured as to produce a very symmetrical movement, at least

of the tip of the scaphognathite during forward beating. Similar symmetries were never observed at other points on the scaphognathite.

From Figs. IV.30 B,C and IV.32 it is clear that the movements of the other parts of the scaphognathite are in general not at all smooth. They are apparently composed of sequences of jumps, and sharp, irregular movements, induced presumably by the activity in the various muscles. In some of the records, definite cyclically recurring points were identified on the movement traces. Analyses were conducted of the time of occurrence of these points in the cycle of the reference muscle (flexor or promotor) with respect to period length. As might be expected, the times were significantly dependent on period length except in the case of events roughly synchronous with, and apparently induced by the activity in the reference muscle.

The results of the above analyses, expressed as phase histograms are related in Fig. IV.33 to the average pattern of activity in the muscles. The latencies and durations of the activity in the different muscles were calculated to give the best fit to all the data presented in the previous chapters. The indicated minimum limits for the starting latencies represent the minimum mean values of  $\phi_L$  from all the experiments on the particular muscle, minus the standard error. The indicated maximum limits represent the maximum mean  $\phi_L$  plus the standard error. Unfortunately, in the interest of economy, analyses were not done on the times in the cycle at which the bursts ended. These therefore had to be estimated from the latencies and burst durations. The limits for the ends of the bursts then, represent the minimum minus one standard error and the maximum plus one standard error of the mean values for  $\phi_D$ . Their positions on the graph were allocated with respect to the position of the average starting latencies.

The positions of the peaks of the histograms in relation to the

muscle bursts bring out some interesting points. Firstly, the start of pronation of the internal tip occurs about midway through the flexor burst, and is probably actuated by the start of activity in the sixtus. The onset of pronation in the external tip is somewhat delayed, occurring about midway in the sixtus burst, and at about the end of the flexor burst. At this point, the activity in both these muscles is maximal (Section V.1). The delay in the rise of the hind portion of the scaphognathite until the hinge and internal segment have already started to fall is important in effecting efficient forward pumping. It ensures that there is a minimal tendency for water to be pushed back into the branchial chamber during the downward sweep of the internal segment. It therefore enhances the forward propulsion of water out of the exhalent channel during the downstroke of the appendage.

During the upstroke also, supination is seen in Fig.IV.33 to begin in the internal segment shortly before the external. The effect obtained is the same, so that the effectiveness of forward pumping on the up and downstroke are enhanced by a similar method. The start of supination is associated with the start of activity in the promotor sub-group, and in particular in the promotor. The promotor therefore appears to serve as an effective antagonist to the sixtus. It is not fully clear what might be the role of the quintus, which on anatomical grounds may be expected to be a more likely antagonist to the promotor. However, pronation does start just towards the end of the quintus burst when the spiking frequency in the muscle appears to be maximal (Section V.1).

'Protraction', a slight rise in the whole appendage just after the end of pronation is seen to be associated with the activity in the primus sub-group, whilst retraction, a similar slight fall after the end of supination, is associated with the start of activity in the quartus sub-group. These two sub-groups therefore perform antagonistic

functions, as might be expected from the anatomy of the musculature.

Fig.IV.34 shows superimposed movement traces from three different points on the scaphognathite, in relation to the average activity in the muscles. This gives a broader, but less detailed picture of the relationship, and is more readily interpreted in terms of the movement of the appendage. The sequence of movements can clearly be seen to be as follows: As the hinge region slows towards the middle of its dorsal movement, the internal tip starts to rise rapidly following the hinge region (the middle of the blade along the radial axis), which reaches its peak only just before the tip. As the hinge reaches its peak, the 'click point' is passed, and the external tip (hind end) rapidly falls. This must be accompanied by some bending about the hinge between the scaphognathite segments since there is but a small drop in the axis and none in the internal tip. It appears that there might at least on occasion, be a brief moment when both the hind and tips and the hinge may all be at the dorsal extremity of the range, and the exhalent channel would be open. This occurs just before the fall of the hind end. The depression of the hind end is followed shortly by a drop in the axis associated with activity in the quartus sub-group. At the start of this drop, the internal and external tips also become slightly 'retracted'. At about three-quarters of the way through the fall, the front end also starts to fall but more rapidly, so that both hinge and internal tip reach the ventral limit of the movement together. There follows a brief period when hind end, tip and hinge are again roughly in line, this time at the ventral extremity of the range, and the branchial chamber is again very briefly open. Almost immediately the hind end flips up into a dorsal position, followed by the hinge and then the internal tip. The cycle then repeats.

The movements of the internal and external extremities of the

scaphognathite are therefore rather like two trapezoidal waveforms about 90 degrees out of phase. They intersect at their maxima and minima and spend most of the cycle at opposite extremes of the range. In a very rough sense also, the movement of the radial axis may be idealized as a sinusoidal wave with its maxima and minima coinciding with the points of intersection of the trapezoidal waves. This idealization is shown in Fig.IV.35.

The movement is clearly very effective, water being both inhaled and exhaled with equal efficiency in both halves of the cycle. Fig. IV.36 shows how the movement traces of Fig.IV.34 would translate into movement in radial profile of the scaphognathite. Each diagram in Fig.IV.36 corresponds to the positions of the tips and hinge at one of the vertical broken lines in Fig.IV.34. The lines represent 25, 50 and 75 percent of the cycle, as well as two other arbitrarily chosen points. Diagram (f) corresponds to the situation at a phase angle of 1.00, just as the promotor starts contracting. Fig.IV.36 shows that each half cycle consists of two phases. The first is the pivoting of the hind region about the external PS axis through the apex of the ECS, up against the limiting wall, and the second, a similar pivoting of the front about the double, internal PS axis. Water is thus progressively pushed forward, first from below the external segment and then from below the internal segment. The mechanical activity of the scaphognathite in forward beating therefore consists of four phases, corresponding largely with the activity in the muscles of the four sub-groups. The activity in the quartus sub-group is analagous to that of the primus sub-group, in that both effect the movement of the hinge region about a longitudinal axis (PR axis). They are different in that the quartus apparently plays no role in the rapid depression (supination) of the hind end about

the radial PS axis, whilst the elevation (pronation) appears to be effected by the interacting activity of the sextus and the muscles of the primus sub-group.

The activity of the promotor and flexor sub-groups appear to be analogous, being concerned respectively with pronation and supination of the internal tip. They differ significantly in the way in which this is effected.

The interactions during reversed beating have been less well analysed because fewer sessions of reversed beating were observed than were forward sessions. No records of the movements of the different portions of the scaphognathite were obtained. The only movement monitored during reversed beating was that of the internal tip, and this was invariably from ventral preparations in which unrestricted ventral movement of the scaphognathite was permitted.

Fig.IV.37 shows the reconstructed average pattern of muscular activity. This represents only a very rough approximation, again because of limited data. Included in the figure are traces of the movement of the internal tip, and as for forward beating, histograms of identified points on the movement traces in the cycle of the reference muscle, the flexor.

These data, in addition to unmonitored observations, indicate that the different sub-groups perform the same functions as they do in forward beating. The flexor and promotor sub-groups are responsible respectively for rapid lowering (pronation) and raising (supination) of the internal tip. The quartus and primus sub-groups still appear to produce little significant movement in the extremities, but are concerned with depressing or elevating the hinge region whilst the tips lie at the extreme of their range. The sequence of movements appears to be as follows:

With the internal segment of the scaphognathite at the dorsal extreme of its range and the hind end still at its ventral extreme, the flexor sub-group contracts whilst the primus sub-group is still contracting. The effect of this overlapping activity in these two sets of muscles is to cause virtually instantaneous movement of the appendage through the 'click point'. The internal tip starts to move downward, but the extreme internal border has probably not yet lifted from the dorsal wall before the hind end has flipped up into the dorsal position. This movement forces water trapped above the scaphognathite backwards into the branchial chamber, and simultaneously pulls water in below the appendage via the normal exhalent channel. It also enhances the downward movement of the internal tip which rapidly reaches the ventral limit of its movement. The quartus sub-group is then activated, depressing the hinge region, forcing the water trapped under the blade backwards, and pulling water in above it. The promotor sub-group then contracts, overlapping with the quartus sub-group. This causes the internal tip to rise but also results in rapid passage through the 'click point' so that the hind end flips down rapidly before the internal tip has risen significantly. The internal tip continues its dorsal movement under the continuing contraction of the primus sub-group, and then the hinge is raised bringing the whole internal segment up against the dorsal roof of the exhalent channel. The cycle then repeats with the overlapping contraction of the flexor sub-group. The histograms in Fig.IV.37 show that the depression of the internal tip is under the action of the flexor sub-group, and so also is the breaking through the click point which rapidly follows. The corresponding click on the upstroke is associated with activity in the promotor sub-group, and the slight protraction in Fig.IV.33 with the onset

of activity in the primus sub-group.

#### IV.5.2. Interim Discussion

In Section III a 'click' mechanism associated with the movement about the radial axes of the scaphognathite was described. From a study of the skeleton it did not appear that this mechanism could contribute much to the forces generated by the muscular activity. It might be worthwhile reconsidering this mechanism now that the movement of the scaphognathite and the activity in the muscles have been described. Fig. IV.34 shows that the hind end of the scaphognathite is rapidly depressed as soon as the activity in the primus sub-group stops. This seems odd at first, but when considered in conjunction with Fig. III.1, the mechanism seems clear. While the secundus is contracting, it pulls on the softer cuticle of the ACS where it joins the ECS. This brings the CLS into such a position that the line through its articulations with the DBS and ECS passes through the body of the sclerite, which acts like a wedge, preventing the rotation of the ESG about the apex of the ECS in response to the contraction of the promotor and remotor. However, the flexibility of the skeleton allows the hinge region where the pull of these muscles is concentrated, to be raised while the hind region remains elevated. The strong overlap between the contractions in the primus and promotor sub-groups will also contribute to this, since the synchronized levation of the ECS on the PR axis, and supination of the ESG about the external radial axis will tend to keep the external tip pressed against the roof of the exhalent channel while the hinge is raised. The click mechanism therefore may merely provide an added safety factor in this context. In addition some of the muscular effort is stored in the elasticity of the skeleton. As soon as the secundus stops firing, the pull on the ACS is released and the CLS is allowed to turn. The energy stored in

the skeleton is rapidly released, and with the continued contraction in the promotor and remotor, causes the hind end of the scaphognathite rapidly to flip down, marginally before the start of the depressor session. Observations on the active appendage indicate that the click mechanism may indeed operate as described, but that at times it may not.

During the downstroke, the synchronized contraction of the quartus, tertius and quintus should have the same effect as the overlap between the primus and promotor sub-groups during the upstroke. The effect would be to keep the external tip pressed against the floor of the exhalent channel as the hinge is lowered. At the extreme supinated position of the ESG the CLS is folded back against the ACS. It appears that it may be difficult for contraction of the depressor muscles to turn it back out of this position. In Fig. III.44 then, it is not until the primus sub-group muscles start to contract that, with the continued contraction of the sixtus, the hind end is allowed to flip up. As on the upstroke, this mechanism may, or at times, may not operate. At times the contraction of the sixtus may be sufficient to cause pronation of the external tip. This probably accounts for the slightly earlier incidence of the click point during pronation in the analyses in Fig. IV.33. The click here might simply be associated with the instabilities described in chapter III. A distinction might therefore be made between an active and a passive 'click' mechanism.

It is interesting to speculate that the active mechanism may be more likely to occur at higher beat frequencies when the inertia of the appendage is more likely to carry the ESG into an extreme, locked position prior to pronation, and to cause 'wedging' prior to supination. This would result in a slight delay in the rise and fall of the external tip at faster beat rates, but would also cause a more

explosive movement when it did occur. The effectiveness of the stroke could therefore be enhanced. In this context then, a study of the volume of water pumped per stroke at different beat frequencies might be of some interest.

From the schematic conceptualization of the movement in Fig. IV.35 it can be seen that theoretically, reversal of the beat direction may be achieved by a shift of the sinusoidal wave (hinge movement) through a phase angle of 0.5 ( $180^{\circ}$ ) with respect to the movement of the front (internal tip). A much smaller relative shift of the trapezoidal wave for the movement of the hind end (external tip) is required, so that the point of alignment of the hind end, hinge region and front end occurs at the completion of levation and depression of the hind end (Fig. IV.35B) rather than just before as in forward beating (Fig. IV.35A). Examination of Figs. 12, 13, 16 and 17B. (Chapter IV.1.3.) shows that the pattern generator probably effects reversal in just this way. The onset of activity in the muscles mainly responsible for the depression (quartus sub-group) and levation (primus sub-group) of the hinge can be seen to shift through a phase angle of the order of 0.5 in going from the forward to the reversed mode (Figs. IV.13 & 16). Those concerned with pronation and supination shift through a much smaller relative phase angle (about half that for the previous set) (Figs. IV. 12, 17B). The actual angle of the shift required in these muscles (promotor sub-group) will clearly depend on the steepness of the falling and rising portions of the trapezoidal waves. In the effecting of this change, the sequence of recruitment of the sub-groups changes from flexor-promotor-primus-quartus (F-P-1-4) to flexor-quartus-primus-promotor (F-4-1-P), so that there is an entire reversal of the sequence of recruitment of the sub-groups.

In reversed beating then, the different muscles function in similar groupings as in forward beating. Only a change in their

relative positions in the cycle, and extents of overlap is sufficient to produce effective reversed pumping. Numerous other changes do however accompany reversal, one of these being a marked change in the fine structure of the bursts in some of the muscles. In some cases, it appears, even the temporal structure of the frequency of spiking in the burst might undergo nearly perfect reversal. The role that the fine structure of the burst may play in regulating scaphognathite movement has not yet been described, although this has been anticipated in the cases of the flexor and sirtus during forward beating. In the next chapter then, the fine structure of the bursts will be described in relation to its role in controlling the movement of the scaphognathite, as well as to the light it might cast on the underlying central pattern generator.

Figure IV. 30 to 32

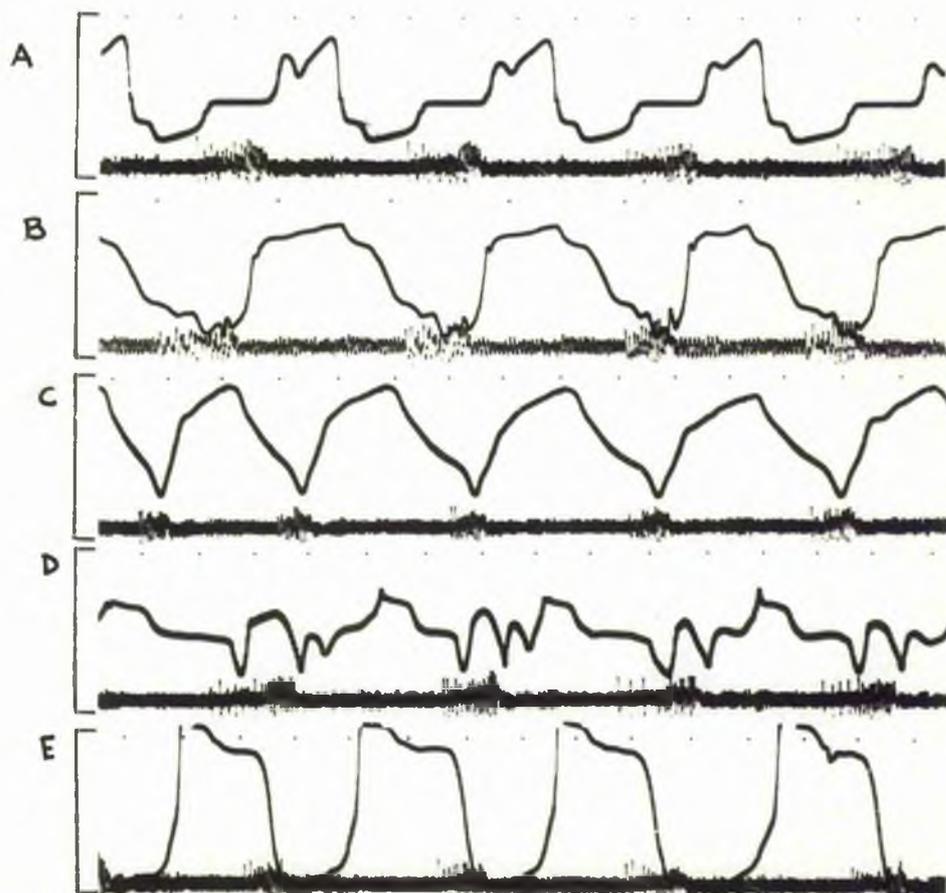
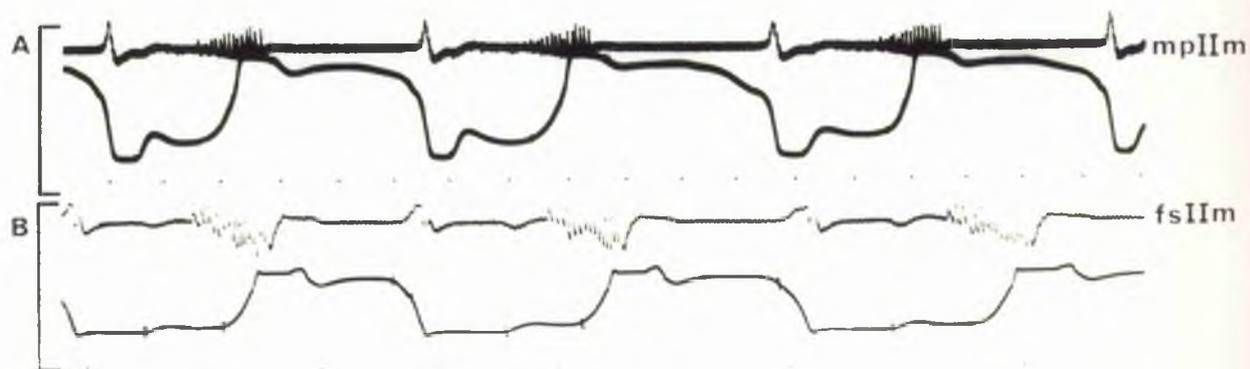
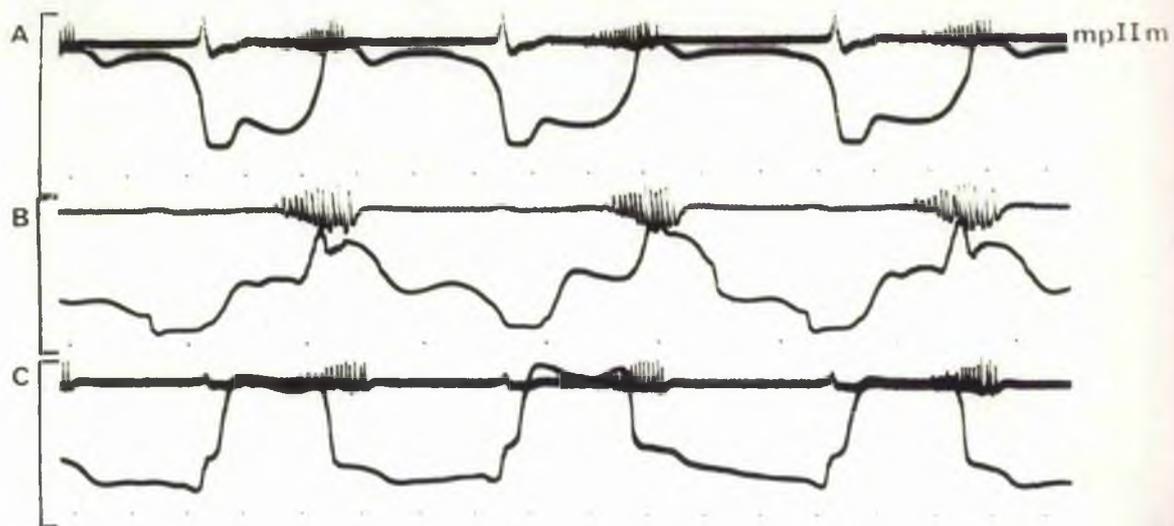
Figures IV. 30 to 32

Figure IV. 30 to 32

Figure IV.30. Movement of the scaphognathite in relation to the bursts in the promotor muscle (mpIIa). A - movement of the internal tip. B - movement of the hinge region. C - movement of the hind end. The promotor bursts in the different strips have been aligned as well as possible to allow the different wave forms to be directly compared. All records were taken during a single experiment with one animal. Notice the repeating pattern: Front down - back up, hinge up front up - back down; hinge down; Front down back up ..... This might be compared with Figs.IV.34 and 35. Time mark: 500 ms. Upward movement of monitor corresponds to levation.

Figure IV.31. Comparison of the dorsal and ventral movements of the internal tip, with reference respectively, to the promotor and the flexor. In A, a rise of the movement trace corresponds to dorsal (upward) movement of the scaphognathite. The reference muscle is the promotor. In B, rise of the movement trace corresponds to depression of the scaphognathite. The reference muscle is the flexor. When displayed in this manner, the ventral going and dorsal going movements of the tip are clearly seen to be of almost indistinguishable form and relative time course (cf. Fig.IV.30A also). Moreover, the relationships of these movements to the flexor and promotor respectively show that these muscles must at least in part, perform corresponding antagonistic functions during depression and levation of the scaphognathite. Time mark: 500 ms.

Figure IV.32. The movement of various points on the scaphognathite with reference to the burst in the flexor muscle. A - hind end. B - pivot at apex of ECS on the external PS axis. C - hinge region. D - angle of the PPS. E - internal tip. Upward movement of monitor corresponds to levation. Notice the extremely irregular movement in D. There is a drop during the phase when the quartus-quintus-tertius could be active; a brief rise as the flexor picks up, perhaps as a result of rotation of the interval segment about the internal PS axis. This is followed by another dip as the sixtus takes over, followed by an irregular rise during the period when the levator muscles would be active and a corresponding fall once more when activity in the quartus sub-group would be expected to start. The movement of the pivot point in B is somewhere between that for the hind end (A) and that for the hinge (C) the other movements have been discussed more fully at other times. Time mark: 500 ms.



The first part of the report deals with the general situation of the country and the progress of the work done during the year. It also mentions the various committees and their work.

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Figure IV.33

The fourth part of the report deals with the work done by the various committees and their work during the year. It also mentions the various committees and their work.

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Figure IV.33. The average pattern of the bursts in the ventilatory muscles reconstructed as described in the text. Forward beating. Compared against this pattern are phase histograms for the occurrence of identifiable points in the movement cycle. Top - Movement of the front end. Middle - Movement of the hinge. Bottom - Movement of the hind end.

- Prn:** Start of pronation. That is, start of depression in the hinge and front end, and of levation in the hind end; Movements resulting in twisting of the scaphognathite about its radial axis.
- cl:** click artefact: Already discussed artefact seen on EMC's as well as in the movement traces where it can usually be seen as a clear discontinuity. Its occurrence is apparently associated with the releasing of the 'click' mechanism and the fast rise or fall of the hind end. Only one click is shown in these cycles, during the depressor phase.
- Sup:** Supination: the reverse of pronation.
- Prt:** Protraction a slight rise in all parts of the appendage associated with onset of activity in the levator muscles.
- Ret:** Conversely an overall drop associated with onset of activity in the depressors.
- Pau:** A pause seen in the rapid pronation of the hind end. The Pause occurs about midway in the total movement.

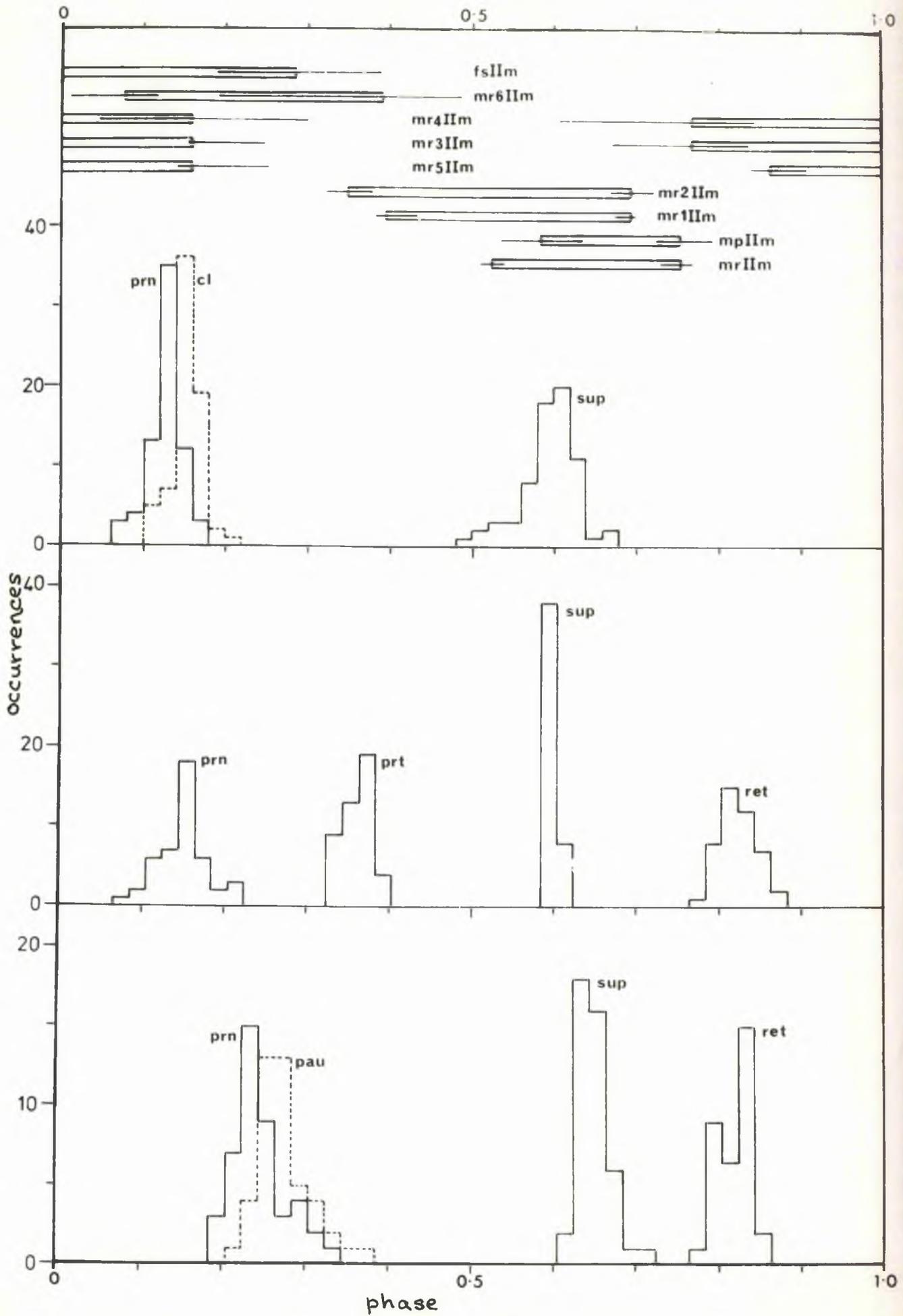




Figure IV.34. The average pattern in the ventilatory muscles as seen in Fig.IV.33, compared with the movement of the front end (solid movement trace), hinge (dotted) and hind end (dashed). The horizontal line *m* represents the mid point about which the excursions occur. Each curve represents the best fit by eye the movement curve from several cycles superimposed by projection and differential enlargement. All data for movements from a single animal in one experiment.

Note the alternating movements of the interval (front) and external tips, and the slower movements of the hinge region.

In spite of the fact that the hinge and hind end are apparently rigidly linked across one of the major radial movement axis, note the large excursion of the hinge whilst the hind end remains stationary at the ventral extremity of its range. This is effected by the co-ordinated activity in the tertius and quartus. As the ECS is lowered due to contraction of the quartus, the PrS pivots about the apical axis, presumably as a result of the activity in the tertius. The combined action results in twisting of the external segment about the point where it meets the ventral wall of the branchial chamber, so that the hinge is lowered forcing water forward. This is then followed by the activity in the flexor and sixtus which lower the interval tip and in the case of the sixtus, contribute to releasing the click mechanism and causing the hind end to rise.

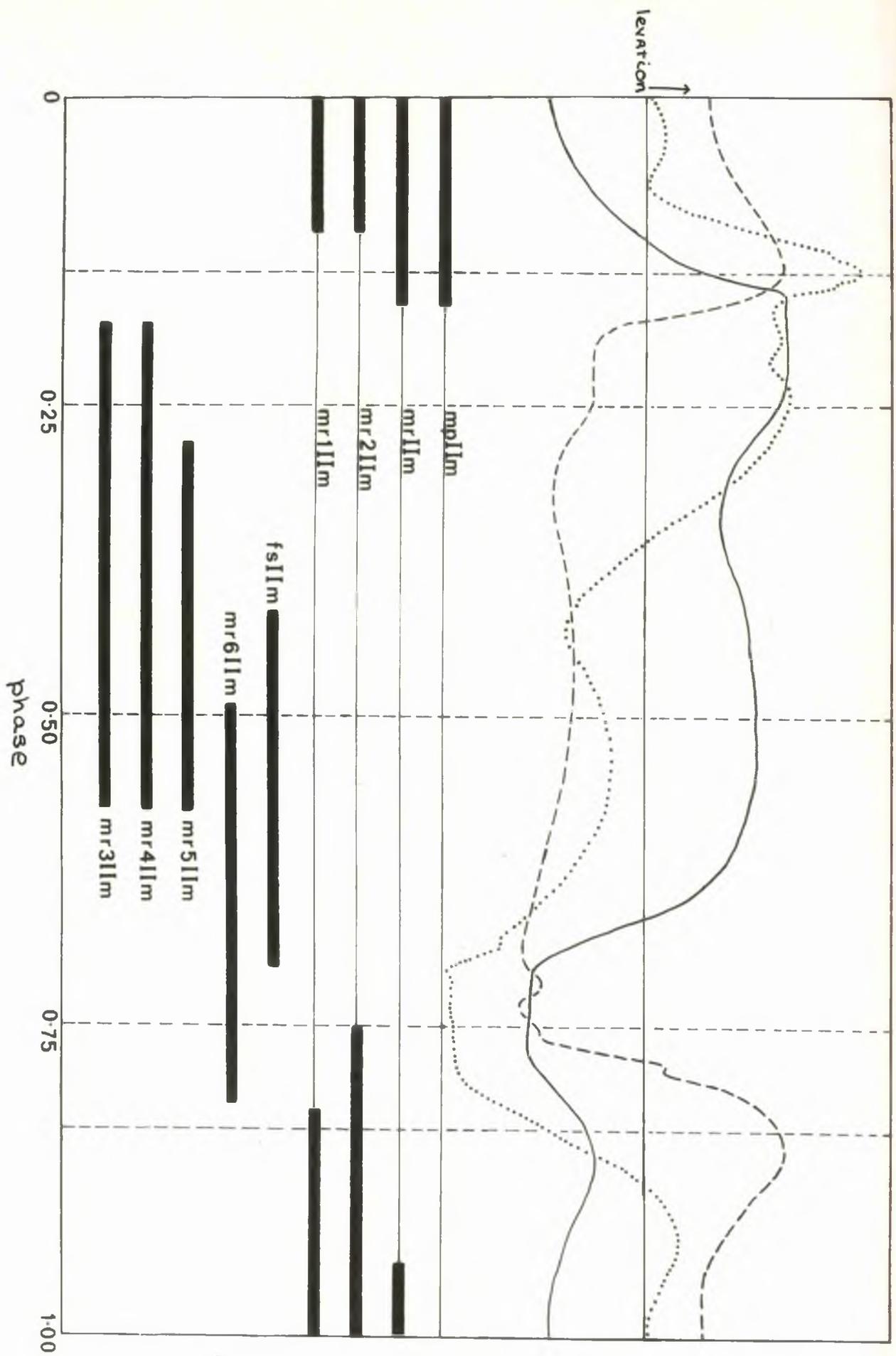
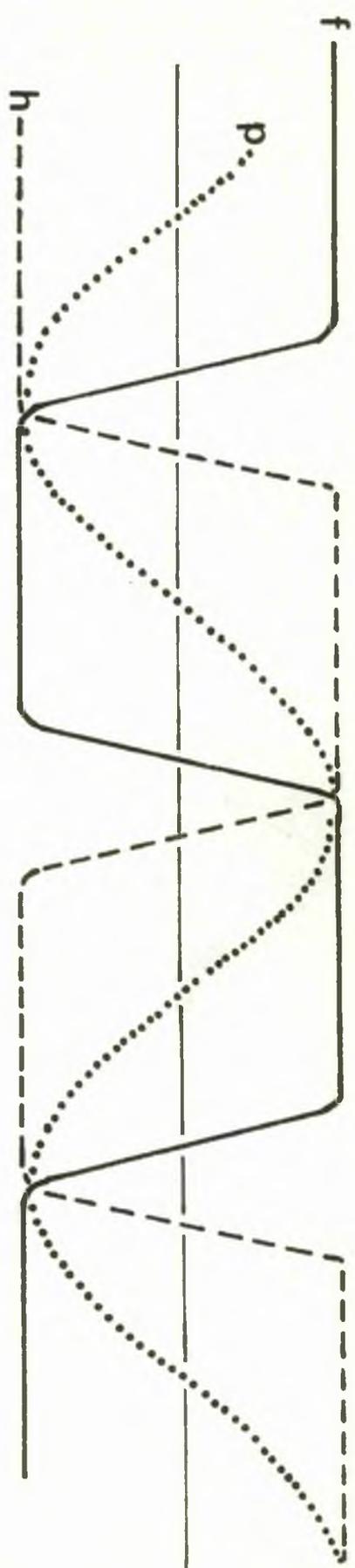




Figure IV.35. Schematic conceptualization of the movements of the front end (f), hinge (p) and hind end (h) of the scaphognathite during forward and reversed beating. The curve for the front end is fixed and the others displaced with respect to it in order to show how the changeover from forward to reversed beating might be effected.

forward



reversed

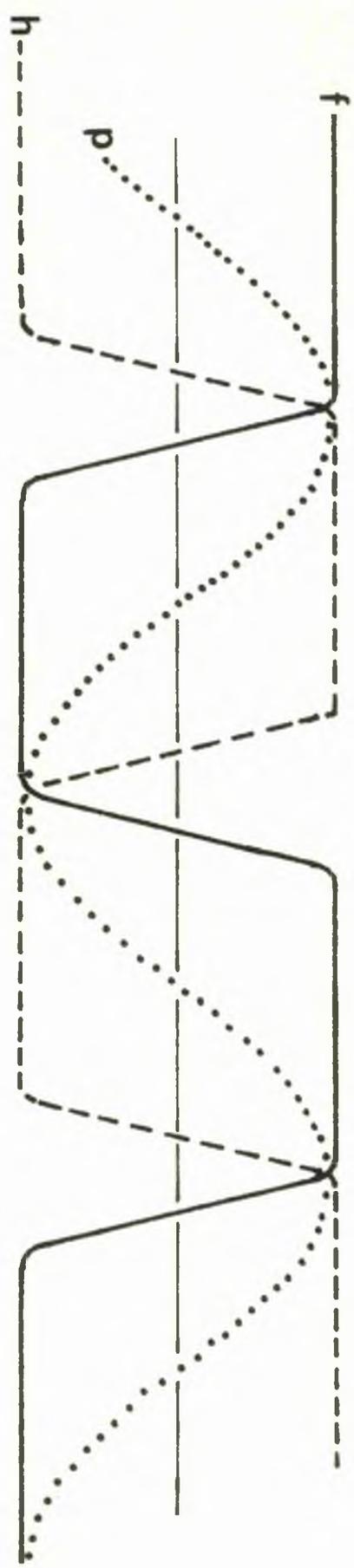


Figure IV.36. A diagram showing the relationship between the horizontal and vertical components of a vector. The diagram consists of a right-angled triangle with a hypotenuse of length 10. The angle between the hypotenuse and the horizontal axis is 30 degrees. The horizontal component is labeled '10 cos 30°' and the vertical component is labeled '10 sin 30°'. The hypotenuse is labeled '10'.

Figure IV.36.

Figure IV.36. Reconstruction from the data of Fig.IV.34 of the dispositions of the front end, hinge, and hind end of the scaphognathite during forward beating.

- a - All three points at or near maxima (most dorsal movement): phase angle of about 0.18 with respect to start of activity in the promotor (see dotted, vertical lines in Fig.IV.34).
- b - phase angle of 0.25 in cycle of promotor.
- c - phase angle of phase angle of 0.50.
- d - phase angle of 0.75.
- e - phase angle of 0.85 in cycle of promotor. All these points at or near minima (most ventral movement).
- d - phase angle of 1.00 in the promotor cycle.

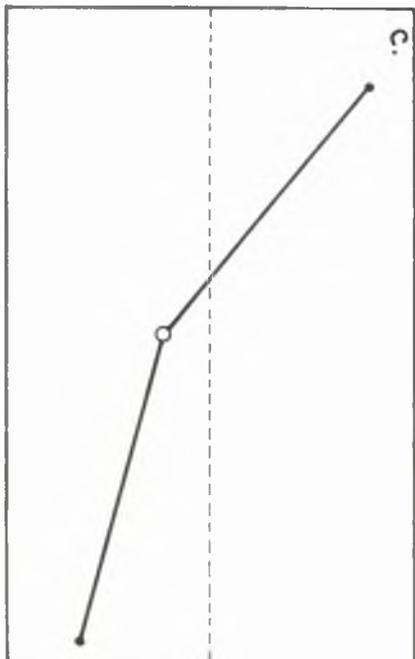
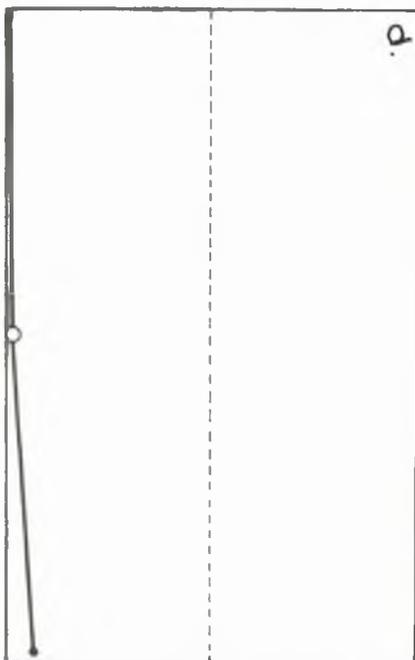
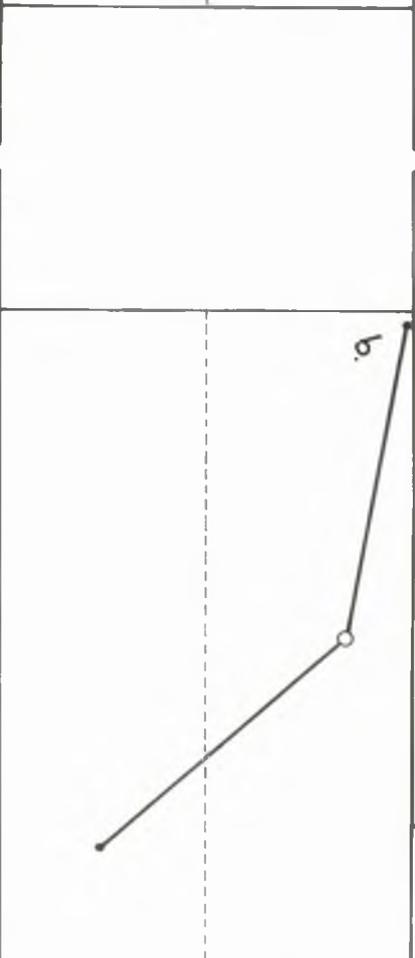
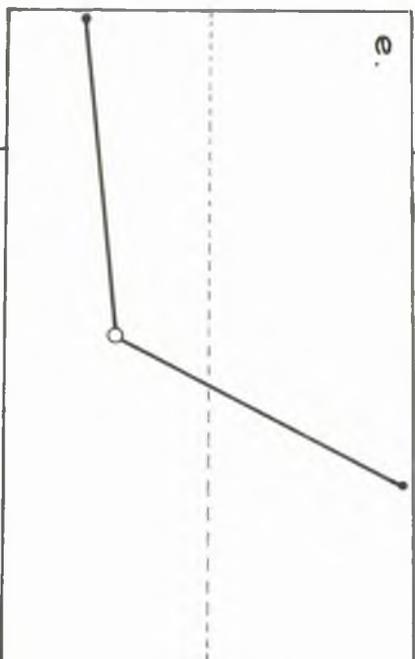
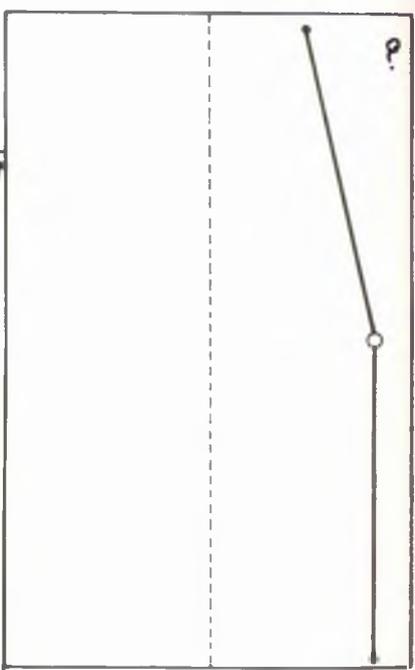
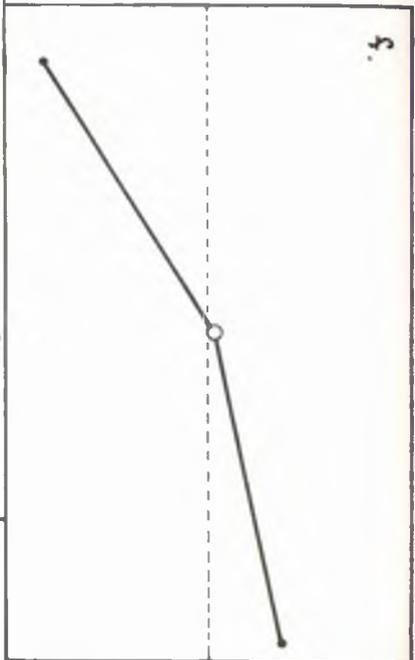


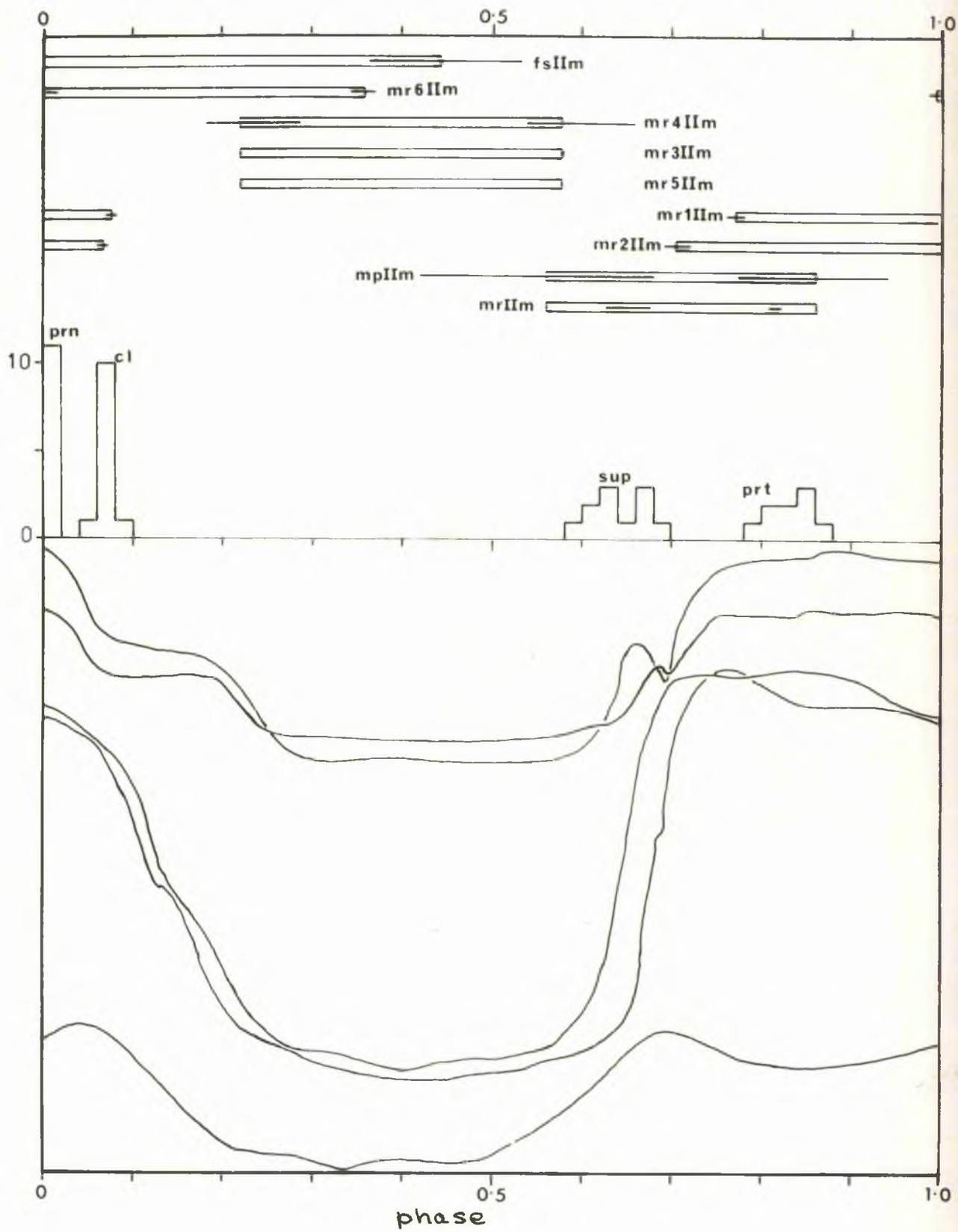
Figure IV.37. A graph showing the relationship between the overall activity in the system and the overall activity in the system. The x-axis represents the overall activity in the system, and the y-axis represents the overall activity in the system. The curve shows a positive correlation between the two variables.

Figure IV.37.

The graph illustrates the relationship between the overall activity in the system and the overall activity in the system. The x-axis represents the overall activity in the system, and the y-axis represents the overall activity in the system. The curve shows a positive correlation between the two variables.

Figure IV.37. Reconstruction (as in Fig.IV.33 for forward beating) of the overall pattern of activity in the ventilatory muscles during reversed beating, compared with phase histograms (middle) of identifiable points in the movement curves (bottom) for the interval tip in the cycle of the flexor. Note the difference in the pattern of the recruitment of the muscles in the different sub-groups. There were no data for the tertius and quintus during reversed beating so that there are no limit lines on these bars. It has been shown that these muscles continue to burst with the quartus during reversed beating. In keeping with overall qualitative results, the remotor burst is represented as coinciding with that in the promotor although the limits found in the records analysed were smaller than the overall means for the promotor. Note that the limits overlap. The large spread of the limits for the promotor result from the inclusion in the analyses of an experiment in which the burst pattern in the promotor was somewhat odd. (Fig.IV.14).

A full reconstruction of the movement during reversed beating was not possible since attempts to record movement of hinge and hind end in reversal were unsuccessful. Movements of the front end recorded on three occasions are included in the lower half of this figure. These can be contrasted with the pattern seen during forward beating. The characteristic undulating wave form for forward beating is markedly altered during reversal. Upward movement of the traces corresponds to levation. Conventions for the histograms are as in Fig.IV.33.



SECTION V

THE FINE STRUCTURE OF THE BURSTS AND  
THE INTERACTIONS BETWEEN THE UNITS IN  
THE VENTILATORY MUSCLES IN CARCINUS.

### INTRODUCTION

It has now been clearly established that the development of tension in Crustacean muscles is in direct relationship with the level of depolarization resulting from the summed, neuro-muscular junctional events (Atwood, Hoyle and Smythe, 1965). In detailed experiments using single fibres of the closer and stretcher muscles of the walking legs in Cancer, Atwood and his co-workers were able to show conclusively that the development of tension was a function of the supra-threshold level of depolarization of the fibres. There was no indication at this unitary level of the lack of direct linkage between electrical and mechanical responses, or of the 'paradox state' (Wiersma and van Harreveld, 1938; Hoyle and Wiersma, 1958) found in some whole muscle preparations.

In the scaphognathite of the decapod Crustacea, muscle fibres appear to fall into the category of 'gradedly responding', showing histological characteristics somewhere between 'Felderstructur' (slow fibre type) and 'Fibrellenstructur' (corresponding to fast fibres) (Pasztor, 1968). The muscles appear to correspond more closely to the 'fast follower' than the 'slow follower' type (Atwood et al., 1965) in that they normally display graded, spike-like depolarizations.

It might be expected that changes in spiking activity associated with facilitation or anti-facilitation as well as with changes in spiking frequency, will affect the development of tension in the muscles and hence the movement of the scaphognathite. Finer details of the patterning of the spikes in the burst also, may significantly influence tension development (Gillary and Kennedy, 1969).

In addition, there is carried in the efferent spike trains, the integrated information relating to the state of the individual motor units, and the variations in input into them. A useful exercise might

be to see just how much of this information is recoverable, and how much can be learnt from the interrelations between the spike trains, about the nature of the integration between units at the motoneurone and pre-motoneurone levels.

## V.1. The general pattern of the interactions.

### V.1.1. Results.

Because the events in the beat cycle tend to occur at more or less fixed phase angles, it is possible to express average patterns as phase histograms, with a reasonable degree of accuracy. The average period of the cycles in all the experiments was of the order of 2000 ms. In a fifty category histogram this gives a resolution of 40 ms, of the order of the average to longer interspike intervals within the bursts. Such histograms then of the phase position of the individual spikes within the bursts of the different muscles in the beat cycle should give a fair picture of the variation in frequency of spiking within the bursts. Moreover, by constructing the histograms for the different units with respect to a fixed reference point, the variation in spiking frequency of these units in the cycle may readily be compared. In cases where phase angle does change with period, this procedure will merely result in a degree of blurring of the relationships.

Figure V.1. combines such histograms for muscles from the different sub-groups during forward beating. Where possible, data were taken (a) from experiments in which the bursts showed single unit activity or in which there were clearly dominant single units; (b) with respect to the first spike in the flexor muscle, and (c) from the same beat cycles e.g. from records of total motor output. In the case of the promotor, the histogram was computed with reference to the first spike in the promotor burst itself (Fig. V.2,A). The pattern with respect to the flexor was obtained by appropriate adjustment of this distribution using a previously determined histogram for the phase position of the first spike of the promotor in the cycle of the

flexor (Fig. V.2,B). In Fig. V.3 similar histograms for reversed beating are shown. In this and in Fig. V.2 the data for the quartus were taken with respect to the first spike in the flexor, but from different cycles from the rest of the data.

From the figures two things are immediately obvious. Firstly, the spiking frequency in the muscles in general tends to start slowly, rise to a maximum, and fall once more. This fits in with the general pattern observed in the lobster swimmeret muscles (Davis, 1969) and in the muscles of the scaphognathite in the crayfish Orconectes (Pasztor, 1968). In the flexor muscle however, as indicated qualitatively, earlier, the pattern is somewhat different. During forward beating, the spiking frequency rises exponentially from an already somewhat high level (partly artefactual - resulting from the fact that the phase position of the flexor spikes was computed with respect to the first spike in the flexor itself), and is sharply terminated at the peak frequencies. During reversed beating the frequency falls off slowly from a relatively high starting frequency. (See note above).

The pattern of the sixtus burst during reversed beating is similar to that of the flexor in the same beat mode, but the rate of decay is more rapid. The spiking frequency apparently falls sharply as, or shortly before, the activity in the quartus starts to rise. This relationship between the activities in the sixtus and quartus can also be seen in Fig. V.1 in the forward mode. Here, the activity in the sixtus begins shortly after that in the flexor, but remains at a relatively low level until the quartus burst begins to fall in intensity. The spiking frequency in the sixtus then rises rapidly to a sharply defined peak and decays rapidly as the activity rises in the levator muscles of the primus sub-group.

This introduces the second interesting aspect of these figures. Although they represent only some of the units and muscles active

during the cycle, it seems clear that the different muscles tend to replace each other continuously, the activity in one set rising reciprocally as that in another set declines. Peaks of activity in one muscle tend to coincide with troughs between others from different sub-groups. It almost appears that a roughly constant level of activity is maintained, but is simply being redistributed amongst the different muscles.

Direct plots of the succeeding interspike intervals in the bursts (Figs. V.4) confirm that the 'shapes' of the bursts are as indicated in the histograms. This is particularly clear in the cases of the flexor, sixtus and promotor. The burst shape in the quintus (Fig. V.4) during forward beating may assume a form similar to that seen in the flexor. This, and the pattern seen in the sixtus correspond with the patterns exhibited in the experiment illustrated in Fig. IV.8,E.

'Bursty' systems appear characteristically, to be modulated by underlying waves of depolarization and repolarization as recorded for example, in the motor units controlling ventilatory muscles in Limulus (Wyse, 1972). Presumably the shapes of the spike trains are determined by the form of the input wave of depolarization. The relationship between this input wave and the pattern of the spike train is illustrated in Fig. V.5. It should be possible to reconstruct from the spike train, the shape of the input wave, at least on a relative scale.\* From the figure it can be seen that the relationship between the extent of depolarization and the interspike interval may be approximated by the equation:

$$V_{n+1} = (V_n + 1) \times \exp^{-Int/t}$$

where  $V_n$  represents the level of depolarization at the  $n$  th spike,  $Int$ ,

---

\* This idea was developed in discussions with Mr. P. R. Balch who deserves credit for its conception and development.

the interspike interval in ms; and  $t$ , the time constant of the membrane potential return. Computing the resulting 'wave of depolarization' with different values of  $t$  showed that within limits ( $t$  varying from 10 to 60 ms), this had little influence on the shape of the resulting wave. Fig. V.6 shows the resulting curves for single bursts in the flexor and quartus in the same cycle during reversed and forward beating. The reciprocal relationship between the 'input waves' into the two muscles can clearly be seen. In forward beating, the depolarization in the quartus rises, settles at a maximum value, then falls off abruptly as the depolarization in the flexor rises to a peak and falls sharply. It is interesting to compare the computed 'wave of depolarization' in the flexor with that recorded intracellularly in the 'A' cells of the stomatogastric ganglion by Maynard (1969), and in the motoneurons in Limulus by Wyse (1972). It remains an open question, whether this abrupt cut-off is an intrinsic property of some motoneurons, or the result of massive, slow ipsp. In reversed beating the depolarization in the flexor rises rapidly then slowly declines as that in the quartus slowly rises to a peak then falls off, gradually, but more rapidly than that in the flexor. The sharp truncation of the bursts evident in forward beating is absent here.

Recording electrodes placed at different points on the quartus muscle may sometimes pick up somewhat different activity, indicating that the muscle is not homogeneously structured or innervated. In Fig. V.7 the 'waves of depolarization' computed from two such records are compared with the 'depolarization' in the flexor. It appears that the activity in one of the units may overlap more extensively than that in the other, with the activity in the flexor. As the depolarization in this unit rises, that in the flexor changes only slightly. The depolarization in the second unit starts later in the flexor cycle,

rises rapidly, and as it rises, the level of depolarization in the flexor falls in inverse proportion to its rise.

In Fig. V.8 similar comparisons are affected between the depolarizations in the flexor, sixtus and two units in the levator complex, probably associated with the primus (1) and secundus (2). It appears that the rise of the depolarization in (1) and the cessation of activity in the flexor may be associated with the fall in the depolarization of the sixtus muscle. The depolarization of the sixtus reaches a peak at approximately the same time, or a little after that in the flexor. It is not however, until the depolarization in the second levator unit begins to rise that the sixtus burst is terminated.

The preceding observations all seem to indicate some sort of reciprocal relationship between the ventilatory muscles. This can in fact be seen in some cases on a qualitative level. Various recordings which illustrate this interaction are shown in Fig. V.9. It appears that different units may be active in the quartus at different times, and with differing degrees of intensity from cycle to cycle. It seems also, that the relationships between the bursts in the flexor and quartus (for example, extent of overlap) may vary considerably depending on which motor units are bursting and the intensity of the bursts. An example of this type of relationship has already been encountered (Fig. IV.8). Here, it appears (Fig. V.9) that a similar, perhaps stronger reciprocal relationship exists between the quartus and sixtus. Changes in the activity in the tertius are not obviously associated with the changes seen in the bursts of the flexor sub-group muscles. Such variable interactions as described above no doubt contribute to the variability of the temporal relationships between the muscle bursts in the cycle, as observed in the preceding chapters.

#### V.1.2. Interim Discussion

From a study on three levels, (a) by qualitative assessment of

the recorded activity in the different motor units; (b) by comparison of the phase angles at which peak discharge frequency occurs in the different muscles, and (c) by comparisons between the overlapping, relative 'waves of depolarization' in some of the units, indications were obtained that there may be negative (inhibitory) interactions between units from different sub-groups, and positive (excitatory) interactions between units from the same sub-group. For example, the flexor and sixtus units in forward beating exhibit peak frequency of discharge at about the same phase angle in the cycle; there is also a rapid, synchronized rise in the computed 'waves of depolarization' for these units, and a fall in that for the sixtus as soon as the flexor burst is terminated. A similar pattern of increasing instantaneous frequency of spiking is seen in the quintus. These relationships may be explained on the basis of positive interactions between collaterals from the same sub-group. On similar grounds Waldron (1967), Waldron and Wilson (1969) and Levine (1973) have postulated positive feedback networks between units governing insect flight. Maynard (1969) has demonstrated electrotonic coupling, acting as a positive feedback system, between the 'A' cells in the crustacean stomatogastric ganglion. These 'A' cells exhibit underlying waves of depolarization resembling those computed here for the flexor during forward beating. Further evidence for the existence of interactions between units to the depressor muscles will be presented in the next chapter.

V.2. The spiking pattern in the bursts, and further interactions between some of the depressor muscles.

V.2.1. Results.

It has been shown that on the whole burst duration in the muscles can be described as a linear function of period. In most cases investigated here, there was also a highly significant, positive linear correlation between burst duration and the number of spikes per burst (Figs. V.10). For a given number of spikes per burst, the burst duration can vary widely. Bursts with larger number of spikes tend towards higher minimum and maximum durations, with higher mean durations, but there is considerable overlap. The relationship was particularly marked in one analysis on the flexor burst, in which the burst durations varied over a relatively wide range, and the spiking frequency was high (Fig. V.10,F). This tended to reduce the effect of small variations such as the loss of single spikes from bursts due to random influences.

In most cases, the relationship between spiking frequency and burst duration was reflected in a similar, though less strong relationship between spike number per burst and period length (Fig. V.11). In the case of the experiment in Fig. V.10,D for the promotor there is significant linear correlation between spike number per burst and burst duration, but the relationship between spike number per burst and period (Fig. V.11,D) appeared quite random, with a linear correlation coefficient of  $-0.270$ . If the period lengths are rounded off to the nearest 50 ms and the resulting data pooled for periods of equal length the relationship appears quite differently, as is shown in Fig. V.11,G. In fact, in this case, the relationships are quite similar to those described by Davis (1969) and Davis and Murphey (1969) in the swimmeret muscles of the lobster Homarus. The resemblance lies

both in the shape of the curve relating number of muscle spikes per cycle to period length and in the absence of any obvious correlation between burst duration and period length. This was the only instance in the present study, in which the above relationships were observed. Moreover, in the same muscle, in a different experiment (Fig. V.12), a more linear type of relationship between spike number per burst and period length has been found, with a significant correlation coefficient of 0.432.

Where spike trains in the different muscles overlap, it has been seen that they appear to exert mutual influence on each other. This sort of interaction seems obvious in say the case of the sixtus and quartus during both forward (Fig. V.1) and reversed (Figs. V.3; V.9) beating. In other cases it is less obvious. The relationships between the 'waves of depolarization' investigated in some of these cases have supported the idea of broad, negative interaction. If spikes in one motor unit exert influences on the wave of depolarization in another, it might be expected that the incidence of a spike in one motor unit during periods of overlapping activity might, by altering the level of the input wave of depolarization, influence the probability of the occurrence of a spike in another. Such influences might be investigated by comparing the histograms for the frequency distribution of the interspike intervals in one unit (used as a reference), with the corresponding frequency distribution of the latencies of the spikes in the second with respect to the first, for periods of overlapping activity. Fig. V.12 shows such histogram pairs for the flexor and sixtus during forward and reversed beating, and for the flexor and quartus during reversed beating. In the flexor and sixtus the frequency distribution of the interspike intervals show pronounced single peaks during both forward and reversed beating. In the quartus

a slightly different distribution is seen, with recurrent, smaller peaks at longer intervals (Fig. V.12). This might be a reflection of the tendency towards grouping of the spikes in this muscle.

In the case of the flexor and sixtus there is a very marked difference in the interactions during forward and reversed beating. Clearly, the close interrelationship demonstrated previously between these bursts during reversed beating extends not just to the starting latencies, but also to the individual spikes in the bursts. During reversed beating there is a pronounced entrainment of the spikes in the sixtus with those in the flexor - the sixtus spikes showing a clear tendency to occur within the first six milliseconds after a spike in the flexor. This shows up also if the data are expressed as a phase histogram of the position of the sixtus spikes in the intervals of the flexor spikes (Fig. V.12e). It should be noted that information concerning non-phasic (latency determined) interactions could be blurred by expressing the data in this way. In the present case the small spread of interspike intervals and relative latencies allow this transformation. It has been used primarily to demonstrate clearly that the group of spikes in Fig. V.12 at longer latencies after the gap, correspond to sixtus spikes occurring within six ms before spikes in the flexor (mean interspike interval in the flexor = 36.69 ms; Number of histogram categories = 20; Mean category = 1.8 ms). It appears then that spikes in the flexor also, may follow closely after spikes in the sixtus but with a smaller probability than for the reciprocal relationship. The latencies involved are apparently of the same order for flexor spikes following sixtus spikes as for sixtus spikes following flexor spikes.

In the case of the flexor and sixtus during forward beating there is no similarly clear relationship. Surprisingly, it appears that the

probability for the occurrence of a sixtus spike just after a flexor spike, is relatively low, whilst the form of the reciprocal relationship for the flexor spikes in the interspike intervals of the sixtus, is more in keeping with what might be expected from a random distribution.

In the flexor and quartus (Fig. V.13), there appears to be a strong tendency for a spike in the quartus to occur just after a spike in the flexor (within 2.5 ms). This is followed by a period during which the probability for the occurrence of a quartus spike appears to be relatively low, but rising again at longer latencies. The low peak at longer latencies in relation to the higher one at shorter latencies might occur simply because there is reduced opportunity for the incidence of latencies of longer durations.

Because of this, it is in fact difficult directly to draw inferences from the data in the present format. In all the interspike intervals in the reference train, test spikes have the opportunity to occur in all latency categories below that for the minimum reference interval. Opportunities for the occurrence of longer test spike latencies arise only when sufficiently long reference intervals permit it. Given the distribution of the reference intervals however, the expected distribution of the test spike latencies when there is no correlation between the spike trains may be derived. The procedure devised for doing this is described in Appendix I. In Fig. V.12 the computed expected distributions are indicated by vertical bars at the mid-class values in each category, for the test spike distribution histogram. A Chi squared test may then be applied to the observed and expected distribution. These tests indicate that the relative distributions of the spikes for the flexor and sixtus in the interspike intervals of each others spike trains are not significant for forward beating. During reversed beating, there is a highly significant

relationship ( $P < 0.01$ ). During reversed beating also, the distribution of the quartus spikes in the flexor interspike intervals is also significant ( $P < 0.01$ ), as is that for the flexor spikes in the quartus interspike intervals ( $P < 0.01$ ). The obvious interpretation of these correlations is that there is interaction between the motor units of these agonist muscles. However, having established this, one cannot readily determine whether the interaction is direct or indirect, although reasonable inferences may be made.

### V.2.2. Interim Discussion

The results indicate that there is significant spike to spike interaction between the flexor and sixtus during reversed beating but not during forward beating, and between the flexor and quartus during reversed beating. In the case of the flexor and sixtus in reversed beating, the connection is strongly suggestive of an excitatory linkage. This could be either ephaptic or synaptic. If the linkage is synaptic, the excitatory influence of the flexor on the sixtus would appear to be stronger than that of the sixtus on the flexor, since the incidence of flexor spikes after sixtus spikes is relatively rare. If it is ephaptic, then the connection must be rectified so that a spike in the sixtus unit will produce a smaller depolarization at the flexor unit than a spike in the flexor unit would cause at the sixtus motor unit. If the connection is synaptic, then interneuronal elements may conceivably be interposed. In view of the very short latencies and the strong correlation between the spikes, the direct, electrotonic linkage seems to be the more likely alternative. (The majority of the sixtus spikes fall into the first category of the latency histogram, indicating a latency of 0 to 2.0 ms). Runaway activity in such a closed, excitatory loop may be prevented by accumulating refractoriness.

In addition, in the present case, inhibition from the quartus unit onto both the flexor and sixtus units may outweigh the mutual excitation. That there is spike to spike interaction between the flexor and quartus during reversed beating is also indicated by the present analyses. The trough in the latency histograms, between 2.5 to 12.5 ms for the quartus spikes in the flexor, and between 2.5 to 10.5 ms for the flexor spikes in the quartus, indicate that the interaction is inhibitory and that perhaps only a single synapse intervenes. It seems also that there is mutual inhibition. From the results of the previous section these relationships could have been expected, and from the qualitative results, it might be expected that there might be even stronger inhibition between the quartus sub-group muscles, and the sixtus. Having said this, it is necessary to note that Kennedy, Evoy and Fields (1966) in collaboration with D.M. Wilson have obtained almost identical results for the interaction between the contralateral abdominal flexor inhibitors in Procambarus. These units are apparently electrotonically coupled (Potter, D.D. and M. Otsuka - personal communication to above authors). The results of analyses indicated a tendency towards near synchrony ( $\pm 3$  ms) and a low probability for 'intervals' (= latency here) between 3 and 10 ms. For electrotonic connection however, one might expect that relationships should be more likely to resemble those for the flexor and sixtus during reversed beating. It is interesting to speculate that in the abdominal flexors, the demonstrated electrotonic linkage may serve to maintain equivalent, contralateral excitability in the neurons, but that additional inhibitory interaction organizes the relationships between the spike trains. It is believed that this could explain the observed relative latency distributions. Certainly, from the results of the previous chapter, one would be surprised if there were excitatory or electrotonic

connection between the flexor and quartus, and the present interpretation of the distribution on its own seems fair. Also, to preempt some experiments yet to be reported, it has been noted, in a digital computer simulation of neuronal networks, that simultaneity of spiking can be exhibited between similar reciprocally inhibiting units with common input. This could explain the significantly high peak occurring in the first category of the histogram.

From the results of the preceding chapter it is surprising that there was no significant spike to spike interaction between the flexor and sixtus during forward beating. In view of the previously postulated electrotonic linkage between these units during reversed beating this may seem odd. Assuming identical interaction between the flexor and sixtus during forward beating, the change in the timing of the bursts could be explained by the shift with respect to the quartus burst so that the quartus sub-group precedes the flexor sub-group. It might be envisaged that the effect now of stronger inhibition from the quartus onto the sixtus than onto the flexor, would be to delay the start of the sixtus with respect to the flexor burst. It is more difficult however, to explain the absence of spike to spike correlation in forward beating on this basis. One possible explanation might be that in reversed beating the correlations involve spikes near the starts of the bursts (see Fig. IV. 1, A and B). The 'waves of depolarization' would still be close to the thresholds of the spike generators and unitary input would probably play a large part in determining whether or not the unit spiked (see Figs. V. 7 and 8). In forward beating, the analyses included spikes near the end of the flexor burst, when the waves would be at a relatively high level. Unitary input although contributing to the general level of excitation would then probably not be very significant in determining the occurrence or non-occurrence

of a spike. This then could explain the lack of significant correlation between the spike trains. The relationship between these units is interesting and will be taken up again in a later chapter.

Figure V.1.

Figure V. 1. Histograms of the phase positions of individual spikes in the different muscles, in the cycle of the flexor forward beating. Phase positions were computed with respect to the first spike in the flexor burst. See text for details.

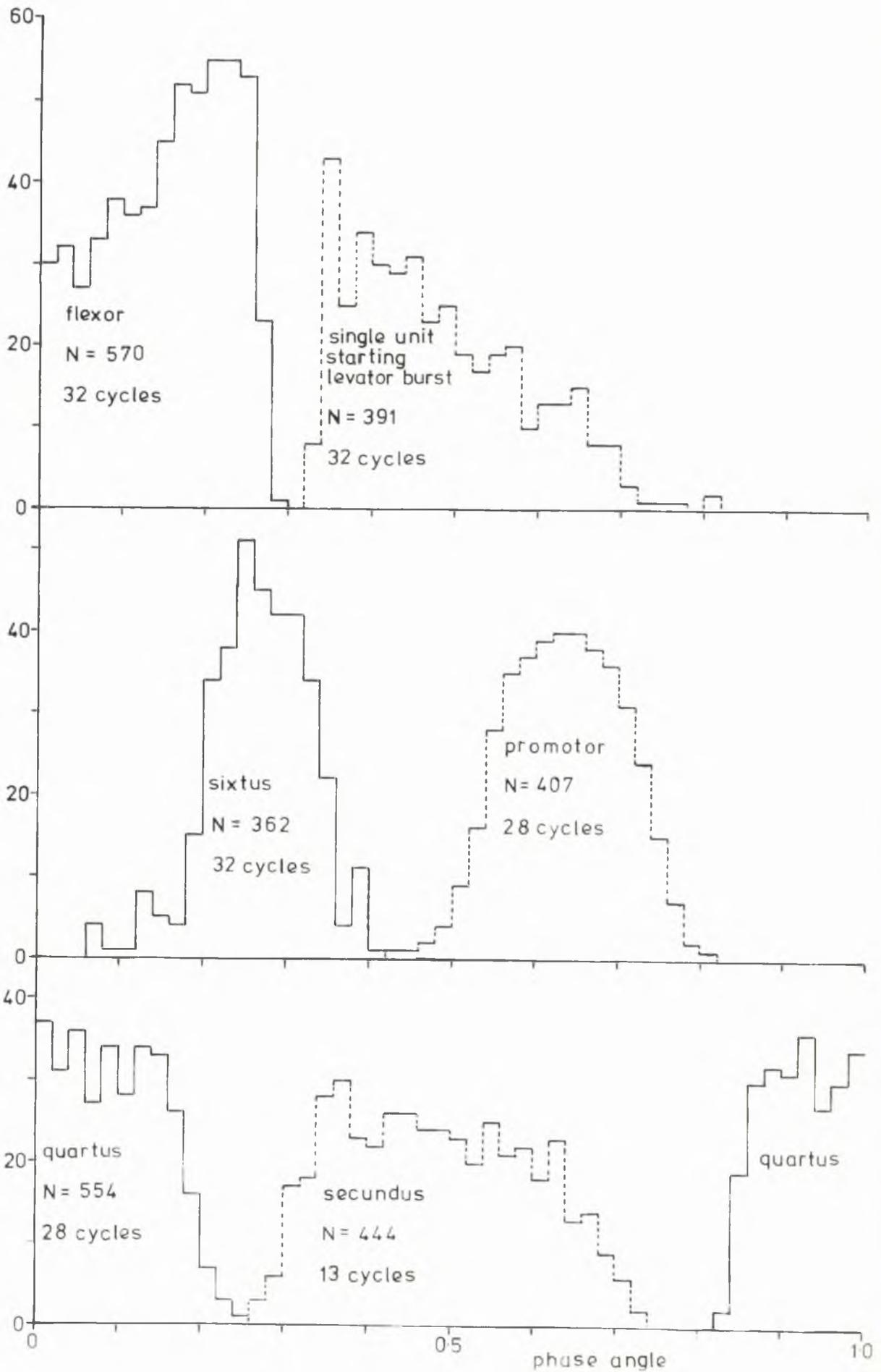




Figure V.2.

Figure V.2. A. Phase position of the first spike in the promotor in cycle of the first spike in the flexor burst. Using this distribution the distribution in B was transformed to that in Fig. V. 1. for the individual spikes in the promotor with respect to the first spike in the flexor.

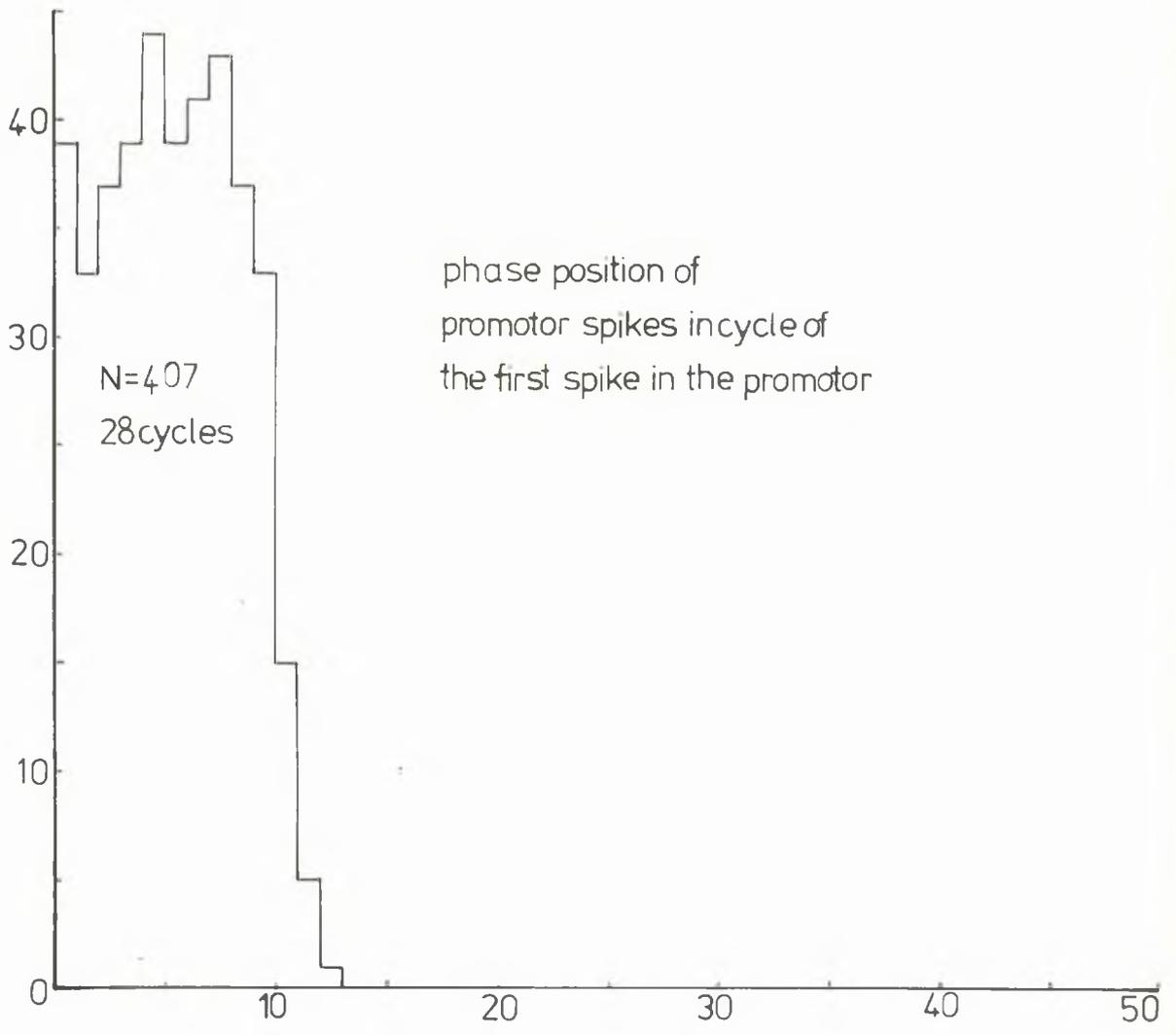
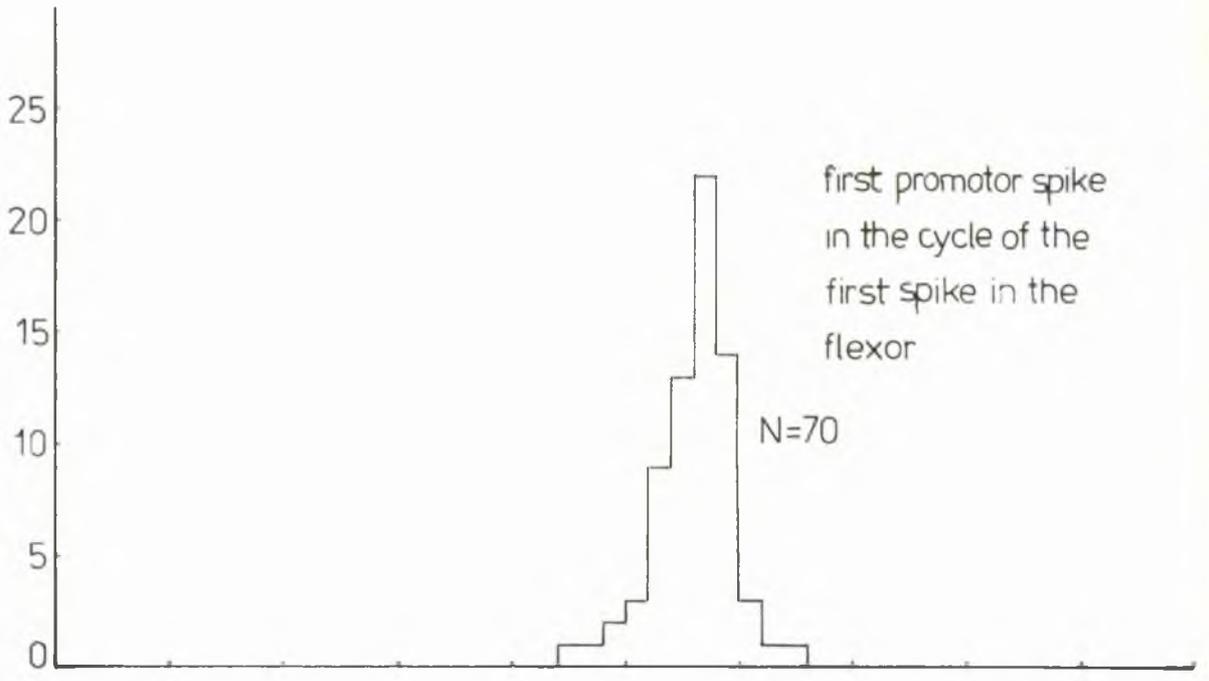




Figure. V.3.

Figure V. 3. Similar histograms to those in Fig. V. 1.  
reversed beating.

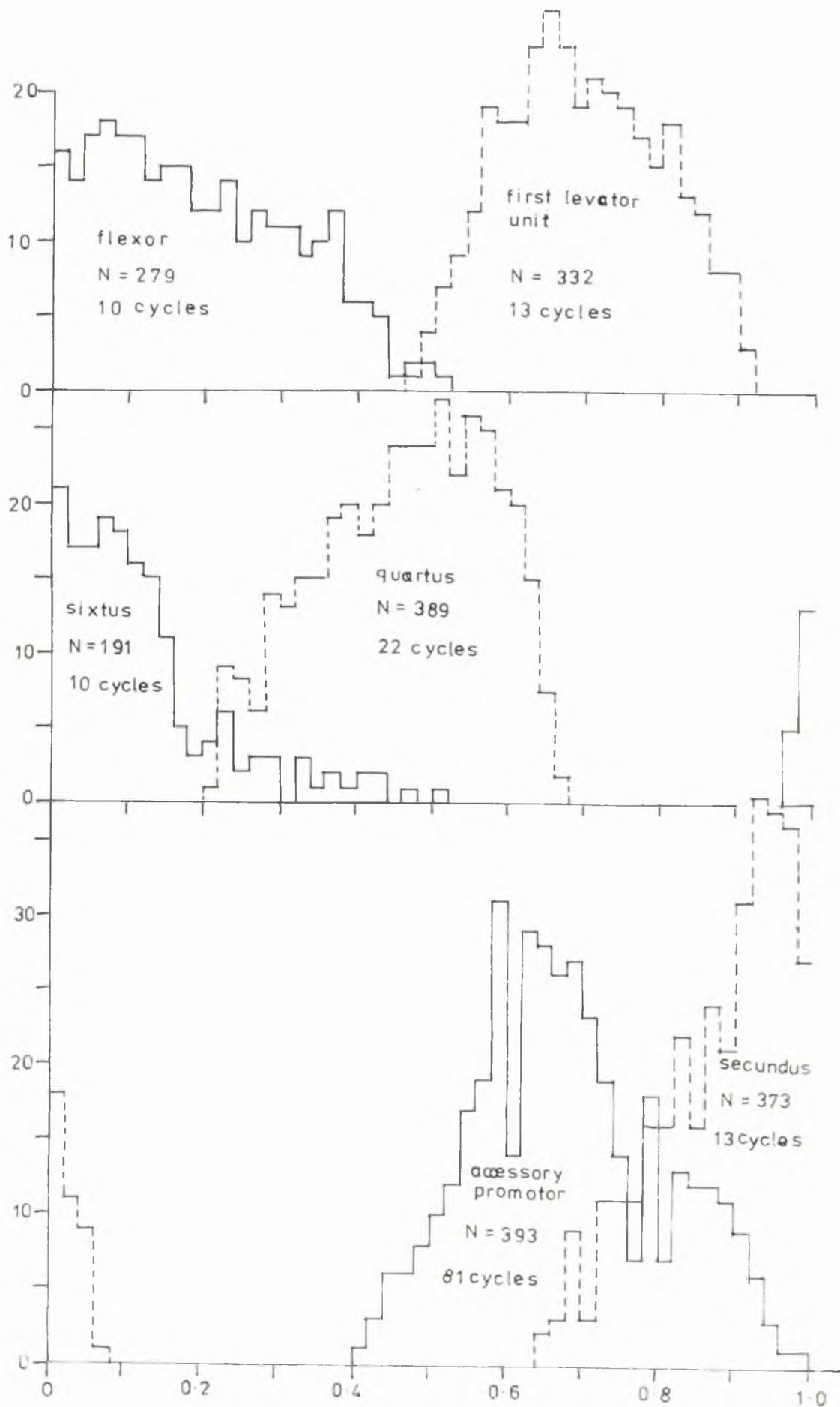




Figure V. 4

Figure V. 4. The 'shapes' of the bursts of activity in different ventilatory muscles in Carcinus. Ordinates: interspike intervals (ms); abscissae: interval number in the burst. The numbers beside the curves indicate the numbers of bursts from which the plotted data were averaged.

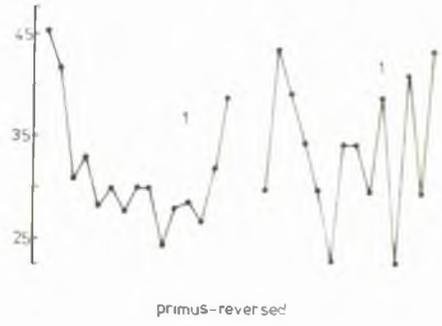
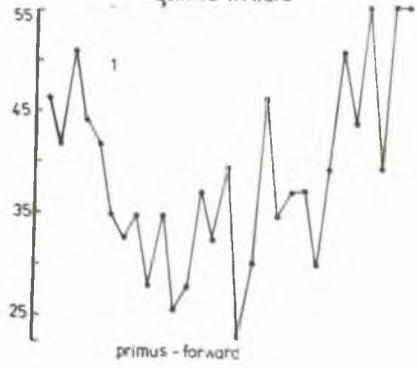
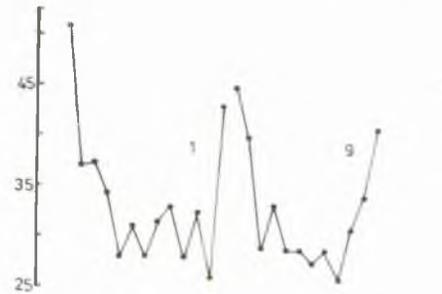
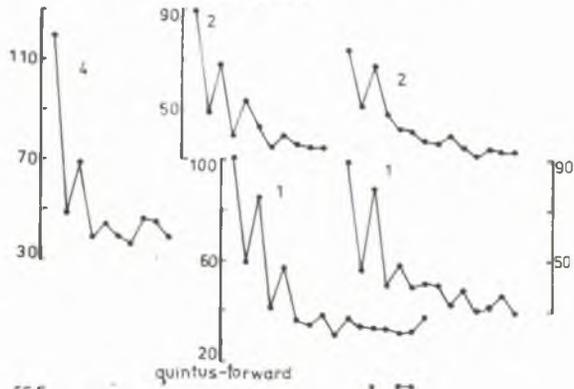
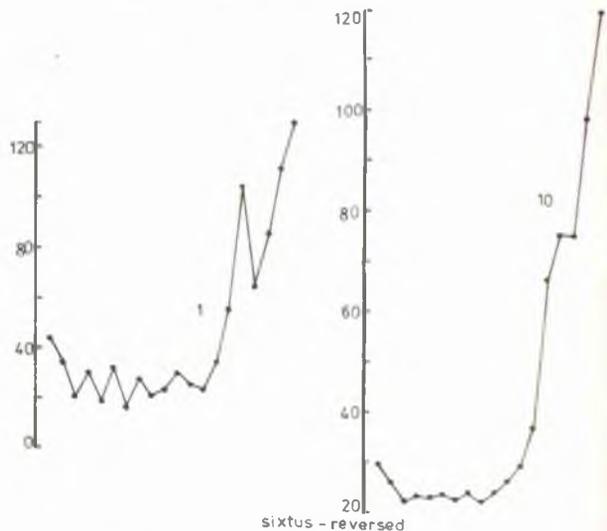
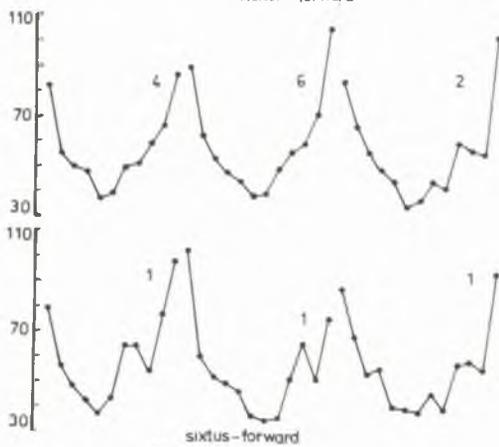
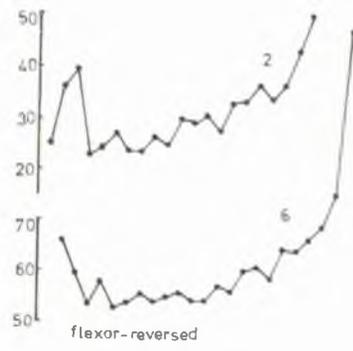
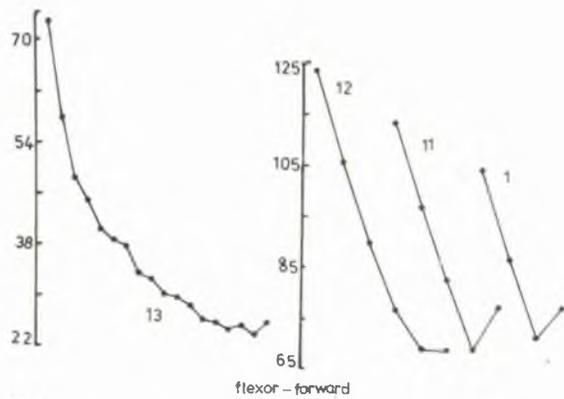
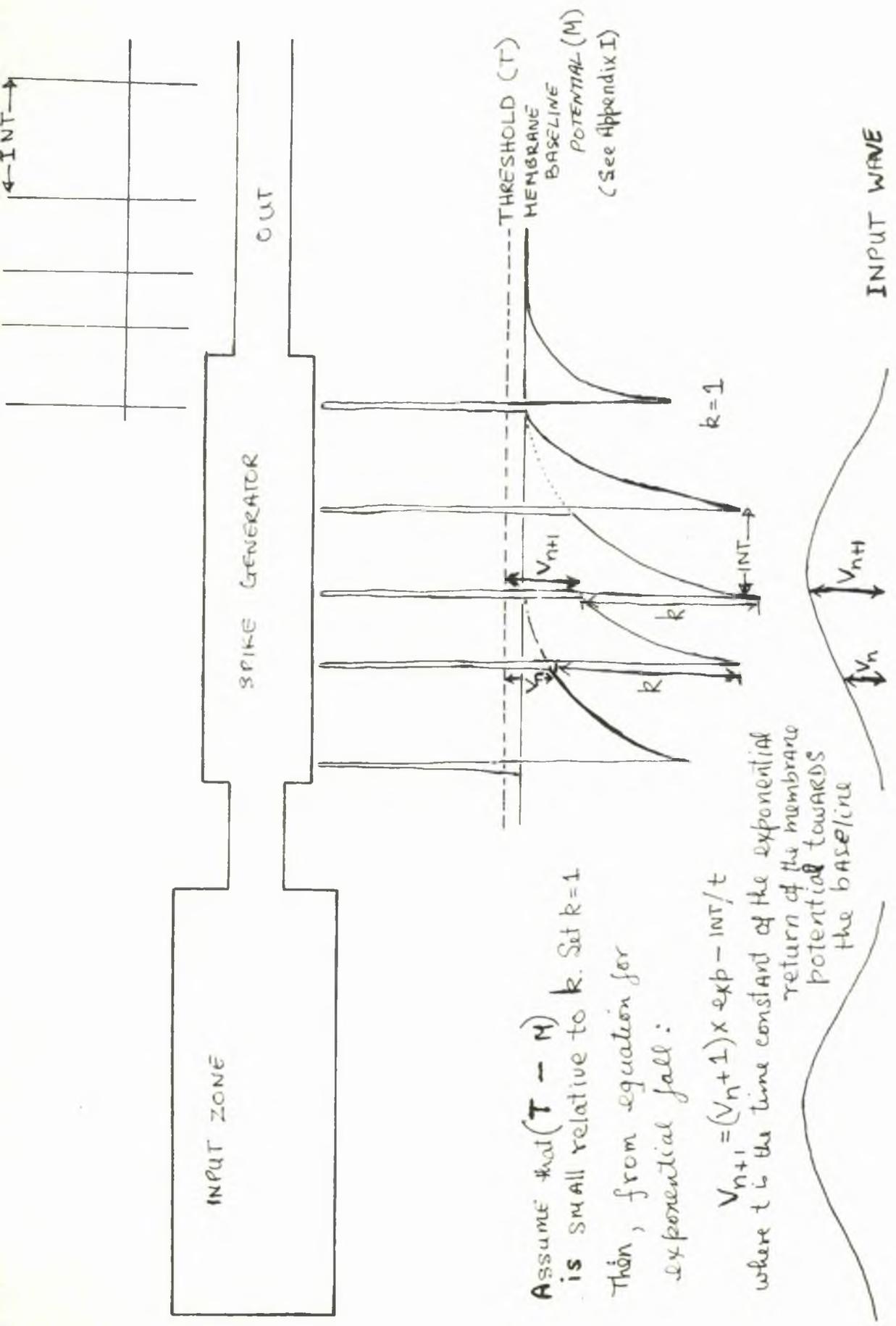


Figure v.s.

Figure V. 5. The relationship between the interspike interval in a bursting neuron, and the input 'wave of depolarization'. It is assumed that the return of threshold to its resting value is relatively fast compared with that for the membrane potential. The membrane baseline potential is assumed to be decreased by a constant from its pre-spike level, when the cell spikes. It should be noted that this assumption differs from that of the digital computer simulation (see Appendix I) which assumes that the membrane baseline potential falls to a fixed level. It is difficult to decide between these two alternatives, which will depend on the nature of the physico-chemical interactions involved in these changes in real cells. This is only poorly understood. The value of the constant fall ( $k$ ) is taken to be 1, since only relative values are required.



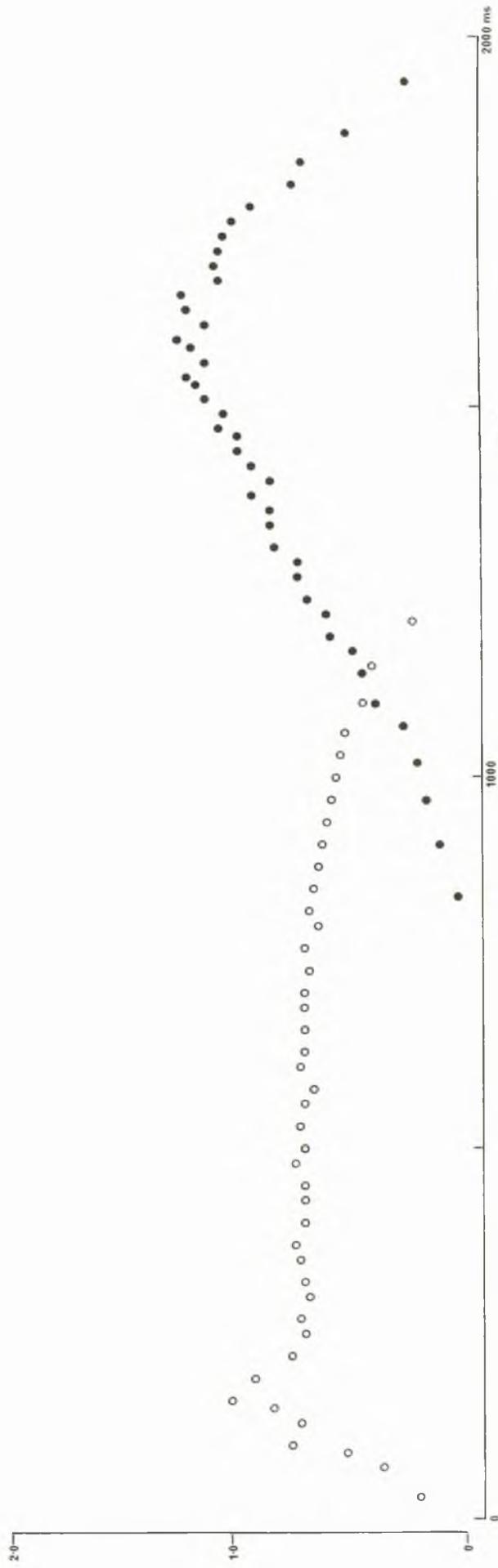
Assume that  $(T - M)$  is small relative to  $k$ . Set  $k=1$ . Then, from equation for exponential fall:

$$V_{n+1} = (V_n + 1) \times \exp(-INT/t)$$

where  $t$  is the time constant of the exponential return of the membrane potential towards the baseline

Figure V.6

Figure V. 6. The computed 'wave of depolarization' in the flexor and quartus muscles. Ordinates: voltage; abscissae: time in ms. since the first spike in the flexor. During reversed beating (A), the flexor burst precedes the quartus burst and slowly falls off, with increasing rapidity as the quartus burst rises in intensity. In forward beating the relationship is reversed. The curves were computed from the sequential interspike intervals as described in Fig. V. 5.



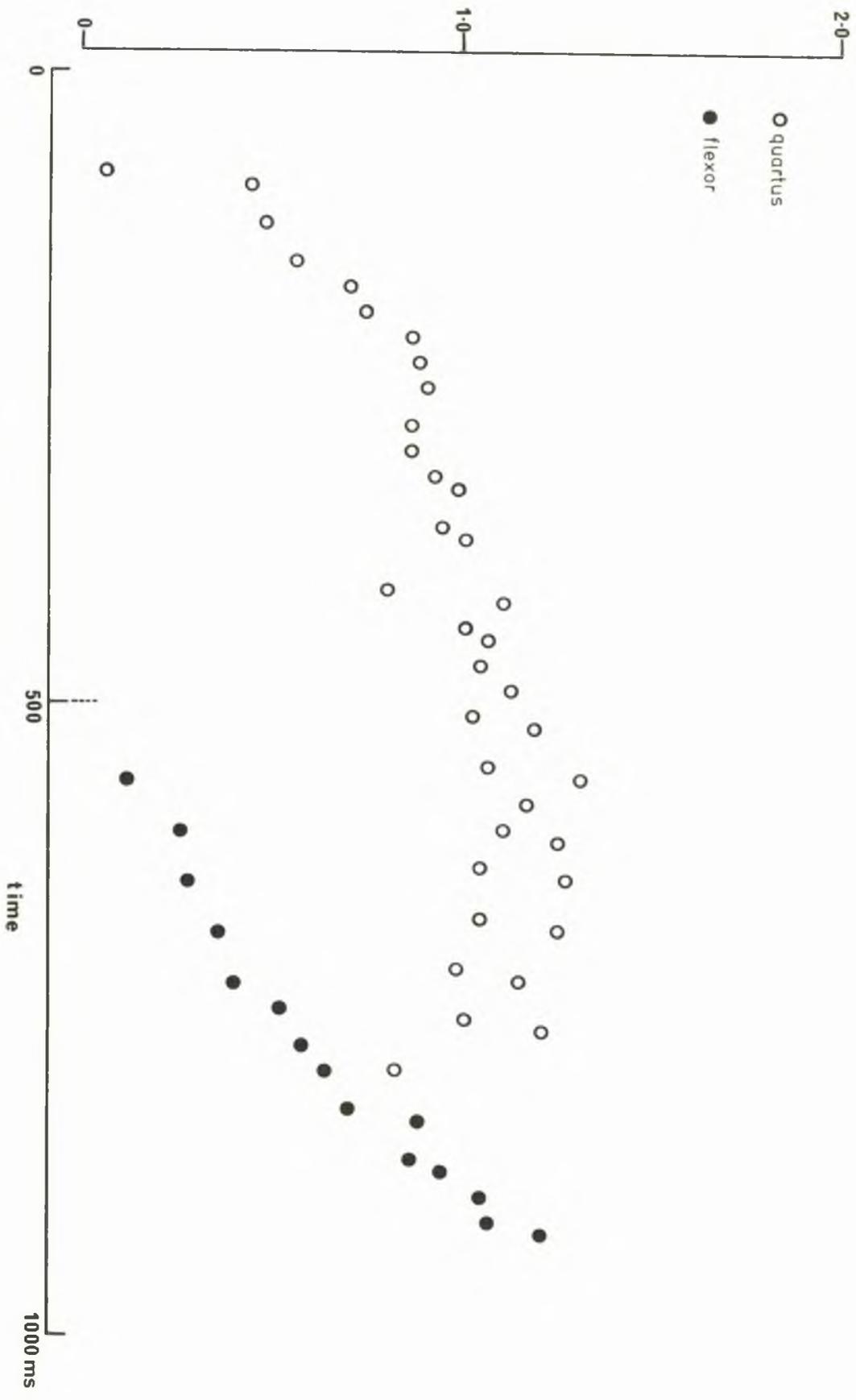
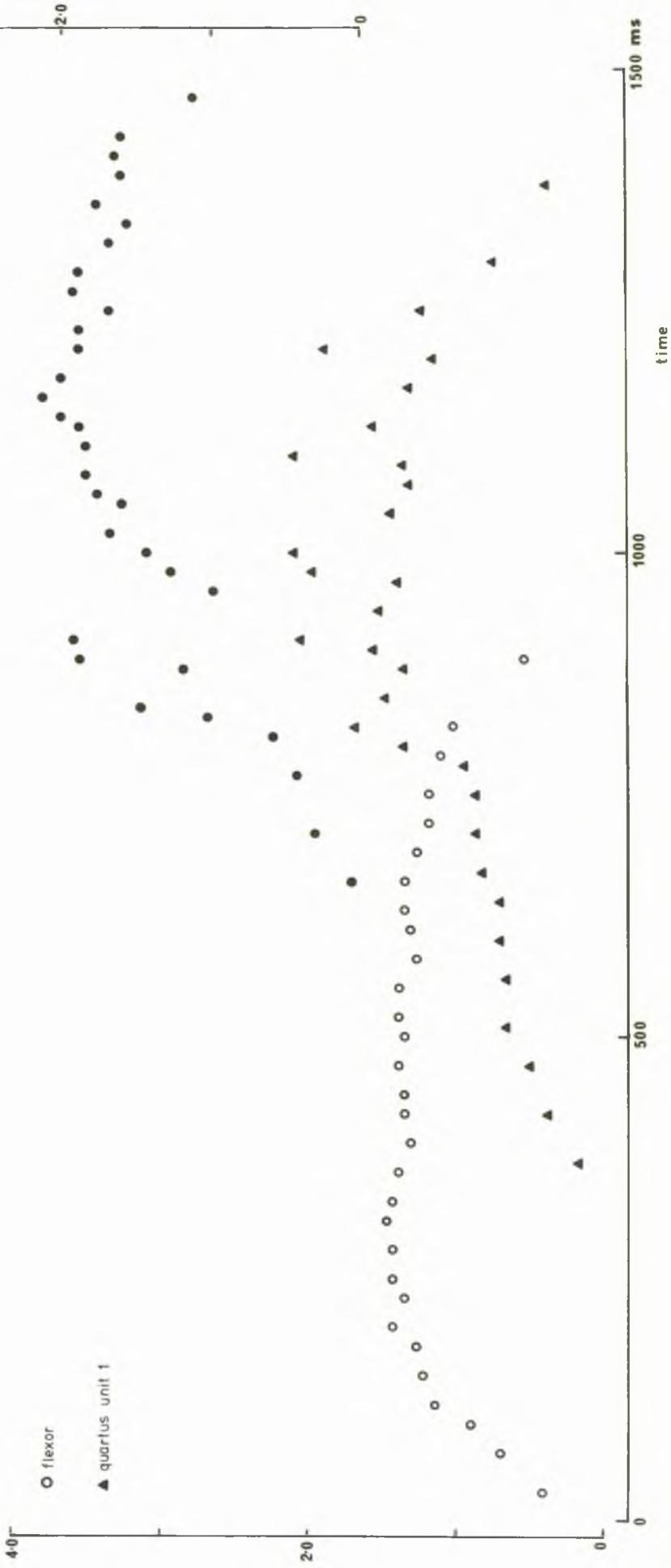


Figure V.7.

Figure V. 7. The 'depolarization' in the flexor unit and in two quartus units during reversed beating. Note that for increased clarity, the origin of the plot for the quartus unit 2 is raised with respect to the others. There appear here to be evident breaks in the curve for the flexor as the two quartus units commence firing. There appear also to be even more pronounced breaks in the quartus waves as the activity in the flexor falls.



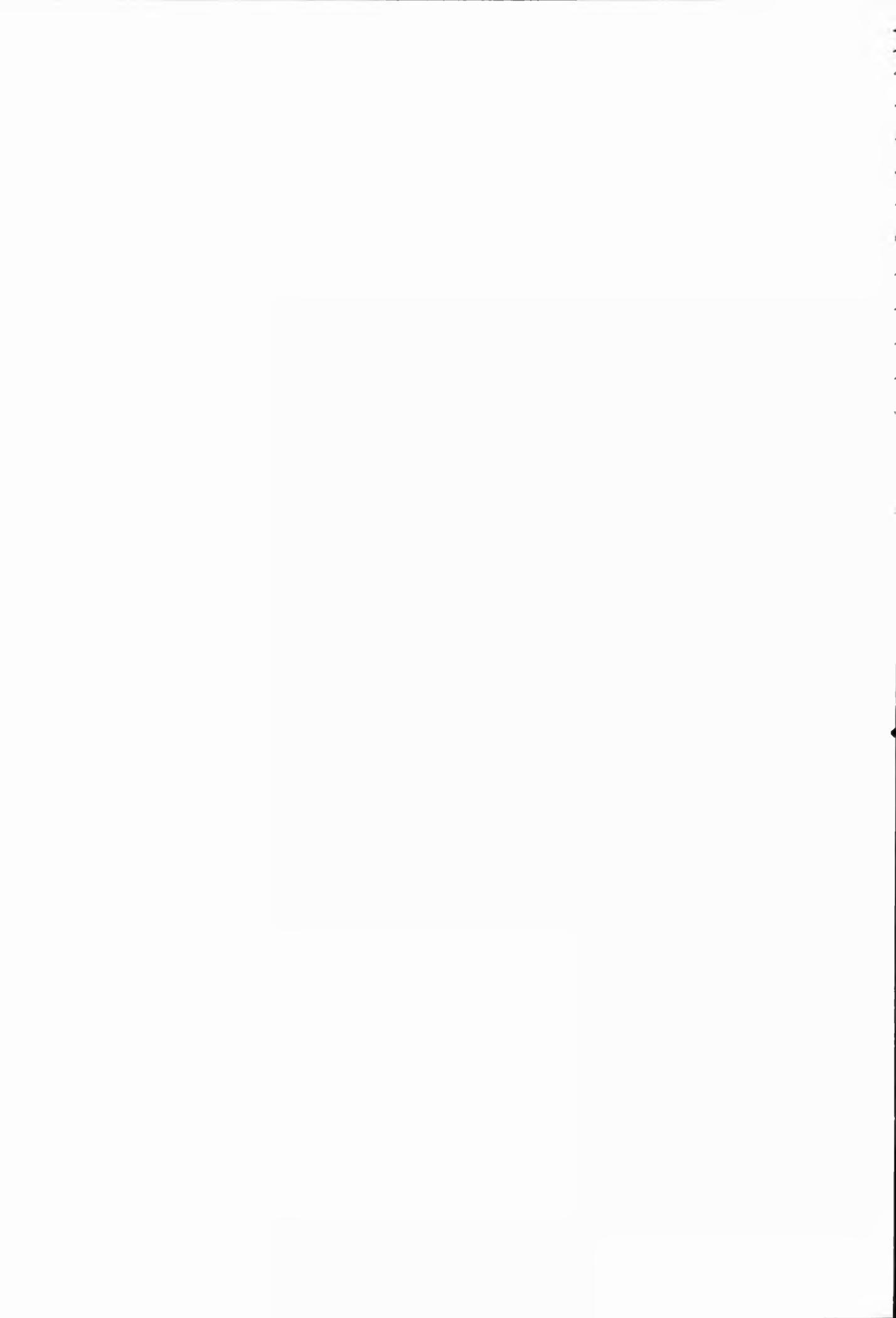


Figure v. 8.

Figure V. 8. Computed 'waves of depolarization' in the flexor and primus sub-groups during forward beating. Notice the clearly displayed, abrupt termination or 'repolarization' in the flexor about 250 ms before the start of the levator session. Only the sixtus and flexor are active at this point in the cycle, so that this repolarization must either be intrinsic, or mediated by an interneuron. Notice the simultaneous peaking in the flexor and sixtus.

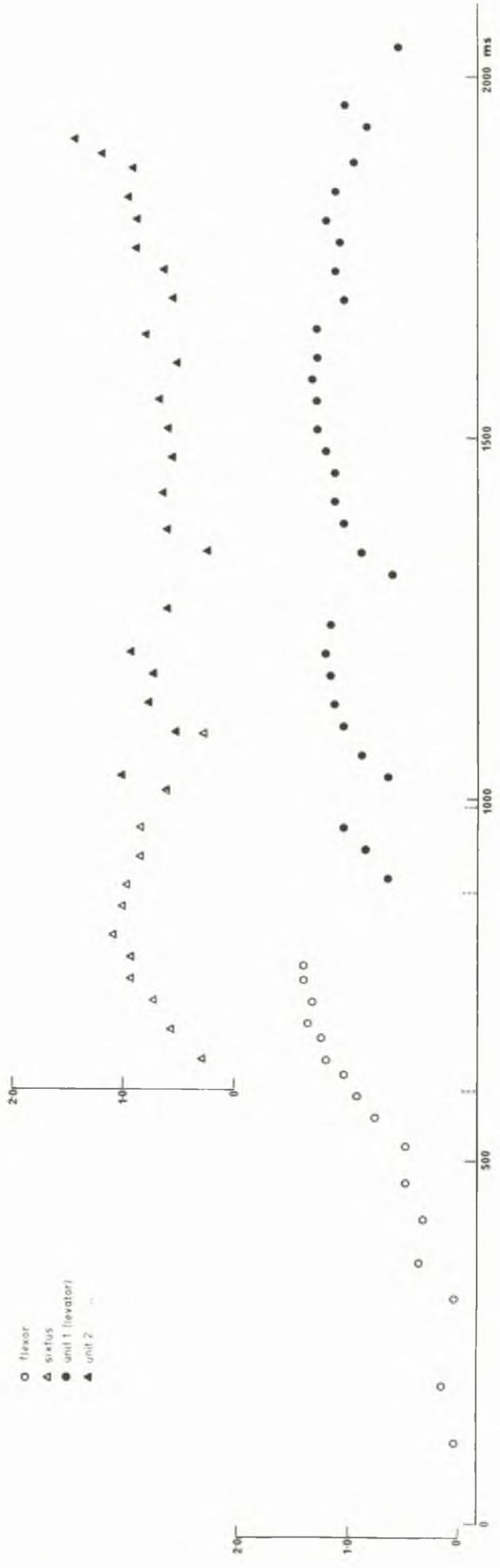




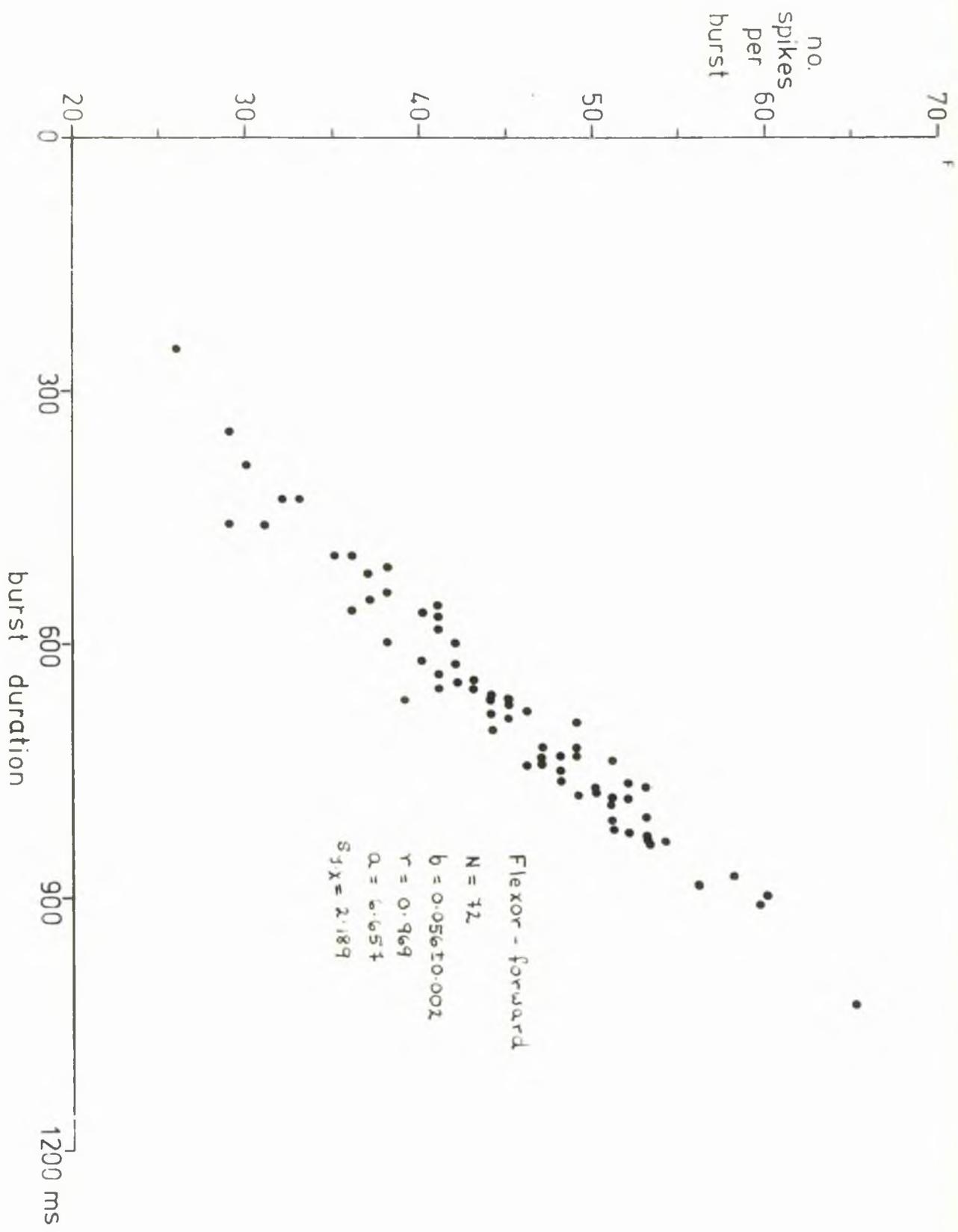
Figure V.9

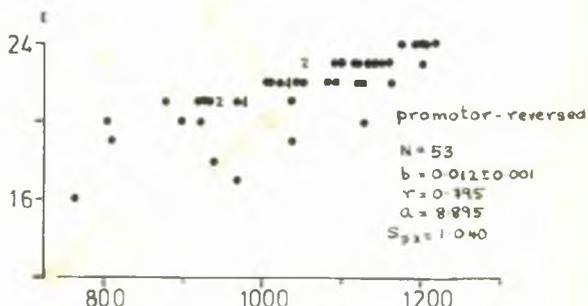
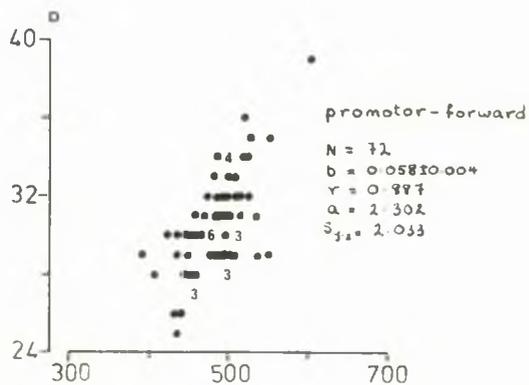
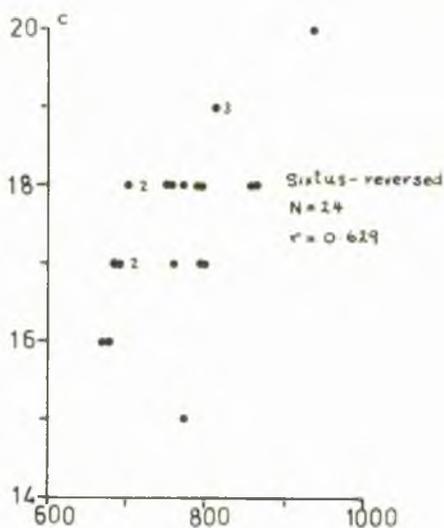
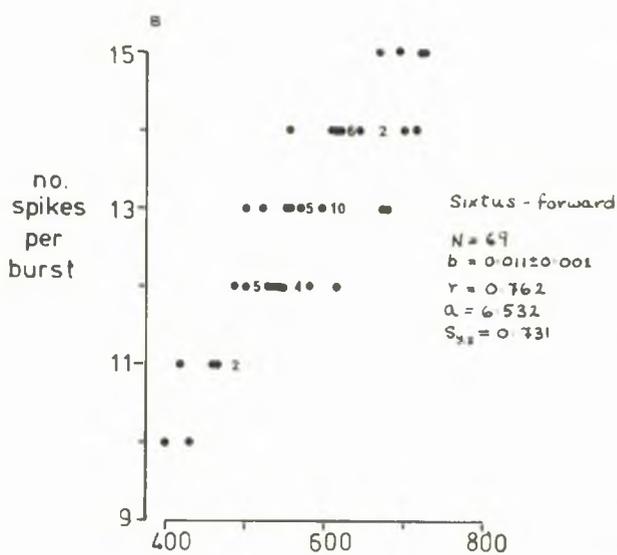
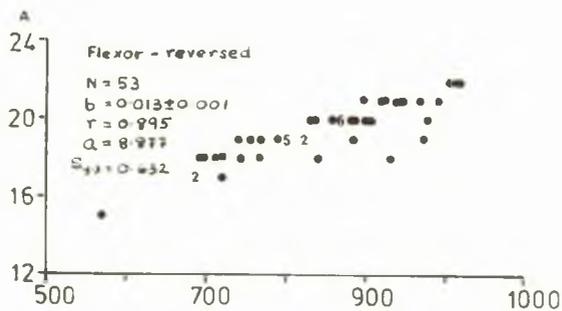
Figure V. 9. Qualitative evidence for negative interactions between some of the motor units in the depressor muscles. Notice in A and O the two types of activity that appear in the quartus, and the associated differences in the flexor bursts. The quartus may burst for an extended period, the intensity of the burst building up slowly as the flexor burst continues, declining slowly in intensity. At times, the quartus burst begins at a high intensity, preceded by what may be a series of large, summing ejp's. When this happens, the flexor burst is disrupted, sometimes reappearing as the intense quartus activity declines. Note how the spiking frequency in the sixtus falls abruptly whenever there is overlap with the quartus.



Figure v. 10.

Figure V. 10. The relationship between burst duration and the number of spikes per burst in some of the ventilatory muscles in Carcinus.

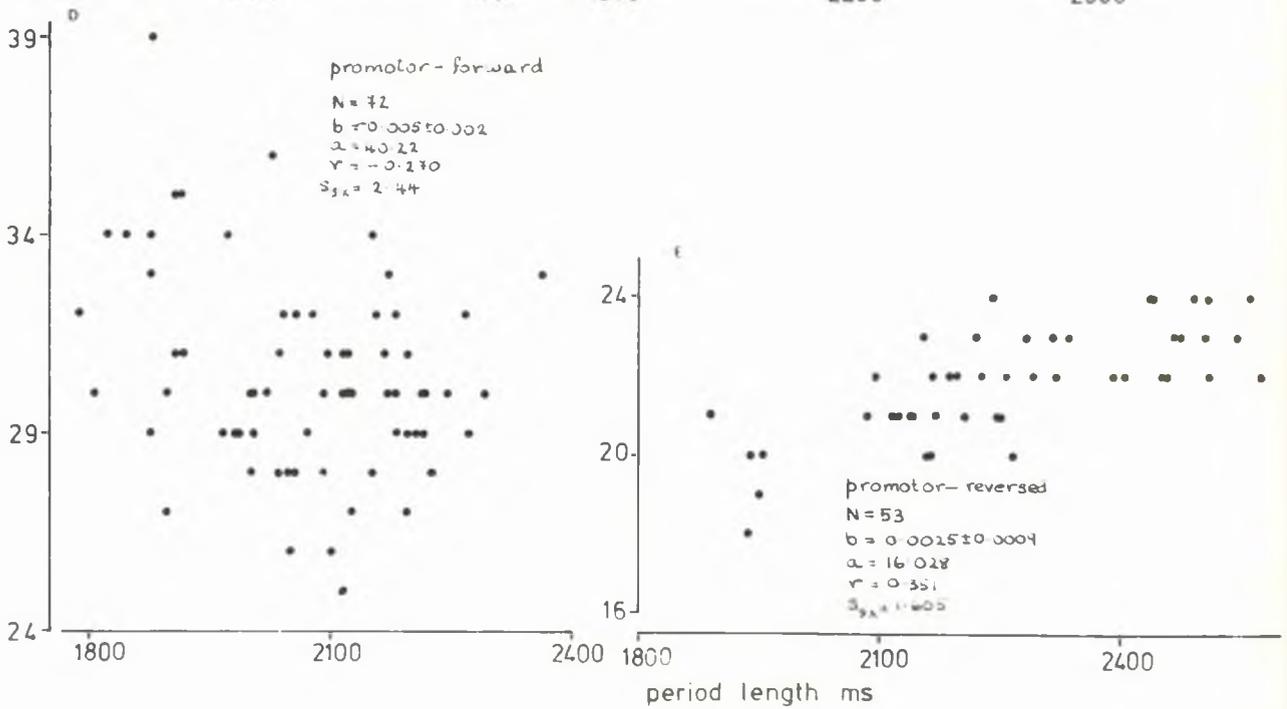
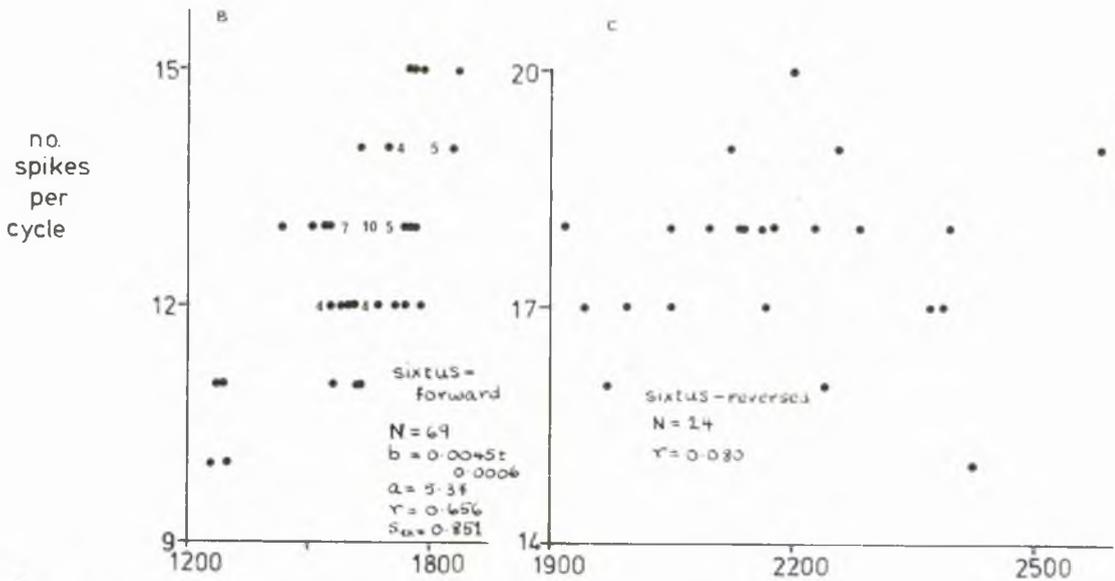
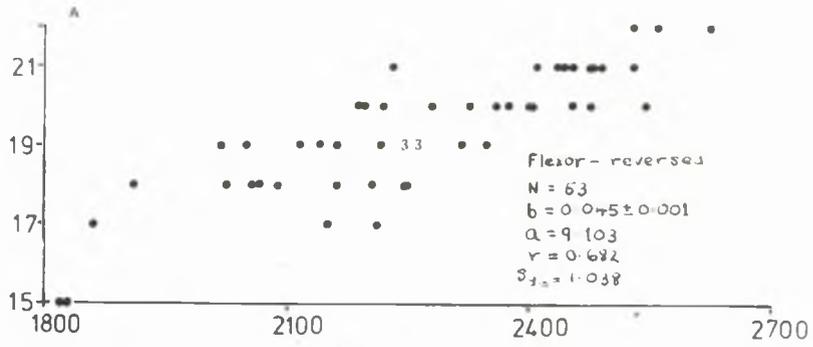


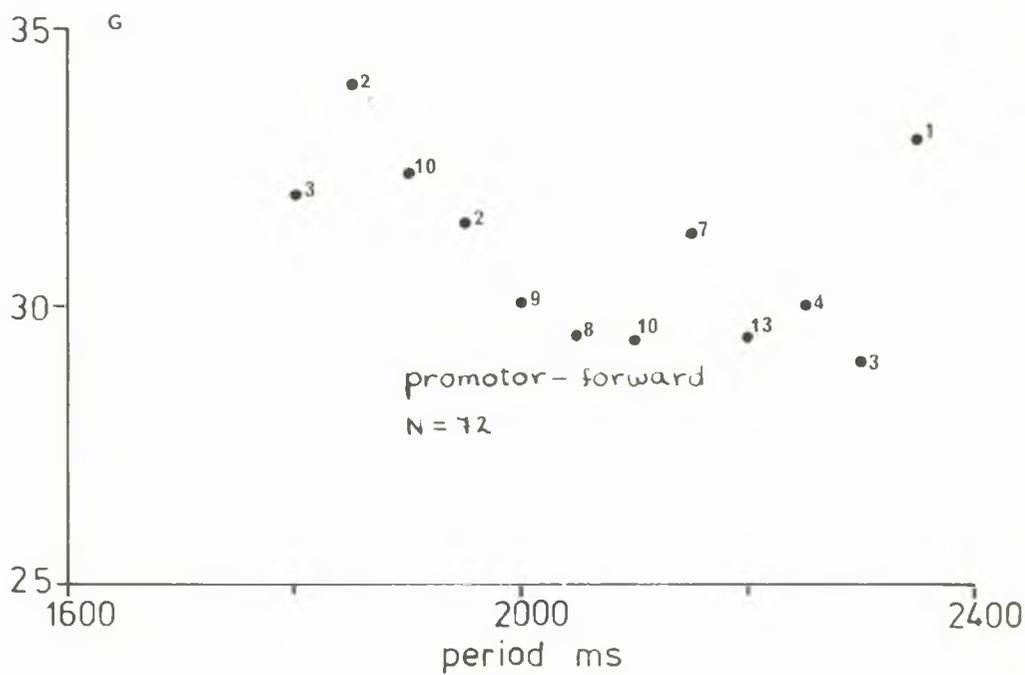
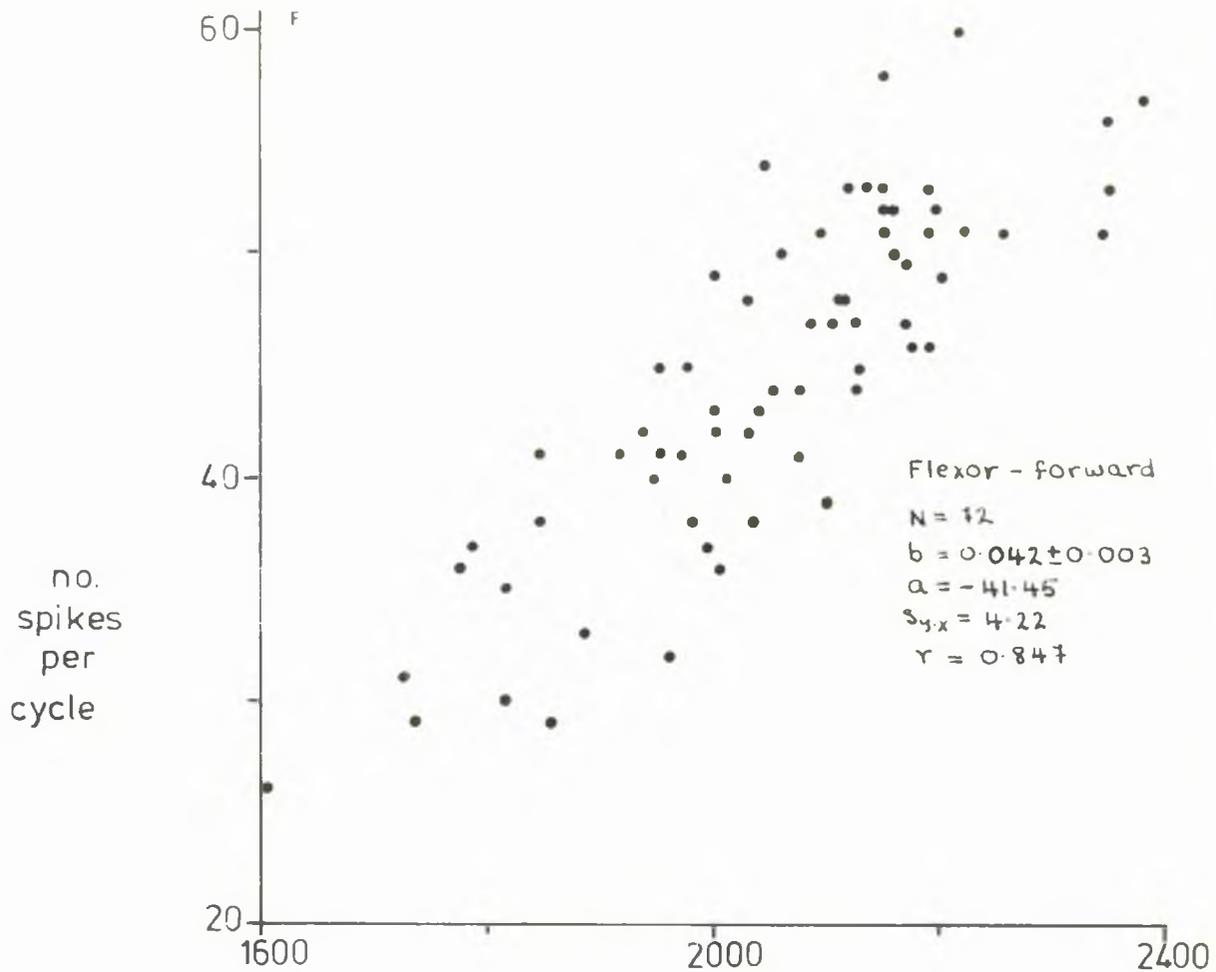


burst duration ms

Figure V-11.

Figure V. 11. The relationship between period length and the number of spikes per cycle in some of the depressor muscles. See text for discussion.





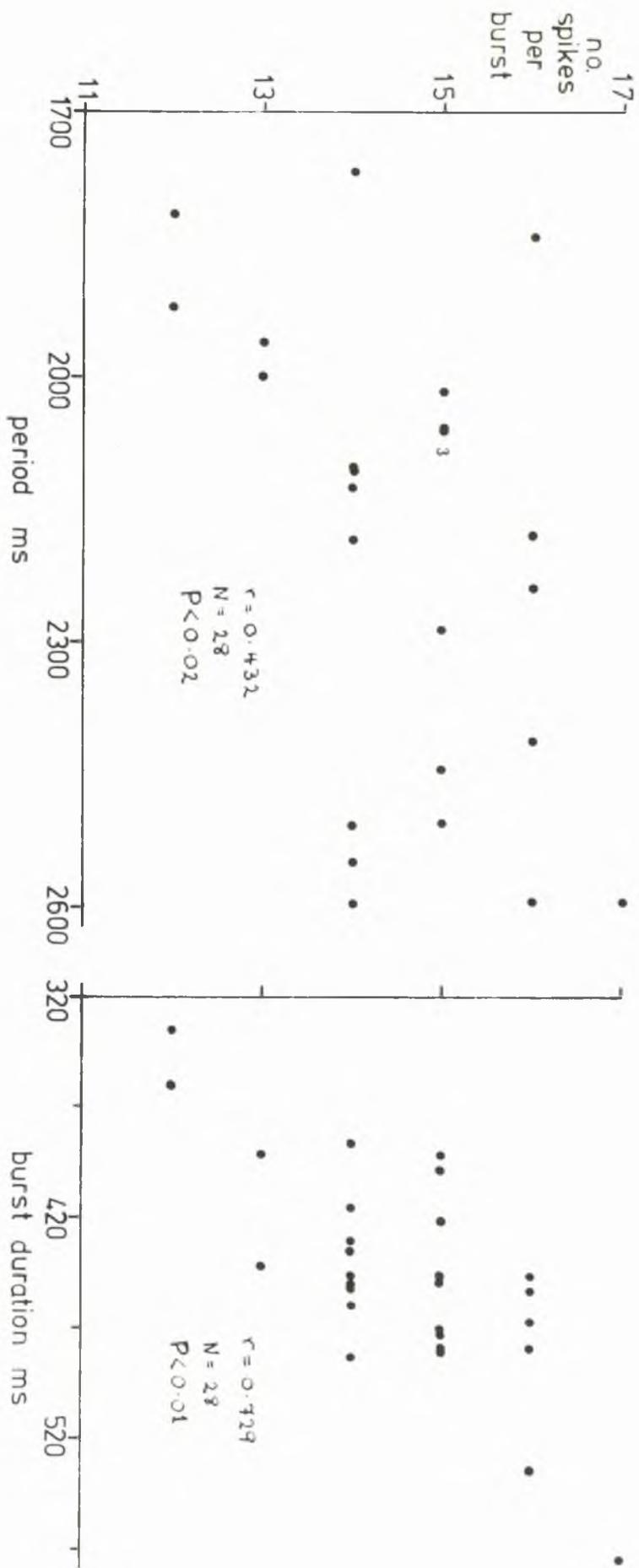
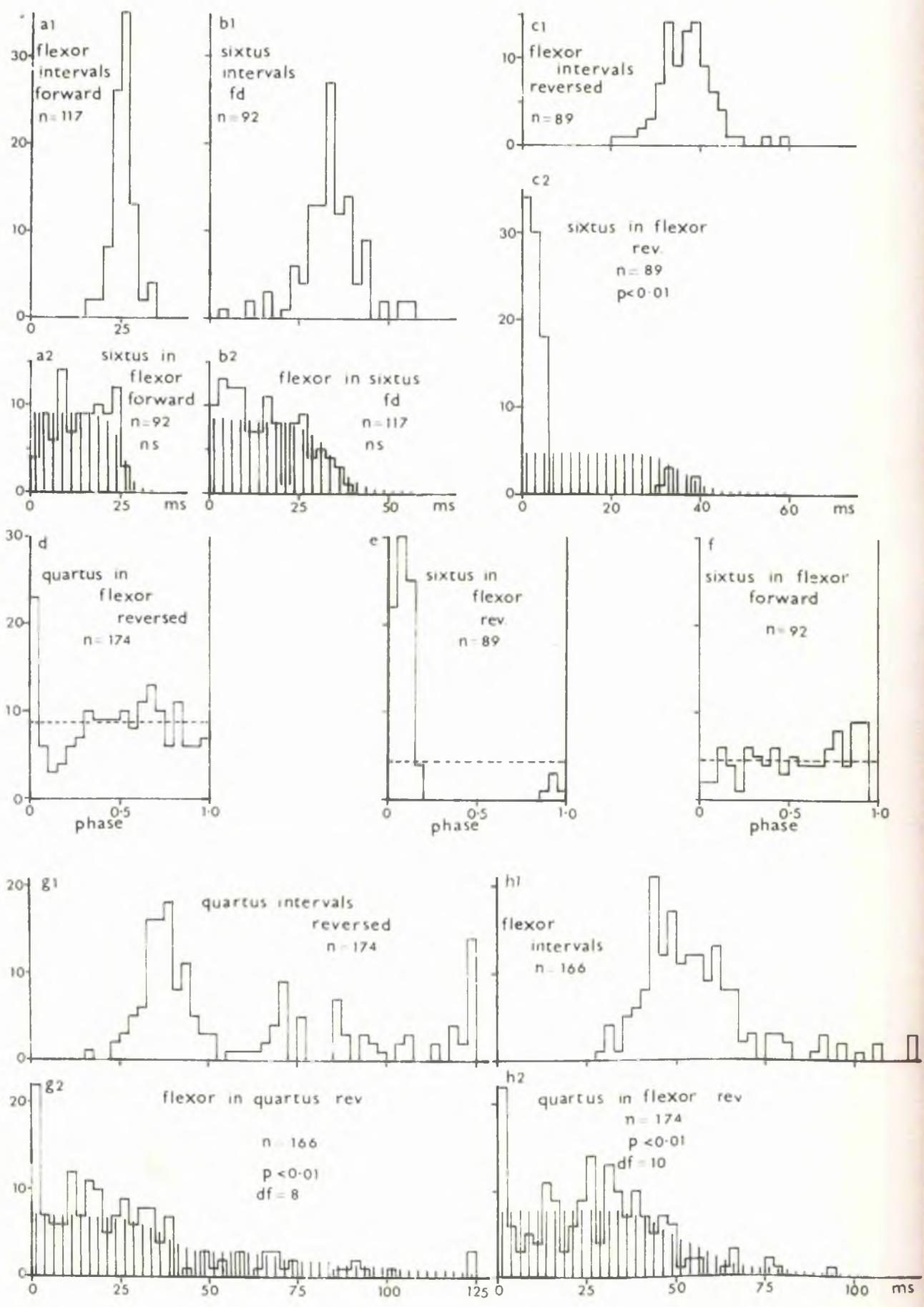


Figure V-12.

Figure V. 12. The interactions between units in some of the depressor muscles during periods of overlapping activity. The histograms are taken in pairs (a1, a2, b1, b2 etc.). The upper member of the pair represents the frequency distribution of the interspike intervals in the reference unit. In the lower member is the observed distribution of the latencies of the spikes in the test unit with respect to the reference unit. The expected distribution in the absence of any interaction is indicated by vertical bars. The expected distributions are derived from the upper distribution as described in Appendix II. d, e and f show phase histograms of the indicated data. In g1, the 122.5 to 125 ms category contains all intervals greater than 125 ms. P is the level of significance for the null hypothesis based on a  $X^2$  test for the observed and expected distribution.



SECTION VI

EXTRA-SYSTEMIC INTEGRATION OF SCAPHOGNATHITE

ACTIVITY AND THE NATURE OF REVERSALS.

### VI.1. Integration between the heart and scaphognathite

Although it constitutes a separately definable system, the single scaphognathite does not function in a vacuum. It forms just one part of a complex organization for ensuring that the organism obtains an adequate supply of oxygen and can eliminate certain metabolic wastes, under a variety of environmental conditions. The rate of oxygen uptake will depend not just on external, but also on internal conditions at the exchange interface (Larimer, 1961; Young, 1973). It is not surprising then, that there may be a marked correlation between heart and scaphognathite activity (Larimer, 1961; Ashby and Larimer, 1964; McMahon and Wilkens, 1971). Detailed investigation of the relationship has not yet been done, and although cardiac regulation by extrinsic neural input has been thoroughly studied by artificial stimulation of the regulator nerves, there have been no studies of the spontaneous activity in these nerves, or of the normal cardiac responses to such activity. The present study deals with some preliminary experiments which extend our knowledge of these interactions and indicate possible courses which more detailed investigations might take.

#### VI.1.1. Methods.

These experiments were conducted on the Norwegian lobster Nephrops norvegicus and the crab Portunus depurator because the regulator nerves were readily accessible in Nephrops and because Portunus was found to survive the dissection better than Carcinus. In order to record from the scaphognathite, the cardio regulator nerves and the heart, specimens of Nephrops were prepared as described by Maynard (1953) for Panulirus argus, but the heart was not perfused. For similar experiments with Portunus, the dissection was as described here for Carcinus when recording from the levator muscles of the scaphognathite (Section II.2.1).

All recordings in these experiments were with suction electrodes. The contralateral cardio regulator nerves were routinely severed in order to eliminate the effects on the heart of the unmonitored activity in these nerve trunks. When recording from the ipsilateral inhibitor only, the ipsilateral accelerator (s) was usually severed also.

### VI.1.2. Results.

A marked correlation was demonstrated here between heart and scaphognathite rates in Nephtrops (Fig. VI.1). Scaphognathite rate was monitored by the photodiode method (Section II.2,3) and heart rate by implanted, insulated copper wire electrodes, bared at the tips. Evidence has been found that the interrelationship may be mediated through units in the extrinsic cardiac nerves in particular, in the inhibitors. En passant recordings from these nerves indicate that under the experimental conditions, there was constant multi-unit discharge in these nerves (Figs. VI. 2 and 3). In the accelerators the activity was of very low frequency and showed no obvious correlation with scaphognathite activity, so attention was concentrated on the inhibitors in which a wider variety of units and more variable activity were exhibited. In both Nephtrops (Fig. VI.2) and Portunus (Fig. VI.3) there is clear correlation between frequency, and to some extent, type of discharge in the cardiac inhibitor and the activity in the scaphognathite. In Nephtrops pauses in scaphognathite beating and disruption of bursts may accompany respectively, intense tonic discharge of medium to large units in the cardiac inhibitor and the discharge in short, intense bursts of a large, phasic unit (Fig. VI.2). During normal bursting in the heart and scaphognathite the frequency of discharge in the medium sized, tonic units remained low at about 5 to 10 Hz. Abrupt increase in the discharge rate of these units to about 25 Hz is followed by cessation of activity in the heart and

scaphognathite. In this experiment (Fig. VI.2), no activity reappeared in the scaphognathite so long as the discharge in the tonic units remained above 12 Hz approximately. Curiously, the heart appears to be less 'responsive' to these discharges than is the scaphognathite. The largest changes in the heart beat usually occur at the onset of the high frequency discharge, when there is a long pause. After about 4 to 6 seconds, even if the discharge continues, heart beats begin to reappear with increasing frequency. If there is then a further rise in the frequency of the inhibitor discharge, the interbeat intervals may once more lengthen. The obvious explanation is that the heart rapidly accomodates to the inhibitor input, but the scaphognathite doesn't. This of course is merely in a manner of speaking, since it assumes that the neurones whose axons are being monitored in the inhibitor also send colaterals to the scaphognathite pacemaker, and influence it in the same way that they influence the cells of the cardiac ganglion. There is no direct evidence that this is the case, and the true relationships may be more complex.

The activity in the cardiac inhibitors (Fig. VI.3) and accelerators in Portunus is very similar to that in Nephrops (Fig. VI.2). However, in Portunus, increased firing frequency in the cardiac inhibitor is normally not accompanied by clear pauses in scaphognathite beating. Here, there is a complex, but quite reproducible response involving changes in beat frequency and amplitude of the muscle spikes in the electromyograms (EMG's) (Figs. VI. 3, 4). The sequence of changes in scaphognathite beating involve first a drop in the interbeat interval (acceleration), followed immediately by a sharp rise (slowing), and a fall once more approximately to the starting level. This then may or may not be followed by relatively irregular beating, or a repetition of the sequence, with smaller peaks (Fig. VI. 4). There are interesting similarities between this response in Portunus and that seen in the last strip on Fig. VI. 2A for Nephrops. It is unfortunate

that in the experiments with *Portunus*, the ecg's were usually anomalous, perhaps as a result of inadequate perfusing procedures.

The relationships between the heart and scaphognathite may extend beyond that of a mere rate linkage. Analyses of fast scan oscillographs from experiments on Nephrops (Fig. VI. 5) indicate that phasic information concerning the scaphognathite cycle might be relayed to the heart via the cardiac inhibitor. Histograms of the phase position of individual units in both the inhibitor and accelerator in relation to the scaphognathite beat cycle in some cases show significant peaks (Fig. VI.5). The relationship was never very pronounced, but it is interesting that significant relationships were at all obtained.

There appears to be little or no feedback from the heart to the central nervous system along the cardiac inhibitor or accelerator in Nephrops. Only rarely were a few, irregular, and somewhat dubious spikes recorded from the proximally sectioned nerve trunks. However, stimulation of the inhibitor might influence scaphognathite activity (Fig. VI.6A). A brief period of stimulation may (strips i,j) or may not (strip k) result in a prolonged pause. In the experiment illustrated in strips i to k, stimulation sessions of less than one full scaphognathite beat cycle failed to elicit any response. The implication of these experiments therefore, is that the normal mechanism for inducing ventilatory pauses is being activated. On the other hand, transient post-stimulus acceleration has also been observed (strip l,m). The responses then, were not clear cut. Such results from the stimulation of the multi-unit nerve trunks, are necessarily difficult to assess. Isolation and stimulation of single units might be more fruitful. There is no indication here, that any of the units in the cardiac inhibitor send colaterals directly to the scaphognathite. The usual reservations concerning antidromic stimulation procedures must apply (Section VII.1.2). Fig. VI.6A demonstrates also, that the discharge in the inhibitor is quite likely to be under the control of higher centres. Stimulation of

the ipsilateral circumoesophageal connective at 20Hz can cause simultaneously, bradycardia and a prolonged ventilatory pause (strips a and b). As perhaps with the antidromic stimulation of the inhibitor, the action is apparently mediated via the mechanisms associated with spontaneous high frequency discharge in the cardiac inhibitor. Strips a and b show that there is an accompanying increase in the discharge frequency in the ipsilateral cardiac inhibitor. This is shown more clearly in strips c and d, from a different experiment. Here, stimulating the connective in a short burst at 25Hz is seen to trigger the normally 'spontaneous' high frequency discharge in the ipsilateral cardiac inhibitor. Paradoxically similar stimuli appear also, to be capable of disrupting the inhibitor discharge (strips e - h). When applied during periods of high frequency inhibitor discharge, such stimuli frequently may be followed by one or more bursts of activity in the scaphognathite. As established previously activity would normally not occur in the scaphognathite during these periods of pronounced inhibitor activity. The paradoxical effects may result from the fact that a mixed population of excitatory and inhibitory command fibres is being stimulated.

Prolonged stimulation of the circumoesophageal connective at low frequencies appears to have an overall inhibitory effect on scaphognathite activity (Fig. VI.6B and C). During stimulation at 2Hz, the rate of activity in the scaphognathite remains low. As soon as the stimulus is stopped, a burst of activity follows. In both cases (B and C) this is interrupted by a brief period of potentiated activity in the inhibitor, which soon subsides, giving way to a relatively high post-stimulus level of scaphognathite activity. During stimulation sessions of ventilatory activity may occur, which are similar to the activity after the stimulation. The effect of the stimulus appears to be a shortening of these sessions of activity, perhaps in two ways. Firstly, the frequency of recurrence and the durations of periods of high frequency activity in the inhibitor may be greater during stimulation. And secondly, even during

some periods of low frequency firing in the inhibitor, normally structured bursts of activity in the scaphognathite appear to be prevented from occurring. There might therefore be a direct effect on the scaphognathite system, as well as an indirect effect, via activation of the system, the activity of which is reflected in the discharge frequency in the cardiac inhibitor. One other point arising from the experiments on *Nephrops* is the occasional occurrence of a rhythmical waxing and waning of the discharge frequency in the cardiac inhibitor. This can be seen in some of the records in Fig. VI.6. The oscillation in inhibitor discharge is often accompanied by periodic disruption of the bursting in the scaphognathite, at a similar cycle time. As a rule periods of higher spiking frequency in the inhibitor may be associated with interruptions of the beat pattern, stronger, more structured bursts occurring in periods of lower inhibitor spiking frequency. This is more clearly shown in Fig. VI.7A. At a much longer period length, a rhythmical pattern of ventilation consisting of batteries of bursts separated by pauses also has been observed in whole animal preparations particularly under conditions of stress, for instance, during prolonged anoxia (Fig. VI.7B). This might probably be related to the periodical resurgence of very high frequency discharge in the cardiac inhibitor as seen in Fig. VI.2. The observation of linked activity in the cardiac inhibitor then, gives some indication of the sort of underlying activity which might be involved in the generation of ventilatory rhythms of longer period than the single scaphognathite beat.

#### VI.1.3. Interim Discussion.

The spontaneous activity in the cardiac inhibitors in *Nephrops* and *Portunus* and the responses of the heart in *Nephrops* to this activity concur remarkably well with earlier artificial stimulation studies (Wiersma and Novitaki, 1942; Maynard, 1953; Florey, 1960). It is interesting that there is a constant background discharge in the single inhibitor at a frequency

(c. 5-15 Hz) below that required to produce a cardiac response to artificial stimulation (studies quoted above). The discharge occasionally rise abruptly to peak frequencies of the order of 25-30 Hz in Nephrops and 30-60 Hz in Portunus. At these frequencies for Nephrops the heart is temporarily inhibited, but appears to accommodate within about 4 to 6 seconds. This is in complete accord with the investigations of Florey (1960) who found that artificial stimulation of single inhibitors at 25-40 Hz could cause complete heart stoppage and that the response rapidly accommodated. His records show that at low frequencies of stimulation (25 Hz) heart beats resumed after about 4 seconds and at higher frequencies (35 Hz) after about 14 seconds in spite of continued stimulation. It is reassuring to find that artificial stimulation of multi-axon nerve trunks can yield results corresponding so closely with the effects of spontaneous activity, even when different species are considered.

The relatively low levels of spontaneous activity in the cardiac accelerators corresponds with the relatively low artificial stimulation frequencies required to produce responses to stimulation of these nerve trunks (studies quoted above). The absence of obvious correlation with either heart or scaphognathite is in keeping with the demonstration by these workers that during simultaneous stimulation of accelerators and inhibitors, the inhibitory influence predominates. It is also in agreement with the findings of Larimer (1964) and Ashby and Larimer (1965) that in the crayfish Procambarus there is a preponderance of coupled inhibitory as opposed to excitatory response in heart and scaphognathite to various applied stimuli.

In Nephrops where sustained reversal of beat direction apparently does not occur, increased frequency of discharge in the cardiac inhibitor is associated with marked ventilatory pauses. In Portunus more complex influences lead to some speculations. It will be shown later (Chapter VI.3) that reversals in Carcinus are associated with transient increases in ventilatory rate (Fig. VI.3) and alterations in EMG amplitude. This is

strongly reminiscent of the changes in beat rate and EMG amplitude associated with high frequency inhibitor discharge in Portunus. Although it seems likely, there is at present no direct evidence that these latter changes are associated with reversal. In addition to the responses to abrupt high frequency discharge, fluctuations at a lower level of inhibitor spiking frequency may be accompanied, particularly in Nepherops, by associated oscillations in ventilatory rate. Assuming therefore that the heart rate is also affected, the pattern of the association in Nepherops is consistent with the close correlation between heart and scaphognathite rate shown in Fig. VI.1.

The occurrence of abrupt frequency changes in the discharge of the cardiac inhibitor and of sharp, phasic bursts of activity, strongly suggests that a command system of some sort governs the coordination of the heart and scaphognathite rates. In fact, Wiersma and Novitski (1942) demonstrated fibres in the oesophageal commissures in Astacus, which when stimulated can elicit inhibition or in other cases, acceleration of heart rate. The inhibitory responses appeared to be mediated via ipsi- and contra- lateral cardiac inhibitors. Wilkens (1973, personal communication) moreover, has found similar fibres in Homarus, which can elicit cardiac as well as ventilatory responses. The present results indicate that stimulation of 'command elements' in the oesophageal connectives can in fact influence the discharge in the ipsilateral cardiac inhibitor, simultaneously affecting scaphognathite activity. It should be interesting now, to see if the responses here, in the cardiac inhibitors are uni- or bilateral and to monitor the activity in the commissures, inhibitors and scaphognathite during spontaneous pauses and reversals. In Carcinus spontaneous reversal may at times occur simultaneously in right and left scaphognathites, and a common, coordinating command input could possibly explain this synchrony.

The records available in the present study are not suitable for analysis of the relative phasing of scaphognathite and heart beats. The demonstration that the heart in Nepherops may receive phasic information concerning the scaphognathite beat cycle indicate that such analyses should be in order. Inspection of film strips recorded at low speeds show no obvious phase

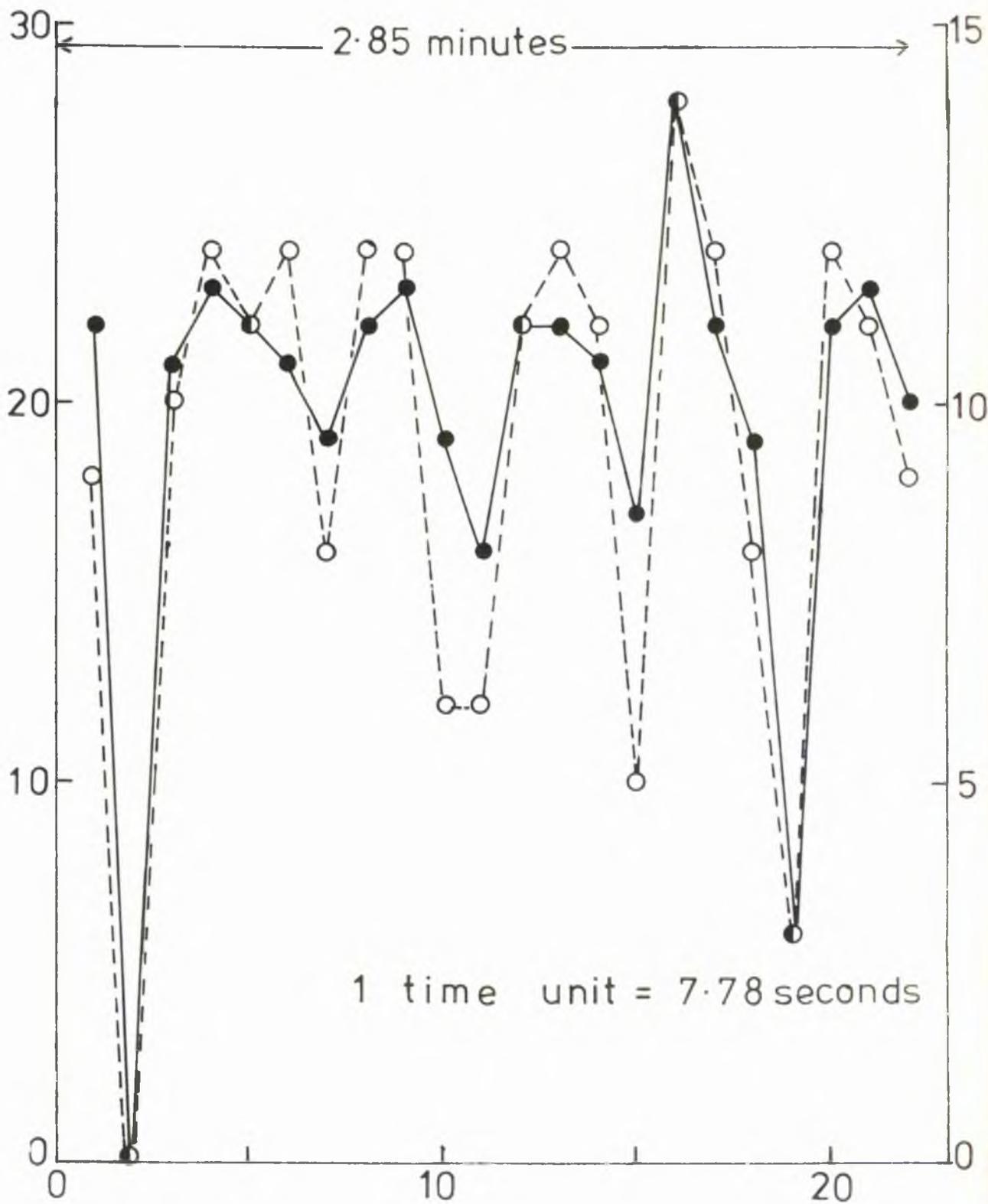
relationship. In the whole animal, the heart response would reflect the integrated input from the inhibitors on both sides. Perhaps therefore, the information becomes useful only when the scaphognathites of both sides are phase locked (see Chapter VI.2). Information of this sort might be responsible for the close coordination between heart and scaphognathite beats in Homarus during 'pauses' in ventilatory activity (McMahon and Wilkens, 1972). It may well be however, that this phasic information might simply be added to the compendium of apparently unused information circulated in the nervous system (Horridge, 1969), and that the phasic aspect of the data is simply ignored or averaged as in the case of the phasic sensory reafference in locust flight (Wilson, 1961).

The rhythmic rise and fall in the discharge frequency in the cardiac inhibitor has implications when viewed in the light of Mendelson's (1971) demonstration of apparently spontaneous sinusoidal oscillators in the sub-oesophageal ganglion in the lobster Homarus, and of Davis' (1969) hypothetical sinusoidal wave of depolarization, passing through the neuropilar network in the abdominal ganglia of the same animal. The activity in the cardiac inhibitors in Nephrops is strongly suggestive of this type of underlying input. The period of the oscillation however, would be several times longer than that associated with scaphognathite or swimmeret beating. Extracellular recording in the neuropile might be one way of further clarifying the nature of these slow oscillations. Elucidation of their origin and mode of generation might be useful in understanding the nature of some slower biological rhythms.

Some of the findings reported in this chapter are of a preliminary nature, and require further analysis and experimentation. They serve firstly, to point out the sorts of external influences that may affect the inter-relationships within the scaphognathite. The 'behavioral unit' is rarely completely isolable from the influence of other, associated units. Secondly, these studies indicate possibilities for further work on the present system from the point of view of the mechanisms of integration between such separate but related units as the heart and scaphognathite.

Figure VI. 1.

Figure VI. 1. The relationship between heart and scaphognathite rates in Nepherops. A - under normal conditions. B - showing coordinated responses during progressively increased hypoxia. The sea water perfusing the bath was percolated down a column packed with chips of porous pot. Deoxygenation was effected by blowing nitrogen gas at measured rates up the column. Notice in both A and B that the ventilatory heart rate ratios were of the order of 2:1. Note also how closely even some of the smallest variations in the two systems are linked.



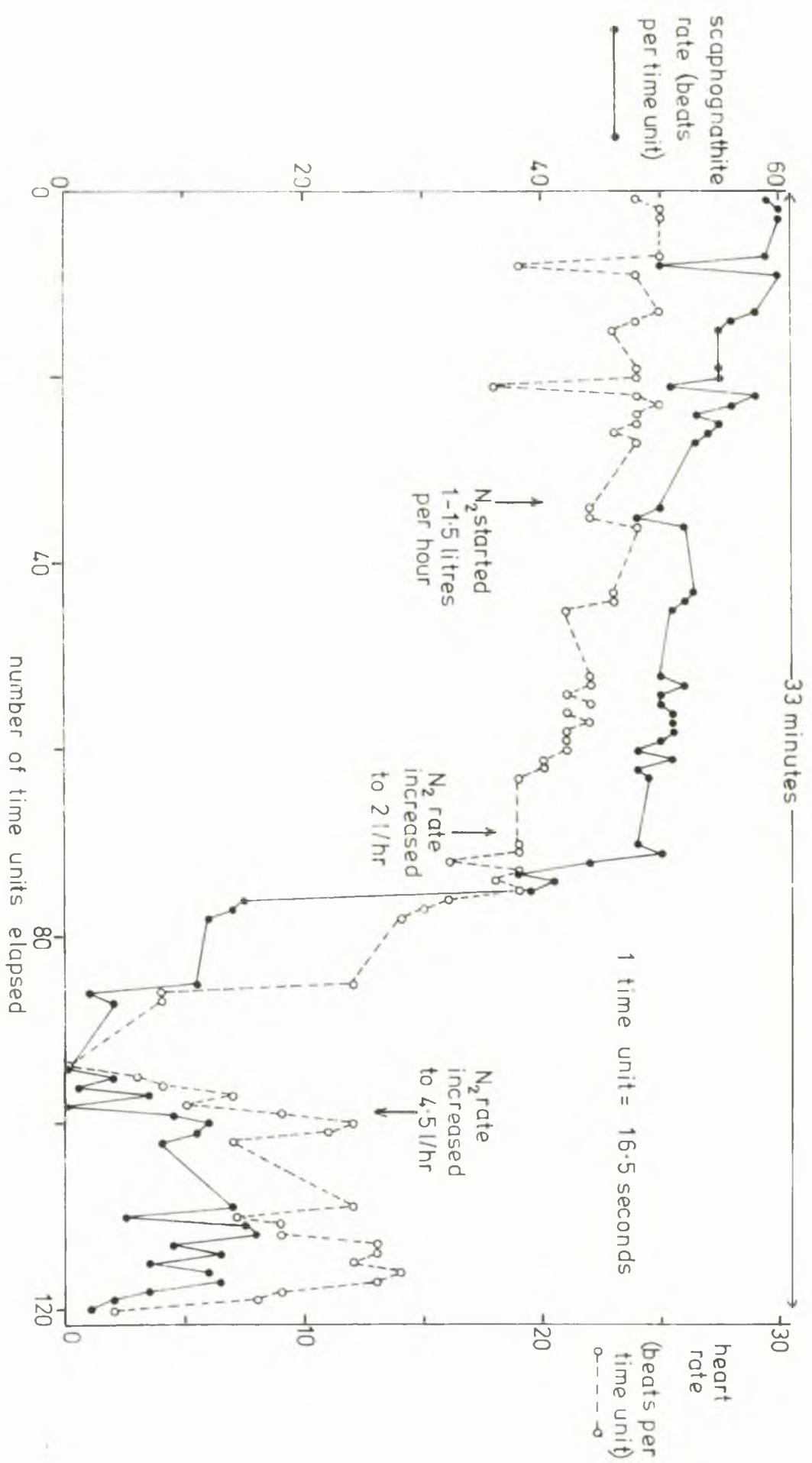
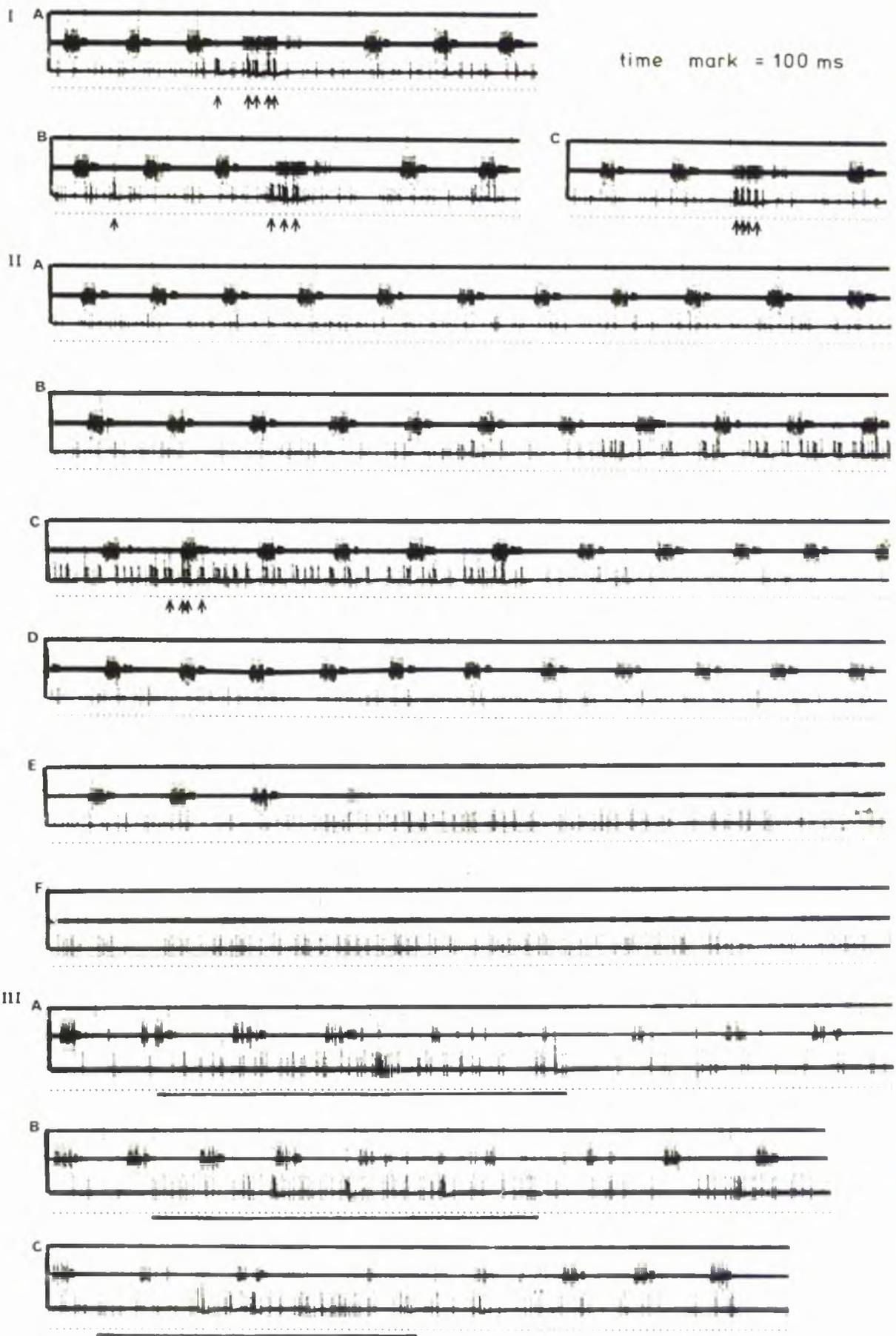


Figure VI. 2.

Figure VI. 2. The relationships between heart and scaphognathite rates and discharge in the cardiac inhibitor in Nephrops. Notice the periodic rise and fall in the frequency of firing in the cardiac inhibitor. Perhaps this sort of activity on a slower scale might explain the periodic rise and fall of the ventilatory rate in Fig. VI. 1A. Notice also change in the muscular activity (perhaps due to changes in the number and type of active units) at the onsets of pauses, and during discharge of a large, phasic unit (arrowed). The numbers between the vertical bars indicate the spiking frequency in the inhibitor (excluding very small and the very large units), during the enclosed period (usually 2 secs). Top trace = eog, middle EMG from an arbitrarily chosen scaphognathite muscles; bottom, cardiac inhibitor. See text for further discussion.



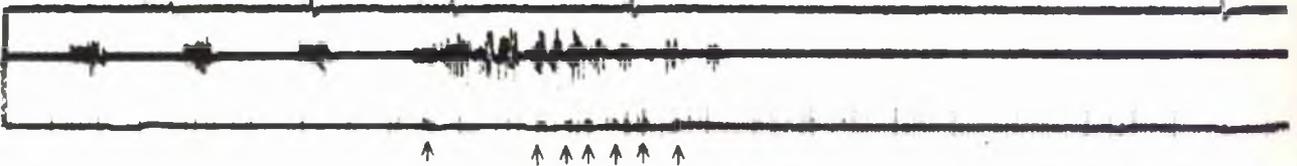
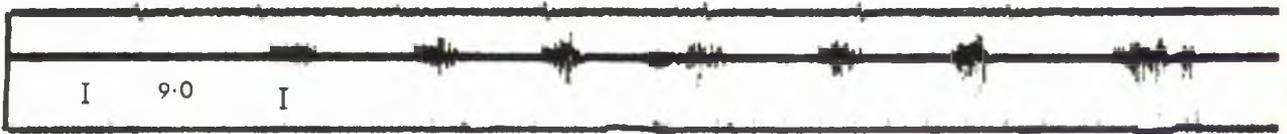
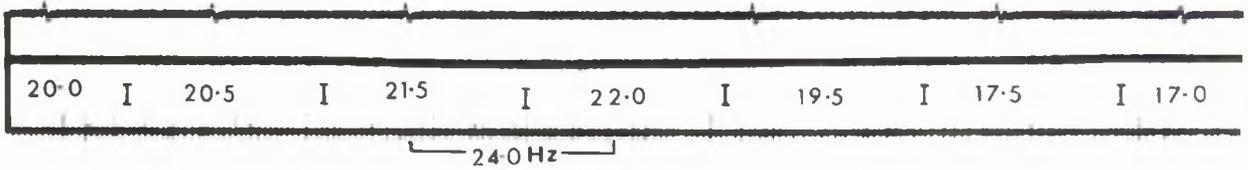
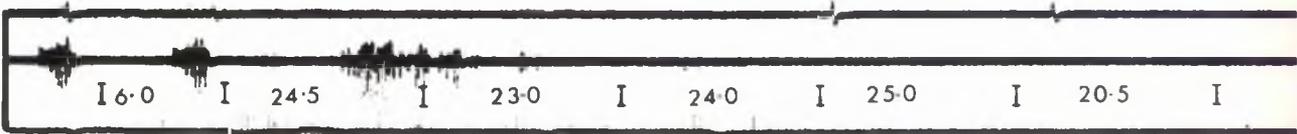
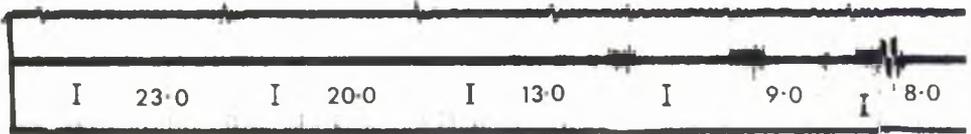
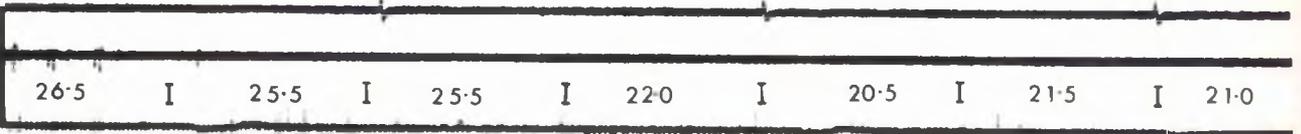
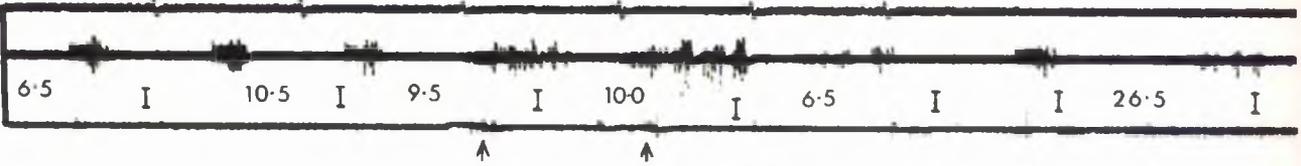


Figure VI. 3.

Figure VI. 3. The relationship between heart and scaphognathite and activity in the cardiac inhibitor in Portunus. The upward pointing arrows roughly indicates the points at which the transient sequence of activity seen in Fig. begins. Three separate experiments are shown (I, II and III). Experiment III is recorded on a faster time base. In I : Top : heart; mid. : inhibitor; bottom : scaphognathite muscle. Notice in I B and in the first part of D that increased activity in some units may not affect the scaphognathite. In II, the heart beat is superimposed on the inhibitor trace (top) as an artefact. There is very little if any effect on heart rate in response to increased inhibitor discharge.

time mark = 500 ms

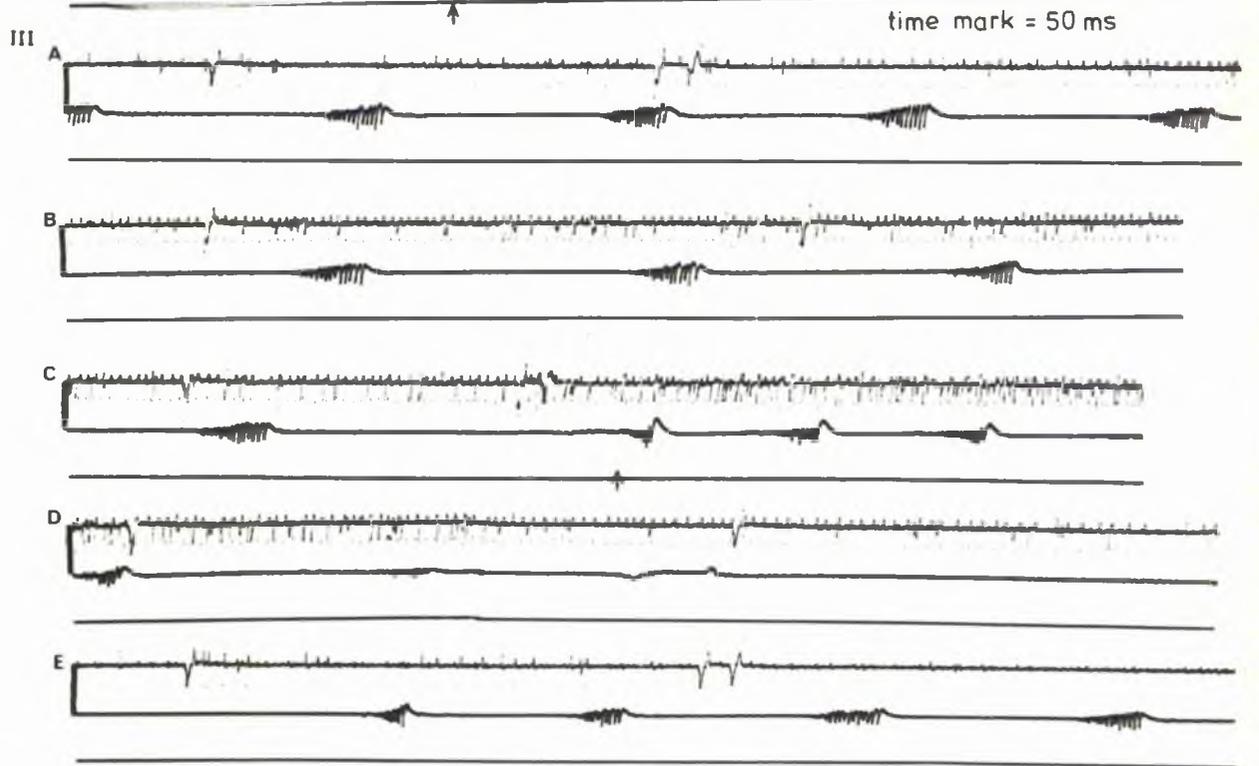
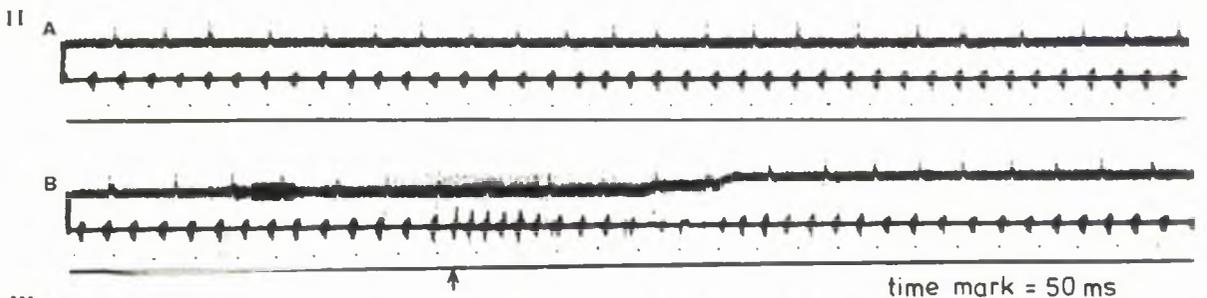
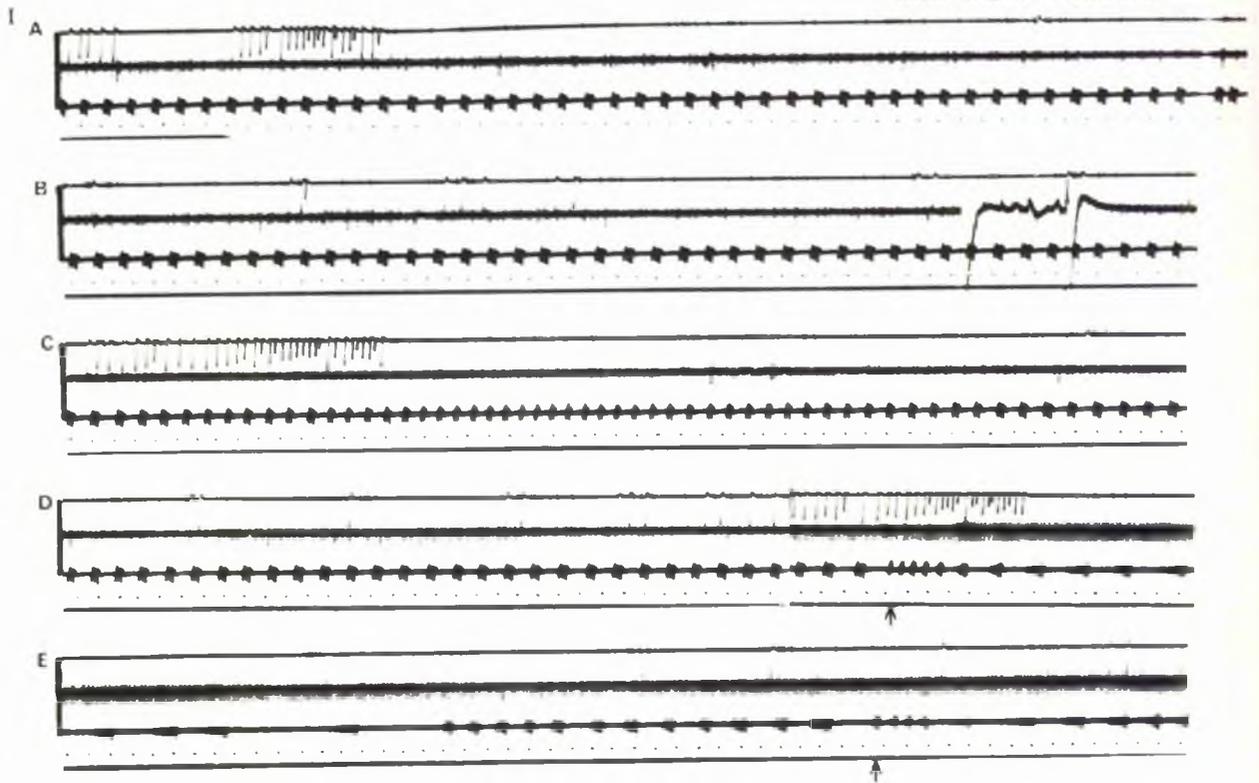
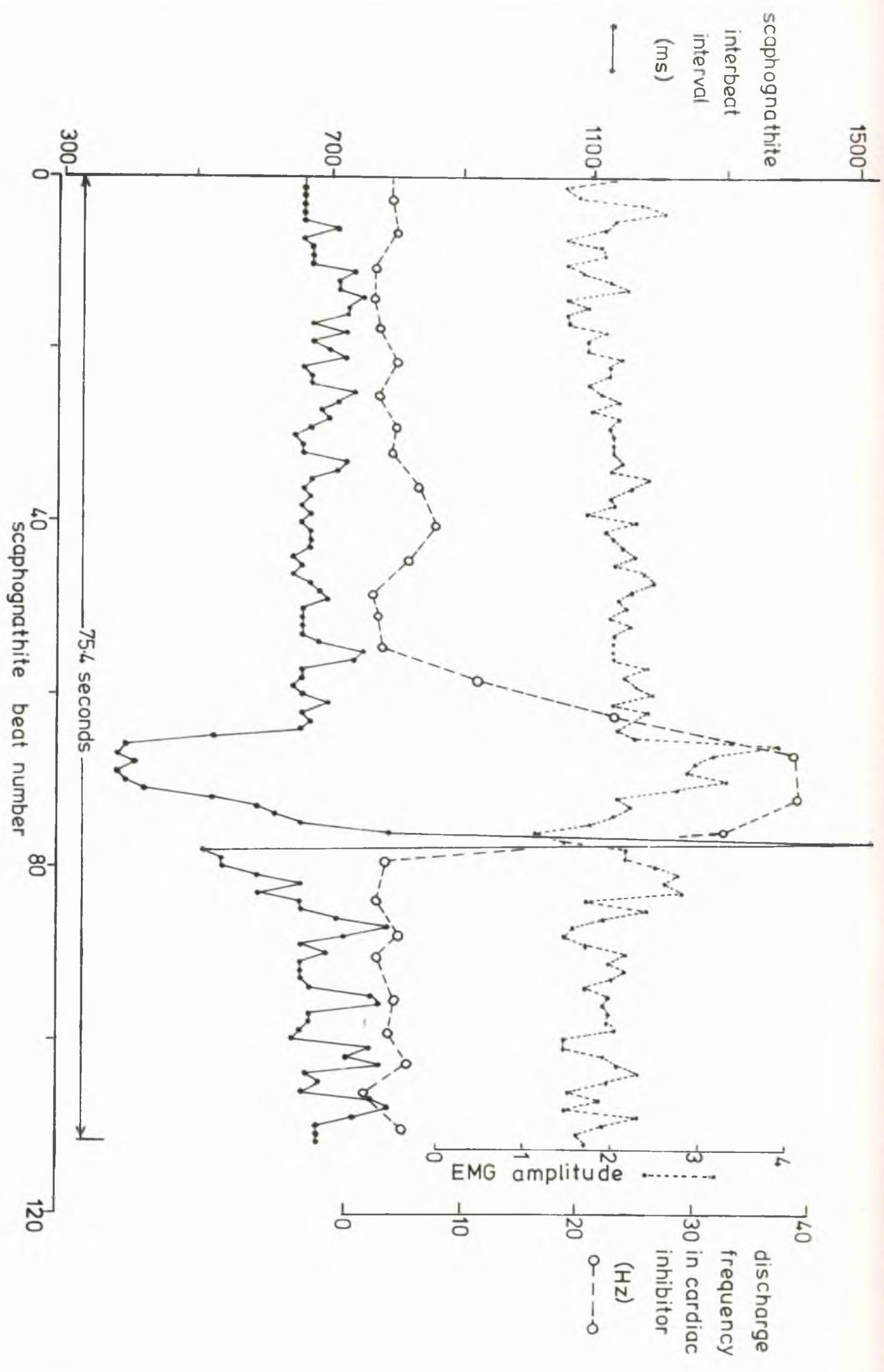


Figure VI. 4.

Figure VI. 4. Relationships between scaphognathite interbeat interval and the firing frequencies in the ipsilateral cardiac inhibitor in Fortunus. See text for discussion.



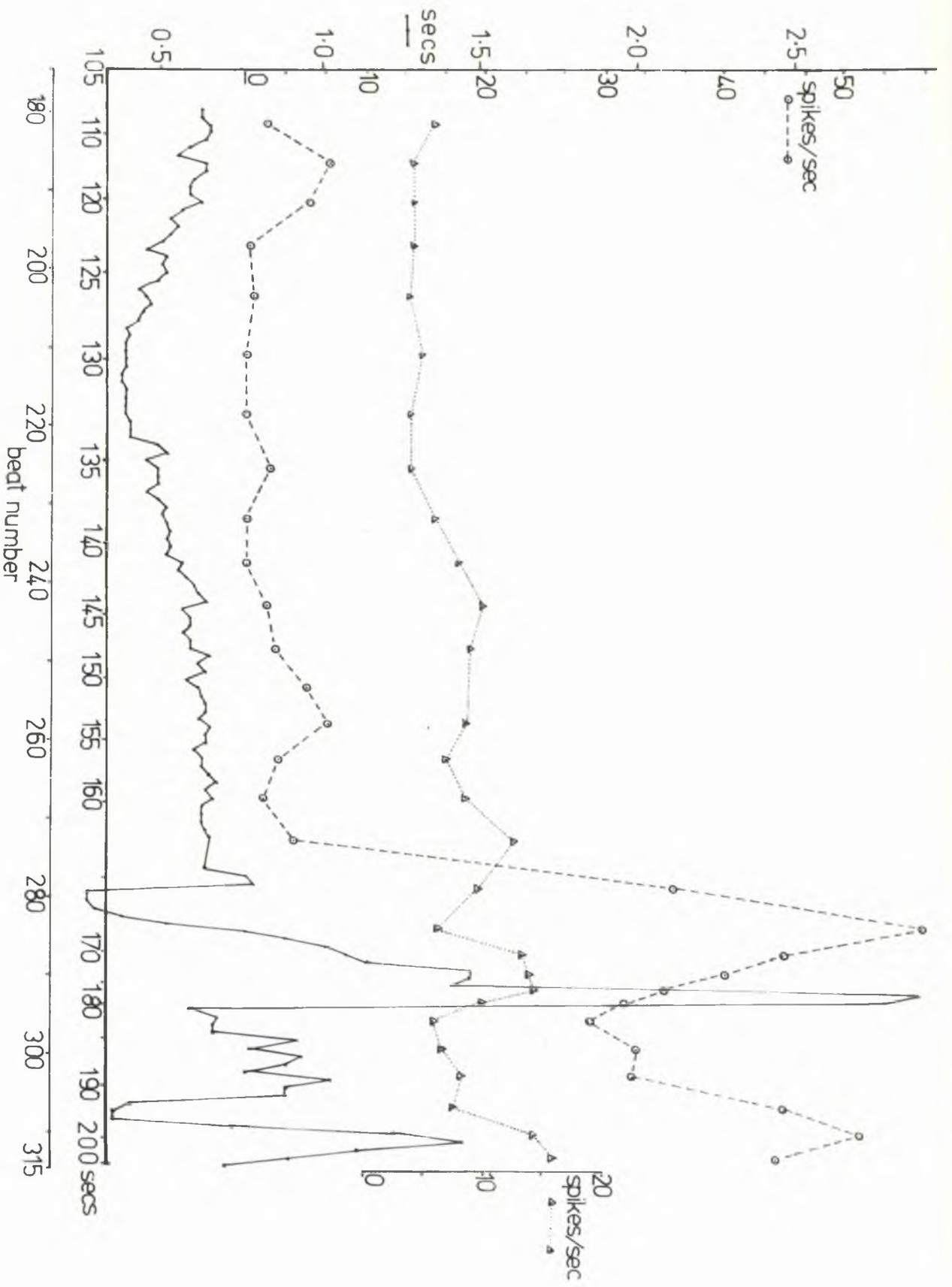
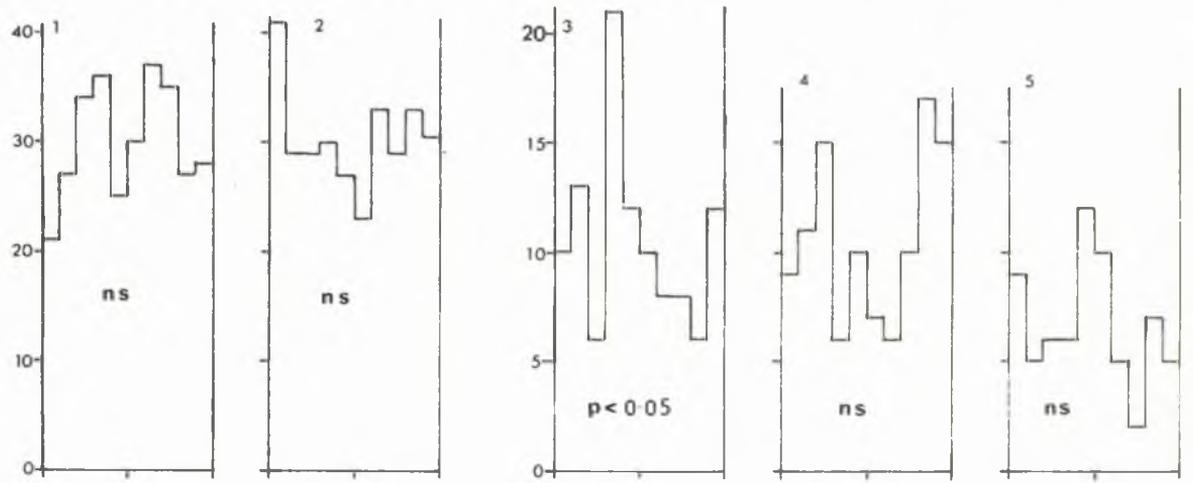
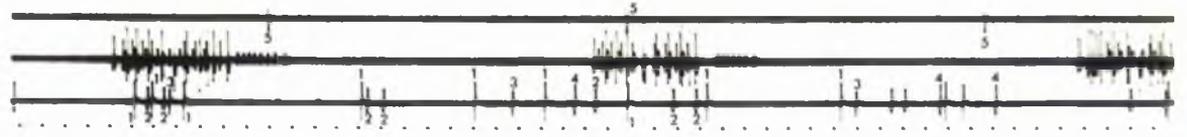
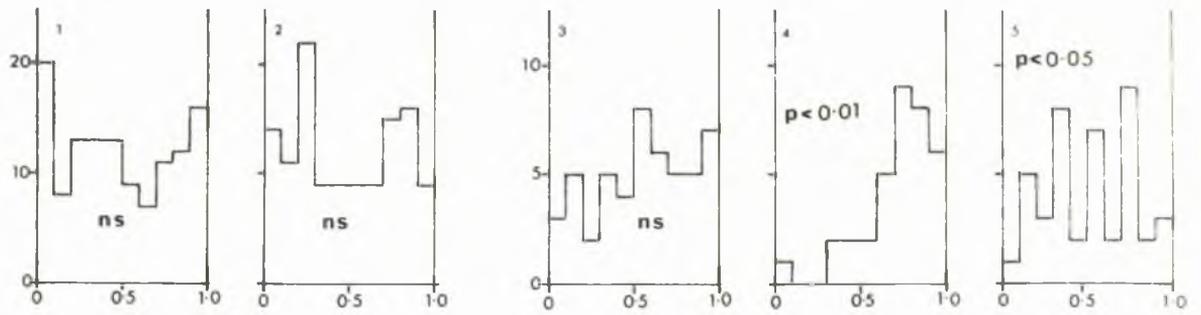


Figure VI. 5.

Figure VI. 5. Analyses of the phase relationships between certain units in the cardiac accelerator and inhibitor in Nephrops, and the scaphognathite beat cycle. The numbers on the histograms refer to the indicated units in the filmstrips below. The unit 4 which shows a highly significant phase relationship with scaphognathite beat, in fact was one of the units showing high frequency discharge during the periods of activity illustrated in Fig. VI.4. A significant relationship was obtained for the accelerator unit (5) in this experiment, showing that although no obvious correlation is seen between scaphognathite and cardiac accelerator discharge, more subtle relationships might exist. See text for further discussion. In each strip: Top : cardiac accelerator; mid. : branch of motor nerve to scaphognathite; bottom : cardiac inhibitor. Time mark = 100 ms.



occurrences

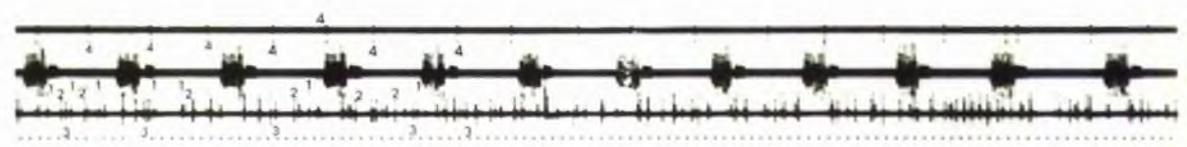
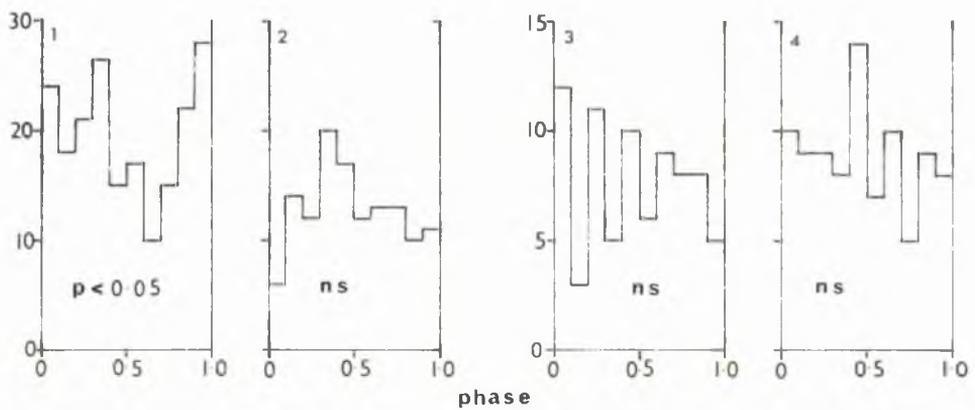
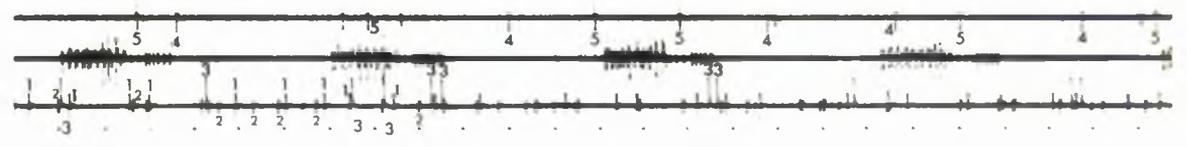


Figure VI. 6A.

Figure VI. 6A. Some artificial stimulation experiments investigating the linkages between heart and scaphognathite and the discharges in the cardiac inhibitor in Nephrops. The numbers indicate the frequency of stimulation during the periods subtended by the rules. (a) to (h) involve stimulating the ipsilateral oesophageal commissure whilst recording from the heart (top trace) the scaphognathite (middle) and the ipsilateral cardiac inhibitor (bottom). In (a) and (b) a muscle in the scaphognathite was monitored and in (e) to (h), one of the incoming motor trunks. (i) to (m) are concerned with stimulation of the cardiac inhibitor whilst recording from the scaphognathite (top) and heart (bottom). See text for further discussion.

A

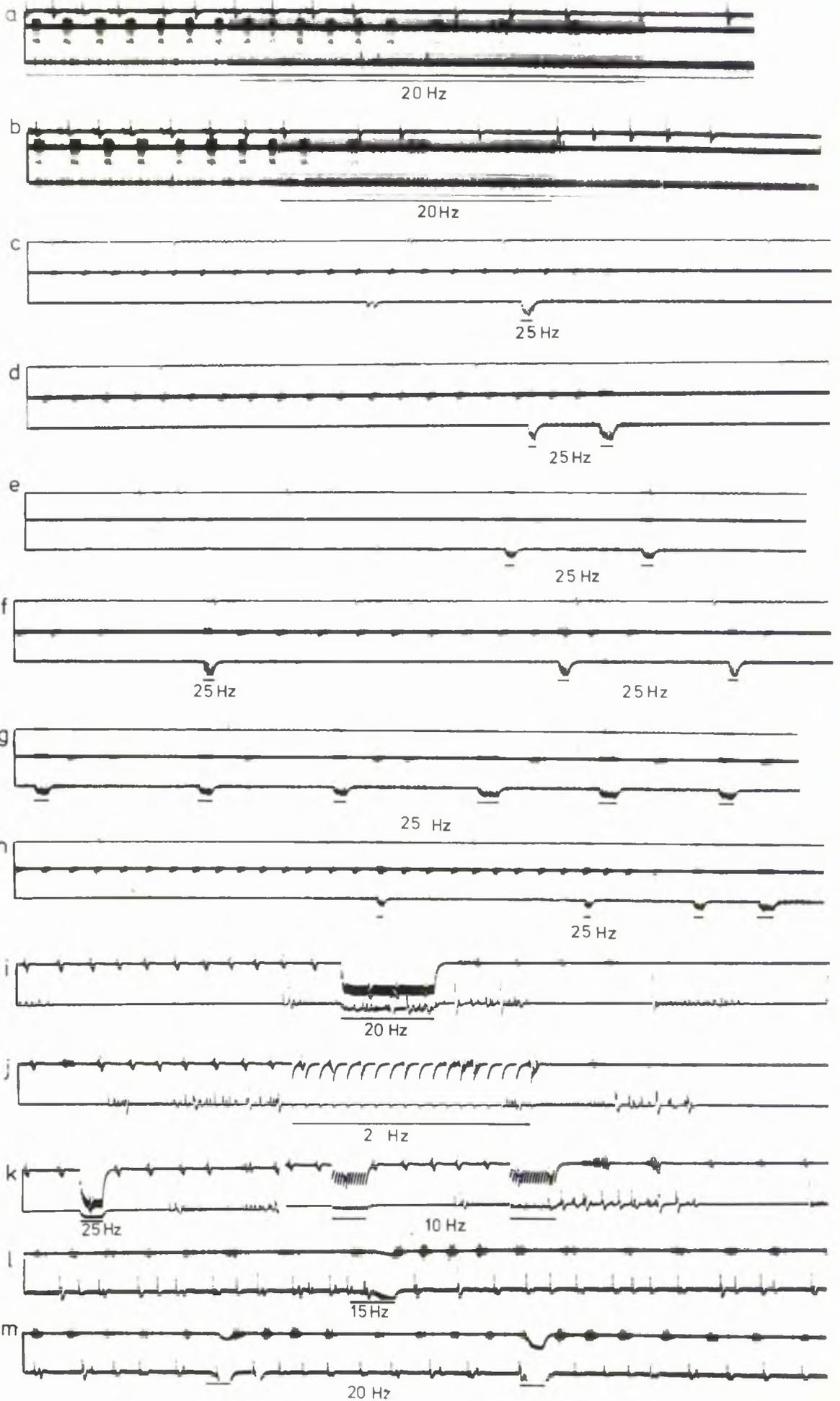
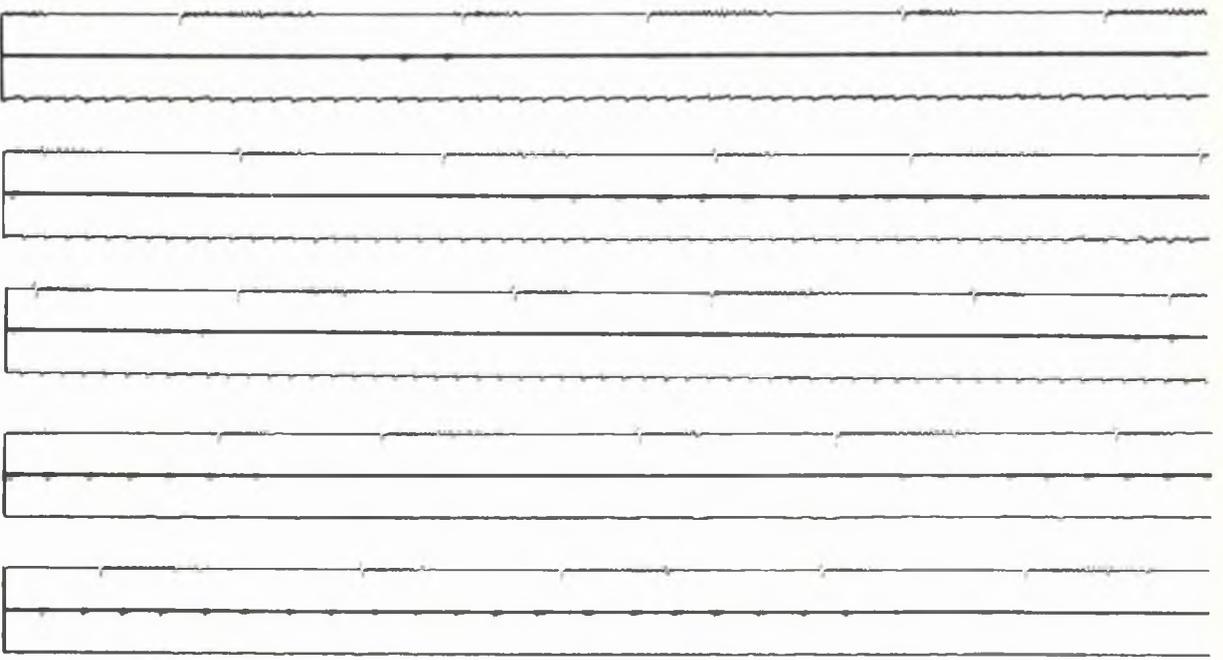


Figure VI. 6 B and C.

Figure VI. 6 B and C. Extended low frequency stimulation of the circumoesophageal connective. Notice in these experiments, the ebb and flow rhythm of activity in the inhibitor during periods when stimulation has stopped.

B



C

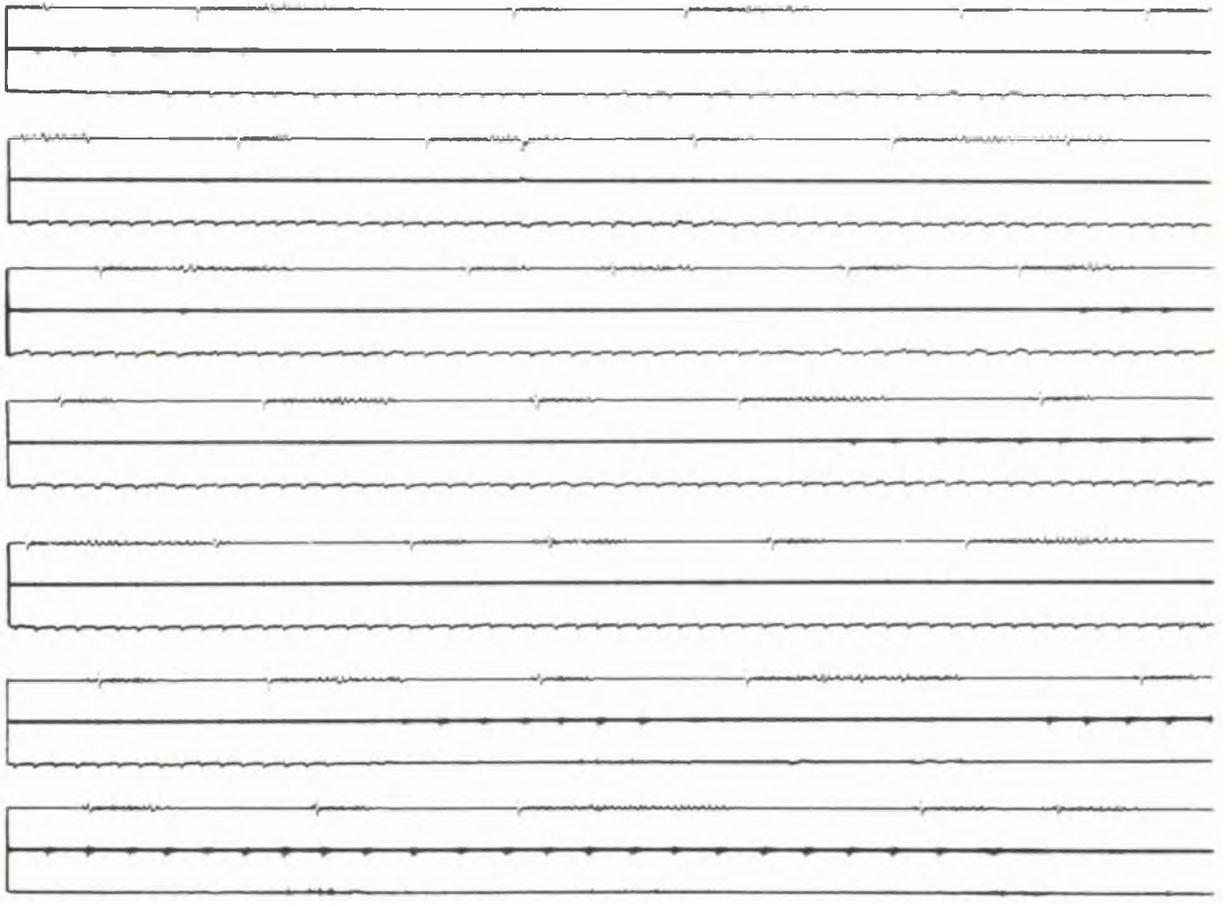


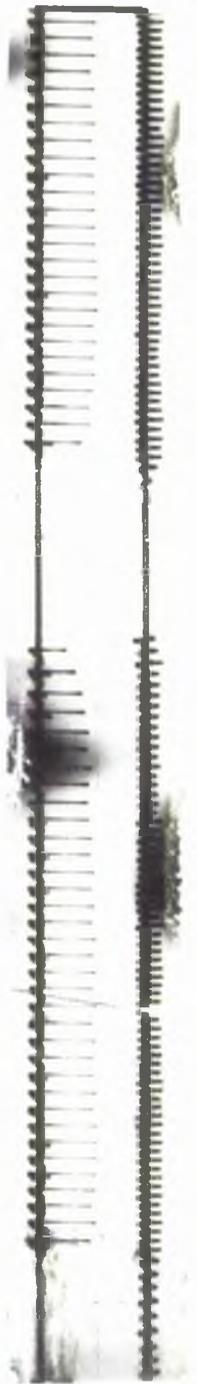
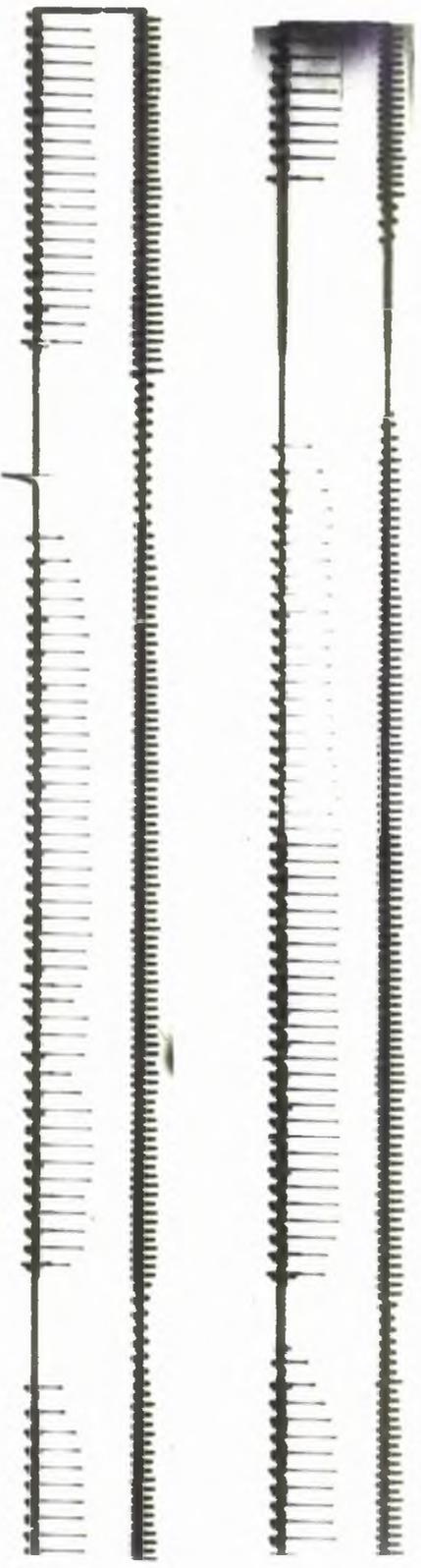
Figure VI. 7.

Figure VI. 7. A. Rhythmic rise and fall in the discharge frequency in the cardiac inhibitor (bottom trace). In this experiment, the accelerator was unusually active. B. A similar rhythm in the recurrence of pauses in scaphognathite activity after prolonged hypoxia, but at a longer period length than that in A.

A



B



5 sec

## VI.2. Relative coordination between the right and left scaphognathites

### VI.2.1. Introduction

In the central nervous system it appears that in many contexts there might be loose coordination between systems that oscillate at approximately the same frequency (Wendler, 1966; Hoyle, 1964). This may be manifested as a locking of the phase relationship between the cycles over certain ranges, and an overall preference for certain relative phase positions although the cycle frequencies may differ (Wendler, 1966). This 'magnet effect' or 'gliding' coordination was first described by von Holst (1934) in connection with fin beating in fish. It is well known that at least in the Macrura, the right and left scaphognathites do not always beat at the same frequency (Segaar, 1934; Johnson, 1936; Larimer, 1964). There has not however, been a detailed analysis of the relative coordination between the beating in the two appendages.

### VI.2.2. Methods

The resistance method for monitoring scaphognathites activity was used in the present studies. The basic design has been described in Section II.2.3. The method provided a good means of monitoring scaphognathite activity in the intact animal. It interfered minimally with scaphognathite activity. In detecting reversals however, it was not so successful because one of the two electrodes on each side frequently did not give a clear signal. Figs. VI. 11 illustrates records from one of the more successful preparations. In the top two channels (right scaphognathite) it is particularly easy to see that changes in (a) the rate of beating and (b) the shape of the waveform in the monitor trace are associated with alterations in the relative

timing between the deflections in the anterior and posterior electrodes. A similar but much less marked change also occurred in the opposite member (bottom channels) about four cycles after the change in the top channels, and persisted until one cycle after the return to the normal pattern in the top channels. Simultaneous observations of the monitor activity and pumping direction confirmed that these changes were associated with incidence of reversals but it is not clear whether they may also accompany mere alterations in beat rate without reversal. These segments of the records may therefore be taken only tentatively to represent transient reversals.

#### VI.2.1. Results

The present studies on Carcinus indicate that here, as in the case cited above, the right and left scaphognathites do not necessarily maintain the same beat frequencies. One may go at 2-3 times the rate of the other, maintaining this difference over many cycles (Figs. VI. 8A and 9A). Usually however, the frequencies are of the same order. Glide coordination then can theoretically be demonstrated as a tendency towards equalization of the beat rates as the appendages pass through certain relative phase angles, so that these angles tend to be held preferentially to all others. Alternatively, adjustments of the relative beat frequencies towards integral multiples at certain phase angles will have the same effect. There will then be more than one 'preferred' relative phase angle.

When the beat frequencies are very different on both sides the phase relationship between the beats shows on the whole the typical drift of uncoordinated oscillators (Figs. VI. 8b and 9b) with no obvious changes in the rate of drift through any given phase angle. Occasionally the beats may become phase locked abruptly, this being accompanied by sharp equalization of beat frequencies (Fig. VI. 8 and 9),

the slower appendage speeding up in order to effect the equalization. The faster appendage may also show increased beat frequency (Fig. VI. 9A). One suspects that this locking might be associated with the abrupt incidence of a common input to both sides from a source external to the immediate system, rather than simply with direct linkage between the two sides. In Fig. VI. 8 it can be seen that after the period of 1:1 phase locking, there follows a sequence in which the beat in B may occur at three different phase angles in the cycle of A, these angles remaining roughly constant over a number of cycles, in which the beat frequencies on both sides differ. Phase histograms in these runs indicate that there is no particular preference for any given phase angle (Fig. VI. 8c and 9c) since periods of coordinated activity are relatively few and occur over relatively brief periods.

In experiments in which the relative frequencies are similar, marked preference for certain relative phase angles is indicated. Phase histograms show significant peaks (Fig. VI. 10c) and as might be expected, the phase plots indicate that there is reduced relative drift about the corresponding phase angles. The preferred phase angles appear to correspond largely with either alternation of the beats on either side (phase angle 0.5) but other relationships are frequently seen. The differences could be related either to true variability or to differences in the point in the beat cycle at which the monitor is affected. There does not appear to be a dominant side as such, whose beat frequency attracts and partially determines that of a subordinate side. Rather, relative phase locking usually appears to be achieved by increased frequency of the slower appendage, or alterations in both. Within a given run the right appendage might sometimes beat faster and at other times, the left, but there appears to be a tendency for one side predominantly to beat at a faster rate than the other (Fig. VI. 10c).

In Figs. VI. 8 - 11 it appears that some periods of marked phase locking might be associated with simultaneous, transient reversals in both appendages (see VI.2.3. Methods). In such cases, the changes in frequency and locking are probably associated with synchronous, common input into both sides. In preparations with the branchiostegites partially removed it was observed that reversals especially when occurring spontaneously, may be synchronous in right and left scaphognathites. The appendages may also reverse independently, especially in response to unilateral, noxious stimuli. If for instance, 20-30 percent alcohol in sea water is applied to one branchial chamber, the ipsilateral scaphognathite may reverse while the contralateral continues to beat in a forward direction. These observations are in some respects consistent with the findings of Wilkens and McMahon (1972) in the lobster Homarus.

#### VI.2.4. Interim Discussion

The relationships described here confirm the suspicion that there might be some degree of relative coordination between the beats in right and left scaphognathites in Carcinus. The form of the relationship is typical of gilding coordination in that phase linkage tends to occur when the beat frequencies in the two scaphognathites are similar and might be associated with particular phase angles (Wendler, 1966). Spontaneous reversal might occur simultaneously in both scaphognathites, and there might be associated equalizing of the beat frequencies and synchronizing of the beats on both sides. This would be consistent with the triggering of reversed beating by a common, synchronizing input into both sides from a higher order command system (see Chapter VI.1.3). Perhaps the hypothetical command system firstly, roughly equalizes the beat frequencies (and perhaps elicits reversal), with the result that a local 'glide coordinating' system is able to effect

phase looking. Bearing in mind the fact that reversals in right and left scaphognathites might be independent, these propositions might be summarized in a model as in Fig. VI.13. The model is not exclusive.

Having proposed this model it is necessary to say that the weaknesses of the data are recognized. The results and model must be evaluated in much the same way as those for the preceding chapter. They indicate possibilities for further experiments which might be designed to test the hypothetical model.

Figure VI. 8.

Figure VI. 8. The relationships between the right and left scapho-nathites when the rates on either side are markedly different (in this case, of the order of 2:1). (a) shows the phase position of the beat in the left scapho-nathite (B) in the cycle of the right scapho-nathite (A), for successive beats in the left appendage. (b) shows on a relative scale, the mean instantaneous frequencies over successive five beat periods in A, and over the most closely corresponding set of beats in B. The relationship is illustrated by the enclosing brackets at the start of the film strip, (d), from which the analyses were done. Notice that phase locking occurs between about beats 75 to 8 in ( ). During this period the beat rate on both sides were abruptly equalized, the right appendage (A) increasing in frequency to beat at the same rate as B. The phase histogram (C) shows no significance since the period of locking was very brief. Further details in text.

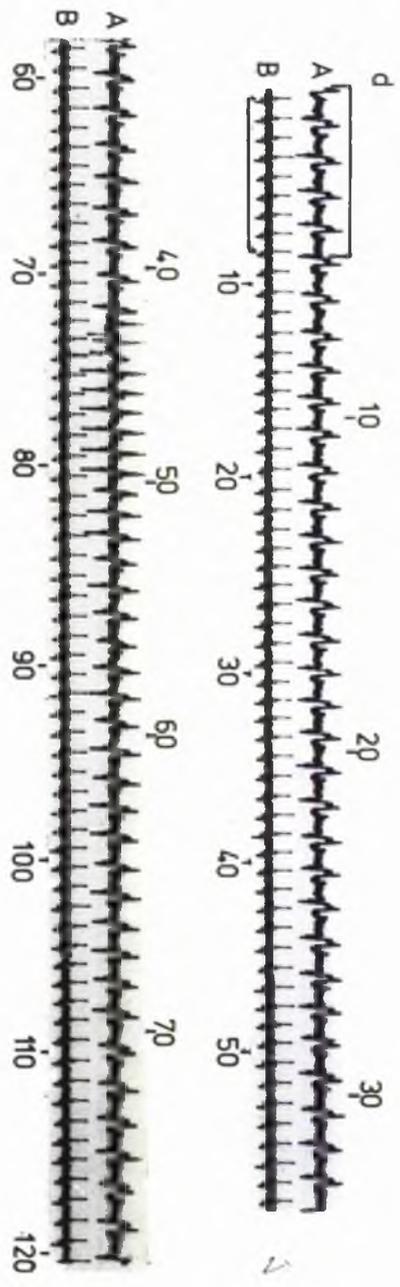
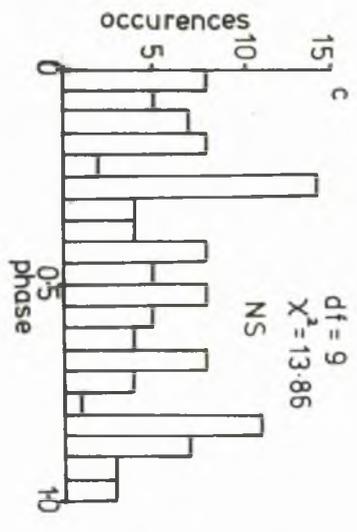
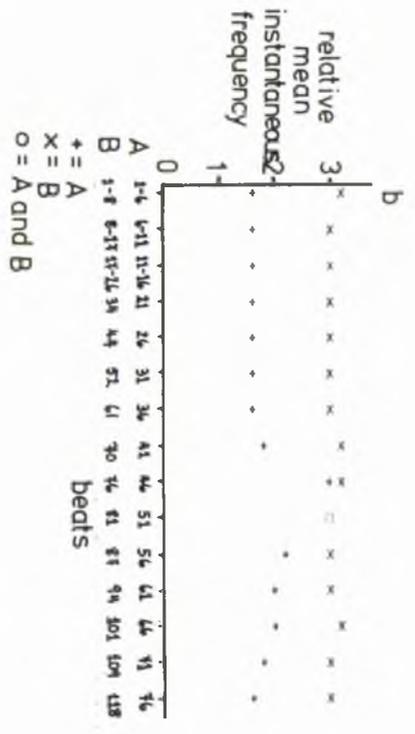
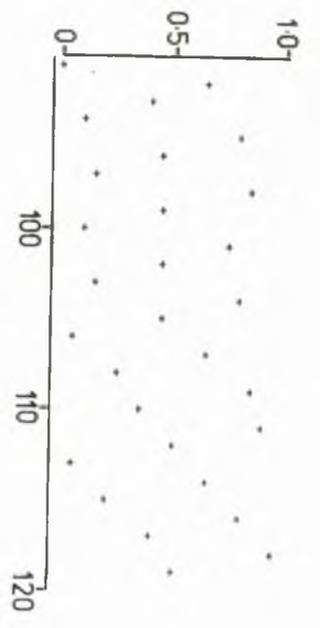
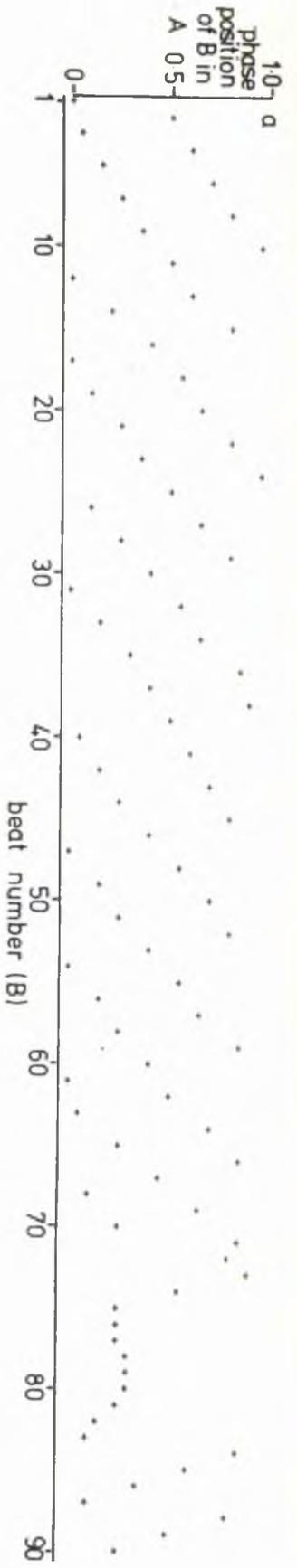
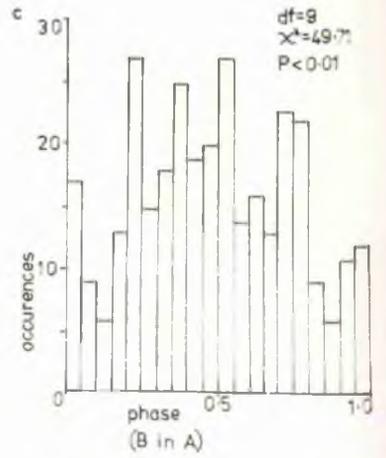
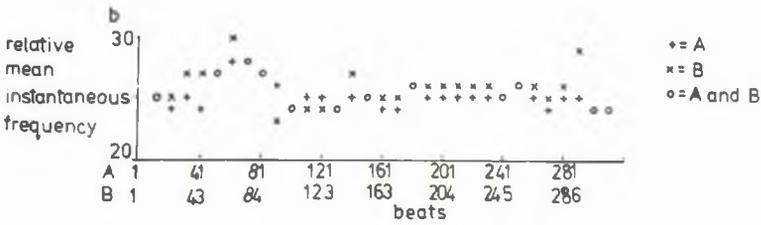
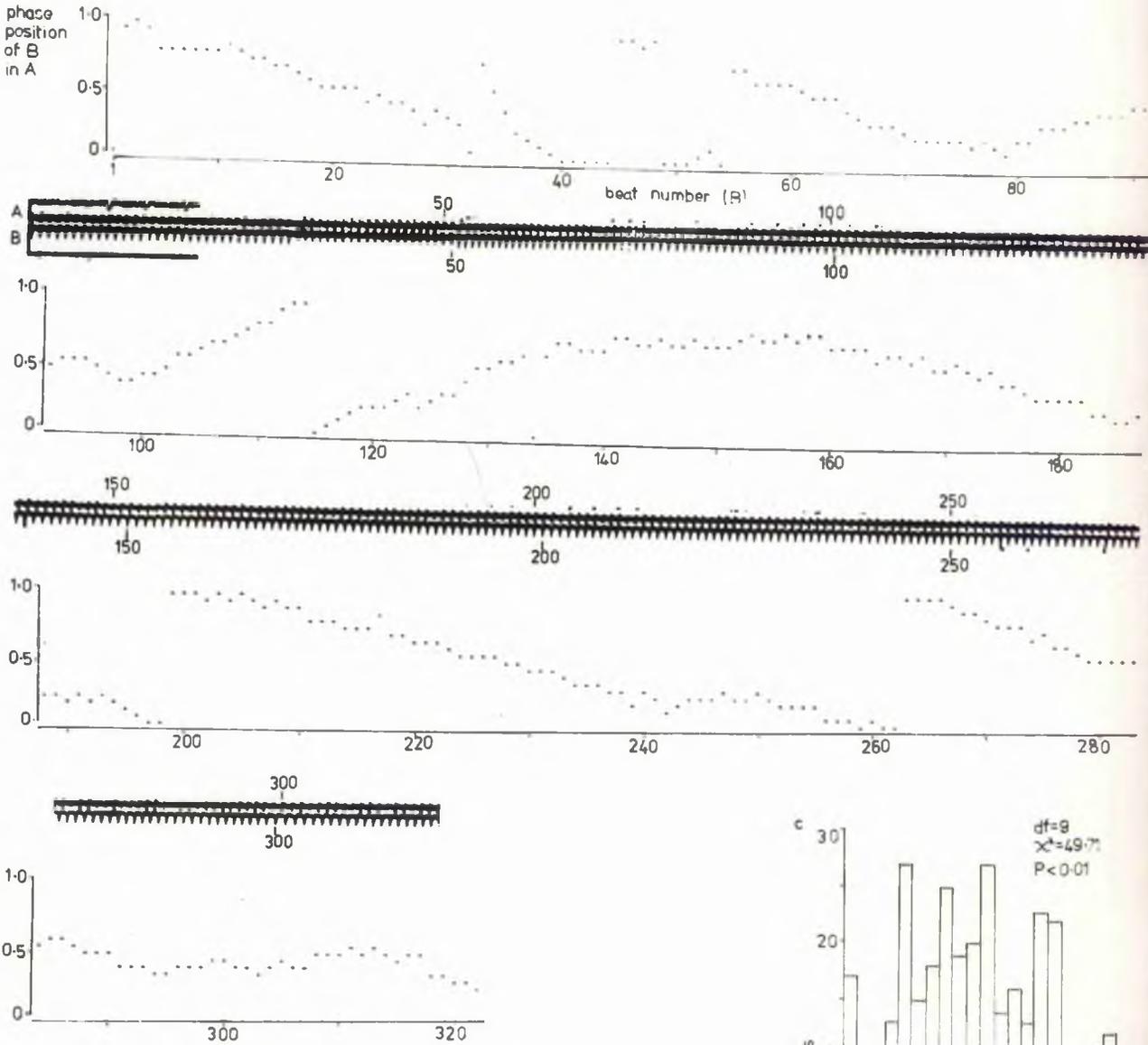
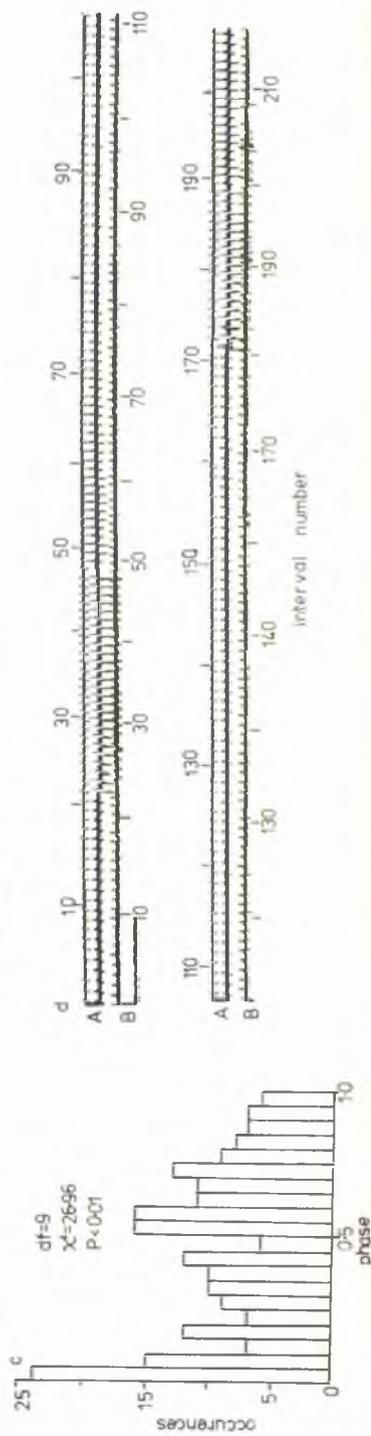
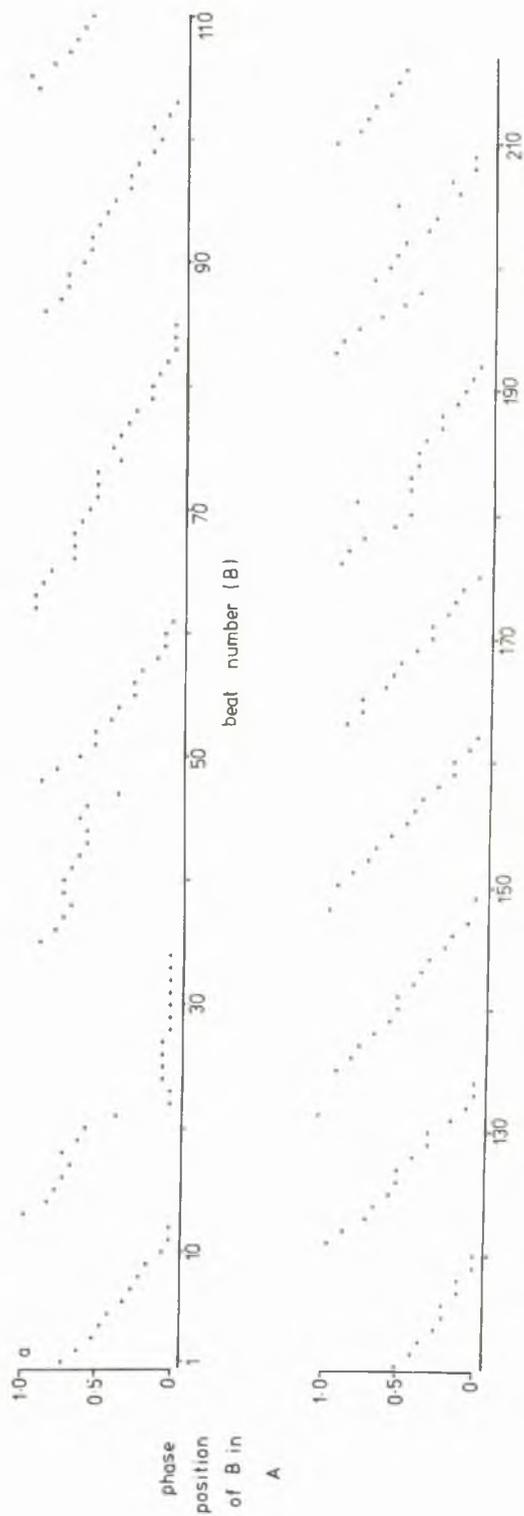


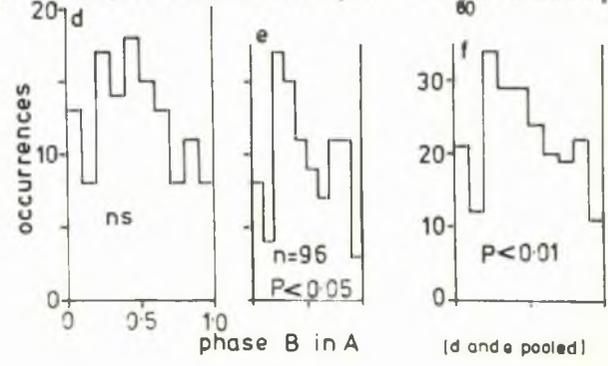
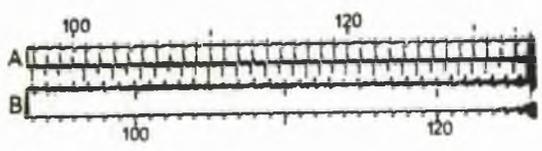
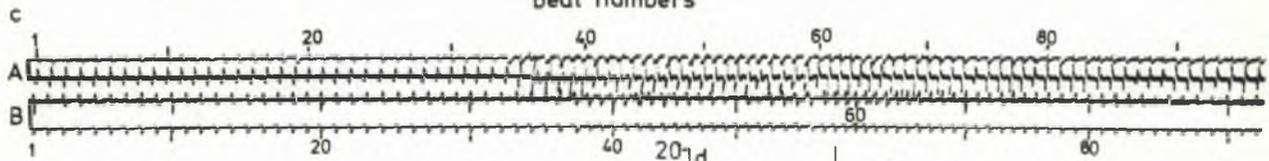
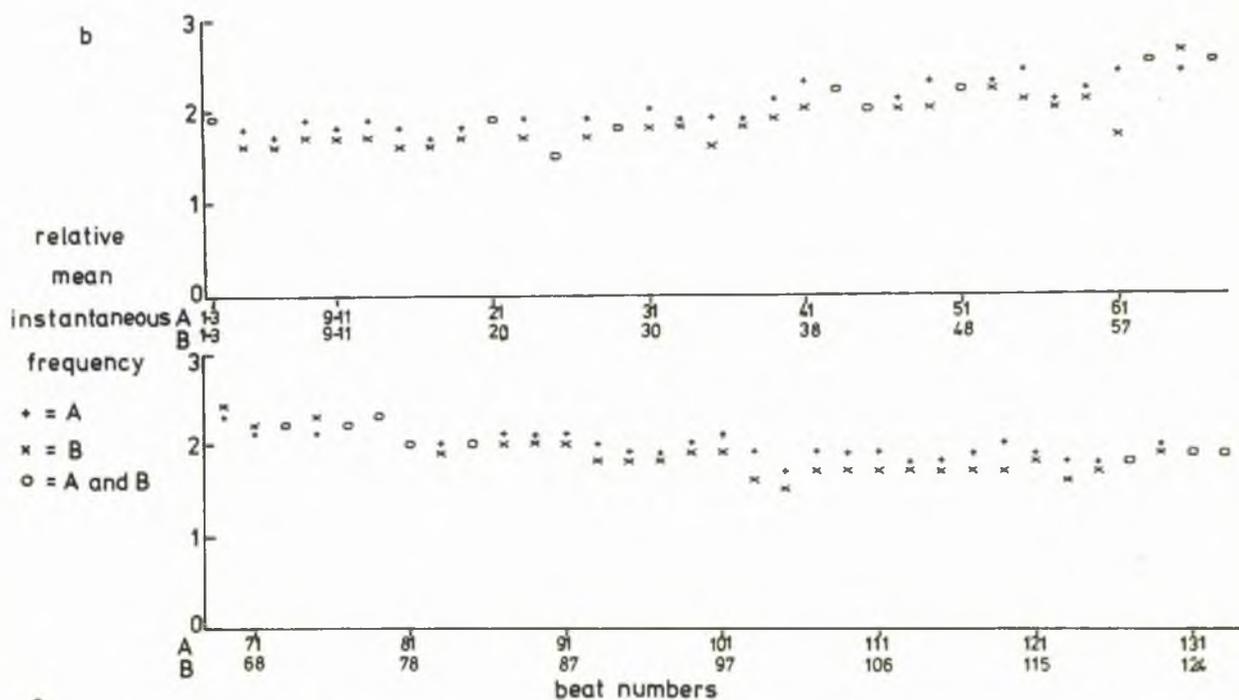
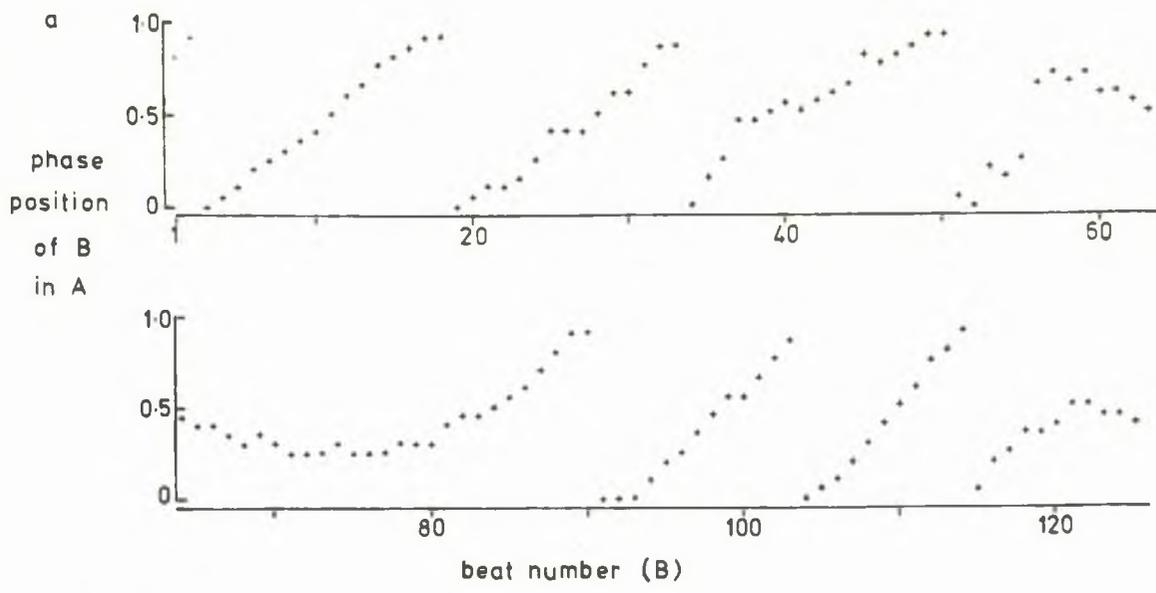
Figure VI. 9 - 11.

Figures VI. 9 - 11. Relationships between the right and left scaphognathite when the beat rates are similar. The histograms have significant peaks and instead of the marked phase progression seen in Fig. 8, there are frequently occurring periods of reduced drift about the preferred phase angles.

a







ay

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Figure VI. 13.

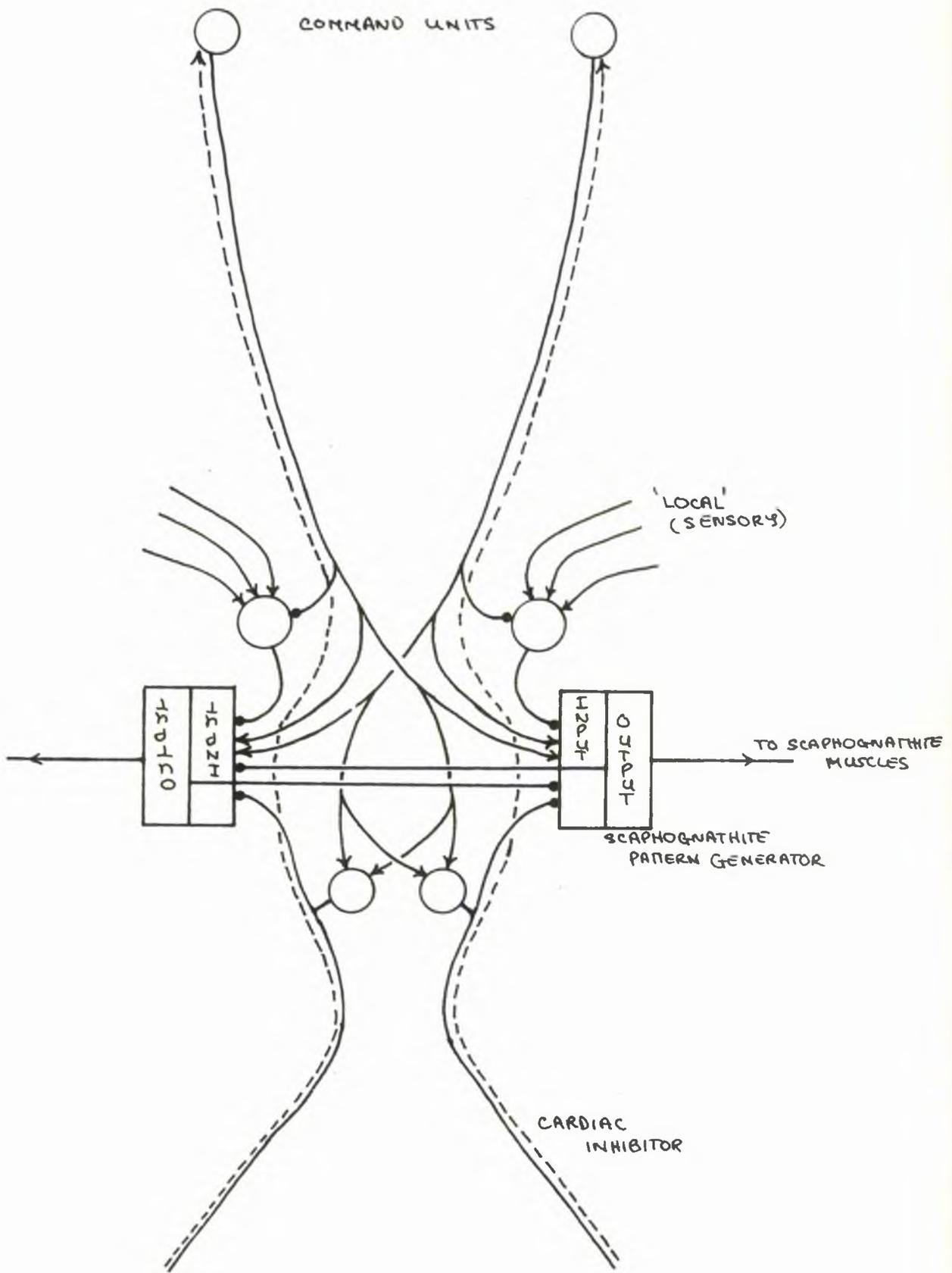
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3

Figure VI.13. A possible model for the extra-systemic linkages of the scaphognathite. The suggested models were constructed bearing in mind the following points:

1. The right and left scaphognathite may show relative coordination, and may become phase locked when the frequencies in both are close: Weak mutual inhibition between contralateral pattern generators could explain this.
2. Abrupt bilateral equalization may accompany equalization of the beat rates on both sides, and phase locking of the appendages.
3. The beats may become phase locked without any obvious overall excitation or inhibition, and there is a tendency towards alternation (relative phase angle of 0.5) under such circumstances.
4. Stimulation of the oesophageal commissure may induce simultaneous heart and scaphognathite stoppage, and may also excite the scaphognathite if applied during a pause, or at times, even when applied during normal activity.
5. Scaphognathite activity is strongly linked with activity in the cardiac inhibitor, stoppage occurring during high frequency inhibitor discharge.
6. In Portunus at least, reversals may be accompanied by a transient period of acceleration followed by inhibition, and high frequency discharge in the cardiac inhibitor is accompanied by similar changes in beat frequency.
7. According to Wierama and Novitski (1942) stimulation of fibres in the oesophageal commissures can influence heart rate via both ipsi- and contra- lateral cardiac inhibitors. J. Wilkens (1973, personal communication) has found similar fibres which influence both heart and scaphognathite rates. The present study was concerned only with uni-lateral effects.
8. Ashby and Larimer (1965) note a preponderance of coupled inhibitory responses between heart and scaphognathite rates.
9. Stimulation of the inhibitor nerve trunk may elicit persistent pauses in scaphognathite beating perhaps by triggering the normal command mechanisms.
10. The scaphognathites on both sides may show independent frequency variations, and may reverse independently.



## VI.3. The nature of reversals

### VI.3.1. Introduction

Up to now a number of differences in scaphognathite activity during forward and reversed beating have been described, and some have been intimated. Primarily, the process involves an inversion of the sequence in which the muscles are recruited, but there are numerous related changes including the appearance of activity during reversed beating, in a muscle (the accessory promotor) which is silent during forward beating. In the present chapter further differences, and the actual process of changing from one mode of beating to another will be examined in detail.

### VI.3.2. Results

The first obvious characteristic of the changeover either from reversed to forward or from forward to reversed beating is a transient acceleration of the beat rate. In the change from forward to reversed beating this acceleration tended to settle at a lower period length than that for the preceding forward session (Fig. VI. 14). In the change from reversed to forward beating the opposite was true (Figs. VI.14). Secondly, as already described, there is a change in the relative sequence of the bursts of activity in the different muscles, and in the temporal structure of the bursts at least in the flexor and sixtus.

The small accessory promotor muscle appears to be active only during reversed beating (Fig. VI.15e) or at times, during pauses (Fig. VI.15e) when it bursts tonically. During reversed beating its activity is phasic and may remain at a low level, consisting of only a few small ejs per cycle. Alternatively, the muscle may be strongly active,

with large ejp's, but at a relatively low frequency compared with that in the other muscles. There is evidence that strong activity in the promotor may be associated with changes in the relative duration of the burst in the primus (Fig. IV. 28) and perhaps in the other levator muscles (Fig. VI. 15e).

Change of beat direction is usually accompanied by changes in ejp size and pattern in most muscles (Fig. VI. 14,15,16). Usually in the levator muscles ejp's during reversed beating were very small in relation to those during forward beating, but the opposite may occur (Fig. IV.14). The reduction in ejp height may occur even when the frequency of the junctional events appears to remain quite high. The reduction may therefore be associated (a) with peripheral inhibition of some sort or (b) with changes in the activating units. In Fig. VI. 16 for the secundus muscle it appears that during reversed beating some of the units active during forward beating have been lost, and the entire burst structure altered. There is no obvious activity in the nerve trunks which might be associated with peripheral inhibition, and at least in the scaphognathite of Orconectes, Pasztor (1968) found no evidence of peripheral inhibition. The second alternative therefore appears to be the more likely.

In Fig. VI. 16 the changes associated with going from forward to reversed beating are particularly well displayed. The first clear indication of incipient reversal here is the suppression of the burst in the flexor in the depressor session preceding the changeover point. One spike in the flexor unit appears in the normal position but the others are eliminated. The following sixtus burst in this session also fails to appear. The levator session following these changes in the depressor bursts assumes the form typical of reversed beating. In the next depressor session it can then be seen that not only do the sixtus and flexor occur at their usual reversed phases in the cycle,

but the units to these muscles are different from those active during forward beating. Curiously, in the flexor, there still occasionally occur in the reversed cycle bursts, single spikes of exactly the same size and shape as those in the forward cycle bursts. These spikes (arrowed in Fig. VI. 16) are perfectly entrained in the sequence of the smaller reversed unit spikes, appearing simply to replace one of the spikes in that unit. There then appears the typical acceleration of the beat rate, but no other obvious changes not perhaps attributable to the general excitation. The changes seen in recordings from the muscles only (Fig. VI. 15) are consistent with these observations. To generalize then, reversal of the beat appears to be associated with overall excitation and specific inhibition of the units activating the flexor and sixtus in the forward mode.

Unfortunately, records similar to that in Fig. VI. 16 were not obtained for the change from reversed to forward beating. In Fig. VI 15 it can be seen that the changeover from reversed to forward beating is not exactly analogous to the opposite change. The reversed mode units to the flexor is apparently not inhibited before the forward unit resumes. Rather it seems, the flexor (and presumably the sixtus also) displays an extra session, the forward unit coming in shortly after the reversed flexor burst preceding changeover. It seems to be generally true that in the change from reversed to forward beating the inhibition on the forward unit to the flexor is first removed, and then the reversed unit inhibited. Certainly, the flexor does not as a rule 'lose' a burst in going from reversed to forward beating. On the contrary, it usually seems to gain an extra burst.

### VI.3.3. Interim Discussion

Bearing in mind the data presented in this and the previous chapters, there appears to be a good case for the proposition that the

occurrence of changes in beat direction is not a random process, but is associated with a reasonably definable sequence of events. It also seems likely that these events might be triggered by some sort of command system, at least under certain circumstances, and that activity in this system might appear directly or indirectly, in the cardiac inhibitors. These propositions are based in some cases on equivocal findings, so that further testing will be required before the model can be regarded as sound. The changes in the motoneurons activating the flexor and sixtus clarifies somewhat the differences in the relationships between the activity the two muscles in forward and reversed beating. However, the differences may be explained on other grounds so that the linkages between the forward units may well be of the same sort as those between the reversed units (Chapter V.2.2). The abolishing of activity in the flexor sub-group is in keeping with the finding that in Homerus (Wilkins and McMahon, 1972) reversed beats display incomplete depressor phases. This is interesting in relation to Pasztor's (1968) demonstration of dual innervation of the muscles in Homarus and Orconectes, but always, of activity in but one unit. It raises questions concerning the comparative evolutionary developments in the motor and command processes concerned with reversal of scaphognathite beat in the Macrura and the Brachyura. Difficult to explain is the occurrence in the reversed flexor spike train of occasional single spikes resembling the spikes of the forward unit. If these spikes are indeed produced by the forward unit, then one wonders what sort of arrangements could permit the escape of single spikes in this unit so that they replaced single spikes in the reversed unit spike train. Further consideration of these points will be taken up in the General Discussion.



Figure VI 14. Changes in beat frequency accompanying transition from one beat made to another. Filled circles represent forward cycles; open circles, reversed. a to d, for Carcinus. e, for Portunus.

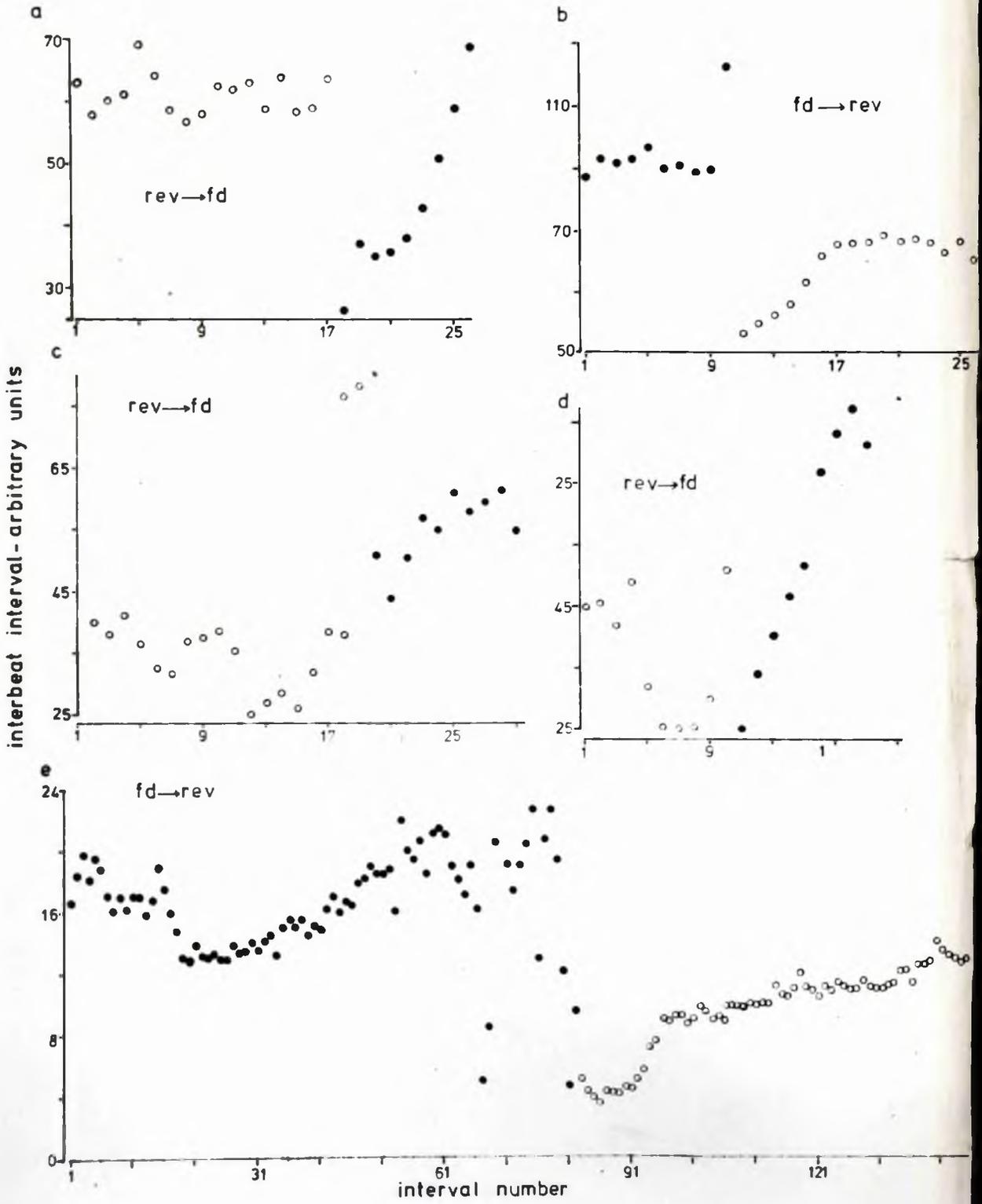


Figure VI. 15.

Figure VI. 15. Muscular activity during changes of beat direction in Carcinus. Strips in the same series are continuous. (a) Shows a transient reversal. Note the 'loss' of a burst in the flexor (expected approximately above the arrow) during the change from forward to reversed beating, and the relative high beat rate during reversed beating. Note also the change in burst structure and the diminution of ejp size during reversed beating, particularly clear in the primus. This can also be seen in (b) in the quartus after the change from forward to reversed beating. In (c) there is on the contrary, intensification of the quartus burst on reversal. (b) and (c) are from the same experiment. In the change from reversed to forward beating, the flexor has an extra burst relative to the other muscles. It is not clear whether this means that the other muscles are inhibited for a cycle, or that the flexor is excited. Note that in (d) the change from reversed to forward beating is accompanied by acceleration, contrary to the norm. In (e) the silencing of the accessory promotor muscle during forward beating is seen. The odd activity in the flexor at the changeover point appears to be a forward mode burst. This sort of activity along with that in the primus and quartus in (a) and (b) make it tempting to suggest that some peripheral inhibition may be acting. The arrows indicate the changeover points.

rev = reversed beating.  
fd = forward beating.

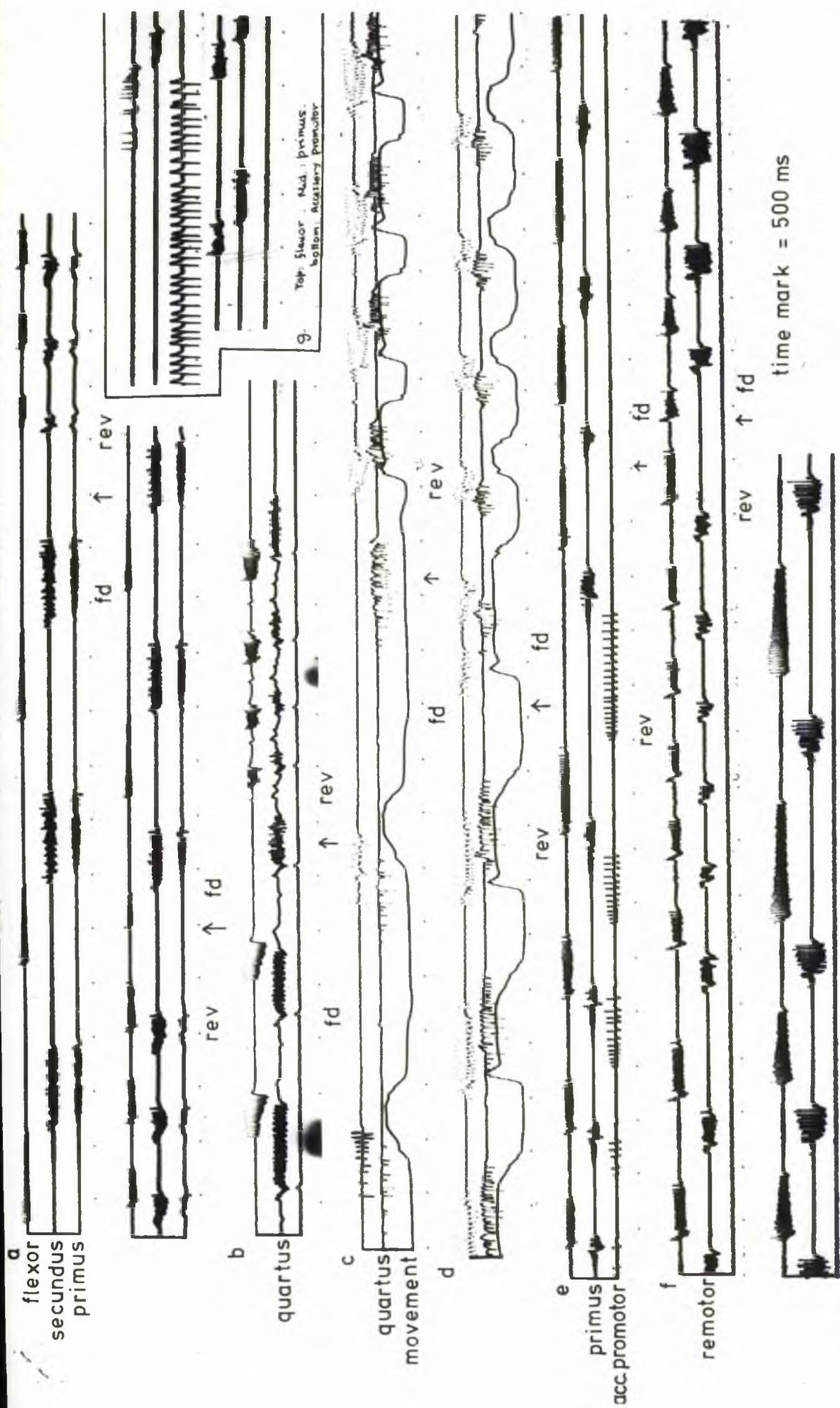
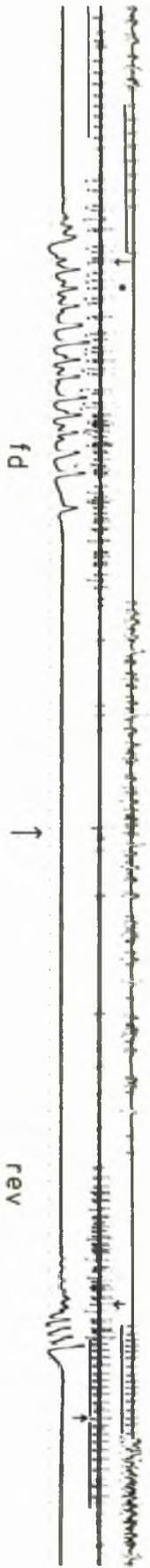


Figure VI. 16.

Figure VI. 16.

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Figure VI. 16. Continuous recording from the secundus muscle (ar2IIa) and the motor nerve trunks to the scaphognathite during the changes from forward to reversed beating. See Fig. IV.1. for the structure of the bursts and identification of the units in these records. Note firstly the increased beat rate after the changeover point (large, upward arrow). Note, above this arrow the incidence of a single spike in the flexor burst (under ruled; middle trace), after which, activity ceases in the flexor during that cycle. The quartus sub-group burst (upper trace) becomes prolonged, and no activity appears in the sixtus, (under ruled; upper trace). The activity in the sixtus muscle appears to have been inhibited prior to reversal. Note the change in the relative positions of the bursts of activity to the muscles of the different sub-groups. It can be clearly seen also that different units are active in the flexor and sixtus sessions during reversed beating. Note in the reversed mode flexor burst however, that occasionally, single spikes are interposed (small, upward arrow) which are of identical size and shape as the forward mode flexor unit. Notice the change in the burst in the secundus muscle. At least one of the activating units (small, downward arrow) seen in the forward mode, persists in the reversed mode (small, downward arrow). At least one (dot above) does not. For further discussion, see text.



time mark = 50 ms

SECTION VII

GENERAL DISCUSSION

## VII. General Discussion

The implications of the present findings have largely been discussed in the respective chapters. The problem now is to synthesize and rationalize a large and variable body of data pertaining to the output characteristics of the neural system governing scaphognathite activity. In order to achieve one of the primary aims of the study, it will be necessary to discover from these data, what is likely to be the nature of the underlying organization. In contemplating this, one feels somewhat overwhelmed by the prospect of finding explanatory systems which will be consistent with the data in broad as well as in detailed terms. The approach therefore, was to construct a basic neuronal network on the basis of the most obvious characteristics of the system, ignoring most of the fine details. The characteristics of this network were then determined by the use of a digital computer program\* designed to simulate the properties of such small neural networks. By appropriate variations in the characteristics of the neurons and their interconnections, and by progressive elaboration of the network one might then determine whether or not the model is capable of 'explaining' the characteristics of the system on a more detailed level. As a prelude to the description of the basic model it will be necessary to review the main characteristics of the system as they are applicable to its construction.

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\* See Appendix I.

VII.1. The main characteristics of  
the pattern generator.

VII.1.1. Timing cues

The results of the analyses on the starts of the bursts in the cycle are disappointingly variable. In general however, the starts of the bursts are clearly not determined by a set of latency dependencies as found, for example, in the coordination of activity in the ipsilateral flagellae of the brachyuran maxillipedes (Burrows and Willows, 1969). The relative timing of the burst starts here is on the whole, a linear function of period. This is more in keeping with the type of organization found between contralateral legs of the same segment and between the protraction and retraction phases in single walking legs in the cockroach (Delcomyn, 1973) and in the lobster (McMillan, ., personal communication). The burst durations also, at least among the depressor muscles, tend to be linear functions of period length. In the levator muscles, particularly during forward beating, there is a tendency towards low or no correlation between burst duration and period length, especially in the promotor sub-group. The phase positions of the starts of the different bursts in the cycle and the phase angle of the burst durations at least in some instances tend to be independent of period length. In cases where there is a significant regression of phase on period length, the rate of change with changing period length is small, except again, in the levator muscles during forward beating. Principally in the muscles of the promotor sub-group, the phase angle of the durations tend to show marked regression on period. The relationship here is similar to that for the timing of the starts of the bursts in the muscles controlling swimmeret beating (Davis, 1969). As perhaps overly stressed already, there is an enormous amount of variability so that

the relationships for none of the muscles could be assigned unequivocally to either category, truly phase locked or phase variant. This might be interpreted as meaning that the moment to moment interrelationships are of little functional importance to the animal, but are allowed to vary 'freely' within limits. Whatever the nature of the timing mechanisms, it seems clear however, that there is the capacity for generating truly phase locked timing cues for both starting times and durations of the bursts, at least in some cases and at some times.

#### VII.1.2. Interactions between units

A number of studies have tried to demonstrate lateral interactions between motor units by antidromic stimulation of motor axons. Although this has been successful in some instances, particularly in the flight muscle units in some insects (Mulloney, 1970; Levine, 1973) it has given negative results in other insects (Bentley, 1969) and in crustaceans (Evoy, Kennedy and Wilson, 1967; Kennedy, Evoy and Fields, 1966), even though in some cases direct interaction was known to exist. Negative results from such studies therefore are not meaningful. As pointed out by Horridge (1968) even a slight widening of an axon may so attenuate the spike depolarization currents as to produce rectification. The present study has concentrated on determining the presence of lateral interaction between units by analysis of spike to spike interactions in overlapping spike trains in bursts from different motor units. These interactions were easiest to study in the flexor and sixtus, but interactions between the flexor and quartus units have also been investigated. The results indicated a direct, positive interaction, perhaps electrotonic, between motor units from the same sub-group, and indirect, negative interaction between units from different, agonist sub-groups.

Comparisons of the computed relative input 'waves of depolarization' in these units support these indications, and the variations in peak spiking frequencies in different units suggest a general, negative interaction between units from different sub-groups. These new methods of investigating interactions between motor units, in particular the comparison of interspike interval/latency histograms and the computation of the underlying input waves, are regarded as potentially very useful. In studying some of the more complex aspects of behaviour, and in investigations on whole animal preparations using implanted electrodes, it is often not possible directly to observe intracellular events in the motor units. It is likely that much of the useful information which can be obtained from intracellular recordings can be reconstructed from analyses on the efferent spike trains. The present analyses represent two relatively simple ways in which this can be done. More exhaustive methods have been devised (Gerstein and Perkel, 1972), but partly because of the relative difficulty of these approaches, their general usefulness is limited. The limitations of the present methods also are recognized, but used in conjunction with other analyses their usefulness might be extended. Of interest in this context also are the methods of Wyman (1969).

#### VII.1.2. Bilateral coordination of scaphognathite rhythms

Glide coordination has been demonstrated between the beating of the right and left scaphognathites. Phase locking has not been observed at beat rate ratios other than 1:1. Even when the ratios are closer to 1:2, locking is normally associated with equalization of the beat rates. The locking of the beats may be associated with the incidence of some common input to both sides. Even when the beat rates are close, locking may at times be accompanied by simultaneous, transient acceleration on both sides, and perhaps by synchronized

reversal. In extended runs with beat rates of approximately 1:1 ratio, significant peaks may be obtained in phase histograms. As clearly described by Wyman (1969) for phase locking between spike trains in dipteran flight motor units, phase locking might occur between identical units receiving common input. Recurrent, transient disturbances will result in continued changes in the adopted phase positions, so that there should be no significant correlation in extended runs. There should be no preferred phase position. The argument may be transferred to the coordination between the two pattern generators of the right and left scaphognathites, but it seems highly unlikely that the characteristics of these two, obviously complex systems should be identical. It is hardly likely therefore that common input could effect exact equalization of the rates and phase locking for more than a few cycles if at all. It seems more probable that weak, direct interactions between the systems may effect locking when common input brings the periods of the cycles close enough for the interaction to be effective. This then, was proposed to explain the significant phase histograms for somewhat extended runs. It is interesting that the interrelationships found here bear remarkable similarity to those between heart beat and ventilatory rates in some fish (Hughes, 1972). The relationship between heart and scaphognathite activity was unfortunately not investigated in the same way as that between scaphognathites, and no studies were conducted on Carcinus. The results for Nephrops and Portunus have already been adequately discussed, and will not be treated here.

### VII.1.3. Reversals

The sequence in which the muscles of the four sub-groups are activated can be completely reversed. In this the system bears some resemblance to that controlling clockwise and counter-clockwise rotation of the tail spine in Limulus (Silvey, 1973) although Silvey

on the basis of the then existing data on scaphognathite activity, did not recognize the similarity. The muscle pairs assigned to each sub-group continue to show near synchrony, but activity in muscles in some cases appears to shift in the different modes to different motor units perhaps with slightly different characteristics and connectivities, thereby altering the finer characteristics of the system. The gross changes on the whole however, appear to be explainable on the basis of the differing temporal juxtapositions of reciprocally interacting units. It may be therefore, that the primary pattern generator remains the same during forward and reversed beating, the changes being confined to units at a lower level where changes have minimal influence on the overall pattern. There is some evidence for a hierarchical system controlling the cueing of the bursts in the different muscles, so that the intergroup cues might be generated at a higher level than the inter-(agonist) sub-group cues, and these, in turn, at a higher level than the intra-sub-group cues.

Scaphognathite beating tends to display different preferred frequency ranges during forward and reversed beating in the region of changeover. Perhaps the systems of connectivity in forward and reversed modes are sufficiently different to result in differing natural oscillation frequencies. If external factors (via higher order interneurons) demand oscillation frequencies too far removed from the natural frequencies (in the hypothetical absence of input) it may be that the interconnections in the system become altered in order to meet the demand. But why reverse? Initially, one might imagine, this was the result of the intrinsic properties of the organization. Benefits such as improved ventilation of the gills in crabs (Aradpragasm and Naylor, 1964a,b;1966; Hughes, Knights and Scammel, 1969) may have led to its active incorporation into the animals' behaviour, and the development of controlling, command

systems to integrate and regulate its occurrence. It may be significant that sustained reversal has not apparently developed in macrurans where reversals might not contribute greatly to improved gill ventilation (Wilkens and McMahon, 1972). In Nephrops high frequency discharge in the cardiac inhibitor is often accompanied by disruption of the scaphognathite activity, followed by a pause. In Portunus this pause is replaced by what may well be a transient session of reversed beating. It may be then that in view of the advantages, some crabs have developed the ability to cope with 'excessive demands on the system' by replacing certain units with others whose characteristics permit sustained reversed beating, at a higher natural oscillation frequency. This would probably have been accompanied by parallel developments in the 'enabling' command system. The macrurans represented here by Nephrops, having less to gain, have failed to exploit fully the predispositions of the pattern generator. One might guess in addition, that the obvious structural advances in the brachyuran scaphognathite might have been prompted by the deficiencies of forward mode ventilation as mentioned above.

#### VII.1.4. Considerations for a model

One system showing interesting analogies with the present has been described by Wyman (1965), in single flight muscles in dipterans. Units to a single muscle may fire in a statistically masked, phasic pattern for extended periods. The pattern will occasionally shift abruptly from one mode to another. Phase histograms of the position of one unit in the period of another may be uni-, bi- or trimodal. Levine (1973) found only uni- and bi- modal relationships in similar units in Drosophila however, the reciprocal inhibition between the units appeared to be asymmetrical. These interrelationships can be modelled readily by means of reciprocally inhibitory networks (Wilson,

1966). The general resemblance to the system governing scaphognathite activity is clear. This therefore seemed to be a good starting point in constructing a model for the present system.

In spite of the similarities, there are marked differences between the organization of the scaphognathite and of the insect flight muscle. Firstly it should be noted that it is the relationships within a single flight muscle that resemble the relationships between the four sub-groups in the scaphognathite. Secondly, the interrelationships in the flight muscle were between single spikes, and not between bursts of spikes. In adapting the model of Wilson (1966) to the present system, the initial problem was therefore to find if it is possible for a four cell, reciprocally inhibitory network with physiologically feasible parameters to generate overlapping bursts of spikes rather than single spikes. Secondly, it was necessary to introduce sufficient asymmetry into the network to produce a bimodal rather than a multimodal system. It seemed desirable also, to attempt to reconcile some of the characteristics of the system with the observation by Mendelson (1971) of sinusoidal oscillations associated with the respiratory rhythm in a number of crustaceans. The development and characteristics of the model therefore, will now be described.

VII.2. The Development and Testing of a Model  
for the Pattern Generator Controlling  
Scaphognathite Activity.

The starting point for the model consisted of four identical 'neurones' having equal inhibitory connections with each other (Fig. VII.1A). All the cells are pacemakers, since the thresholds are set below the membrane resting potentials, (i.e. on their own would fire continuously). Fatigue is simulated by very weak self-inhibition of very long time constant (Weiss, 1962; Wilson, 1966). In order to determine whether this system could produce alternating bursts of spikes rather than single spikes per cycle (Wilson, 1966) an analog model was first used. The basic unit ('neurone') of this model is shown in Fig. VII.1B and an example of the output in Fig. VII.1C. The relative values of the relevant parameters (threshold, cross and self-inhibition, membrane resting potential etc.) were determined and fed into the digital simulation program (Appendix 1). With appropriate adjustment of the parameters, the network produced overlapping bursts of spikes. The strengths of the cross inhibitions were then adjusted as shown in Fig. VII.2 so that the cells formed two groups with greater overlap between members of the same group than between members of different groups. There is no pause in the cycle. As shown in Fig. VII.2, the basic network has been extended also, by the addition of an upper 'layer' of cells. These may be regarded here as simulating the non-spiking cell bodies found in the crustacean central nervous system. If so, then the lower 'layer' can be regarded as the spike generating zones. The thresholds of the upper layer cells, the driver cells, are set at a very high value so that they do not spike. They are connected electrotonically to the corresponding lower layer cells, the follower cells. The electrotonic connections are such that the

membrane potential of the follower cell always asymptotically approaches a voltage equal to the average of the membrane resting potential of the follower cell and the membrane potential of the non-spiking driver cell. The time-constant of this asymptotic approach is the time-constant of the membrane potential of the follower cell. Changes in the membrane potentials of the follower cells will have no effect on the driver cells. From the arrangement of the connections in this network - all inter-sub-group inputs go onto the driver cells - it can be seen that the variations in the membrane resting potentials of the follower cells will reflect the variations in the membrane potentials of the corresponding driver cells. The membrane potentials (of the follower cells) will follow the membrane potentials of the driver cells but they cannot exceed their threshold; at the threshold spiking activity will begin. The situation is analagous to that shown in Fig. .

It can be seen in Fig. VII.2 that there is now but one symmetrical path of lowest inhibition. The cells should fire with equal likelihood in the order 5-6-8-7-5— or 5-7-8-6-5——. Fig. VII.3 shows how inhibition on to one of these units can result in reversal of the beat direction. Excitation (Fig. VII.4) can also result in reversal of the sequence. Fig. VII.5 shows the variation in inter-spike interval during the burst in some typical bursts. These might be compared with the results for the actual system (Fig. V. 4). Fig. VII. 6 shows the fluctuations in the membrane potentials of the non-spiking portions of the cells in a series of cycles. The 'waves' of depolarization and repolarization are roughly sinusoidal, and are reminiscent of Hendelson's (1971) sinusoidal oscillators. Depolarization of one of these cells by setting the membrane resting potential to  $-40.0$  mv results in tonic activity in that cell whilst all activity ceases in the others. Assuming an logy with the system in the

scaphognathite, this would be equivalent to tonic firing in the units of one sub-group and stoppage in the other three sub-groups. Hyperpolarization of one cell in the simulated network does not abolish rhythmic activity, but silences the activity in the hyperpolarized cell only. The burst durations in the other cells increases markedly, but the period length is not greatly affected. On the whole, these observations might be reconcilable with Mendelson's (1971) findings (see Section I.2). However, Mendelson (1971) also observed in the silent, non-bursting system, that hyper- and de-polarization of the 'oscillator' resulted in respective tonic discharges in the nerve trunks bearing antagonist units. This is more difficult to explain away, and more difficult to simulate.

In the scaphognathite, the activity in each sub-group is composed of recurrent, overlapping spike trains from different units. From the records, it appeared that different units within each muscle may be active for a variable portion of the burst or not at all, without disruption of the overall pattern. One might attempt to simulate this at the present level by using each 'upper layer' cell to drive two follower cells which are reciprocally inhibitory (Fig. VII.7A). When this is done the upper layer of cells can be regarded as separate interneurons or alternatively, it would be possible to use two driver cells for each pair of follower cells and still obtain the same result. The overall burst duration will be determined by the total activities in each of the units, whilst the relative durations of the respective units in the pair will depend largely on their thresholds, and on the strengths of the cross inhibition. The output from a typical run is shown in Fig. VII.7B.

In Fig. VII.7A, the cell 9 represents an input cell which may inhibit or excite equally, all the driver cells. The period of the cycle is dependent upon the frequency of input, paradoxically increasing

as the level of excitation increases (Fig. VII.8). In Figs. VII.9 to 13 some of the frequency dependent parameters of this system are exhibited. These may be compared with the corresponding plots for the real system (Section IV). There are clearly elements of similarity even at this crude level of the simulation. The 'shapes' of some of the bursts are displayed in Fig. VII.14. In addition to the resemblances to some aspects of the scaphognathite activity, the present system in a number of ways is reminiscent of the activity in the swimmeret motor output system (Davis, 1969). This will not be discussed here.

In order to obtain output that more closely resembles that of the scaphognathite, the system need not be extended greatly, but some alterations in the interconnections will be necessary. At the time of writing, the simulation lags somewhat behind the understanding of the actual system, and some of the inferred characteristics of the real system have not been built into the model. The final state of the model is shown in Fig. VII.15A and the typical output in Fig. VII. 15B. There is clearly a strong resemblance between the outputs of the real system during forward beating and the model, cell 5 being analagous to the flexor, and cell 15 to the sixtus; cell 7 to the quartus/tertius and 17 to the quintus; cells 6 and 16 to the primus and secundus and cells 8 and 18 to the promotor and remotor. The cells 10 and 20 are necessary to simulate the sharp truncation of the flexor burst shortly before the start of the levator session, and the pause after the termination of promotor/remotor activity before the start of the depressor session. Cells 5 and 15 (flexor and sixtus analogues) are in a positive feedback loop, as indicated by analyses on the actual system; however, the interconnections in the model are synaptic rather than electrotonic. Contrary to the indications in the real system, the quintus analogue does not form a part of a similar positive feedback loop. There is weak reciprocal inhibition between the quintus and

quartus analogues. Shortcomings such as these and a few others, need updating in the model, but they will probably not greatly influence the overall characteristics.

As it stands the model displays several characteristics which are different from the simpler versions. The tendency towards reversal for example, is diminished, and attempts to induce reversal tended to result in disruption of the pattern and eventual settling back into the initial sequence. If the actual system functions in a similar way, this might 'explain' the necessity to change the units active in the sextus and flexor as a prelude to reversal. The failure of the model as it stands to reverse is probably also connected with the persistence of the terminating bursts in the 'interneurones' 10 and 20. From the absence of pauses between the levator and depressor sessions in the actual system during reversal, one might suspect that these 'terminating' units either are inhibited during reversal, or the units inhibited by them are replaced by other uninfluenced units. Experiments are now in progress in which two additional cells with slightly different characteristics are placed in parallel with cells 5 and 15. Their thresholds are slightly lower than those of cells 5 and 15, and they are strongly inhibited by cells 5 and 15. They should therefore presumably remain inactive unless cells 5 and 15 are inhibited by input from an external source. It should be interesting to see how this affects the characteristics of the system, and to see if input inhibition on to cells 5, 15, 10 and 20 can effect reversal in a manner analogous to that seen in the actual system.

In addition to its reluctance to reverse, the model appears to show diminished sensitivity to input excitation. The reasons for this are not clear, but again the answer may lie in the terminating effects of the interneurones 10 and 20. It may also be that with relatively low inhibition between cells in agonist sub-groups, increasing overall

excitation simply results in greater overlap, and more extensive truncation of the bursts, so that the period length is not so markedly affected. Fig. VII.16 shows how period length varies with input excitation, and Figs. VII.17 - 20 illustrate some of the frequency dependent parameters of the model. An interesting point to note in these plots is the marked deviation of the intercepts from zero. This would be consistent with earlier speculations on the effect of truncation of the bursts on the regressions of relative latencies and burst durations on period length in the real system. Also, with reference to the statistical analyses, it might be noted that in the present model, the period length clearly cannot be considered as a truly independent variable, and must be determined by factors such as burst durations, extent of overlap, and relative latencies. It might in fact, best be regarded as the dependent variable, determined on the basis of a number of independent variables. Perhaps this possibility should be taken into account in future analyses on similar systems. In cases with a controlling sinusoidal oscillator the period length might more reasonably be considered as an independent variable.

One important point not yet tested, concerns the relative variances of the intra-sub-group, inter-agonist sub-group, and inter-antagonist sub-group latencies (Table V). This was taken to imply a hierarchical organization (Fig. IV.22B). The model, except in the case of intra-sub-group relationships implies no hierarchy. Thus, one might expect reduced variance in the latency between the starts of bursts in the same sub-group. But it is not clear whether the variance of the starts of the bursts in agonist sub-groups should be expected to be less than between the starts of bursts in antagonist sub-groups. This may well be so, as a result of weaker reciprocal inhibition between units in agonist rather than between those in antagonist sub-groups, but the hypothesis remains to be tested. Another one possibility is illustrated in Fig. VII.21. This hinges on the concept

that the period is not a true independent variable, but has been treated as such. The variance at each stage, and the variance of the period length, would be affected by the variances at each preceding stage. The cumulative effect might not be a simple arithmetic sum, as there might be significant covariance due to subtle interactions, and compensations. However, untangling such interrelationships would appear to demand somewhat abstruse and sophisticated statistical procedures. For the present, one might simply state that intuitively, such a system seems capable of explaining the observed rise in the variances of the relationships at the different levels.

One of the main difficulties in building a hierarchical model with reciprocal interactions is the difficulty of coordinating the intrinsic frequencies of oscillation at each level, so that the period of the oscillation (cycle time) at each succeeding level is reasonably close to that in the preceding level. Each level tends to have a different intrinsic cycle time, and a different response curve to input stimuli. It was difficulty of this kind that defeated attempts to integrate a reciprocally interacting pool of followers with a sinusoidally oscillating driver, whose activity would determine the period length. Problems arise also, in effecting a reversal of the system. It would not however, be impossible to construct a reasonable qualitative model along these lines.

Altogether, it is believed that the present model, with the previously indicated extensions, and a few other minor alterations, is the best approximation to the pattern generator in the scaphognathite as we now understand it. More detailed analyses of the characteristics of the model are clearly required, and perhaps even a reanalysis of the characteristics of the actual system. The finer details of the interrelationships in the model are apparently very susceptible to alterations in input frequency. Relative latencies burst durations

and period lengths seem to require several cycles to settle at a given value when the input is altered. There is a great deal of quasi-random scatter, even though the model is fully deterministic (i.e. there is no randomness in the connections, thresholds and membrane resting potentials). It might not be surprising therefore, that the analyses on the actual system should yield such variable results. With this in mind however, perhaps future experimental procedures could be so designed as to yield greater reproducibility and more meaningful characteristics of the different aspects of the system. Simultaneous monitoring of the activity in all the muscles would certainly be helpful.

### VII.3. Summary

The usefulness of overt modelling is now quite well accepted. Many workers use overt models to express clearly, concepts which in abstraction might be more difficult to state or to grasp, and to summarize succinctly, extensive and unwieldy sets of data concerning the characteristics of various systems. But the usefulness of modelling extends beyond precis. Models, by giving form to ideas can serve as nuclei for the crystallization of ideas which might otherwise have escaped notice. Models may even suggest the existence of previously unsuspected mechanisms, and might so prompt a search for these mechanisms. This has so far been rare in the biological sciences.

Static models can fulfill all of the above requirements, but have inherent deficiencies, in that they remain intuitive, and are not subject to immediate testing. Functional models on the other hand, provide immediate tests for the intuition (Wilson, 1966), and display a greater range of measurable characteristics. A given output might quite conceivably be generated by several basically different models. But the larger the number of points of agreement found, the smaller must be the number of possible alternatives. In the present case, the suggested model resembles the real system in many respects, but the points of deviation are numerous. This is almost inevitable in so complex a system, in which various parameters are assigned arbitrary values, and other parameters are completely ignored. By their nature, all models are approximations, and in particular, neural network models, in dealing with systems whose characteristics are only poorly understood, and in many cases can only be guessed at, are particularly imprecise (Harmon, 1964).

But how many systems, constructed on completely different bases, one might ask, could give as reasonable an approximation to the scaphognathite motor pattern as found here? Wilson (1966) points out

that systems based on excitatory couplings, either synaptic or electrotonic, produce output in which phase separations are strongly dependent of frequency, or in which events are synchronous. Mutual inhibition produces relationships in which phase separations tend to be independent of frequency. With the indications of mutual interactions between units found in the present study, this would seem to imply that any model for the pattern generator is likely to be based, necessarily, on reciprocally inhibitory couplings. That the relationships in the real system need not be truly phase locked is consistent with the observation in the model that intercepts of relative latency and burst duration plots against period length need not pass through the origin. With the pattern of reversal seen in the four sub-groups in the real system, it seems likely that the model will not be hierarchical.

It remains then to test more rigorously the characteristics of both the real system, and the model, and to improve the model through less speculative determination of the more detailed parameters. This will probably not be possible without intracellular experiments on the motor and inter-neuronal elements involved in the pattern generation. With the present knowledge of the system, such experiments, if at all possible, are likely to be more successful than hitherto. It is believed that one of the more serious drawbacks in previous intracellular experiments on similar systems (Mendelson, 1971; Wyse, 1972) has been a lack of detailed understanding of the relationships between the individual elements, and their integration into the whole pattern. The availability of a testable hypothetical network, should in addition, allow more telling experiments to be performed at the unitary, intracellular level.

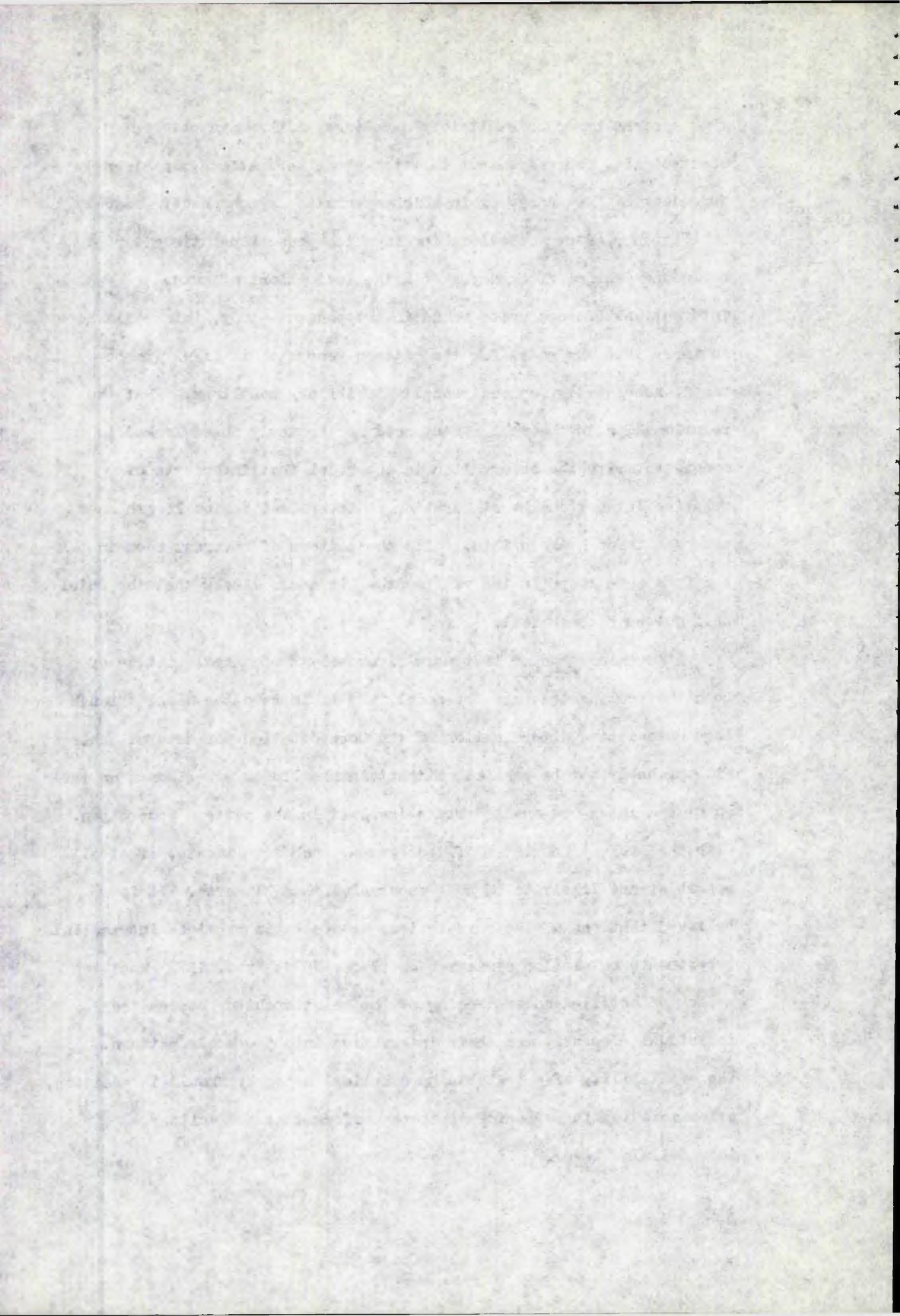
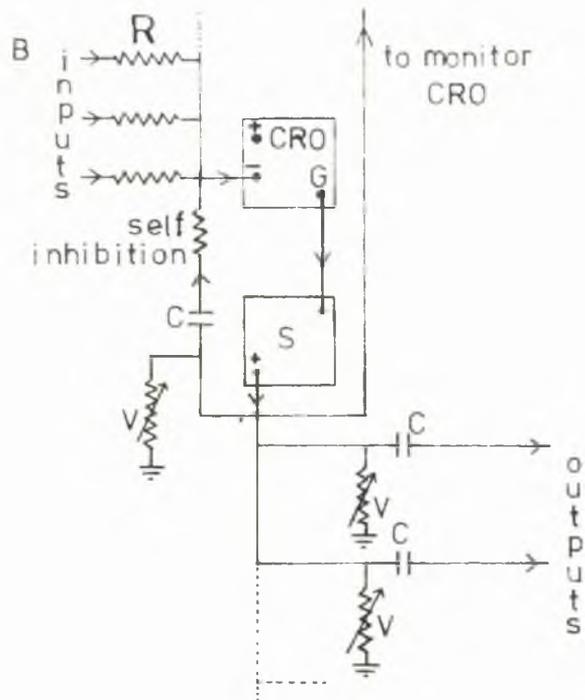
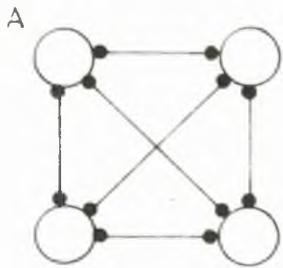


Figure VII. 1.

Figure VII. 1. A. The four cell reciprocally inhibitory network of Wilson (1966). All the 'cells' are identical pacemakers and the reciprocal interconnections are equal.

B. Analog circuit used to determine the basic characteristics required in a system such as that shown in A, in order to generate alternating bursts of activity in the four 'cells'. The triggering level of a cathode ray oscilloscope (CRO) horizontal scan was used to simulate threshold. When the input exceeded the triggering level ('threshold') the beam scan was triggered, along with the 'gate out' pulse (G). This was used to trigger in turn, an electronic stimulator. The variable resistor (V) and capacitor (C) couplings were used to shape the square wave produced by the stimulator, and to regulate the time constant of the output pulse ('ipsp') decay. The outputs from each 'cell' were led as required, to the other three 'cells'. The magnitude of the resistances R and V also determined the magnitude of the pulse. The positive outputs were fed into the negative input of the oscilloscope. The outputs from the four 'cells' were monitored on a four channel oscilloscope. The system was designed by Mr. P.R. Balch.

C. Examples of the output from the four, reciprocally inhibiting analog 'cells' described in B, when the parameters were adjusted to allow 'burst' production. Each dot represents a 'spike' in the indicated 'cell'.



C

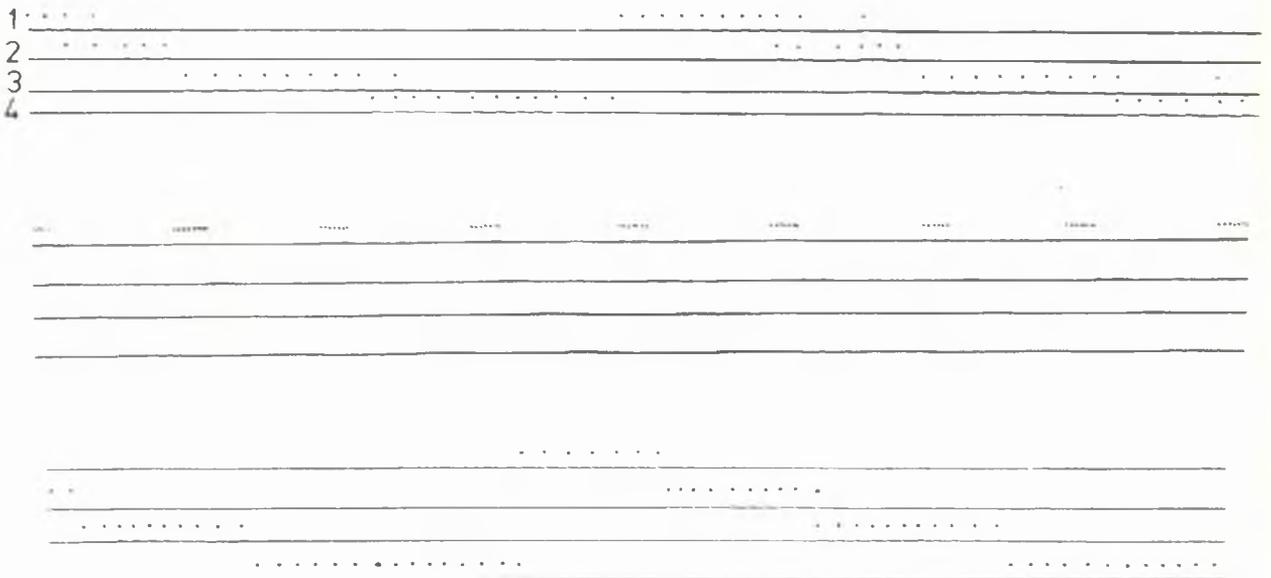


Figure VII. 2.

Figure VII.2. A. Modified Wilson (1966) network with inhibitions between 1 and 3 and between 2 and 4 relatively weak. With the network as indicated, the model should display an equal likelihood to fire in the order 5 - 7 - 6 - 8 ---; 5 - 7 - 8 - 6 ---; 8 - 6 - 7 - 5 ---; 7 - 5 - 6 - 8 ---. If the reciprocal connections between 5 and 4, 8 and 1 and between 7 and 2, 6 and 3 are increased, then the sequences 5 - 7 - 6 - 8 --- and 8 - 6 - 1 - 5 --- are rendered less likely than the remaining two, and the system becomes effectively bimodal.

b. An example of the output from the network diagrammed in A. Notice that 'cells' 5 and 7 and cells 5 and 8 form two strongly overlapping pairs with less overlap between members from the opposite pairs than between members from within the pair.

T is the threshold resting potential of the respective cells, and  $\mu$ , the membrane resting potential. The voltages represent the 'ipsp' height and the times, the time constants of the 'ipsp's' for the respective connections.





Figure VII. 3.

Figure VII. 3. The network of Fig. VII. 2, modified as suggested to restrict the number of likely modes to two, induced to reverse by means of a burst of inhibition. Inhibition is from cell 9 (an 'input' cell - see Appendix I) inhibits cells 3 and 4. The inhibitory connection to cell 3 was of  $-0.8$  mv with a time constant of 20 ms. That to cell 4 was  $-0.5$  mv with time constant, 20 ms. The input cell fired for 705 ms at a frequency of 200 Hz. The effects of this are firstly to delay firing of cell 7 so that the burst in 5 is prolonged (see Fig. VI. 16); secondly, cell 8 gets a very short session. This seems to prevent accumulation of fatigue, so that when the inhibition stopped, cell 8 got another abnormal session. This results in reversal of the sequence (see Fig. VII. 15d).





Figure VII. 4.

Figure VII. 4. Reversal caused by excitation. Network as in Fig. VII. 3, but cell 9 (input cell) excites cells 2 and 3 instead of inhibiting them. This causes cell 7 to start earlier than normal, shortening the burst in 5. Cell 6 also appears where cell 8 would be scheduled, and over-rides the burst in cell 8. This causes 8 to fire after, instead of before 6. Having become quite fatigued, cell 7 fails to appear, and 5 gets a session in spite of the strong inhibition between 8 and 1. 7 follows, but the strong inhibition between 7 and 2 now prevents 6 from firing. 8 is apparently still fatigued, and has also been receiving relatively strong inhibition from 7, so 5 gets an extra session. A reversed sequence proceeds from this point. This changeover is clearly less like anything seen in Fig. 15 than is the changeover in Fig. VII.3. Clearly many other ways of achieving reversal are possible, one of the most obvious being inhibition on to one of the four cells only (see Fig. VI. 16). This however has not been tried.



Figure VII. 5.

Figure VII. 5. The structure of the bursts in the 8 cell network. In (a) the membrane resting potentials of the follower cells were set at  $-70.0$  mv; in b and c they were set at  $-65.0$  mv. As models for the bursts in the scaphonathite muscles these clearly leave much to be desired, but show promise when compared with the bursts in Fig. V. 4.

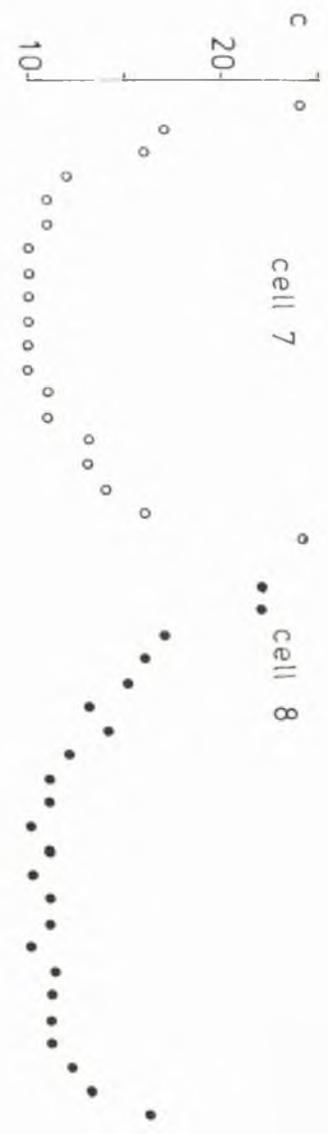
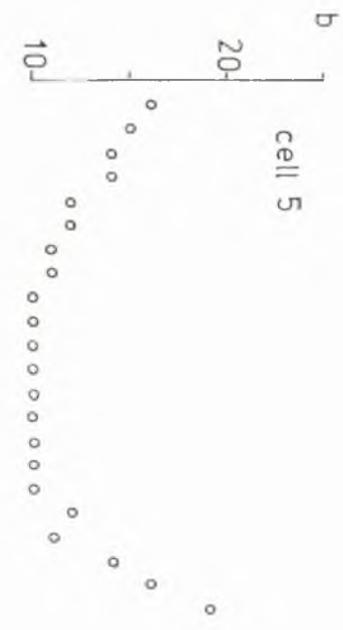
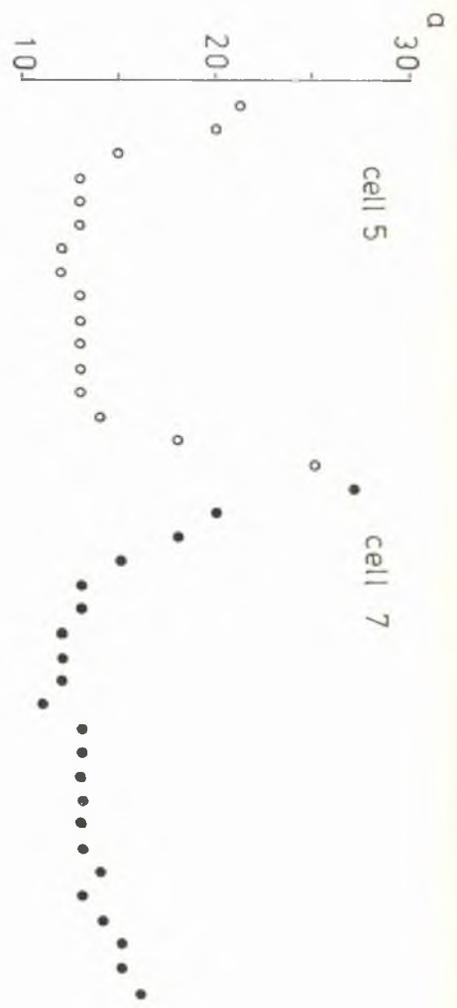




Figure VII. 6A.

Figure VII. 6A Variations in the membrane potentials of the driver cells in the 8 cell reciprocally inhibitory network. If one considers the existence of spatial attenuation and the smoother time course of psp's in a real system, as compared with the simulation, it can easily be envisaged that these variations could assume the form of 'sinusoidal oscillations'. The membrane resting potential of the follower cells (5, 6, 7, 8) follow with a lag, the membrane potentials in the driver cells. When threshold is exceeded, the follower cells spike. The driver cells membrane potentials never exceed their own threshold and so these cells never spike.

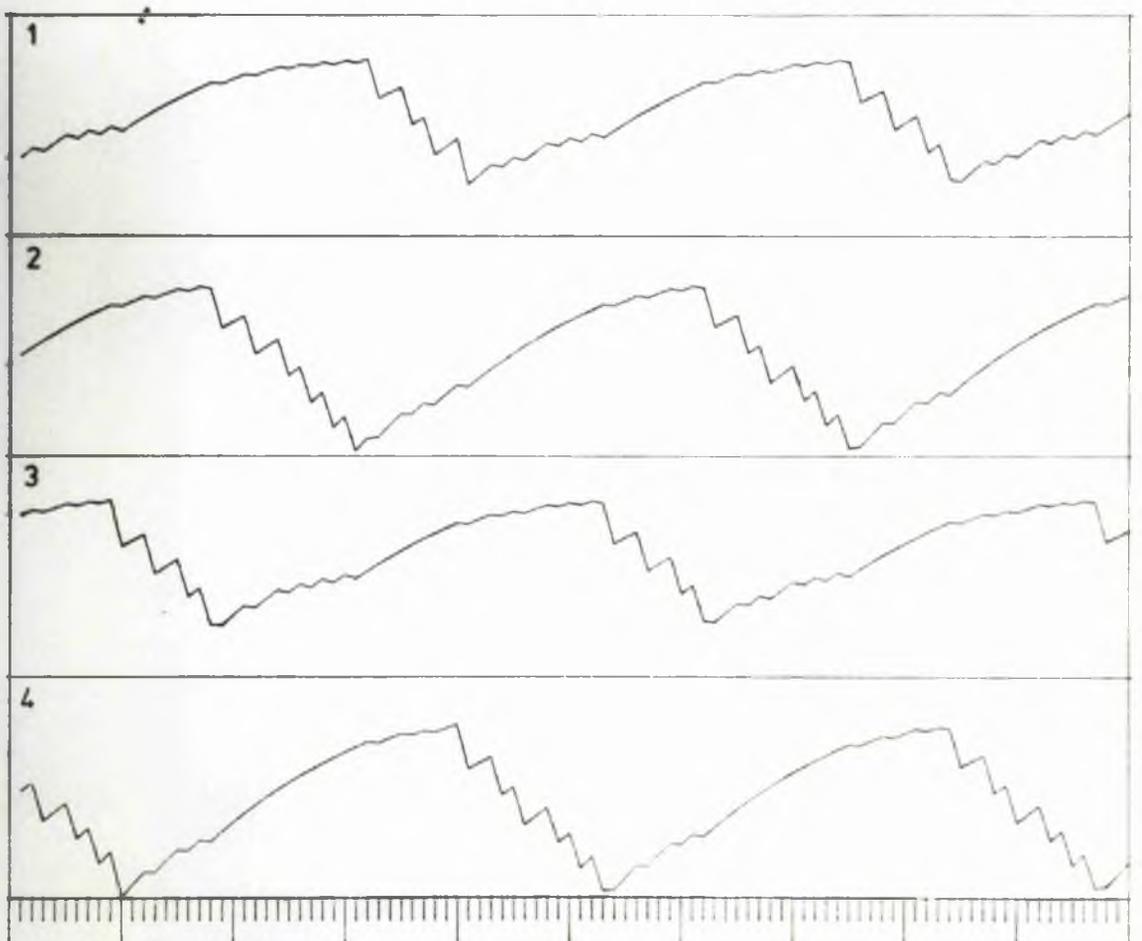
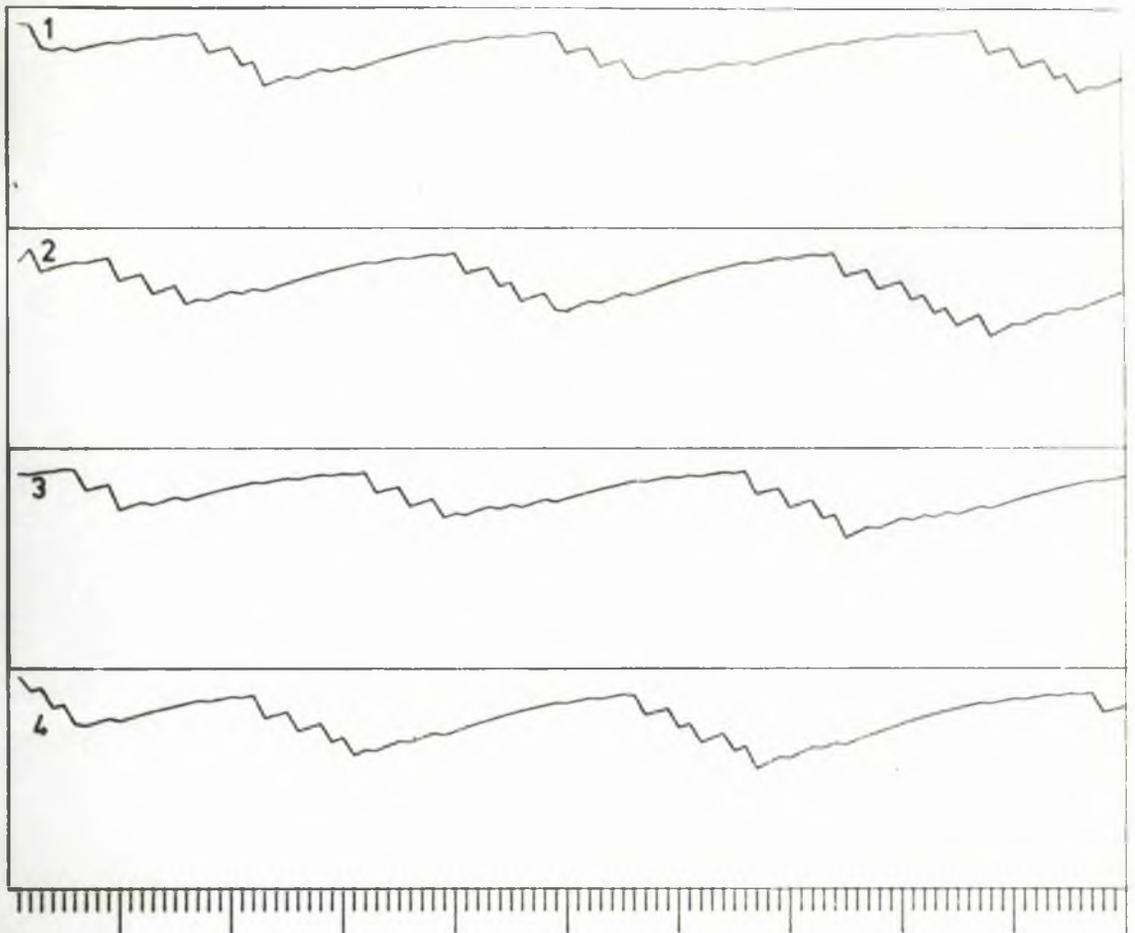






Figure VII. 6B.

Figure VII. 6B. The effect of hyper- and depolarizing cell 1 on the firing pattern of the 8 cell network. (a) shows the basic pattern. It is continuous with (b) in which, at the upward pointing arrow, the membrane resting potential of cell 1 was raised to -40 mv and held there, as a result of this, cell 1 takes over completely and the other cells remain silent. In (c) the cell (1) has been hyperpolarized to a membrane resting potential of -99 mv. Rhythmic activity does not stop, but cell 5 is silenced. Cell 8 shows little change compared with its characteristics in (a), but cells 6 and, especially 7, show abnormally long bursts. See text.



Figure VII. 7.

Figure VII. 7A. Twelve cell reciprocally inhibitory network. By adding a second follower cell to each driver cell, the system is made more closely to resemble that in the scaphognathite. The two follower cells (5 and 15 in the illustration) make identical connections, except with each other. Cell 15 has a threshold resting potential of  $-79.0$  mv, and places on 5, ipsp of  $-3.0$  mv and 50 ms time constant. Cell 5 has a lower threshold resting level of  $-80.0$  mv, and its 'ipsp' on to 15 are of  $0.7$  mv and 60 ms time constant. All four units are updated in this way, so that, for instance, the relationship between cells 7 and 17 will be equivalent to that between 5 and 15, and so on. With such strong inhibition between them, the two followers do not really simulate the individual scaphognathite muscles very well. They do however show some resemblance in behaviour to single units in a given muscle. B. Typical output from this network. Because of the strong total cross inhibition, there is very little overlap. Cell 19 is an input cell which places equal excitation of  $0.5$  mv and 100 ms time constant onto cells 1 to 4. In the example shown, the input frequency is 250 Hz.



Figure VII. 8.

Figure VII. 8. The dependence of period length on input frequency in the 12 cell network. Negative input values indicate inhibitory input.

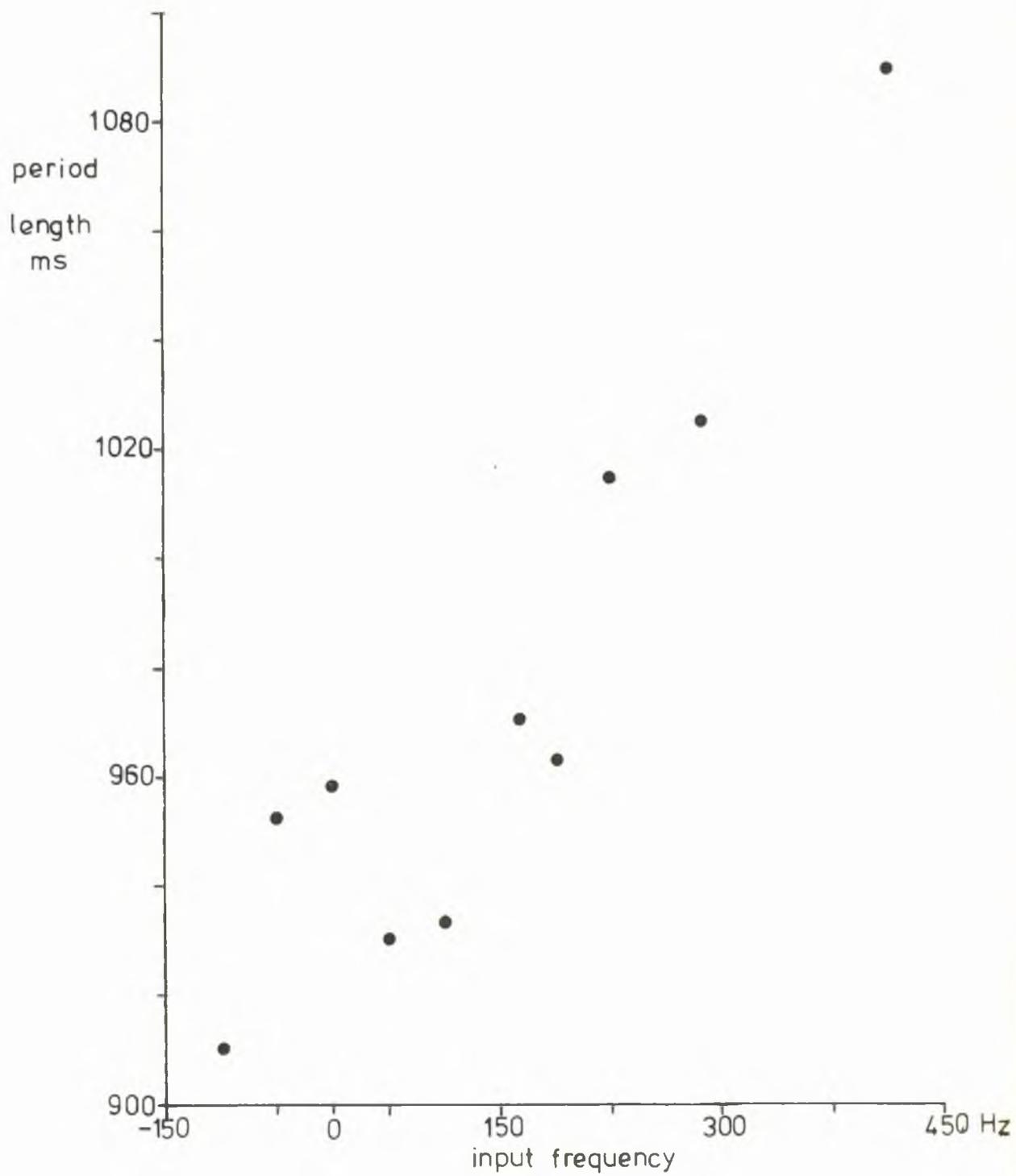


Figure VII. 9.

Figure VII. 9. Relative latency ( $L$ ) and  $\phi$ , for the starts of the bursts in cells 5 and 8 against period. This is the correlate of the inter-group relationships for the real system. Note that there is true phase locking.

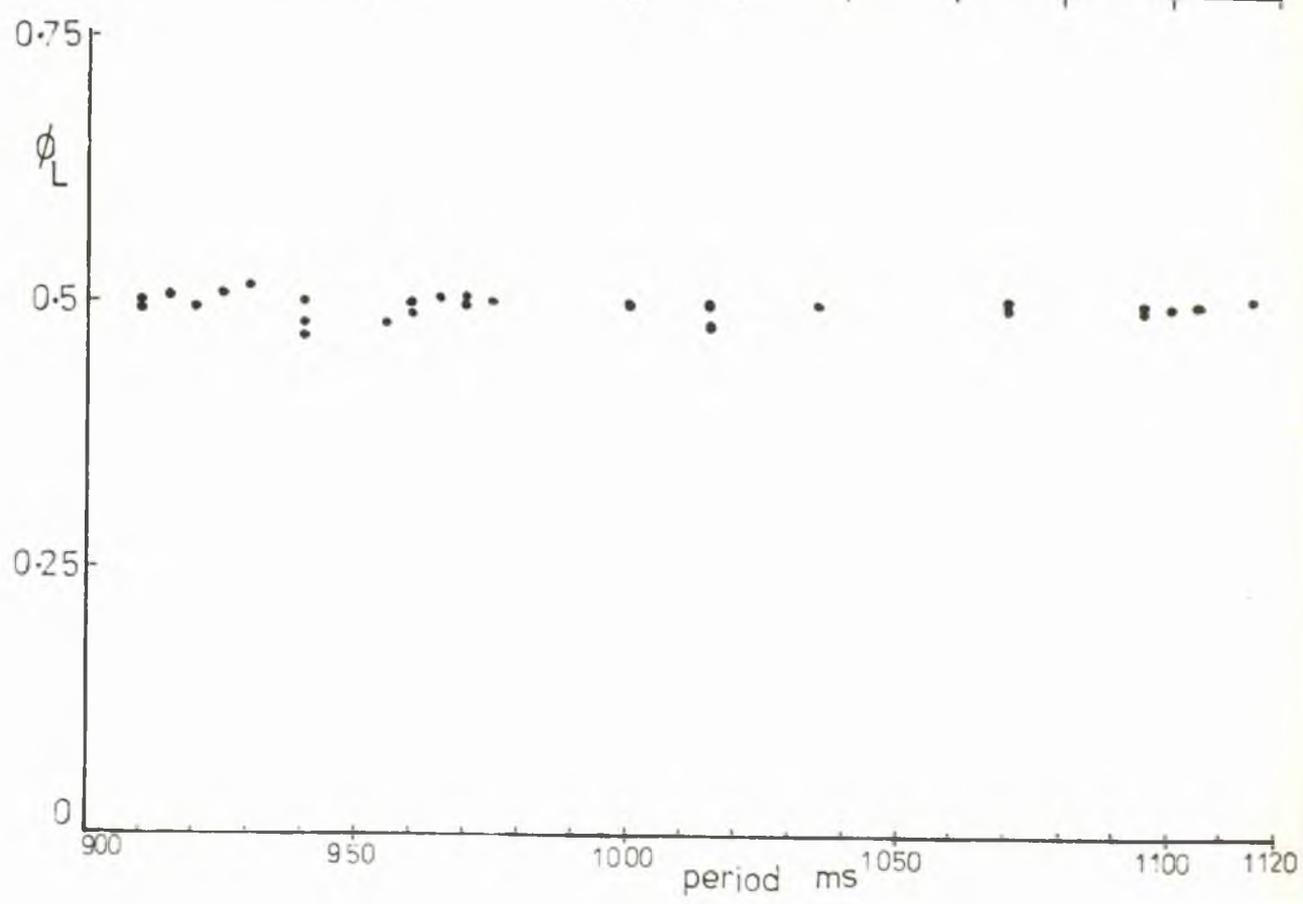
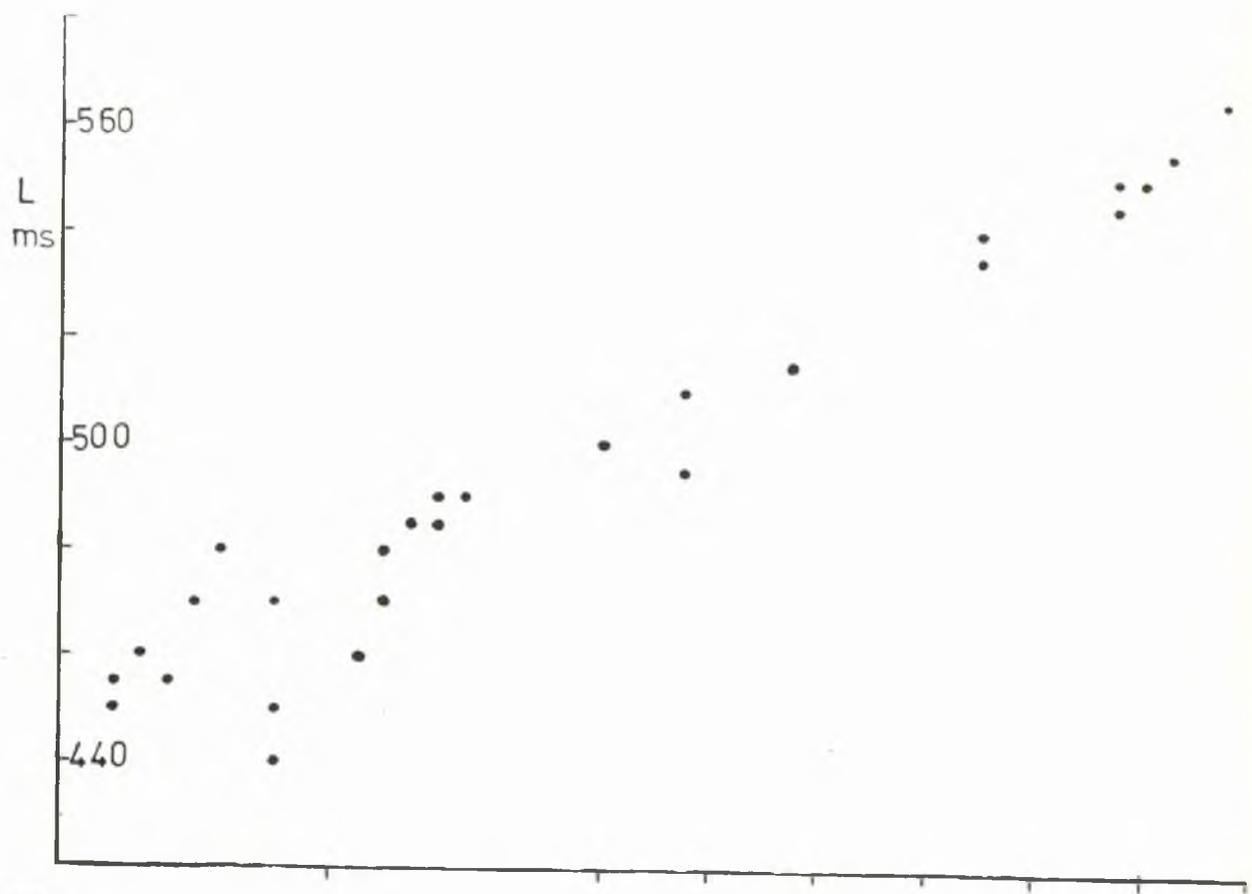


Figure VII. 10.

Figure VII. 10. Relative latency and  $\phi_I$  for the starts of the bursts in cells 5 and 7 against period. There may be a slight tendency towards diminished phase angle at longer periods. It is difficult to say without an analysis, whether this relationship is linear or asymptotic. This relationship is the correlate of inter-agonist sub-group relationships in the real system, and shows interesting similarities with the relationships for the flexor and quartus during reversed beating (Fig. IV. 15B).

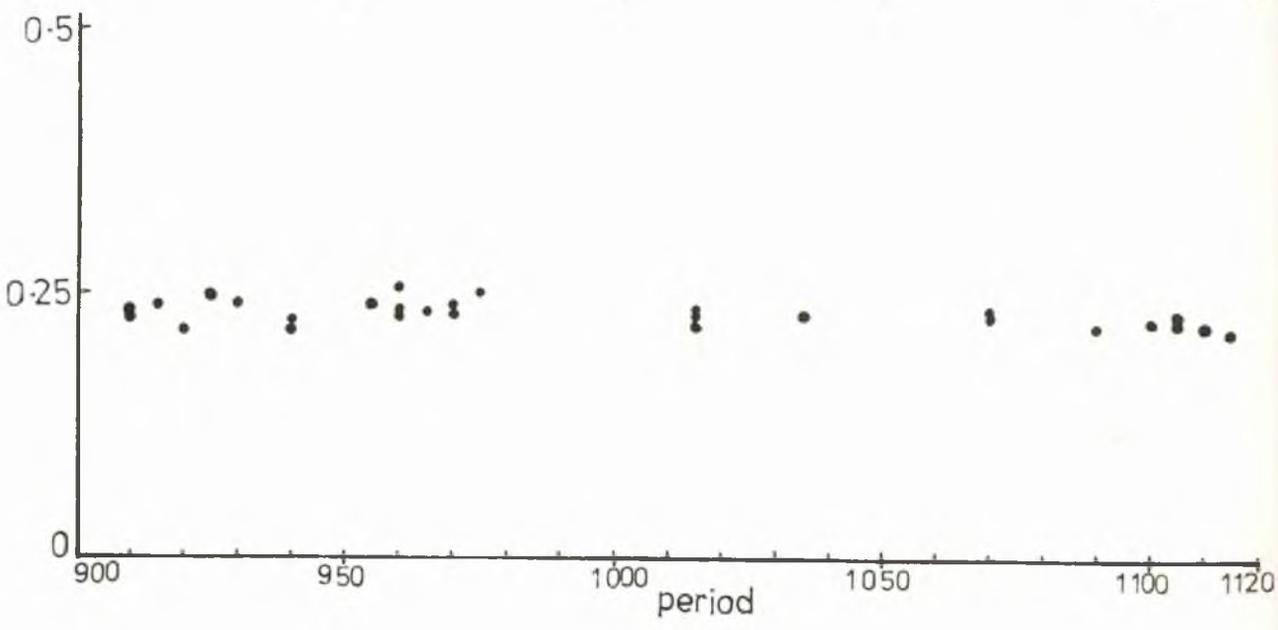
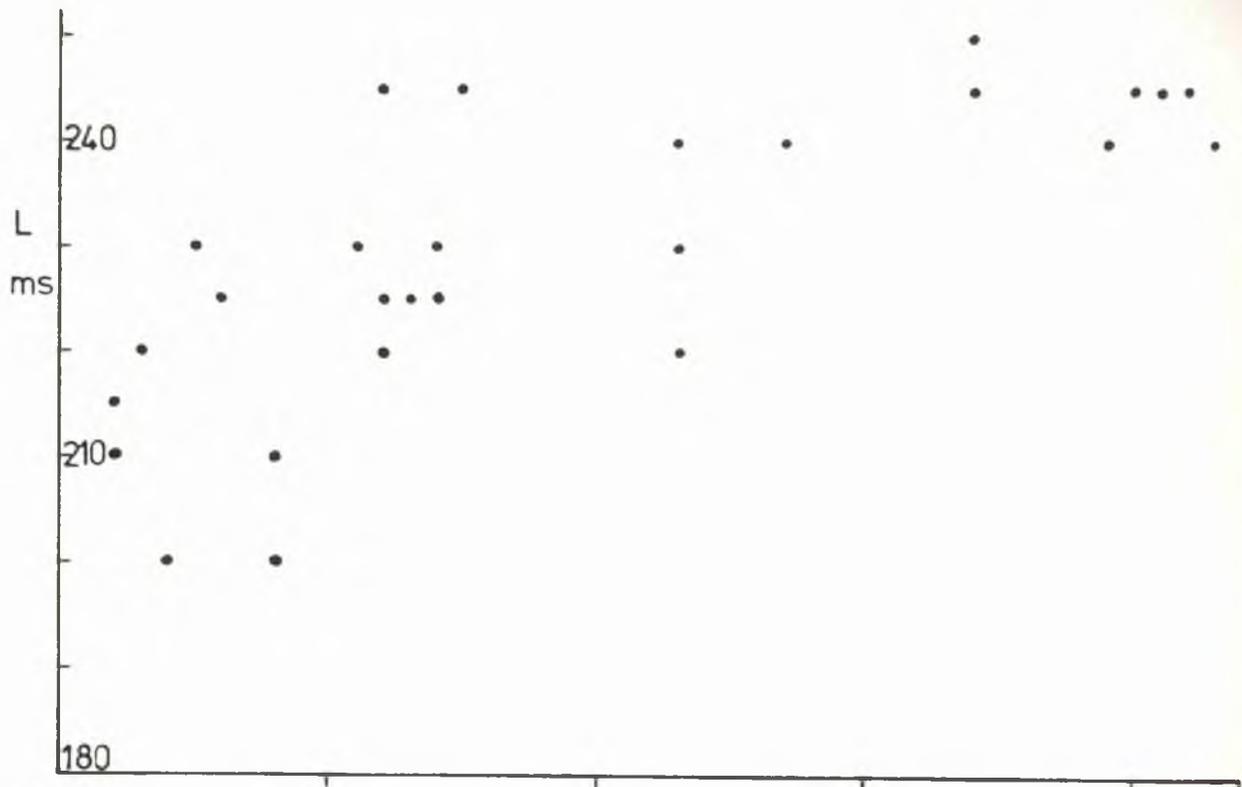


Figure VII. 11.

Figure VII. 11. The relationship between period length and total duration ( $L_D$ ) and  $\phi_D$ , for bursts in cells 5 and 15. Here again the relationship shows phase constancy.

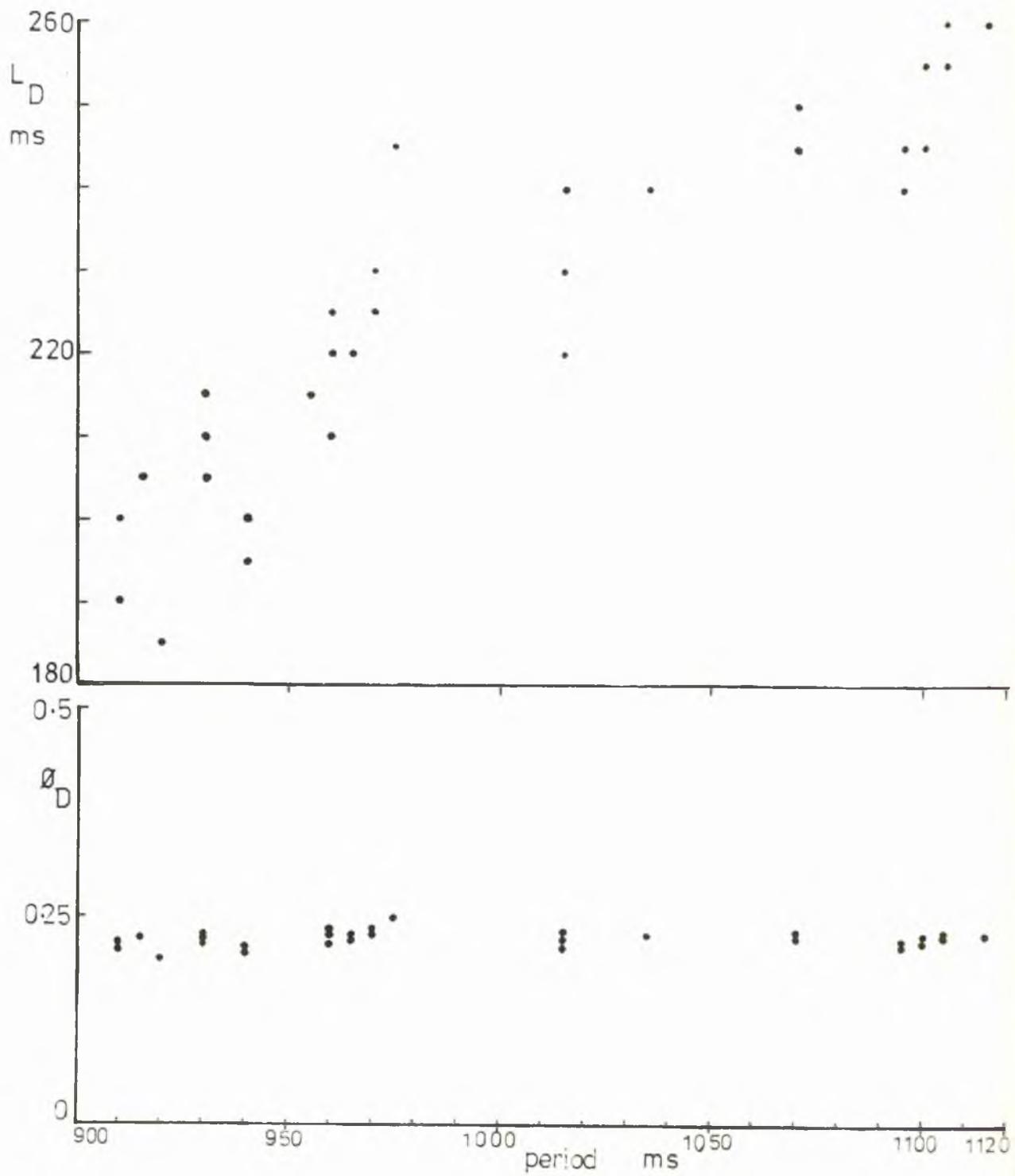


Figure VII. 12.

Figure VII. 12. The relationships between burst duration on period length in cell 15. These two muscles, because of their connections, divide the full session for the leader cell number 1 between themselves. The exact nature of the individual relationships will depend on the relative thresholds, and strengths of mutual inhibition. For cell 5 (open circles) a hyperbolic relationship would seem possible here. Bearing in mind the linear relationship between number of spikes per burst and burst duration (Fig. VII. 13B) the relationship between period length and number of spikes per cycle might be similar to that in Fig. V. 11G for the promotor.



Figure VII. 13.

Figure VII. 13. A. Total number of spikes per cycle in cells 5 and 15 as a function of period length - a linear relationship. B. A similar, but more defined relationship between number of spikes per burst and burst duration. This corresponds to the condition found here in most of the scaphognathite muscles but differs from that found in lobster swimmeret muscles (Davis, 1969).

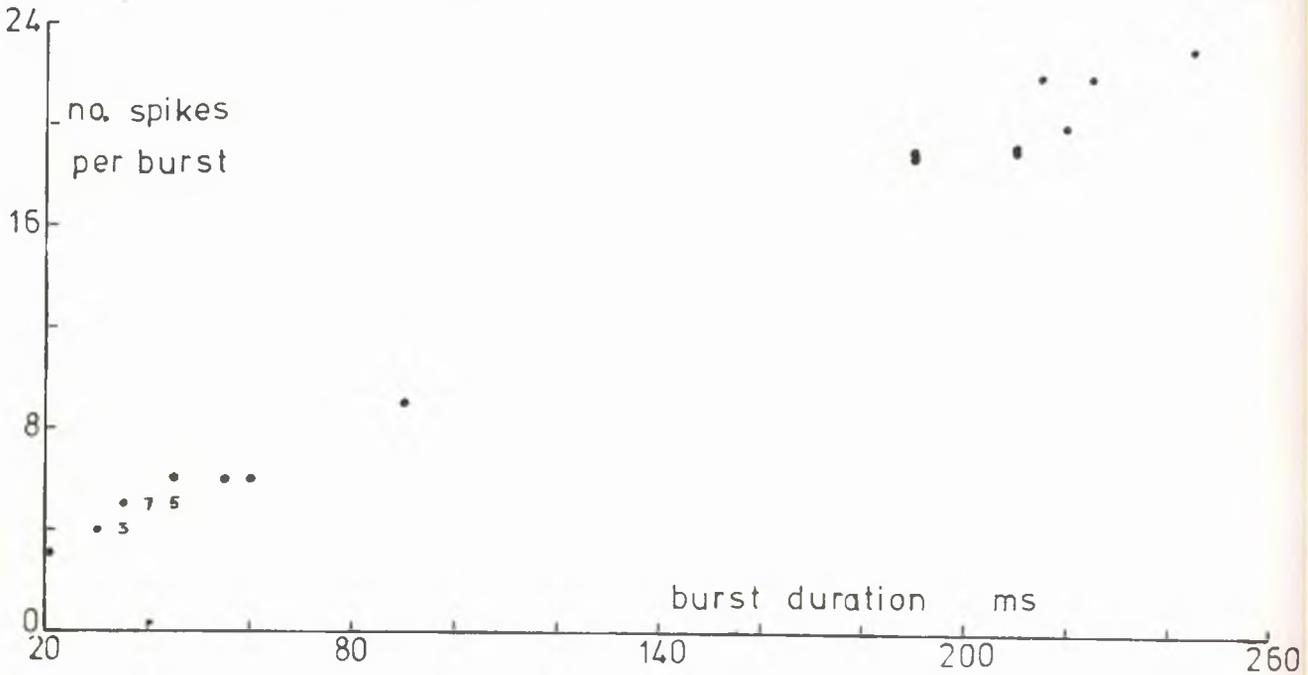
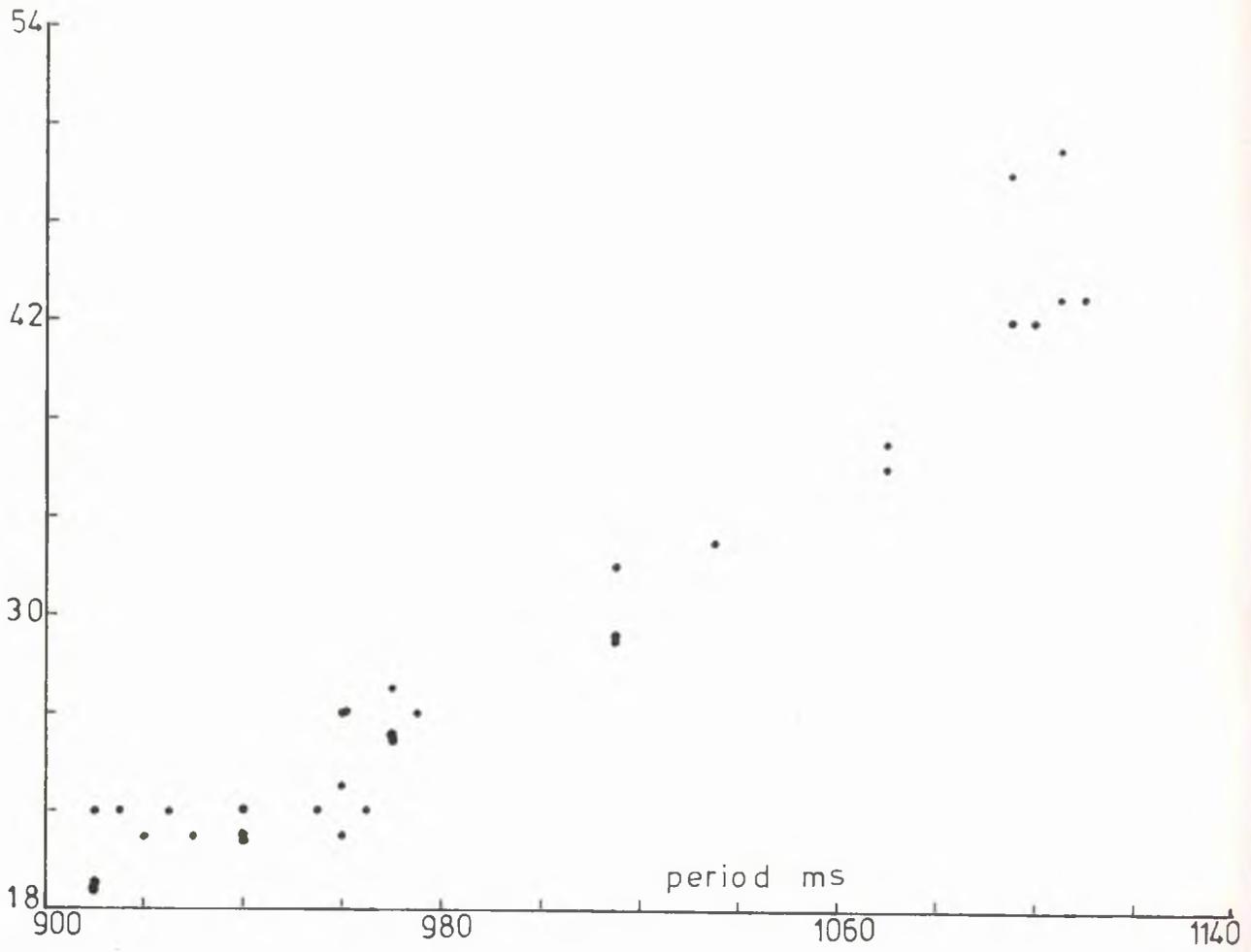


Figure VII. 14.

Figure VII. 14. The 'shapes' of some of the bursts in the neural analogus for the final state of the model.

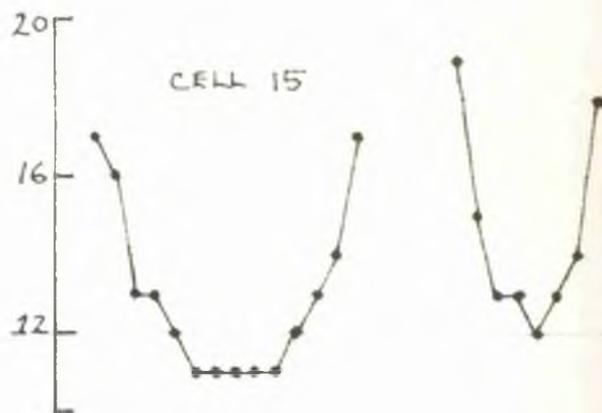
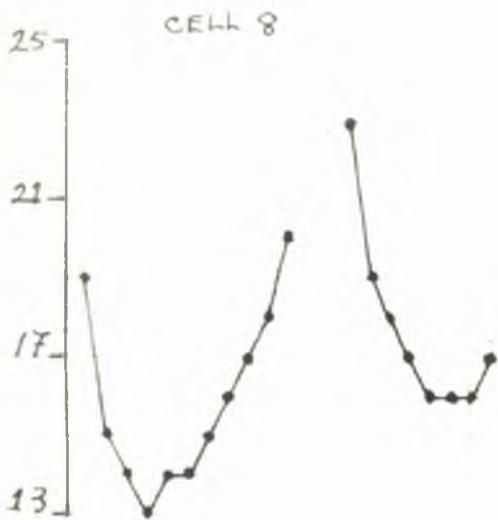
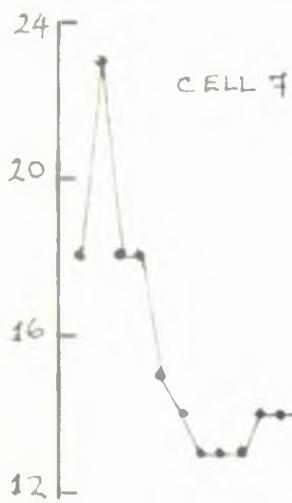
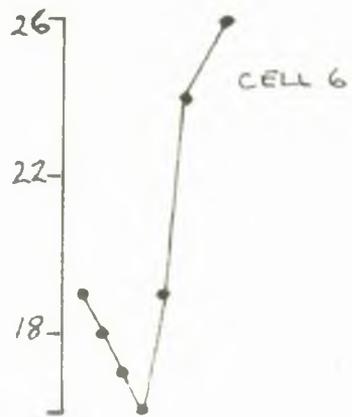
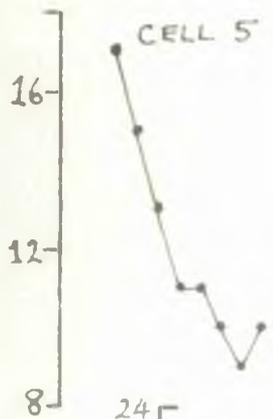




Figure VII. 15. (A) ... (B) ... (C) ... (D) ... (E) ... (F) ... (G) ... (H) ... (I) ... (J) ... (K) ... (L) ... (M) ... (N) ... (O) ... (P) ... (Q) ... (R) ... (S) ... (T) ... (U) ... (V) ... (W) ... (X) ... (Y) ... (Z) ...

... (A) ... (B) ... (C) ... (D) ... (E) ... (F) ... (G) ... (H) ... (I) ... (J) ... (K) ... (L) ... (M) ... (N) ... (O) ... (P) ... (Q) ... (R) ... (S) ... (T) ... (U) ... (V) ... (W) ... (X) ... (Y) ... (Z) ...

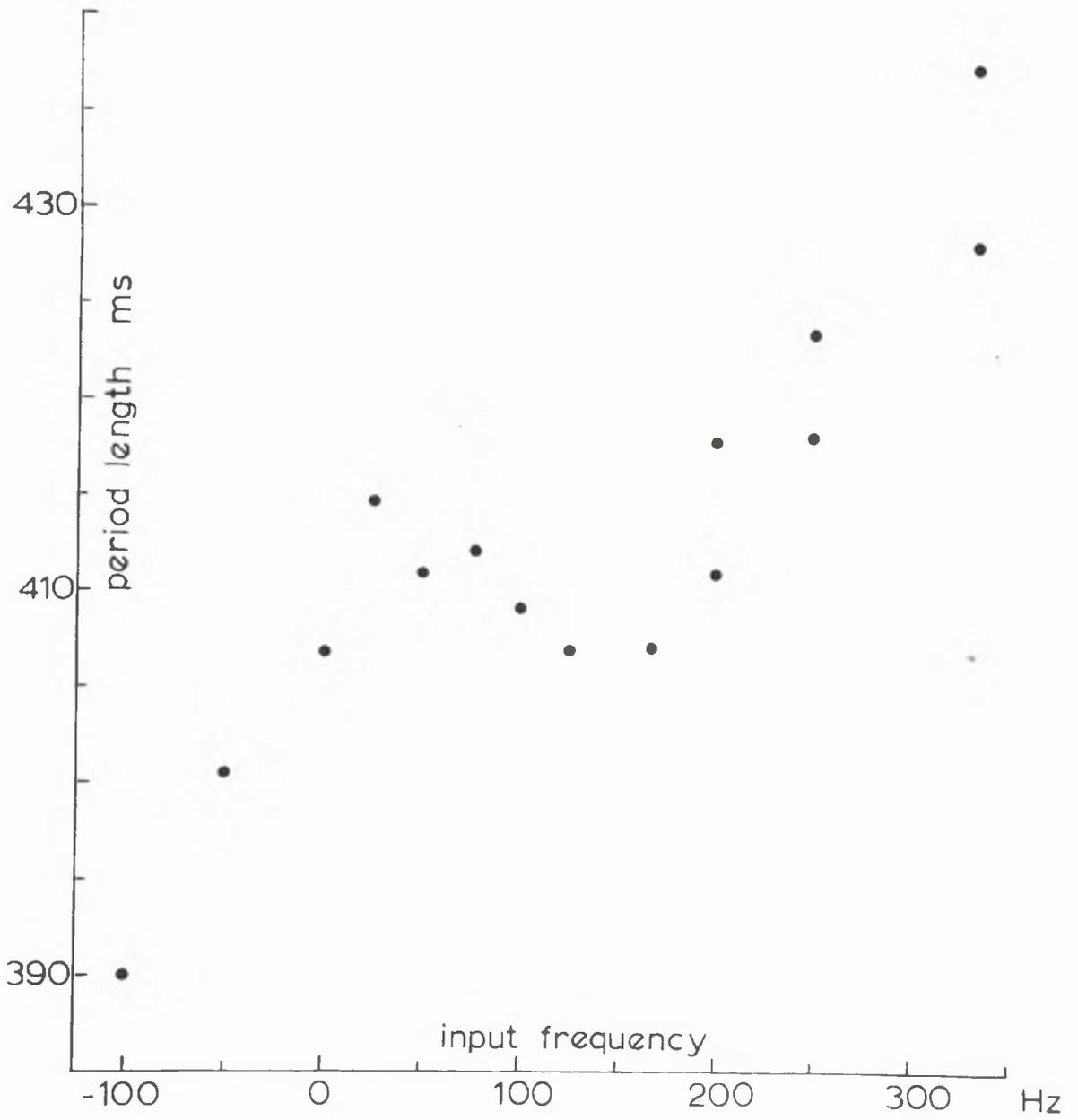
Figure VII. 15. (A) Diagram of the final state of the model. The network and connectivities are essentially the same as in Fig. VII. The representation here is more chematized however, for clarity. Cells 5 and 15, 6 and 16, etc., are the analogues of the units active in the four different subgroups. Notice that there is a closed excitatory loop between cells 5 and 15 (flexor sub-group analogue). This arrangement could be replaced by an electronic connection between the two cells. Between cells 7 and 17 (quartus sub-group analogue) there is weak reciprocal inhibition, and between the units in the levator sub-group analogues there are no interactions. For sub-group C, the relative thresholds of the motor unit analogues and the burst-terminating 'interneuron' (cell 10) are shown. The effect of this arrangement is to terminate the burst in the antagonist group before the start of activity in either group, giving rise to a pause. In the case of sub-group B, cell 10 inhibits cells 5 (flexor analogue) very strongly, but gives very weak inhibition to cell 15 (sixtus analogue), which continues firing into the levator phase of the cycle.

(B) The output pattern of the network with input excitation of 77 Hz (top) and 333 Hz (bottom) from a 'command' cell (19) onto cells 1 to 4. The bursts in the different units have been blocked in for clarity. The 'interneurone' bursts are enclosed by broken lines.



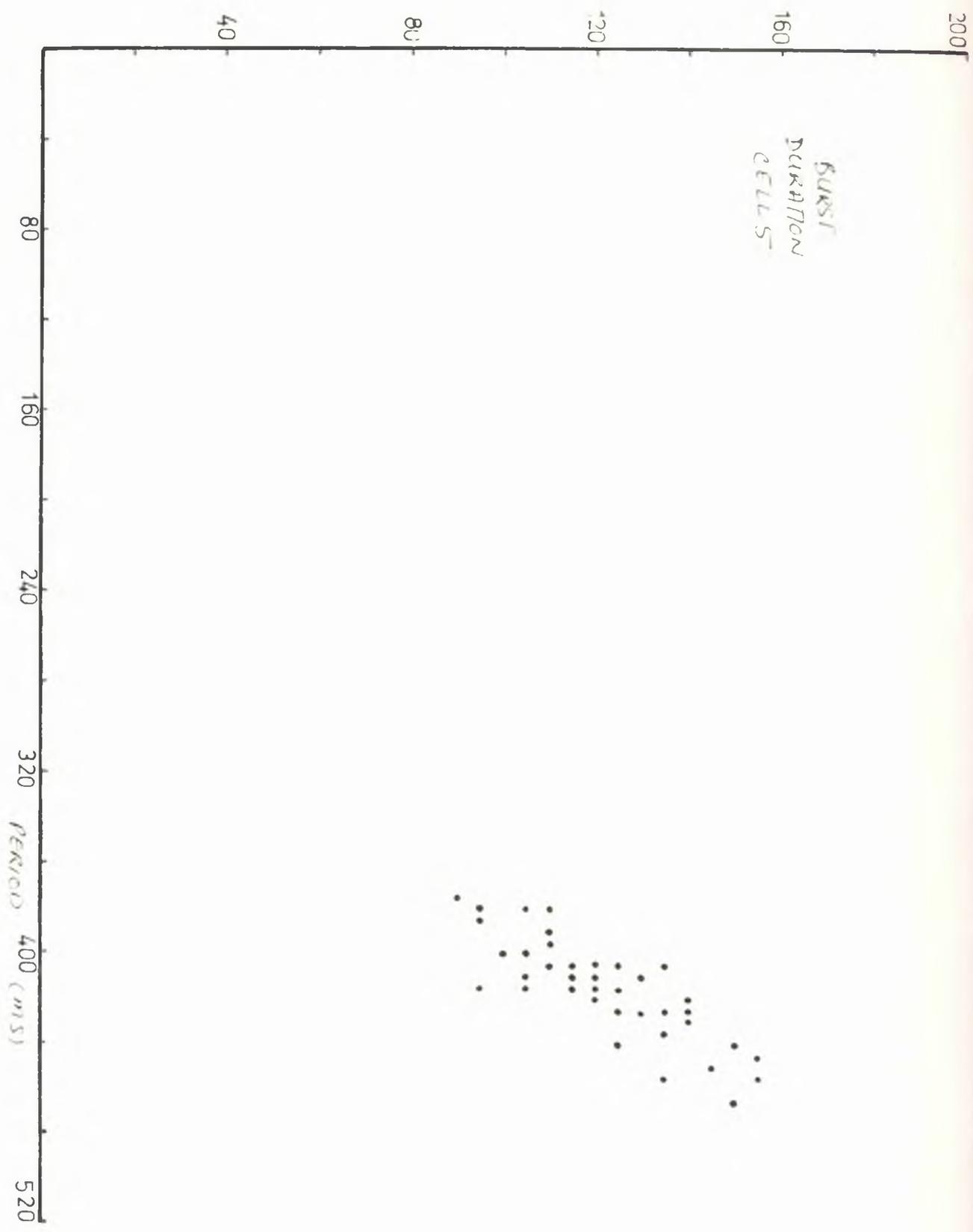
Figure VII. 16.

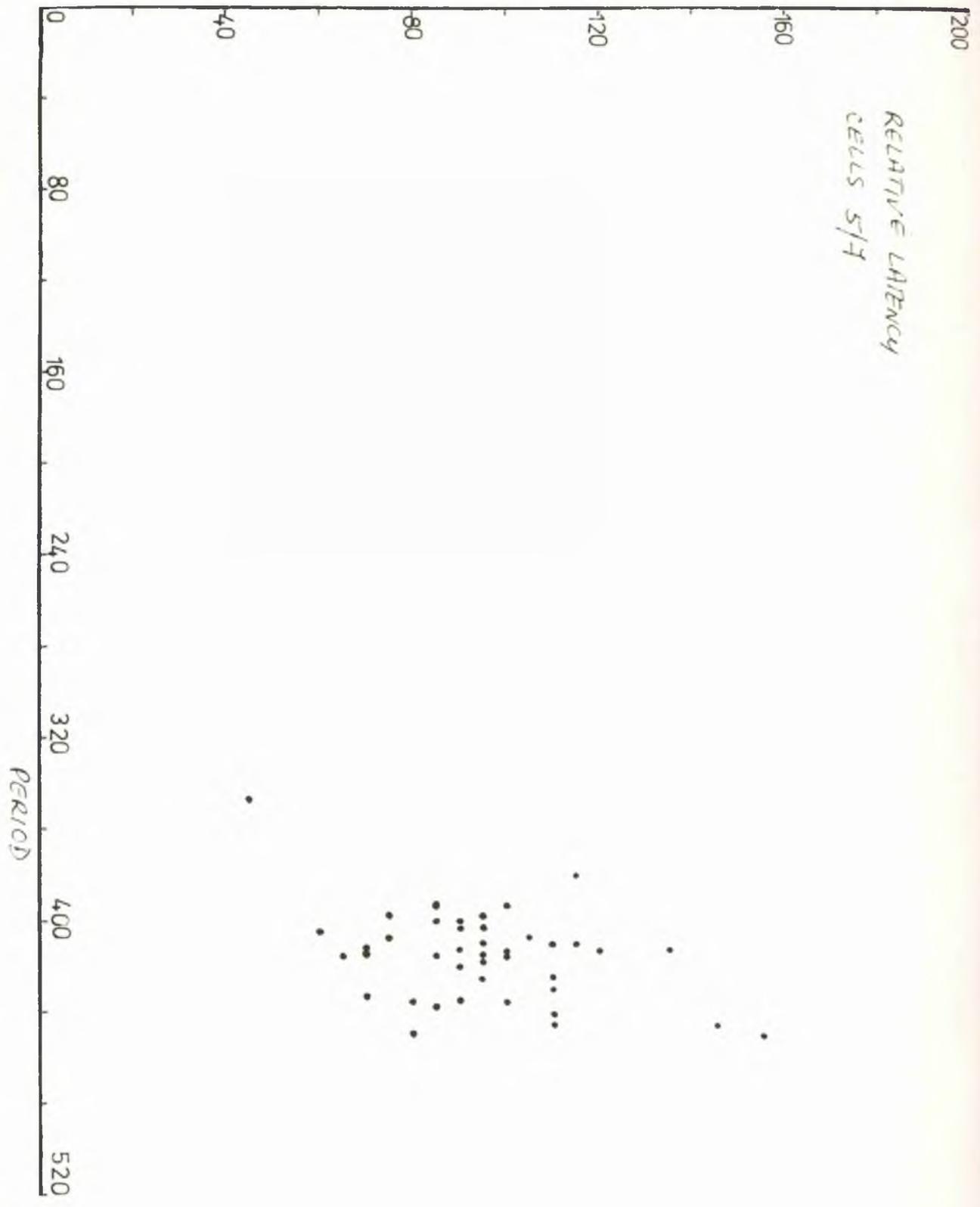
Figure VII. 16. The dependence of period length in the final network (Fig. VII. 15) on input frequency. Negative input represents inhibition; positive, excitation. There is a break in the relationship between +50 to +150 Hz input frequency, but otherwise, the relationship is similar in form to that for the simpler network. The break is probably related to the point at which the bursts in the different groups no longer are self-terminated, but are terminated by the inhibition from the 'interneurones', cells 10 and 20 (see Fig. VII. 15).

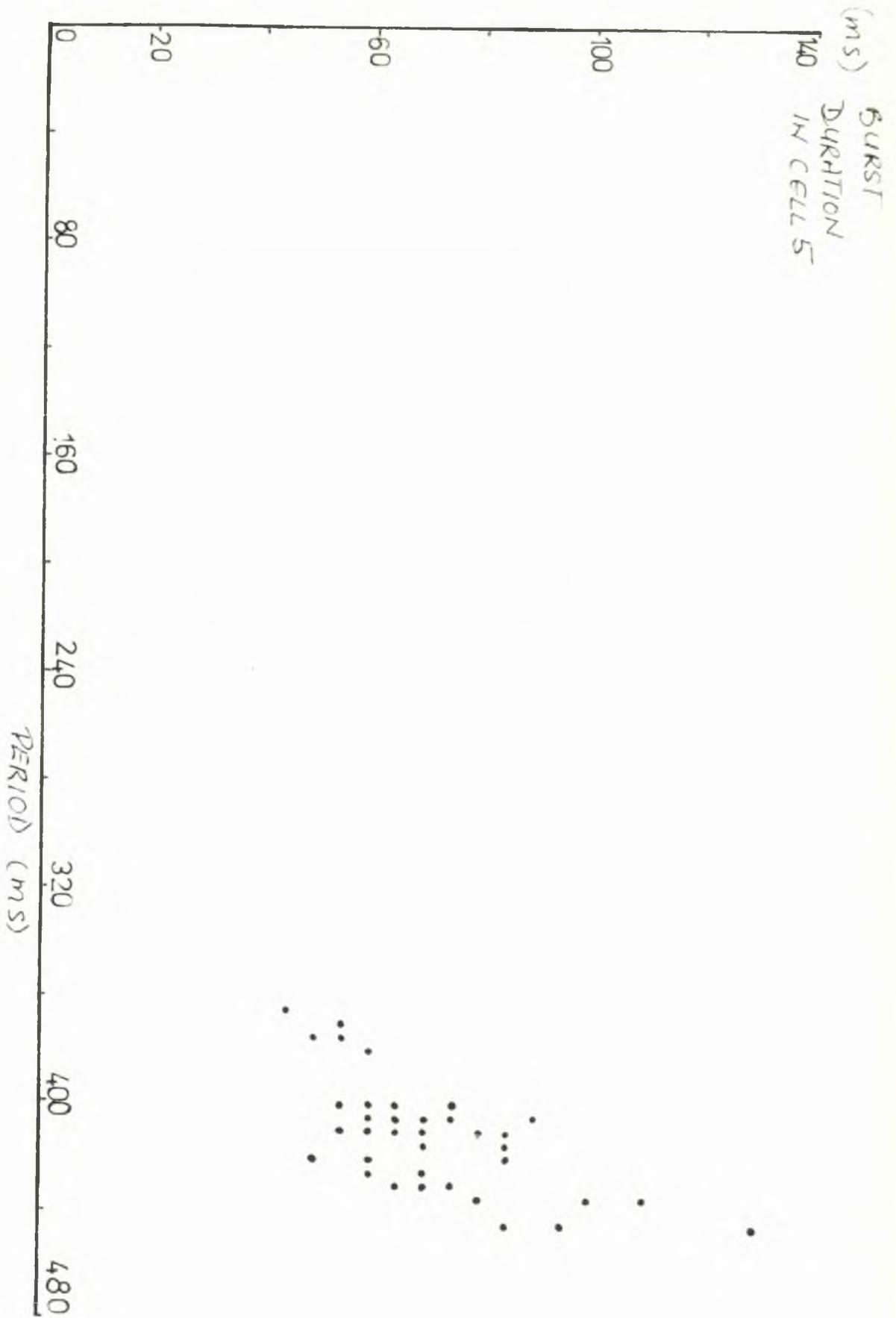


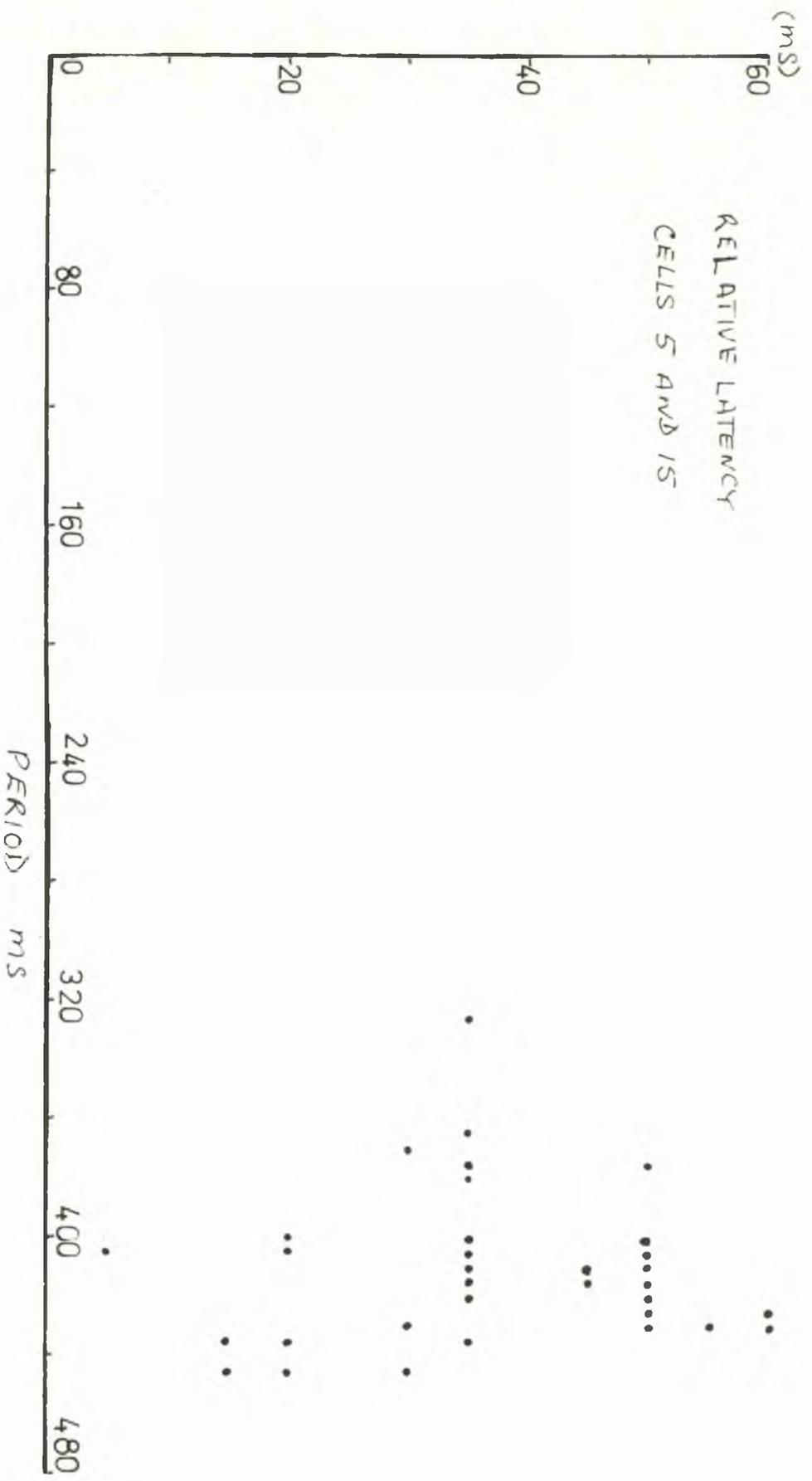
Figures VI. 17 - 21.

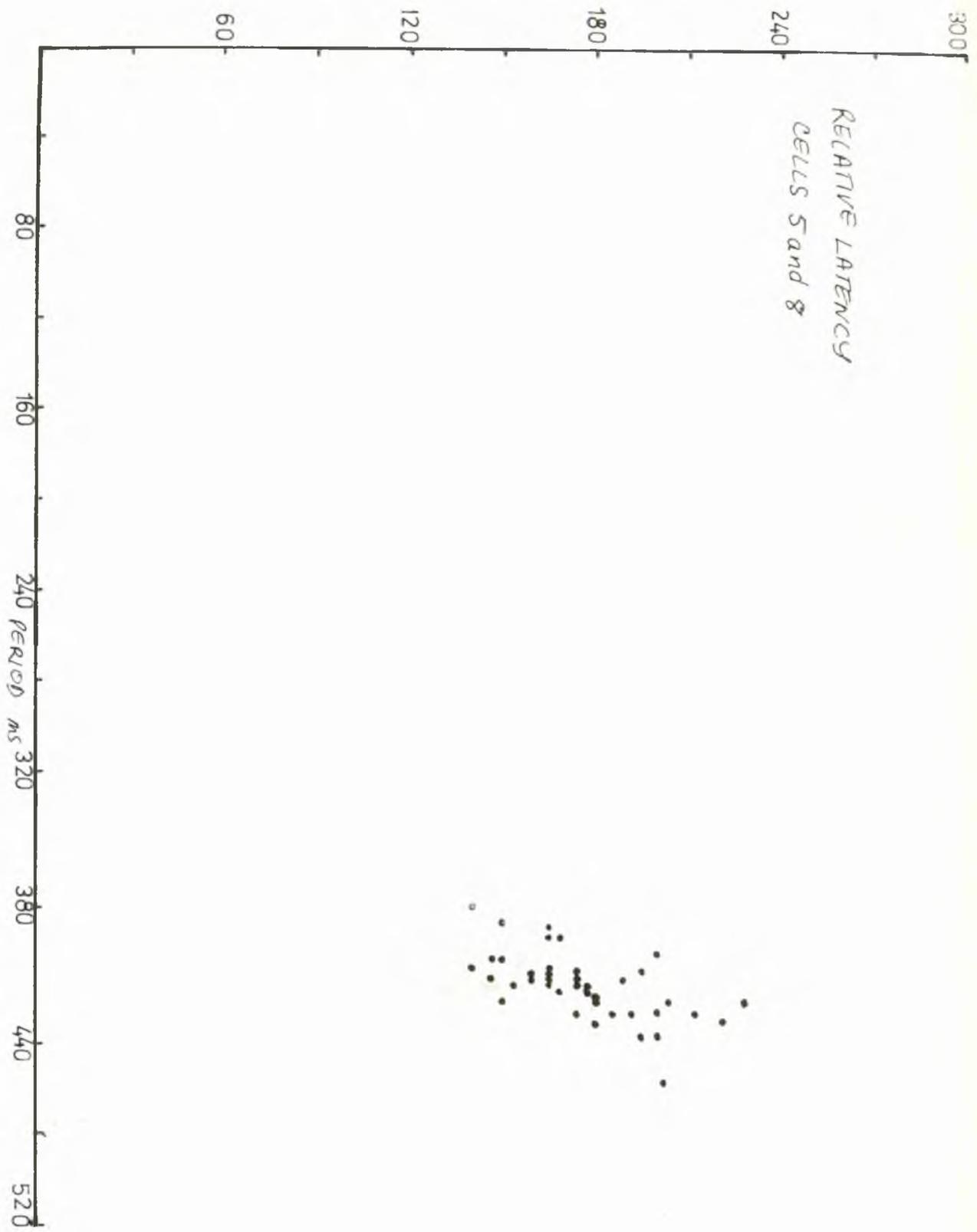
Figures VII. 17 - 21. Some frequency dependent characteristics of the bursts in the final model.











## VIII. CONCLUSIONS

The approach of this thesis has been to examine in detail, the activity in the scaphognathite and to some extent, its integration with peripheral systems, the aim being to construct a picture of how the rhythmic beating is generated, coordinated and controlled. A degree of progress has been made on all of these fronts, but the findings are subject to some qualifications. The main findings are listed below, along with some qualifying remarks where necessary.

(1) The brachyuran scaphognathite is composed of two main segments, one internal (anterior) and one external (posterior). The two segments are joined along the radial axis of the appendage by a flexible hinge. There are three main axes for movement. One is longitudinal, and about it, the whole appendage, in particular the external segment, can be raised and lowered. The other two are radial and pass through the internal and external segments, allowing twisting (pronation/supination) of the respective segments.

(2) The beating of the scaphognathite in Carcinus is effected primarily by nine muscles, five ventral and four dorsal. The dorsal and ventral groups of muscles can each be separated in to two sub-groups on the basis of their activity (two muscles to each dorsal sub-group and two and three in the two ventral sub-groups). The muscles in each sub-group show nearly synchronous bursts of activity in each beat cycle. There is a variable degree of overlap between the bursts of activity in the different sub-groups.

(3) . By virtue of the coordinated activity in the muscles of the four sub-groups, the internal and external tips of the scaphognathite execute movements which might be described roughly by two trapezoidal wave-forms, about  $90^{\circ}$  out of phase. The hinge region simultaneously

displays an approximately sinusoidal movement, the maxima and minima corresponding with the points of intersection of the waves described by the tips.

(4) By a peculiar skeletal arrangement, a 'click' mechanism is established, which assists in the timing of the movements of the different parts of the scaphognathite, and which might enhance the degree of acceleration displayed during the rise and fall of the external tip of the scaphognathite.

(5) Reversal of the beat direction is effected by inversion of the sequence in which the four sub-groups of muscles are activated.

The effect of this on the movement is to shift the sinusoidal wave-form for hinge movement through about  $180^{\circ}$ , and the trapezoidal wave-form for the movement of the hind end through a smaller angle, about  $90^{\circ}$ , with respect to the wave for the front end. By these changes, the direction of pumping is reversed.

(6) In the changeover from forward to reversed beating, the single units active in the two flexor sub-group muscles appear to be inhibited and replaced during reversed beating, by different units. There may be a change in the relative extents of activity in different units active in the other, multiply innervated muscles. During the process of reversal, the flexor sub-group muscles appear to 'lose' one burst, relative to the other muscles. The changes during changeover from reversed to forward beating are less clear, but usually the muscles of the flexor sub-group seem to gain an extra burst. In the region of the changeover, there appears to be a tendency towards longer period lengths during forward, and shorter during reversed beating. The changeover from forward to reversed beating may therefore be accompanied by specific inhibition of the forward mode flexor and sixtus units, and overall excitation. Reversal would perhaps be associated with release

of the inhibition to the flexor sub-group forward units (which might in turn be envisaged to inhibit the reversed mode units), and overall inhibition.

(7) The relative timing of the starts and durations of the bursts in the different muscles are in general, linear functions of the period of the beat cycle. This indicates that the cueing of the starts and terminations of the bursts is not dependent on a set of latency dependencies. In fact, there appears to be a tendency towards phase constancy, although there are several cases in which the phase relationships change slightly with changing frequency, and some even, in which the phase relationships change quite markedly. The timing of the relative starts and durations of the bursts might best be described by the relationship  $L = \underline{a} + \underline{b}$  (period) in which  $\underline{a}$  may or may not be significantly different from zero. When  $\underline{a}$  is not significantly different from zero, truly phase locked relationships result; when it is, there will be a significant regression of phase on period. The muscles of one of the levator sub-groups, the promotor sub-group, may not conform to this relationship during forward beating. There is a great deal of variability in the finer aspects of the timing of the bursts. It was difficult to obtain reproducible results on a given muscle in different animals, and there is indication that even in a given animal, the relationships may vary from time to time. This variability makes some of the above generalizations only tentative, and subject to a degree of doubtfulness.

(8) There is some indication that the variance of the relative latencies between muscles of the same sub-group might be less than that between muscles of different sub-groups. In turn, the latencies between muscles from different sub-groups in the same group may show lower variance than those between muscles from different groups (levator and

depressor). This might be consistent with a hierarchical system of cueing, but other systems could also explain the differences.

(9) Comparisons of (a) the changes in spiking frequencies in the bursts in the different muscles at different phase angles in the cycle of the flexor burst (b) the computed, underlying 'waves of depolarizations' in some of the units, and (c) spike to spike interactions in overlapping units from different bursts, strongly suggest that there might be reciprocal, negative interactions of some sort (reciprocal inhibition) between units from muscles of different sub-groups, and at least in some cases, positive interactions (reciprocal excitation) between units in muscles from the same sub-group.

(10) Digital computer simulations of models based primarily on reciprocally inhibitory neuronal networks, indicate that several aspects of the patterning of the activity in the scaphognathite muscles may be consistent with a pattern generator of this type. The model implies no hierarchy between group and sub-group levels, but the organization into sub-groups occurs at a higher level than that for the individual muscles. The output of the final model can, at least qualitatively, mimic that of the scaphognathite pattern generator. Further development of the model on a fine scale is clearly required, and further testing of its characteristics is necessary. However, it is believed to give the best approximation to the system controlling scaphognathite activity as we know it.

(11) Glide coordination has been demonstrated between the right and left scaphognathites in Carcinus. The appendages may beat at widely differing frequencies, and show no phase locking. Equalization of the beat rates and phase locking may be abruptly initiated, and this might possibly be associated in some cases, with transient simultaneous reversal on both sides. In other cases it may not be. Phase histograms

of extended runs show significant peaks. It appears then, that phase locking may be effected by two mechanisms, which may act together. Firstly, common input perhaps from command elements, may bring the rates of beating on both sides close together. Weak inhibitions between the pattern generators on both sides could then aid in equalization of the rates, and phase locking.

(12) There is strong correlation between activity in the scaphognathites and in the cardiac inhibitor, in Nephrops and Portunus. Abrupt high frequency tonic discharge in some medium to large units in the cardiac inhibitor in Nephrops is accompanied by pauses in heart and scaphognathite beating. Heart beats tend to resume within 4 - 6 seconds in spite of sustained inhibitor discharge. The scaphognathite tends to remain silent until the discharge rate returns to normal. Stimulation of the ipsilateral circumoesophageal connective can elicit a similar sequence of activity in the heart, scaphognathite and cardiac inhibitor.

Paradoxically similar stimulation during the ventilatory pauses described above, can also cause disruption of the inhibitor activity, and may be followed by bursts of activity in the scaphognathite. The opposing effects are ascribed to activation of different command units due to stimulation of the whole connective. Abrupt phasic bursts in a large unit in the inhibitor are frequently accompanied by disruption of the burst pattern in muscles of the scaphognathite. High frequency tonic discharges in the cardiac inhibitor in Portunus are accompanied by a complex, but reproducible sequence of changes in beat frequency and EMG amplitude in the scaphognathite. It is suspected that these changes might be associated with transient reversal.

(B) There is some indication that in Nephrops the heart via the cardiac inhibitors and accelerators may receive phasic information concerning the scaphognathite beat. It is not known whether this information is

used, or if it is, under what circumstances it may be used.

(14) The systems studied here offer interesting possibilities for further study. The inherent variability and the poor survival rates of the preparations are disadvantages. However, these might be overcome. With the present knowledge of the organization of and the interrelations between the motor units, and with the functional model as a testable hypothesis, it is believed that intracellular studies if possible, might be extremely rewarding. The findings concerning the linkages between the heart and right and left scaphognathites in Nephrops and Portunus are of some interest. They indicate that these systems might provide interesting material for a more detailed study of the integration between separate behavioral units on a local as well as a command level. There are also, interesting implications concerning the evolutionary development of motor and command processes. The Macrura do not as a rule show sustained reversal, whilst the Brachyura may. Increased activity in the cardiac inhibitor results in a sustained ventilatory pause in Nephrops, while Portunus responds in a more complex fashion, probably including a transient period of reversed beating. A comparative study of the organization and control of the pattern generators in these two types of animal should be of some interest.

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## APPENDIX I

### The Main Features of a Digital Computer Simulation Designed by Mr. F.R. Balch\* to Simulate the Characteristics of Small Neuronal Networks.

The characteristics of the model (Chapter VII.2) were studied by means of a digital computer program designed, and kindly loaned to the author by Mr. P.R. Balch of the Gatty Marine Laboratory. The program enables simulation of neuronal characteristics and interconnections. Up to twenty neural analogues may be specified and interconnected to give rise to an interacting network. The usefulness of the program lies in the fact that beyond the simplest networks, it is difficult, if not impossible, to predict with any certainty what the resultant output of such a network might be. By the use of such a simulation, one is given a means of evaluating the characteristics of a particular network.

The simulation is basically similar to that of Perkel (1965), but includes a number of extensions and modifications which make it more flexible and, to some extent, more realistic. Each neural analogue is identified by a number from one to twenty, and is defined by a set of specifiable constants, and two main state variables, threshold and membrane potential. Whenever membrane potential in a given 'neuron' exceeds threshold, the 'neuron' spikes, and after a specifiable delay, the synaptic and axonal delay a wave of specified starting height, sign and time course is added to the membrane potential of each 'neuron' to which the spiking cell is connected via a "chemical" synapse. The firing cell enters an absolutely refractory period followed by a relatively refractory period, and return to normalcy. This in brief is the principle of the simulation. A more detailed description is given below.

Each 'neuron' in the simulation has the following properties:

(1) A membrane resting potential which is continuously approached, exponentially by the (a) membrane baseline potential at any given time.

(2) A threshold resting potential which is exponentially approached by the (b) threshold at any given time. The (c) membrane potential is equal to the algebraic sum of the baseline potential and the input EPSP's and IPSP's from other connecting cells. Whenever the membrane potential exceeds threshold the cell fires. When the cell fires, the membrane baseline potential rises to a fixed value, the (3) spike-height, and after (4) the absolutely refractory period falls to a fixed value, the (5) post-spike undershoot, returning exponentially towards the membrane resting potential at a rate determined by the (6) time constant of the membrane baseline return. On spiking the threshold also rises to a very high level (infinity), and after the absolutely refractory period, is set at a fixed value above its prespike level, from which it falls exponentially towards the threshold resting potential at a rate determined by the (7) time constant of threshold return. The values of the time varying parameters (a, b and c) are calculated at discrete intervals or (8) time steps. The parameters with numeric indices are specified at the beginning of the simulation run and remain fixed throughout the run.

The interconnections between cells are specifiable, and may be excitatory, inhibitory or electrotonic. For synaptic connections, when a cell spikes, PSP's of specified magnitude, time constant and sign (positive for excitatory and negative for inhibitory) are added to the membrane baseline potential of the cells to which the firing cell is connected, after a specified delay, the synaptic and axonal delay. The rise time of the PSP's is instantaneous, and the decay exponential, towards zero. A single cell may connect with several other cells and each connection may be different. A single cell therefore may simultaneously make excitatory, inhibitory and electrotonic connections

(3) Regular - in which the cell spikes at a regular, specified frequency.

Input cells may be used to simulate higher order command activity, or may be used simply as an electronic stimulator.

The state of the various cells may be obtained at desired intervals in terms of

- (a) whether or not the cell is spiking
- (b) the membrane potential
- (c) the interspike intervals
- (d) the reciprocal interspike intervals (instantaneous frequency).

(a) is routinely printed for each of the cells in use. If the cell is silent, its state is represented by a hyphen at the stated printout interval. If it is spiking, then the number of spikes which occurred during the printout interval is printed. Corresponding plots of membrane potential, interspike intervals or reciprocal interspike intervals at the stated printout times may also be obtained.

In the essence of the computer simulation is the absence of random variability, characteristic in biological systems. This randomness may be introduced by specifying a standard deviation for the membrane baseline potential. This will cause a random factor to be added at each time step, to the membrane baseline potential. This parameter was not used in the present study as its computation proved to be somewhat time consuming.

The computer simulation described is more flexible than most earlier simulations of similar design. It is also easy to use. However, it possesses certain deficiencies shared by such simulations, which are imposed by restrictions of the algebra, of our understanding of intra- and inter- neuronal activity, and of availability of computing time. For example, a 'neuron' is treated as a dimensionless point, so that

of differing magnitude, time constant and sign (in the case of synaptic connections). After a cell fires, the actual magnitude of IPSP will be determined by the difference between the membrane base-line potential and an arbitrary, fixed value equal to 1.5 times the post-spike undershoot. As the baseline potential returns towards the testing level, the height of the PSP approaches the set value. Similar adjustments apply for EPSP. These computations may be improved, since the adjustments are made only after the cell spikes. A more accurate approach would permit continuous adjustments depending on the time since the last input from that source (see Hartline and Cooke, 1969).

Electrotonic connections may be rectified or two way, and of differing strengths. The strength of the connection is a weighting factor between 0 and 2. In the case of the rectified connections used here, the connecting cell is uninfluenced by the connected cell. In the connected cell however, the membrane baseline potential, instead of approaching the resting potential, now approaches the mean of the membrane potential of the connecting cell times the weighting constant, and its own membrane resting potential. In the present simulations the strengths of the electrotonic connections (the weighting constants) were always 1.

Cells may be specified as input cells as opposed to the standard cells described above. As input cells, the neurones may make connections, but may not receive them. Their activity is specified in separate statements determining the timing and the nature of the activity. The activity may be

(1) Sinusoidal - in which the membrane potential varies sinusoidally with specified amplitude, about a specified origin, and with a given frequency.

(2) Random - in which the cell spikes randomly at specified average frequencies during the times of specified activity.

there is no opportunity for differential spatial attenuation and summation; the cable properties of the neurone are particularly difficult to simulate. To some extent the use of a non-spiking driver neurone, electrotonically connected to a spiking follower, and receiving all the input, simulates a spatial effect. It tends to integrate, and smoothe the input onto the spiking cell, but some of the more subtle properties of spatial summation and dendritic integration would be impossible to simulate at this level. The rise time of PSP's, also, is instantaneous. This and many other similar difficulties arise as a result of difficulties in establishing readily computable approximations of non-linear relationships. However, the simulation does provide a reasonable approximation of neuronal activity and interconnections as we know them.

## APPENDIX II

Derivation of the expected random latency distribution for the occurrence of a test spike in the interspike intervals of a reference spike train, given the frequency distribution of the reference intervals.

If two spike trains overlap as shown in Fig. II.1, then in the region of overlap, interactions between the units might be expected to influence the relative dispositions of the spikes in each train, with respect to the spikes in the other. Such interactions might be revealed therefore by inspection of the frequency distribution of histograms for the latencies of say, spikes in B with respect to spikes in A, and vice versa, in the region of overlap. In order to determine whether the resulting histograms are indicating significant interactions or no interactions, it is first necessary to determine the distribution which might be expected if there were no interactions between the units. The significance of the deviation from this 'expected' distribution can then be tested using statistical procedures such as the chi squared or the Kolmogorov-Smirnov tests. The 'expected' distribution for the case of no interaction may be derived from the frequency distribution histogram for the inter-spike intervals in the 'reference' train. The derivation devised for the present purposes is described below.

For the uncorrelated condition, whenever a spike occurs in the A train (a reference spike), a spike in the B train (a test spike) is given the opportunity to occur, equally, in all latency categories below that for the minimum reference interval. Opportunities for the occurrence of longer test spike latencies arise only when sufficiently long reference intervals permit it. If for instance, a reference interval falls into the tenth category of the histogram, then test

spikes have been given the chance to occur in any category from 1 to 9. Spikes in the tenth category may occur shortly before the reference spike, when they would be allocated to category 10. They may also coincide with, or come shortly after the reference spike, when they would be assigned a different reference interval. On the whole then, test spikes would have been given the chance to occur in about 9.5 categories. If the number of reference spikes in a category  $n$  is designated  $R_n$ , the total number of options given to a test spike will therefore be,

$$n = N$$

$$R_n(n-0.5),$$

$$n = 1$$

where  $N$  is the category of the longest reference interspike interval. The distribution of these opportunities differs in the different categories. As above, if a reference spike occurs  $R_n$  times in category  $n$  it may in fact, fall anywhere between the upper or lower limit of the class mark. The chance of a test spike occurring in that category will therefore, to the best approximation, be  $R_n/2$ . In each category below the  $n$  there will simultaneously be  $R_n$  opportunities, so that the total number of opportunities for a spike to occur in a category  $n$  must include the total number of reference intervals which occurred in categories greater than  $n$ . This is given by

$$\sum_{n+1}^N R_n \quad \text{so that the total number of chances}$$

will be

$$(R_n/2) + \sum_{n+1}^N R_n$$

The fraction of the opportunities occurring in each category then, will be given by,

$$\left( \frac{R_n}{2} + \frac{R_n}{n+1} \right) / \frac{R_n}{1} (n-0.5)$$

In order to obtain the expected frequency for the occurrence of test spikes in each category, this proportion is multiplied by the total number of test spikes that occurred. A Chi squared test may then be applied to the observed and expected distributions.

### References.

- ARUDPRAGASAM, K. D. and NAYLOR, E. 1964 a. Gill ventilation and the role of reversed respiratory currents in Carcinus maenas (L). J. exp. Biol. 41, 299 - 307.
- ARUDPRAGASAM, K. D. and NAYLOR, E. 1964 b. Gill ventilation volumes, O<sub>2</sub> consumption and respiratory rhythms in Carcinus maenas (L). J. exp. Biol. 41, 309 - 321.
- ARVANITAKI, A. and CHALAZONITIS, N. 1967. Electrical properties and temporal organization in oscillatory neurons. Symp. Neurobiol. Invert. (Edited by Salanki J.) 169 - 199.
- ASHBY, R. A. and LAMMER, J. L. 1965. Modifications of cardiac respiratory rhythms in crayfish following carbohydrate chemoreception. J. cell comp. Physiol. 65, 373 - 380.
- ATWOOD, H. L.; HOYLE, C.; SMYTHE, T. 1965. Mechanical and electrical responses of single innervated crab-muscle fibres. J. Physiol. 190, 449 - 482.
- BENTLEY, D. R. 1969. Intracellular activity in cricket neurons during generation of behaviour patterns. J. Insect. Physiol. 15, 671 - 701.
- BOHRADATIL, L. A., 1922. On the mouth parts of the shore crab. J. Linn. Soc., Zool. 35, 115 - 142.
- BOHRADATIL, L. A., 1926. Notes upon crustacean limbs. Ann. and Mag. Nat. Hist., Ser. 9, 17, 193 - 213.
- BURNS, H. D. and LAURICIGHI, C. C. 1960. Repetitive firing of respiratory neurones during their burst activity. J. Neurophysiol. 23, 27 - 46.
- BURROWS, H. and WILLOWS, A. D. 1969. Neuronal co-ordination of rhythmic maxilliped beating in brachyuran and anomuran Crustacea. Comp. Biochem. Physiol. 31, 121 - 135.

- CARPENTER, D. O. 1967. Temperature effects on pacemaker generation, membrane potential, and critical firing threshold in Aplysia Neurons, J. gen. Physiol. 50, Part I. 1469 - 1484.
- CHALAZONITIS, N. 1963. Effects of changes in CO<sub>2</sub> and PO<sub>2</sub> on rhythmic potentials from giant neurons. Ann. N. Y. Acad. Sci. 109, 451 - 479.
- COCHRAN, D. H. 1935. The skeletal musculature of the blue crab, Callinectes sapidus Rathbun. Smithsonian Misc. Collections, 92, No. 9, 76 pp.
- DAVIS, W. J. 1968. Quantitative analysis of swimmeret beating in the lobster. J. Exp. Biol. 48, 643 - 662.
- DAVIS, W. J. 1969. The neural control of swimmeret beating in the lobster. J. exp. Biol. 50, 99 - 117.
- DAVIS, W. J., and MURPHY, R. F. 1969. Discharge patterns of swimmeret motoneurons in the lobster, simulated with a digital computer. J. exp. Biol. 50, 119 - 128.
- DELCOMYN, F. 1971. The effect of limb amputation in locomotion in the cockroach, Periplaneta americana. J. exp. Biol. 54, 453 - 469.
- EVOY, W. H., KENNEDY, D., and WILSON, D. M. 1967. Discharge patterns of neurones supplying tonic abdominal flexor muscles in the crayfish. J. exp. Biol. 46, 393 - 417.
- EVOY, W. H. and COHEN, M. J. 1971. Central and peripheral control of Arthropod Movements. Advances in Comp. Physiol. 4, 225 - 266.
- FIOREY, E. 1960. Studies on the nervous regulation of the heart-beat in decapod crustacea. J. Gen. Physiol. 43, 1061 - 1081.
- GENSTEIN, G. L. and FIEKEL, D. H. 1972. Mutual temporal relationships among neuronal spike trains. Statistical techniques for display and analyses. Biophys. J. 12, 453 - 473.

- GILLIARY, H. L. and KENNEDY, D. 1969. Neuromuscular effects of impulse patterning in a crustacean motoneuron. *J. Neurophys.* 32, 607 - 612.
- GRAY, J. 1950. The role of peripheral sense organs during locomotion in the vertebrates. *Symp. Soc. exp. Biol.* 4, 112 - 126.
- HARTLINE, D. K. and COOKE, I. M. 1969. Postsynaptic membrane response predicted from presynaptic input pattern in lobster cardiac ganglion. *Science* 164, 1080 - 1084.
- HARMON, L. D., 1964. Problems in neural modelling. *In Neural Theory and Modelling.* Ed. R. F. Heiss, Stanford U. Press, Stanford, California. p. 9 - 30.
- HOLST, E. von. 1939. Über relative Koordination als Methode zentralnervöser Functionanalyse. *Ergeb. Physiol.* 42, 228 - 306.
- HOLST, E. von 1943. Über relative Koordination bei Arthropoden. *Pflügers Arch. ges. Physiol.* 246, 847 - 865.
- HORRIDGE, G. A. 1968. Interneurons: their origin, action, specificity, growth and plasticity. Freeman & Co., (London and San Francisco). 436 pp.
- HOYLE, G. 1964. Exploration of neuronal mechanisms underlying behaviour in insects. *In Neural Theory and Modelling.* Ed. R. F. Heiss, Stanford Univ. Press. pp. 346 - 376.
- HOYLE, G. and WIKESMA, C. A. G. 1958. Coupling of membrane potential to contraction in crustacean muscles. *J. Physiol. Lond.* 143, 441 - 453.
- HUGHES, G. M., 1972. The relationship between cardiac and respiratory rhythms in the dogfish, Scyliorhinus canicula L. *J. exp. Biol.* 57, 415 - 434.
- HUGHES, G. M., KNIGHTS, B. and SCHEFFEL, C. A. 1969. The distribution of  $PO_2$  and hydrostatic pressure changes within the branchial chambers in relation to gill ventilation of the shore crab Carcinus maenas L. *J. exp. Biol.* 51, 203 - 220.

- HUGHES, G. M. and WILMSMA, A. G. 1960. The coordination of swimmeret movements in crayfish. *J. exp. Biol.* 37, 657 - 670.
- IKEDA, K. and WILMSMA, C. A. G. 1964. Autogenic rhythmicity in the abdominal ganglion of the crayfish: the control of swimmeret movements. *Comp. Biochem. Physiol.* 12, 107 - 115.
- JOHNSON, H. L. 1936. Respiratory regulation in crustaceans. *J. exp. Biol.* 13, 467 - 475.
- KALMUS, H. 1930. Untersuchungen über die Atmung de Flusskrebases Potamobius astacus Leach. *Z. vergleich Physiol.* 12, 725 - 759.
- KANDEL, E. R. 1969. The Organization of subpopulations in the abdominal ganglion of Aplysia. *In The Interneuron*. Ed. M.A.B. Brazier. UCLA. Univ. Calif. Press. p. 37 - 70.
- KENNEDY, D. 1969. The control of output by central neurons. *In The Interneuron*. Ed., M.A.B. Brazier, UCLA. Univ. Calif. Press. p. 21 - 35.
- KENNEDY, D., EVOY, W. H., FIELDS, H. L. 1966. The unit basis of some crustacean reflexes. *Symp. Soc. exp. Biol.* 20, 75 - 109.
- LARIMER, J. L. 1961. Measurement of ventilation volume in Crustacea. *Physiol. Zool.* 34, 158 - 166.
- LARIMER, J. L. 1964 a. The patterns of diffusion across the crustacean gill membranes. *J. cell. comp. Physiol.* 64, 139 - 148.
- LARIMER, J. L. 1964 b. Sensory-induced modifications of ventilation and heart rate in crayfish. *Comp. Biochem. Physiol.* 12, 25 - 36.
- LARIMER, J. L. and GOLD, A. H. 1961. Responses of the crayfish, Procambarus simulans, to respiratory stress. *Physiol. Zool.* 34, 167 - 176.
- LEVINE, J. 1973. Properties of the nervous system controlling flight in Drosophila melanogaster. *J. comp. Physiol.* 84, 129 - 166.
- McMAHON, B. R. and WILKINS, J. L. 1972. Simultaneous apnoea and bradycardia in the lobster Homarus americanus. *Can. J. Zool.* 50, 165 - 170.

- MATHIEU, P. A. and ROBERGE, P. A. 1971. Characteristics of pacemakers and oscillations in Aplysia neurons. *Can. J. Physiol. and Pharm.* 49, 787 - 795.
- MAYNARD, D. M. 1953. Activity in a crustacean ganglion. I. cardio-inhibition and acceleration in Panulirus argus. *Biol. Bull.* 104, 156 - 170.
- MAYNARD, D. M. 1961. Thoracic neurosecretory structure in Brachyura. *Biol. Bull.* 121, 316 - 329.
- MAYNARD, D. M. 1969. Excitatory and Inhibitory processes. Discussion, in *The Interneuron*. UCLA. 37 - 40. Ed. W.A.B. Brazier.
- MELLON, B. 1969. The reflex control of rhythmic motor output during swimming in the scallop. *Z. vergl. Physiol.* 62, 310 - 336.
- MENDELSON, M. 1971. Oscillator neurones in crustacean ganglia. *Science*, 171, 1170 - 1173.
- MILL, P. J., and HUGHES, G. M. 1966. The nervous control of ventilation in dragonfly larvae. *J. exp. Biol.* 44, 297 - 366.
- MILLER, F. L. 1960. Respiration in the desert locust. I. The control of ventilation. *J. exp. Biol.* 37, 224 - 236.
- MILLER, F. L. 1966. Central nervous control of respiratory movements. *In The physiology of the insect central nervous system*. Ed. Treherne, J.E. and J.M.L. Beaumont, Acad. Press, pp. 141 - 156.
- MULLONEY, B. 1970. Organization of flight motoneurones in Diptera. *J. Neurophysiol.* 33, 86 - 95.
- PANTIN, C.F.A. 1948. Notes on microscopical techniques for Zoologists. Cambridge University Press.
- PASZTOR, V. M. 1968. The neurophysiology of respiration in decapod Crustacea. I. The motor system. *Can. J. Zool.* 46, 585 - 596.

- FASZTOR, V. M. 1969. The neurophysiology of respiration in decapod Crustacea. II. The sensory system. *Can. J. Zool.* 47, 435 - 441.
- PERKEL, D. H. 1965. Applications of a digital computer simulation of a neural network. In *Biophysics and cybernetic systems*. Ed. Maxfield et al., Washington. Spartan Books.
- PILKINGTON, J. B. and SIMMERS, A. J. 1973. An analysis of bailer movements responsible for gill ventilation in the crab Cancer noval-zelandiae. *Mar. Behav. Physiol.*, 2, 73 - 95.
- PEINCLE, J. W. S., 1949. The excitation and contraction of the flight muscles of insects. *J. Physiol. Lond.* 108, 226 - 232.
- REISS, R. F., 1962. A theory and simulation of rhythmic behaviour due to reciprocal inhibitions in small nerve nets. *Am. Fed. Inf. Process. Soc., Proc. Spr. Joint Computer Conf.* 21, 171 - 194.
- SALMOIRAGHI, G. C., HUBBS, B. D., 1960. Notes on the mechanism of rhythmic respiration. *J. Neurophysiol.* 23, 14 - 26.
- DEGAAR, J. 1934. Die Atmungsbewegungen von Astacus fulvialilis. *Z. Vergleich. Physiol.* 21, 492 - 515.
- SHERINGTON, C. S. 1906. *The integrative action of the nervous system*. New Haven: Yale University Press.
- SILVEY, G. E., 1973. Motor control of tail spine rotation of the horseshoe crab, Limulus polyphemus, *J. exp. Biol.* 58, 599 - 625.
- TAUB, E., BACON, R. C., BERMAN, A. J. 1965. Acquisition of a trace-conditioned avoidance response after deafferentiation of the responding limb. *J. comp. physiol. Psychol.* 59, 275 - 279.
- TAUB, E. and BERMAN, A. J. 1963. Avoidance conditioning in the absence of relevant proprioceptive and exteroceptive feedback. *J. comp. Physiol. Psychol.* 56, 1012 - 1016.

- THOMAS, H. J. 1954. The oxygen uptake of the lobster Homarus vulgaris.  
J. exp. Biol. 31, 228 - 257.
- TINBERGEN, N., 1950. The hierarchical organization of nervous mechanisms  
underlying instinctive behaviour. Symp. Soc. exp. Biol. 4, 305 - 312.
- WALDRON, I., 1967. Mechanisms for production of motor output pattern in  
the flying locusts. J. exp. Biol. 47, 201 - 212.
- WALDRON, I. and WILSON, D. 1969. Latency relationships between closely  
co-ordinated locust flight motor neurone. J. exp. Zool. 170, 293 - 300.
- WEISS, P. 1950. Biological mechanisms in animal behaviour. Experimental  
analysis of co-ordination by the disarrangement of control-peripheral  
relations. Symp. Soc. exp. Biol. 4, 92 - 111.
- WENDLER, G., 1966. The co-ordination of walking movements in arthropods.  
Symp. Soc. exp. Biol. 20, 229 - 250.
- WIERSMA, C. A. G. and NOVITSKI, B. 1942. The mechanism of nervous  
regulation of the crayfish heart. J. exp. Biol. 19, 255 - 265.
- WILKENS, J. L. and 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 - 480.
- WILSON, D. M. 1962. The central control of flight in a locust. J. exp.  
Biol. 38, 471 - 490.
- WILSON, D. M. 1964. The origin of the flight-motor command in grasshoppers.  
In Neural Theory and Modelling. Ed. R. F. Reiss, pp. 331 - 345. Stanford:  
Stanford Univ. Press.
- WILSON, D. M. and GETTRUP, E. 1963. A stretch reflex controlling wingbeat  
frequency in grasshoppers. J. exp. Biol. 40, 171 - 185.
- WILSON, D. M. and WYMAN, F. J. 1965. Motor output patterns during random  
and rhythmic stimulation of locust thoracic ganglia. Biophys. J. 5, 121 - 143

- WOLVERKAMP, H. P. and WATKINMAN, T. H. 1961. Respiration In the Physiology of crustacea. I. Metabolism and Growth. pp. 35 - 100.
- WYMAN, R. J., 1965. Probabilistic characterization of simultaneous nerve impulse sequences controlling dipteran flight. Biophys. J. 5, 447 - 475.
- WYSE, G. A. 1972. Intracellular and Extracellular motor neuron activity underlying rhythmic respiration in Limulus. J. Comp. Physiol. 81, 259 - 276.
- YOUNG, R. E. 1973. Response to respiratory stress in relation to blood pigment affinity in Goniopsis cruentata and (to a lesser extent) in Carollisoma guanhum J. exp. Mar. Biol. Ecol. II, 91 - 102.