STATOCYST INPUT, MULTIMODAL INTERACTIONS, AND THEIR EFFECTS ON MOTOR OUTPUTS IN THE NORWAY LOBSTER, *NEPHROPS NORVEGICUS* (L.)

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Oh, the depth of the riches of the wisdom and knowledge of God!
How unsearchable his judgements, and his paths beyond tracing out!
"Who has known the mind of the Lord?
Or who has been his counsellor?
Who has ever given to God, that God should repay him?"
For from him and through him and to him are all things.
To him be the glory.

...... so that in everything he might have the supremacy.

Declaration :

I declare that this thesis represents, except where a note is made to the contrary, work carried out by myself and that the text was composed by myself.

Paul C. Knox

1st. December 1986

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Summary.

The statocyst organ in Decapod crustaceans detects body tilt. It is a powerful input to various equilibrium pathways. A range of techniques have been used to study the transmission of statocyst information and the effects of this information on thoracic and abdominal motor systems in *Nephrops norvegicus*.

Interneurons carrying information from the statocysts were recorded in the circumoesophageal connectives. Three different types of interneuron were recorded; of these, one responded best to tilt in the roll plane, while the other two responded best to tilt in the pitch plane but with opposite polarities. Combinations of pitch and roll, produced responses in these interneurons which, while maintaining the same phase position, decreased in size as the preparation was moved out of the preferred plane of tilt. These interneurons showed little or no response to various types of leg stimulation.

The responses of intact animals to body tilt were investigated by tilting animals in a large seawater tank; responses were recorded on videotape. Tilt in the roll plane produced a range of responses, the most prominent of which was an asymmetric leg response. Legs on the lowered side cycled in a patterned manner while legs on the raised side were held in a protracted and levated position. This response is distinct from previously reported patterns of leg activity such as walking and "waving". After leg autotomy, this pattern of leg activity was abolished.

Coordination was found between the cycling of the legs and the beating of the swimmerets during tilt in the roll plane. This was compared with the coordination between legs and swimmerets during

walking.

Tilts in the pitch plane resulted in systematic alterations of abdominal posture. Head-down tilt produced a marked abdominal flexion and head-up tilt produced extension. An examination of the responses of swimmerets to tilt in this plane showed that the angle of the powerstroke during head-down tilt was intermediate between the laterally directed powerstroke seen during tilt in the roll plane, and the rearward beat seen during head-up tilt. Recordings made in the swimmeret system revealed at least one tonically active returnstroke motoneuron which received input from the statocysts. Pitching the preparation head down caused an increase of firing frequency in this unit. This unit has been anatomically and physiologically identified as one of the returnstroke motoneurons.

Recordings made from the slow abdominal flexor motoneurons revealed a sensitivity to tilt in the pitch plane. However, they responded in an opposite manner to the tonic returnstroke unit. The peripheral inhibitor to this muscle was also recorded. It fired in phase with the tonic returnstroke unit.

Interactions between statocysts, legs and swimmerets were investigated at the behavioural level. Substrate contact prevented expression of asymmetric swimmeret responses to roll. However it was possible to demonstrate that this effect is not due to physical contact between the substrate and the terminal segment of the leg, the dactylopodite.

In the absence of substrate contact, swimmeret beating was nevertheless inhibited when leg cycling was prevented by blocking leg movement at the proximal joints. Blocking leg movement on one side of the body was sufficient to suppress the swimmeret responses on both sides.

In conclusion, information from the statocysts is carried by unimodal interneurons. This information is available to both the swimmerets and the abdominal posture system. In the absence of swimmeret beating, statocyst information continues to reach the swimmeret system where it determines the output of a tonic returnstroke motoneuron. Substrate contact radically alters the effect of statocyst input. This finding poses specific questions as to the precise nature of the interaction between descending statocyst information, leg input and output patterns, and the swimmeret and abdominal posture motor systems. This may be an ideal system for investigating interactions between the pattern generators of two different systems, specifically the legs and swimmerets, and the role of different types of sensory input in these systems.

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Chapter 1:

GENERAL INTRODUCTION.

1. Introduction.

The role of the nervous system in the initiation, control and termination of behaviour has long been the subject of intensive study. To enable such study to take place it has been necessary to find preparations which allow access to the nervous system while still producing recognizable patterns of behaviour. The mammalian nervous system is extremely complex and many processes take place within a relatively inaccessible central nervous system. A preparation which allows neurophysiological recordings to be made from specific neurones may need to be dissected to an extent that prevents the expression of recognizable behaviour. Perhaps for this reason much of the early work performed using mammals looked at aspects of the peripheral nervous system such as control of muscle and reflexes involving peripheral receptor systems such as muscle spindles, tendon organs and joint receptors (eg Creed et al 1932; Matthews, 1933; Laporte and Lloyd, 1952; Boyd, 1954). This work has more recently been complemented by recordings from the spinal cord and higher control centres (Harrison and Jankowska, 1985; Shik and Orlovsky, 1976). Lower vertebrates exhibit similar problems as their nervous systems are still relatively inaccessible (see Wallen and Williams, 1986). Frog preparations for example were used to study spindle physiology and neuromuscular transmission, but very little work has been done on the nervous control of behaviour. Intact human studies have been possible with the advent of microneurography (Vallbo and Hagbarth, 1967,1968). Studies in clinically abnormal human nervous systems have also made a contribution (Halliday, 1967; Hallett, 1979).

Invertebrate preparations, by contrast, have a relatively accessible nervous system. In comparison to the mammalian nervous system there are relatively small numbers of neurons involved. Processing within the central nervous system is distributed between segmental ganglia rather than concentrated at one site. Furthermore, even in a dissected state the invertebrate nervous system will continue to produce recognizable motor output. The behavioural repertoire of some invertebrates is extremely limited, so attention has focussed on particular groups which do show easily recognizable patterns of behaviour. As early as the latter decades of the nineteenth century the value of arthropods, and in particular crustaceans, was recognized. The crustaceans exibit a range of complex behaviours such as various types of locomotion, mating behavour, escape reactions and even social behaviour. They also have many of the advantages discussed above in terms of an accessible nervous system, relatively few neurons (compared to mammals) and good survival in a dissected state.

2. Historical Perpsective.

By the end of the 19th century, basic concepts of nervous activity were being worked out using the crayfish claw. Using such a preparation Richet, upon finding that increased stimulation of the opener muscle caused the strength of contraction to diminish, rightly invoked the concept of inhibition (Richet, 1879). Others extended these findings (Biederman, 1889). The picture was further clarified when, in the early years of this century, Lucas showed that two excitatory processes were occurring in the same muscle (Lucas, 1907, 1917).

By the 1930's more subtle effects were becoming clear. The timing of pulses and their temporal patterns were shown to be of great importance (Blaschko et al, 1931; Wiersma 1933). This was later investigated using microelectrodes to study single fibres (Fatt and Katz, 1953).

But perhaps of greatest significance was the technique developed by Wiersma at this time of splitting nerve bundles to the point where only single axons were being stimulated. Fast and slow axons innervating the crayfish claw closer muscle were demonstrated by van Harreveld and Wiersma (1936). It was not long before the inhibitory axon to the same muscle was also demonstrated (van Harreveld and Wiersma, 1937).

The technique of testing single axons, whether stimulating motor axons to produce a muscle contraction or sensory axons recorded during various types of stimulation, was and is of the greatest importance, particularly with the advent of single unit recording using intracellular microelectrodes.

As well as being used to look at neuromuscular mechanisms, crustacean preparations were from the outset used to study the central control of behaviour. Central neurons were found to elicit recognizable behaviours when stimulated electrically (Wiersma, 1938; Wiersma and Novitski, 1942). This led to the concept of the command neuron (Wiersma and Ikeda, 1964) which has proved to be one of the most powerful concepts in comparative neurobiology over the last few decades.

Another important aspect that was beginning to be stressed was the importance of comparison between species. This was particularly due to Wiersma and his co-workers. The need was recognized to argue from the general to the specific, rather than the other way around. Here again, crustaceans played a role, as it was possible to compare a wide range of species, from various ecological niches, all with a fundamentally similar nervous system (Wiersma, 1941). This has continued to be a valuable approach.

3. Behavioural Context.

Before continuing this review of the current state of knowledge concerning various aspects of invertebrate neurobiology there is another important factor to be borne in mind. With advances in our understanding of neural mechanisms has come a danger of seeing these as entities in their own right, separate from the behaviours they control and underlie. However, components that have been discussed above, namely sensory input systems, centres of integration and motor output systems, are all blended together. This blend is the behaviour that we observe in the real world. Moreover, very often, not merely the behaviour, but the context within which that behaviour takes place also has to be taken into account before a true understanding of the behaviour is possible.

4. The Control of Movement.

The control of movement has continued to excite great interest, and has been aided by advances in technique. A wide range of species, from humans to arthropods, can now be compared and contrasted.

Data obtained for mammalian systems has been complemented by that obtained utilising invertebrate preparations. In some cases, concepts developed using invertebrate preparations have been successfully transferred to vertebrates, notably the concept of central pattern generation (see below).

a. Locomotory Systems.

Within the general context of the control of movement, the study of locomotion has been of great significance. Here again the use of invertebrates has been crucial.

Insects have been used for the study of both flight and walking. Various approaches have been used. Fixed, dissected preparations have been used to look in detail at reflexes involved in the control and coordination of leg movements (eg cockroach walking leg, Wilson, 1965, 1966; Delcomyn, 1971). But this approach has the disadvantage that patterns of activity recorded may not occur during normal movement, and therefore there is some doubt as to the relevance of such findings to the actual situation in walking or flight. So free-walking preparations have also been developed, allowing either study of the gross movements using high speed cine techniques or video, or study of particular muscles and nerves in the performing animal (Runion and Usherwood 1966; Burns, 1973).

These approaches have been used to gather data on basic mechanisms in various species. Locusts and grasshoppers were used to investigate the control and coordination of leg movements (Hoyle, 1964; Usherwood et al,1968) as were cockroaches (Hughes, 1952 and 1957) and stick insects (Wendler, 1965).

b. Central Pattern Generation.

An early observation of the greatest importance was that the flight system of the locust was capable of generating rhythmic motor activity in the absence of sensory feedback (Wilson, 1961); this led to the concept of central pattern generation. This concept has been widely applied both to other systems in insects (eg. cockroach walking, Pearson and Iles, 1970, 1973) and crustaceans (eg. crayfish swimmerets, Heitler and Pearson,1980) and also to other non-arthropods including higher species such as the cat and man (Egger and Wyman, 1969; Grillner and Zangger,1974; Forssberg, 1986).

c.Importance of Sensory Input.

It has become increasingly clear that the concept of central pattern generation alone is not capable of explaining the locomotory behaviours observed in those systems studied so far. Nor does it explain how the nervous system deals with unexpected events such as would occur due to some external blockage of a movement or unexpected change in the environment.

In the flight system of the locust the pattern or rhythm recorded in completely deafferentated preparations is different from that observed in the presence of sensory feedback (Pearson, 1985). Furthermore the difference is not that the absence of tonic sensory input simply slows down an otherwise normal pattern, but rather that the whole pattern is altered. There is undoubtedly important information reaching the nervous system which is phasic in character (Möhl, 1985b) and it has become clear that phasic sensory input from various sense organs alters the timing of the motor activity (Neumannet al, 1982).

The focus of attention has gradually moved to the role of sensory input in the production of useful motor patterns. Knowledge of the sense organs themselves, their structure and function, is clearly necessary. Specific stimulation or ablation with a view to observing gross effects on the pattern of movement under consideration also has a role. In studying the interactions of sensory input and motor output, other types of patterned movement have been studied besides rhythmic locomotory patterns. Of importance here are various types of reflexes.

d. Interaction of Sensory Modalities.

In many of the systems discussed above, sensory input will usually originate from more than one source. Where there is the clear involvement of several modalities the question arises as to how and where they interact, such that the final response of the target motor system is consistent with requirements. Two possibilities emerge. In one case the sensory information could converge onto a restricted set of multimodal interneurons, which essentialy serve to integrate the balance of inputs before synapsing onto motor neurons. The other possibility is that the sensory information could be carried down unimodal channels to some integrating centre such as the brain or a segmental ganglion, where the information would be processed and the requisite instructions sent to the motor system.

In the locust, the jump employed as an escape response is triggered via an interneuron receiving visual, auditory proprioceptive and tactile input (M-interneuron, Pearson et al, 1980). This system could be compared with the crayfish escape system where the medial giant fibres respond to tactile and visual stimuli. The non-giant escape system responds to a wide range of modalities but no single interneuron has yet been identified which produces this escape response.

Recent work in the locust flight system, shows quite clearly the involvement of multimodal interneurons, receiving sensory input from any two or all of ocelli, compound eyes and head hairs (Rowell et al, 1986). These interneurons only respond to specific combinations of stimuli to the three modalities and in effect carry out an editing process. These correct combinations are found to occur when behaviourally compatible stimuli are given, ie the three modalities reinforce each other by signalling a similar course deviation. When these interneurons fire, they control appropriate corrective steering. So these interneurons are acting in an integrative fashion. Clearly then there is evidence that the first strategy outlined above is used in certain situations. However, as will be shown, this strategy is not necessarily used in all situations.

5. Crustaceans as Model Systems.

Crustaceans offer advantages in terms of the requirements and themes discussed above. They possess many sense organs the location, structure and in many cases the function of which are known (Alexandrowicz and Whitear, 1957; Whitear, 1962; Wales et al, 1970; Hartman and Austin, 1972). The movements of crustacean appendages, for example the legs and antennae, can in most cases be broken down into the constituent movements of a series of single joints, the movement of which is limited to one plane. This means that many movements can be quantified with relative ease (see for example chapter 3, leg movements; chapter 4, abdominal movements). Crustaceans also exhibit a wide range of both locomotory and reflex behaviours.

Crustacean walking has been used as a model system to study interactions between centrally-generated motor patterns and feedback from the periphery (Clarac, 1982, 1986). Most if not all of this feedback is phasic in character eg feedback from receptors detecting substrate contact will only operate during the stance phase in a given leg (eg see Klarner and Barnes, 1986). Therefore there may be similarities with systems such as the locust flight system where much of the feedback is also phasic. The implications of this are being explored in the walking system of crayfish (eg. Sillar and Skorupski, 1986).

The swimmeret system of crayfish and lobsters is another rhythmic system which has been studied in some detail (Davis, 1968; Heitler and Pearson, 1980; Miyan and Neil, 1986; Neil and Miyan, 1986). While in some species the swimmerets have ceased to be important for locomotion, (eg *Jasus*), in others they are still important, particularly within the context of equilibrium reactions (eg *Homarus, Nephrops*).

a.The Equilibrium Reactions of Decapods.

The study of equilibrium reactions has played an important part in understanding the control of movement, and more specifically the role of sensory input. Several sensory modalities may be involved in the control of a particular reflex, allowing questions involving the interaction of sensory inputs to be investigated. Several motor systems may be involved, raising questions concerning interactions on the output side of the response.

Equilibrium reactions have been divided into two catagories: compensatory and righting responses (Davis, 1968). Compensatory responses, which counteract movements away from the normal orientation, have been exhaustively studied, particularly at a behavioural level (Stein and Schöne, 1972; Schöne et al, 1976; Neil and Schöne, 1977). Righting responses serve to restore normal body orientation by the coordinated movement of legs, swimmerets and uropods. These responses have received comparatively little attention (but see Newland, 1985).

The reactions themselves are usually fairly stereotyped. It is often possible to manipulate the sensory input which gives rise to them. Different sensory modalities, such as the eyes, statocysts and leg proprioceptors are often involved and it is possible to study each modality individually and in concert with the whole system. So it is possible to build up a complex picture from several less complex components.

There is already a large body of data available on various of these components. In many decapod species input from the statocysts is known to be very important. Consequently, the statocyst organ, its structure, function and output connections, has been the subject of much study. Much of this work has been done on the crayfish, *Procambarus clarkii* (Takahata, 1981; Takahata and Hisada, 1979; Yoshino et al, 1980; Hisada and Neil, 1985) although there is some information available for *Homarus* (Cohen, 1953,1961) and also the fine structure of the statocyst in *Astacus* (Schöne and Steinbrecht, 1968).

In *Procambarus* four pairs of descending interneurons have been located which respond to statocyst stimulation. The interneurons respond in a phaso-tonic manner to tilt; the tonic component of the response codes the magnitude of the tilt (Takahata et al, 1982). The connections from the sensory hairs in the statocyst to the interneurons have been examined (Takahata and Hisada, 1982) as well as the output connections to uropod muscles (Takahata et al, 1985).

Several sensory modalities in addition to the statocysts are involved in detecting variations in orientation. The legs are known to monitor body position with respect to the ground in decapods (Alverdes, 1926; Schöne et al, 1976) as in many species other species (Magnus 1924; Sherrington, 1947; Wendler, 1975). The eyes also play a role (Neil et al,1983).

It has become clear that the various sensory modalities interact. Substrate contact is known to have an important influence on responses to gravity and light (Kuhn, 1914; Alverdes 1926; Schöne et al, 1978; see also chapter 5). The compensatory responses of eyes and antennae are influenced by leg proprioceptors and inputs from statocysts (Schöne et al, 1983; Priest, 1983).

Interneurons similar in some respects to those in *Procambarus* have been reported in the crabs, *Carcinus maenas* and *Scylla serrata* (Fraser, 1975). However these interneurons, called equilibrium interneurons, also received input from leg proprioceptors and a non-specific input involving optic pathways. The *Procambarus* statocyst interneurons responded poorly if at all to other inputs.

Important differences emerge between the interneurons discussed above which occur in crustacea, and those discussed earlier which are found in the flight system of the locust. The integrative function performed by the locust interneurons has yet to be demonstrated in crustaceans. Furthermore, the statocyst interneurons of lobsters and crayfish respond poorly if at all to other sensory modalities; they are not as clearly multimodal as are the crab and locust interneurons (Takahata and Hisada , 1982; see ch2).

There are other important differences in terms of the output of the flight system as compared with swimmerets or legs in decapods. The flight system output is complex in that even small temporal or spatial changes can have major consequences. In contrast the swimmerets, while a rhythmic system, are fairly stereotyped in their movements, although as will become clear perhaps not as stereotyped as once thought (see chapter 4). Their capacity, and indeed their need to make fine adjustments is limited. There are also differences in the time course over which changes in output need to be made. In the flight system course or orientation correction has to be made quickly, perhaps within a few milliseconds. In the crayfish escape pathway, where there is a time constraint of a similar magnitude (though for a different reason ie the need to avoid capture by a predator), a degree of mulitmodality operates. In the equilibrium pathways of decapods, corrections which take place over several seconds are sufficient. For this reason equilibrium pathways may utilise other strategies for dealing with the problem of obtaining and integrating the relevant sensory information and passing instructions to the relevant motor systems.

6. The Norway lobster, *Nephrops norvegicus*

There are four major families of lobsters, the Palinuridae (or spiny lobsters), the Synaxidae (or coral lobsters), the Scyllaridae (or slipper lobsters) and the Nephropidae (or clawed lobsters). As its name suggests *Nephrops norvegicus*, the subject of the experiments reported in this thesis, belongs to the last family.

Nephrops is widely distributed on the continental shelf of Europe; its geographical range extends from Morocco to Iceland, and in the Mediterranean as far east as Egypt. In commercial terms it is now the most important shellfish species in the United Kingdom (Howard, 1982).

a. Ecology.

Much of the research so far carried out on *Nephrops* has been fisheries-related, aimed at understanding its ecology and behaviour in the field. Its distribution is related to the availability of a substrate composed of fine cohesive mud in which it can construct burrows. These burrows extend 20 to 30cm beneath the sediment surface and can be quite complex. The animal spends much of its time inside the burrow which provides shelter and protection (Howard, 1982). It emerges to feed and to mate. The emergence rhythms are reflected in the peak catches recorded and have been related to the light intensity at the sea bed. At depths of between 40 and 50m peak catches occur at dusk and dawn. As the depth increases the times of peak capture shift towards midday (Arechiga and Atkinson, 1975; Atkinson and Naylor, 1976).

The *Nephrops* eye has been found to be very sensitive to light. Indeed exposure to daylight for only short periods of time, or to artificial light, bleaches the pigment in the retina causing irreversible damage (Loew, 1976; Shelton et al, 1985). As animals used in this study had been exposed to both daylight and artificial light over a long period, visual inputs into the various equilibrium pathways played no role in the experiments described in this thesis.

Laboratory experiments have complemented the finding that in the field, water currents affect catches. Experiments performed in a flume tank have demonstrated that animals do indeed have a preferred orientation in a water current (Newland, 1985). They adopt a head down current orientation with respect to the water movement.

b. Neurobiology.

The nervous system of *Nephrops* follows the familiar Decapod plan (for review, see Sandeman, 1982): a chain of segmental ganglia which are joined by paired connectives. These are separate in the thorax, but share the same sheath in the abdomen.

Cross-sections of the nerve cord at various levels show some degree of organisation (Fig. 1). The clearest features are the medial and lateral giant fibres. The general rule of larger fibres lying dorsally and smaller fibres lying ventrally can also be seen to apply (Skobe and Nunnemacher, 1971). In the spinal cord of mammals, clear fibre tracts can be distinguished functionally and anatomically (Rexed, 1952,1954). In *Nephrops* while there is not the same clear distinction into discrete tracts, there is some evidence to suggest that similar types of activity are found in the same area of the nerve cord (Priest, 1982; chapter 2; see also Sandeman, 1982).

Nephrops has been the subject of three major neurobiological studies to date. These have investigated the neuromuscular basis of swimmeret equilibrium reflexes (Miyan, 1982; Miyan and Neil, 1986; Neil and Miyan, 1986), the role of leg proprioceptors in the control of antennal reflexes (Priest, 1983), and the control of escape behaviour (Newland, 1985). A considerable body of data has been built up on this species, on its various sensory and motor systems and on its equilibrium responses.

Two sensory systems have been examined in these studies. The CB chordotonal organ at the base of each walking leg gives rise to a large group of both primary afferents and interneurons which run in the nerve cord. These units, which ascend to the brain, respond to either levation or depression of the legs at intermediate to low frequencies (0.01-0.5Hz). This system, which provides information to the antennae, may also feed onto other reflex systems (Priest, 1983). Similar units have been recorded which descend into the abdomen (Wiersma and Bush, 1963; see chapter 5).

The structure and effects of the statocysts have also been studied. They were found to provide powerful inputs to the swimmerets (Neil and Miyan, 1986) and also the uropods (Newland, 1985). However, these findings raised the question of how the statocyst input affected these systems, and also where and how it interacted with other sensory modalities. Thus the study of descending statocyst pathways served as a starting point for the experiments in this thesis (chapter 2). Clear differences emerged between *Nephrops* and the published work on crayfish in terms of the mode of transmission and effects of statocyst information.

The effects of statocyst input on various motor systems was examined. Particular attention was paid to responses in the pitch plane which, unitl now, have received comparatively little attention. This proved to be a particularly fruitful avenue of study showing that statocyst input has specific and important effects on legs (chapter 3) and abdominal posture (chapters 4&6), as well as previously unreported effects on the swimmerets (chapter 4). These findings allowed further study of interactions between descending statocyst input and sensory inputs from the legs (chapter 5).

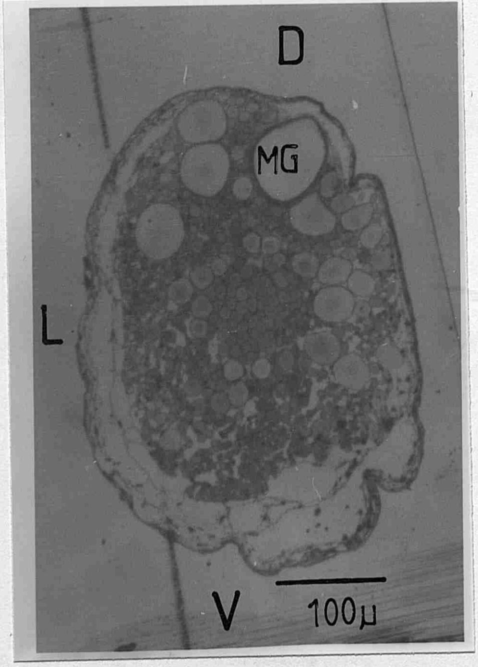
Fig. 1

Cross-sections of the nervous system of *Nephrops norvegicus*.

A. Circumoesophageal level.

B. Thoracic level.

C. Abdominal level.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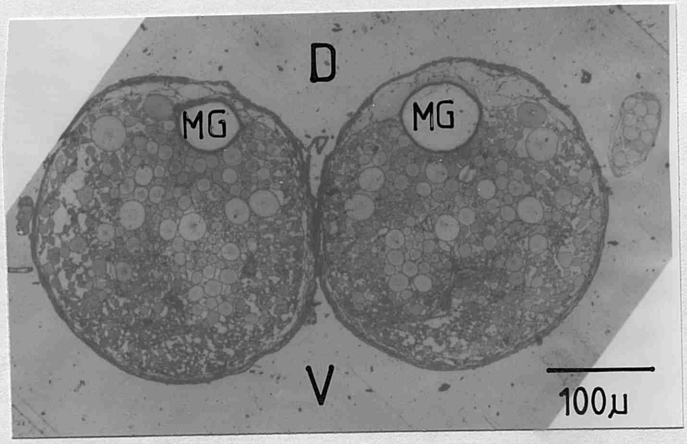
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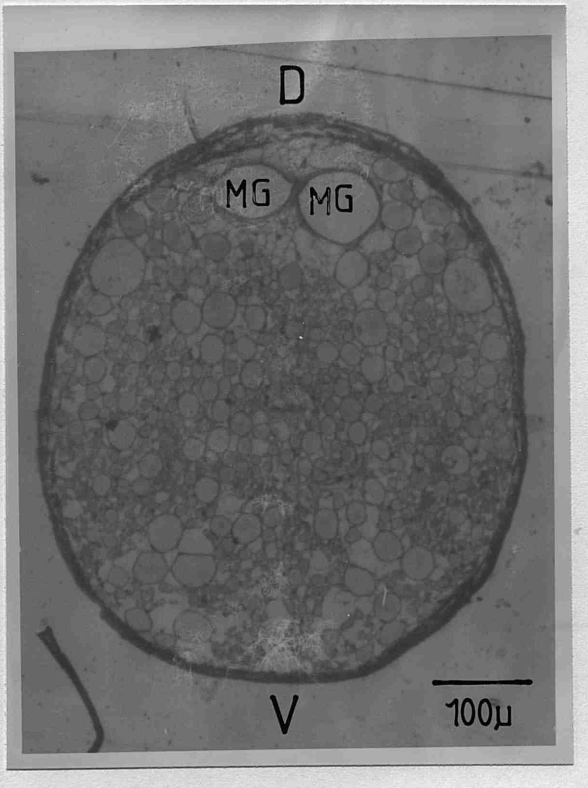
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Chapter 2:

TRANSMISSION OF TILT INFORMATION.

A. Introduction.

Many of the most important sensory systems in macrurous decapod crustaceans are at the anterior end of the body, while most of the motor systems they influence lie caudally. Thus there is a need for the transmission of information obtained by these sensory systems to the relevant integrating or motor centres.

The statocysts are one such cephalic sensory system. They lie in the bases of one of the cephalic appendages, the antennules and have been shown to provide information for various equilibrium reactions involving abdominal motor systems such as the swimmerets (Neil and Miyan, 1986) and uropods (Yoshino et al, 1980; Newland, 1985). Thus the statocyst system provides opportunities for studying the means by which information is transmitted to motor systems.

Other problems can also be addressed by studying the statocyst system. The reflexes in which they participate involve other sensory systems. In the case of swimmeret reflexes, substrate contact alters the effect of statocyst information on the swimmeret system (chapter 5). The question arises as to where the two types of information, one from the legs and one from the statocysts, interact. As was pointed out in the Introduction, various sites or means of interaction are possible. By investigating the statocyst pathway, characterising its operation and then investigating other inputs it has been possible to reject one of these possibilities and to suggest the likely strategy adopted by *Nephrops* for bringing together various sensory modalities.

Therefore a search of the nervous system of *Nephrops* was undertaken in an attempt to locate neurons responsible for transmitting the statocyst information to the motor systems such as the swimmerets, abdomen and uropods. Both circumoesophageal and abdominal connectives were investigated. Once found, other inputs to these interneurons were investigated.

B. Materials and methods.

a.Statocyst stimulation.

The statocysts were stimulated physiologically by tilting in various directions and at various frequencies. Two preparations were used to allow tilting while recording from connectives. One of these also offered the advantage of also allowing controlled stimulation of the legs as well as a stable recording site for intracellular penetrations.

(i) Whole animal tilts.

The preparation which was used most frequently, and indeed yielded the bulk of the results reported here, allowed tilting of the whole animal while recording from the ventral nerve cord, and particularly the circumoesophageal connectives. This was achieved by clamping the animal in an inverted position into a tilting frame mounted on an annulus. By rotating the frame relative to the annulus the axis of rotation about which the animal was tilted could be altered (Fig 1). Tilt about the longitudinal axis (ie roll, Fig. 1B), the transverse axis (ie pitch, Fig. 1C) and various intermediate axes in between were used to provide a detailed picture of the response of statocyst interneurons. These various axes of tilt were expressed in terms of the angle adopted by the animal in the yaw plane. Thus tilt about the longitudinal axis was denoted as tilt at 0° . Movement of 90° in a clockwise direction meant tilt about the tranverse axis.

Once fixed at a particular position the annulus on which the frame rotated was oscillated. The parameters of this oscillation were set using a microprocessor-based function generator (Strathkelvin Instruments), which allowed either sinusoidal or ramp tilts to be administered over a wide range of frequencies and angular velocities. The amplitude could also be controlled. The maximum amplitude available was 20°but the amplitudes most commonly used were between 10° and 15° . This range of amplitude was adequate for eliciting interneuron responses to tilt. The output from the function generator was fed to a galvanometric movement device which was connected by drive rods to the annulus and provided a feedback signal of position. Thus the actual movement rather than the intended movement of the apparatus could be monitored.

This apparatus was placed in a tank which contained cooled circulating saline.

(ii). Head tilt.

To allow investigation of interactions between statocysts and sensory input from the legs, and also to facilitate stable intracellular penetrations of the abdominal connectives, a preparation which allowed tilt of the head and therefore the statocysts, while keeping the rest of the thorax and the abdomen firmly anchored, was used (Miyan, 1982). This entailed cutting round the cuticle approximately 0.5cm posterior to the eyes and detaching this anterior portion of the cephalothorax (or the "head") from the rest of the cephalothorax. The head could then be clamped into the tilting apparatus and tilted in roll, independently of the rest of the body. As well as stimulating the statocysts, leg proprioceptors could also be stimulated. To accomplish this the legs of one side were autotomised and the joints of the legs on the intact side were immobilised such that movement was only possible at the CB and TC joints. Each leg was then glued to a small piece of rigid plastic tubing* which was slipped over a bar. This allowed depression and levation of all or some of the legs on the side being stimulated while preventing movement at the TC joint.

The head tilt and the movement of the legs were driven by separate galvanometric devices, which were controlled by the ramp/sine generator described above. One of the outputs could be inverted with respect to the other, allowing in-phase and anti-phase stimulation of the two sensory systems.

b. Investigation of other inputs to responding units.

Tilting of the whole animal was the most effective method for recording statocyst interneurons. In this preparation other inputs to these interneurons were also investigated. In several cases the ventral surface of the antennules was scratched or tapped to ascertain the response of a particular unit to vibration as opposed to tilt. The antennules were also deflected to ensure that the responses recorded were not generated by movements of the antennules.

To investigate the effects of leg inputs, legs were moved either singly or in various combinations about the CB joint. An attempt was made to discriminate between this stimulus and the tactile stimulus produced by holding the legs.

c. Recording techniques.

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* At point 3. CH5 FIG 1A

Most of the successful interneuron recordings were made in the circumoesophageal connectives, slightly anterior to the oesophageal commissure. The connectives could be reached by either a ventral or a dorsal route.

The ventral dissection was used in most cases. The advantage of this dissection was that it left the main artery to the brain intact. Thus the onset of anoxia in the brain was delayed. The dissection entailed the removal of the mouthparts, oesophagus and various other tissues to ensure clear and unrestricted access to the connectives. A black metal platform coated with "Sylgard" was placed under the connective which was to be used for recording. This improved visibility and aided desheathing. Most of the recordings were made from the desheathed surface of the connective. It could also be split if necessary and pinned out. Despite the seemingly extreme nature of the dissection, in most cases the preparation was viable for several hours provided the temperature was maintained around 9°C.

Extracellular recordings were made using suction electrodes. Tips were hand pulled in a bunsen flame from "Portex"* tubing. Tips down to 50um were used to obtain recordings of single or few units. The electrodes were held in micromanipulators which were mounted on the tilting frame.

Signals from the suction electrodes were amplified, displayed on an oscilloscope and stored along with the output of the movement monitor on a FM tape recorder.

It also proved possible to obtain intracellular recordings from the abdominal connectives with the head tilt preparation. Electrodes were pulled from 1.5mm OD glass capillaries (Clark Electromedical). These were filled with 3M KCl and used the same day. The electrodes were mounted in a 1D Narishige hydraulic

* Polythene ID 0.86 × OD 1.27.

manipulator which itself was attached to a coarse manipulator operating in three dimensions. This allowed both coarse and fine contol of the microelectrode. The recorded signal was fed into a Neurolog DC preamplifier. The amplified signal was displayed and stored as previously described for extracellular recordings.

d.Analysis.

Data was analysed using a laboratory microcomputer system. Spikes were fed via an interface to the computer. They were counted automatically and time histograms constructed. These were stored on disc. A second programme analysed the data. Where the stimulus was sinusoidal, circular statistics were employed to analyse phase histograms constructed from the data. Various parameters were calculated such as circular mean, standard deviation and magnitude of the mean vector (Batschelet, 1981) and "X" (see Priest, 1983). This allowed detailed comparisons to be made of the responses of the units recorded.

Ramp-and-hold stimuli allowed examination of different components of the responses. Tonic levels of firing during the plateau segments of the stimuli were investigated statistically by calculating mean bin heights and comparing these using a "D" test.

Impulse frequency was measured using an instantaneous ratemeter (Neurolog NL250). The output of the meter was summed over several cycles to show systematic variations of the firing frequency of a unit during different parts of the stimulus.

C.Results.

1.The preparations.

As outlined above, both animal preparations used in the study of interneuron responses were inverted. The various equilibrium responses that the statocysts control, particularly the swimmerets and uropods, have been shown to operate in a consistent manner at inverted positions. This has been established at both a behavioural level (Newland, 1985; see also chapter 3) and for the swimmerets at the neuromuscular level (Miyan, 1982).

Most of the identifiable responses to tilt were recorded from the circumoeophageal connectives. It proved difficult to record statocyst interneuron responses in the abdominal cord. Reasons for this are discussed below.

Attempts to record intracellularly from statocyst interneurons in the abdominal cord were unsuccessful. However stable penetrations of the neurons in the abdominal connectives were achieved, suggesting that this preparation could be used for intracellular recording of more readily located interneurons.

Fig. 2 shows two types of activity recorded from the connectives, slightly anterior to the third abdominal ganglion. The unit in Fig. 2a fired on penetration at a relatively high rate. This declined over several minutes to a lower tonic rate of firing. Other units did not fire on penetration.

Fig. 2b is an example of sub-threshold activity recorded from a similar electrode position in a different preparation. The resting membrane potential in this case was approximately -65mV.

2. Types of interneuron.

The various types of interneuron encountered in the circumoesophageal connectives will be described in the following sections. Interneurons which were recorded in the abdominal connectives will be dealt with later.

Various types of statocyst interneuron were identified on the basis of their responses to different types of tilt. The response of a particular interneuron to tilt in different planes was tested over several cycles of stimulation at fixed amplitude and frequency. None of the interneurons tested responded equally well when tilted in different planes; each showed a preferred plane of tilt. Three different types of interneuron were found. Two of these responded to tilt in the pitch plane. These could be distinguished from each other as one responded to head-up pitch while the other responded to head-down pitch. These interneurons showed very little response to roll. On several occasions they were recorded simultaneously (see Fig 11.). The third type of interneuron responded to tilt in the roll plane (Fig. 9).

The tonic level of firing observed in these interneurons varied from experiment to experiment, and also in any particular interneuron, frequently during the course of an experiment. As the frequency of firing increased the modulation due to tilt tended to decrease. This was taken to be a sign of fatigue or damage and a unit which showed such activity was left to recover for a short time before being tested again. If no improvement could be seen its responses were disregarded.

In some cases, particularly after a prolonged series of tilts, the response of a unit declined and even disappeared. In such a case the preparation was also left to recover for a short time after which the response sometimes reappeared. The response could also be restored on some occasions by giving a general mechanosensory stimulus such as scraping the thorax or moving the legs.

2(i). Nature of response.

The pitch interneurons responded in a phaso-tonic manner to ramp stimuli ie an interneuron fired at a high rate during the movement phase and this rate declined to a resting level once the new position had been reached. The unit shown in Fig. 3a clearly fires at a higher rate during the ramp than during the hold. Fig. 4a. shows the plot of instantaneous frequency against time for this data. The tonic component of the response seemed more prone to habituation than the phasic component. Problems such as this were avoided by tilting over a limited number of cycles (ten cycles unless otherwise stated).

To investigate these responses further various tests were performed. To investigate effects of alteration in absolute position, the set point about which the animal was tilted was altered. Fig. 3 shows the result of such a test. This is a unit which responds to head-up tilt, and in all three traces the two components of the response can be clearly seen. Positive and negative angles denote positions above and below horizontal respectively. Therefore in a. the animal is held head-down on both plateaus. There is a very little effect on either the tonic level of activity or the phasic response on the ramp. The plots of instantaneous frequency (Fig. 4) suggest slight increase in the firing level as the test is performed at increasingly head-up angles. The slope of the response during the ramp is similar in all three plots.

Factors which might influence the phasic component of the response were investigated. One of these was the angular velocity of tilt. Tests were carried out over a limited range of velocities. Fig. 5 shows an example of the activity seen at different velocities. These traces also illustrate the finding that on some occasions the tonic element of the response habituated while the phasic response remained clear. The histograms in Fig 6. were constructed by superimposing ten cycles at each angular velocity for the unit depicted in Fig 5. These suggest that the peak frequency decreased with decreasing velocity of tilt.

It should be noted that the amplitudes used in these tests are relatively small (of the order of 10°). It is not surprising that effects of absolute angle of tilt are relatively small, while other elements of the response, such as the phasic response during the movement are quite clear.

The frequency response of these units was also investigated using sinusoidal tilts. Use of this type of stimulus allowed the data obtained to be analysed using circular statistics. Typical results are shown in Figs. 7 and 8. A small number of test frequencies were used ranging from 0.14 Hz to 0.63Hz. Over this limited range the effects observed were small. The magnitude of the mean vector remained relatively constant, while "X" decreased with increasing frequency. An empirical investigation into this use of these two parameters by Priest (1983) showed that with the magnitude of the mean vector greater than 0.5, a decrease in "X" signified a decrease in the strength of the response. Thus these two taken together suggest a slightly stronger response in these interneurons to tilts at the lower frequencies. This is similar to what has been found for other populations of sensory interneurons in *Nephrops* (Priest, 1983).

2(ii).Response at different angles.

Tilting the animal in either pitch or roll planes allows a picture of typical responses to be built up and various other parameters to be investigated. It is however limiting in that the statocyst must, under natural conditions, be responsive to tilt in combinations of these planes. To build up a complete picture of the operation of the interneurons, these intermediate angles must be investigated. The bulk of the experiments performed on the statocyst interneurons were of this type. Once a unit which responded to tilt was located, its response at other angles was tested. Approximately twenty interneurons were held long enough to be tested at a range of angles. Eighty tests performed on these interneurons yielded clear information on the responses of units about particular angles.

Two examples are shown; the unit pictured in Fig. 9 responded best to roll, whereas the two units shown in Fig 11 responded best to pitch. However it is clear from these figures that these interneurons are also firing at intermediate angles. The unit tested in Fig. 9 (see also the instantaneous frequency plots in Fig. 10), responded to tilt in the roll plane, but also to tilt 45° either side of roll. The frequency with which it fired was very similar at all three test angles (Fig. 10).

As the two units shown in Fig. 11 were recorded simultaneously they clearly lie close together in the connective. They were tested round 360° , moving from 270° (pitch starting head-down) in a clockwise direction (see Fig. 1). This meant the response at 270° was tested twice, once at the beginning and once at the end of the series. While the best responses occur at 270° for both units, they carry tilt information over a wide range of angles.

Sets of phase histograms for these interneuron responses are shown for unit 1. in Fig. 12 and for unit 2. in Fig. 13. The phase of the responses switches by 0.5 units around 0° . They maintain a phase difference relative to each other of approximately 0.5 phase units at all angles.

These two units were recorded in several preparations, although not always simultaneously. On all occasions they responded in an almost identical manner. The phase position at 270° serves to illustrate this. The circular mean of unit 1 in Fig. 12 is 0.89+/-0.02. This particular unit was tested on eight occasions in different preparations. The circular mean was similar on all these occasions only varying from 0.87+/-0.01 to 0.92+/-0.04. The other unit also showed little variation from preparation to preparation.

Data collected from these two interneurons in a number of preparations was pooled to give a more complete picture of their responses to tilt. The phase position of the circular mean was plotted against the angle of the plane of tilt in which the animal was tilted (see Fig. 1). In any one experiment it was difficult to test a particular unit at all angles. Over a number of experiments however, a complete composite picture was obtained.

Fig. 14 shows the response of the head-up interneuron (see Fig. 13) at the various angles. This represents one experiment, with a large number of tests being done over the first 180° . The phase shift in the response occurs at 10° . This interneuron fired when the body was tilted in the roll plane. Although the modulation was limited, it was sufficiently systematic to allow a circular mean to be calculated. Fig. 15b also represents the response of this interneuron, although this plot is constructed from two experiments. Again, there were responses to roll (0° and 180°); the phase shifts in the response occur after these points.

Fig. 15a. shows the plot of the response of the head-down interneuron (see Fig. 12); this was constructed from data obtained in three experiments. The response is clearly inverted relative to the response in the other interneuron (Fig. 15b). This interneuron was either completely tonic when tilted in the roll plane, or as in Fig. 11, did not fire at all.

Tilt around any axis therefore causes a combination of these interneurons to fire eg tilt in a plane 45° out of pitch will cause a pitch interneuron to fire (depending on the direction of tilt) as well as perhaps at least one roll interneuron (eg Fig. 9). However for any single pitch interneuron the strongest responses are seen in pure pitch.

2(iii). Abdominal units.

The interneurons discussed above were all recorded in the circumoesophageal connectives. However, two units of interest were recorded in the abdominal connectives while using the head tilt preparation outlined above.

Fig. 16 shows the response of two units to head tilt (ie roll). Both of these units fired tonically. However there were significant differences between them. The unit shown in Fig. 16A was switched on by tilt to the right and off by tilt to the left. Apart from this it showed no modulation. It was therefore unlike any of the units recorded at the circumoesophageal level. The unit in Fig. 16B was tonically active. Its frequency of firing was increased by tilt to the left. It is very similar to the unit shown in Fig. 9.

3. Investigation of other inputs.

Various other inputs to the statocyst interneurons were investigated. The most important of these was leg input. After an interneuron had been tested and its response to tilt defined, legs were manually levated and depressed in various combinations. None of the interneurons which responded to tilt responded to this leg stimulation.

In other experiments units were found which did respond to leg stimulation (Figs. 17&18). In Fig. 17 the first burst is due to contact with the dactyl. Subsequent depression of the leg produces modulation of the activity. The units shown in Fig 18. responded best to depression of all the legs on the left side. Depression of the legs on the right side while producing some spikes, is not as effective. However, tilting these preparations produced very little response. So while leg interneurons exist in very close proximity to the statocyst interneurons the two types are distinct in terms of their input modalities.

Scratching the leg bases had little or no effect on statocyst interneurons, although on occasion it did serve to alter the tonic level of firing. This was assumed to be a general effect.

One other type of unit was demonstrated and is of interest in the present context. The statocysts sit within the base of the antennules which can move in a dorso-ventral direction. Although in *Nephrops* such movement is slight, it is important in other species (Neil, 1982). In preparations where statocyst interneurons had been recorded, the antennules were deflected by pulling ventrally. This produced little or no response in the statocyst interneurons but did clearly stimulate other neurons, possibly from proprioceptors at the basal joint of the antennules. One such unit is shown in Fig 19. This received input from both left and right antennules.

The statocyst interneurons showed a poor response to vibration either of the whole animal or of the antennules. This did however occasionally recruit large phasic units which fired with only one or two spikes.

4.Statocyst structure.

Although not the main focus of attention in this study, the structure of the statocyst itself was briefly examined in an attempt to further understand the operation of the interneurons. Also for comparison the statocysts of another of the lobsters (*Homarus americanus*) and a crayfish (*Pacifastacus leniusculus*) were looked at. Examples of the statocyst of these species are shown in Fig. 20 after removal of lith material.

The statocyst itself is a chitinous sac containing two main elements, a crescent of sensory hairs and a statolith. The hairs form a number of rows and tend to be slightly bent towards the floor of the sac. The statolith sits on top of the hairs. Tilt causes the lith to move in a characteristic manner. As the sensory hairs are morphologically and functionally polarised towards the centre of the crescent (Stein, 1975; Takahata and Hisada, 1979), particular hairs detect tilt in particular planes. Thus tilt is detected in terms of the pattern of excitation of hairs around the crescent.

While the statocysts of all three species are constructed on the same basic plan, clear differences emerge. The major division seems to be between the lobsters and the crayfish. The *Pacifastacus* statocyst is quite similar to the statocyst in *Procambarus* which has received most of the attention in the literature to date.

Of the several rows of hairs in the *Nephrops* statocyst only the inner two rows are in contact with the lith. The outer rows are particularly well developed in *Nephrops*. In *Homarus*, while the two inner rows can be seen, outside of these the hairs form small clumps with three to five hairs per clump.

The overall shape of the sensory crescent in the two lobster species is "rectangular" when compared with the more rounded crescent in *Pacifastacus*, and indeed *Procambarus* (Takahata, 1980). In the two lobster statocysts there are a large number of sensory hairs which could be assigned to either pitch or roll categories.

Pacifastacus also lacks the triangular patch of hairs at the anterior end of the crescent. However as most of the patch is not in contact with the lith this probably has no bearing on the operation of the different interneurons in these species.

D. Discussion.

Several types of statocyst interneuron have been identified in the course of these experiments. Of these the pitch interneurons have been extensively studied. The interneurons differ chiefly in terms of the planes of tilt in which they respond, rather than the manner in which they respond to tilt.

Comparison with the responses of statocyst interneurons in other species is now possible. The statocyst interneurons of *Procambarus clarkii* have received a good deal of attention (eg. Takahata and Hisada, 1982a,b). While there are similarities between the interneurons of *Procambarus clarkii* and those described in this chapter for *Nephrops*, there are also differences. One source of difficulty in comparing the two species is the difference in the methods used to study them.

The tilts used by Takahata and Hisada (1982) were of large amplitude (typically 180°) and limited to only two planes of tilt, pure roll and pure pitch. Data was presented in terms of single cycles of stimulation. They found the phasic component of the response fluctuated and this was not investigated. They did show clearly that the tonic component coded for magnitude of tilt. Interestingly they found that in one interneuron the phasic component was more marked in pitch than in roll. They related their various findings to the properties of the sensory hairs in the statocyst.

How similar are the statocyst interneurons of *Procambarus clarkii* and *Nephrops* ? There is clearly a phaso-tonic response to tilt in the pitch interneurons of both species. However, those units which responded to roll in *Nephrops* responded in a tonic manner, ie there was no phasic peak at or during the movement to which they responded (see Figs. 9&10). It is likely, although it was not tested here, that the interneurons in *Nephrops* would respond in a similar manner to large amplitude tilts. Tilt information is certainly available to various motor systems outside the angular ranges tested here (Newland, 1985; see chapter 3). For example, asymmetric responses in the roll plane are maintained through to inverted positions. The response only switches to the other side once this position is passed.

There is also the possibility that the interneurons reported here are only a sample of a larger population of interneurons responding in essentially the same manner, outside of the range tested here. The apparatus used did not allow many of these aspects to be investigated. However within the constraints imposed by the method employed important new aspects of the response of those units which were found were examined ie the reponse of units to intermediate angles of tilt.

Difficulty in locating statocyst interneurons at the level of the abdominal connectives could be due to several factors. Cord splitting was not utilised in these experiments as it greatly reduced the survival times of the preparation. It may be that the statocyst units are smaller in the abdominal connectives and therefore their extracellular spikes are more easily obscured by other activity. Indeed in the work on statocyst inteneurons in the crayfish most of the tests were carried out on interneurons recorded in the circumoesophageal connectives (Takahata and Hisada, 1982). Although the same units were located in the abdomen they seemed to be more dispersed.

Of the units recorded in the abdomen which did respond to head tilt, only one responded in a similar manner to units recorded at the circumoesophageal level (the unit shown in Fig. 16b). The response of the other unit, of simply switching on and off, is sufficiently different to suggest that it be regarded with caution, at least for the moment.

Results obtained allow us to build up a picture of how the system operates in a complex three-dimensional context i.e. not limited to only pitch and roll. Takahata and Hisada (1982a) describe the function of their interneurons in terms such as head-up, same side down, the implication being that the interneurons, eg. C1, responded equally well to both these angles of tilt. If this is indeed the case, then what of intermediate angles? An interneuron responding in this fashion has an acceptance angle of at least 90° . However, as the results reported in this chapter show, the interneurons in *Nephrops* respond to tilt 45° either side of the most sensitive angle. If the same holds for the crayfish system the interneurons will respond round a full 180° in the yaw plane. All of the interneurons reported in *Procambarus* have similar wide ranging sensitivity and therefore similar extremely wide acceptance angles.

Contrast this with the interneurons in *Nephrops*. The pitch interneurons clearly responded most strongly to pitch. In the roll plane these interneurons either did not fire, or were almost tonic. Also they responded to either head-up or head-down tilt, never both. In *Procambarus* the two interneurons responding to pitch (C2 and I1) responded to both head-up and head-down tilt.

Many of these differences are almost certainly due to the difference on the structure of the statocysts as discussed above. The crayfish with smaller numbers of sensory hairs and with a more rounded crescent is not able to resolve differences between pitch and roll as clearly as *Nephrops*. Each sensory hair will have a relatively large influence on the interneuron (or interneurons) with which it synapses. Therefore, the input of those hairs whose plane of polarisation is congruent with either pitch or roll will make up only part of the input to a particular interneuron. Other inputs from hairs whose planes of polarisation lie at different angles will influence and even dominate the output of the interneuron. In *Nephrops* with the more "rectangular" crescent, lith hairs may fall into two almost distinct types, those whose plane of polarisation falls in or near the roll plane and those whose plane of polarisation falls in or near the pitch plane. Furthermore, with the greater number of hairs, the influence any single hair may have will be reduced. Hence the importance of input from hairs which have an intermediate polarisation plane will be less than is the case in *Procambarus clarkii*.

Comparison of the statocyst systems of the two species suggests that *Nephrops*, and by implication the other clawed lobsters, possess an advanced tilt detecting system, whereas *Procambarus*, and perhaps the crayfish in general, possess a relatively undeveloped system. In the spiny lobster, *Palinurus* the system is more primitive yet, with an ill-defined sensory crescent, and instead of one lith a collection of separate grains (Neil, 1985). Clearly there is a need for more comparative study of both structure and function of statocysts from a wider range of species.

The statocyst interneurons in *Procambarus* were named on the basis of the main input statocyst. This was not investigated here as the inverted preparation used did not allow easy stimulation of individual statocysts either electrically or by deflection of the antennule. Furthermore attempts to electrically stimulate the sensory nerve by entering the base of the antennule dorsally in an upright preparation were defeated by the extremely short length and small size of the statocyst sensory nerve. Therefore the precise inputs to these interneurons remain to be investigated. Development of a more dissected preparation allowing manipulation of the sensory hairs themselves, and access to the sensory nerve, as well as recording from the circumoesophageal connectives would be a helpul progression. It should be noted however, that in pitch the information coming from either statocyst will be substantially similar, whereas in roll, each statocyst will provide very different information; the side up statocyst will show a marked increase in activity, while the side down statocyst shows a decrease.

Other inputs to the statocyst interneurons in *Nephrops* have also been investigated. Inputs from CB joint receptors effectively allow the nervous system to detect changes in body orientation relative to the substrate. Stimulation of these receptors elicits equilibrium reactions which are similar to and consistent with those seen during tilt. In an extensive study of interneurons carrying this information from the legs, Priest (1983) found only weak, if any, inputs from the statocysts. However the stimulus used was not tilt, but deflection of the antennule. This type of stimulus may not sufficiently stimulate the statocyst to produce activity in the statocyst interneurons.

In this study it is clear that statocyst interneurons receive only very weak if any input from the legs. Furthermore, interneurons which clearly do respond to leg input do not respond to tilt. And tactile inputs from the legs, which will be shown later to have wide ranging effects on the expression of tilt driven reflexes (chapter 5), also have very little effect on the statocyst interneurons.

At this point it is wise to bear in mind that multimodal interneurons in another context, the locust flight system, which deal with orientation, or more specifically course correction, respond to only very specific combinations of inputs (Rowell et all, 1985). This was taken into account in this study by using "sensible" combinations of leg stimulation e.g. in the case of an interneuron responding to pitch, the legs were levated and depressed in combinations that would occur in pitch.

The picture which emerges in *Nephrops* is one in which sensory information is carried down essentially unimodal channels. In *Procambarus* weak leg input to one of the statocyst interneurons was found (Takahata and Hisada, 1982a) but it was variable and the experimental arrangement was such that it could be either prorioceptive or tactile input. Visual stimuli were also investigated and found to have weak effects. The experiments conducted above were on blinded animals and so this could not be looked at in *Nephrops*.

The similarity between the statocyst interneurons and the leg interneurons is not confined to their unimodality. Both populations also show a similar frequency response. Priest (1983) found that the strength of the response of leg interneurons to leg levation and depression decreased at higher frequencies. One motor system that both these sensory systems feed onto is the oculomotor system (Neil, 1982). Both substrate tilt and body tilt cause compensatory eyestalk movements. It has been shown in *Palinurus elephas* that the responses of the eyestalks to substrate tilt decrease at higher frequencies. The same is true of the response to body tilt in crayfish (Fay, 1975). This type of frequency response is clearly manifested at the level of the sensory interneurons in both the legs and the statocysts in *Nephrops*.

The frequency response of the compensatory responses of the eyestalks, and by implication of the the sensory systems that subserve these responses, has been related to the behavioural context in which they take place. Long-bodied macruran Decapods are relatively unstable in roll (Alexander, 1971). In its habitat *Nephrops* may be subject to lateral disturbances of frequencies in the range within which the legs and the statocysts operate (see Neil, 1982).

The other context in which the statocystsplay a major role is in righting reactions, particularly after a bout of escape swimming which takes the animal up into the water column (Newland, 1985). Under these cicumstances the animal is observed to stabilise its position in roll and then swim down to the substrate at a slightly head-down angle. Clearly, accurate information from the statocysts is vital in controlling this process as even small deviations if not corrected could have serious consequences. This is perhaps a situation analagous to that in the locust flight system except that most of the sensory information comes from one system, i.e. the statocysts. It is vital that the resolution offered by the detecting apparatus is not dissipated by an inefficient transmission system. As has been shown, the resolution at the level of the interneurons is clear enough to distinguish between pitch and roll, and certainly in pitch to distinguish between head-up and head-down. The system at all levels is capable of controlling orientation in a complex three-dimensional environment.

Fig. 1

Apparatus used in whole animal tilt experiments (see Methods for detailed description).

A. Animal was held in central frame which could be rotated relative to annulus. Thus the effective plane in which the animal was tilted could be systematically altered.
Angles were assigned as shown. Thus tilt of the animal in position diagrammed, at 270° represents tilt in the pitch plane. Thick arrow represents animal with arrowhead at anterior end. Thin arrow represents axis of tilt for apparatus.

B. Tilt in the roll plane, ie around longitudinal axis (a). (Assigned angle 0/360°)

C. Tilt in the pitch plane, ie around tranverse axis (a). (Assigned angle 270 °).

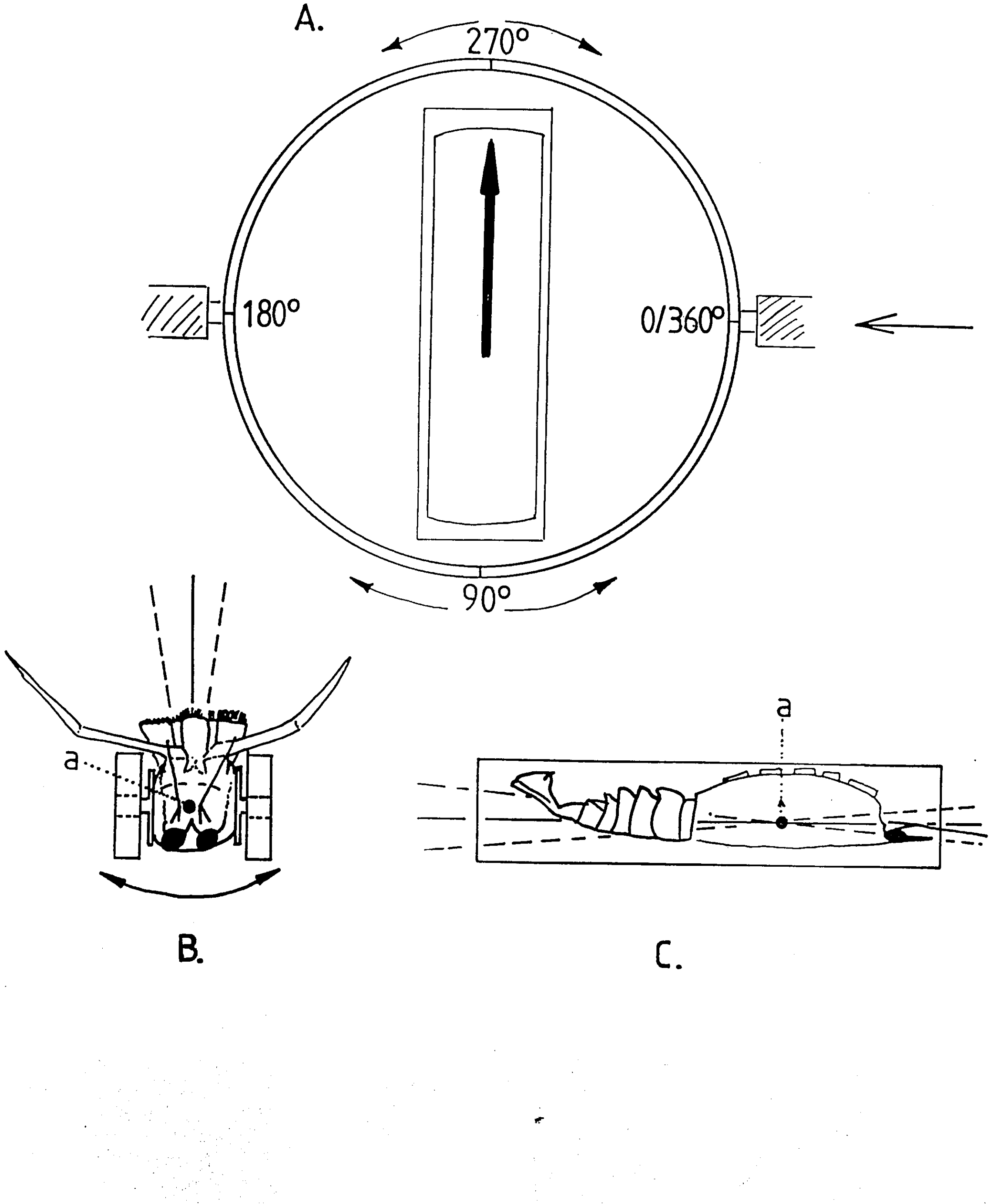

A.
270°
180°
0/360°
90°
a
B.
a
C.

Fig. 2

Examples of intracellular activity recorded in connectives.

a. Spikes fired in response to penetration.

b. Subthreshold activity.

Scale identical for a and b.

Detailed description in text.

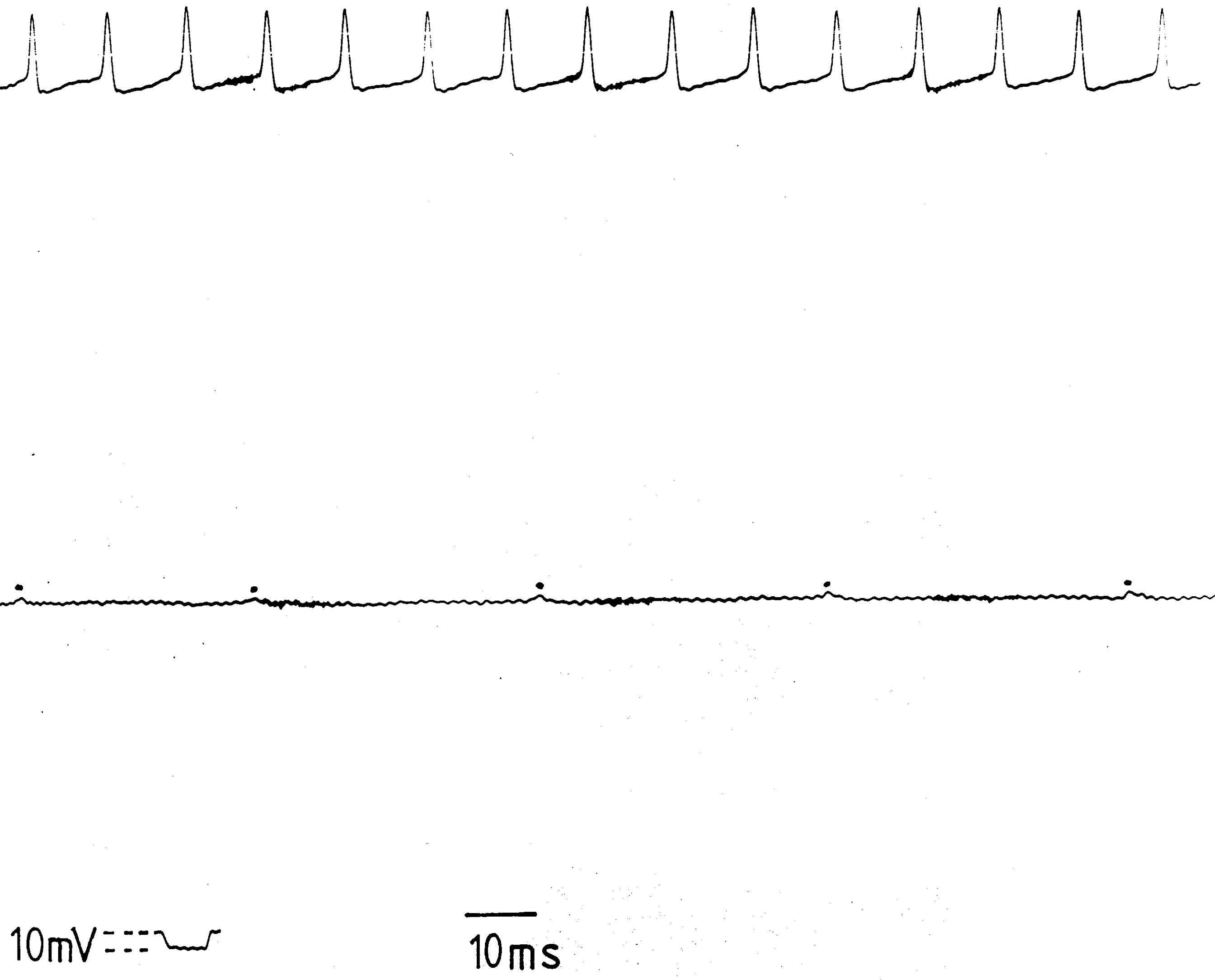

10mV
10ms

Fig. 3

Effect of absolute position on pitch interneuron. Upward deflection in movement trace denotes head-up tilt. Negative angles denote head-down range of tilt.

a. Response of unit to tilt in head-down range.

b. Response of unit to tilt around 0° (ie 5° head-down to 5° head-up.

c. Response of unit to tilt in head-up range.

(Activity recorded en-passant from left circumoesophageal connective).

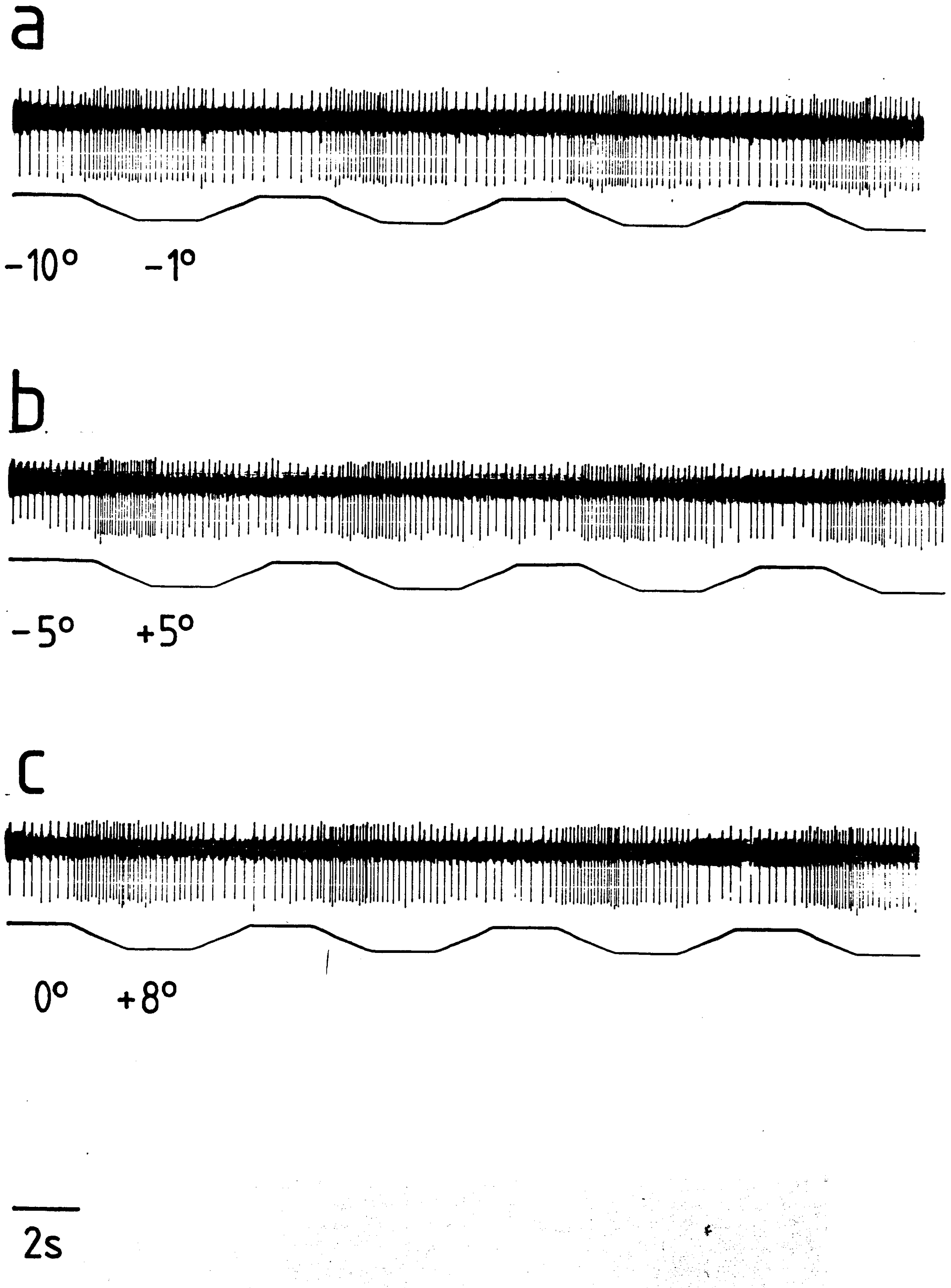

a
-10°
-1°
b
-5°
+5°
c
0°
+8°
2s

Fig. 4

Instantaneous frequency of firing of unit shown in Fig. 3. Data collected over ten cycles.

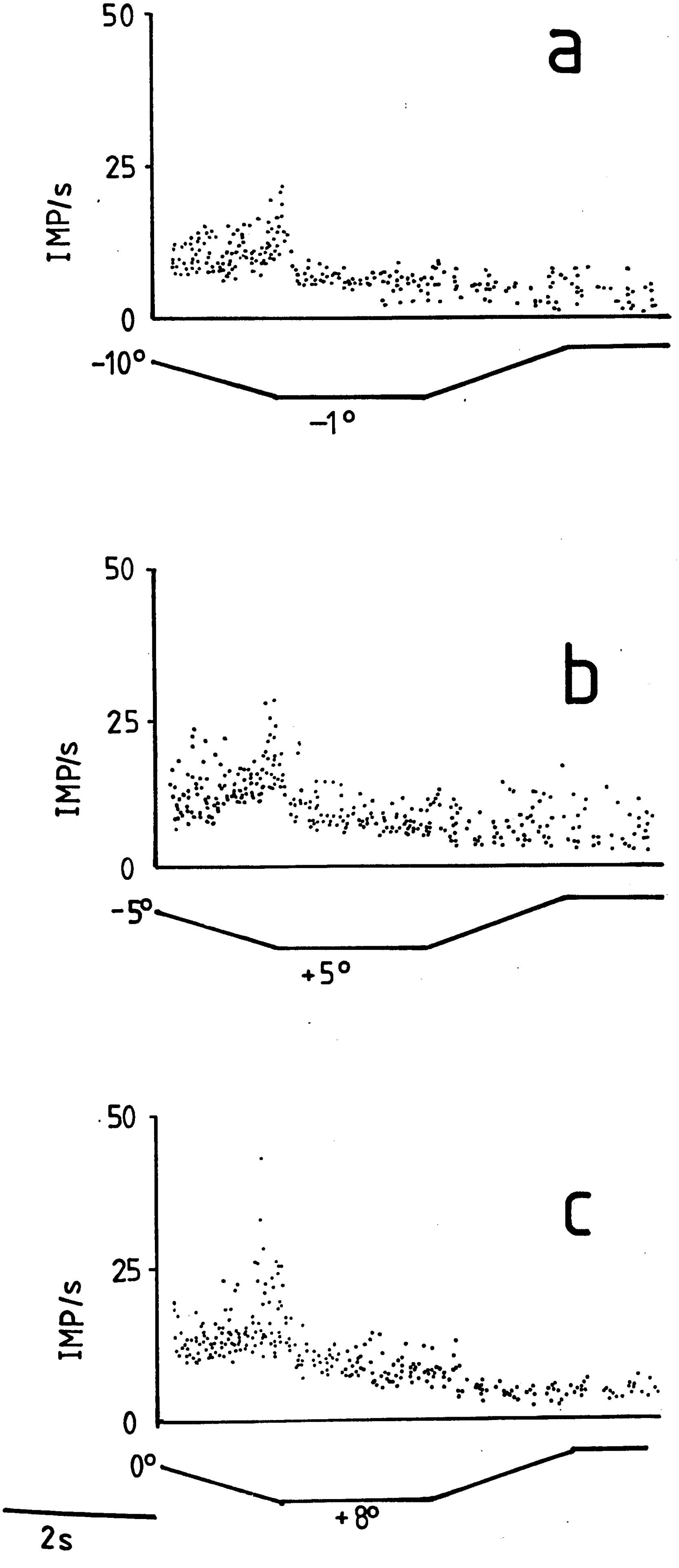

50
a
25
IMP/s
0
-10°
-1°
50
b
25
IMP/s
0
-5°
+5°
50
c
25
IMP/s
0
0°
+8°
2s

Fig. 5

Effect of tilt at different angular velocities. Amplitude approx

10° . Velocities as shown.

Downward deflection denotes head-up tilt.

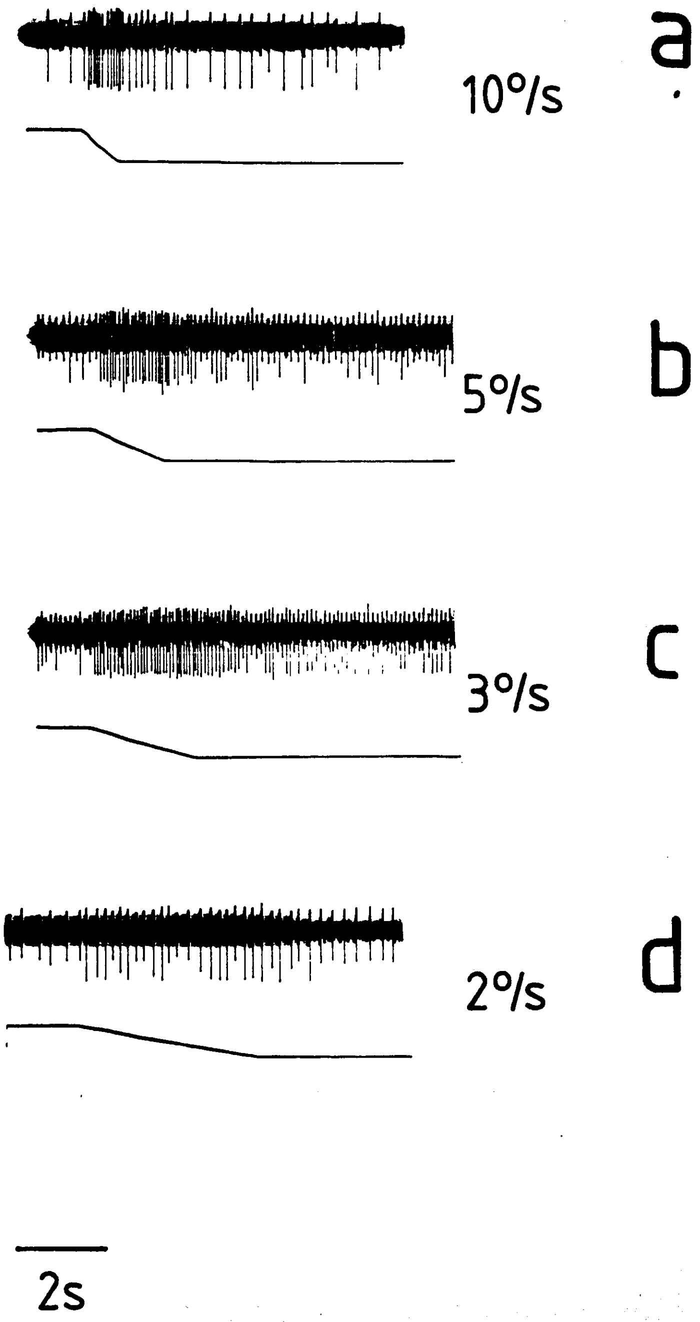

a
10°/s
b
5°/s
c
3°/s
d
2°/s
2s

Fig. 6

Histograms constructed from ten cycles of data in Fig. 5

Velocities as in Fig. 5.

Downward deflection denotes head-up tilt.

Spikes were fed through a window discriminator and counted into 100 histogram bins.

Bin width= $\frac{\text{cycle time}}{100}$

(In this example 0.2s)

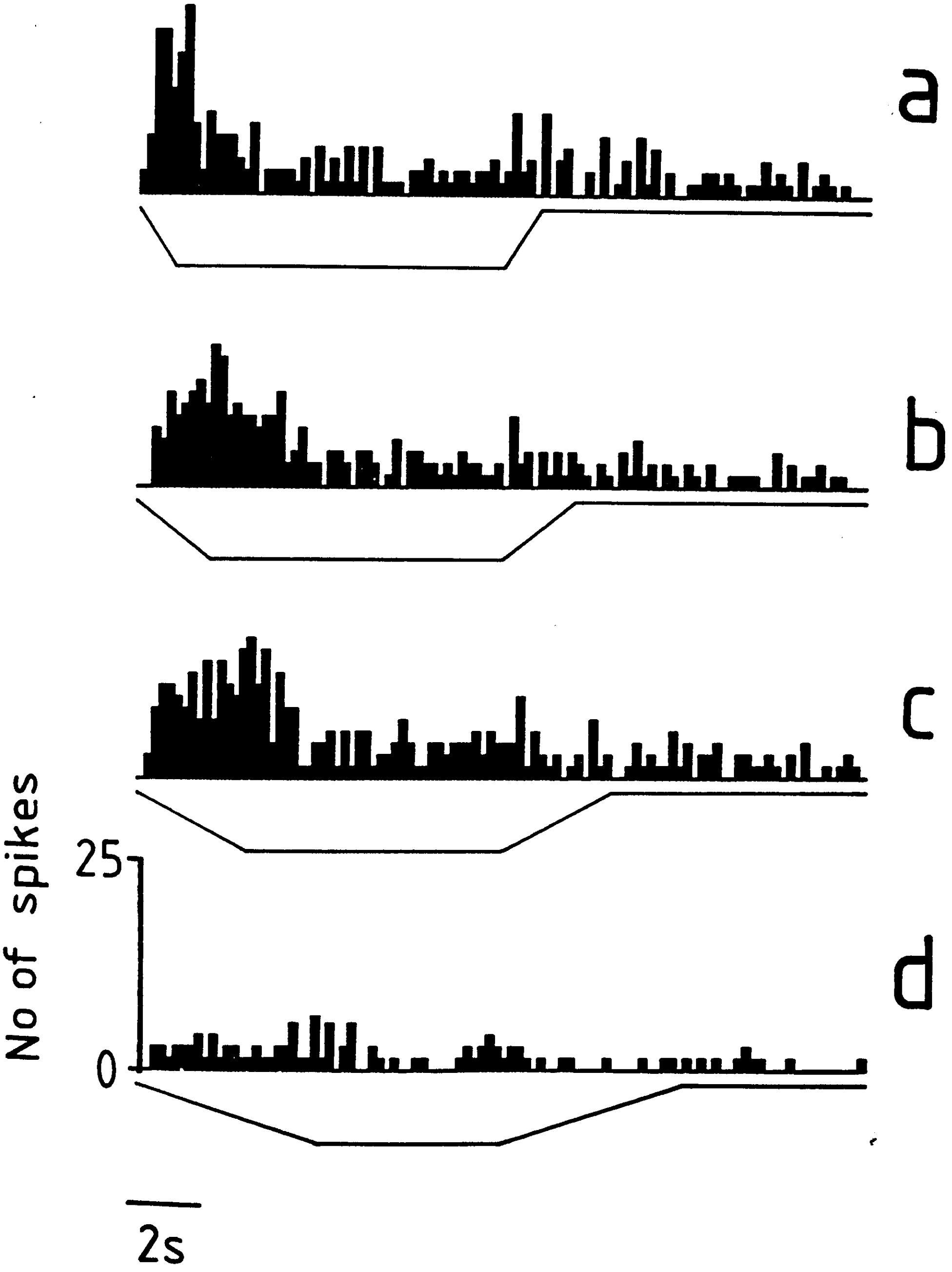

a
b
c
No of spikes
25
0
d
2s

Fig. 7

Example of response of pitch interneuron(s) (which respond to head-up tilt) to tilt at different frequencies.

a. 0.63Hz.

b. 0.50Hz.

c. 0.33Hz.

d. 0.25Hz.

e. 0.14Hz.

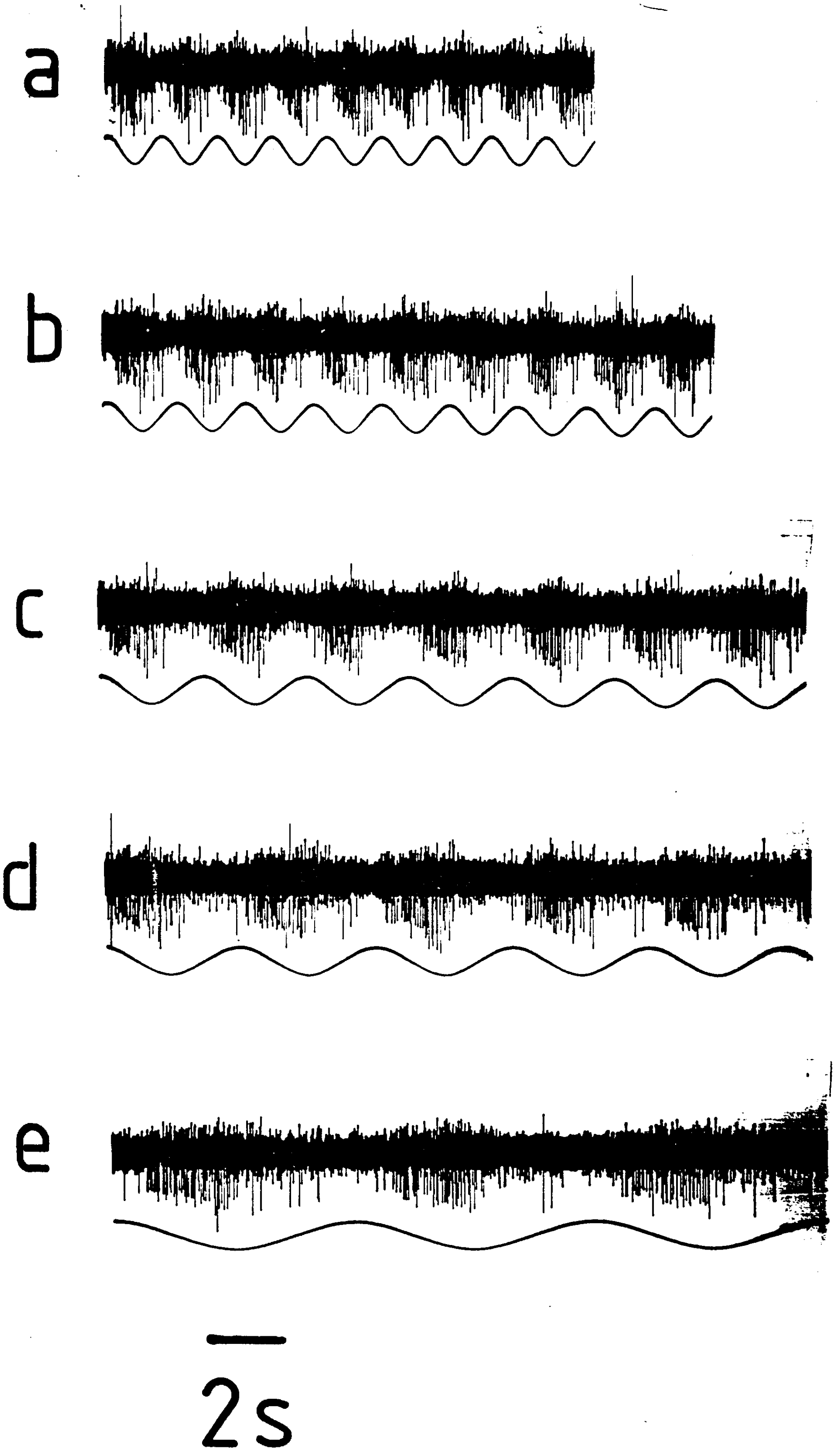

a
b
c
d
e
2s

Fig. 8

Plot of magnitude of mean vector and statistical parameter "X" against frequency for data shown in Fig. 7 (see Methods for details of these parameters).

Circles and dotted line: magnitude of mean vector.

Squares and dashed line: "X"

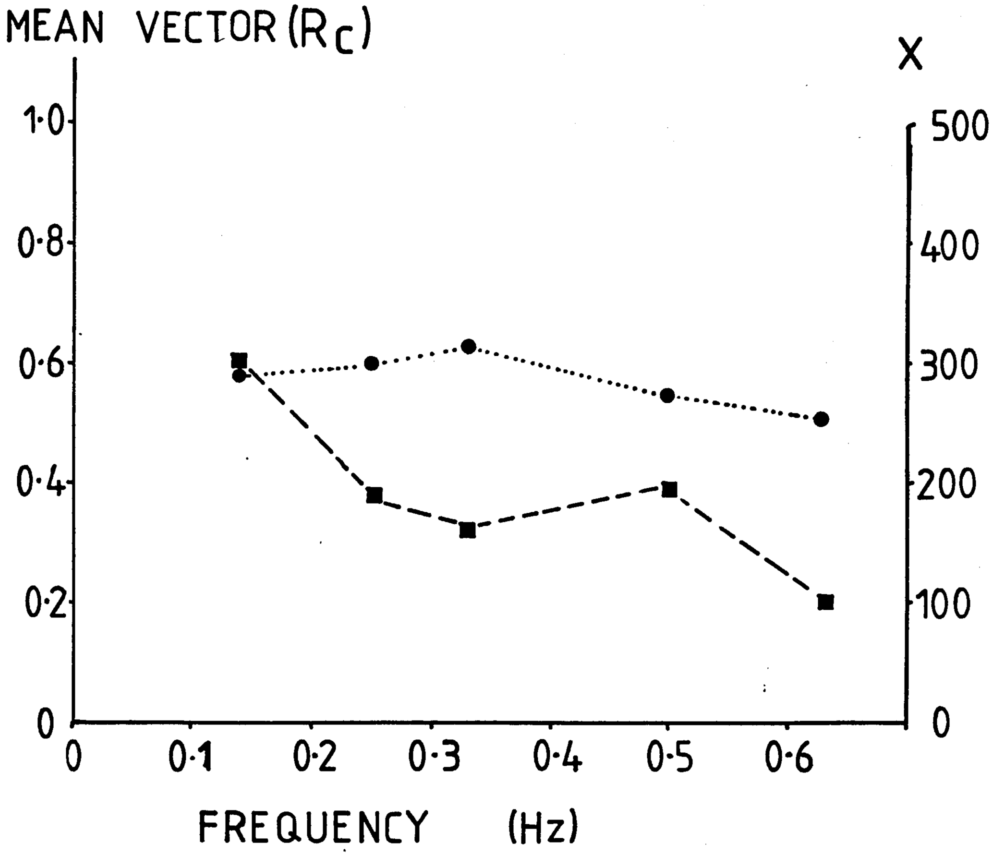

MEAN VECTOR (R_c)
X
1·0
0·8
0·6
0·4
0·2
0
500
400
300
200
100
0
0
0·1
0·2
0·3
0·4
0·5
0·6
FREQUENCY (Hz)

FIG. 9

Response of a roll interneuron recorded in the left connective to tilt in different planes.

a. 315° .

b. 0° .

c. 45° .

(For explanation of angles see Fig. 1 and text.)

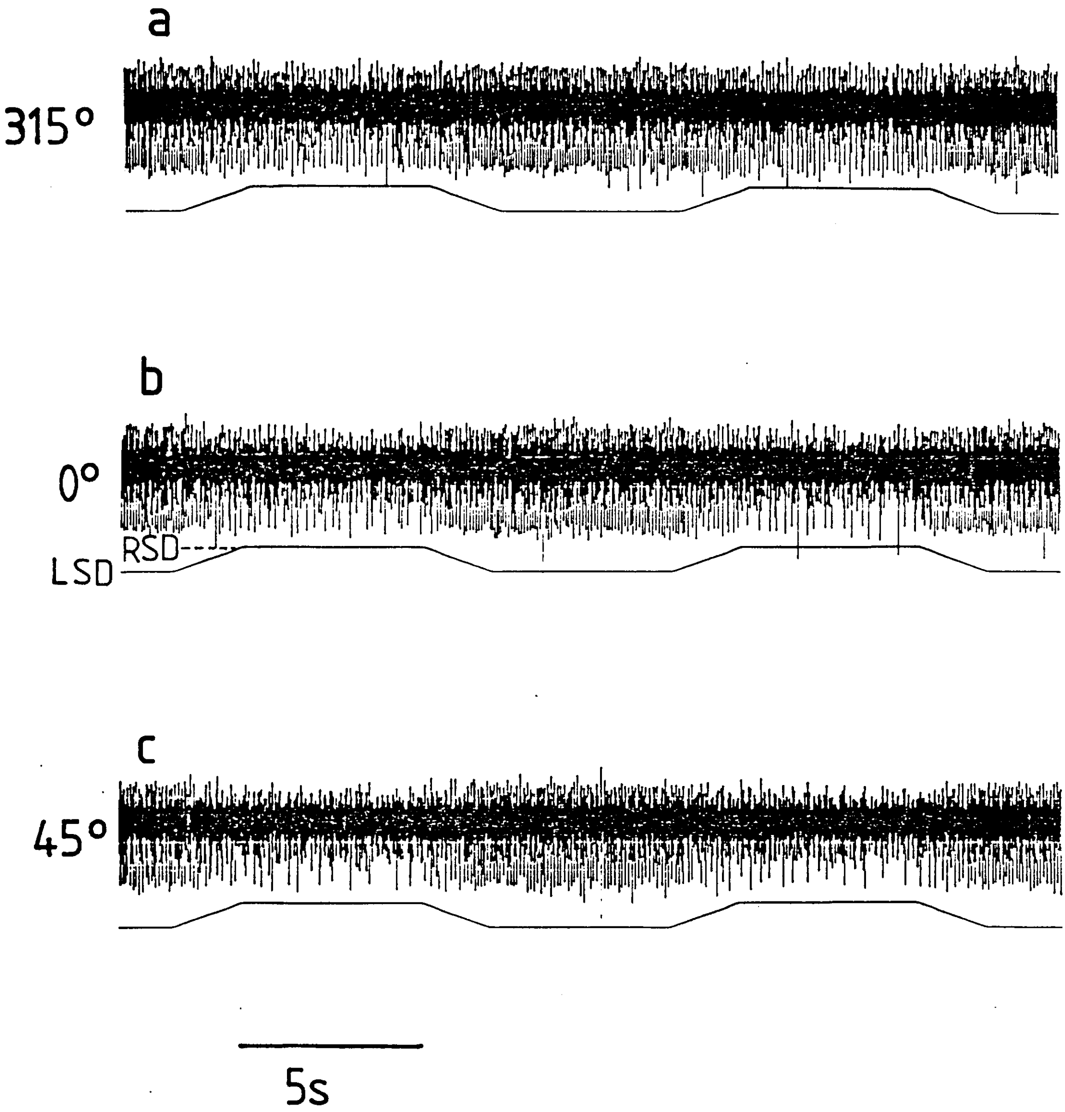

a
315°
b
0°
RSD
LSD
c
45°
5s

Fig. 10

Instantaneous frequency plots for data shown in Fig. 9. Angles in a,b&c as in Fig. 9.

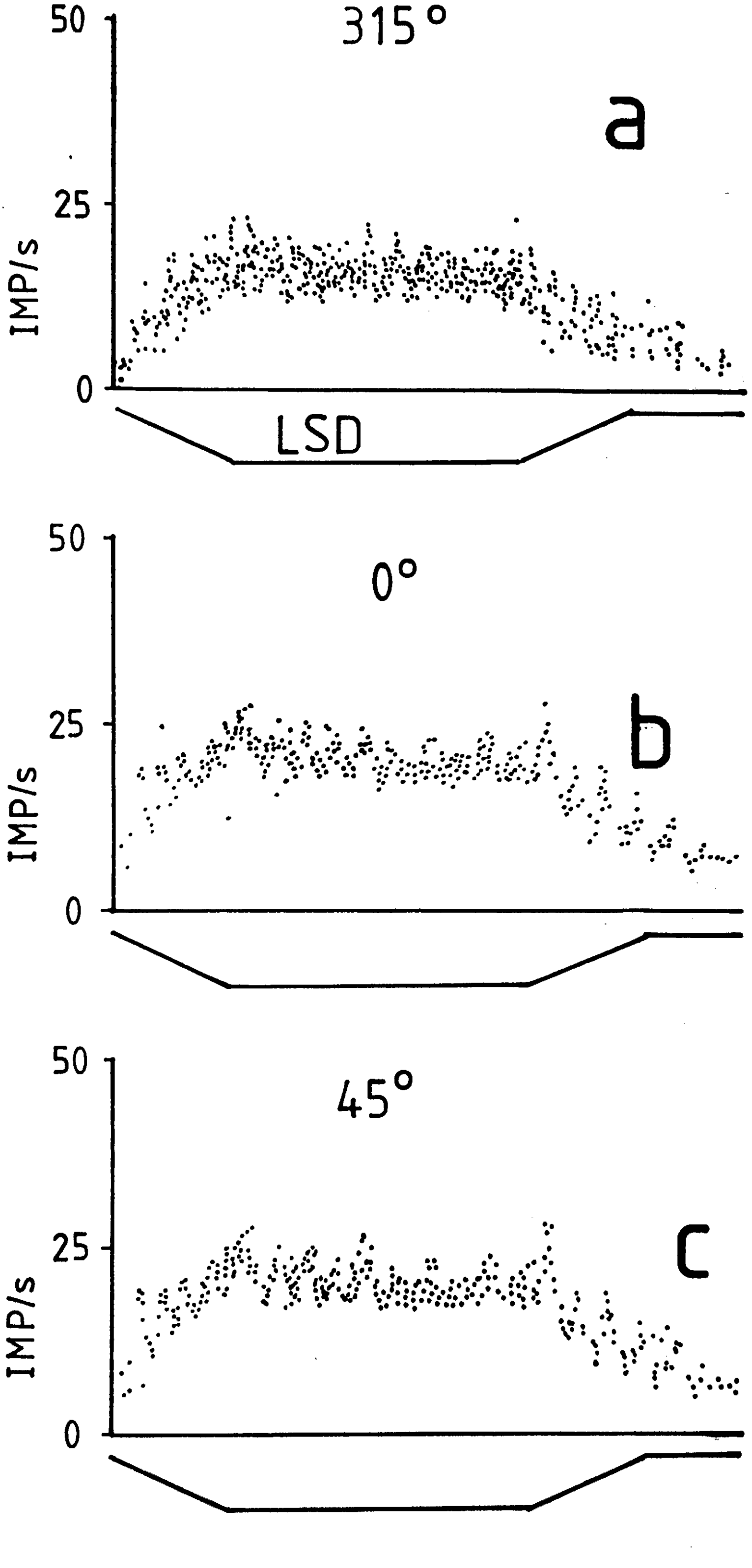

50
315°
a
25
IMP/s
0
LSD
50
0°
b
25
IMP/s
0
50
45°
c
25
IMP/s
0
2s

Fig. 11

Two units responding to tilt in the pitch plane tested in other planes. Unit 1 (large spikes) responds to head-down tilt, unit 2 (small spikes) responds to head-up tilt. Upward deflection on movement trace in a. denotes head-down tilt.

Preparation tested at following angles :

a. 270°.

b. 315°.

c. 0°.

d. 45°.

e. 90°.

Alteration in spike size is an artefact produced when apparatus is rotated to each angle. Same units are firing throughout.

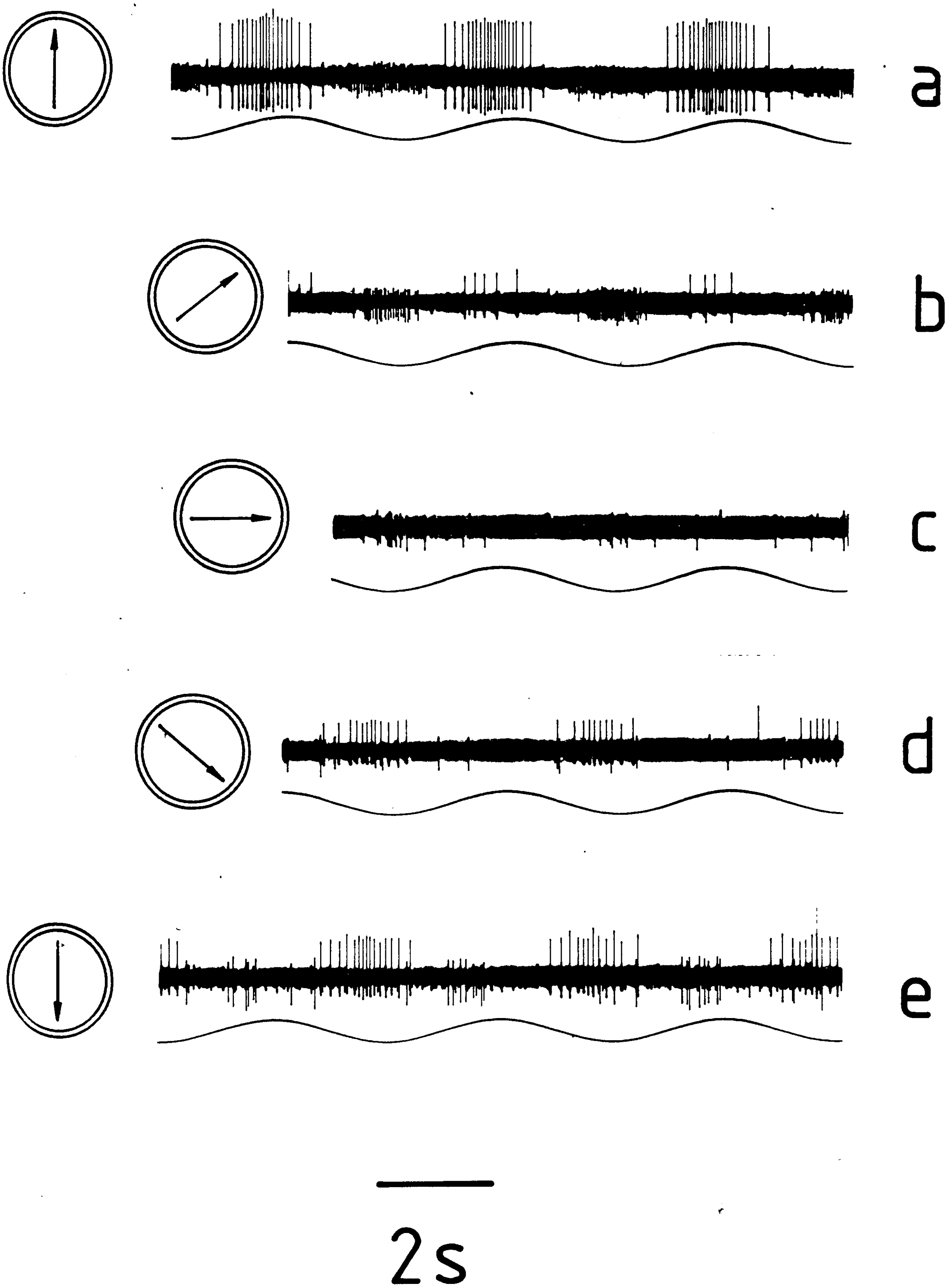

a
b
c
d
e
2s

Fig. 12

Phase histograms of activity of unit 1. Data collected over te cycles at each angle (detailed description in text).

Statistics were calculated on these histograms such as the phase position of the circular mean. These are plotted on subsequent figures.

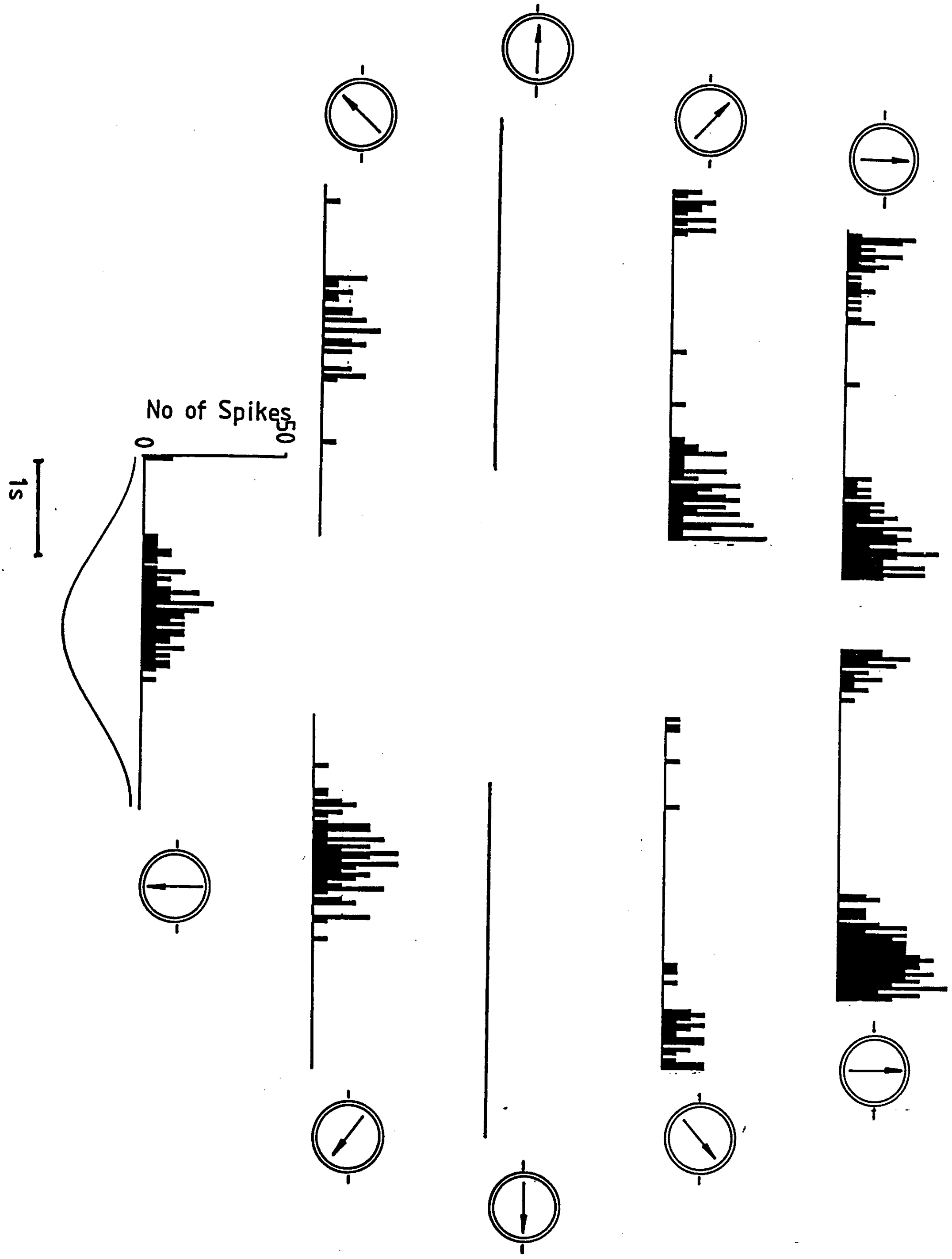
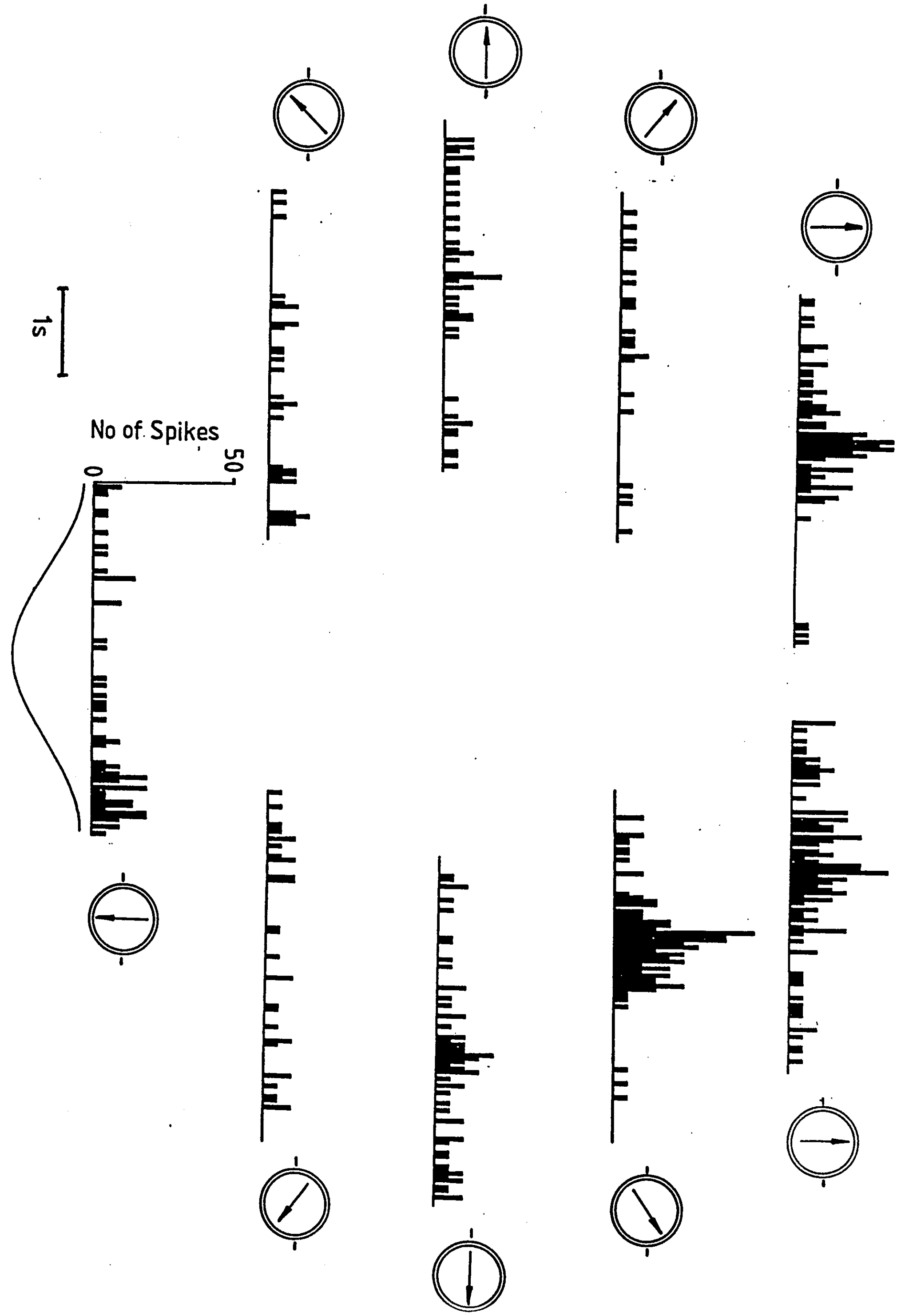

No of Spikes
0
50
1s

Fig. 13

Phase histograms of activity of unit 2. Data collected over ten cycles at each angle (detailed description in text).



No of Spikes
50
0
1s

Fig. 14

Response of a head-up interneuron to tilt. Phase position of the circular mean (+/-circular standard deviation) plotted against angle of the plane of tilt (see Fig. 1). This unit responded in a manner similar to unit 2 (see Figs 11&13).

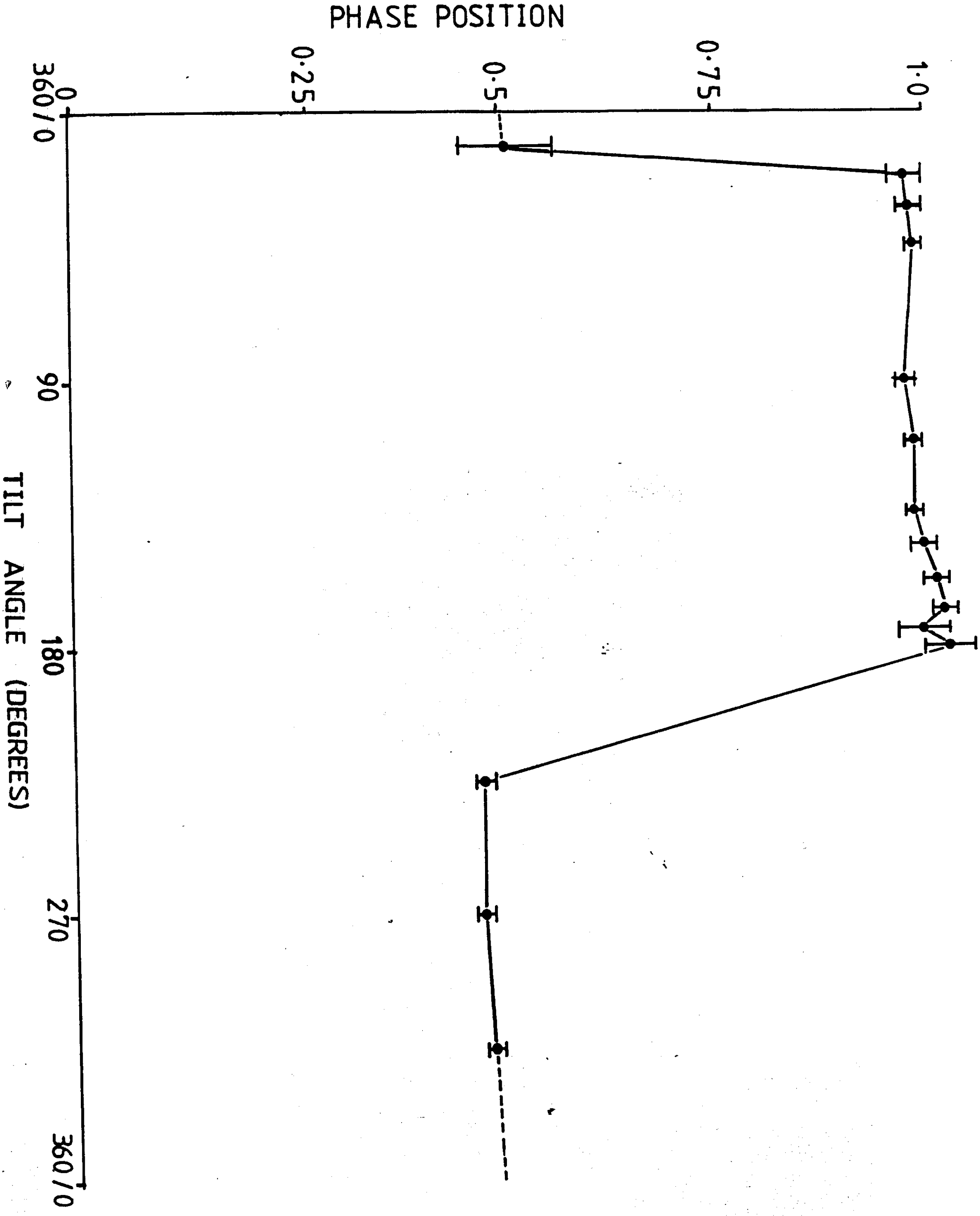

PHASE POSITION
1·0
0·75
0·5
0·25
0
360/0
90
180
270
360/0
TILT ANGLE (DEGREES)

Fig. 15

Composite figures of responses of pitch interneurons to tilt in different planes. Phase position of circular mean (+/-csd.) plotted against angle of plane of tilt (see Fig. 1).

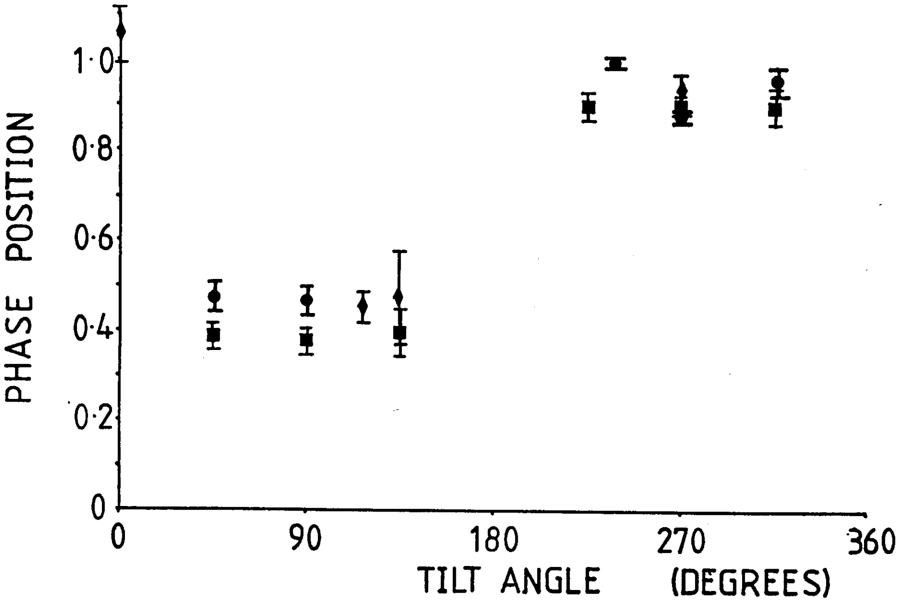
A. Head-down interneuron (see Fig. 11, unit 1).

B. Head-up interneuron (see Fig. 11, unit 2).

In each plot, a particular symbol represents result obtained in a particular experiment.

Detailed description in text.

A


PHASE POSITION
1·0
0·8
0·6
0·4
0·2
0
0
90
180
270
360
TILT ANGLE (DEGREES)

B

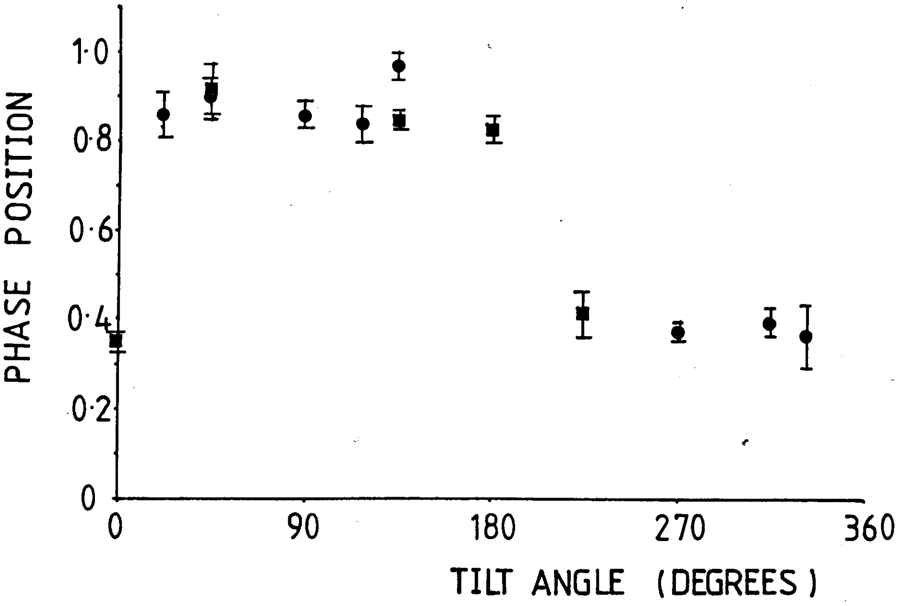

PHASE POSITION
1·0
0·8
0·6
0·4
0·2
0
0
90
180
270
360
TILT ANGLE (DEGREES)

Fig. 16

Units recorded from abdominal connectives using head-tilt technique (see Methods). Upward deflection on movement trace denotes head-tilt to the left. Timescale identical in A&B.

A.

1. Effect of ramp stimuli (compare with Fig. 3).

2. Effect of holding tilted to the right.

3. Effect of sine stimuli (compare with Fig. 12)

B.

Tonic unit responding to head-tilt to the left (compare with Fig. 9b).

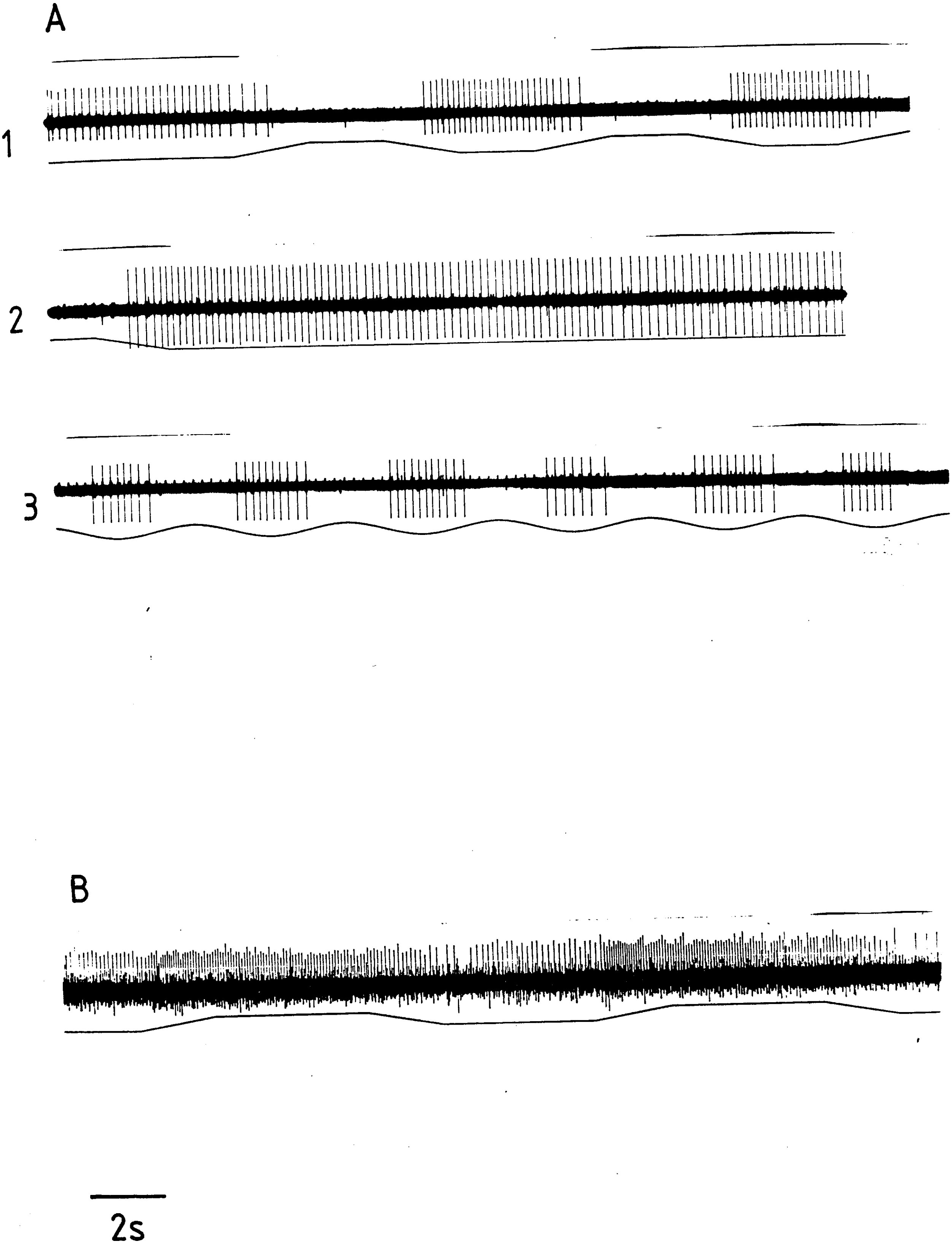

A
1
2
3
B
2s

Fig. 17

Response of unit recorded in the left circumoesophageal connective to leg movement and tilt.

a. Response to depression (DEP.) of legs on the left side.

b. Example of response to roll tilt. Upward deflection in movement trace denotes tilt to right side. Unit was also tested in pitch with similar result.

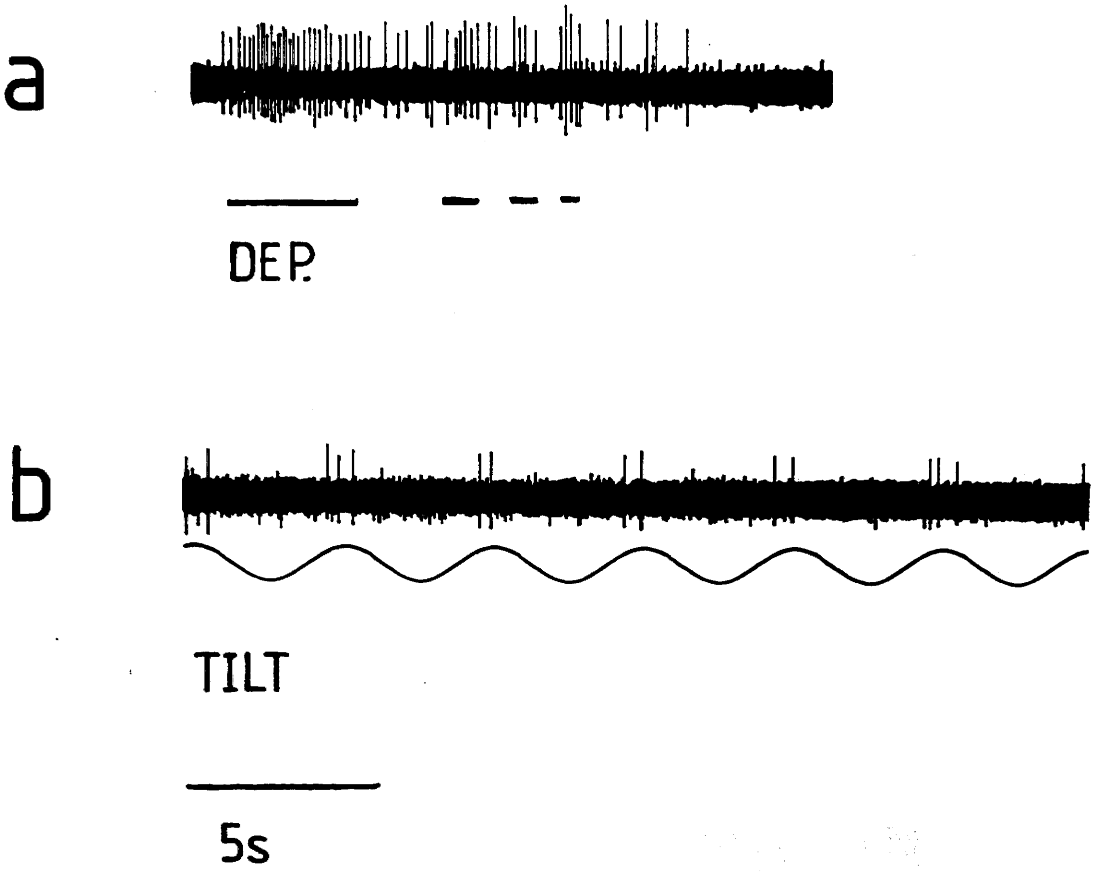

a
DEP.
b
TILT
5s

Fig. 18

Responses of circumoesophageal connective units to depression of combinations of legs. Bars indicate depression.

a. Depression of various legs on the left side.

b. Depression of all legs on the right side.

c. Depression of all legs on the left side.

d. Response to tilt. Upward deflection denotes head-down.

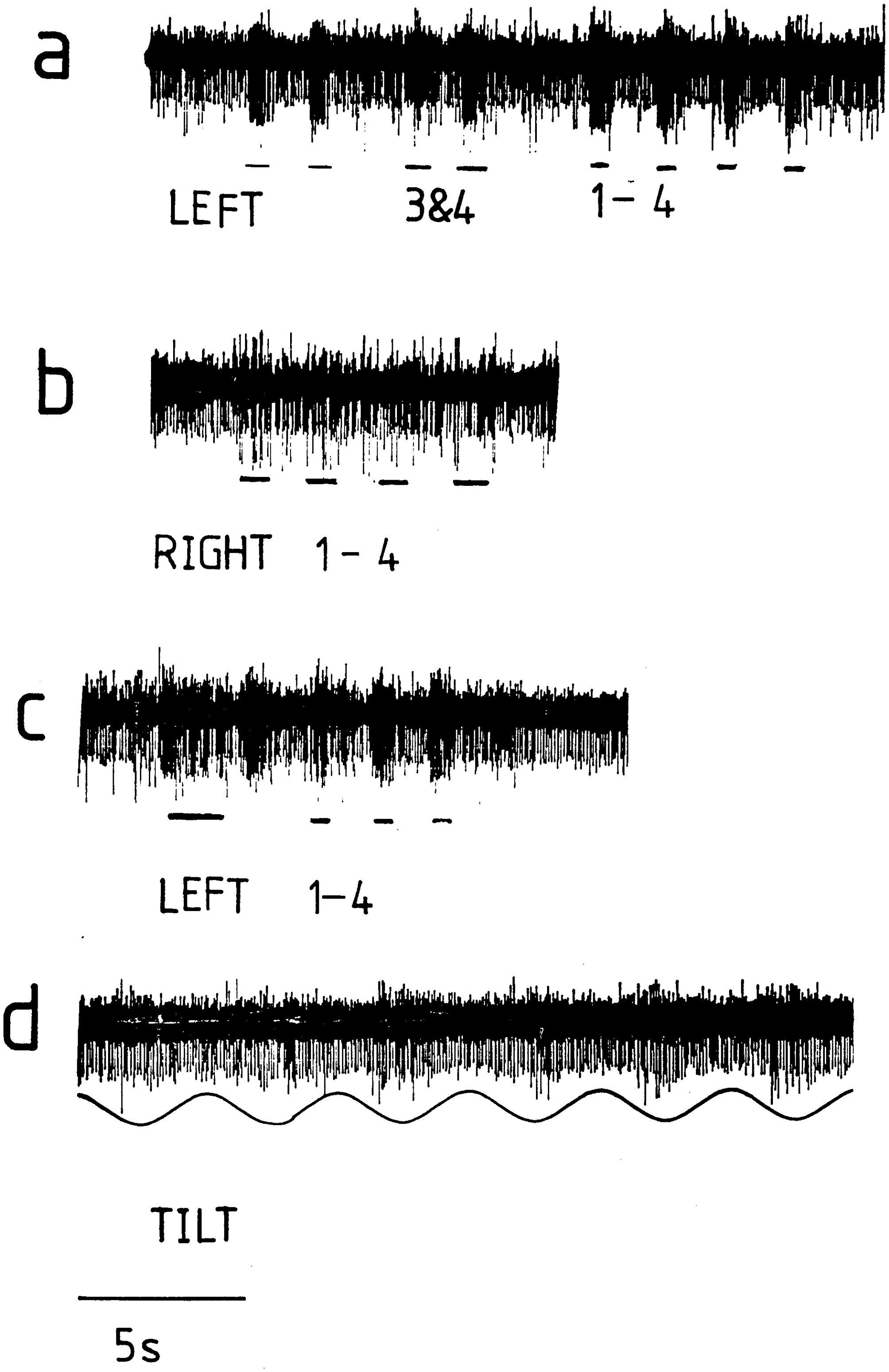

a
LEFT
3&4
1- 4
b
RIGHT 1 - 4
c
LEFT 1-4
d
TILT
5s

Fig. 19

Response of left circumoesophageal unit to ventral deflection of antennules. Bars represent ventral deflection as shown.

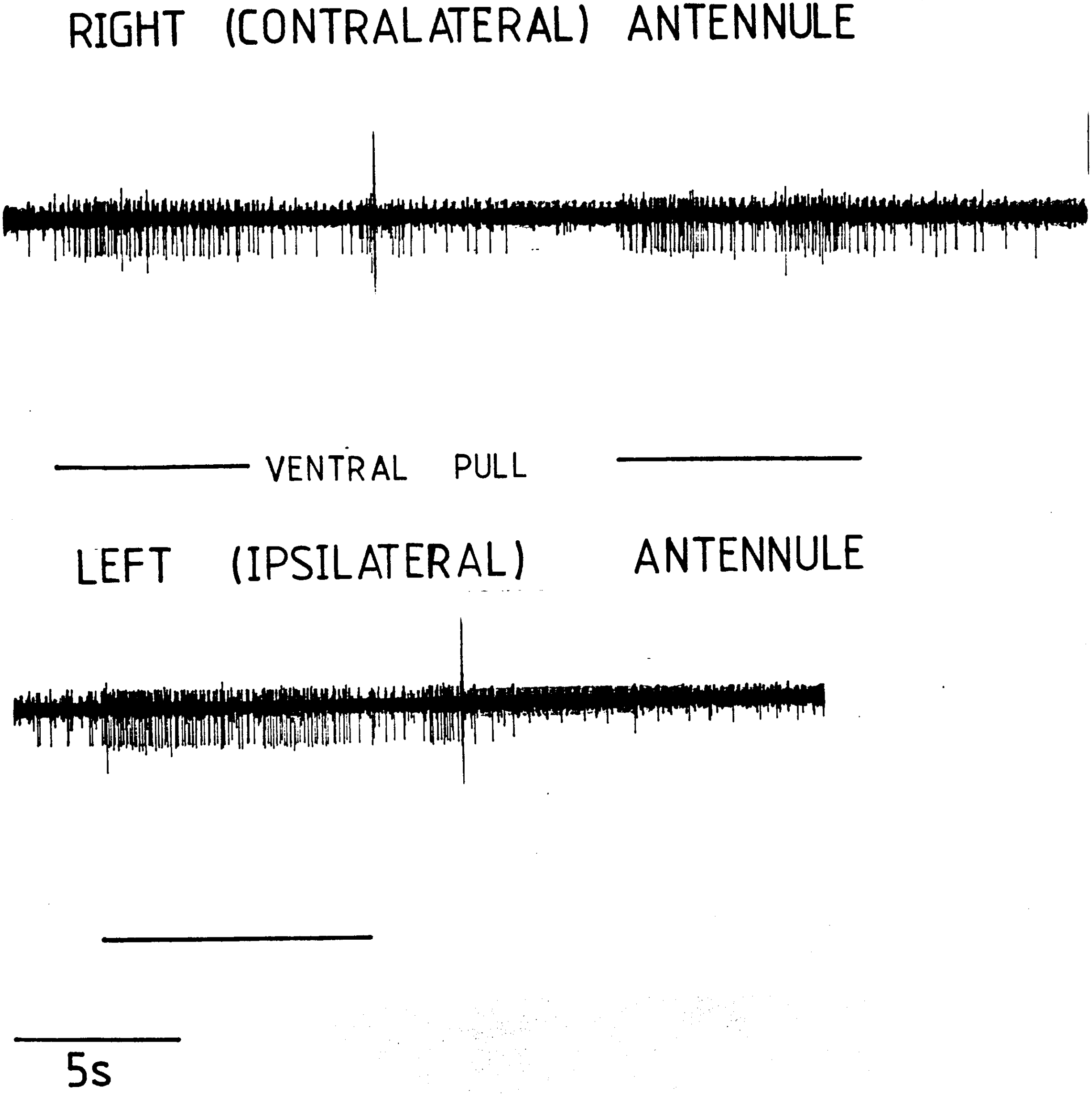

RIGHT (CONTRALATERAL) ANTENNULE
VENTRAL PULL
LEFT (IPSILATERAL) ANTENNULE
5s

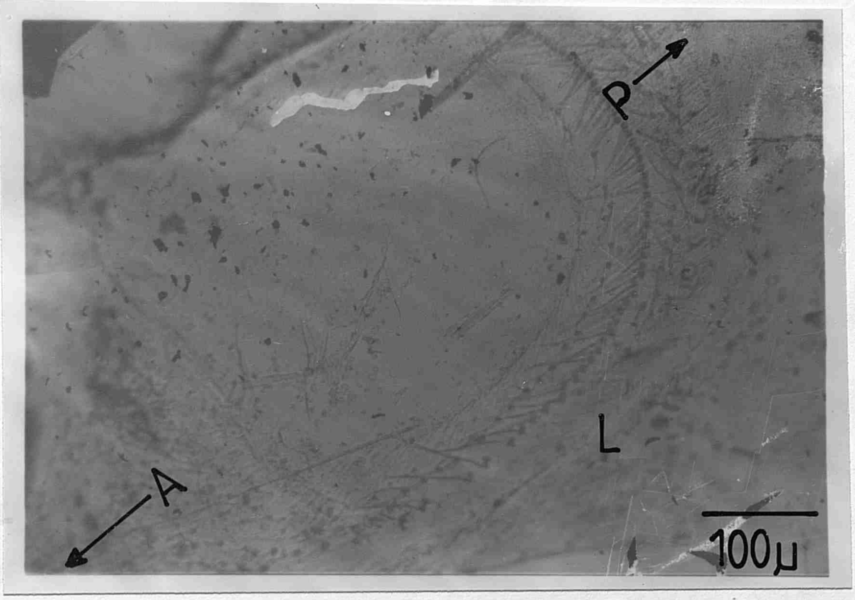
Fig. 20

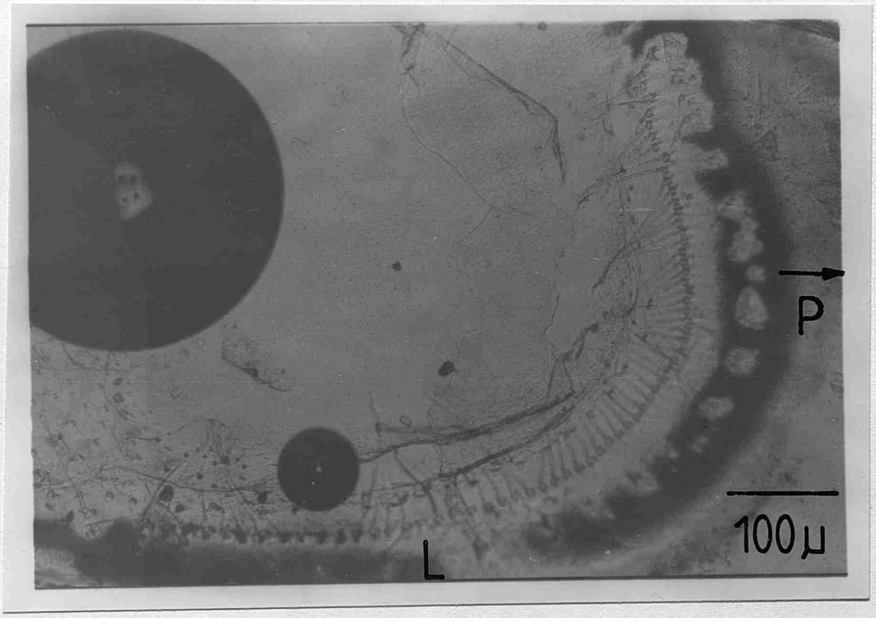
Statocyst sensory crescents of :

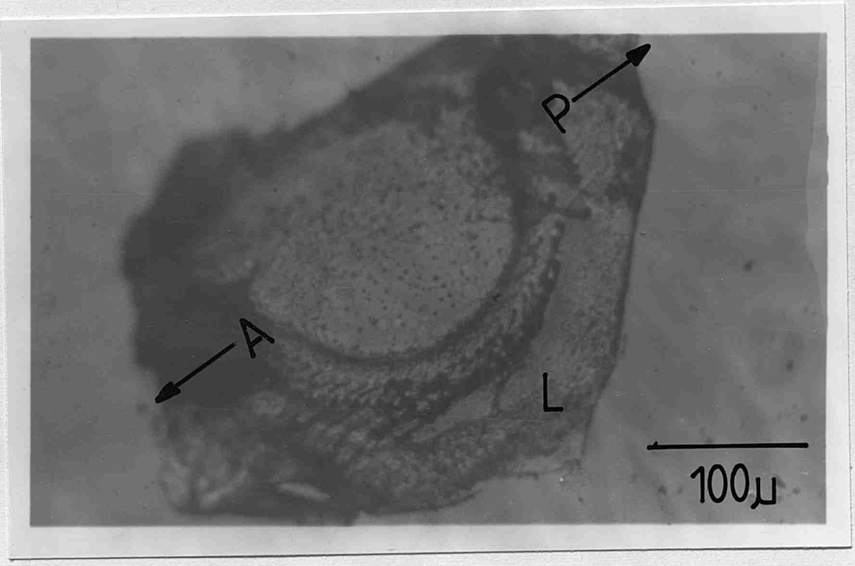
A. *Nephrops norvegicus*

B. *Homarus gammarus*

C. *Pacifasacus leniusculus*


P
L
A
100µ


P
L
100µ


P
A
L
100µ

A. Introduction.

In many decapod crustacean species, imposed movements of the body with respect to gravity, produce two types of response. These are righting responses, so called because they act to restore normal orientation, and compensatory reponses which act to compensate for the imposed movement without restoring normal body orientation. Crustaceans, and in particular crayfish and lobsters, have been widely used in studying these responses. Most attention has focussed on responses involving abdominal appendages such as swimmerets (Davis, 1969; Miyan 1982) and uropods (Yoshino et al, 1980; Hisada, 1985), and cephalic appendages such as the antennae (Priest, 1983), and the eyes (see Neil 1982).

The most important appendages for normal substrate-bound locomotion in the adult forms of most of the lobsters are the legs. Decapod crustaceans show great flexibility and variety in the patterns of leg activity used in walking (Clarac and Barnes, 1985). Most are capable of backward, forward and sideways walking over a wide range of velocities. The activity of the legs in relation to walking has been the subject of much study (eg. Ayers and Davis, 1977; Clarac, 1982). The mechanisms responsible for coordination between legs are also many and varied, with both central and peripheral mechanisms involved. The concept of central pattern generation has been invoked to explain the underlying pattern of leg activity in walking in insects (particularly cockroaches, Pearson and Iles, 1970, 1973). However, this idea has come increasingly under attack (notably by

Pearson himself, Pearson, 1985), as the motor output produced by completely deafferented preparations differs in important aspects from that seen in preparations where sensory feedback is present. In crustaceans, as it has been difficult to demonstrate segmental central oscillators in completely deafferented thoracic preparations (but see Sillar and Skorupski, 1986), the importance of sensory feedback from the periphery has been stressed (Evoy and Ayers, 1982).

The role of sensory input in the initiation and control of thoracic motor systems has figured heavily in many studies. (for reviews see Evoy and Ayers, 1982; Clarac and Barnes, 1985; Clarac, 1985). However this is usually sensory feedback from the legs themselves, from receptors such as the the thoraco-coxal muscle receptor organ (TCMRO) or the cuticular stress detectors (CSD's). Some of this feedback has been recorded chronically in behaving animals (eg for CSD's see Klärner and Barnes, 1986) and some is inferred from behavioural experiments (see Clarac, 1985). Any interplay between these sources of sensory information and other sources outwith the legs has involved looking at a third system, such as the eyes (eg Schone et al, 1983).

Comparatively little attention has been directed towards looking at the effects of "extrinsic" sensory input on the legs. Work on the caudal photoreceptor in crayfish would perhaps come into this category (Prosser, 1934). The reasons for this lack of attention may be that in walking, the effects of such extrinic inputs are dominated by the type of inputs described above, "intrinsic" to the walking system itself. Other patterns of leg activity are therefore needed to investigate the effects of extrinsic inputs.

A pattern of leg activity, different from walking in both the velocity and phase relationships of the movements, has been reported for several species of lobster. It takes the form of slow leg waving and is thought to aid gill function (Pasztor and Clarac, 1983). However these authors reported that this activity was not observed in *Nephrops*.

Another distinct pattern of activity is induced when the animal is subjected to tilting in the roll plane. Although known for some time (eg see Schone, 1964), no quantitative study of this pattern of movements has been made. Rather, most attention was directed to the righting responses of the swimmerets and uropods which occur under these conditions. The characteristics of this leg response to tilt will be described for *Nephrops* in this chapter, and compared with both walking, and with waving which has been observed in this species.

B. Materials and Methods.

Adult male *Nephrops* were attached to a tilting frame as follows. A stainless steel rod, 8cm long x 4mm diameter, was glued halfway along the cephalothorax in the midline using dental cement. The rod was then attached to the tilting frame using an x-block. The frame could be tilted by hand, such that the animal was tilted about its longitudinal axis by up to 30° either side of the normal upright position (Fig 1).

Leg movements were observed and recorded using a video system. This mixed a timing signal with the video signal, allowing movements to be timed in hundredths of seconds if necessary.

To allow more quantitative analysis, particularly of phase relationships between active legs, a movement monitor system was used. A length of copper wire (diameter 0.003 in.) was taken and one end melted in a bunsen flame to form a small sphere. The wire was then attached to a leg by gluing with "Cyanoacrylate C3" such that the sphere lay at the distal end of the meropodite. This usually entailed wrapping the wire around the legs several times, gluing it in place, and also gluing it to the dental cement "saddle". Provided sufficiently long lengths were used, movement was not impeded in any way (see Fig. 1).

Two aluminium plates were attached to the tilting frame, parallel to the long axis of the animal such that they were equidistant from the midline (Fig. 1B). These were connected to a signal generator and a signal of 47kHz and 5V passed between them. The detector wire on the leg produced a voltage proportional to its position between the plates. The plates and the animal were tilted together, keeping their same relative positions.

The signals from up to four appendages could be recorded at any one time. These were recorded on a Racal "Store 4" FM tape recorder. To produce a hard copy the tape was replayed and the signals fed via amplifiers to a Devices four channel pen recorder. The information was digitised using a bitpad. The topmost points of each cycle, which corresponded to the leg at its most levated position, were used to calculate cycle and cumulative period times of up to fifty cycles of four curves. A program, written in MBasic calculated and produced a printout of the period of each cycle; and for up to three curves the cycle-to-cycle phase relationship of movements within the reference curve. This data could then be further analysed to produce information on mean periods and mean phase relationships for various combinations of legs.

Data is plotted in two forms to illustrate the various relationships. Linear plots show the variations observed with time and circular phase plots show variations of phase in relation to the period of movement.

C. Results.

1. General anatomy of the walking legs.

Although all decapod crustacean walking legs are constructed to the same basic pattern, there is still great variability and in some species they have become specialised to perform certain tasks. The basal leg anatomy has already been described in some detail for *Nephrops* (Priest, 1983). There is also information on a more general level for various other species (see Page, 1982; Evoy and Ayers, 1982). A brief description of the gross external anatomy of the legs in *Nephrops* will be provided. To prevent confusion with other published work, the legs, or strictly pereiopods, will be numbered in five pairs from anterior to posterior. The four pairs of walking legs (pairs 2 - 5) consist of seven segments and six joints (see Fig 2). These are the T-C, C-B, I-M, M-C, C-P and P-D joints (Fig. 2B). The joint between the basipodite and ischiopodite is fused. Each joint allows movement in one plane, coordinated actions at more than one joint producing a resultant complex movement in three dimensions. The degree of movement possible at each joint is shown in Fig. 3 and compared with other published data.

When animals performed spontaneous walking, the anterior pairs of walking legs, (L2&3) tended to take up a more protracted position. This meant that much of the movement was produced by flexion and extension of MC. The posterior pairs (L4&5) were held out at 90° to the body, and protraction and retraction was accompanied by a lesser degree of flexion and extension of MC.

2. Asymmetric leg cycling response.

2.(i). Position of the legs in the absence of tilt.

In the absence of tilt and substrate, active animals positioned the legs in a characteristic manner. The meropodite was held horizontal and the MC joint slightly flexed. Legs were held such that the tips were approximately equally spaced around the thorax. This position was also adopted as the animal passed through 0° between tilts.

In less active or fatigued animals, or during bouts of thanatosis (death feigning), the legs hung beneath the animal, ie they were more depressed relative to the position described above.

2.(ii).Response to tilt in the roll plane.

Tilt in the roll plane produced a clear asymmetric response of the legs in most preparations, although there was a degree of variability in other aspects of the response, particularly in fatigued animals.

The asymmetric leg response was elicited by relatively small tilts away from the normal upright position. Measurements showed that the response was triggered by tilting the animal more than approximately 10° (10.31° +/-3.15°, n=15) either side of 0° (normal upright position). The effect of tilt at other angles is discussed below.

The response of the legs that were tilted side-up was postural and "tonic" in character. The response of the legs tilted side

2.(iv) Side-down legs.

Side-down legs moved in a characteristic patterned manner (Fig. 4b). They were held out from the thorax, in contrast to the side-up legs.

Activity during the cycling was confined to the T-C, C-B and M-C joints. Muscle action at these joints produces protraction-retraction, levation-depression and extension-flexion movements respectively.

The main features of the response are illustrated in Fig. 6. This shows the response of legs 2,3&4 on the right side. Side-down tilt produced a lateral movement as the legs moved out from the thorax. Cycling continued while side-down tilt continued. Side-up tilt caused the legs to be held against the thorax, producing a medial movement. Note that in this particular case, leg 2 cycled weakly while tilted side-up.

The clearest component of the cycling is the large degree of levation/depression. However there is also flexion/extension of the MC joint (Fig. 7). Measurement from video tape showed that the meropodite levated through an arc of 80.75+/-16.80° due to action of the CB joint, while at the most levated position the MC angle was 40.50+/-6.20° . During depression of the leg the MC joint extended, and at the limit of the depression the MC joint angle was 144.0+/-13.90° .

The protraction and retraction of the leg was more difficult to resolve. However, most of this movement seemed to occur with the leg at the limit of levation.

3. Quantitative analysis of cycling pattern.

3.(i). Period of cycling.

The period of the cycling pattern was defined as the time taken for a leg to move from the limit of levation to the limit of the successive levation. The period was not only stable in any particular animal, but was very similar from animal to animal. Mean values for the period of movement are shown in Table 1; these were calculated from 200 individual period times. The two middle pairs of walking legs (L3&4) seemed to cycle slightly more slowly than L2 and L5.

To investigate any systematic variation in the period with time the test animal was held at a tilted position for up to 30s. During this time the period was fairly constant. However there was a slight oscillation in the values. If the animal remained tilted for a matter of minutes all the righting responses began to wane, including those of the uropods and swimmerets. They could be restored by raising the level of activity. This was usually done either by tapping the bar to which the animal was attached, or by administering a tactile stimulus to the animal by gently tapping the thorax.

The period was plotted against time for one set of data on which further analysis was carried out (Fig. 8.). This shows data for approximately 25 cycles. While L2,4&5 show relatively little variation, the cycling of L3 is both slower and more irregular.

3.(ii). Phase relationships in cycling.

The phase relationships of the legs were examined to attempt to reveal any coordination between legs.

The plots show clearly that some coordination between legs does occur. However the precise nature of this coordination is more difficult to define. Phase values were calculated for L3,4&5 in L2 (Fig. 9), and also for each leg in its anterior neighbour (Fig. 10). Between L2 and L3 there seems to be very little relationship. However both L4 and L5 show coordination. The pattern which emerges is very different from that which would be expected if there was no coordination between the legs. Perhaps most revealing is the pattern seen for L4 in L3 (Fig. 10). L3 cycles with a very unstable period, yet there is still evidence of coordination between it and L4.

The circular plots reveal the stability of the period of the the legs with the exception of L3. Both of the lower plots in Figs. 11 and 12 show that L4 and L5 have preferred phase positions relative to both L2 and also their anterior neighbour. Furthermore, Fig. 12 suggests that where differences in the period of two adjacent legs produce more than one cycle of the test leg within the base leg, some coordinating influence forces the extra cycle into a particular phase position 0.5 phase units, or 180° , from the preferred phase position.

4. Effect of different types of tilt.

4.(i). Range of roll tilts.

The asymmetric leg response was switched on at 10° as stated above.* The animal was rolled through 360° to investigate the response at other roll angles. The response did not switch again until 180° was reached. Here again there was a region of 10°

* See 2(ii)

either side of inverted where there was no response. At these angles where the animal was inverted the response was weaker but still recognizable. The strongest response was seen in the first 90° either side of the normal position. However clearly directional information was available at all points round 360° .

Occasionally with the animal held inverted at 180° the leg response would switch from one side to the other. There was never an appearance of an intermediate response or the response seen in the absence of tilt. Significantly this "hunting" in the leg response was always accompanied by hunting in the uropod and swimmeret responses.

4.(ii). Tilt in other planes.

The response of the legs was also investigated by tilting the animal outwith the roll plane. Tilting the animal in the pitch plane produced very little response in the legs. Pitch head-down did produce a small degree of protraction, and head-up a small degree of retraction, but these responses were very variable from animal to animal.

The response of the legs to tilts at intermediate angles was more revealing. The asymmetric response persisted as the animal was moved away from pure roll. It continued except in a narrow region each side of pure pitch. The width of this region was an arc of between 10 and 20° either side of pure pitch.

5. Effect of lith removal.

The above findings led to the speculation that the leg system would respond in the same manner as the swimmerets and uropods when the lith from one of the statocysts was removed. In these other systems the removal of the lith of one of the statocysts causes a 30° shift in the response to tilt.[*] This means that at the normal position an asymmetric response is elicited. This can be corrected by rolling 30° away from the operated side. The same was observed for the asymmetric leg cycling response.

When the liths from both statocysts were removed the asymmetry in the leg response was abolished. The legs were held in a position similar to that observed in the unoperated animal in the absence of tilt.[**] On some occasions weak cycling was observed. It was of very small amplitude, and only involved basal joints.

6. Effect of leg autotomy.

It was not possible to look in detail at the effect of autotomy of single legs on the cycling of the other legs. On several occasions however, all the legs on one side were removed. In most cases this had very little effect on the response of the intact side to tilt. On on occasion the pattern of the remaining legs was changed markedly. The legs on the intact side still cycled strongly, but in a very depressed position. The effect of this was to bring the tips of the legs under the animal and almost to the midline.

The effect of autotomising all legs was looked at in two animals. There was no clear response to tilt. When the stumps moved, they seemed to move together either in phase, or with only very small phase differences between stumps. In general however, this procedure seemed to depress all activity.

[*] Miyan 1982. [**] See 2(1)

7. Comparison with other patterns of leg activity.

Two other patterns of leg activity will be compared here with leg cycling. These are the patterns seen during walking and leg waving.

7.(i). Walking.

Data on the period of movements and phase relationships of legs during walking was obtained from cine film of *Nephrops* walking on a treadmill. It should be noted that in cycling and in leg waving (see below) the active legs carried out essentially the same movement; in walking different legs are moving in different ways. The anterior pair of walking legs produce forces by flexion and extension of MC, pulling the animal forward during the powerstroke. In contrast L4 and L5 tend to be held at 90° to the body; movement at the TC joint is important for producing forces and the more distal segments act as struts.

The period of movement is both stable (Fig. 13) and similar for all the legs. The middle pairs (L3 and L4) step slightly slower than the other pairs (L2 and L5). The step period in walking is very similar to the period of cycling, and very much faster than the period of movement for waving (table 1). The plots in Figs. 14 and 15 show good coordination between legs during walking. There is a delay of approximately 0.2 phase units betweenL3 and L4, and L4 and L5, with L3 showing a larger delay relative to L2.

7.(ii) Leg waving.

Leg waving was observed on several occasions, and it proved possible to record this pattern with monitor wires attached to the legs. Fig. 16. illustrates the pattern of activity observed. The animal slowly protracted and retracted the three anterior pairs of walking legs, while the most posterior pair were used for support.

The necessary condition for this pattern to be expressed was that the animal was left undisturbed. If during the waving, the bar to which the animal was attached was tapped lightly, the normal stance was taken up.

Period and phase plots were constructed as for cycling and walking to allow comparison. Three features emerged from this analysis. Firstly the period of the movement is very much more irregular than for cycling and walking (Figs. 17&18). Secondly the period is much longer than that for cycling and walking. Thirdly, there is only a very small phase difference between the movements of the legs (table 2).

The circular mean and circular standard deviation for each leg in each of the three patterns are compared in Fig. 19. These plots serve to emphasise much of what has been discussed above. In waving, circular means of legs 3 and 4 are close together, i.e. a small phase difference between leg movements. The small standard deviations suggest close coupling between the different legs. In walking the circular means are further apart and there is a larger degree of variability in the phase relationships between legs. The new pattern analysed in this chapter, leg cycling, is more closely related to walking than waving. The circular means of the legs are relatively widely separated, with large standard deviations suggesting a greater degree of variability in the phase relationships adopted than in either waving or walking.

D. Discussion.

The leg cycling response described in this chapter clearly should be regarded as a patterned movement. Quantitative analysis so far carried out provides evidence of this and allows comparisons to be drawn between leg cycling and both walking and leg waving. In their paper on leg waving Pasztor and Clarac (1983) state that the only pattern seen when the animal is removed from the substrate, apart from struggling, is slow leg waving. They also failed to find leg waving in *Nephrops*. These authors were not concerned with the effect of tilt and the term struggling was not defined. It should be noted that in this study undefinable struggling movements were infrequent. In the absence of tilt and substrate active animals adopted a characteristic posture rather than struggled.

Three main types of movement have been defined for decapods so far in the literature. These are locomotory movements, such as walking or rearward swimmeret beating, compenstory responses, such as demonstrated for the eyes and antennae, and righting responses, such as demonstrated by uropods and asymmetric swimmeret beating. Clearly there is considerable overlap between these various categories. In the Spiny lobsters, movements of the antennae may function as effective righting responses (Neil, 1985), and the swimmerets clearly contribute to locomotion when they beat asymmetrically.

The question arises as to which category, if any, the leg response described above fits. This raises a further question as to its function. Other statocyst-induced responses such as those of the swimmerets and uropods produce a righting torque. However the legs of *Nephrops* show no signs of the specialisation that might be expected if the legs were to produce effective forces against water. Such specialisation of walking legs does exist in those species which use their legs for swimming (eg *Callinectes sapidus*, Spirito, 1972). The legs of *Nephrops* probably produce very small amounts of righting torque. Furthermore, the direction of cycling is only appropriate during the first 90° of tilt either side of 0° . While the chelipeds with their greater mass might be thought to be of some assistance, experiments elsewhere have shown their effectiveness in aiding righting to be minimal (Newland, 1985). It is also difficult to conceive the leg response as a compensatory response.

However, there is another possibility that was indicated by one of the results reported above. When the legs on one side were autotomised, this affected the pattern of the intact legs in an unexpected manner. The cycling demonstrated by the intact legs when tilted side-down would in effect compensate for the absence of cycling caused by the autotomised legs. This leads to the proposal that the leg cycling response performs the function of searching for substrate.

As will be shown later (chapter 5), the presence or absence of substrate is a crucial determinant for the type of reactions displayed to various stimuli. *Nephrops* has been shown previously to respond immediately to any appendage detecting substrate by adopting the normal stance (Newland, 1985). The appendages best placed to detect substrate are the legs, and the cycling may increase the opportunities for detecting substrate. The pattern of cycling means that at any one time, at least one leg would be at its most depressed position.

The pattern of motor output which produces the cycling might be that produced by a thoracic chain of central pattern generators. Until recently, the crustacean thoracic system had not been demonstrated to be able to produce rhythmic motor output in the absence of sensory feedback (but see Sillar and Skorupski, 1986). Assuming rhythm generation in the thorax is similar to that in the abdomen, each hemiganglion in the thorax would have circuitry capable of generating rhythmic motor output (Miyan, 1982). However, the pattern of activity seen in the asymmetric response to tilt gives us further clues as to the operation of these centres. While legs tilted side-down express a rhythmic motor output, any rhythmic output is suppressed in the side-up legs. This situation is perhaps analagous to that in the swimmeret system where the side-up swimmerets beat with a strong lateral beat, whereas the side-down swimmerets tend to stop beating. In the case of the asymmetric leg response, it may be that the lack of excitation of the segmental hemiganglion, coupled with what might be termed a postural input, prevents a rhythmic motor output on the side-up. This suggests that coupling across the ganglion is relatively weak; one half ganglion can produce rhythmic motor output while the other is prevented from doing so.

Much interest has been shown in the influence of sensory feedback on rhythmic systems. Indeed this aspect has been particularly important in studies on the control of crustacean walking (Clarac, 1982, 1985). Clearly there will be differences in the feedback reaching the nervous system in the three types of leg movement described in this chapter. In leg cycling and leg waving, much of the feedback from receptors in the legs themselves must be reafferent, that is feedback which is produced by the movements the animal itself makes. In both of these conditions the only external force the legs act against is the resistance of the water. In walking, much of the sensory feedback is phasic in nature, generated by receptors detecting load during the powerstroke of legs (eg see Klarner and Barnes, 1986). It has been suggested that in the presence of sensory feedback, the walking pattern is selected, whereas a diminution in feedback causes the waving pattern to be selected (Pasztor and Clarac, 1983). The role that the sensory feedback serves in walking is suggested by these authors to increase the general level of excitation, alter interlimb coordination, and alter the output of the CPG.

Cycling clearly is more analagous to the walking pattern both in terms of the period of movement and the coordination seen. Yet in terms of the feedback generated it is closer to waving. However there is one type of sensory input feeding into the system during cycling that is not present during waving, that is the statocyst input.

The role of the statocyst input may be to raise the level of exitation of the thoracic oscillators, a role played by other inputs in walking such as contact and load on the legs.

In the swimmeret system there are interneurons running between ganglia which produce the metachronal pattern seen in swimmeret beating (Paul and Mulloney, 1986). Any two ganglia demonstrate the appropriate delay. However the legs are clearly not as stereotyped in their movements as the swimmerets. To produce the degree of coordination seen in the leg system it has been thought that the whole range of sensory input is necessary. Load has been shown to be one of the most important factors (Clarac and Barnes, 1985). In leg cycling there is essentially no load on the legs, and this may be sufficient to explain the greater variability in cycle to cycle period and phase position seen in cycling than in walking.

Postural control of legs has been explained in terms of command fibres. Interneurons have been stimulated electrically to produce flexion or promotion of the legs (Page, 1982). The side-up legs respond posturally and in a manner very similar to that seen in the tail-flick. There are various possibilities as to how the system might be constructed to produce this response. The statocyst interneurons carrying the directional information could synapse onto premotor interneurons that produce this response. In the tailflick the giant fibres which are involved may use the same premotor system to produce the desired posture of the legs.

One other observation deserves comment. As mentioned above, hunting was occasionally observed at angles where the animal was inverted. The hunting involved the uropods, swimmerets and legs. A switch in one was always accompanied by a switch in all. This suggests both that any confusion that arises at inverted positions is not rooted in the motor systems themselves and that all of these systems are driven by similar inputs from the statocyst system, perhaps the same interneurons. As the responses of statocyst interneurons were unambiguous to tilt at inverted positions, the presence of hunting suggests the involvement of other systems in these equilibrium pathways. Other candidates for the source of "noise" in the system which might produce this confused motor output are perhaps the premotor networks of local interneurons. These are known to have an important involvement in both the swimmeret and uropod systems (Nagayama et al, 1984; Paul and Mulloney, 1985).

Table 1.

Mean (+/-sd.) period times for the three patterns of leg activity described in this chapter.

Table 2.

Circular mean (+/-csd.) of phase values of legs measured in L2 for the three patterns of leg activity. R= concentration parameter (Batschelet, 1981).

1.

L	WAVING	WALKING	CYCLING
2	3.36 ± 0.67s	0.69 ± 0.10s	0.54 ± 0.16s
3	3.37 ± 0.66s	0.78 ± 0.14s	0.69 ± 0.18s
4	3.36 ± 0.62s	0.75 ± 0.12s	0.60 ± 0.12s
5	———	0.69 ± 0.18s	0.56 ± 0.15s

2.

	WAVING		WALKING		CYCLING	
L	ϕ	R	ϕ	R	ϕ	R
3	0.03 ± 0.04	0.97	0.58 ± 0.10	0.79	0.48 ± 0.20	0.18
4	0.87 ± 0.04	0.96	0.32 ± 0.12	0.70	0.58 ± 0.15	0.54
5	———	0.99	0.13 ± 0.15	0.53	0.94 ± 0.14	0.63

Fig. 1

Tilt apparatus.

A. Tilt bar.

B. Monitor plates.

C. Monitor wires.

D. From signal generator.

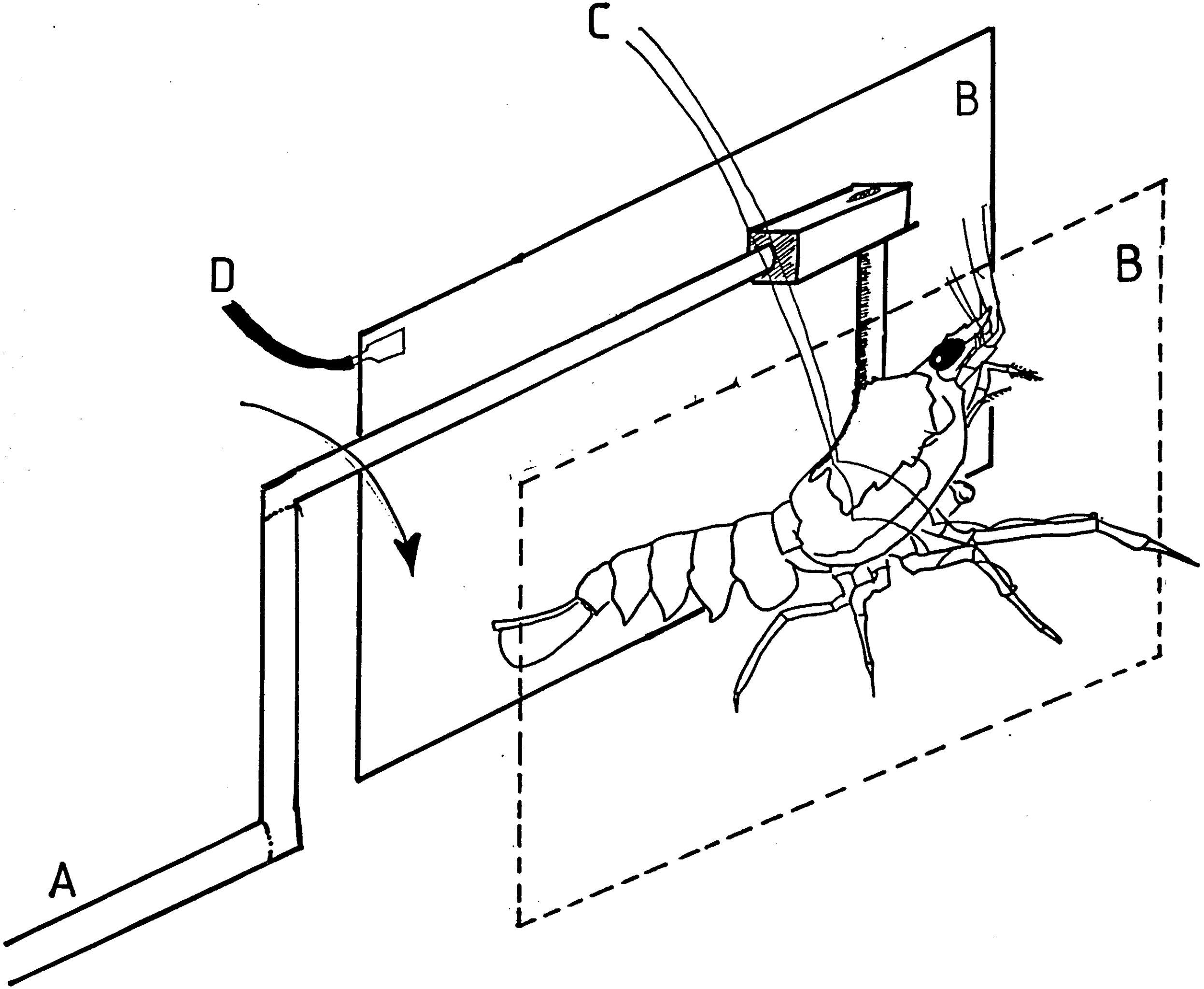

C
B
D
B
A

Fig. 2

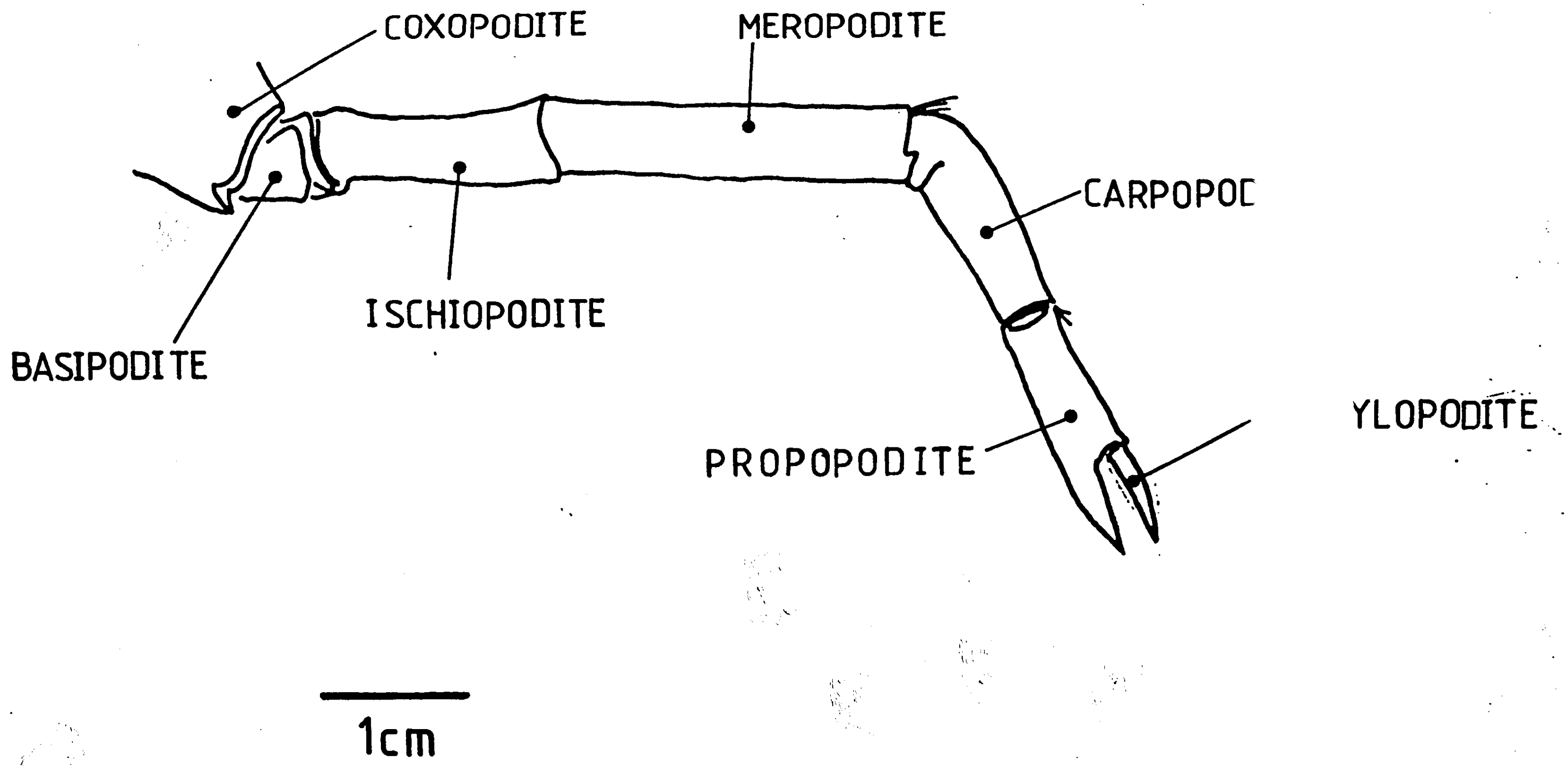
A.

Front view of the second left walking leg of *Nephrops*.

B.

Abreviations used in the text to refer to joints.

A



TC : Thoracicocoxal joint.
CB : Coxabasal joint.
IM : Ischiomeral joint.
MC : Merocarpal joint.
CP : Carpopropal joint.
PD : Propodactal joint.

B

Fig. 3

Range of movements at the joints of the walking leg in *Nephrops* as compared with *Homarus gammarus* (Page, 1982). Measurments were obtained by hand on a single medium-sized male animal. * indicates those joints which are active in cycling.

	TC	CB	BI	IM	MC	CP	PD
NEPHROPS	90°	160°	—	40°	150°	100°	5°
						135°	65°

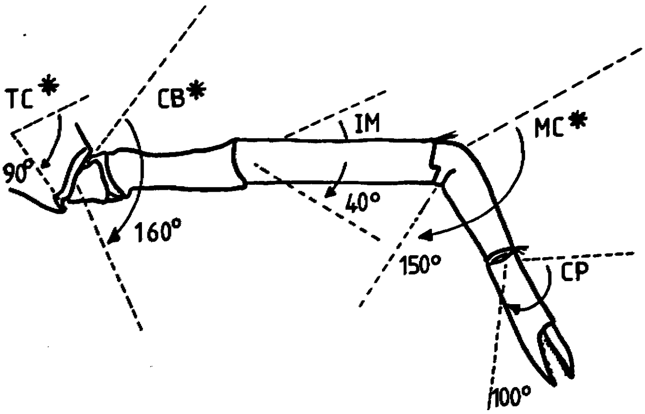
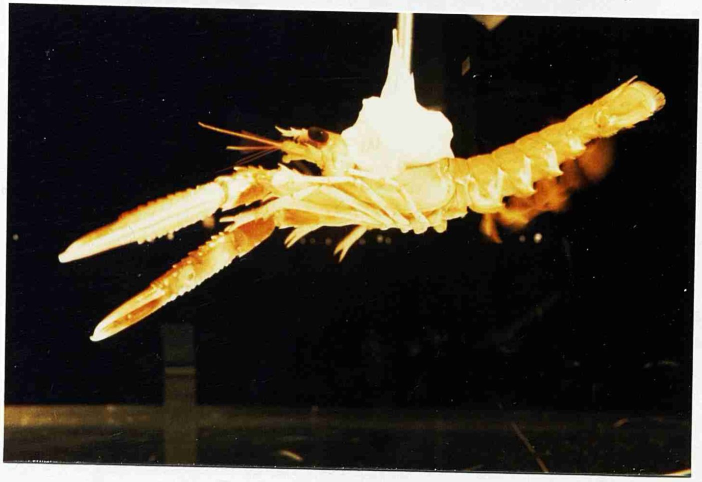


Fig. 4.

The asymmetric leg cycling response.

A. Side-up legs.

B. Side-down legs.



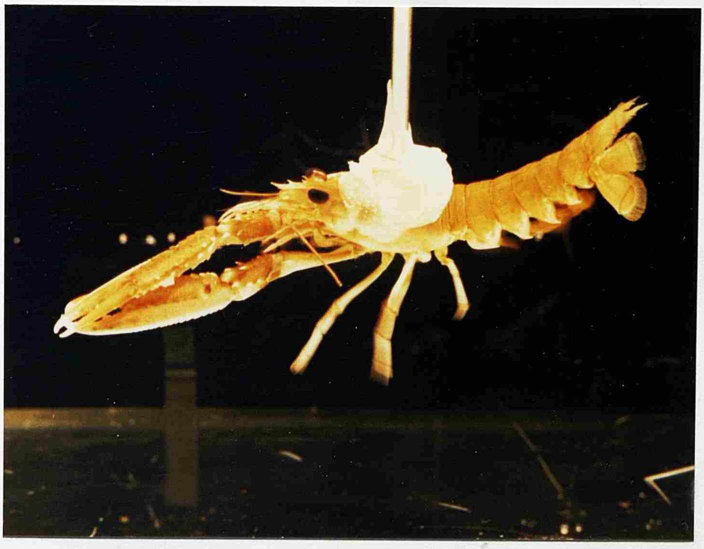
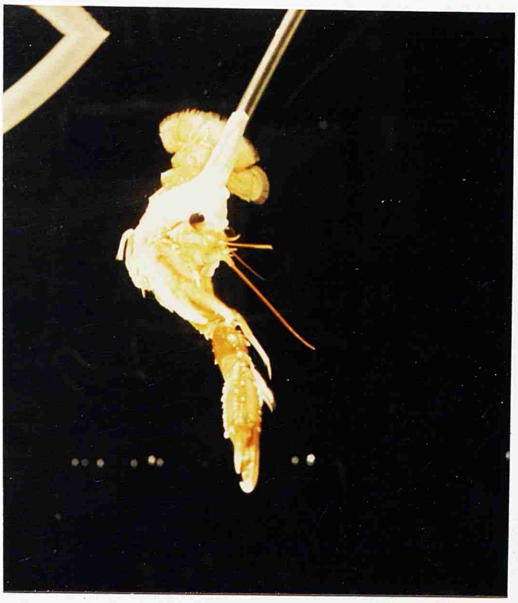


Fig. 5

Effect of roll on chelipeds.



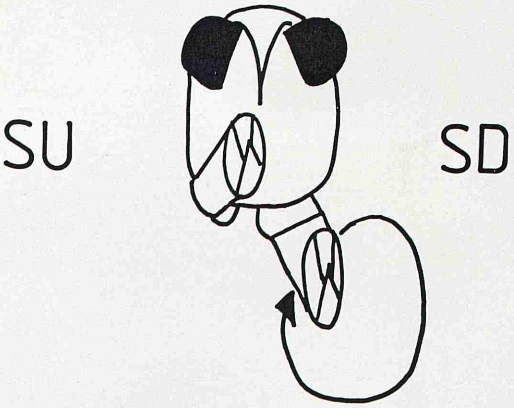

SU
SD

Fig. 6

Effect of roll on legs 2-4 on the right side.

LSD, left side down; RSD, right side down; MED, medial; LAT, lateral.

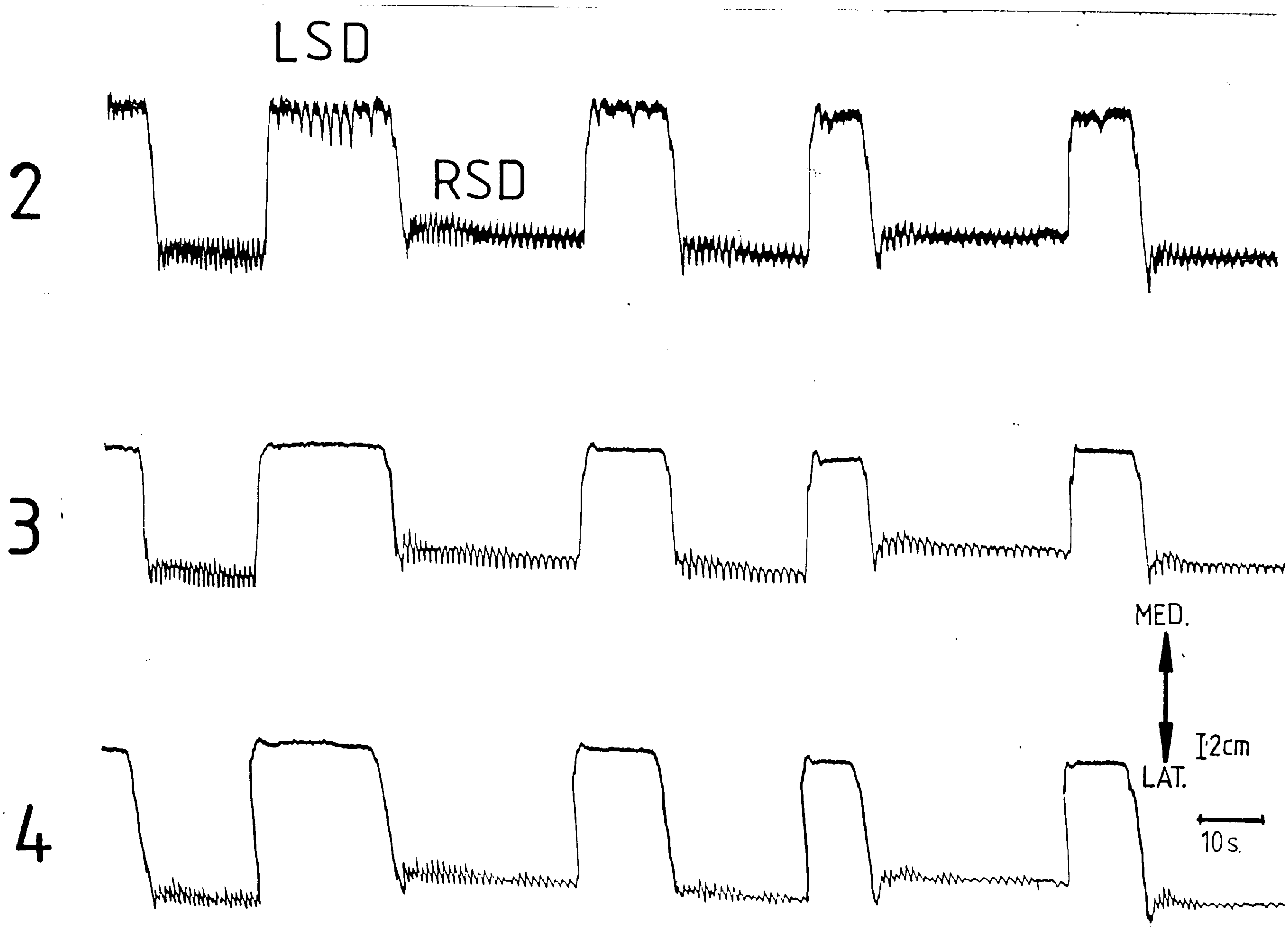
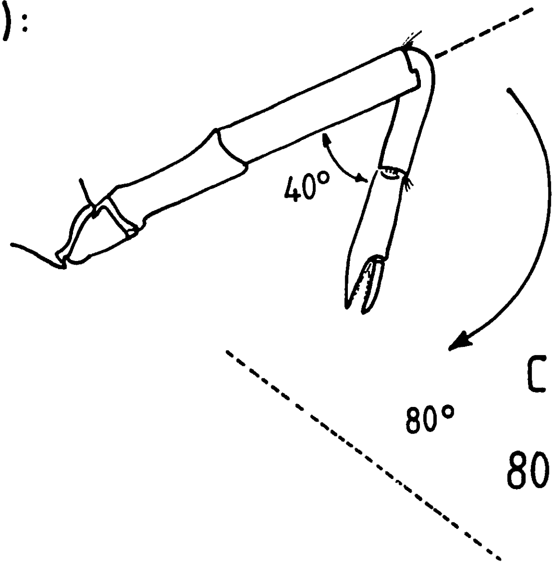

LSD
2
RSD
3
4
MED.
LAT.
2cm
10s.

Fig. 7

Joint angles (mean +/-standard deviation) during cycling. Frontal view of left L2.

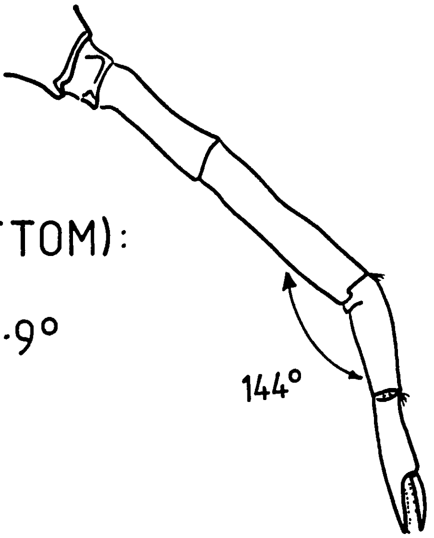
MC (TOP):

40·5±6·2°



CB ANGLE:

80·75±16·8°



MC (BOTTOM):

144·0±13·9°

Fig. 8

Plots of period against time for L2-5 during cycling ie tilted side-down.

a : L2

b : L3

c : L4

d : L5

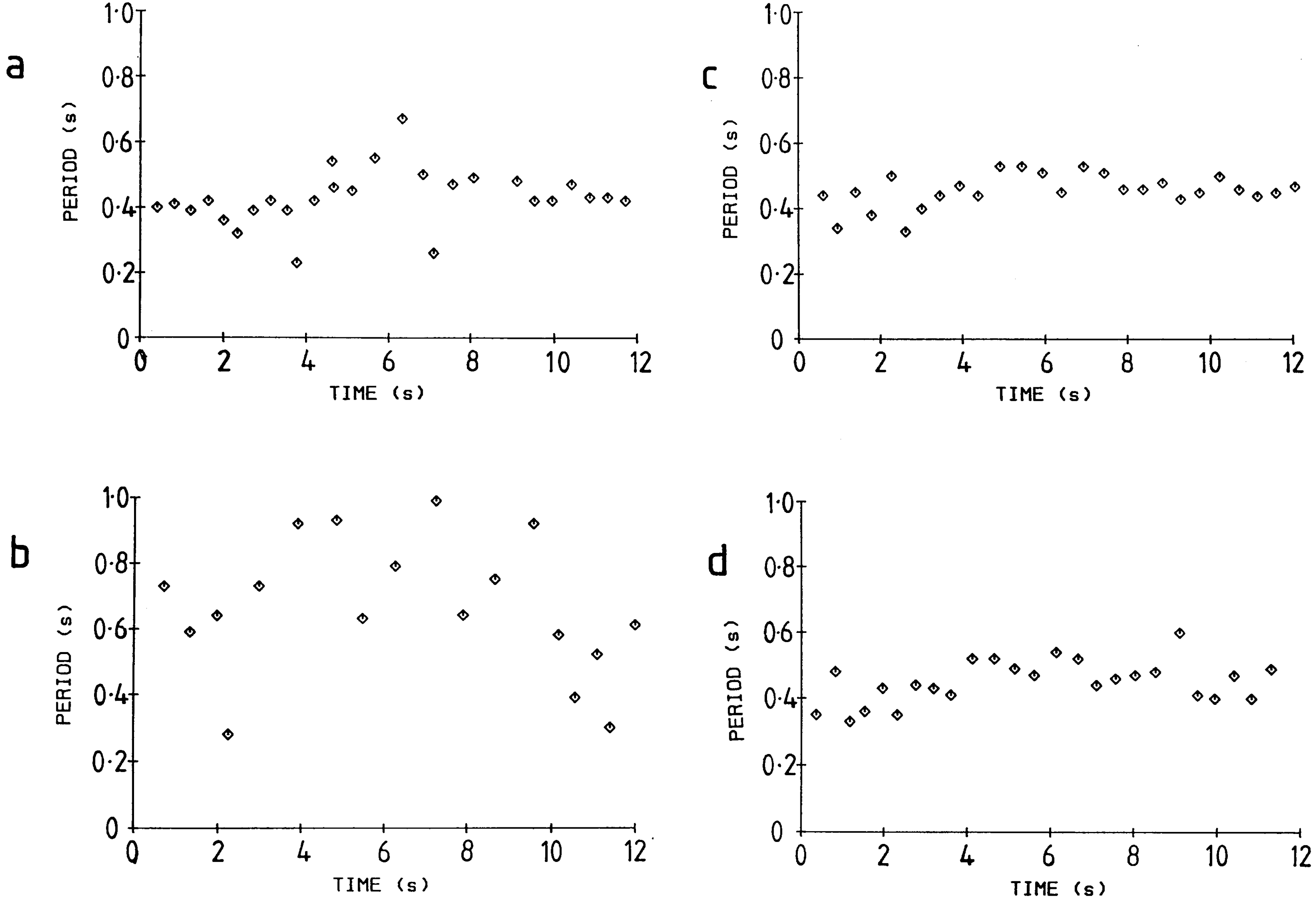
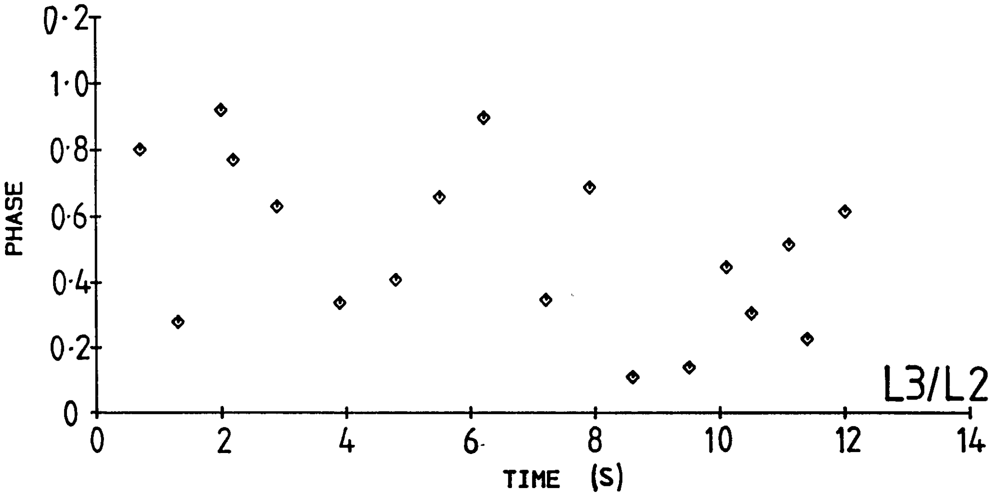
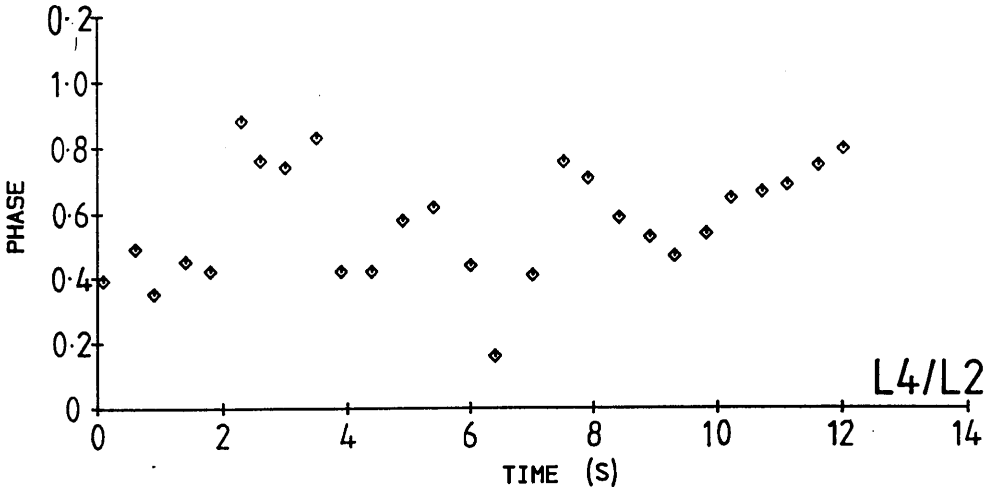

a
b
c
d
PERIOD (s)
TIME (s)
0
0·2
0·4
0·6
0·8
1·0
0
2
4
6
8
10
12

Fig. 9

Plots of phase against time for L3-5 in L2. Same data set as shown in previous figure.


PHASE
0·2
1·0
0·8
0·6
0·4
0·2
0
0
2
4
6
8
10
12
14
TIME (S)
L3/L2


PHASE
0·2
1·0
0·8
0·6
0·4
0·2
0
0
2
4
6
8
10
12
14
TIME (S)
L4/L2

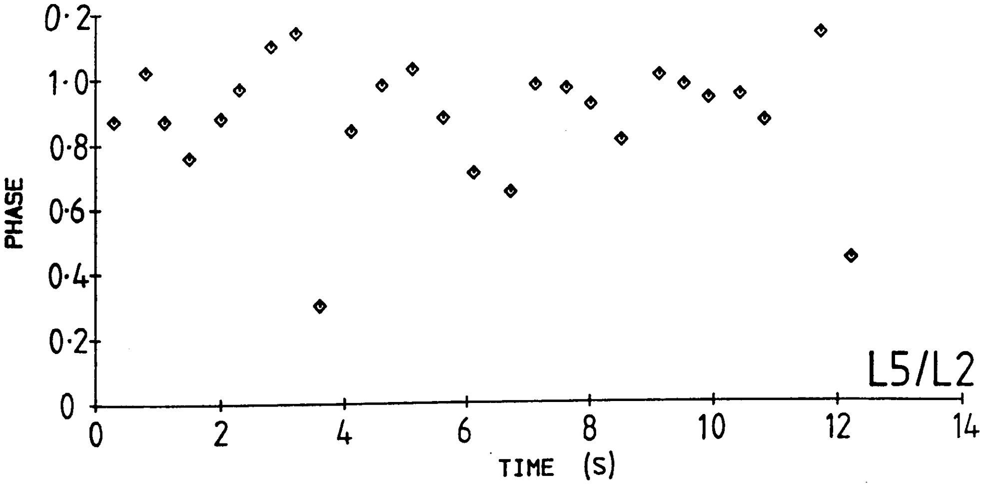
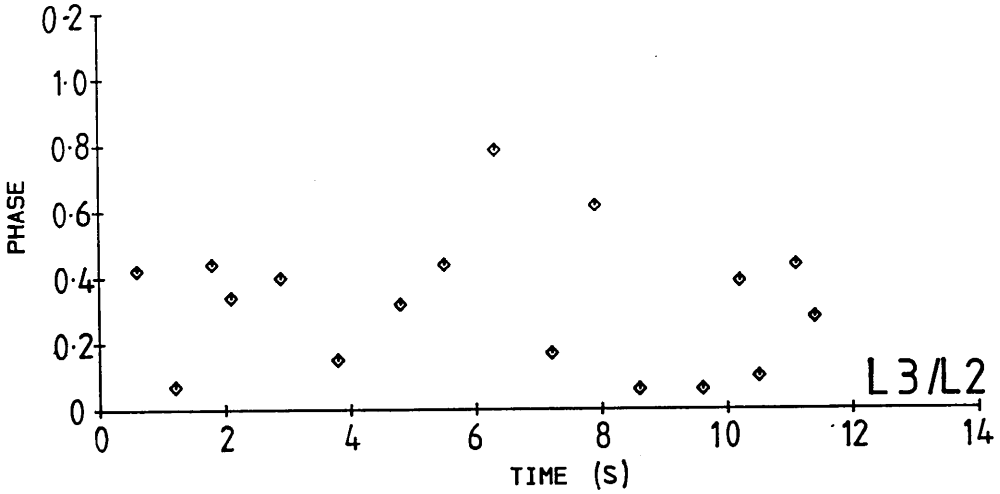
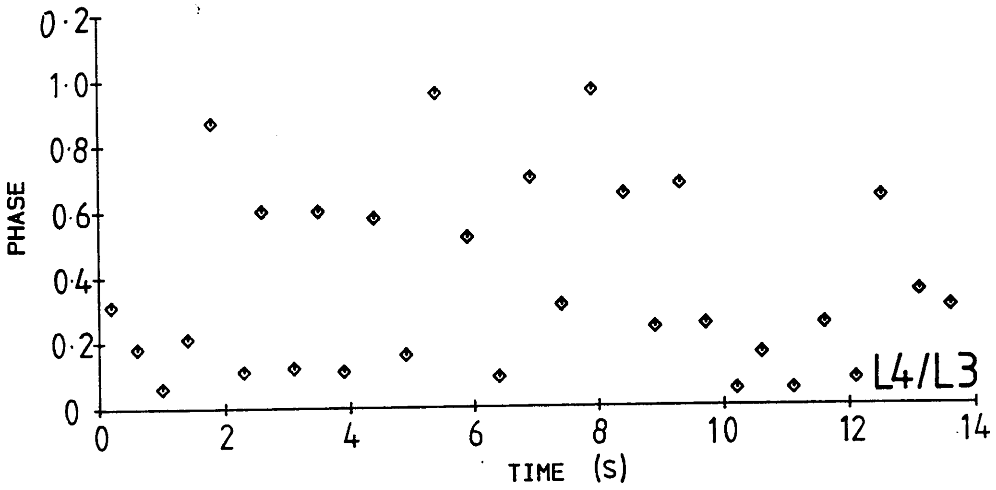

PHASE
0·2
1·0
0·8
0·6
0·4
0·2
0
0
2
4
6
8
10
12
14
TIME (S)
L5/L2

Fig. 10

Plot of phase against time for L3 in L2, L4 in L3, and L5 in L4.

Same data as shown in Figs. 8&9.


0·2
1·0
0·8
0·6
0·4
0·2
0
PHASE
0
2
4
6
8
10
12
14
TIME (s)
L3/L2


0·2
1·0
0·8
0·6
0·4
0·2
0
PHASE
0
2
4
6
8
10
12
14
TIME (s)
L4/L3

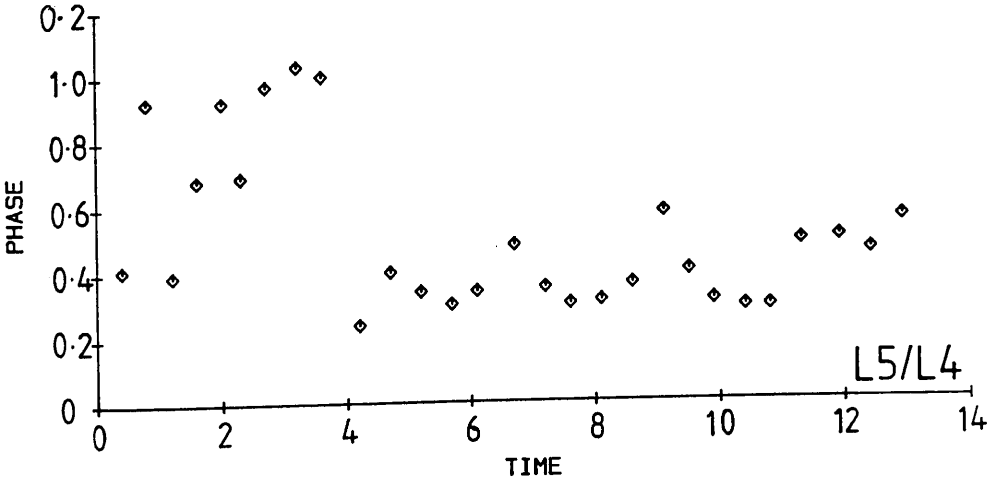
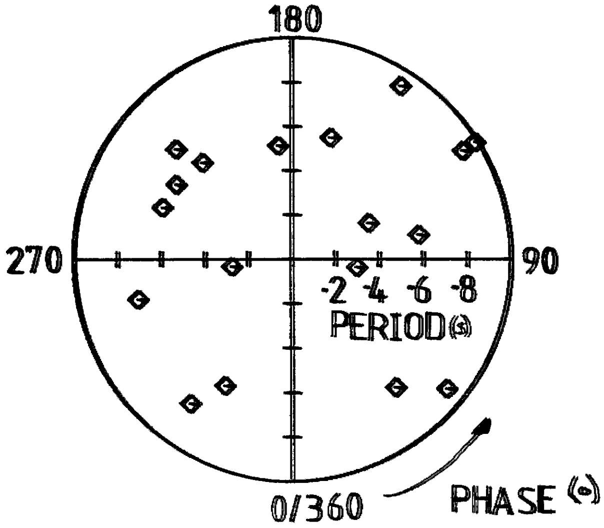

0·2
1·0
0·8
0·6
0·4
0·2
0
PHASE
0
2
4
6
8
10
12
14
TIME
L5/L4

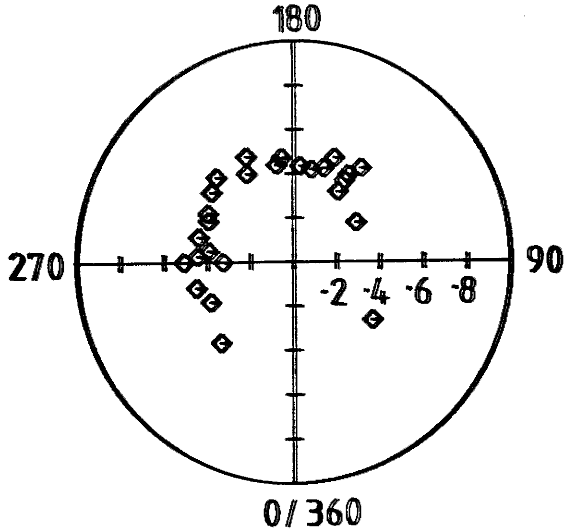
Fig. 11

Same data as shown in Fig. 9, replotted as circular plot. Axes, period (s). Angle, phase position.$^{(\circ)}$

L3/L2



L4/L2



L5/L2

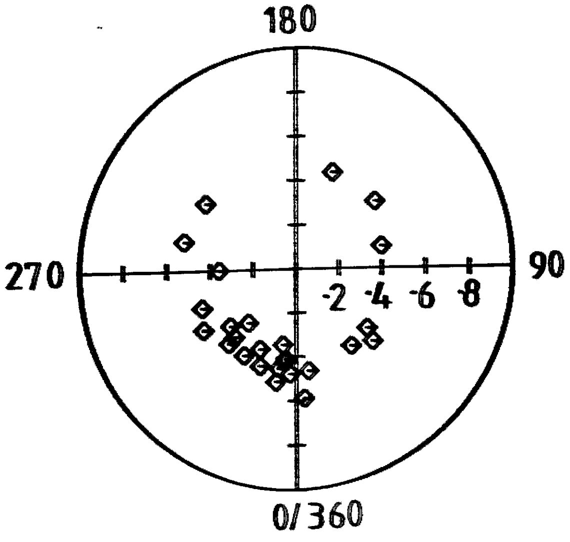
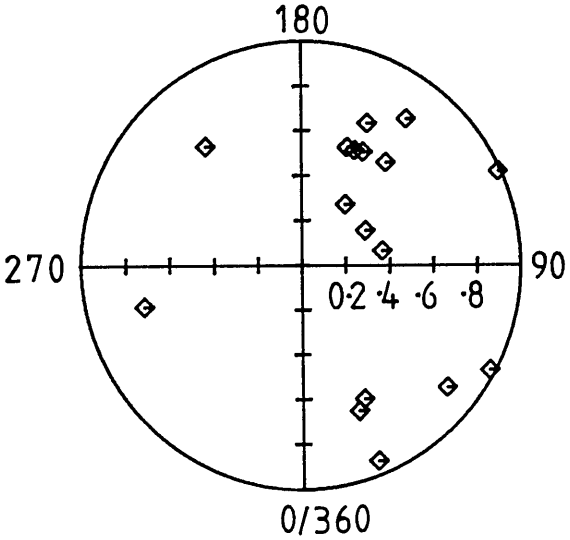


Fig. 12

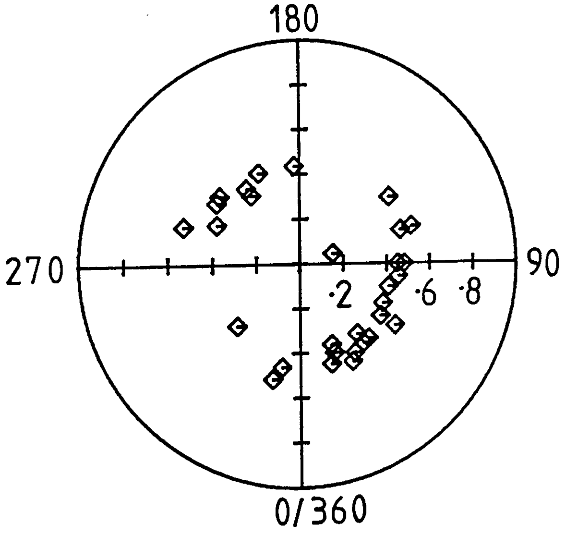
Data from Fig. 10 replotted as circular plot. Axes, period* (s). Angle, phase position.$^{(o)}$

(* of test appendage)

L3/L2



L4/L3



L5/L4

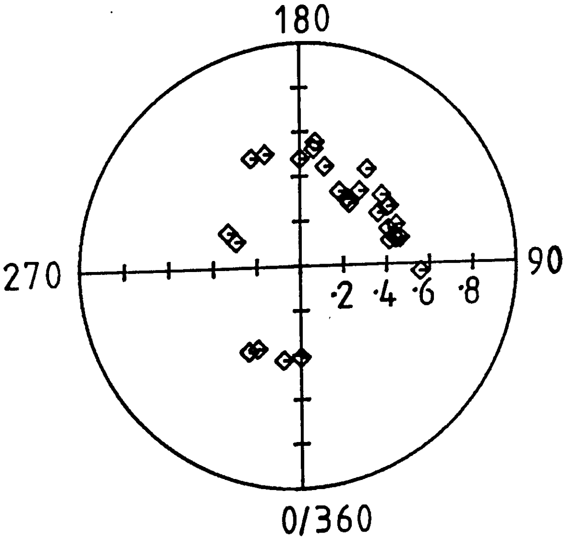


Fig. 13

Plots of period against time for L2-5 during walking.

a : L2

b : L3

c : L4

d : L5

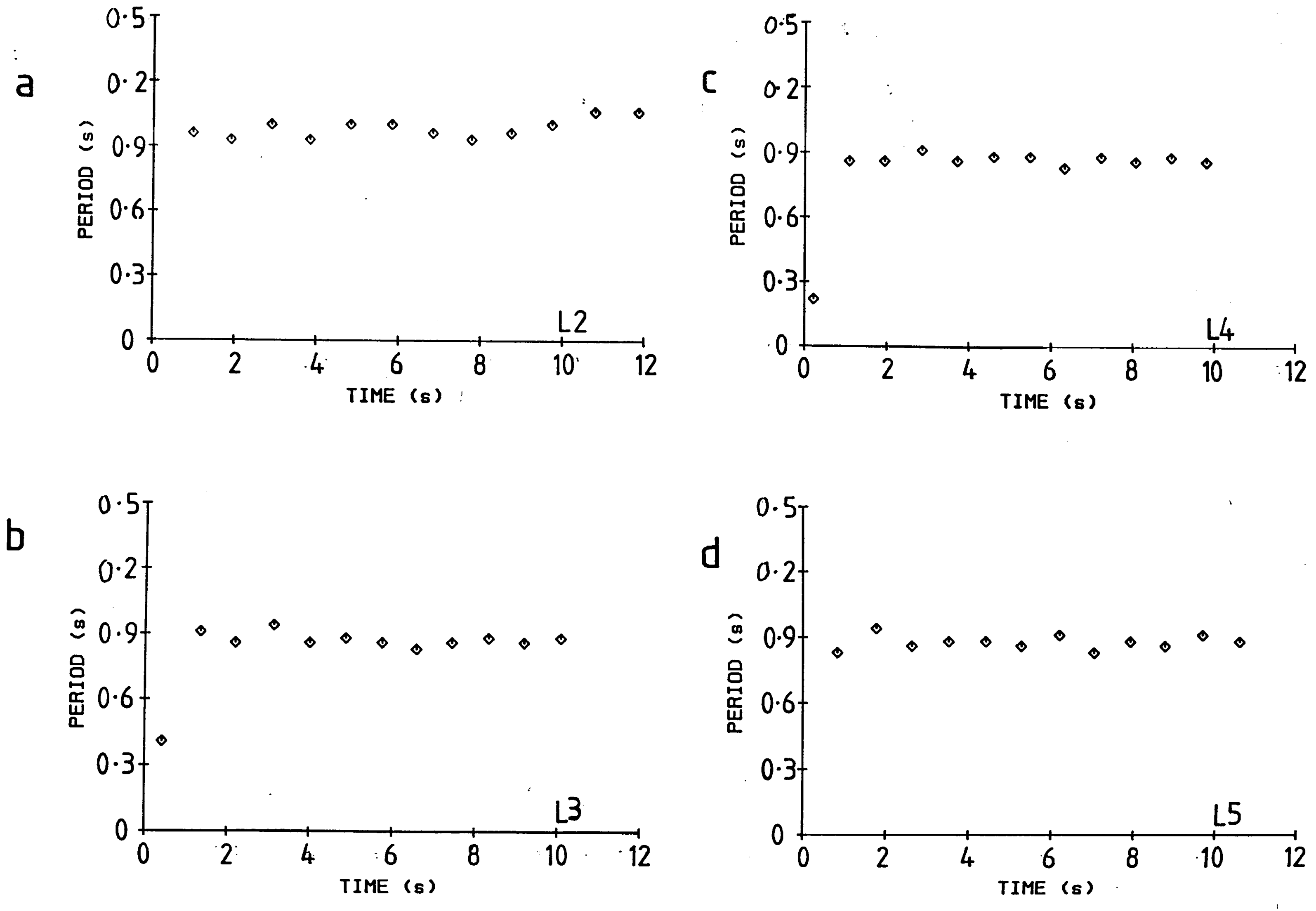
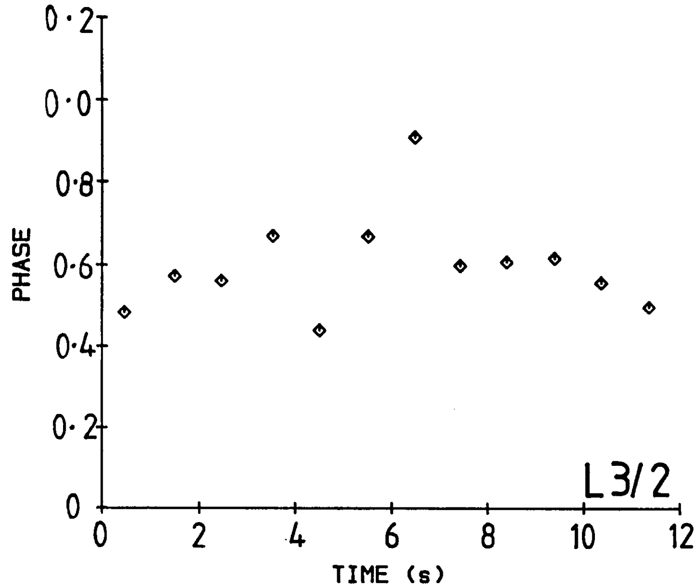
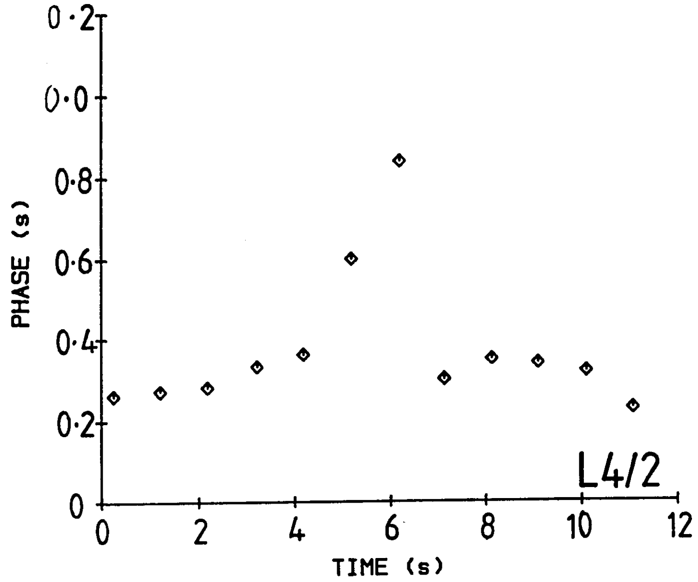

a
PERIOD (s)
0·5
0·2
0·9
0·6
0·3
0
0
2
4
6
8
10
12
TIME (s)
L2
c
PERIOD (s)
0·5
0·2
0·9
0·6
0·3
0
0
2
4
6
8
10
12
TIME (s)
L4
b
PERIOD (s)
0·5
0·2
0·9
0·6
0·3
0
0
2
4
6
8
10
12
TIME (s)
L3
d
PERIOD (s)
0·5
0·2
0·9
0·6
0·3
0
0
2
4
6
8
10
12
TIME (s)
L5

Fig. 14

Plots of phase against time for L3-L5 in L2; same data set as Fig. 13.


0·2
0·0
0·8
0·6
0·4
0·2
0
PHASE
L3/2
0
2
4
6
8
10
12
TIME (s)


0·2
0·0
0·8
0·6
0·4
0·2
0
PHASE (s)
L4/2
0
2
4
6
8
10
12
TIME (s)

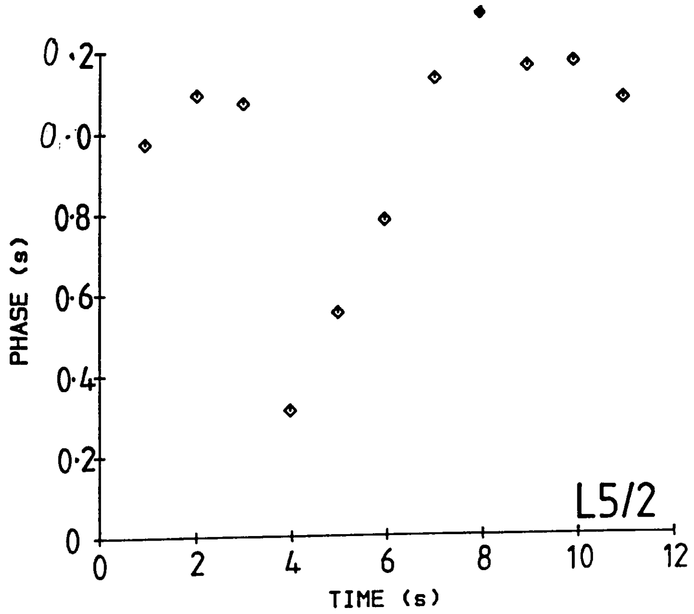
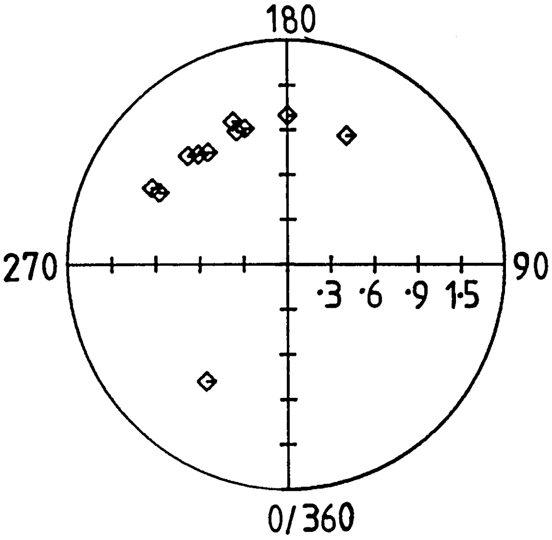

0·2
0·0
0·8
0·6
0·4
0·2
0
PHASE (s)
L5/2
0
2
4
6
8
10
12
TIME (s)

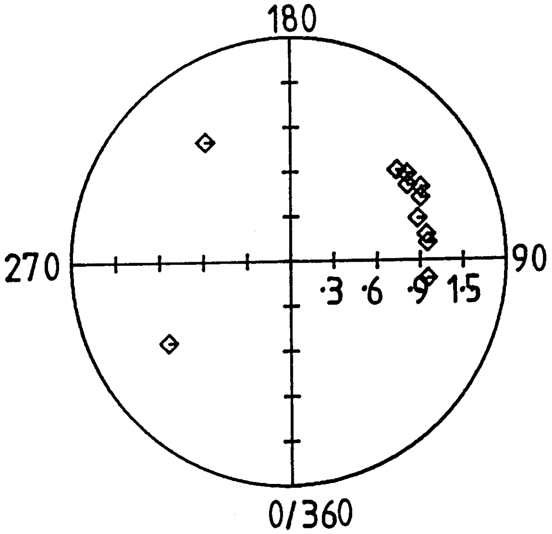
Fig. 15

Circular plots of the data in Fig. 14. Axes, period (s). Angle, phase position.

L3/L2



L4/L2



L5/L2

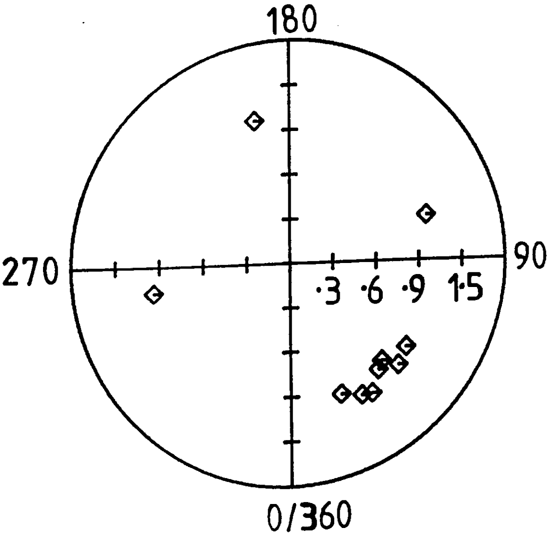
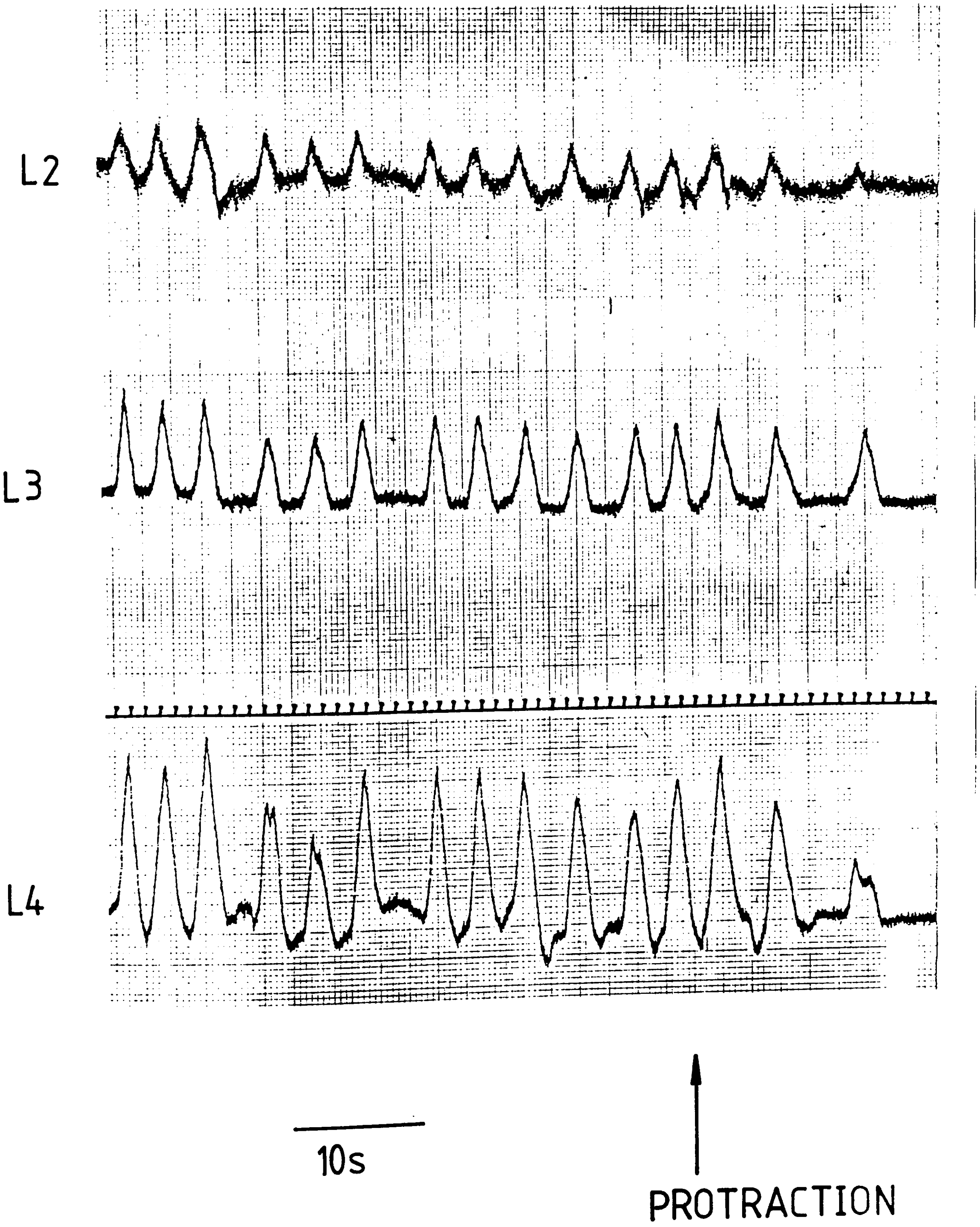


Fig. 16

Slow waving activity in L2-4. L5 remained on the substrate.



SLOW WAVING

Fig. 17

Plots of period against time, and composite plot of L3&4 in L2 during slow waving.

a : L2

b : L3

c : L4

d : Phase plot. (L3 & L4 in L2)

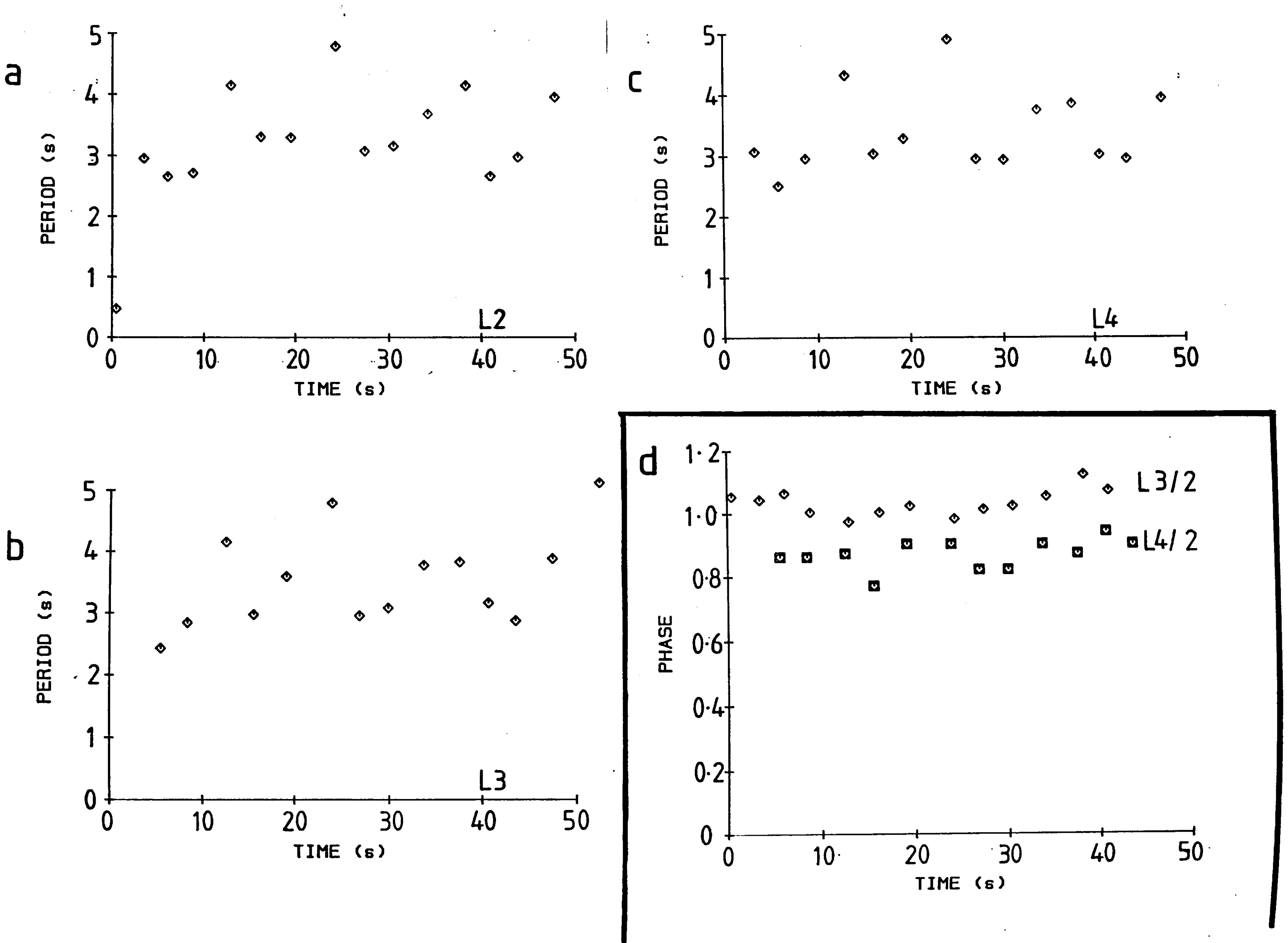
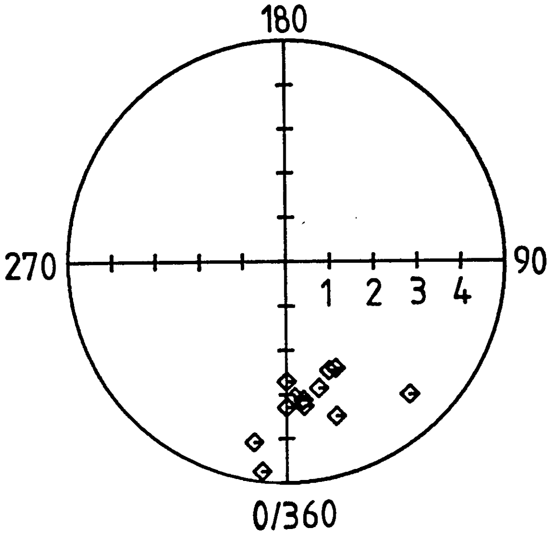

a
PERIOD (s)
TIME (s)
L2
b
PERIOD (s)
TIME (s)
L3
c
PERIOD (s)
TIME (s)
L4
d
PHASE
TIME (s)
L3/2
L4/2

Fig. 18

Circular plots of phase data in Fig. 17. Axes, period (s). Angle, phase position.

L3/L2



L4/L2

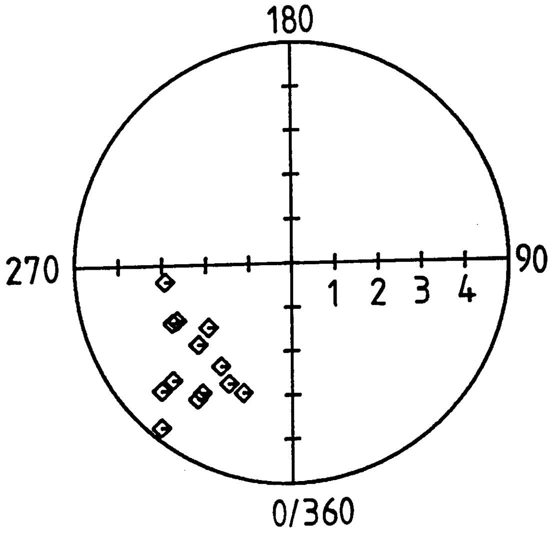
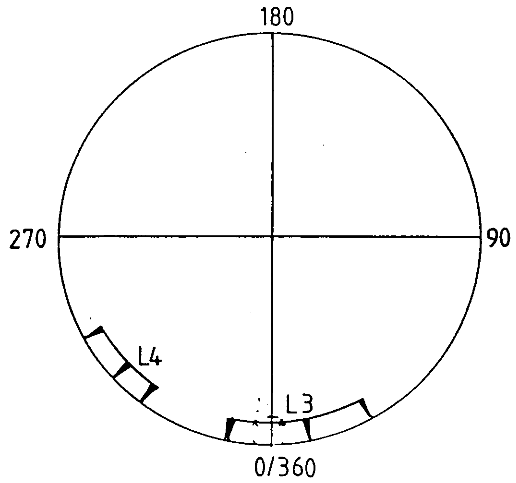
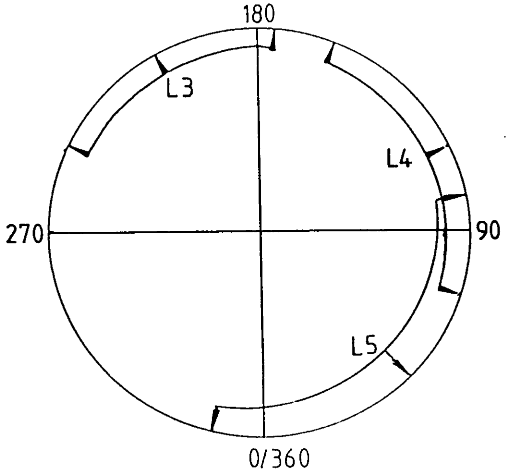


Fig. 19

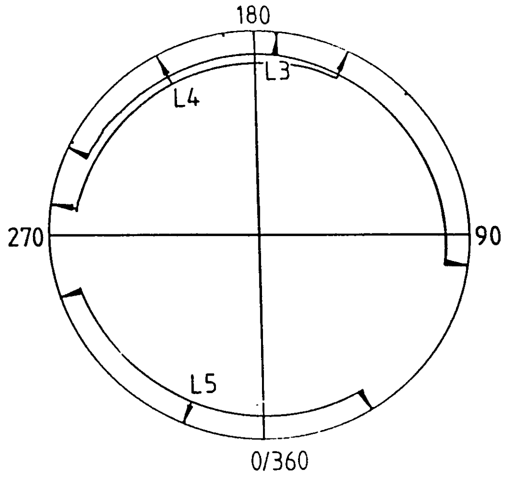
Comparison of mean phase relationships of legs during the three patterns of leg activity reported in this chapter. Circular plots of circular mean +/- circular standard deviation for each active leg.



WAVING



WALKING



CYCLING

Chapter 4 :

EFFECT OF TILT ON ABDOMINAL SYSTEMS.

A. Introduction.

Abdominal motor systems will be discussed in this chapter. The uropod responses in both pitch and roll have been described elsewhere and will not be considered in any detail here (see Newland, 1985). Swimmerets have received some attention in regard to their responses in the roll plane (Miyan, 1982), but in this chapter their responses in the pitch plane will be examined. The other system of importance is the axial abdominal musculature itself and the effect of tilt on abdominal posture will be dealt with in some detail.

Abdominal motor systems have been studied from various points of view. Perhaps the best known work has been on the escape reflex of the crayfish which comprises single or sequential fast flexions and extensions of the abdomen (Wine and Krasne, 1982). Work on this particular aspect has now been extended to other species, notably *Nephrops* (Newland, 1985) and *Galathea strigosa* (Sillar and Heitler, 1985a,b&c).

The axial muscles of the abdomen may be divided into two main groups: the slow or tonic muscles which are utilised for postural movements, and the fast or phasic muscles which are involved in escape responses. Both groups may be further divided into those muscles which are responsible for flexion, and those which are responsible for extension of the abdomen.

Various methods of stimulation have been employed to activate both the fast and the slow muscle systems. Tactile inputs are important for eliciting the escape reflexes of both crayfish (Wine and Krasne, 1972) and *Nephrops* (Newland, 1985). Tactile inputs also play a role in the abdominal postural system (Kotak and Page, 1986). However the most widely used technique has been that of command fibre activation. The command fibres for escape responses are the giant fibres, which run the length of the nerve cord. Because of their large size, they are relatively easily stimulated electrically (Johnson, 1926; Larimer et al, 1971). The response to electrical stimulation is very similar to that produced by tactile stimulation (Wine and Krasne, 1982).

Electrical stimulation was also used to discover interneurons in the circumoesophageal connectives which produced a range of abdominal postures (Bowerman and Larimer, 1974).

One method for producing abdominal extension that has become widely used is the removal of substrate contact (Page, 1975; Williams and Larimer, 1981; Takahata and Hisada, 1985). As the results in this chapter will demonstrate, in *Nephrops* this response is dependent on tilt as well as the loss of substrate contact.

Another finding reported in the literature is of interest. That is the interaction of abdominal posture with other abdominal motor systems. It was noted by Yoshino et al, (1980) that uropod responses to tilt were only expressed when the abdomen was either extended or in the process of extending. However, the statocyst information necessary for the uropod response has been shown to be present regardless of the abdominal posture (Takahata and Hisada, 1985). It is still uncertain as to where the interaction between the two systems takes place. Similar observations have also been made for the swimmerets (see also chapter 6). It is not clear whether the observed interaction between abdominal posture and swimmerets is mechanical or neuronal in nature.

Most studies of abdominal extension have been carried out from the point of view of command fibre involvement (eg. Williams and Larimer, 1981; Miall and Larimer, 1982). This chapter will report the effect of tilt on the abdomen.

In some species rotation about the thoraco-abdominal joint has been observed in response to tilt in the roll plane. This rotation of the abdomen has not been observed in *Nephrops*. However it will be shown that there is a clear abdominal postural response in the pitch plane.

The swimmerets are well known for their asymmetric response to tilt in the roll plane (Davis, 1968). As a system they have also attracted attention from the point of view of central pattern generation (Heitler and Pearson, 1980; Heitler, 1985), command fibre control (eg. Ikeda and Wiersma, 1964; Davis and Kennedy, 1972) and intersegmental coordination (Davis, 1969; Stein, 1971; Paul and Mulloney, 1986). Recently a model of the system was proposed (Neil and Miyan, 1986). The importance of statocyst input in this model is clear, but there has been an assumption that the output of the system is stereotyped. The swimmerets are assumed to exhibit only two type of beat, either lateral, in response to roll, or rearward back in most other circumstances. However until now the swimmeret responses to tilt in other planes have not been systematically investigated. As will be shown, the types of swimmeret beat observed in response to tilt in the pitch plane suggest modifications in the model of Neil and Miyan (1986).

B. Materials and methods.

The responses of both abdomen and swimmerets to tilt were recorded using a video system which allowed single frame analysis from tape. The tilting apparatus used was the same as that described in the previous chapter. To allow measurement of abdominal posture, the animal was viewed from the side during tilting. When the reponses of swimmerets were recorded the camera was hand held such that it was perpendicular to the most caudal pair of swimmerets. To improve visualisation of the swimmerets, the last pair were painted black using a mixture of black paint and cyanoacrylate C2 glue. It was found that the paint alone flaked off after a short time. The midpoints on the two most posterior sternal ribs were also painted as calibration points.

The video data obtained allowed quantitative measurements to be made of various aspects of the abdominal posture. The coordinates of various points on the screen were obtained from single frames using a video analyser, comprising crosshairs controlled by a joystick. Coordinates obtained in this manner were dumped via an interface to a computer, where they could be stored on disc or printed.

Fig. 1 shows the eight points of which coordinates were taken. The coordinates were plotted, and joined by lines in the manner shown in Fig 1. The angle of tilt was measured as the angle between horizontal and a line drawn from the tip of the rostrum to the dorsal end of the cephalo-thorax (points 1 and 2, angle of tilt is angle a, Fig 1). The angle between each line joining successive points from 2 to 8 and the line defining the angle of the thorax (between points 1 and 2) was measured. This effectively provided a measure of the angle of each abdominal segment 3-6 and the telson. The positions of the thoraco-abdominal joint and the first abdominal joint could not be resolved separately. However, the angle of line 2-3 provided the resultant of the angles of these joints. Thus in the figures of segmental angles the segments are designated 1/2,3,4,5,6 and the telson T. As the final position adopted by the telson is the cumulative resultant of the angles of the individual segments, the angle between the telson (line 7-8) and the thorax (line 1-2) provided a useful parameter for preliminary investigation of the effect of tilt on posture. Flexion is denoted by negative values in degrees and extension by positive values.

Two types of analysis were performed on the swimmerets. Using the coordinate system described above, the coordinates of the points shown in Fig. 9a. were obtained ie the coordinates of the ends of the rami (Fig. 9a. points 1&2) the distal end of the basipodite (3) and the calibration points (4&5). The angle of the powerstroke phase of the beat was defined as the the angle between the bisector of angle 1-3-2 and the midline (the extension of line 4-5).

The second type of analysis performed on swimmerets was temporal analysis of the period of beating. A timing signal mixed with the video signal provided individual times for each frame to the nearest 0.02s. This was adequate for resolving the time taken for both the power- and returnstrokes.

Information on the period of the beat was also obtained using the movement monitor system described in the previous chapter. This method allowed the simultaneous monitoring of several swimmerets, and thus swimmeret phase relationships under various conditions could be examined.

In some experiments the statolith, ie the clump of loosely aggregated sandgrains which lies on the floor of the statocyst sac was removed as follows. The animal was removed from the tank and clamped, by means of the rod attached to is back, underneath a dissecting microscope such that the dorsal surface of the base of the antennule could be seen. The eye on the side to be operated upon was held out of the way by a loop of thread. The dorsal surface of the statocyst sac was removed with a scalpel and a fine jet of water directed into the lumen of the sac. This was usually sufficient to remove all the lith material.

C. Results.

1. Abdominal posture in the absence of tilt.

The abdomen of *Nephrops* consists of six segments and the post-segmental telson. Segments 2-5 bear paired swimmerets and segment 6 the paired uropods. The uropods are homologous with the swimmerets. Contractions of axial muscles in each segment produce flexion and extension about each segmental joint.

Animals were observed in holding tanks and walking freely on the bottom of the experimental tank to obtain some impression as to the normal abdominal posture. Animals standing quietly held the abdomen slightly flexed. This usually meant that the hairs along the posterior fringes of the uropods were in contact with the substrate. The cephalothorax under these conditions was tilted head-up (Fig. 1).

During unrestrained walking the cephalothorax was also held head up. The abdomen retained the same posture as that seen during quiet standing on most occasions, but sometimes, particularly when the animals were moving quickly, the abdomen extended.

As has been reported in the literature for crayfish (Williams and Larimer, 1981), and for *Homarus* (Davis, 1968), the removal of substrate also caused a marked abdominal extension in *Nephrops*. In the absence of tilt, the abdomen flexed after a short time and swimmeret beating ceased.

2.(i). Effect of tilt on abdominal posture.

The patterns of postural activity that will be described could be elicted from all animals with no great difficulty. Although no asymmetric abdominal rotation was observed in response to roll, rolling an animal about the longitudinal axis did produce an extension of the abdomen along with the other characteristic equilibrium responses, e.g. asymmetric responses of the swimmerets, uropods and legs (chapter 3). When the animal was brought back to the normal position the extension posture was held and symmetrical swimmeret beating continued for a few seconds (or up to a minute in fresh animals), followed by abdominal flexion and the cessation of beating.

There was a clear postural response of the abdomen to tilt in the pitch plane. The head-up response consisted of a marked flexion of the abdomen, whereas the head-down response involved a clear abdominal extension (Fig 2).

These tilt reponses in pitch were abolished with slight tilt in the roll plane (as small as 5° in some cases). The relevant roll response accompanied by abdominal extension then appeared. It seemed that the head-up response was more easily dominated by roll responses than the head-down response.

2.(ii). Relationship between posture and tilt.

The total extent of flexion/extension of the abdomen (as defined in Methods) was plotted against the magnitude of tilt (Fig. 3). The difference between head-up and head-down is clear.

While there is some variability in the response, it can be seen that increases in the extent of tilt either head-up or head-down does not produce greater flexion or extension. Rather the response seems to be "all or nothing". Tilting head down by only a few degrees produces extension. To produce flexion, the animal must be tilted head-up by more than 10° . Head-up tilt of less than 10° produces extension, so that the switch between the two responses occurs not at 0° but at approximately 10° head-up.

2.(iii). Differences between segments.

To further study these postural responses, the state of extension/flexion of individual segments was studied during tilt at various angles. Once again there is a degree of variability, but a clear pattern emerges.

The angle measured for the abdominal segments and telson (as defined in **Methods**) relative to the thorax is illustrated in graphical form in Figs. 5&6. These exclude data from tests of angles of less than 10° head-up which is dealt with separately. Given that the overall posture is not dependant on the magnitude of tilt but only whether it is head-up or head-down, the data from a number of tests at different angles are superimposed for each of these two conditions (Fig. 4a & 5a). A mean and standard deviation for each segment was calculated and plotted (Figs 4b&5b). These plots show that although the overall posture looks very different in the two conditions, ie flexion or extension, the underlying state of the individual segments relative to each other is similar in both head-up and head-down tilt. Looking at the pattern along the segments, the more posterior segments tend to be less extended or more flexed. Comparing the two curves, any segment will be less extended or more flexed in head-up tilt compared with head-down tilt. Fig. 7 shows the mean values for each segment. When these are compared using the standard t-test the differences between the means are all highly significant, with the exception of segments 3 and 4.

Four tilts of less than 10° head-up were plotted and analysed in the same manner as discussed above (Fig 6, see also Fig. 7c). It is clear that the pattern seen at these angles is very similar to the head-down pattern. Indeed, t values obtained for comparisons between these tilts and head-down are low showing no significant difference, whereas those obtained for the comparison with head-up tilts are high giving significant differences for segments 1/2,4,6 and the telson (for details of Fig. 7. see figure legend).

2.(iv). Effect of statolith removal.

The liths were removed bilaterally from the statocysts and the animals tilted as before. There was no difference in the normal resting posture when the legs were in contact with the substrate. However there was no postural response of the abdomen to tilt.

The posture adopted by the abdomen in the absence of normal statocyst input consisted of extension of the anterior segments and flexion of the posterior ones. This resulted in a posture which was an extension in terms of the criterion used above. Two examples are shown (Fig.8, see also Fig.3, diamonds). Fig. 8a. shows the comparison of a 10° tilt head-down with the liths removed (solid line) with the similar response before lith removal. Fig. 8b. compares tilts of 36° before and after statolith removal. The effect of the procedure is to produce a posture intermediate between that normally seen in response to the two types of tilt.

3. Effect of tilt on swimmerets.

The neuromuscular basis of the response of swimmerets to tilt in the roll plane has already been described in some detail elsewhere (Neil and Miyan, 1986). However no quantitative measure of the beating itself was made. Thus for the purpose of comparison between the responses to tilt in the pitch and roll planes various parameters of the swimmeret beat were measured under three conditions: roll, head-up pitch and head-down pitch.

3(i). Roll.

The most noticable feature of the swimmeret response to roll is the asymmetry of the response. The side-up swimmerets beat laterally, and the side-down swimmerets either stop beating, or beat straight back. The angle of the asymmetric lateral beat was measured (Fig. 9, see Methods), and was found to be 41+/-6° (see Fig. 22).

As well as the angle of the beat, the period of the beat is also of interest. The overall mean period calculated for lateral beating during roll was 0.36+/-0.04sec (n=126; see also Fig. 22). However, variations of period of the beat were found with time. Fig. 10. shows the period of the lateral beat plotted over twenty seconds and comprises fifty-five beats following side-up tilt of 30° . Clearly in this case as time proceeds the period increases. This phenomenon of the beat slowing occurred frequently. However there was also evidence in other cases of the beat period decreasing with time (eg see Fig. 11). Only very rarely in roll did the side-up swimmerets stop beating completely, and when this did occur, it was in fatigued animals.

3.(ii). Head-up pitch.

The swimmerets were active during tilt in the pitch plane. As might be expected, the type of beat observed was always symmetrical. Head-up pitch produced a form of beating in which the swimmerets moved directly backwards and forwards on each side. The angle of the powerstroke (as defined in methods) was 3+/-8° (see Fig. 22).

The mean period of swimmeret beating during head-up pitch measured over a large number of samples in several animals was 0.36+/-0.07 sec. This was not significantly different to that for roll (see Fig. 22). However on several occasions a very slow form of beating was observed; and in some cases there was no beating at all.

Information about the swimmerets in head-up pitch was also obtained using the movement monitor system described in chapter 3. The major advantage of the movement monitor was that it allowed several swimmerets to be studied simultaneously. Thus it was possible to gather some data on phase relationships between swimmerets in both head-up and head-down beating. Fig. 11. shows a plot of period against time for three swimmerets, S2, S3 and S4, during head-up tilt. The mean period for each swimmeret is 0.58sec (see Fig. 17).

Phase values were calculated for S3 and S4 in S2 and these were plotted against time (Fig. 11.). The circular plot of this data is shown in Fig. 12. The coordination of the system is demonstrated by the close packing of the data points. The phase difference between S3 and S4 is approximately 0.5 phase units.

3.(iii). Head down pitch.

Pitching the animal head-down always produced swimmeret beating. The beating usually continued until the animal was returned to the normal position. The beating was bilateral ie. the swimmerets beat with a laterally directed powerstoke. The angle of the beat was 25+/-8° (Fig. 9a). This angle of beat is very much less than that seen in asymmetric beating in response to roll (41+/-6° Fig. 9c, Fig. 22).

The period of beating calculated from the video was again very similar to the other two types of beating at 0.37+/-0.05 sec. The period calculated on data obtained using the movement monitor was greater than this at 0.47sec. (Fig. 17).

There is again a good deal of variation in the period when plotted against time and also variation in the phase values calculated for S3 and S4 in S2 (Fig. 13). The circular plots show the same general pattern as in head-up beating (Fig. 14).

Figs 15. and 16. are circular plots of the circular mean and circular standard deviation of the phase positions of the swimmerets during both head-up and head-down tilts. Note that in both cases S3 and S4 are separated by approximately 0.5 phase units.

4. Power and returnstroke variation.

To further investigate the various types of swimmeret beat observed, the relationship between power- and returnstrokes was examined. It is of interest to discover whether the variation and the slowing of the beat, is accomplished by systematic variation of one or other part of the cycle.

The plots of the values obtained for asymmetric beating in roll are shown in Fig. 18 and the regression values are shown in Fig. 21. Both the returnstroke and powerstroke durations increase with increasing period. However, the returnstroke gradient is steeper than the powerstroke (Fig. 21).

The same result is found for both head-up beating (fig. 19) and head-down beating (Fig.20; see Fig 21). In all three types, the gradient of the regression line is steeper for returnstroke than powerstroke. The correlation coefficient is also greater for each returnstroke plot than the corresponding powerstroke plot. However these differences are not statistically significant.

D. Discussion.

The results reported in this chapter show that tilt in the pitch plane produces effects not yet described in the literature, which complement the findings already made on the effects of tilt in the roll plane (eg. swimmeret reflexes, Davis, 1968; Miyan, 1982; uropods Yoshino, 1980; Newland, 1985). Some of these effects are clear eg. the effect of tilt on abdominal posture and the angle of the swimmeret beat. However, some of the results are not clear and these will be discussed first.

Measurements of swimmeret beating parameters using the movement monitor system entailed increased load on the swimmerets used due to the monitor wires. This may explain the increased period of the beats and the cycle to cycle variation or"jitter" in the period measured. However, trends in the drift of period with time appeared not only in the data obtained using this method, but also that obtained from the video (eg Figs. 10&11).

Data concerning phase relationships of swimmerets was obtained using the monitor system. However in this case, all the swimmerets are subjected to similar conditions, and the comparison is between these swimmerets. The period is very similar for the three swimmerets in each of the two experiments illustrated (Fig. 17), although it is longer than the mean periods calculated from the video data. The phase calculations show that S2 and S3 beat in phase or 0.25 units out of phase in head-up and head-down beating respectively. However, the relative difference between S3 and S4 is very similar in both conditions (approxiamtely 0.5 phase units). Qualitative observation of the phase relationships did suggest that particularly in head-up beating the swimmerets did beat almost in antiphase with their neighbours. Thus the phase relationships between swimmerets may be different in certain conditions from the value of 0.25 phase units, which has appeared in the literature (eg. Laverack et al, 1976; Paul and Mulloney, 1986).

The overall function of statocyst-mediated extension and flexion of the abdomen, along with the action of the swimmerets under these conditions, clearly has to be seen in terms of the control of body orientation in concert with the equilibrium reflexes of uropods, swimmerets and perhaps also the legs (see chapter 3).

The swimmeret beating in head-up tilt occurs along with the abdominal flexion. However the flexion is never so pronounced that it mechanically impedes swimmeret beating to any extent, except perhaps in the case of the most caudal pair of swimmerets. The forces produced by the beating which is directed straight back will be significantly shaped by the posture of the abdomen and the telson and uropods. This positioning may be designed to redirect the forces in a downward direction, helping to correct for the head up attitude. The uropods are symmetrically open during head-up tilt and this may aid redirection of the flow produced by the beating of the swimmerets.

The type of beating observed in head-down tilts is perhaps of greater significance. This type of "half-lateral" beating is distinct from the fully laterally directed beat seen in roll. If the latter is assumed to be to produce a righting torque to correct for the imposed tilt, then the function of the half-lateral beat may not simply be to produce forces which would act to bring about the normal posture. Other studies have shown that when *Nephrops* is released in mid-water and allowed to "free-fall", it will often control orientation in the roll plane, and then swim to the substrate with a slightly head-down attitude. Thus the function of the type of beat observed in head-down tilt may be to produce forces which will both stabilise and propel.

The lateral beat is produced by the contraction of particular muscles (M9,10&13) in the basipodite of the swimmeret in response to descending statocyst input (Miyan and Neil, 1986). It may be that statocyst input in pitch is organised to produce only a certain degree of contraction in these muscles, not the full extent seen in roll. This poses various questions about the organisation of the neuromuscular apparatus as it operates in pitch.

Protraction and retraction of the swimmeret are monitored by a sensory receptor known as strand B; this receptor is involved in a negative feedback reflex with powerstroke motoneurons. Lateral beating however is monitored by the twisting muscle receptor (TMR) (Miyan and Neil, 1986). This receptor is involved in a positive feedback reflex with muscle 10, one of the main swimmeret twisting muscles, and it is suggested that this reflex ensures complete lateral beating in response to roll. However, clearly in pitch, the positive feedback does not act to produce the magnitude of lateral beating observed in roll. The angle of the half lateral beat is constant, which suggests there is a specific degree of excitation to the muscle. Therefore the effectiveness of input from the TMR must be reduced either by efferent control of the TMR itself, or centrally in the segmental ganglion.

There is a precise switch between these patterns, with no intermediate stage, as soon as a few degrees of roll are introduced into the stimulus tilt . This switch suggests that weak input from roll interneurons is able to override strong input from pitch interneurons.

The results of experiments on abdominal posture also provide new insights into the operation of this system. Instead of considering a single joint about which flexion and extension takes place we are dealing with several joints. If in a particular condition the joints assume a particular degree of flexion and extension we are dealing with spatial patterning. This raises questions concerning the neuronal machinery for achieving the underlying motor output. Other neuronal systems for producing temporal patterns of motor output (eg locust flight, cockroach walking) have shown the importance of interaction between central and peripheral elements. In the abdominal posture system, these same elements may be involved in producing the pattern of motor output to the various segments which make up the overall posture.

Studies on the control of abdominal posture in crustaceans have looked at command fibres for abdominal posture (Evoy and Kennedy, 1967; Bowerman and Larimer, 1974; Page, 1975) and how the large numbers of fibres that have been found interact to form an efficient abdominal positioning system (Jellies and Larimer, 1985, 1986). Many of these studies were performed on isolated abdominal nervous systems using electrical stimulation. Other studies looked at the effect of sensory input into the system. Recently the finding that the loss of substrate contact causes abdominal extension (Larimer and Eggleston, 1971) has been exploited in experimental situations (Williams and Larimer, 1981; Takahata and Hisada, 1985). Tactile inputs from swimmerets have also been examined (Kotak and Page, 1986). Within the abdomen itself there are a range of reflex effects which contribute to the development of posture. In *Nephrops* flexion of the joint between segments four and five causes a marked flexion of the telson (personal observations). Fields (1966) found that discharge of the tonic (lateral) abdominal muscle receptor organ (MRO) produced firing in one of the five motoneurons supplying the tonic superficial extensor muscles. This same motoneuron had a low threshold to tactile stimulation.

We may be able to bring this information together to form an overall picture of the operation of the various elements which make up the abdominal positioning system. Thus statocyst inputs resulting from head-up or head-down tilt may act as command signals to trigger particular sub-systems for either flexion or extension postures. These sub-systems may generate the specific segmental motor output required, which may in turn be modulated where necessary by input from abdominal MRO's.

The finding that the angle of tilt in the pitch plane is an important determinant of the posture adopted by the abdomen has implications for some of the published work on this system. Most of this work has been carried out on the crayfish *Procambarus clarkii*. The normal alert posture of this species is similar to *Nephrops*, ie with the cephalo-thorax held head-up (Wine et al, 1974). When *Nephrops* is held in this position in midwater the abdomen remains flexed. If the animal is tilted until the carapace is level, then the abdomen extends. Page (1975) shows drawings of the postures adopted by *Procambarus* when the substrate is removed. An extension of the abdomen is observed. However, all the drawings show the animal held with the carapace level. The extension is discussed by Page in terms of an increased level of excitation within extension motor centres in the abdomen caused by the difference in afferent feedback due to the loss of substrate contact. However, an alternative explanation is suggested by the experiments described here: maintained abdominal extension could be due to the expression of statocyst input into the abdominal positioning system (see also chapter 5).

Fig. 1

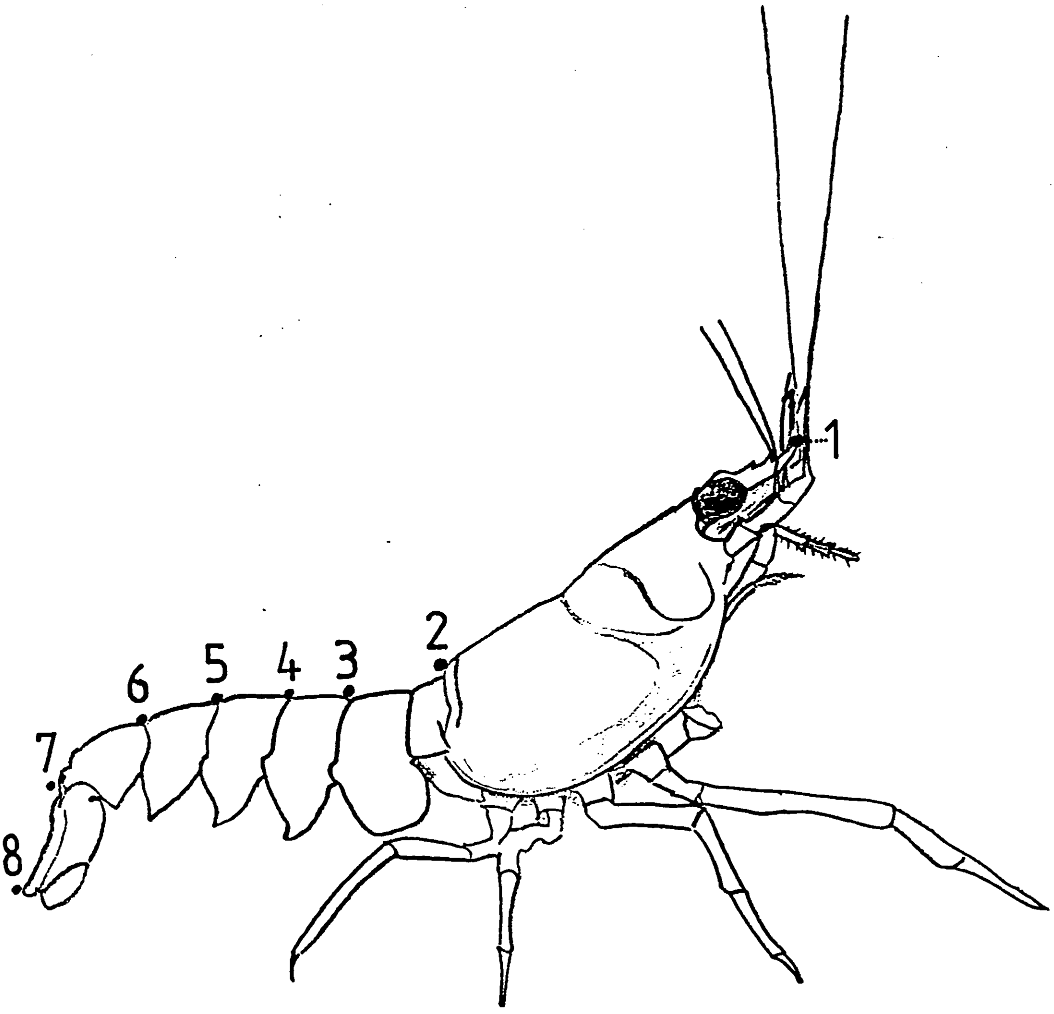
Top.

Sketch of *Nephrops* in normal posture. Note head-up position of cephalothorax. Chelipeds not shown.

Coordinates were taken at numbered points shown.

Bottom.

Points were plotted and joined. Angle of tilt and the angles of the various segments were then measured by hand. Further details in Methods.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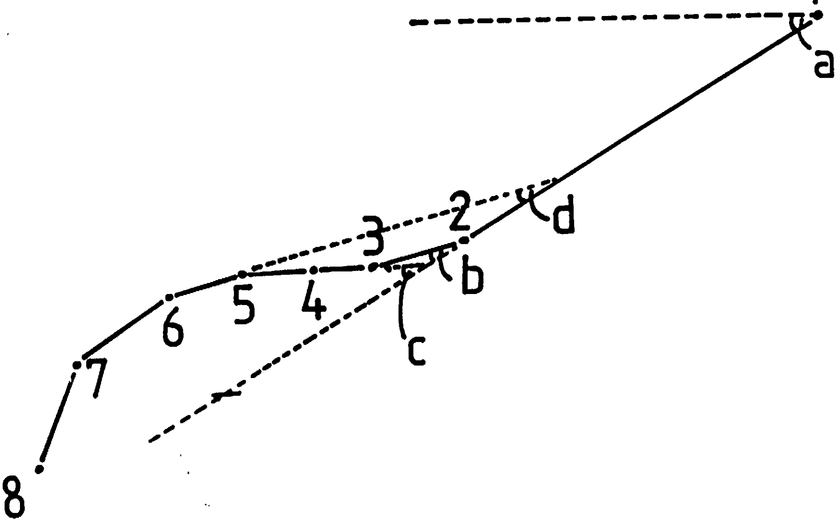

1
a
d
2
3
b
5
4
6
c
7
8

Fig. 2

Postures adopted at three postions, head-up, normal (ie slightly head-up, see Fig. 1) and head-down. Traced from single video frames of same animal and superimposed.

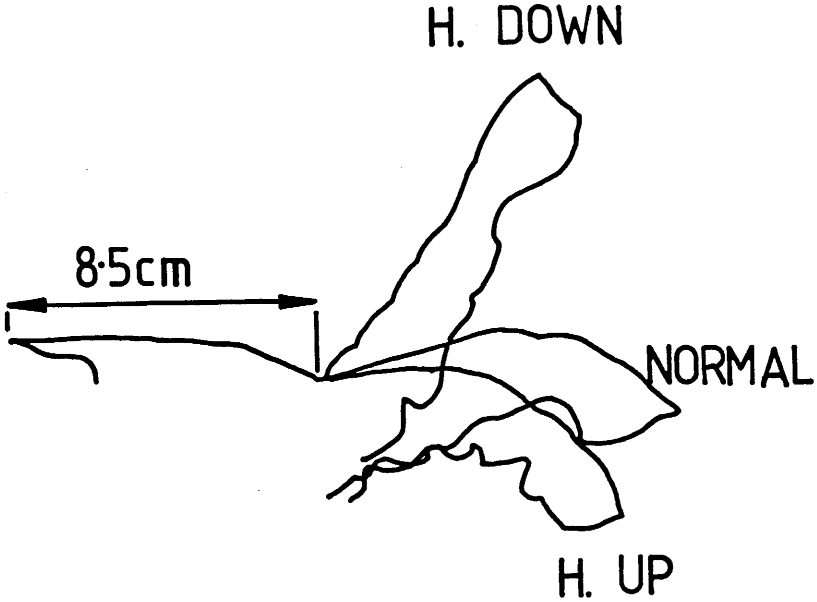

H. DOWN
8·5cm
NORMAL
H. UP

Fig. 3

Effect of tilt on overall angle of abdomen (as defined in Methods). Head-up tilt produces flexion; note that at less than 10° head-up the response switches to an extension. Head-down tilt produces extension.

Diamonds represent the results of tilting after lith removal.

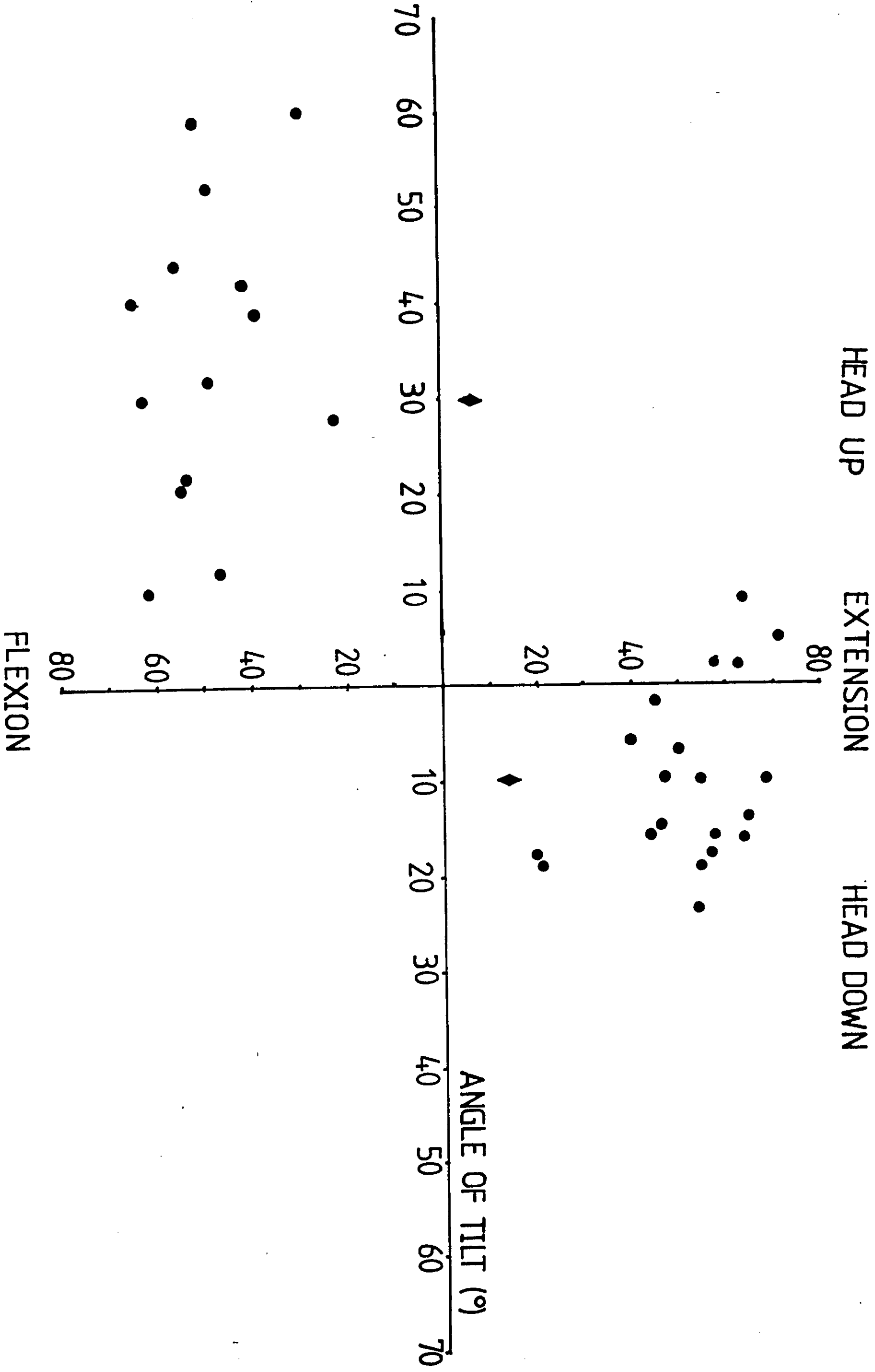
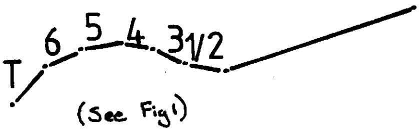
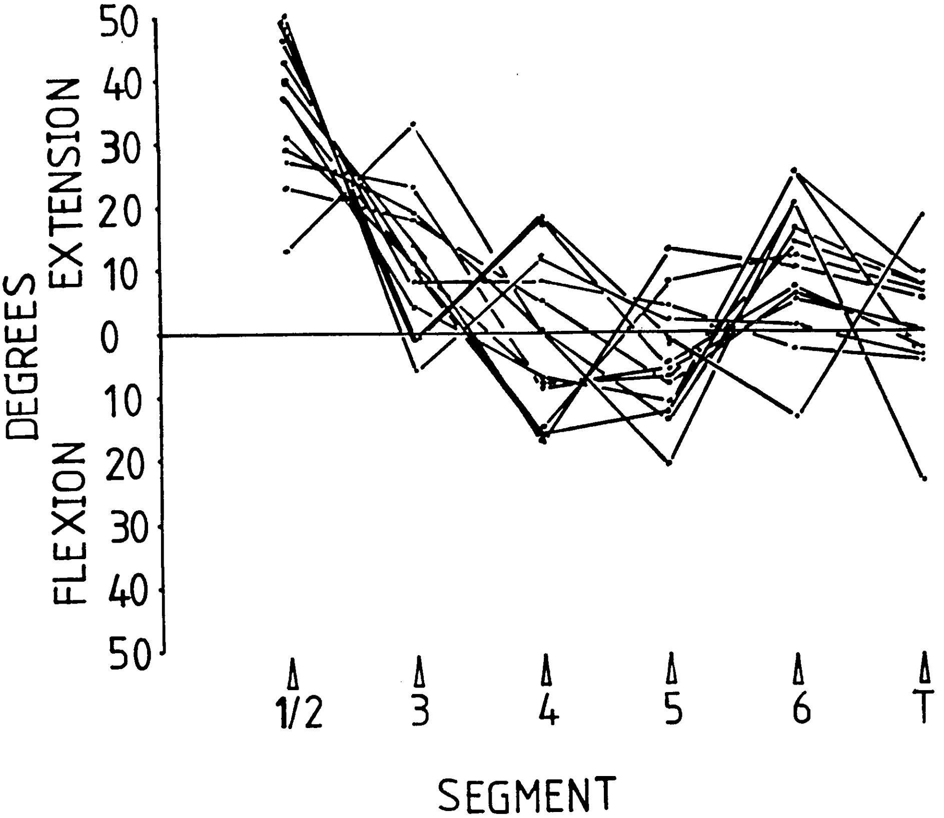

HEAD UP
HEAD DOWN
EXTENSION
FLEXION
ANGLE OF TILT (°)
70
60
50
40
30
20
10
10
20
30
40
50
60
70
80
40
20
20
40
60
80

Fig. 4

a. Angles measured for segments 1/2, 3-6, and telson during head-down tilt at various angles.

b. Mean (+/-sd.) of above angles for each segment.




DEGREES
EXTENSION
FLEXION
50
40
30
20
10
0
10
20
30
40
50
1/2
3
4
5
6
T
SEGMENT

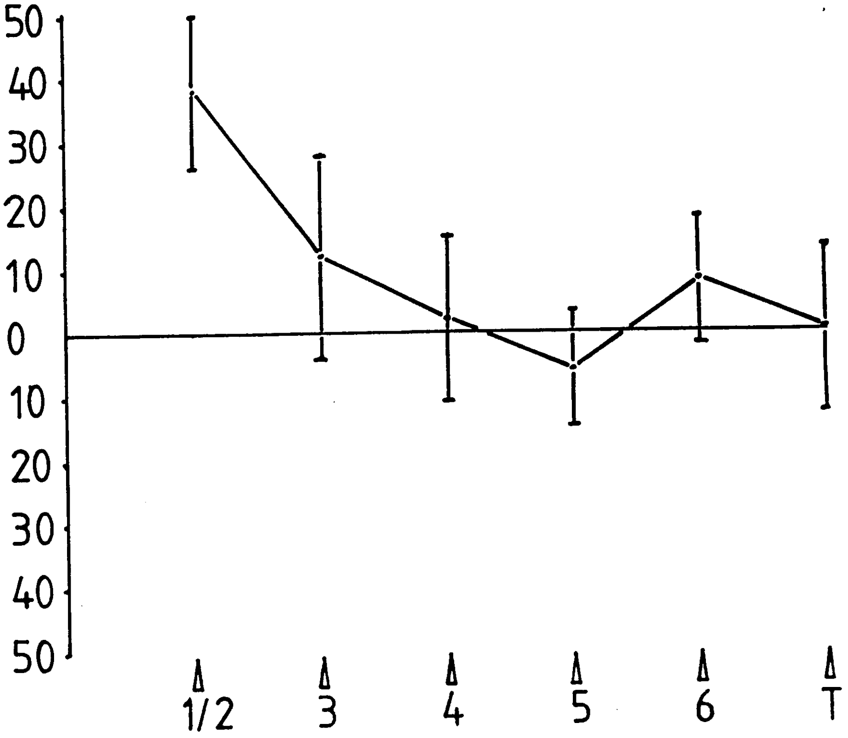
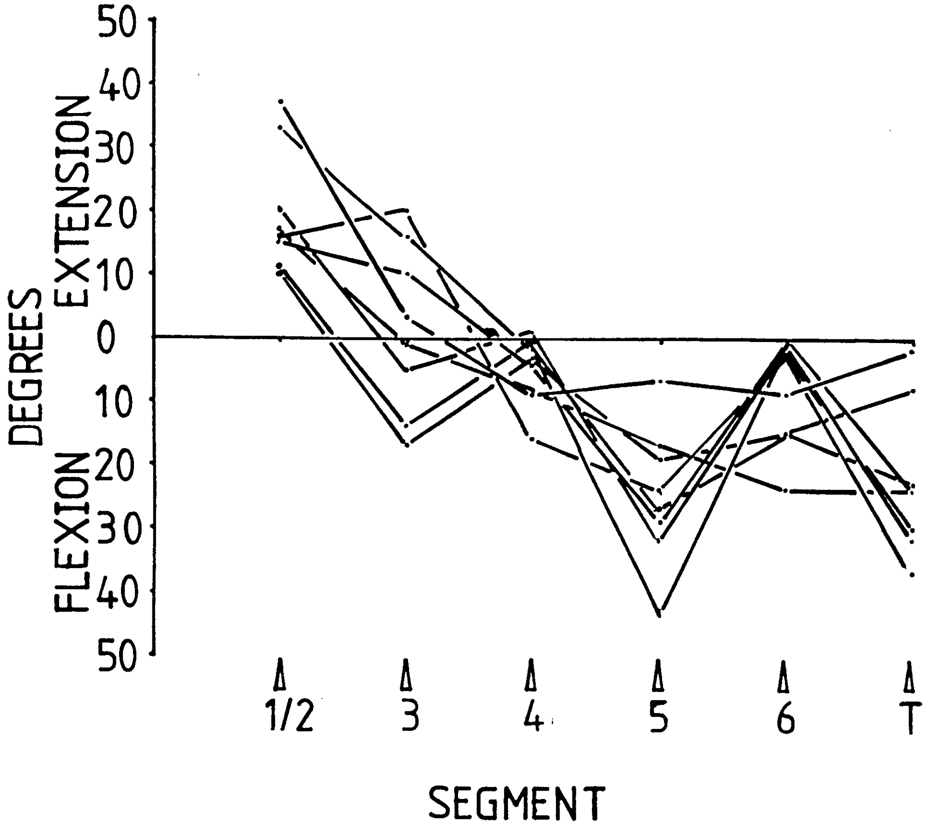

50
40
30
20
10
0
10
20
30
40
50
1/2
3
4
5
6
T

Fig. 5

a. Angles measured for segments 1/2, 3-6, and telson during head-up tilt at various angles >10°

b. Mean (+/-sd.) of above angles for each segment.


DEGREES
EXTENSION
FLEXION
50
40
30
20
10
0
10
20
30
40
50
1/2
3
4
5
6
T
SEGMENT

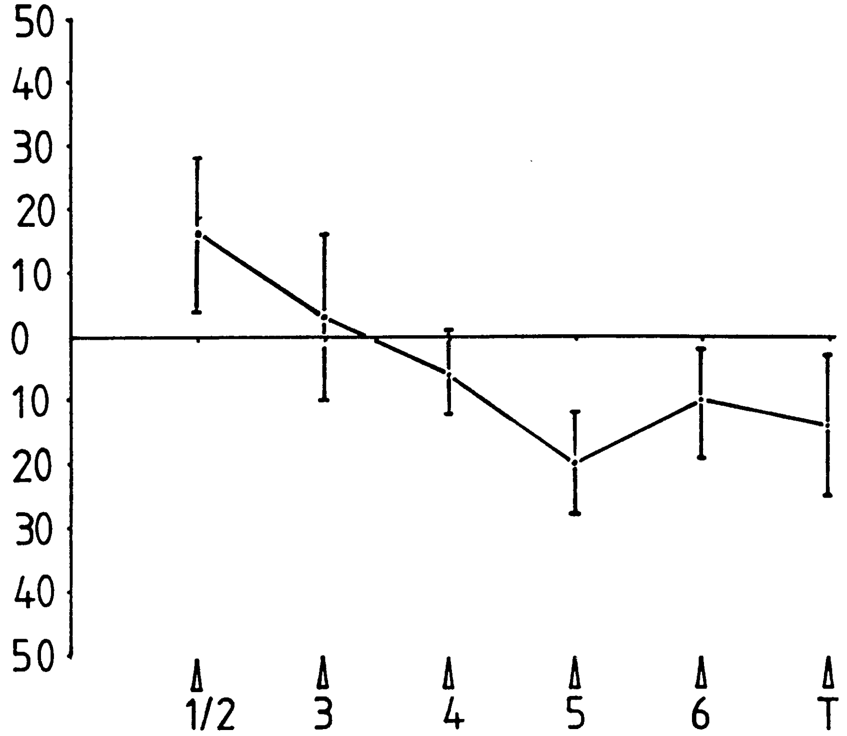

50
40
30
20
10
0
10
20
30
40
50
1/2
3
4
5
6
T

Fig. 6

a. Angles measured for segments 1/2, 3-6, and telson during head-up tilt at four angles of less than 10° .

b. Mean (+/-sd.) of above angles for each segment.

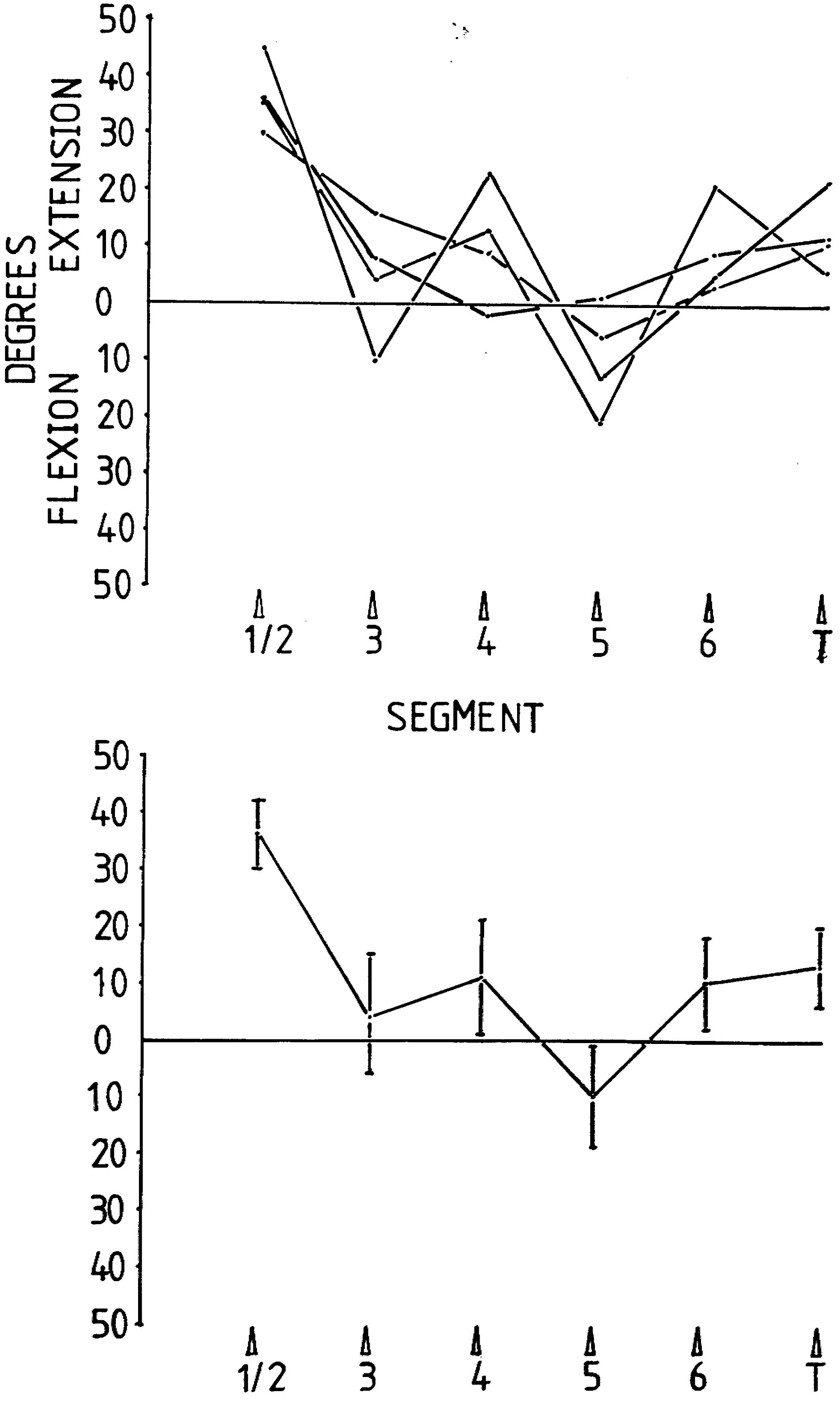

DEGREES
EXTENSION
FLEXION
50
40
30
20
10
0
10
20
30
40
50
1/2
3
4
5
6
T
SEGMENT
50
40
30
20
10
0
10
20
30
40
50
1/2
3
4
5
6
T

Fig. 7

a. Mean (+/-sd.) angles for segments 1/2, 3-6 and telson (T) during head-up tilt. Negative angles denote flexion.

b. Mean (+/-sd.) angles for segments 1/2, 3-6 and telson (T) during head-down tilt.

c. Mean (+/-sd.) angles for segments 1/2, 3-6 and telson (T) during head-up tilt at four angles of less than 10° .

d. T. values calculated on above data.

  a. Comparison of head-up and head-down.

  b. Comparison of head-down and head-up <10° .

  c. Comparison of head-up <10° with head-up (ie a.).

	SEG	1/2	3	4	5	6	T
HEAD UP	$\bar{X}$	16	3	-6	-20	-10	-14
	SD	12	13	7	8	8	11
	N	9	15	15	15	15	15

	SEG	1/2	3	4	5	6	T
HEAD DOWN	$\bar{X}$	38	12	2	-6	8	0
	SD	12	16	13	9	10	13
	N	16	16	16	16	16	16

	SEG	1/2	3	4	5	6	T
HEAD UP <10°	$\bar{X}$	36	4	11	-10	10	13
	SD	6	11	10	9	8	7
	N	4	4	4	4	4	4

SEG	1/2	3	4	5	6	T
a	4·02	1·59	1·56	3·70	4·53	3·18
b	0·16	0·92	0·56	0·78	0·27	1·88
c	3·02	0·05	3·84	1·94	3·38	3·89

Fig. 8

Effect of statolith removal (see also Fig. 3).

a. Effect of tilt 10° head-down.

b. Effect of tilt 36° head-up.

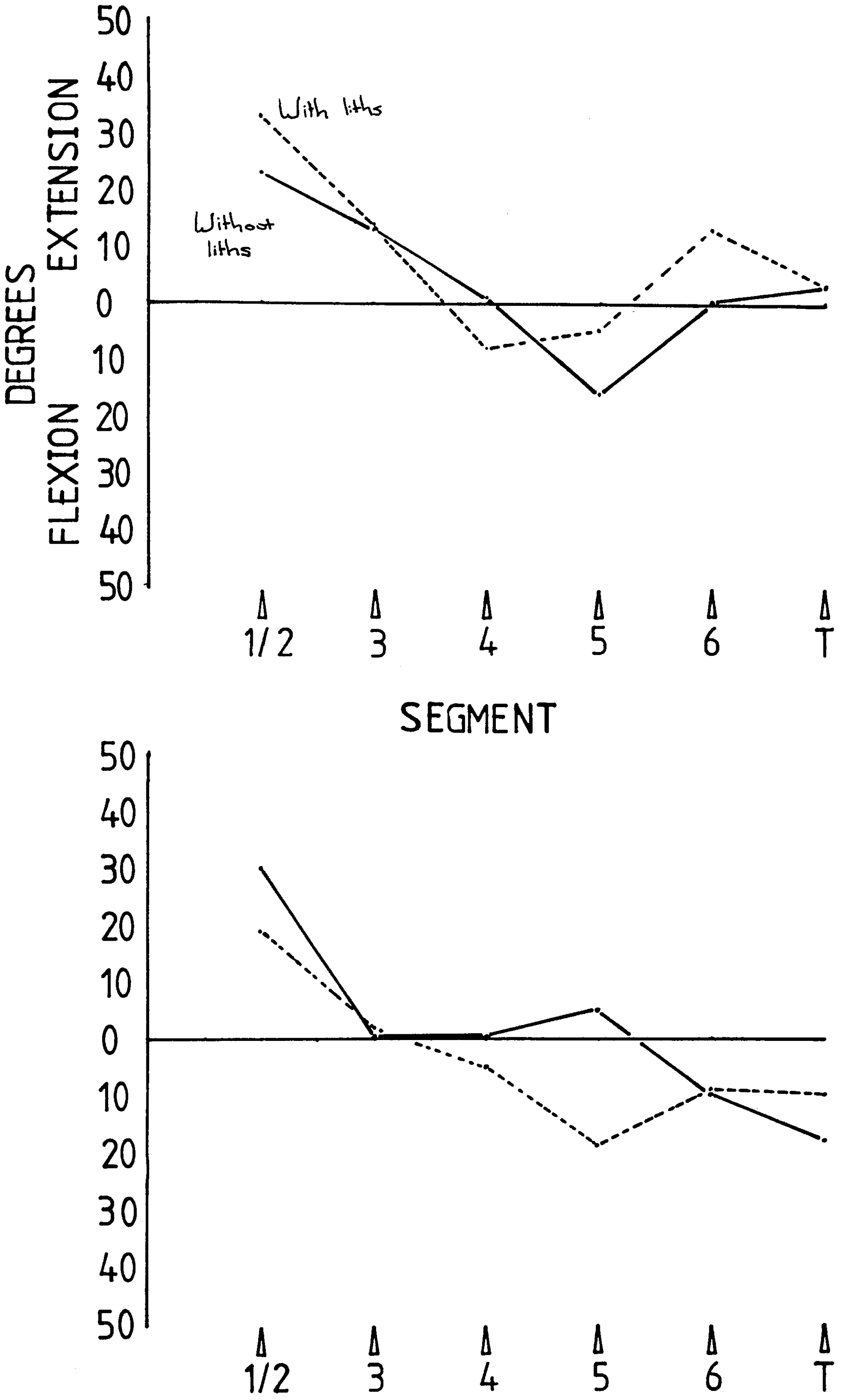

DEGREES
EXTENSION
FLEXION
50
40
30
20
10
0
10
20
30
40
50
With liths
Without liths
1/2
3
4
5
6
T
SEGMENT
50
40
30
20
10
0
10
20
30
40
50
1/2
3
4
5
6
T

Fig. 9

Effect of tilt in the pitch plane on swimmerets.

A. Angle of powerstroke during head-down tilt. Note uropod response (Newland, 1985). Coordinates were taken at numbered points shown. Angle of the beat was defined as angle a. (see Methods).

B. Response to head-up tilt.

C. Response to roll, left side-up.

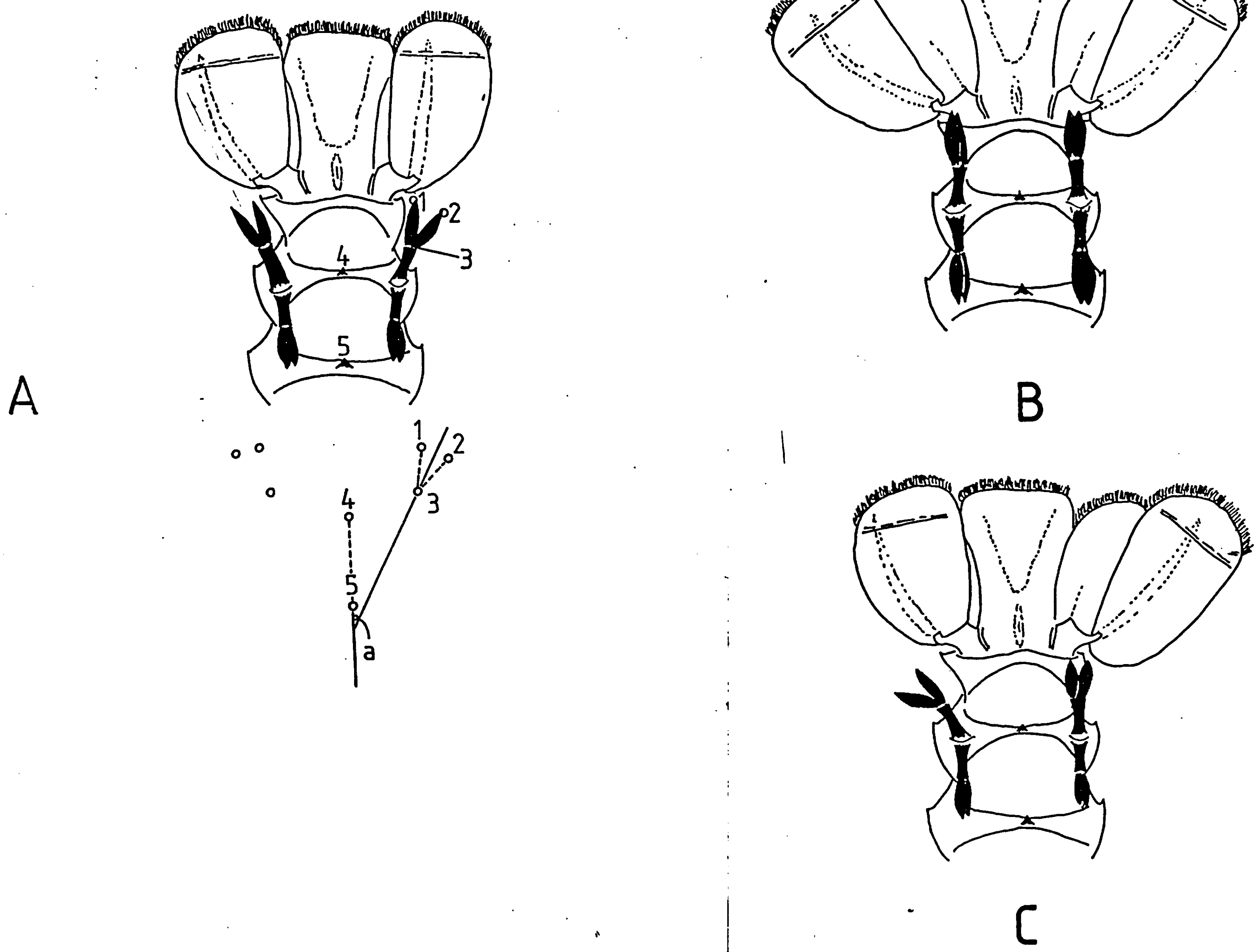

A
1
2
3
4
5
1
2
3
4
5
a
B
C

Fig. 10

Plot of period (s) against time (s) during side-up tilt of S5.

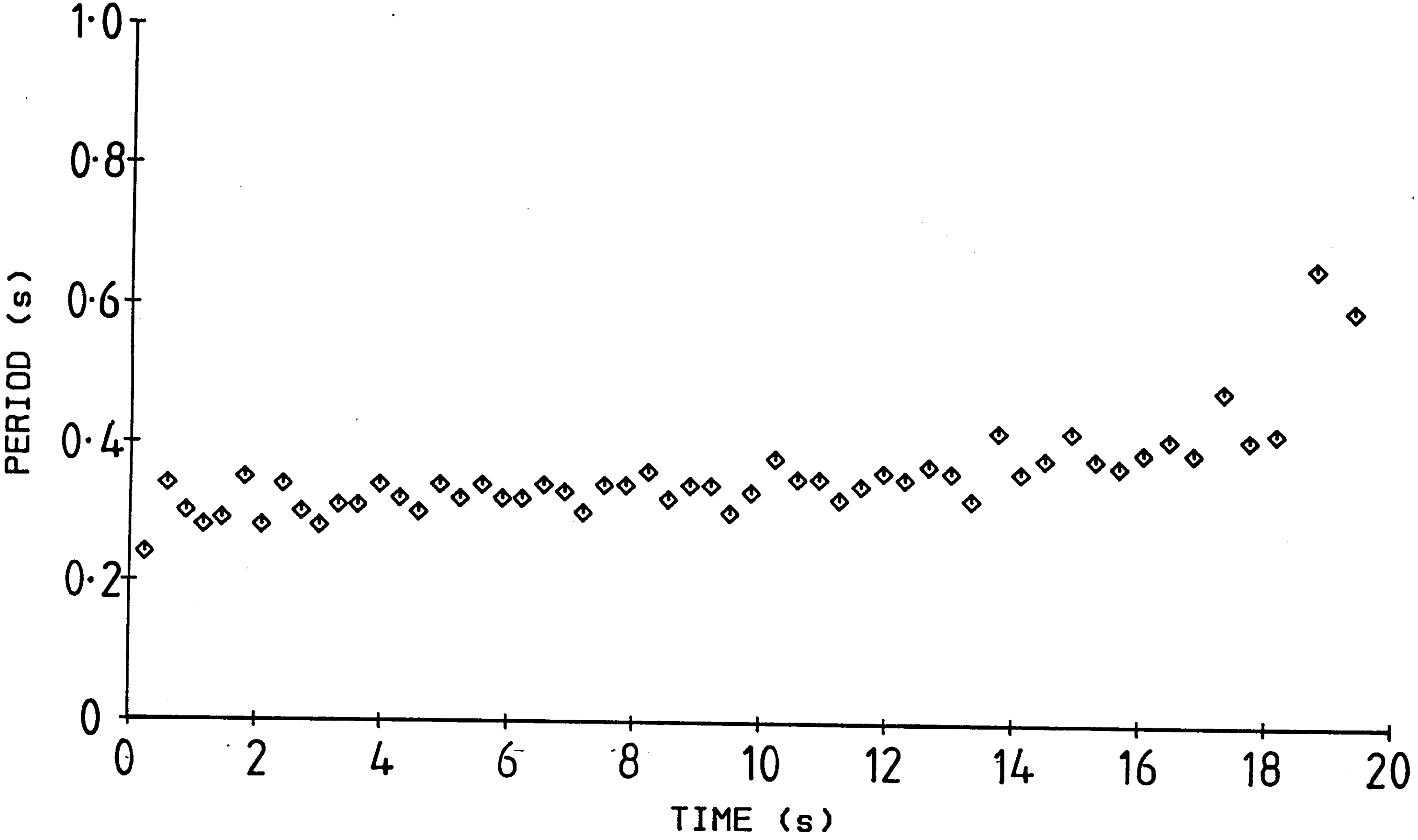

PERIOD (s)
1·0
0·8
0·6
0·4
0·2
0
0
2
4
6
8
10
12
14
16
18
20
TIME (s)

Fig. 11

Plots of period against time and composite plot of phase against time for S3 and S4 in S2 during head-up tilt.

a : S2

b : S3

c : S4

d : Phase plot.

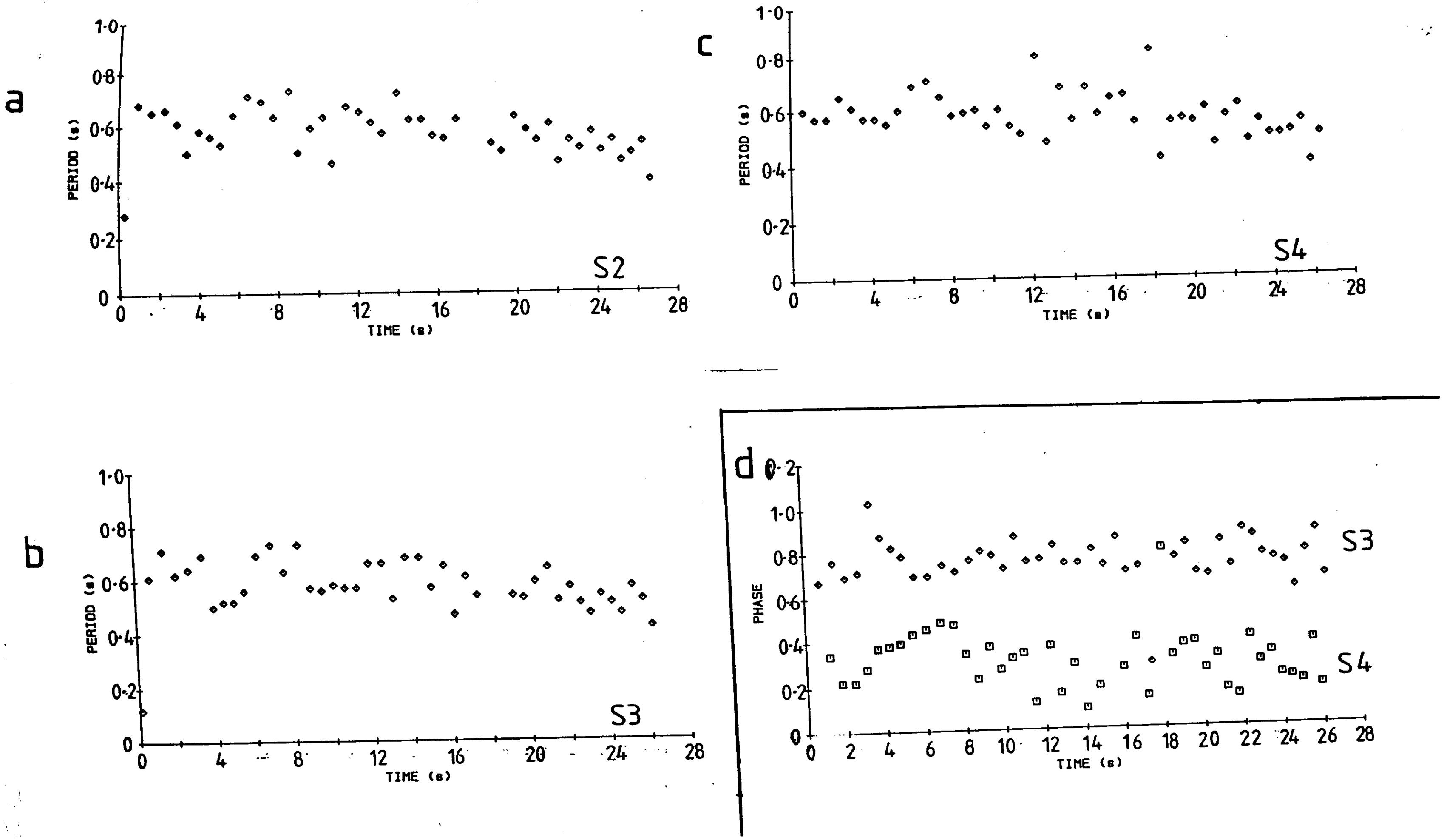
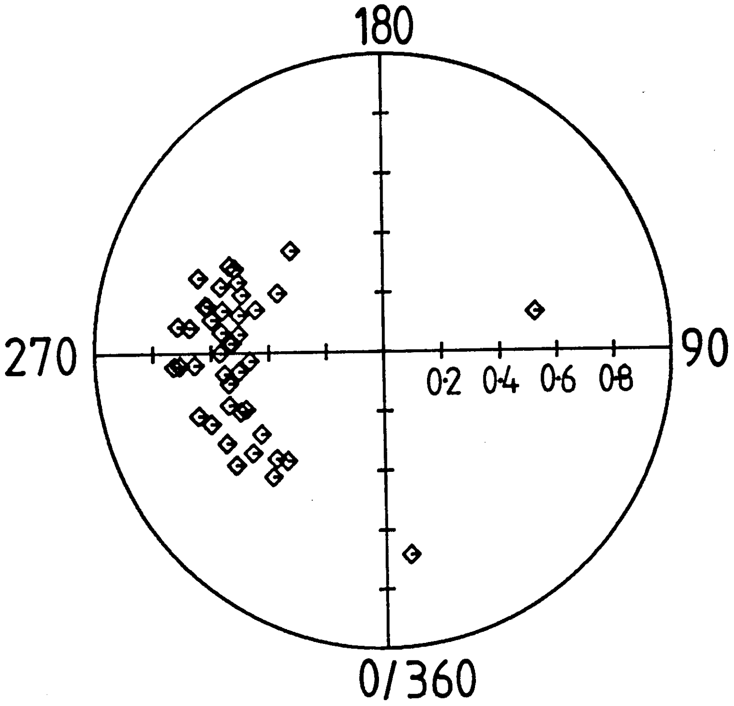

a
PERIOD (s)
1·0
0·8
0·6
0·4
0·2
0
TIME (s)
0
4
8
12
16
20
24
28
S2
c
PERIOD (s)
S4
b
PERIOD (s)
S3
d
PHASE
1·2
1·0
0·8
0·6
0·4
0·2
0
TIME (s)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
S3
S4

Fig. 12

Circular plots of phase data in Fig. 11. S3 and S4 in S2.

HEAD UP

S3



S4

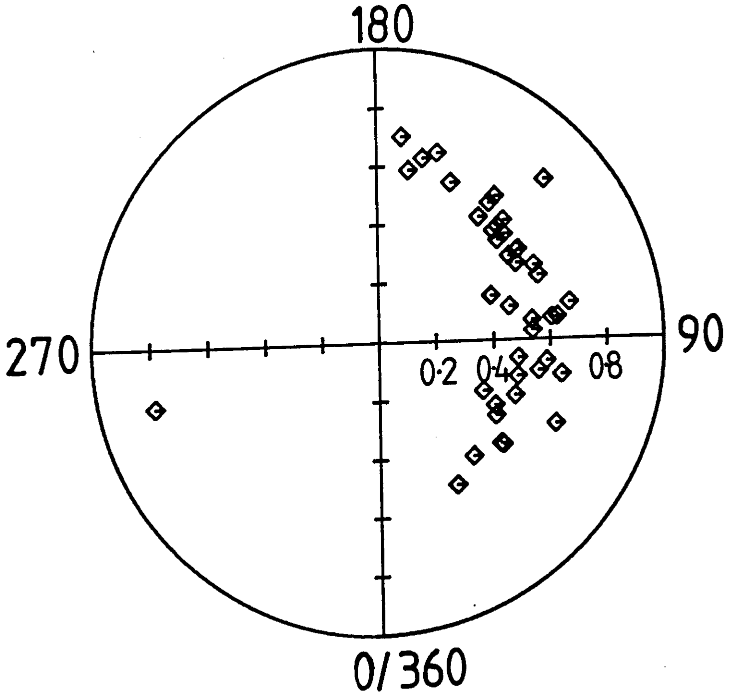


Fig. 13

Plots of period against time and composite plot of phase against time for S3 and S4 in S2 during head-down tilt.

a : S2

b : S3

c : S4

d : Phase plot.

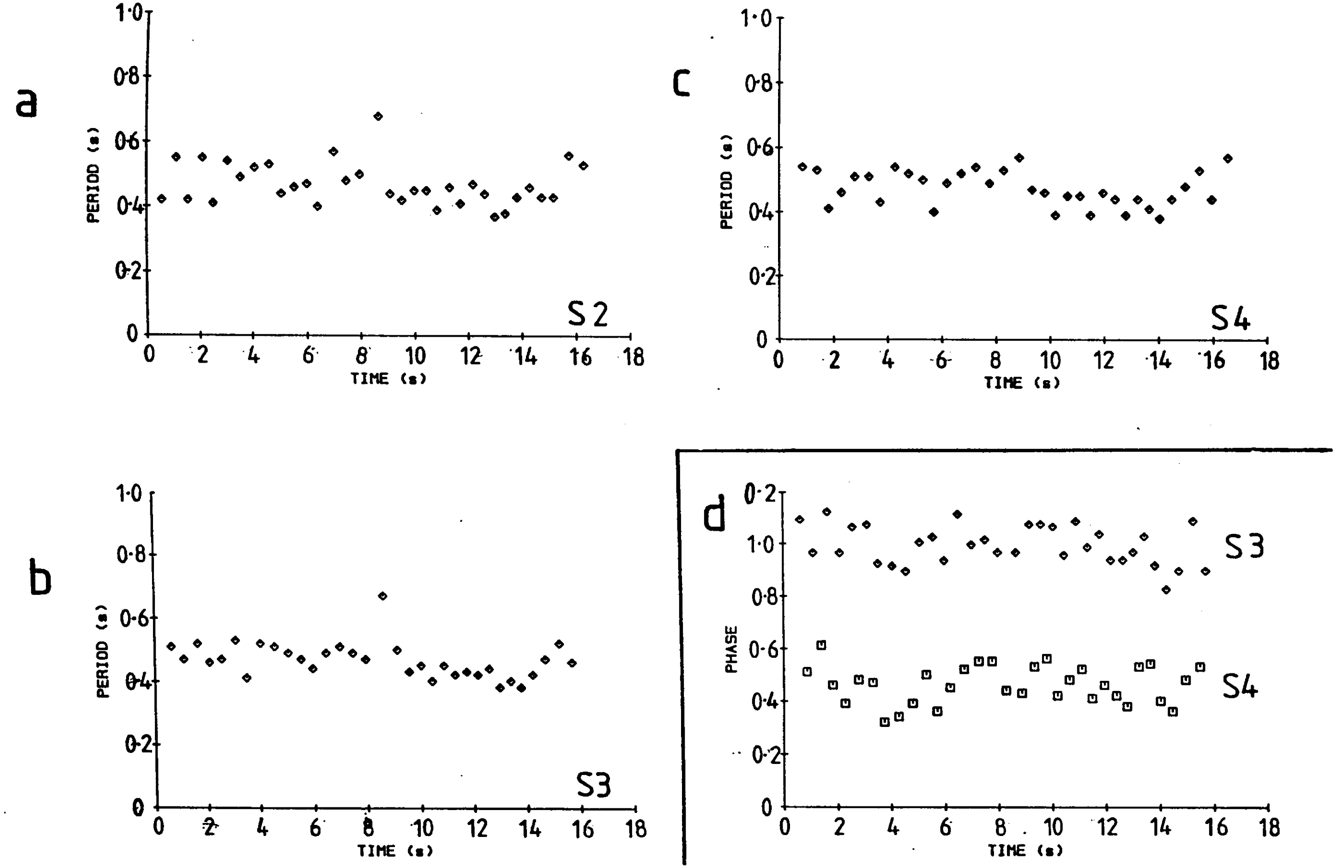
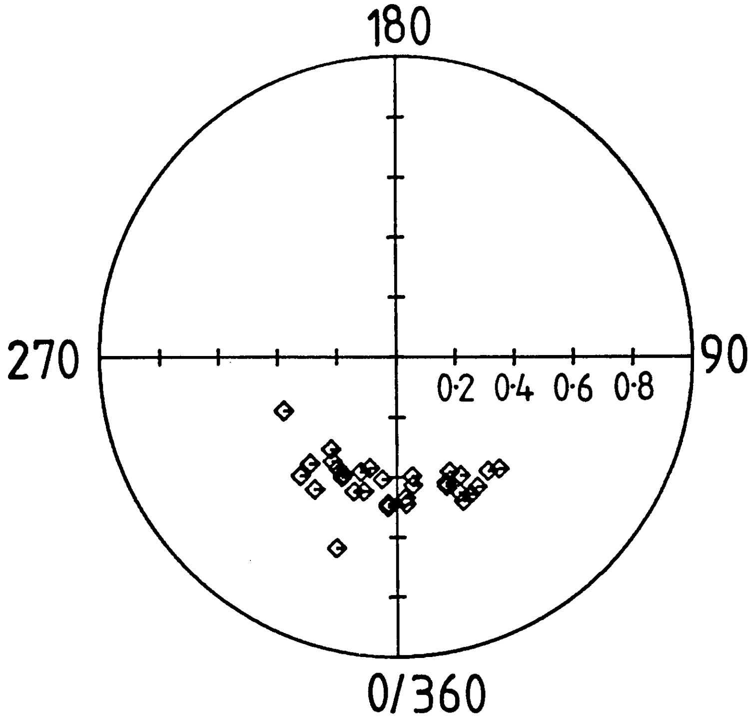

a
PERIOD (s)
1·0
0·8
0·6
0·4
0·2
0
TIME (s)
0
2
4
6
8
10
12
14
16
18
S2
c
PERIOD (s)
1·0
0·8
0·6
0·4
0·2
0
TIME (s)
0
2
4
6
8
10
12
14
16
18
S4
b
PERIOD (s)
1·0
0·8
0·6
0·4
0·2
0
TIME (s)
0
2
4
6
8
10
12
14
16
18
S3
d
PHASE
0·2
1·0
0·8
0·6
0·4
0·2
0
TIME (s)
0
2
4
6
8
10
12
14
16
18
S3
S4

Fig. 14

Circular plots of phase data in Fig. 13. S3 and S4 in S2.

HEAD DOWN

S 3



S 4

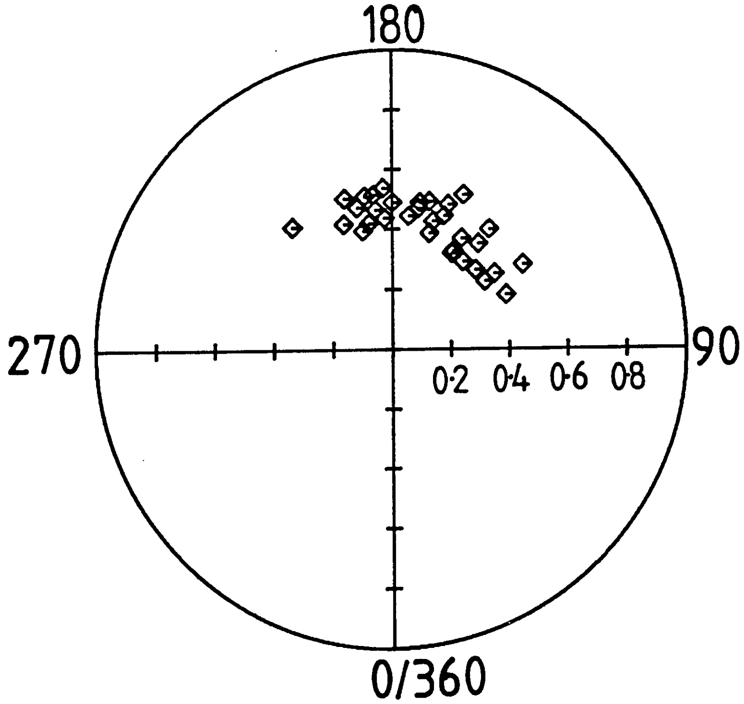
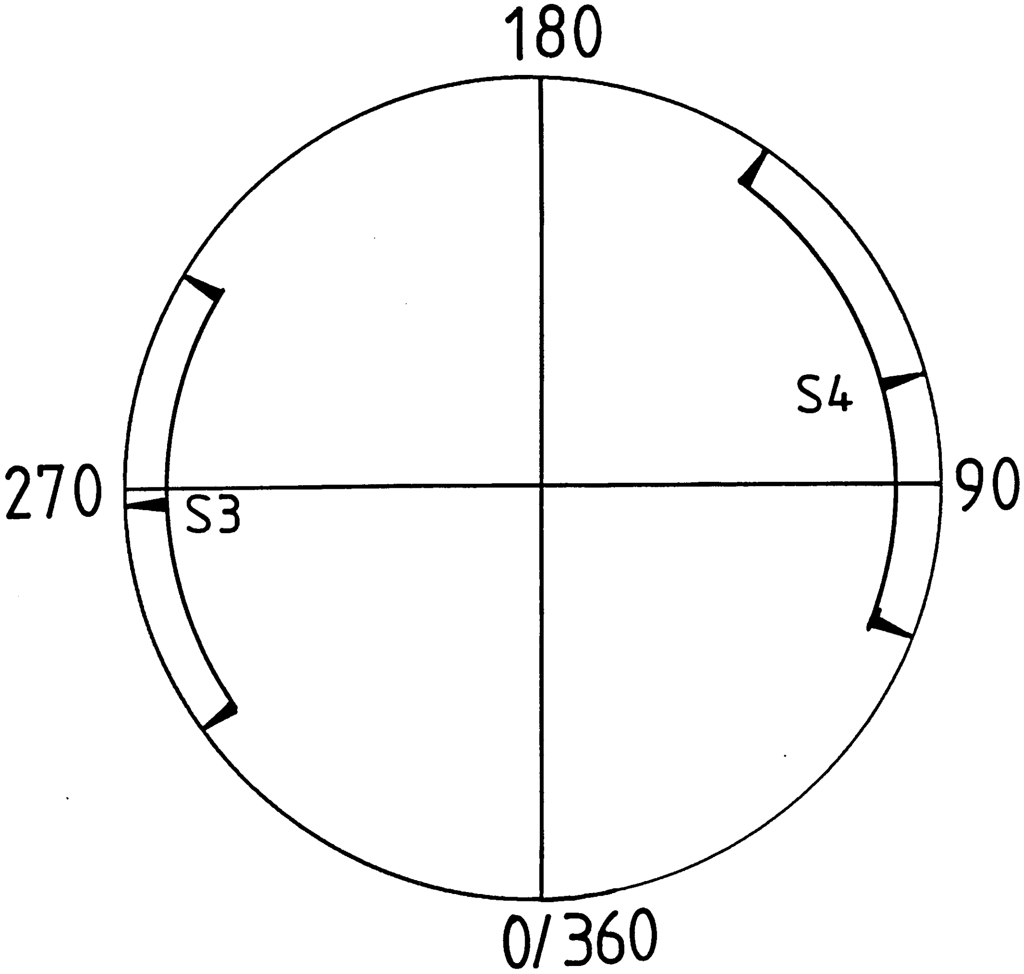


Fig. 15

Mean phase positions of S3 and S4 in S2 during head-up tilt.

HEAD UP

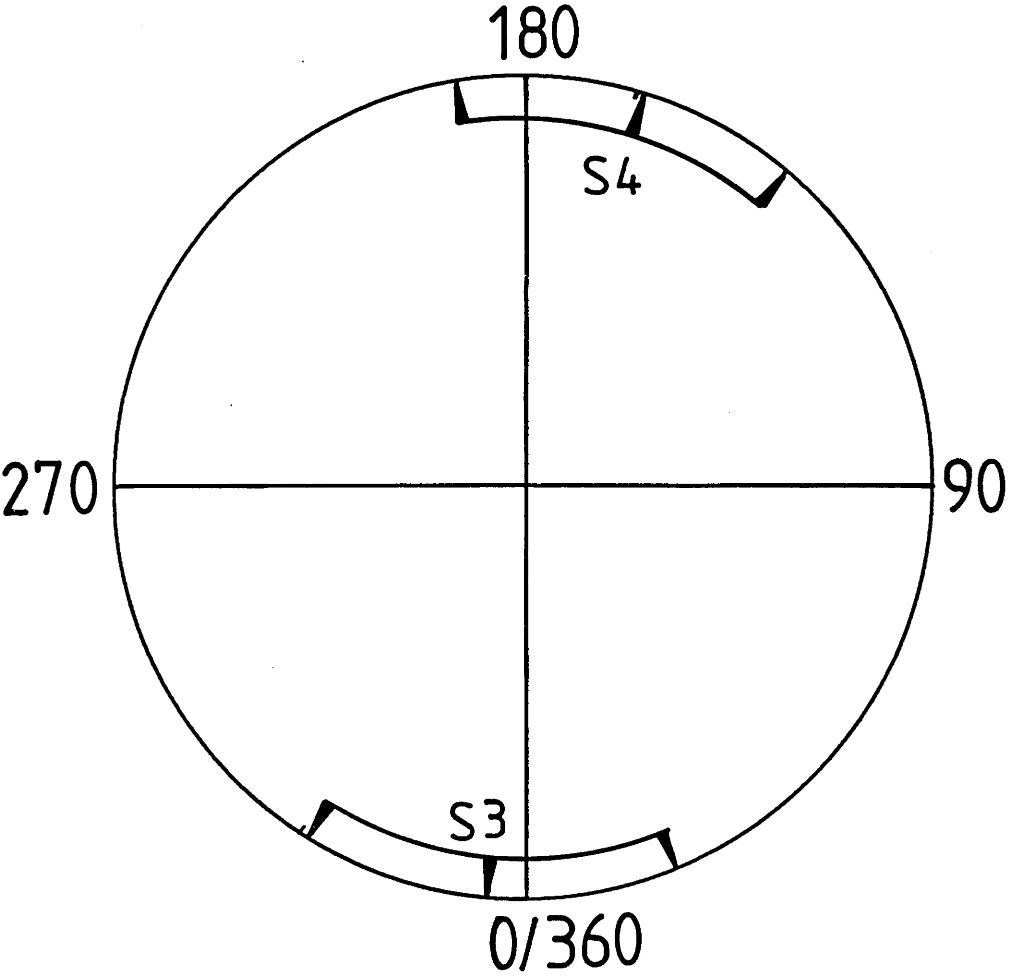


	S3	S4
$\bar{X}$	273°	107°
SD	31°	39°
R	0·85	0·77
N	42	43

Fig. 16

Mean phase positions of S3 and S4 in S2 during head-down tilt.

HEAD DOWN



	S3	S4
$\bar{X}$	355°	165°
SD	27°	25°
R	0·89	0 90
N	33	32

Fig. 17

Mean period times for S2-S4 during tilt in the pitch plane. These figures were obtained using the movement monitor system (see text for details).

HEAD UP

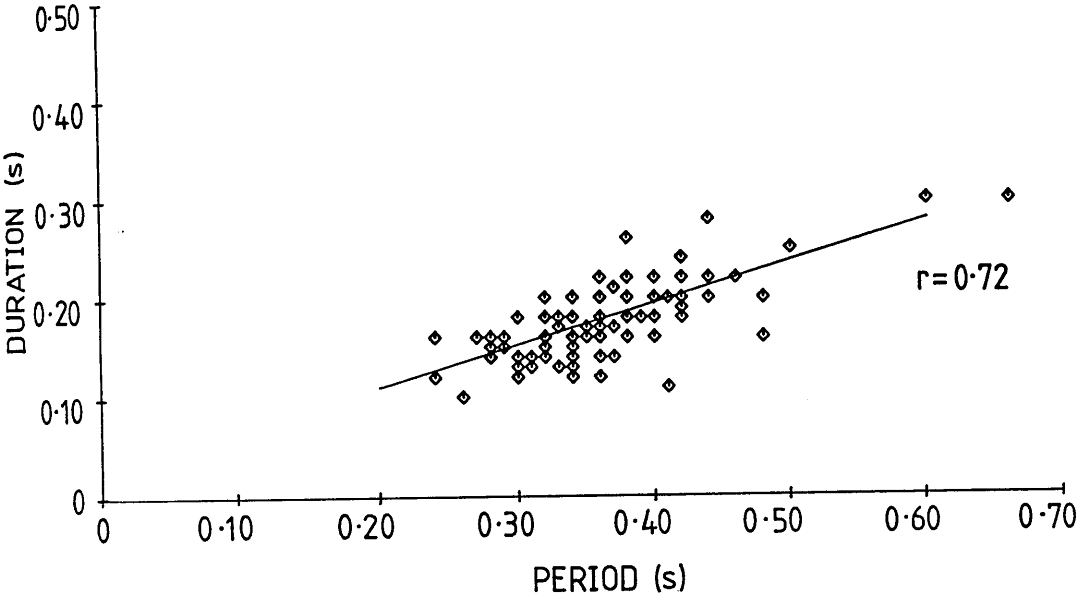
S2	S3	S4
0·58±0·08 (44)	0·58±0·07 (44)	0·58±0·08 (44)

HEAD DOWN

S2	S3	S4
0·47±0·07 (33)	0·47±0·06 (33)	0·46±0·07 (32)

Fig. 18

Plots of duration against period for powerstroke (top) and returnstroke (bottom) during asymmetric swimmeret beating in roll. Regression parameters in Fig. 21.


0·50
0·40
0·30
0·20
0·10
0
DURATION (s)
0
0·10
0·20
0·30
0·40
0·50
0·60
0·70
PERIOD (s)
r=0·72

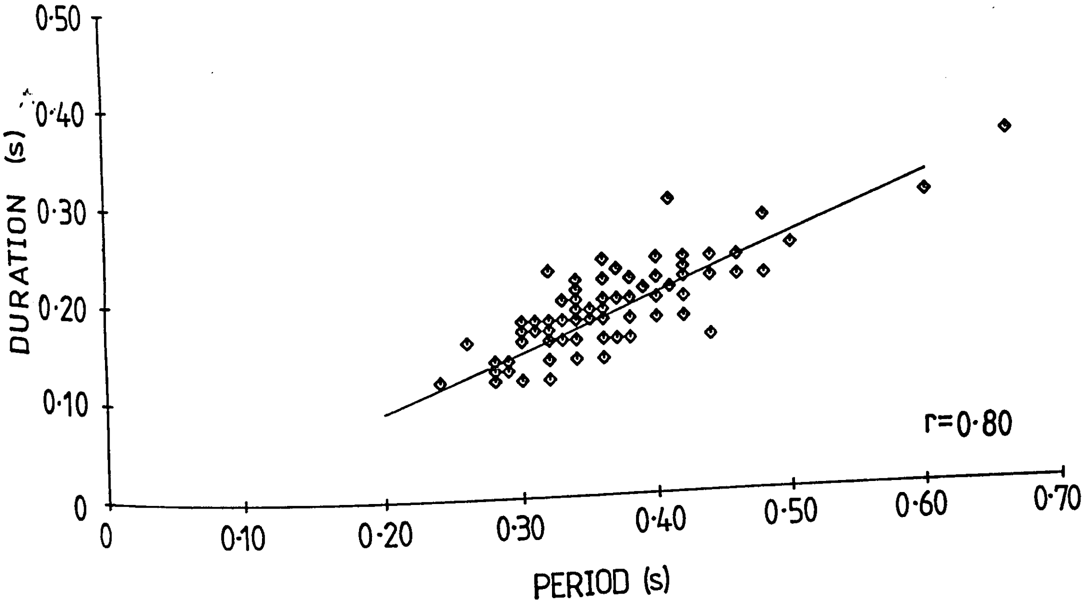
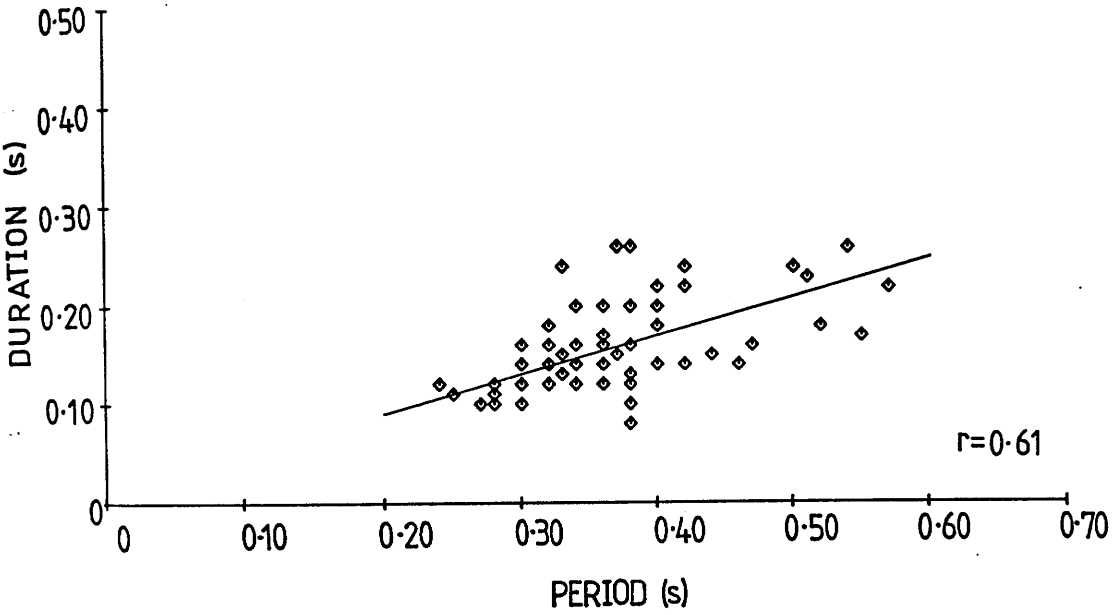

0·50
0·40
0·30
0·20
0·10
0
DURATION (s)
0
0·10
0·20
0·30
0·40
0·50
0·60
0·70
PERIOD (s)
r=0·80

Fig. 19

Plots of duration against period for powerstroke (top) and returnstroke (bottom) during head-up tilt. Regression parameters in Fig. 21.


0·50
0·40
0·30
0·20
0·10
0
DURATION (s)
0
0·10
0·20
0·30
0·40
0·50
0·60
0·70
PERIOD (s)
r=0·61

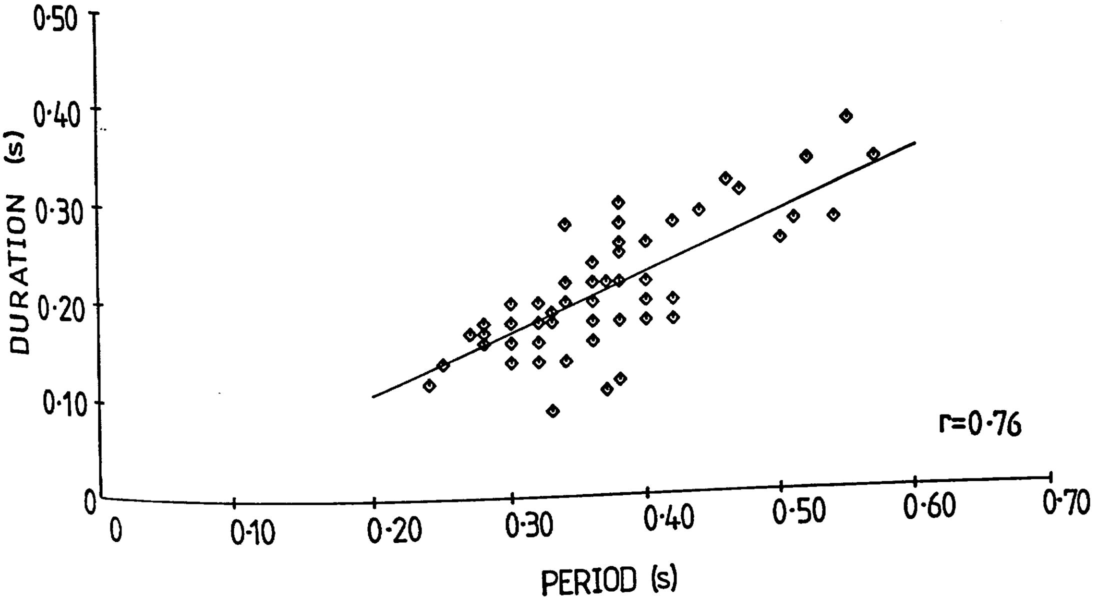
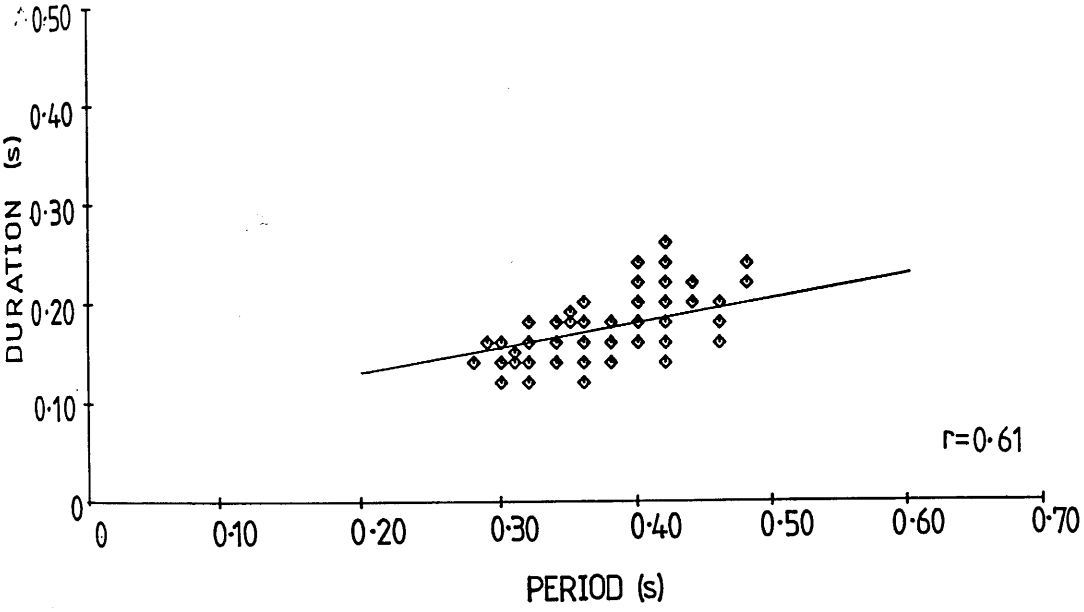
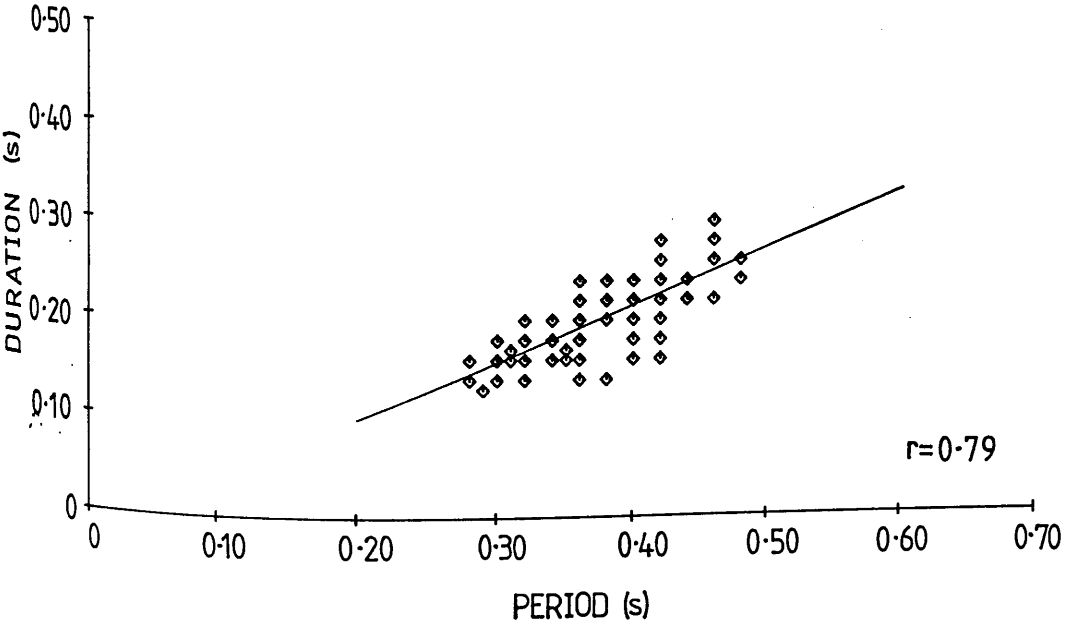

0·50
0·40
0·30
0·20
0·10
0
DURATION (s)
0
0·10
0·20
0·30
0·40
0·50
0·60
0·70
PERIOD (s)
r=0·76

Fig. 20

Plots of duration against period for powerstroke (top) and returnstroke (bottom) during head-down tilt. Regression parameters in Fig. 21.



DURATION (s)
0·50
0·40
0·30
0·20
0·10
0
0
0·10
0·20
0·30
0·40
0·50
0·60
0·70
PERIOD (s)
r=0·61



DURATION (s)
0·50
0·40
0·30
0·20
0·10
0
0
0·10
0·20
0·30
0·40
0·50
0·60
0·70
PERIOD (s)
r=0·79

Fig. 21

Regression parameters for previous figures.

POWERSTROKE

	a	b	r
ROLL	1·98	0·43	0·72
HU	1·65	0·38	0·61
HD	7·58	0·26	0·61

RETURNSTROKE

	a	b	r
ROLL	-1·60	0·53	0·80
HU	-1·34	0·61	0·76
HD	-1·47	0·57	0·79

Fig. 22

Summary of efects of tilt on both swimmerets and abdominal posture.

CONDITION	TYPE OF BEAT	ANGLE	PERIOD	STATE OF ABDOMEN
ROLL	UNILAT.	41±6° n=12	0·36±0·04s n=126	EXT.
HEAD DOWN	BILAT.	25±8° n=12	0·37±0·05s n=119	EXT.
HEAD UP	STRAIGHT	3±8° n=12	0·36±0·07s n=84	FLEX.

Chapter 5 :

INTERACTIONS BETWEEN SYSTEMS.

A. Introduction.

The nervous system of any organism has to deal with many types of interaction. It has to deal with sensory input from many different systems at the one time. This information has to be brought together in an organised and controlled manner and processed. The nervous system also has to deal with sensory feedback from movements that the organism itself initiates. Information as to whether the desired movement or action has been achieved has to be collated and the appropriate alterations in output produced.

Many of these interactions occur in the initiation and control of crustacean equilibrium reactions. As these reactions often involve appendage movements (eg legs, antennae, swimmerets), they allow investigation and analysis of underlying neuronal interactions at a behavioural level. Results obtained using this experimental approach are reported in this chapter. Of particular interest were interactions of statocyst-induced responses of the swimmerets and the abdomen with various types of sensory information provided by the legs.

Three types of interaction involving sensory inputs from the legs which have effects on other motor systems have been reported in the literature. Firstly, removal of substrate contact has been noted to produce alterations in abdominal posture (Larimer and Eggleston, 1971; Sokolov, 1973; Page, 1975). Substrate contact is also known to reduce the response of the eyestalks in spiny lobsters to tilt ie statocyst input (Schöne et al, 1978).

Substrate contact receptors have been reported in the crab, *Carcinus maenas* (Zill, et al, 1985), and have been implicated in the switching between the motor programs for walking and swimming seen when intact animals lose contact with the substrate (Bevengut et al, 1986). Thus it was of interest to investigate the effect of substrate contact on the output of various motor systems in *Nephrops*, particularly those already known to exhibit statocyst-driven reflexes, eg. the swimmerets.

Secondly, movements of various joints in the legs have been reported to have particular effects. Imposed joint movements produce resistance reflexes (Bush, 1962) which are dependent on activation of chordotonal organs and act against the imposed movement of the particular joint. But movements at particular joints also have effects at other joints in the same leg, and also in other legs (for review see Page, 1982). Joint movements also have effects on other motor systems. Flexion of the MC joint in *Procambarus clarkii* has been shown to have an excitatory effect on motoneurons supplying the postural muscles of the abdomen (Page and Jones, 1982). However, Neil (1985) has argued that this effect is a type of "startle" response, and should not be confused with true proprioceptive reflexes. These reflexes, particularly those arising from receptors at the CB joints (CB chordotonal organs), are involved in antennal reflexes and compensatory movements of the eyes (eg antennal responses, Barnes and Neil, 1982; compensatory eye movements, Scapini et al, 1978).

There are also what might be termed "dynamic" interactions between the walking legs and the swimmerets. Cattaert and Clarac (1984), have shown in *Homarus gammarus* that the pattern of beating in the swimmerets is altered when the animal begins to walk. Therefore, the discovery of another dynamic pattern of leg activity in *Nephrops* (chapter 3), leads to the question as to whether this activity has any effect on the swimmeret system. Further interest is added as both the leg cycling and the asymmetric swimmeret beating are initiated and controlled by the statocysts.

Thirdly, evidence will also be presented for another type of interaction. Field studies have shown that *Nephrops* orientates in a specific manner in a water current with respect to the direction of water movement (Newland and Chapman, 1985). These field observations have been complemented by laboratory investigations (Newland, 1985). Orientation towards water currents has also been studied in crayfish (Ebina and Wiese, 1984). There are fields of sensory hairs on various appendages and on the body of Decapods which detect water movement (Laverack, 1962; Wiese, 1976). These feed on to large numbers of interneurons which trigger escape and orientation responses (Ebina and Wiese et al 1984). The question then arises as to the role this system might play in the absence of substrate contact and how it might interact with the statocyst information in maintaining and correcting orientation in midwater.

To complement observations of behavioural responses of *Nephrops*, some attempt has been made to obtain recordings of various neuronal elements that may play a role in the responses.

B. Materials and Methods.

Most of the materials and methods employed in the work reported in this chapter have already been described in other chapters. Responses of intact animals were studied utilising the system outlined in chapter 3. To examine the effect of substrate contact on the various responses a footboard was also attached to the frame, providing support for the animal and allowing it to adopt a normal posture. The footboard could be fixed and tilted with the whole assembly so that no relative movement occurred between the tilt bar, animal and footboard. The footboard could also be tilted alone, causing levation and depression of the legs.

Two types of leg/swimmeret interaction were investigated. The first of these was the interaction between leg cycling and swimmeret beating. The movement monitor system was used to obtain information about the timing of leg cycling and swimmeret beating in the same animal. The phase position of a series of beats from particular swimmerets was calculated within the cycle of the leg movements. The method used was identical to that outlined in chapter 3. This was compared with the pattern observed in walking.

The effect of reducing the extent of leg cycling was investigated in two ways. In early experiments, movements of legs on one or both sides were prevented by wrapping stiff wire around these legs and attaching the wire to the frame. Leg cycling was also reduced by selectively blocking particular joints. Movement at joints was prevented by gluing a small balsa wood spar at particular points. In practice the particular joint being examined was blocked in all legs. Thus all the CB joints could be blocked by securely attaching the spar across the joint itself on each leg ie at points 1 and 2 in Fig. 1a. TC movement was prevented by attaching a spar down the longitudinal axis to each leg base at point 1 in each segment. It could also be blocked distally by attaching the spar between the meri of each leg, slightly proximal to the MC joint (point 3). The animal was tilted in mid-water and the response of the swimmerets to tilt noted.

The interaction between leg contact with the substrate and swimmeret responses to tilt was investigated by tilting the animal both with and without the footboard. Physical contact was prevented by fitting small plastic funnels over the ends of the legs (Fig 1b). These were attached to the propus and did not impede the PD joint in any way, but prevented any physical contact between the end of the leg and the substrate. Animals were then tilted both with and without the footboard in place.

Two other preparations provided electrophysiological data reported in this chapter. The inverted "head-tilt" preparation outlined in chapter 2 allowed recording in the ventral connectives between the last thoracic ganglion and the first abdominal ganglion (ie the T5-A1 connective) while applying levation and depression movements to the legs. This preparation also allowed a brief investigation to be made of the effects of stimulation of various mechanosensory hairs by both direct mechanical stimulation and water movement. The recording techniques involved in this were outlined in chapter 2.

Data from the legs and swimmerets was recorded and analysed as described in chapter 3. However the circular plots of the phases of swimmeret beats within the leg cycle show a bimodal pattern of distribution. Thus it is not valid to perform simple circular statistics on this data. However methods are available for dealing with such distributions allowing the calculation of simple statistical parameters.

The method used here assumes that the two clusters of points are 180° at opposite ends of an axis through the circle (Batschelet, 1981). The two groups can then be combined to allow the calculation of the angle of the axis around which the points are clustered and the spread of the points on either side of it. Effectively this is the calculation of the circular mean and the circular standard deviation for this data.

Using this method, the circular mean and circular standard deviation of the data shown in Fig. 4 was calculated and the results depicted in Fig. 5. Note that each circular mean could be plotted at two points, one 180° shifted relative to the other. For clarity however only one is plotted for each set of data.

C. Results.

1. Interactions between legs and swimmerets.

1(i). "Dynamic" interactions.

Clear evidence was uncovered of a strong correlation between the cycling activity of the legs and the beating of the swimmerets. Fig. 2 shows plots of the phase positions of swimmeret beats of swimmerets 2 and 3 (S2 and S3) within the cycle of movement of the second walking leg (L2), plotted against time. Circular plots of the data are also shown (Fig 4); the angle of a particular beat represents its phase position, and the distance from the centre of the plot its period. It should be noted that the leg cycling response involves the side-down legs, whereas the swimmeret response to tilt involves the side-up swimmerets. Table A shows the mean periods of the movements of leg and swimmerets.

A comparison of the mean periods shows that the swimmeret period is approximately 45% that of the leg cycling period. The periods are 0.28+/-0.05s and 0.29+/-0.06s for S2 and S3 respectively, compared with 0.66+/-0.12s for the leg. However the period times themselves do not explain the relationship between the beats and the leg cycle.

Fig. 2 suggests that the preferred relationship is that of two swimmeret beats to each leg cycle. This is emphasised by the bimodal distribution of points in the circular plots (Fig. 4). Some of the details of the relationship begin to emerge when the plots of phase against time (Fig. 2) are compared with those of period against time (Fig. 3). At 2, 4, 6 and 8s in Fig. 2 there are three S2 beats within one leg cycle. Fig. 3 shows that these points correspond to the slowest of the leg movements which occur every few cycles. At 10s where there are four swimmeret beats, there is not only a slow base cycle, but also several swimmeret beats with very short periods. Just after this point (from 11s on), the leg cycling settles down over five cycles to a steady period. This is matched in the plots of swimmeret phase by a segment of exactly two beats to each cycle.

The pattern for the third swimmeret is not as clear. The plot of period against time for this swimmeret (Fig. 3. S3) shows a greater degree of variability than that for S2. There is some evidence to suggest that one of the beats occupies a fairly constant phase relationship within the base cycle, with the other showing a high degree of variabilty. The circular plot (Fig. 4. S3) shows one group of points rather spread out while the other is well concentrated. Compare this with the plot for S2 which shows both groups of points well concentrated. At the end of this set of data, where both the swimmeret and the leg settle into a more even rhythm, there are two beats within the cycle.

The circular plots (Fig. 4) show this clearly. The period of the beats remains relatively constant over the whole range of phase values. For each swimmeret the two phase positions are approximately 180° apart.

Fig. 5 shows the circular mean and circular standard deviation calculated for the two swimmerets. The difference between these is 92° or 0.25 phase units.

The limited data available for walking (see chapter 3) was analysed from the point of view of the interaction between swimmerets and legs. Plots of phase against time are shown in

Fig. 6, and period against time in Fig. 7. Table B. shows the mean period times for leg and swimmerets. In this case the beating of S2, S3 and S4 is compared with the cycle of the fifth walking leg, ie the one closest to the swimmerets.

The movement of the leg and the beating of the swimmerets is slower than found in cycling. However the swimmeret periods are now only 35% of the leg period, compared with 45% in cycling. The periods are also less variable with time. The phase plots (Fig. 6) show a drift in the phase positions occupied by the swimmeret beats in the leg cycle. Over the first few cycles the drift is relatively slow but from 2s onwards it increases. Examination of Fig. 7 shows that the period of the beat lengthens in all three swimmerets while the period of the leg movement remains constant.

Comparing walking and cycling, it becomes clear that there is coordination between the legs and swimmerets in cycling which is not present during walking. While there is evidence of phase "jitter" in the relationship between swimmerets and legs in cycling, there is some factor or factors which draw the swimmeret beats into particular phase positions.

1(ii). Effect of prevention of cycling on swimmeret responses to tilt.

When leg cycling was prevented by wrapping wire round the legs on each side and the animal was tilted, there was very little swimmeret beating and complete absence of asymmetric swimmeret responses. When the wire was removed from one side, the swimmeret response on both sides was slightly improved, asymmetric beating occurring during and just after tilt. However, this response was transient and when the body was held in a tilted position the swimmerets ceased to beat within a short space of time. The response of the unimpeded legs to tilt was also reduced (see Fig. 8).

Removal of the wire from the remaining side restored the normal tilt responses of legs and swimmerets. This procedure of binding then freeing the legs was repeated between five and ten times on several animals, with the same result in each case.

To further investigate these findings, movements were prevented at various joints (Fig. 8). Blockage of all the MC joints tended to reduce the leg cycling response while having only a small effect on the swimmerets. However, marked reductions in swimmeret responses were produced by blocking either the TC or CB joints. When both TC and CB joints were blocked, preventing leg cycling, swimmeret responses to tilt were abolished. When all the CB, TC or CB and TC joints were blocked on one side, the effect on the swimmerets was a general decrease in activity on both sides. These effects were completely reversible: when the joints were unblocked individually there was a gradual return of the swimmeret response to tilt, until when all the joints were freed there was a full response.

These results are presented in tabular form in Fig. 8. The monitor of the effectiveness of the joint blockage was the strength of swimmeret beating on the side-up during tilt. The normal response was scored as ten points (see Fig. 9).

1(iii). Effect of substrate contact on swimmeret responses to tilt.

The animal always stood on the substrate when this was provided, irrespective of the angle of the substrate relative to the body, or the orientation of the body to gravity. When the animal was tilted in roll with its legs in contact with the footboard, there were no asymmetric swimmeret responses. On some occasions there were bouts of beating, particularly during the tilt movement, but this appeared to be bilateral beating. The exact nature of this beating was not determined and it was not possible to establish whether this was the type of beating seen in head-down tilt (see chapter 4).

In order to investigate the basis of the effects of substrate contact, physical contact between the dactyl and the footboard was prevented by fitting funnels over the ends of the walking legs (Fig 1b). Animals still sought and maintained "contact" with the footboard. Thus although there was no physical contact between the dactyl tip and the footboard, the forces generated by the legs against the footboard remained.

Animals prepared in this way were tilted in roll both with and without the footboard in place (Fig. 9). In both cases the responses were identical to those seen in the unoperated animal. With the footboard in place there was still complete suppression of the tilt responses. Therefore, inhibition of the tilt responses did not depend on tactile contact between dactyl tips and the substrate. Without the footboard the normal asymmetric responses occurred.

1(iv). Effect of footboard tilt.

The effect of footboard tilt, ie. imposed levation and depression movements of the legs, was also examined. No clear directional response was seen in the swimmerets. During the tilt, the swimmerets did beat, but beating ceased very quickly. The type of beating was difficult to resolve. Both lateral beats and straight beating was observed, but no clear pattern of response emerged.

It was possible to record from the swimmeret muscles during footboard tilts. Fig. 10 compares the effect of body and footboard tilt on the returnstroke muscle myogram. Tilting the body side-up produced clear rhythmic activity (Fig. 10a). With footboard tilts of a similar magnitude there was a small amount of activity on both ramps, but no sustained or rhythmic activity (Fig. 10b).

2. Descending leg information.

During the course of these experiments it was possible to record descending leg activity. Three examples are shown in Fig. 11. All of these neurons responded to depression of the legs on one side of the body. They were recorded between the thorax and the first abdominal ganglion, with the connectives below the first abdominal ganglion cut. Fig. 11a shows two units which only responded during the movement (note also the small unit in 11c). They show a strong response to levation and also an "off" response on depression. In contrast the larger units shown in Figs. 11b&c are more tonic in their response to levation. The unit in Fig 11b does not produce an "off" response. Similar units have previously been recorded in *Nephrops* which ascended from the thorax and were recorded in the circumoesophageal connectives (Priest, 1983; see chapter 2).

3. Other types of interaction.

3(i). Effect of directed water jets.

One other stimulus was observed to interact with tilt in a significant manner. When an intact animal was held level in midwater, there was normally no asymmetric activity or disposition of appendages ie no leg cycling, or asymmetric swimmeret beating. In practice it was sometimes difficult to find this position as relatively small tilts from 5° to 10° were sufficient to produce the whole range of righting responses. However, with the animal sitting quietly in midwater, asymmetric responses of swimmerets and legs could be produced by directing water jets from appropriate angles at the animal. A jet of water directed from above and in front of the animal down its right side produced lateral beating in the swimmerets on the right, and leg cycling from the legs on the left ie the normal right side up response. If the jet was directed down the midline, no asymmetric responses were produced. In this situation there was sometimes a clear abdominal flexion. In cases where no clear response was produced by water jets, the general excitability of the animal was nevertheless increased.

If an animal was tilted a few degrees, such that asymmetric responses began to appear, these could be switched to the inappropriate side by directing the water jet to the opposite side as outlined above.

When water jets were directed at animals standing on the footboard the only effect was a general excitatory one.

3(ii). Responses of sensory hairs to mechanical stimuli and water movements.

Of the various sensory receptors which may provide the input to the above responses, sensory hairs, particularly those on the uropods and telson were the most easily studied. Various aspects of these receptors were investigated.

Fig. 12 shows the responses of interneurons recorded in the *connectives to mechanical stimulation of the uropod. Fig. 12a shows the response produced by pressing on the right endopodite. There is a clear increase in the activity of two spontaneously active units. A more dramatic effect was produced by pressing down on the right exopodite. The activity in both units is inhibited. The activity gradually returned to the resting state after several seconds. There is some evidence that such stimuli produce effects in the swimmeret system.

Other interneurons were recorded which received input from the thick fringe of sensory hairs which is found at the end of the telson and uropods. In Fig. 13 it can be seen that the strongest responses in these units were produced by gently stroking the hairs of the endopodites on both sides (Fig. 13a&b) with weaker responses being produced by stroking the exopodites (c&d). However, these units were relatively insensitive to water movements. Fig. 14 shows the effect of disturbing the water both adjacent to the tail (Fig 14a) and the head (Fig. 14b). This does not produce firing of the larger units which responded to the direct stimulation. Rather a smaller unit is recuited. This unit is of a similar size as the smaller unit which can be seen firing in Fig.13b & c.

Units responding to water movement were recorded usually from the ventrolateral area of both *connectives. Other units of this type are shown in Fig. 15. These units responded best to water movements on the ipsilateral side of the body. Disturbing the

* abdominal

water adjacent to the tail (Fig 15a) caused the firing of both large small units together. When the water was disturbed near the head (Fig 15b), the large unit fired, but the smaller unit fired after a short delay. The smaller unit fired spontaneously, in the absence of any stimulus, at a relatively low but constant frequency. When the whole animal was tilted, its firing pattern was disrupted. While the tilt could not be said to produce a modulation in the firing pattern, it did have an effect. In fact in other experiments, "contamination" due to units responding to water movement rather than tilt, was a major difficulty.

3(iii). Centrally generated rhythms.

In view of the interaction reported above between leg cycling and swimmeret beating it is of interest to note that rhythmic activity was recorded in the connectives at the T6-A1 position. As cutting the connectives below the recording site had no effect, this was established as decending activity. Two examples are shown in Fig 16. The mean periods are very different; 0.4s for a and 1.5s for b. Both of these examples continued for many minutes with little or no variation. The source of this activity is unknown.

D. Discussion.

The asymmetric leg cycling response has offered important new opportunities for the study of interactions between different rhythmic motor systems and also interactions between various types of sensory feedback. There are clear interactions between the rhythmic movements of the legs and swimmerets, and also interactions between sensory information provided by various receptors in the legs and descending statocyst information.

Coordination between swimmeret beating and walking has been reported previously (Cattaert and Clarac, 1983). These authors reported two types of coordinated activity which they labelled "loose relative" and "tight relative" coordination. The former occurs when the swimmeret beating is relatively rapid, with approximately three swimmeret beats to each step of the leg. Sequences of "stable preferential phase" were interspersed with "phase glide". This is very similar to the pattern observed in the leg cycling experiments reported above.

The tight relative coordination was only seen during bouts of slow leg movement and slow swimmeret beating. In these circumstances the swimmerets and legs exhibited a 1:1 pattern of activity, with a 2:1 pattern occasionally being seen.

The period of both the tilt-driven leg cycling and swimmeret beating seen in *Nephrops* is very stable and there were no bouts of significantly slower activity observed.

The particular set of walking data presented here showed very little evidence of coordination between the two systems. This could be because it is taken from a relatively short bout of walking, and the two motor systems do not have an opportunity to settle into a coordinated pattern. However, coordination during walking does tend to be weak and only appears in certain conditions (Cattaert and Clarac, 1983).

The basis of the coordination between the leg cyclng and swimmeret beating is not yet clear. However two suggestions made by Cattaert and Clarac (1983) as the basis for the interaction they demonstrated in walking lobsters may well apply here. They proposed either an anatomical connection between the two systems by way of interneurons, or identical sensory inputs perhaps via the command fibre system. There is reason to believe that both of these mechanisms are involved in the coordination of swimmeret beating with leg cycling. Clearly both systems receive input from statocyst interneurons. Furthermore, it appears from the results in chapter 3, that this input comes from very similar, perhaps identical statocyst interneurons. However, it would be difficult to account for the ongoing coordination by this mechanism alone as the descending statocyst input has to act on two rhythmic systems cycling with different periods. Specific sensory inputs from the legs may provide another element in the control of the interaction. A large number of interneurons receiving information from CB joint receptors have been recorded in the circumoesophageal connectives in *Nephrops* (Priest, 1983). Similar interneurons have been recorded here, but descending into the abdomen (see also Wiersma and Bush, 1963). These neurons and others perhaps carrying feedback from the TCMRO, could be involved in the coordination.

There is also the possibility that specific coordinating interneurons, such as those which regulate the intersegmental swimmeret rhythm (Paul and Mulloney, 1985), carry information from oscillatory circuits in the leg system to swimmeret oscillators. This possibility is supported by the recordings of descending rhythmic activity from the abdominal connectives.

Sensory input from the legs to the swimmerets not only has an important role in coordinating swimmeret beating, but it is also important in determining whether swimmeret beating will occur at all. This was a completely unexpected but unambiguous result of experiments aimed at reducing or preventing leg cycling. The sensory feedback coming from the cycling legs will originate from various receptor systems. Most of the movement is levation and depression at the CB joint, accompanied to a lesser degree by extension/flexion of the MC joint, and protraction/retraction at the TC joint (chapter 3). Such movements will produce a wealth of sensory information. Chordotonal organs such as those at the CB and MC joints are able to provide information concerning direction and velocity of joint movement, as well as monitor joint position (for review see Bush & Laverack 1982). In leg cycling, a complication is that much of the information will be of a phasic nature. Studies on this type of sensory feedback have involved predominantly the TCMRO. Reflexes evoked by stretch of the TCMRO are phase dependent. During activity of the promoter muscle, TCMRO stretch produces a resistance reflex, but during activity in the remotor muscle stretch produces an assistance reflex (Sillar and Skorupski, 1986). Thus the type of reflex produced is dependent on the relationship of the sensory feedback to the on-going rhythmic activity.

Effects of proprioceptive feedback that are dependent on the phase of on-going activity have also been demonstrated at the behavioural level. If the leg of a walking rock lobster is blocked during the powerstroke movement, stepping of the leg is inhibited, the leg exerts force against the block, and stepping in the other legs is reduced. If the leg is blocked during the returnstroke movement, stepping continues at reduced amplitude, very little force is exerted against the block, and the other legs are hardly affected (Clarac, 1985). Thus sensory feedback has different effects at different points in the cycle.

The blockage used in the leg cycling experiments was maintained at all parts of the cycle. However, the effect of the blockage will be to disrupt phasic sensory feedback. It may be that the absence of this feedback prevents proper patterning of the motor output to the legs, and perhaps also abolishes phasic input from the leg oscillators to the swimmeret system. Certainly there is still feedback from the periphery to the CNS, but it will no longer be phasic. It may also be that feedback from systems not normally active, such as those which might detect stresses on the cuticle due to the wire used to wrap the legs, or the spars at the joints, actually inhibits swimmeret beating.

The pattern of sensory feedback to the CNS when the animal is in contact with the substrate will of course be dramatically different. While the animal is standing quietly sensory feedback will be tonic rather than phasic. The experiments with the funnels have shown that feedback from contact receptors at the end of the leg is not the basis of contact effects. This finding provides an interesting contrast with findings in the crab *Carcinus maenas* where units in the dactyl have been recorded during locomotion and shown to be capable of monitoring both external forces such as those caused by contact, and internally generated forces generated by muscle contraction (Zill et al, 1985). Feedback from these units has been shown to be important in determining whether the motor programs for walking or swimming are employed (Bevengut et al, 1986).

The basis of the substrate contact effect in *Nephrops* may actually be the detection of load or force at points on the leg other than the dactyl. Klarner and Barnes (1986) have shown that in crayfish the cuticular stress detectors (CSD's) which are found in the basal part of the leg, can monitor contact of the leg with the substrate, and forces during the powerstroke of the leg. If these or similar receptors were responsible for the observed substrate contact effects, this would explain why physical contact is not important, but contact in terms of transmission of load is essential.

An extension of this would be that the procedures used to block joints or prevent legs from moving may well have simulated the load present on the leg when substrate is present. Against this is the finding that, when cycling is prevented, swimmeret beating is abolished, whereas substrate contact only removes the asymmetric responses of the swimmerets without necessarily preventing swimmeret beating.

The effects observed by directing water jets at animals suspended in mid-water also merit further comment. Mechanosensory hairs so far studied are distributed over much of the dorsal surface of both crayfish and lobsters. The most carefully studied are the hairs which are found on the rostrolateral region of the telson of the crayfish. These have a dual innervation and respond to currents in both rostral and caudal directions (Wiese et al, 1976). In *Palinurus* the hairs on the body lie approximately parallel to the rostrocaudal axis, while those on the legs lie parallel to the longitudinal axis of the segment on which they are found (Vedel and Clarac, 1976). To produce the responses observed from both the swimmerets and the legs, information as to direction of the water jet must be available. Hairs on the telson in *Procambarus* respond maximally to currents along the longitudinal axis (Wiese et al, 1976). However, the afferents from hairs on the telson are arranged such that they project to side specific interneurons, and these are organised such that they are excited by water movements on their own side and inhibited by movements on the contralateral side (Wiese et al, 1976; Reichert et al, 1982). Thus interneurons in this system are already known to extract some directional information from waterborne stimuli. It may be that these interneurons contribute to higher order interneurons integrating information from a wider area of the body surface and feeding the information obtained into the relevant equilibrium pathways.

Substrate contact clearly interacts with descending statocyst input. It gates out the statocyst input to the swimmerets, and also the abdomen. It must also interact with input coming from the sensory hair fields. The results of the water jet experiments show that in the absence of substrate contact, inputs from systems detecting water currents, almost certainly the sensory hairs sensitive to water movements, reinforce or even act against statocyst input to produce the asymmetric responses of the legs and swimmerets. However, water currents directed at an animal free to move and in contact with the substrate produce orientaion to the current (Newland, 1985; Ebina and Wiese, 1984). It would be of interest to know whether, under these circumstances, asymmetric responses of the swimmerets and uropods can be observed. Thus the presence or absence of substrate determines not only the effectiveness of the statocyst input to various motor systems, but also the effect of inputs from sensory hairs.

Table A

Mean (+/-standard deviation) period times for L2 and S2 and S3 during cycling.

Table B

Mean (+/-standard deviation) period times for L2 and S2,S3&S4 during walking.

A

LEG 2	SWM 2	SWM 3
0·66±0·12	0·28±0·05	0·29±0·06

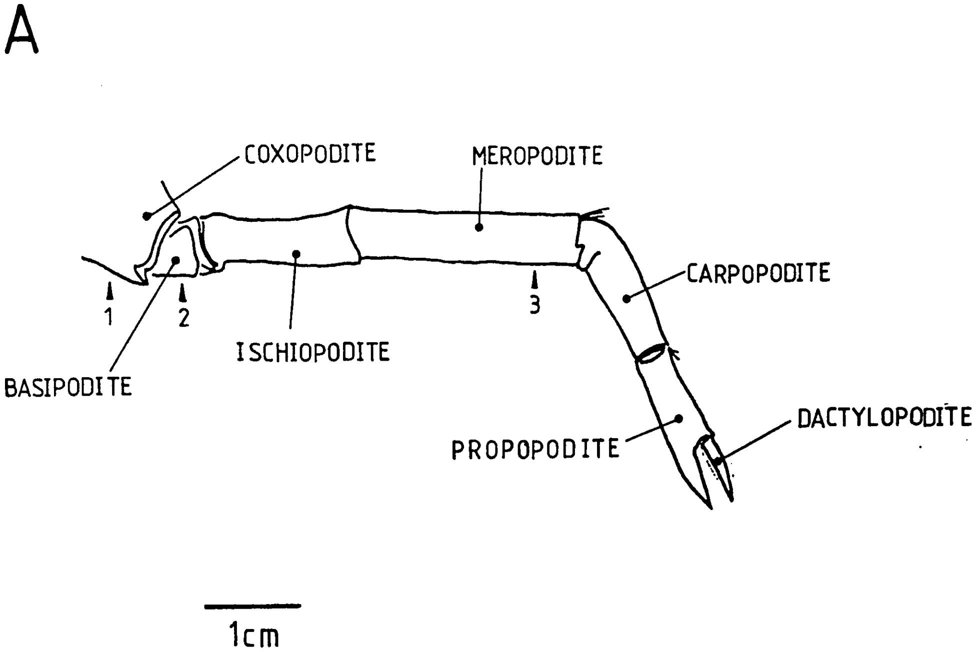
B

LEG 5	SWM 2	SWM 3	SWM 4
1·08±0·31	0·37±0·08	0·37±0·02	0·35±0·08

Fig. 1

A. Frontal view of L2. Points 1,2 and 3 show points of attachment of spars in joint blockage experiments (see text for details).

B. Attachment of funnel to leg such that there is no contact between the dactyl tip and the substrate.


A
COXOPODITE
MEROPODITE
CARPOPODITE
1
2
3
ISCHIOPODITE
BASIPODITE
DACTYLOPODITE
PROPOPODITE
1cm

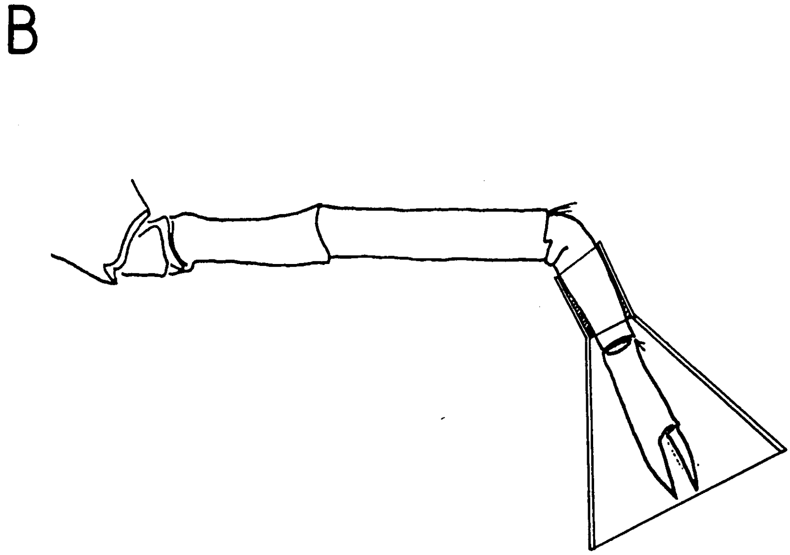
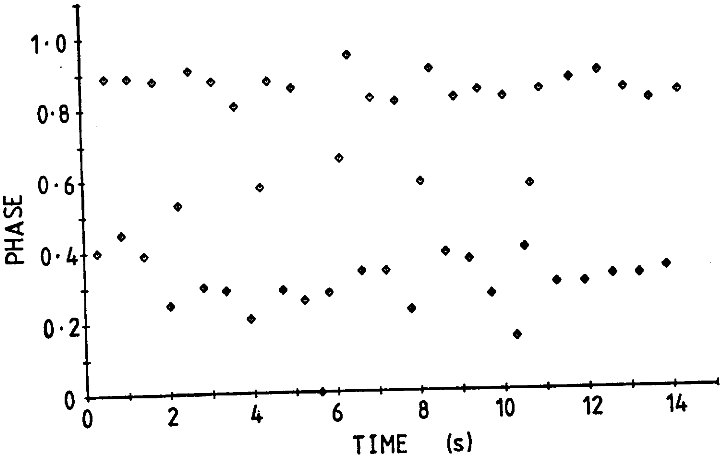

B

Fig. 2

Plots of phase against time for S2 and S3 in L2 during cycling.

S2


PHASE
1·0
0·8
0·6
0·4
0·2
0
0
2
4
6
8
10
12
14
TIME (s)

S3

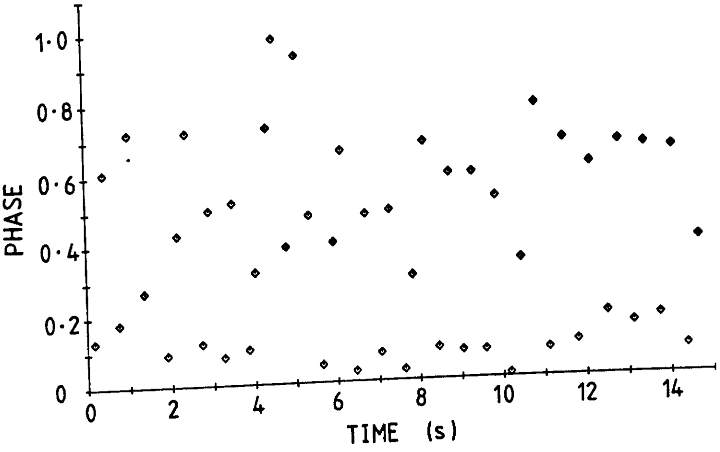
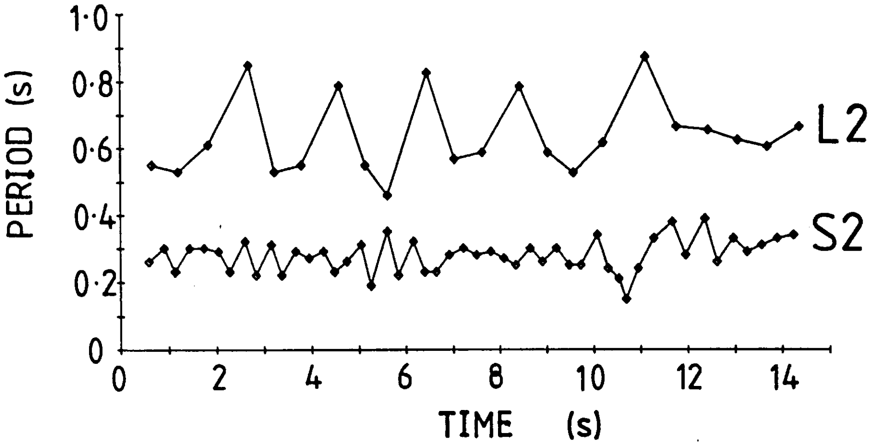

PHASE
1·0
0·8
0·6
0·4
0·2
0
0
2
4
6
8
10
12
14
TIME (s)

Fig. 3

Plots of period against time for S2 and S3 and L2 during cycling.


PERIOD (s)
1·0
0·8
0·6
0·4
0·2
0
0
2
4
6
8
10
12
14
TIME (s)
L2
S2

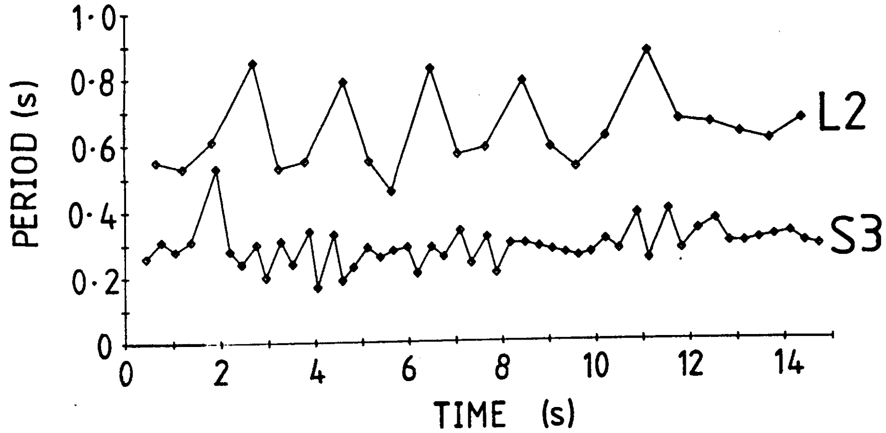
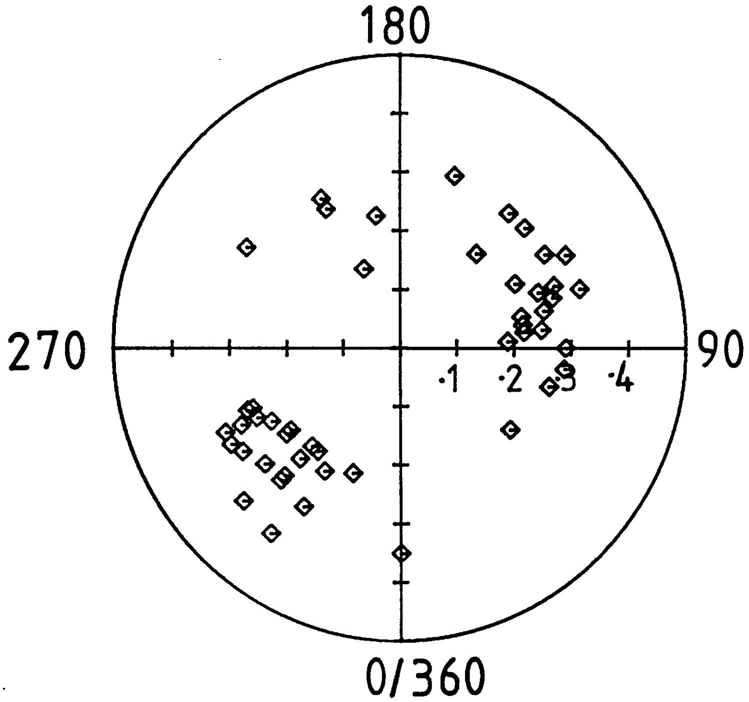

PERIOD (s)
1·0
0·8
0·6
0·4
0·2
0
0
2
4
6
8
10
12
14
TIME (s)
L2
S3

Fig. 4

Circular plot of data in Fig. 3. Axes, period of beat (s). Angle represents phase.

S2



S3

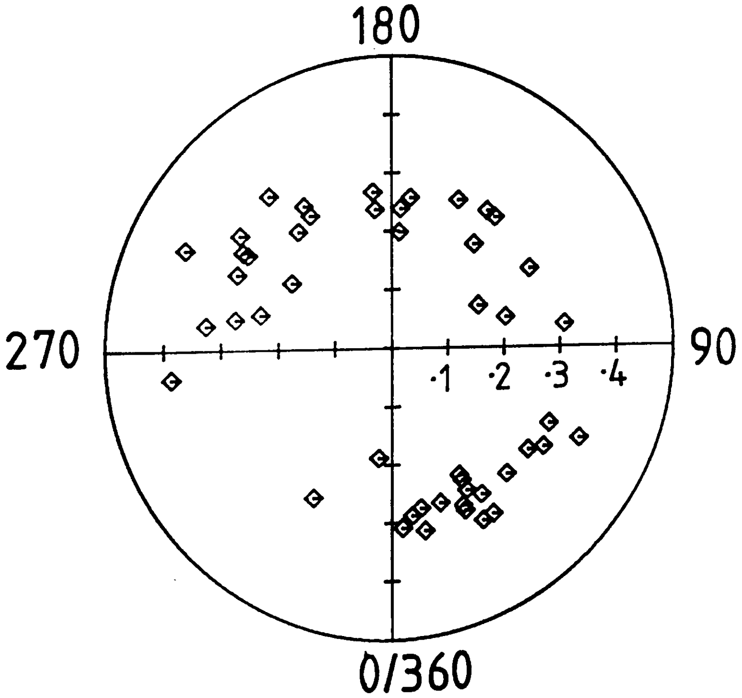
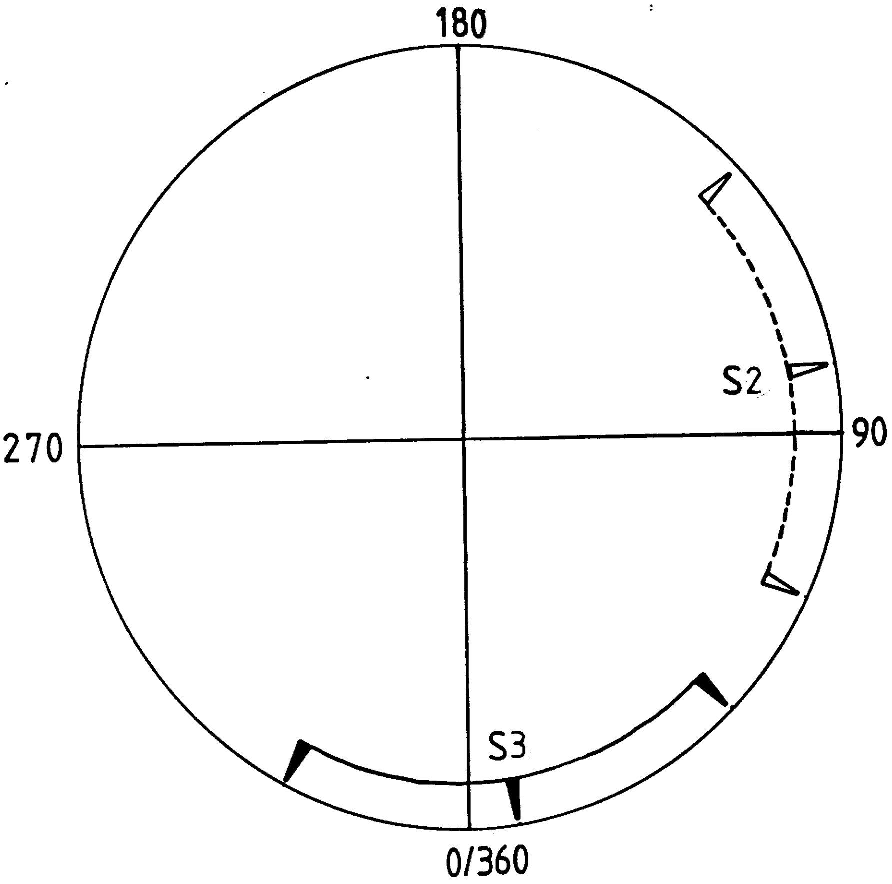


Fig. 5

Mean phase positions of S2 and S3 in L2 during cycling.

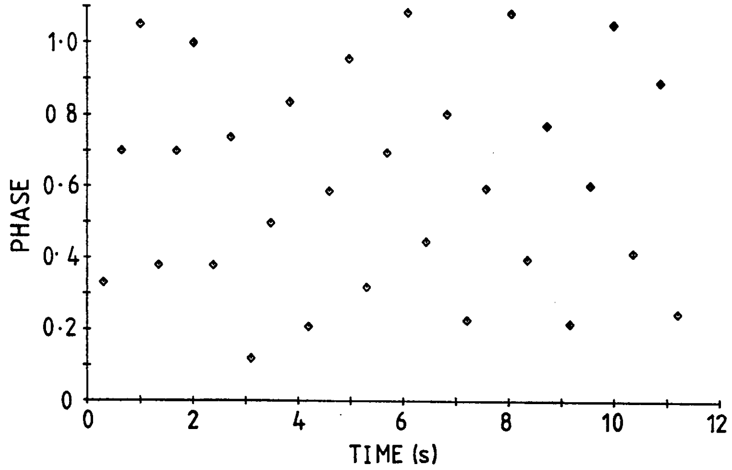


	S2	S3
$\bar{X}$	100°	8°
CSD	35°	37°
R	0·27	0·16
N	49	49

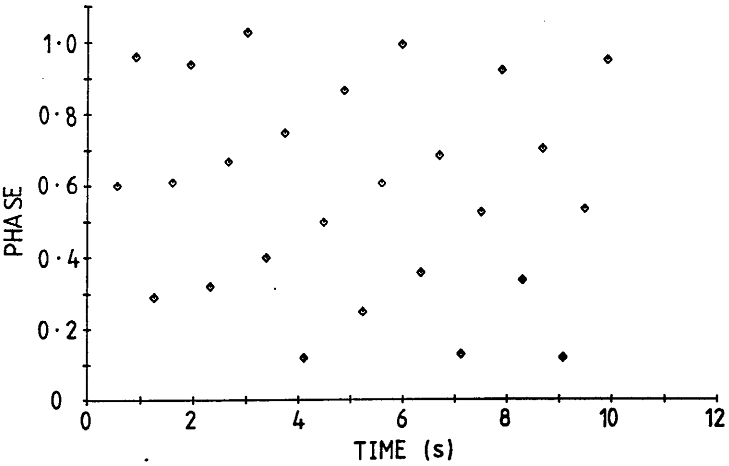
Fig. 6

Plots of phase against time for S2-S4 in L2 during walking.

S2


PHASE
1·0
0 8
0·6
0·4
0·2
0
0
2
4
6
8
10
12
TIME (s)

S3


PHASE
1·0
0·8
0·6
0·4
0·2
0
0
2
4
6
8
10
12
TIME (s)

S4

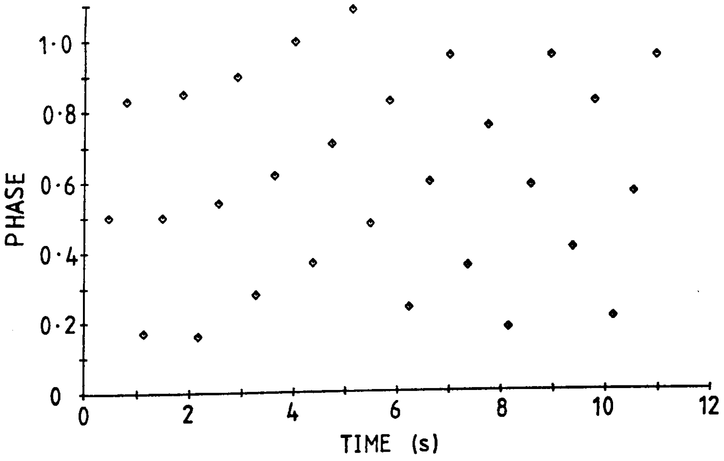
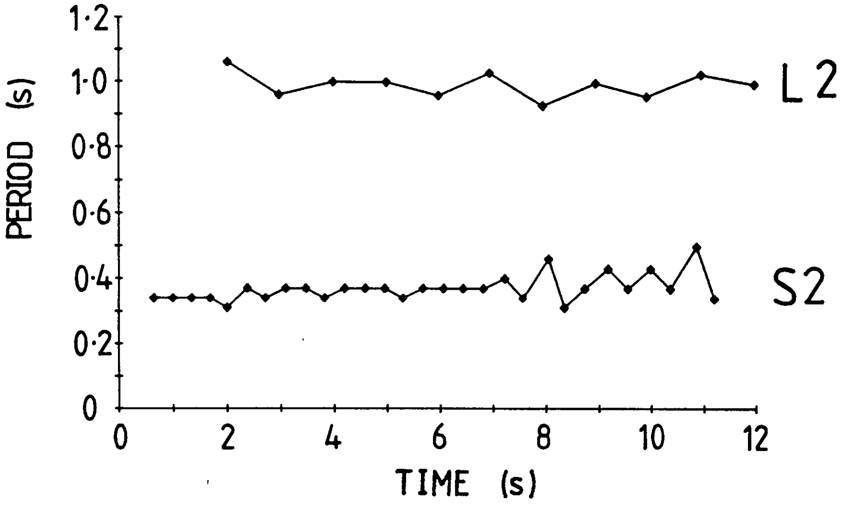
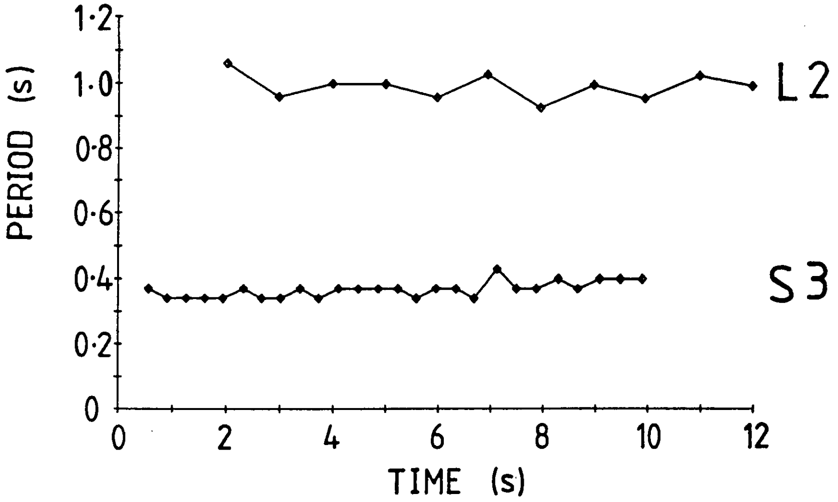

PHASE
1·0
0·8
0·6
0·4
0·2
0
0
2
4
6
8
10
12
TIME (s)

Fig. 7

Plots of period against time for S2-4 and L2 during walking.


1·2
1·0
0·8
0·6
0·4
0·2
0
PERIOD (s)
0
2
4
6
8
10
12
TIME (s)
L2
S2


1·2
1·0
0·8
0·6
0·4
0·2
0
PERIOD (s)
0
2
4
6
8
10
12
TIME (s)
L2
S3

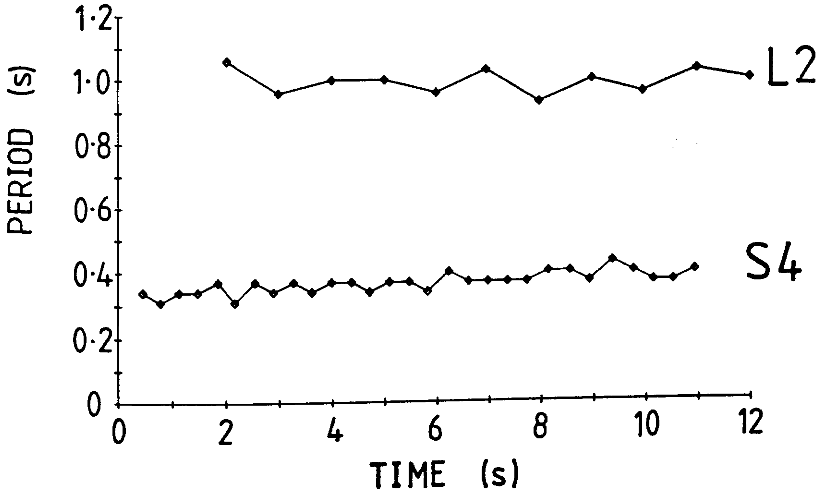
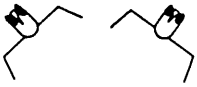
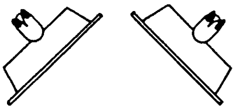
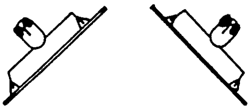

1·2
1·0
0·8
0·6
0·4
0·2
0
PERIOD (s)
0
2
4
6
8
10
12
TIME (s)
L2
S4

Fig. 9

Effect of substrate contact on swimmeret responses to tilt in the roll plane. Full response was scored as ten points (eg as in body roll alone).

Fig. 8

Effect of joint blockage on swimmeret responses to tilt in the roll plane. Full response scored as ten points.

TEST	SWIMMERET BEATING
BODY ROLL 	●●●●●●●●●●
CO-ROLL 	●
BODY ROLL WITH FUNNELS 	●●●●●●●●●●
CO-ROLL WITH FUNNELS 	●

JOINT	BILATERAL BLOCK	UNILATERAL BLOCK
MC	●●●●●●	●●●●●●●●
CB	●●●	●●●●●
TC	●●●	●●●●●
CB/TC	—	●
WIRE	—	●

Fig. 10

Comparison of effects of body tilt and footboard tilt on swimmerets. Myogram of swimmeret muscle activity.

A. Effect of body tilt. Upward deflection in movement trace denotes tilt side-up.

B. Effect of footboard tilt. Upward deflection denotes left side up.

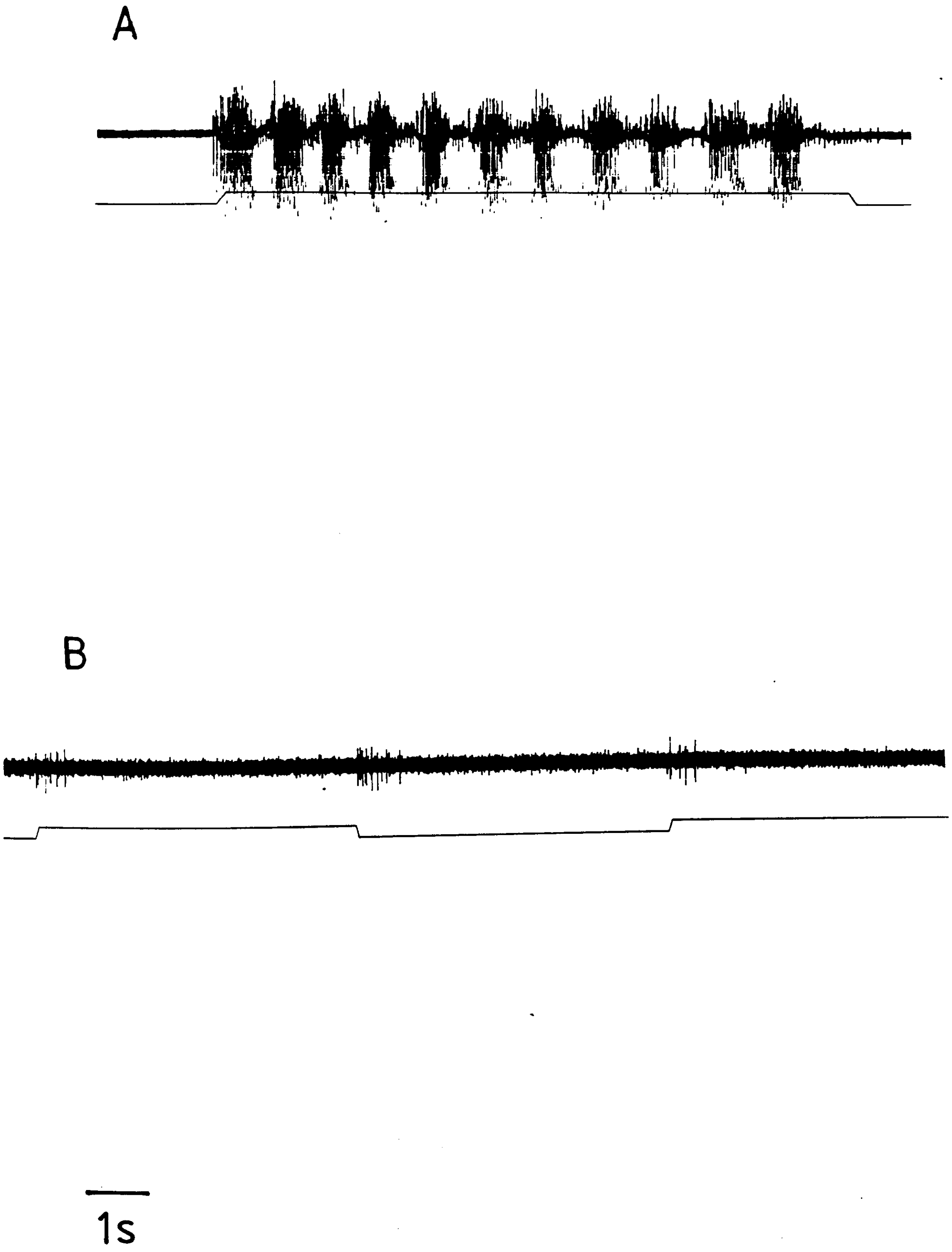

A
B
1s

Fig. 11

Recordings of descending leg activity. Upward deflection denotes leg levation.

A. Phasic units.

B. Tonic unit.

C. Tonic unit.

Further description in text.

(Recorded from T5/A1 connective)

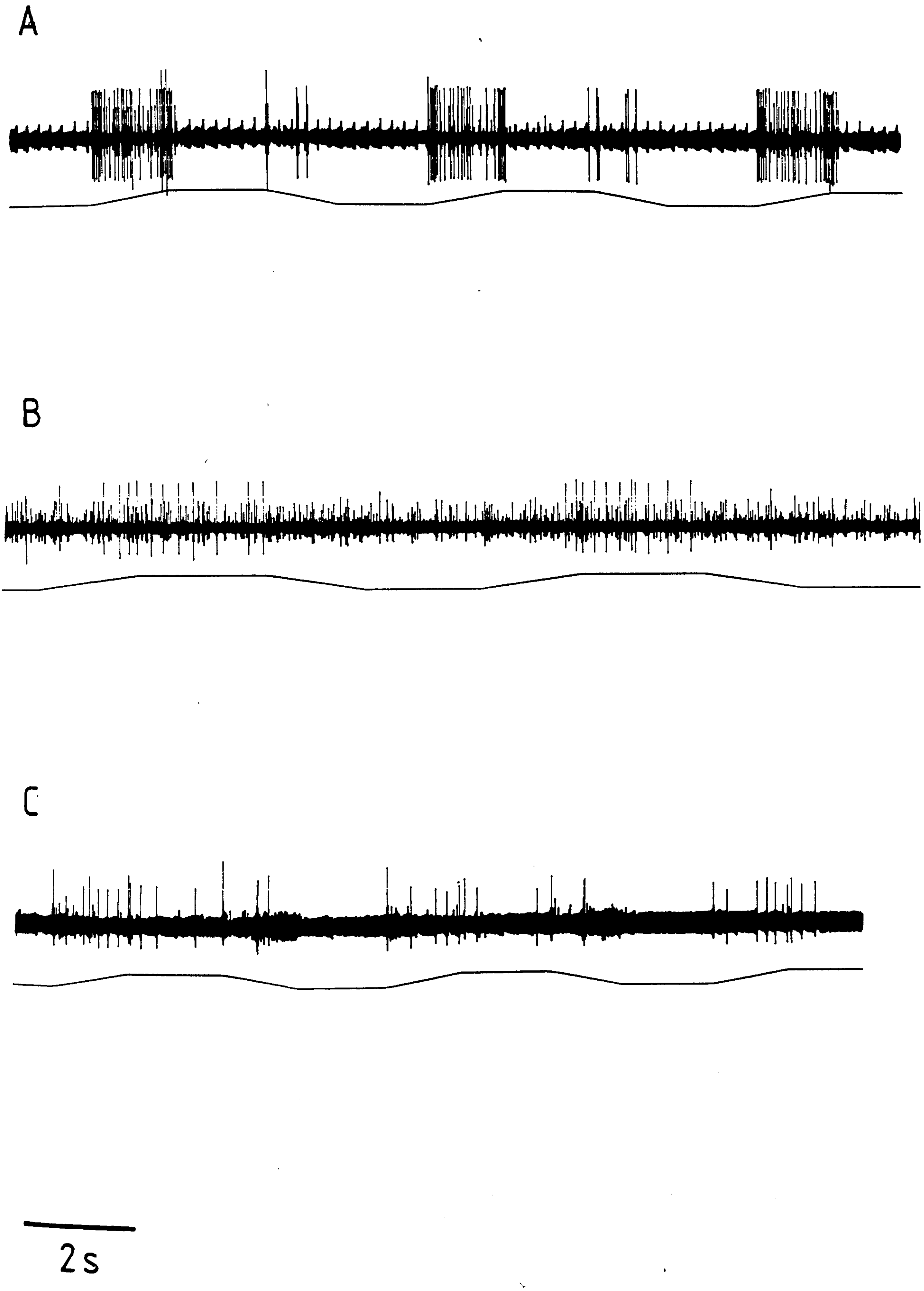

A
B
C
2s

Fig. 12

Units responding to mechanical stimulation of the uropod. Bar represents pressing on ventral surface of:

A. Right endopodite.

B. Right exopodite.

(Recorded from T5-A1 connective)

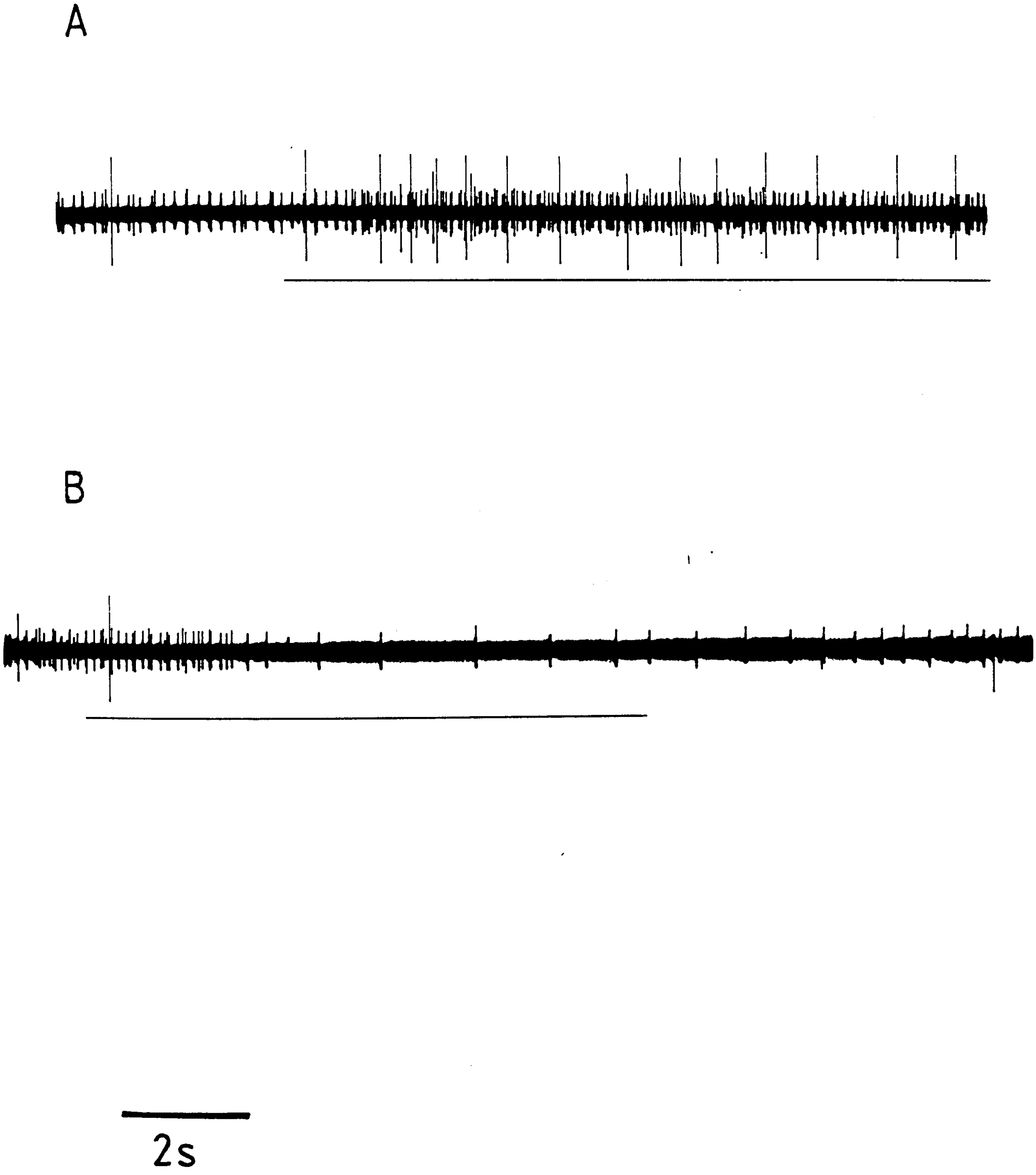

A
B
2s

Fig. 13

Responses of units to stroking fringe hairs of uropods.

A. Right endopodite.

B. Left "

C. Right exopodite.

D. Left "

(Recorded from T5-A1 connective)

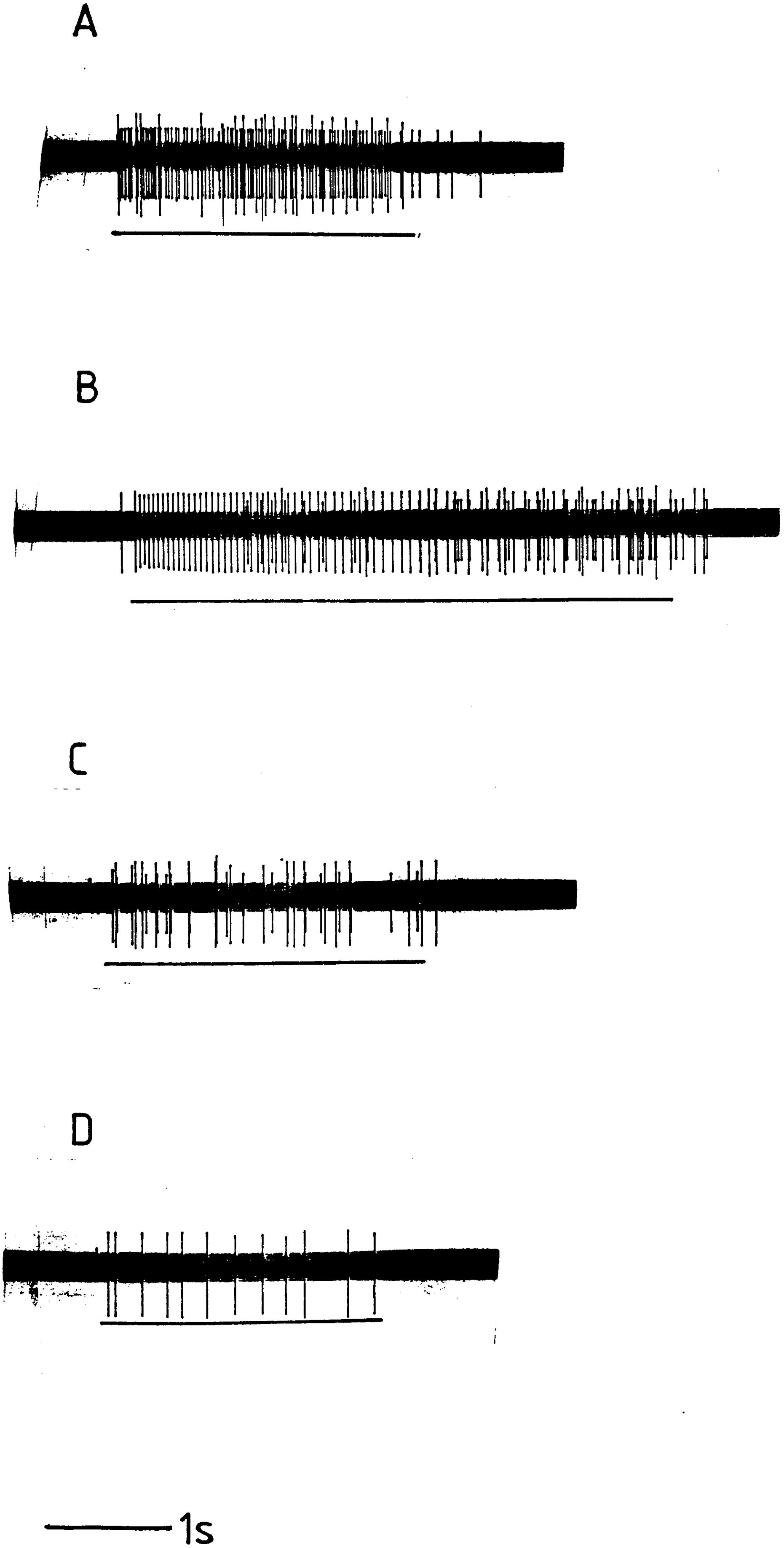

A
B
C
D
1s

Fig. 14

Response to water movement. Same recording as in Fig. 13.

A. Water disturbance at tail.

B. Water disturbance at head.

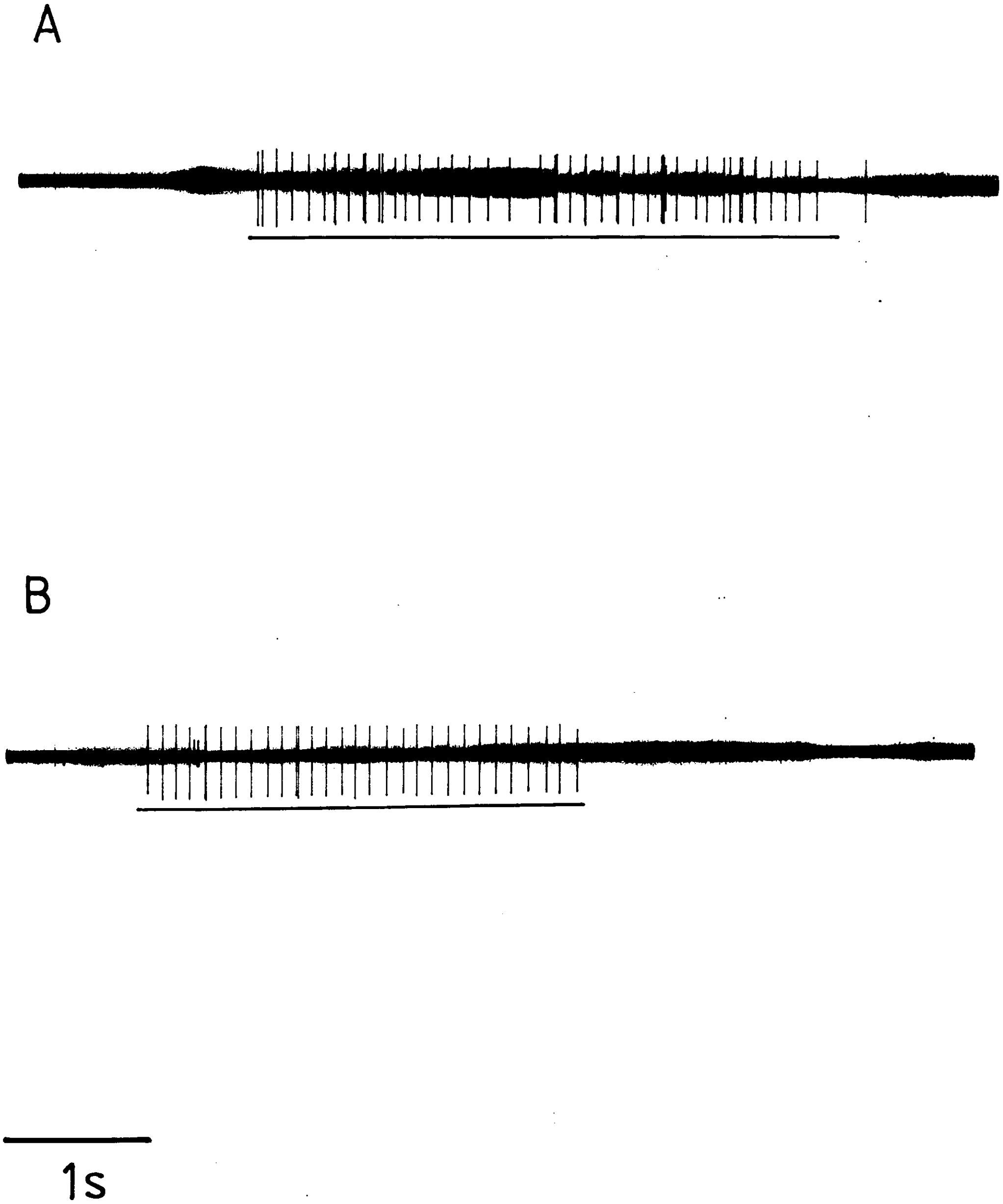

A
B
1s

Fig. 15

Response of units to water movement and tilt.

A. Water disturbance at tail.

B. Water disturbance at head.

C. Tilt in the pitch plane. Upward deflection denotes head-down tilt.

(Recorded from T5-A1 connectives)

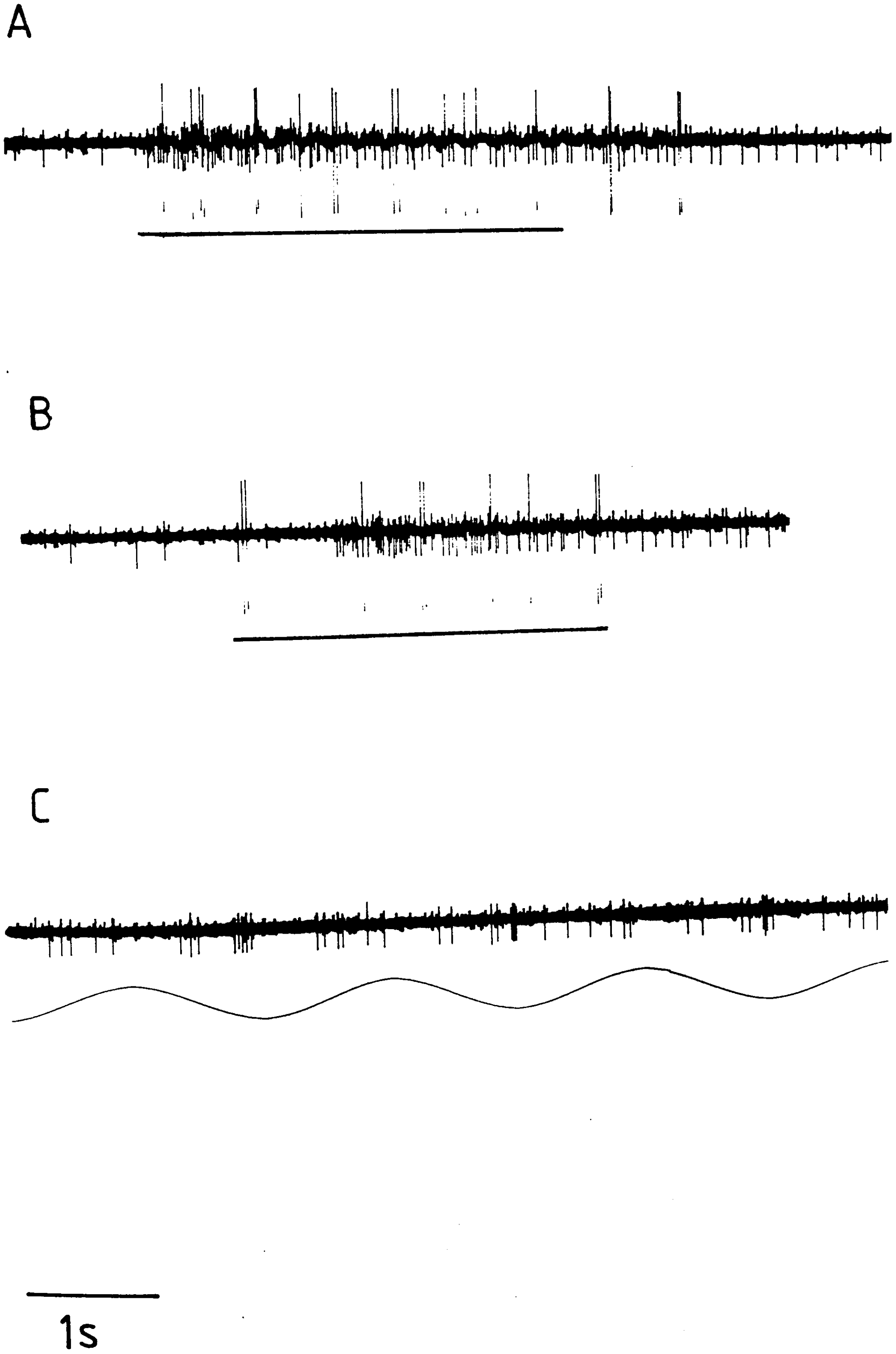

A
B
C
1s

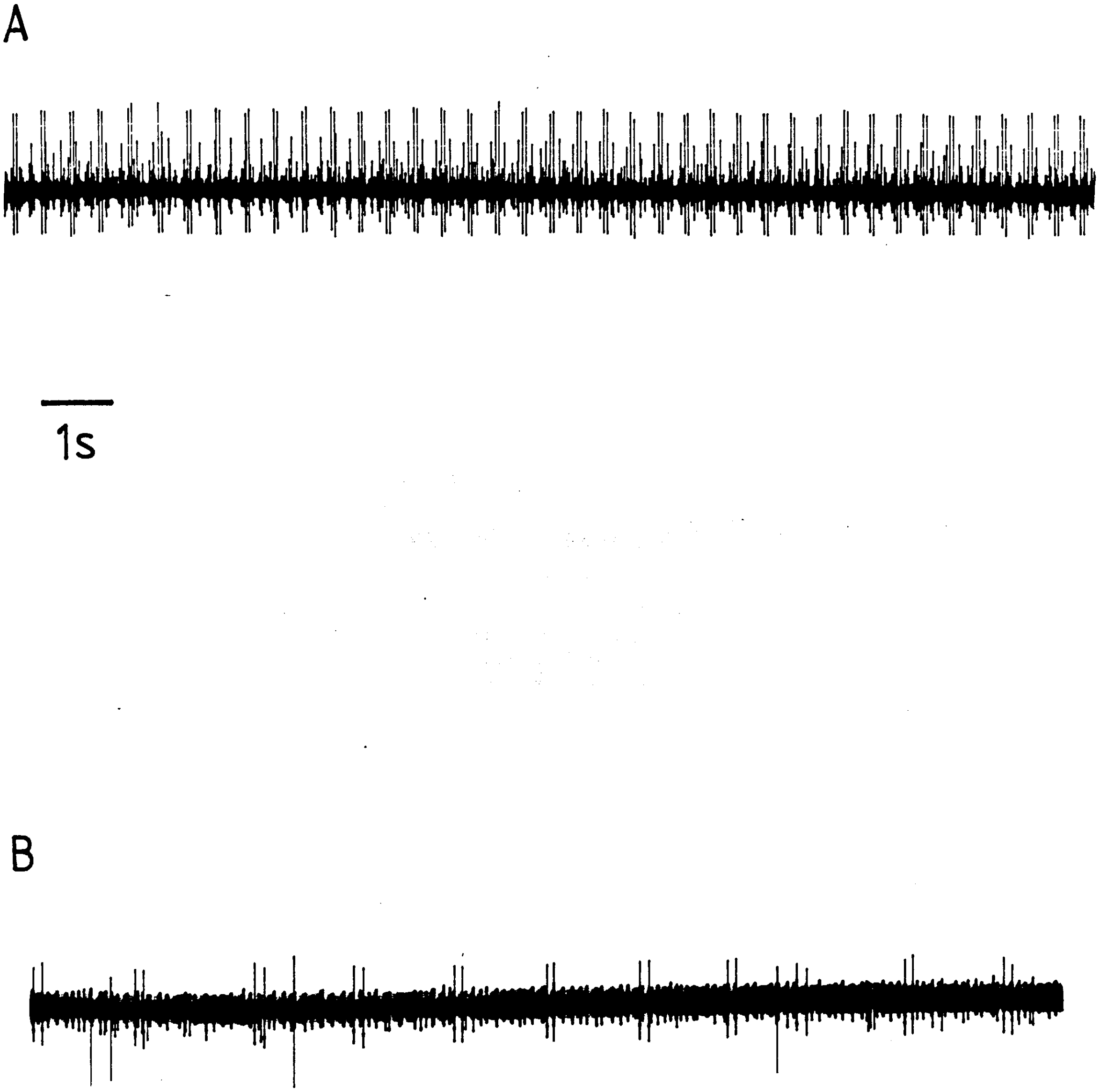
Fig. 16

Recordings of centrally generated rhythms.

A. Period approx. 0.4sec.

B. Period approx. 1.5sec.

(Recorded from T5-A1 connectives)


A
1s
B

Chapter 6 :

EFFECT OF TILT ON ABDOMINAL MOTOR OUTPUTS.

A. Introduction.

The reactions of the abdomen and swimmerets to tilt have already been described at a behavioural level (chapter 4). It was shown that certain responses observed to tilt in the pitch plane are distinct from those in the roll plane. It was suggested that these systems might offer opportunities to study interactions of various types. The most obvious of these is the interaction between the postural state of the abdomen and the expression of swimmeret reflexes.

The existence of interactions between the postural state of the abdomen and other motor sytems has been known for some time. Yoshino et al, (1980) noted that uropod steering responses only occurred when the animal, *Procambarus clarkii* in this case, was actively extending or flexing its abdomen. A similar relationship also exists in *Nephrops* (Newland, 1985). It has been suggested that facilitation from command interneurons for abdominal extension is necessary to allow statocyst inputs to reach uropod motoneurons (Takahata and Hisada, 1985). Interaction between swimmerets and abdominal posture has received some attention also. Williams and Larimer (1981) noted in *Procambarus* that extension of the abdomen produced by removal of substrate (or a combination of substrate removal and tilt as argued in chapter 4) was accompanied by swimmeret beating in 86% of trials. More recently it has been reported that mechanosensory stimulation of the swimmerets in *Homarus* produces abdominal extension (Kotak and Page, 1986). Thus an examination of the nature of the interaction between abdominal posture and swimmerets in *Nephrops* was undertaken.

Recording from both the swimmerets and the motor neurons to the tonic superficial flexor muscles (SFM's) will reveal whether the interaction between the two systems is mechanical, i.e. that the swimmeret system is suppressed when the abdomen flexes because it is mechanically impeded, or neuronal. In the first case the motor activity in the swimmeret system might not be expected to decline until after abdominal flexion commences, whereas in the later case the two effects would be expected to occur simultaneously.

The major factor which governs both abdominal posture and the expression of swimmeret reflexes, in the absence of substrate contact, is input from the statocyst system (chapter 4). Therefore it is reasonable to hypothesise that under such conditions the statocysts may also control the interaction.

Another aspect of the interaction is that of a rhythmic system (the swimmerets) interacting with a tonic system (the abdomen). In fact such an interaction occurs within the swimmeret system itself. As well as a large number of motoneurons which are only active during beating, there is at least one tonically active returnstroke motoneuron which continues to fire in the absence of beating (Miyan, 1982; Neil and Miyan, 1986). This unit merits detailed study for two reasons. Firstly, in the absence of beating, it continues to receive input from the statocysts, and is modulated in its activity in a predictable manner. As such it acts as a useful monitor of statocyst input in the absence of beating. Secondly, when beating occurs this unit fires along with the main returnstroke group, while still exhibiting tonic characteristics. Thus it is also a monitor of the output of the swimmeret central pattern generator (CPG).

Activity may be recorded from both the swimmeret and the SFM motoneurons using the inverted preparation outlined in chapter 2.

Furthermore, the use of this particular method allowed the same type of analysis to be carried out on these motor systems in relation to tilt around different axes as was performed on the statocyst interneurons. Such an analysis takes us much further than the behavioural analysis described in chapter 4. There it was demonstrated that pitch responses are only expressed in a narrow region at or near pure pitch. However, the pitch interneurons discussed in chapter 2 carry information outwith this narrrow band. By recording from the motor systems themselves it should be possible to throw further light on this apparent discrepancy.

Much is known already about the anatomy of both the swimmerets (for *Homarus* see Davis, 1968; *Nephrops* see Miyan, 1982) and the SFM's (for *Procambarus* see Wine et al, 1974; for *Homarus* see Thompson and Page, 1982). For completeness the central anatomy of these two systems in *Nephrops* has also been briefly investigated here.

B. Materials and methods.

The preparation used was the same as that described in the Methods for chapter2 (Ba(i)).Only the dissection will be described here.

The abdominal nervous system of *Nephrops* consists of a chain of six segmental ganglia. The paired inter-ganglionic connectives run within a common outer sheath just beneath ie dorsal to the ventral membrane. The ganglion in each segment lies in the midline beneath or slightly anterior to the sternal rib. The ganglionic root innervating the swimmeret muscles, ie root 1, runs along the surface of the abdominal musculature just below the rib (see Fig. 2). Therefore to obtain access to the root the rib had to be removed. In early experiments the rib was removed along its whole length. However as attention focussed on the more distal branches, the bulk of the rib was left intact. Instead both the swimmeret itself and the cuticle at its base were removed revealing the nerve branches to both power- and returnstroke muscles.

Access to the motor supply of the SFM's, the superficial branch of root 3 (3s), was obtained by removing the ventral membrane from between two sternal ribs. No other dissection was necessary.

In most preparations only one of the motor systems was examined. In some cases however, simultaneous recordings were made from both root 1 and root 3s.

To reveal the central anatomy of the motoneurones supplying the two systems, the cut motor axons were backfilled with cobalt chloride. The branch to be filled was carefully cleaned of surrounding tissue and any blood that had collected. A small piece of Parafilm was place under it, and a pool constructed by applying a Vaseline/paraffin oil mixture from a syringe. Two viscosities of Vaseline were used; one to actually construct the pool (approximately 50:50 Vaseline/paraffin oil) and the other to run down the outside of the pool to seal it (approximately 20:80 Vaseline paraffin oil). A solution of 100mM CoCl was then placed in the pool. The pool was sealed and left in a refrigerator, usually overnight although some were left for as long as 42 hours. The protocol used to develop the backfill is shown in table 1. The preparations were examined before being mounted in thick aluminium slides using the mounting medium "Histomount" for storage and photography.

C. Results.

The general anatomy of the abdominal ganglionic roots is shown in Figs 1 and 2. The swimmeret motor axons leave the ganglion by root 1, and the motor axons to the SFM's by the superficial branch of root 3 (3s). All three ganglionic roots are clearly visible when viewed from the ventral surface. While two divisions of root 1 are visible a short distance from the ganglion, particularly when the root is flattened against underlying structures, they run together as far as the base of the swimmeret. Only at this point are clearly separate branches visible (Fig 2). Hence removal of the swimmeret and the distal portions of the rib was sufficient to gain access to the branches of interest. A numbering system was adopted to aid in the identification of various branches. A note was made of the position at which various types of activity was recorded using this system. Of the two main divisions, the anterior branch supplies the returnstroke muscles and the posterior branch the powerstroke muscles; these were called branches a and p respectively (fig 2). Branch a2 curves to follow the powerstroke branch at the base of the swimmeret, while branch a1 continues in a more anterior and dorsal course and gives rise to finer branches supplying various returnstroke muscles. Branch a122 continues laterally towards the abdominal wall.

1. Central swimmeret anatomy.

Backfills of the whole of root 1 show two features of interest. Two distinct groups of cell bodies can be seen which lie ventro-laterally in the ganglion (Fig 3 but see also Fig 5). Of the two the posterior group is more closely packed while the anterior group seems more spread. The other feature of interest is that two fibre tracts curve round to the dorsal region of the ganglion. When the anterior branch 1a is backfilled, only the anterior group of cell bodies can be seen, in addition to the anterior fibre tract (fig 5). Axons can be seen running anteriorly from the region of the anterior fibre tract (fig 6). At a higher magnification it appears that there are several, possibly as many as four, axons running together. In some specimens there is a separate projection, also running anteriorly, but along a path that is ventral to that of the other fibres (fig. 7).

2. Swimmeret motor activity.

2.(i) Rhythmic responses.

Beating of the swimmerets is produced by alternating bursts of activity in power- and returnstoke motoneurons. Spontaneous bursting, ie rhythmic activity, of this type was recorded in some active preparations from branches 1a and 1p in the absence of any stimulus. Such spontaneous activity is shown in Fig. 8A. This activity was recorded from branches 3R1a (Fig. 8A1) and 3R1p (Fig. 8A2). When the electrode is attached medially to branch 1a, tonic activity is usually present in the form of one unit which is smaller than the units involved in the bursts (Fig. 8A1). The tonic unit in the returnstroke group becomes rhythmic when spontaneous beating occurs (Fig. 8b and c).

It has been shown that tilt is a very effective way to induce beating in the swimmeret system (Miyan, 1982). When the preparation was tilted head-down the swimmeret tonic returnstroke motoneuron fired rhythmically; when tilted head-up the tonic pattern was exhibited (Fig. 9). This activity was recorded from a distal branch (3R1a12) and hence the spike amplitude of the the tonic unit is increased relative to the other units. The rhythmic bursts of the tonic unit are not as tightly coupled as that of the more phasic units.

When histograms are constructed for this response in pitch, a clear pattern emerges. Rhythmic activity only occurs in one part of the cycle, at head-down (Fig. 10a). For comparison a histogram constructed from rhythmic activity recorded in the powerstroke branch is also shown. In this case the stimulus for beating was provided by side-up roll (Fig. 10b). The appearance of distinct peaks in both histograms demonstrates that the bursts which make up the rhythmic activity occur at the same points within each cycle. This has implications for our view on the effect of tilt on the CPG for the swimmeret system (see Discussion).

2.(ii) Tonic responses to tilt.

The tonic swimmeret returnstroke unit is of particular interest as it continues to receive input from the statocysts in the absence of beating. Therefore the tonic response of this unit to tilt was investigated. The most noticeable response is an increase in activity due to head-down tilt. On some occasions side-up tilt also produced a tonic increase in activity.

The effect of tilt in the pitch plane at different velocities was investigated. The result of one such series of tests is shown in Fig. 11. and the histograms constructed from the data in Fig.

12. The amplitude of tilt was the same in each test. At the highest velocity (20 °/s) the first head-down ramp caused several beats. This effect was less pronounced or absent in the subsequent cycles at the same velocity. The response consisted of four main components: a phasic peak on the head-down ramp, a decline to a tonic level of firing, a smaller phasic peak on the head-up ramp, a tonic level on the head-up plateau lower than that seen during the head-down plateau. In each case, and in other tests of the same type, the tonic level of firing on the head-down plateau was higher than that on head-up. However as the examples in Figs. 11&12 demonstrate, the velocity of tilt clearly has a bearing on the tonic level as well as the height of the peak at the beginning and end of the response. Over a number of cycles the activity in the unit tends to decrease. In other recordings this decrease continued until the activity was confined to a response to the movement.

Bilateral recordings of tonic returnstroke activity were made from branches 1a1 on both sides of the third abdominal ganglion. Spontaneous beating in these recordings occurred in synchrony demonstrating the bilateral symmetry of the system under these conditions (Fig 13a). Head-down tilt in the pitch plane, which did not induce beating, did produce an equal increase in the tonic level of firing on both sides (Fig. 13b). When held in a head-down position, the firing continued at a high level with no modulation (Fig. 13c).

The effect of tilting this preparation in different planes was investigated and the results are shown in Figs. 14 and 15. The recording from the right side is clearest throughout the various tests (top trace). Comparison of Fig. 14a with 14b shows that the response on the right side is no longer concentrated in one part of the cycle. Rather it has both spread over a wider area, and its mean phase position has moved (Fig. 15b top histogram). On the left side the response is complicated by the appearance of weak rhythmic activity. It is significant that this appears in a particular part of the cycle where the effects of head-down pitch and side-up roll might be expected to combine. On the left side there is no shift in the phase position of the response. Thus at the first intermediate position the tonic response in the two roots is now in antiphase.

Side-up roll produces beating in active preparations and a tonic increase in activity in more inactive preparations. Thus response to roll seen in the bilateral recording is what might be expected. The activity on both sides is increased on side-up (Fig. 14c and 15c). The response is less clear on the left side, but the histogram shows a concentration of activity in antiphase to that seen on the right.

The phase position of the response on the right side remains unchanged through the remaining planes of tilt. Comparing the response in roll (Fig. 15c) with the response in the intermediate plane of tilt (Fig 15d) the activity is increased. On the left side the response is spread, although the phase position remains substantially unaltered. However between here and the last test there is a phase shift such that the two sides show bilateral symmetry in the last test.

The phase position of the response for each side was plotted against the angle of the plane of tilt with respect to yaw. The result is shown in Fig. 24 and discussed later.

All modulation seen during tilt was abolished by cutting the connectives anterior to the recording site.

3. Central anatomy of motor axons to SFM's.

The other motor system that was investigated in these experiments was the superficial flexor muscles (SFM's). The bilaterally symmetrical motor roots to these muscles leave the abdominal connectives by way of the superficial branch of the third root (3s). Cobalt chloride backfills revealed five axons which travelled anteriorly to the ganglion of that segment, and one axon which travelled posteriorly. The anterior-going axons travelled in the dorsal region of the ipsilateral connective and gave rise to five cell bodies in the ventral region of the ganglion (fig. 16). The cell body of the axon that projects posteriorly was observed to be located in the anterior region of the next posterior ganglion. This corresponds well with the homologous system in both *Procambarus* (Wine et al, 1974) and *Homarus americanus* (Thompson and Page, 1982).

4. SFM motor activity.

Spontaneous activity in the superficial branch of the third root (3s) usually consisted of one to three units. However, of these the smallest unit fired throughout experiments, whereas the others tended to drop out. Up to four units were recorded on any one occasion (Fig. 17). Unit a. was usually present, and this unit responded to tilt as described below. Firing in the other units in this recording, labelled b to d on the basis of spike amplitude, could be elicited by mechanosensory stimulation.

The motoneurons in this system are conventionally labelled f1 to f6. However, it has been shown that f6 activity in *Homarus* is difficult to obtain by natural stimulation and that f1 and f2 spikes usually have very similar amplitudes (Thompson and Page, 1982). Thus if the unit labelled a in Fig. 17 represents either f1 of f2, and f6 is assumed not to be firing, it is reasonable to assume that b represents f3, c f4, and d f5. Thus d must also be the peripheral inhibitor. Further evidence for the identification of the peripheral inhibitor will be presented below.

The smallest of the SFM units responded to head-up tilt (Fig. 18). During the first series of tilts in pitch two other units responded; the largest unit fired with the small unit, i.e. to head-up tilt, while the other unit of intermediate spike amplitude fired in antiphase to the small unit, ie it responded to head-down tilt.

The response of the small unit was recorded during tilt in different planes (Fig 18). The modulation in the activity due to tilt clearly decreases as the plane of tilt moves away from pitch until, when tilted in the roll plane, there is a tonic level of firing but not modulation. As the unit continues to fire during roll as it does in the absence of any kind of tilt, the reduction in activity seen during head-down tilt may be due to inhibition. Fig. 19 shows the histograms constructed from this data. Also shown are the histograms constructed from activity recorded at planes of tilt intermediate to those shown in Fig. 18. These demonstrate that the modulation in activity is present well away from pure pitch and that the switch in the phase of the response occurs at or near roll.

The activities of both the motor axons to the SFM's and the tonic swimmeret returnstroke motoneuron were recorded simultaneously to confirm similarities and reveal differences in their respective responses. This data is shown in Fig. 20 and the histograms constructed from it in Fig. 21. In Fig. 20 the top trace (labelled 1) is the activity recorded from branch 3R1a1, and the bottom trace (labelled 2) is the activity recorded from 3R3s.

It is clear that both the tonic swimmeret returnstroke motoneuron and the larger SFM unit in the recording from branch 3R3s respond to head-down tilt. This provides further evidence that the larger SFM unit is in fact the peripheral inhibitor, as during head-down an abdominal extension is normally observed. Careful examination of the rest of the activity in the lower trace suggests that there may be two units responding to head-up tilt. As the function of this activity is to produce flexion of the abdomen in response to head-up tilt, this will be treated as one entity i.e. flexion activity. The histograms in Fig. 21(2) represent the combined activity of these two units.

There is a high degree of synchrony in the response of the tonic swimmeret returnstroke and flexor inhibitor units to head-up tilt, although careful examination shows that the relationship is not one-to-one. At the slower velocities where influences other than tilt may begin to have effects, increase in the discharge of the tonic returnstroke motoneuron is accompanied by an increase in the flexor inhibitor, eg Fig. 20e. Both units fire together, possibly stimulated by an accidental tactile stimulus.

The discharge patterns of all three types of activity are best at the higher tilt velocities. Both the tonic returnstoke motoneuron and the flexor inhibitor show "off" responses while the flexor excitatory motoneurons shows no such response. Of the two types of activity recorded from branch 3R3s, the flexor motoneurons maintained a high level of activity at all velocities although the clarity of the response is reduced at lower velocities. The inhibitor activity decreased at the lower velocities. The activity of the tonic returnstroke motoneuron also decreased at the lower velocities although a tonic level of activity was always maintained. The response in the tonic swimmeret returnstroke motoneuron disappears at velocities below 2.5° (Fig. 21e1) while the SFM motoneuron responds at the lowest velocities.

As noted above, modulation of tonic activity by tilt was abolished by cutting the connectives anterior to the recording site. However, tonic activity continued at both recording sites.

5. Interactions between systems.

To further investigate the basis of the interaction noted above the motor roots involved were stimulated electrically. The stimulus was sufficient to produce contraction in flexor muscle fibres. No response was seen in the activity of the tonic returnstroke unit to stimulation of 3R3s, and there was no response recorded in 3R3s to stimulation of 3R1a1.

The legs were manually stimulated either by pinching or by levating and depressing them about the CB joint. The clearest effects were general in character, taking the form of a rise in the tonic level of activity. In the case of the swimmeret motoneurons, stimulation of the legs was sometimes sufficient to produce phasic activity.

Fig. 22 shows the effect of pinching the dactyls of contra- and ipsilateral L2. In Fig. 22a the contralateral leg with respect to the side being recorded was used; in 22b the ipsilateral leg is stimulated. The stimulus causes a rise in the tonic activity in both the tonic swimmeret returnstroke unit (3R1a1 large unit) and the SFM unit (3R3s small unit). The activity rises in both systems simultaneously. Other SFM units are recruited in response to the stimulus. In another recording of SFM motoneuron response to leg stimulation (Fig. 23), three units are recruited. However, the smallest unit (which responds to tilt) shows the clearest response, firing at a high frequency for a considerable period.

[illegible] by the [illegible]
[illegible] and particularly [illegible]
[illegible] the [illegible] systems both [illegible]
[illegible] and neurally (Kotak and Page, [illegible]
[illegible] differences between the [illegible]
[illegible] and abdominal flexor muscles. [illegible]
[illegible] in pitch where their line of [illegible]
longitudinal axis of the animal, [illegible]
[illegible] Outside of this plane they [illegible]
[illegible] the results reported above indicate, [illegible]
[illegible] by tilt. This reflects the [illegible]
[illegible] statocyst interneurons. In chapter [illegible]
[illegible] continue to provide [illegible]
[illegible] Fig. 18 demonstrates the [illegible]
[illegible] to be modulated by tilt [illegible]
[illegible] modulated by tilt in the [illegible] plane.
[illegible] input from only the pitch [illegible]
[illegible] in chapter 2.

D. Discussion.

The results reported in this chapter have implications for various types of systems. The patterns of activity demonstrated by the swimmeret system must be compared and contrasted with other information on the same system in the literature (particularly that of Miyan, 1982), and also with ideas concerning other rhythmic systems (eg Pearson, 1985). The activity manifested by the tonic superficial flexor system must also be borne in mind particularly as there is a very close relationship between the two systems both behaviourally (Williams and Larimer, 1981) and neuronally (Kotak and Page, 1986).

There are basic differences between the action of the swimmeret muscles and abdominal flexor muscles. Behaviourly the SFM's are only active in pitch where their line of action, which lies along the longitudinal axis of the animal, coincides with the plane of tilt. Outside of this plane they serve no clear function, and as the results reported above indicate, they cease to be modulated by tilt. This reflects the input they receive from descending statocyst interneurons. In chapter 2 it was shown that pitch interneurons continue to provide information outwith the pitch plane. Fig. 18 demonstrates that the motor output to the SFM's continues to be modulated by tilt outwith the pitch plane, but is not modulated by tilt in the roll plane. The SFM's may therefore receive input from only the pitch interneurons already described in chapter 2.

The outputs of both motor systems are anatomically bilaterally symmetrical. The motor output pattern of the motor axons to the

SFM's on both sides of the midline would be expected to be symmetrical at all angles of tilt. The tonic output of the swimmeret system is clearly not bilterally symmetrical under all conditions. As with the motor output to the SFM's, the tonic response of the swimmerets to tilt in one direction in the pitch plane is symmetrical, while in roll the side-up fires while the side down is silent. At intermediate planes of tilt two aspects of the response are of interest; one is where the phase shifts in activity take place, the other is the way the nature of the activity changes whithin the cycle of movement.

The activity of the swimmerets in the roll plane is probably initiated by statocyst interneurons carrying information about tilt in the roll plane. Such interneurons have been recorded in *Procambarus* and are implicated in uropod steering (Takahata et al, 1985). Thus in accounting for the bilateral pattern of tonic activity there are now two routes by which excitatory inputs reach the swimmeret system; both derive from the statocysts, but this does not necessarily mean that their effects will always be congruent. In fact the results presented in this chapter suggest that when both effects are combined they add together to provide a greater degree of excitation to the swimmeret system than would otherwise be the case. When they occur at different points they can be seen separately. It may be that in the intact animal either effect separately is sufficient to produce beating in the swimmeret system whereas in a dissected and fixed preparation their combined effects are necessary to produce beating.

The phase shifts exhibited by the two motor systems are very different. This again may be the effect of two types of statocyst input combining to produce the pattern seen in the swimmeret system, whereas only one type of input drives the SFM's (Fig 24).

Whereas the phase shift in the SFM motoneuron occurs about roll, in the swimmeret system it occurs at or about pitch. While the pattern exhibited by the SFM motoneuron is consistent with the hypothesis of it being driven by pitch interneurons, these interneurons alone could not produce the pattern seen in either of the plots for the tonic swimmeret returnstroke motoneurons (see also chapter 7).

We are now in a position to comment on the interaction between the swimmeret system and the abdominal posture system in *Nephrops*. The close connection between the responses of the swimmeret tonic returnstroke motoneuron and the SFM peripheral inhibitor suggest that there are neuronal connections between the two systems. The electrical stimulation experiments show that this is probably not in the form of collaterals from one set of motoneurons synapsing on the other set and vice versa. However one possibility is that the interaction occurs at the level of separate pools of premotor local interneurons which separately receive statocyst input, as well as input from other systems such as the legs. Such local interneurons are known to exist in the swimmeret system (Paul and Mulloney, 1985) and are also of importance in the uropod steering system (Nagayama et al, 1984).

The functional significance of the responses discussed is not yet clear. It is probable that the responses of the abdomen to tilt should be seen within the context of control of mid-water equilibrium (see chapter 4). Alterations of abdominal posture could have two important effects. Firstly, abdominal posture will affect the flow of water around the body and thus itself promote righting and control of equilibrium. Secondly however abdominal posture will interact with the effectiveness of other systems which have a role within this context ie the swimmerets and uropods.

The significance of the tonic swimmeret returnstroke activity again is not clear. Tonic activity of the returnstroke muscles would serve to hold the swimmeret in a protracted position against the ventral surface of the abdomen. Behavioural observations of such effects have been reported (Miyan, 1982). While this may affect righting and equilibrium, such a contribution would be expected to be small.

The finding that tilt is a major factor in the production of abdominal posture also has wide implications, as has already been discussed (chapter 4). The findings in this chapter complement those already discussed and extend them. Various factors emerge from the motor records which were unclear from the behavioural studies. The records suggest that in pitch the motor activity recorded from motoneurons supplying the superficial tonic flexors does not begin until later in the imposed head-up tilts than, for example, the tonic swimmeret motor activity occurs in the head-down tilts. The timecourse of the behavioural response was not studied. However in some cases it was clear that in the case of both head-up and head-down tilt abdominal movement continued to a final position after the end of the imposed tilt.

The peripheral inhibitor, present in some of the traces, which fires in an opposite manner to the small unit in the flexor recordings and in the same manner as the tonic returnstroke unit is of particular interest. Abdominal posture is the product of two systems, only one of which has been looked at in these experiments, ie the SFM's and the slow extensor muscles (SEM's). The implication of the results reported in this chapter and those of chapter 4, is that the activity in these muscles too is modulated in the pitch plane by tilt. However, as they respond in the opposite manner to the SFM's, the flexor inhibitor may serve as being representative of the SEM activity.

As was shown in chapter 4, in considering abdominal posture, we are considering a patterned movement; it is a pattern in space as much as in time. Each of the jointed segments assumes, in a given condition, a particular state of flexion/extension with respect to its neighbours. The motor activity recorded in these experiments has to be regarded as only a preliminary step, as it constitutes a "snap-shot" of the overall pattern. It is of interest that the motor activity in the fourth segment is slightly delayed relative to the tilt. Of further interest is the timing of the output in other segments during similar tilts.

There is another element in the control of abdominal posture that has not been studied in these experiments. Each abdominal segment posseses a pair of muscle receptor organs (MRO's). One of these, the lateral or tonic MRO, has a high sensitivity to stretch and a low rate of adaptation. It responds readily to abdominal movements produced by the SEM's and SFM's (Fields, 1966). This receptor is known to be involved in the reflex control of abdominal posture (for review see Page, 1982). However the preparation used to study SFM motoneuron responses to tilt was fixed, therefore reflex effects were not seen. However the existence of such a sensory system would allow the production of meaningful patterns of motor output to the abdomen, which could be monitored and controlled.

Two types of neuronal element are included in theoretical models of many motor and equilibrium systems. One is the command interneuron, and the other is the central pattern generator. For various reasons, the orthodox understanding of what these terms mean has come under attack recently (for review see Davis, 1985, and Pearson, 1985). The reasons for this will be dealt with in more detail in chapter 7. However, the results described in this chapter, for both systems, have implications for ideas concerning these elements.

The tonic swimmeret returnstroke unit is both tonically modulated by statocyst input in the absence of beating, and is affected by rhythmic output from the swimmeret oscillator circuitry which itself is affected by the same statocyst input. It has been suggested that this unit is isolated from the output of the oscillator. However, Figs. 8,9&10 suggest a clear if variable involvement. What is also clear is that the effectiveness of the rhythmic drive onto this particular motoneuron is not as great as for the other motor units in the system. This is manifested as a low intra-burst frequency and small inter-burst interval. The spikes of the other motoneurons are closely packed in the bursts with a relatively longer inter-burst interval. The response of the tonic unit is, however, somewhat variable, both from preparation to preparation, and within one preparation throughout the experiment.

The tonic modulation of the unit is less variable, and acts as a monitor of decending statocyst input into the system. Thus in the absence of rhythmic activity statocyst information is still available to the motor system. This finding clearly has implications for the central pattern generator (CPG) for this system. When there is no rhythmic output, the CPG is either not operating, or is operating but is disconnected at a premotor level from the output of the system. In this case, a parallel pathway must exist by which statocyst information bypasses the CPG to reach the tonic motoneuron (Neil and Miyan, 1986).

However, tilt not only initiates beating, but must reset the

CPG. To produce the type of histograms illustrated in Fig. 10 the rhythmic activity has to start at the same point and proceed with the same frequency during each cycle, otherwise clear peaks would not occur. The set of data obtained by Neil and Miyan (1986) in roll over a wider range of stimulus frequencies than employed here shows the same effects. Therefore, statocyst input into the swimmeret CPG must both gate and reset the rhythm of the CPG and couple it to the motoneurons, or switch on the CPG itself.

In intracellular studies of neurons in rhythmic systems one of the criteria used to determine if a particular neuron is part of the CPG is to establish whether it is capable of resetting the rhythm of the motor output. On this basis, if the first of the options presented above is accepted then descending statocyst input, perhaps even particular statocyst interneurons, must be firmly included as part of the CPG.

If the second of the options presented above is preferred then the statocyst input is clearly acting as the command signal for beating. The interneurons carrying the information must therefore be described as command interneurons or elements. Furthermore, in the control of abdominal posture, another area dominated by command fibre concepts, the statocyst input clearly has a command function. Head-up pitch interneurons act as command iterneurons for abdominal flexion, and head-down interneurons act as command interneurons for abdominal extension.

These conclusions demonstrate a problem that is also emerging in other areas of interest to invertebrate neurobiology. Regardless of which of the above schemes is shown to be the case by further study, distinctions between cells are beginning to break down. Therefore the idea of the CPG as a distinct group of cells within an abdominal hemiganglion is properly being replaced by the concept of a large number of cells having a role in the determination of the pattern of motor output. There is clear evidence that hemiganglionic centres operate in the crayfish swimmeret system (Paul and Mulloney, 1985). But it is also clearly accepted that each centre is influenced by inputs both from the contralateral hemicentre and extra-segmental centres. Thus the suggestion that descending statocyst interneurons act not simply as an input to but as a major determinant of the pattern of motor output in the swimmeret system fits into the currently accepted concept of pattern generation.

Part of the difficulty concerni assigning certain functions to particular parts of the system is that each part may be responsible for or involved in more than one function. These involvements may be obscured rather than revealed by the use of isolated preparations. Throughout these experiments an almost intact preparation has been used, and more importantly, the stimulus employed has been a physiological stimulus shown at a behavioural level to have effects consistent with those described here. Thus it may be that in this preparation, the interneurons carrying information from the statocysts act both as sensory interneurons and command interneurons. The precise function assigned to them may depend on the context within which they are viewed. Thus, within the context of a consideration of the swimmeret system they act as command interneurons, whereas within the context of tonic reactions of the uropods they convey directional information but are dependant on the command signal of another system (that for abdominal extension) for the information they carry to be expressed (see Takahata and Hisada, 1985).

Table 1.

A. Protocol for development of $CoCl$ backfills (see text for details).

A. Protocol for Developing Cobalt Chloride Backfills.

Remove tissue and pin out in "Sylgard" lined dish.

1. Add fresh saline + 2-3 drops conc. ammonium sulphide (10 mins-1 hour).
2. Wash in fresh saline (3x5 mins).
3. Fix in acetic alcohol (10 mins).
4. 95% alcohol (10 mins).
5. 70% alcohol (10 mins).
6. 2% sodium tungstate soln. (10-30 mins).
7. Intensification soln. (see below). Remove after 10 mins. or on appearance of non-specific deposition of silver.
8. Wash in distilled water (3x5 mins).
9. 0.1M sodium hydroxide. Remove once tissue cleared or after 5 mins.
10. Wash in distilled water (3x5 mins).
11. 30% alcohol (10 mins).
12. 50% alcohol (10 mins).
13. 70% alcohol (10 mins).
14. 100% alcohol (10 mins).
15. 100% alcohol (10 mins).
16. Clear in "Histoclear", mount in thick slides using "Histomount".

Table 1.

B. Intensification solution (see A.).

B. Intensification Solution.

Soln. A.

Distilled water	355ml
1% Triton X 100	15ml
Sodium acetate	1.5g
Acetic acid (Glacial) ...	30ml
Silver nitrate	0.5g

(Keep refridgerated)

Soln. B.

5% Sodium tungstate.

Soln. C.

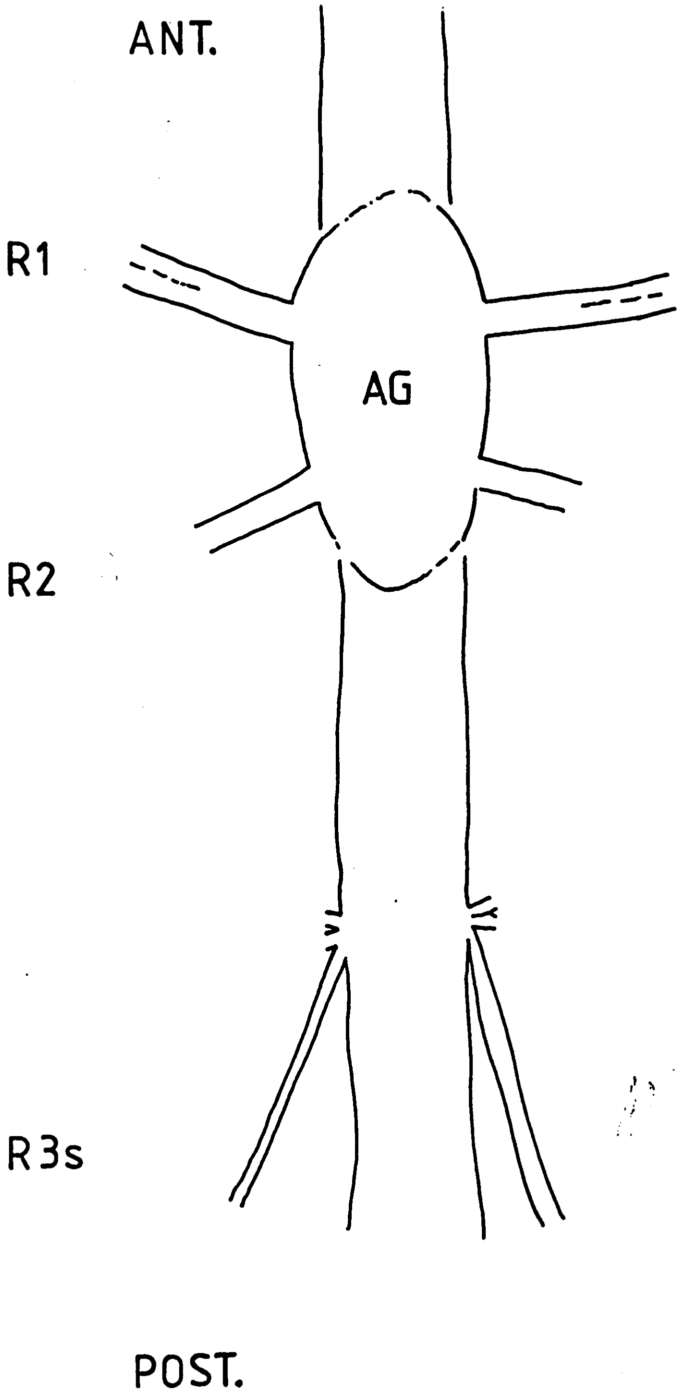
0.25% ascorbic acid. (Must be freshly made)

These solutions are mixed in the following proprtions 8A:B:C.

(Technique modified from Davis, 1982)

Fig. 1

General anatomy of an abdominal ganglion showing the ganglionic roots. Root 1 is the motor supply to the swimmeret system and root 3s the motor supply to the tonic superficial flexor muscles (SFM's).



VENTRAL
VIEW

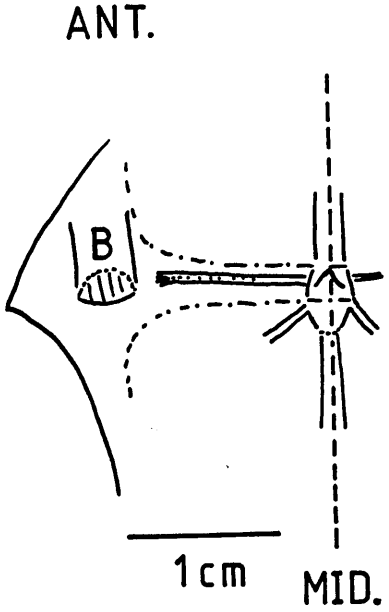
Fig. 2

Top.

Relationship between the ganglion, root 1 and gross external structures such as the sternal rib and base of the swimmeret (B).

Bottom.

Division of root 1 into finer branches at base of the swimmeret (B). Explanation in text.


ANT.
B
1cm
MID.

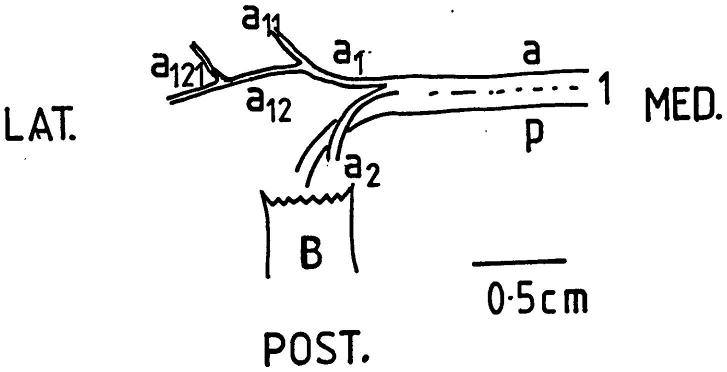
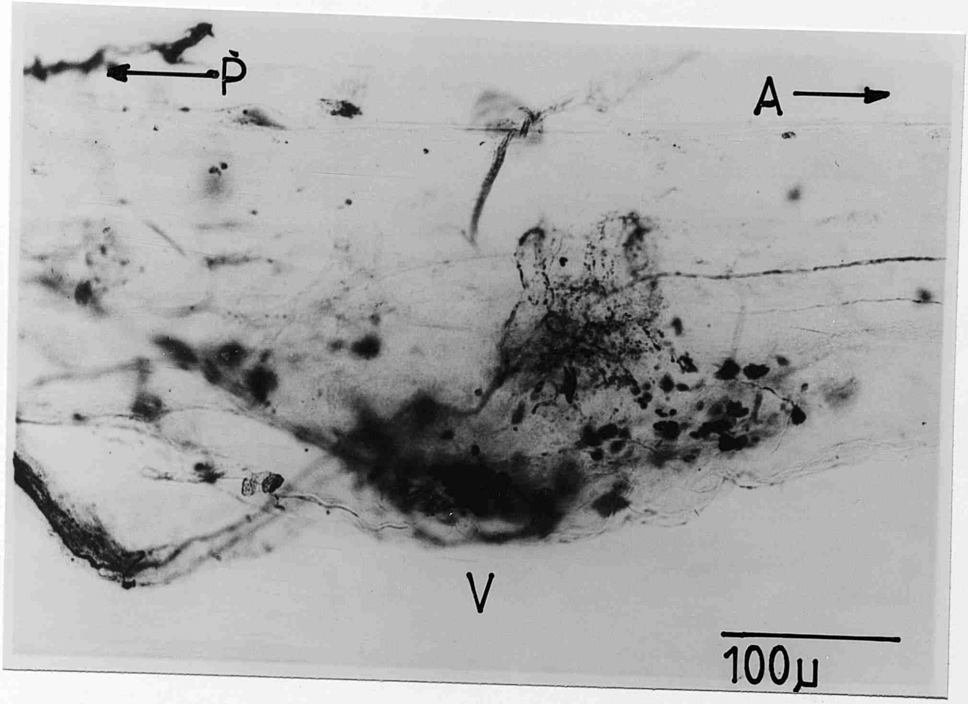

a_{11}
a_1
a
a_{121}
a_{12}
1
LAT.
MED.
p
a_2
B
0·5cm
POST.

Fig. 3

Backfill of whole of root 1. Detailed description in text.

Fig. 4

Camera lucida drawing of above.


P
A
V
100µ

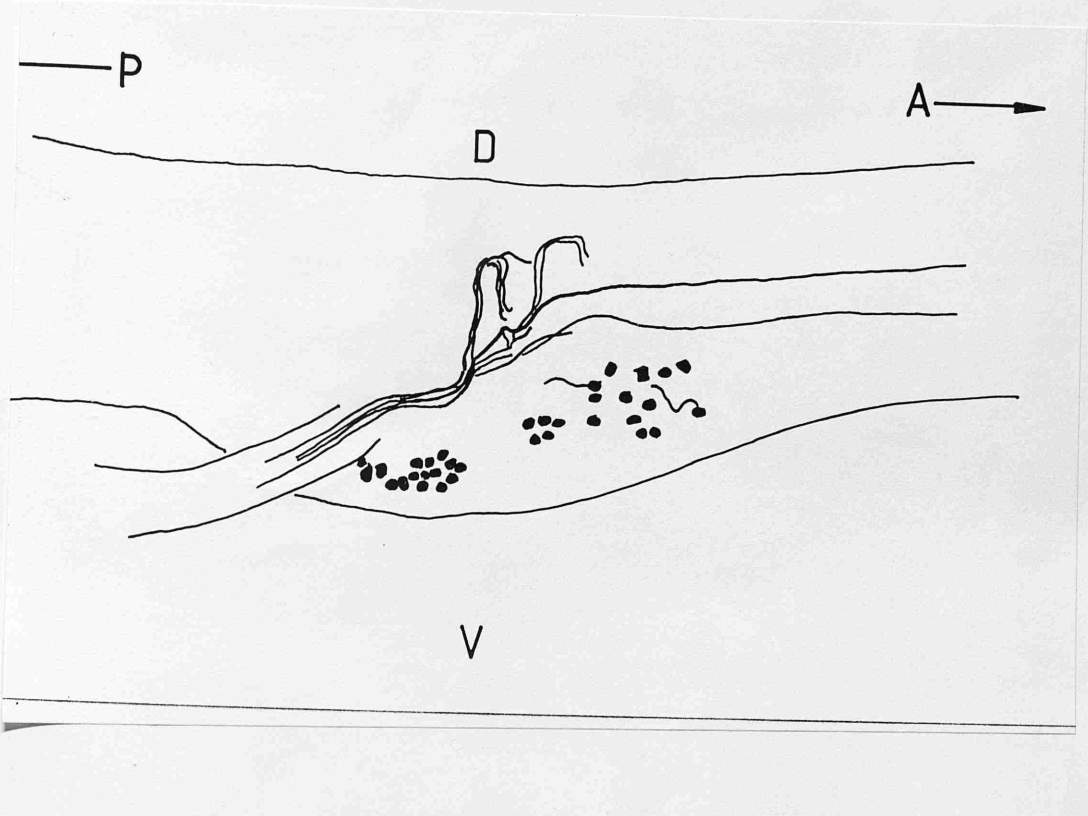

P
A
D
V

Fig. 5

Backfill of anterior branch, branch a. Detailed description in text.

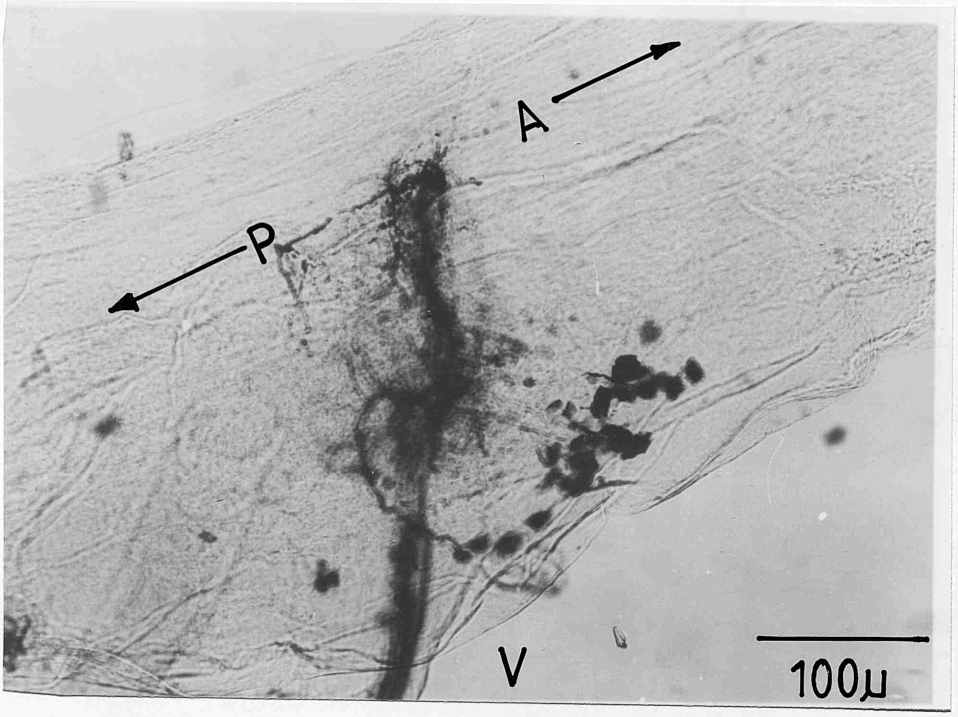
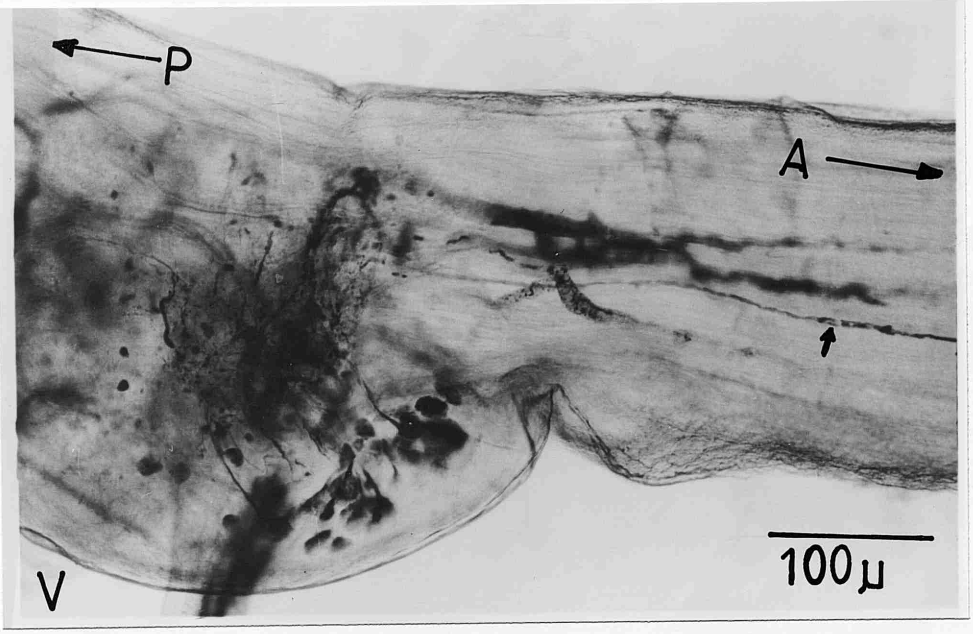

A
P
V
100µ

Fig. 6

Dorsal projections from swimmeret motoneurons.

Fig. 7

Ventral projections from swimmeret motoneurons.


P
A
V
100µ

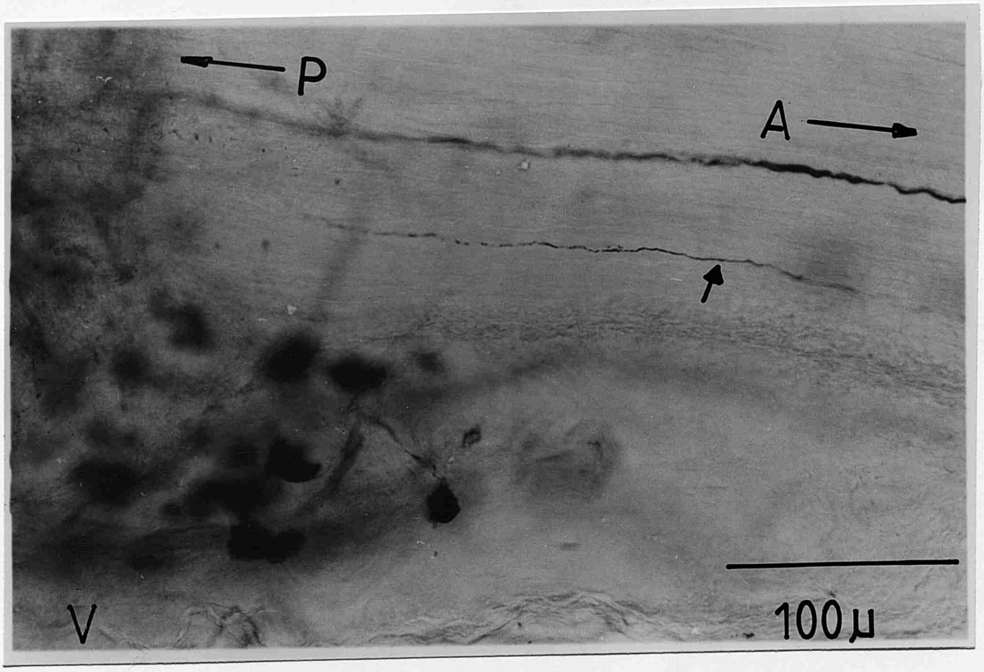

P
A
V
100µ

Fig. 8

A. Spontaneous activity recorded from root 1 on the right side of the third abdominal ganglion. (Recorded en-passant)

A1 : Recording from 3R1a.

A2 : Recording from 3R1p. (See Fig. 2)

Arrows indicate activity in the tonic returnstroke unit.

B&C. Magnification of returnstroke (3R1a) recording.

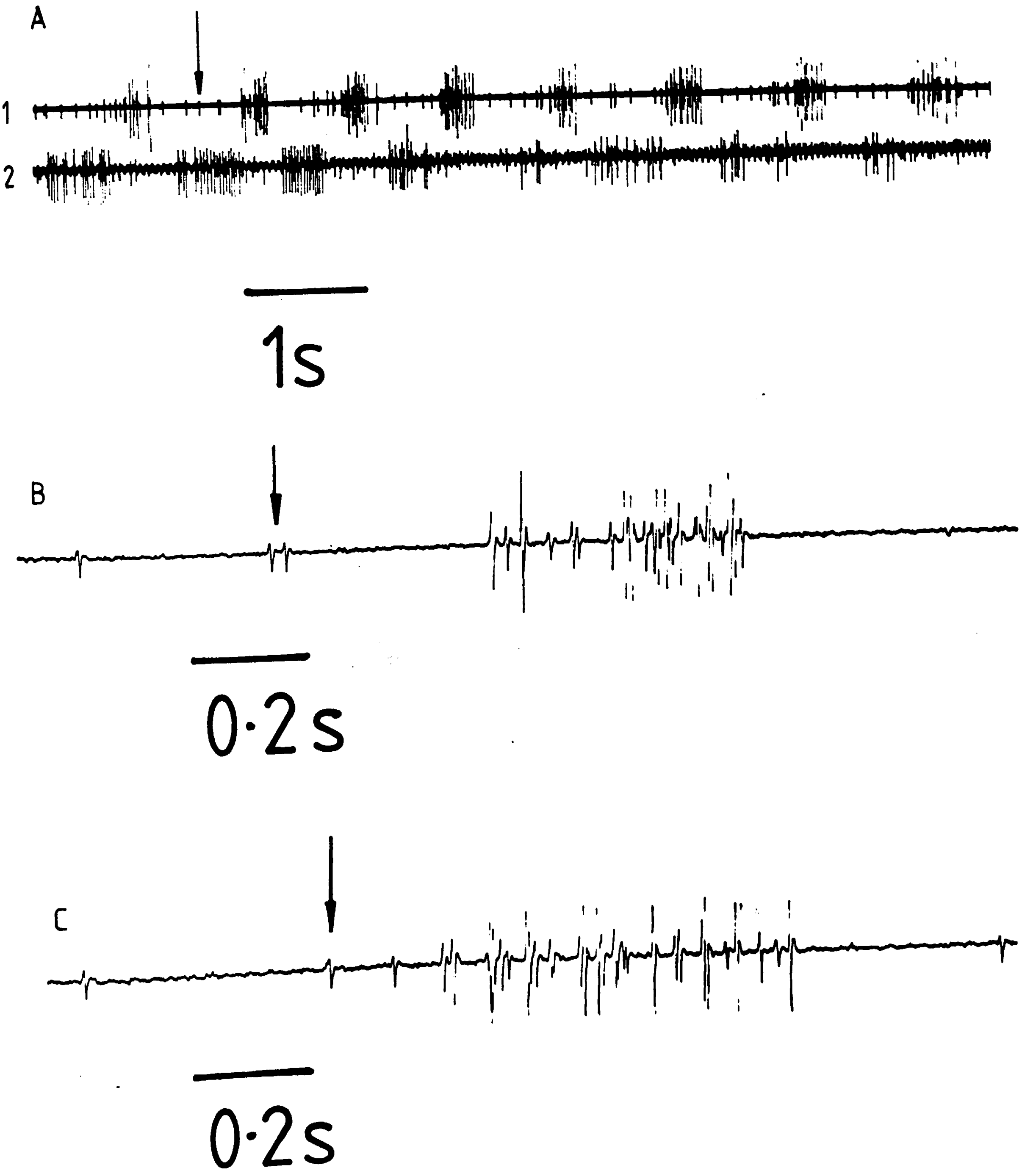

A
1
2
1s
B
0·2s
C
0·2s

Fig. 9

A&B. Effect of tilt in the pitch plane on activity of tonic returnstroke unit.

Upward deflection denotes tilt head-up.

C. Same record, faster sweep speed.

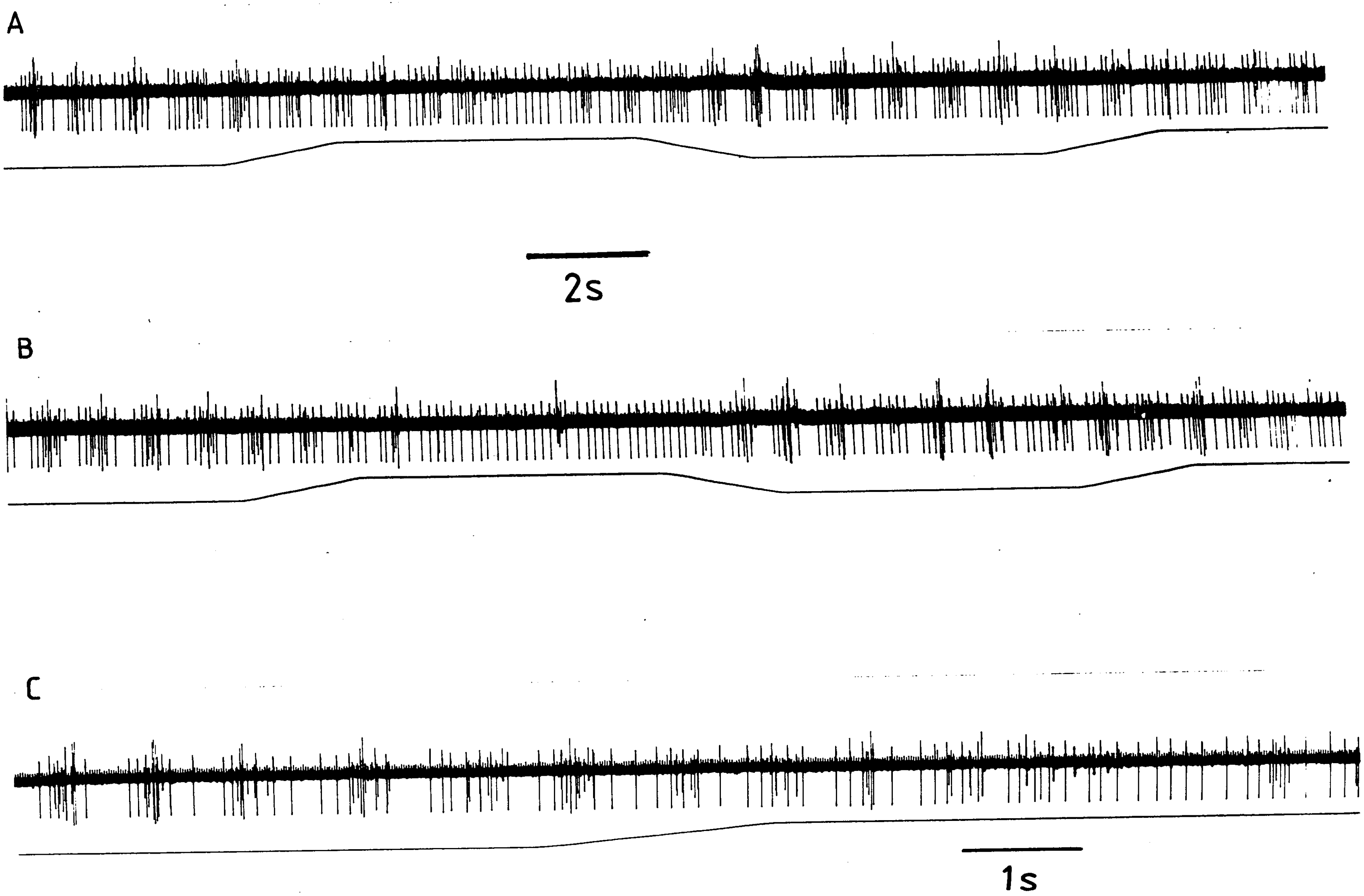

A
2s
B
C
1s

Fig. 10

A. Histogram of activity in tonic returnstroke unit constructed over ten cycles during tilt in the pitch plane. Upward deflection denotes head-up.

B. Histogram of powerstroke activity constructed over ten cycles during tilt in the roll plane. Upward deflection denotes side-up.

Detailed description of both histograms in text.

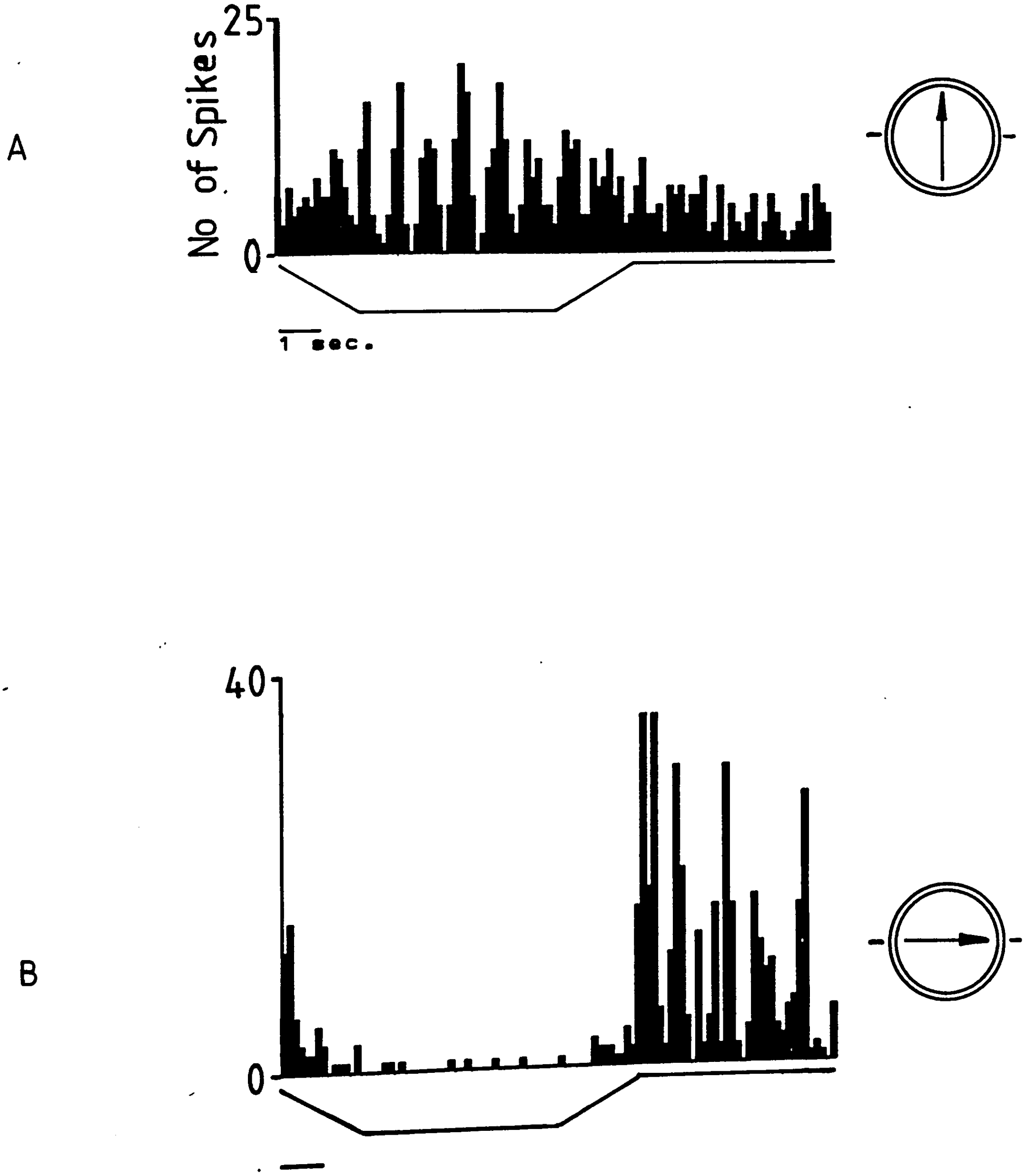

A
25
No of Spikes
0
1 sec.
B
40
0

Fig. 11

Effect of tilt in the pitch plane at different velocities on tonic returnstroke unit.

Velocities :

a. 20° /s.

b. 10° /s.

c. 5° /s.

d. 3°/s.

Upward deflection denotes tilt head-up.

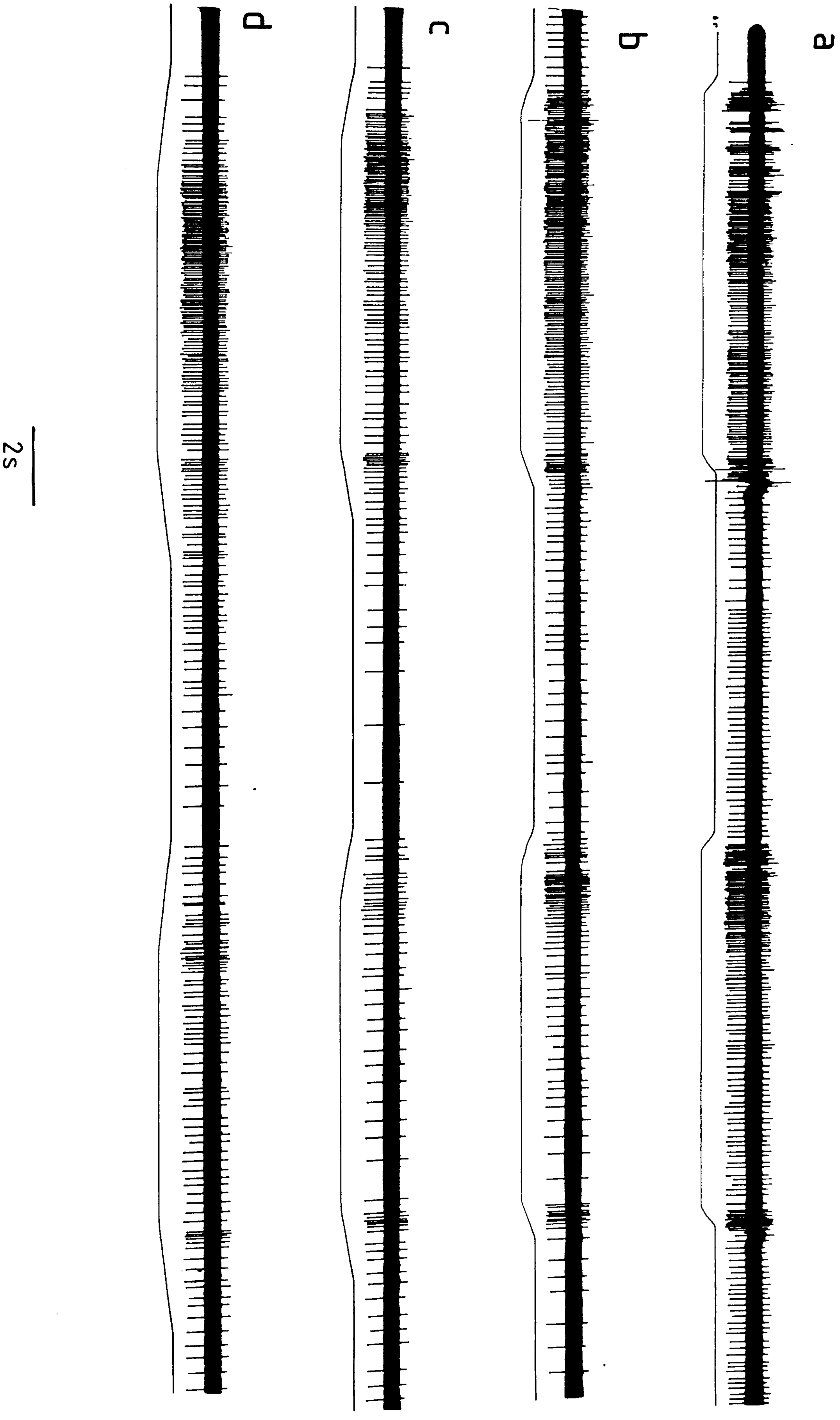

a
b
c
d
2s

Fig. 12

Histograms constructed from data in Fig. 11. Data collected over ten cycles. Velocities as shown.

Upward deflection denotes tilt head-up.

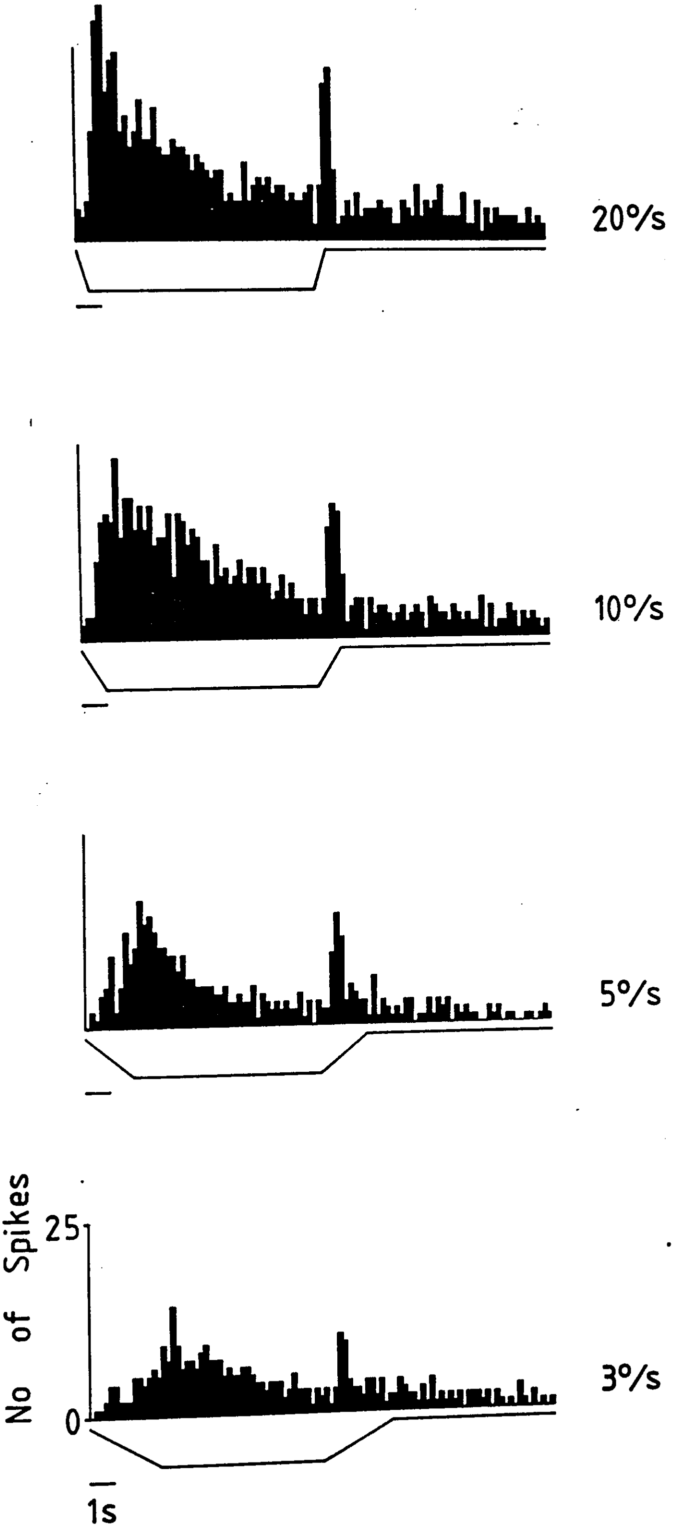

20°/s
10°/s
5°/s
No of Spikes
25
0
3°/s
1s

Fig. 13

Bilateral recording (from branch 1a1 on both sides) of tonic returnstroke activity. Top trace : right side.

a. Spontaneous activity.

b. Response to tilt in the pitch plane. NB. Upward deflection denotes head-down tilt.

c. Response to being held head-down at the end of series of tilts.

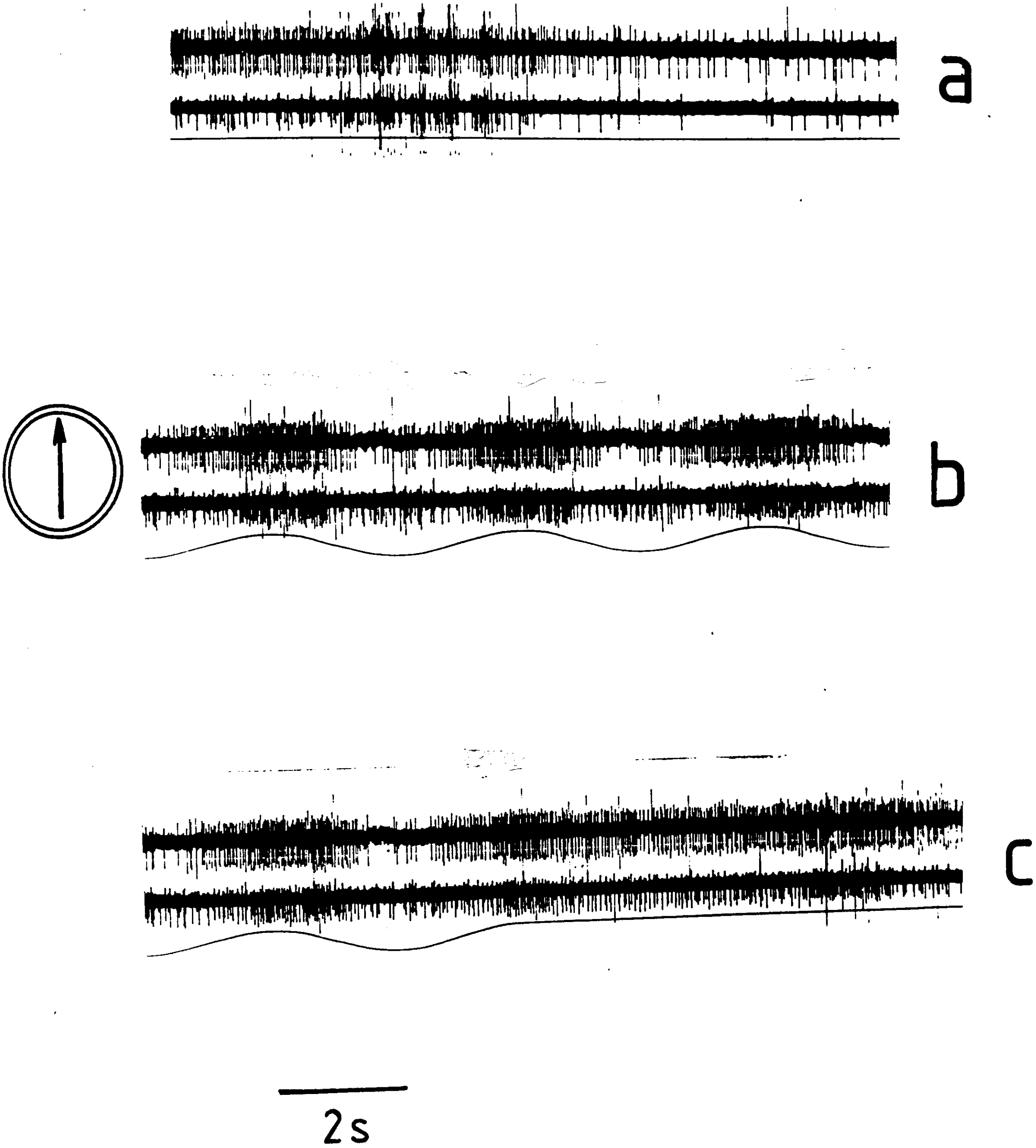

a
b
c
2s

Fig. 14

Bilateral recording of response of tonic returnstroke motoneuron to tilt in different planes. Top trace : right side.

A. 270° (Pitch; see chapter 2, Fig. 1) Upward deflection: head down

B. 315°

C. 0° (Roll). Upward deflection denotes left side up.

D. 45° .

E. 90° (Pitch). Upward deflection: head up

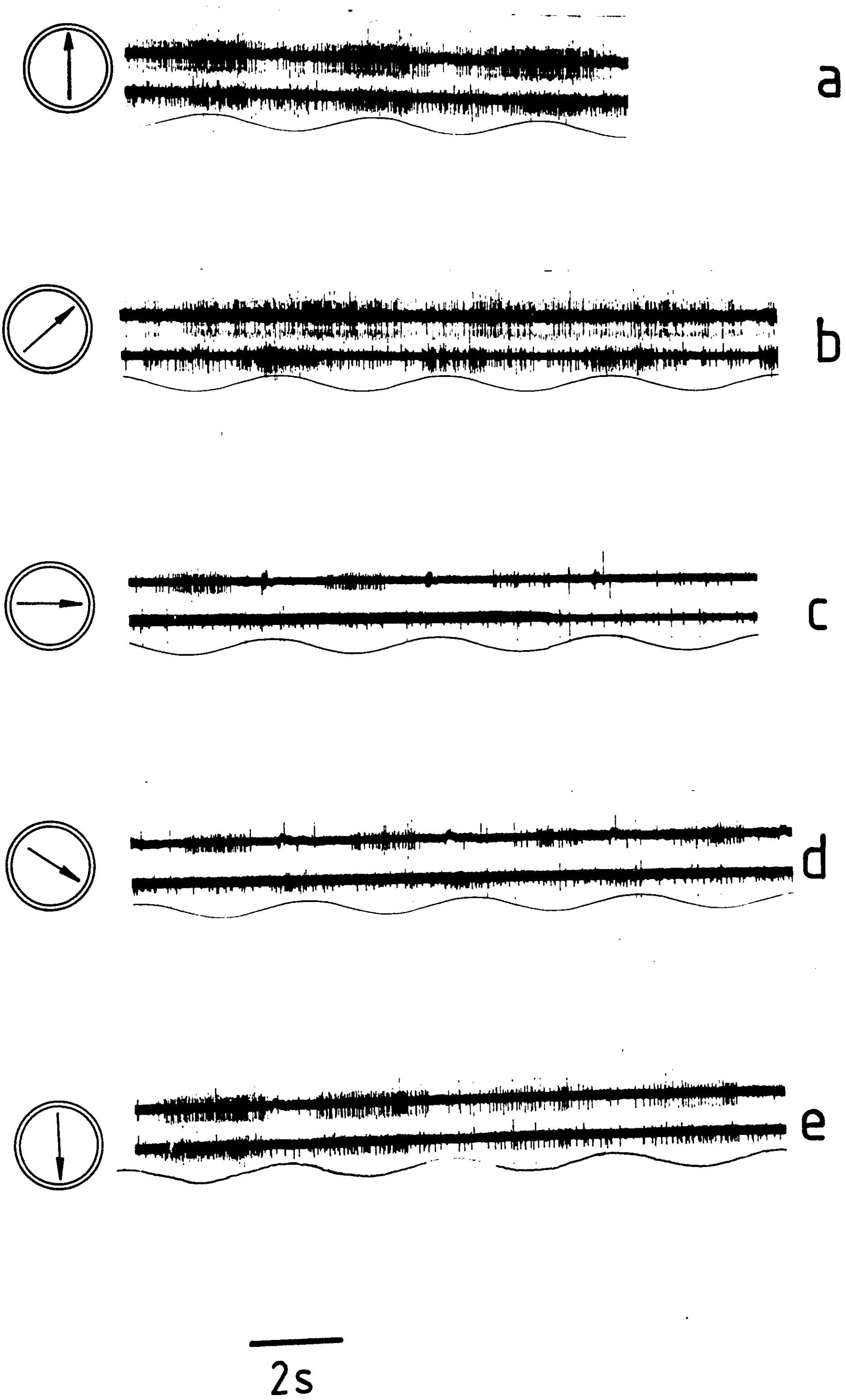

a
b
c
d
e
2s

Fig. 15

Histograms constructed from the data shown in Fig. 14, collected over ten cycles. Top histogram : right side.

a. 270° (Pitch; see chapter 2, Fig. 1)

b. 315°

c. 0° (Roll).

d. 45° .

e. 90° (Pitch).

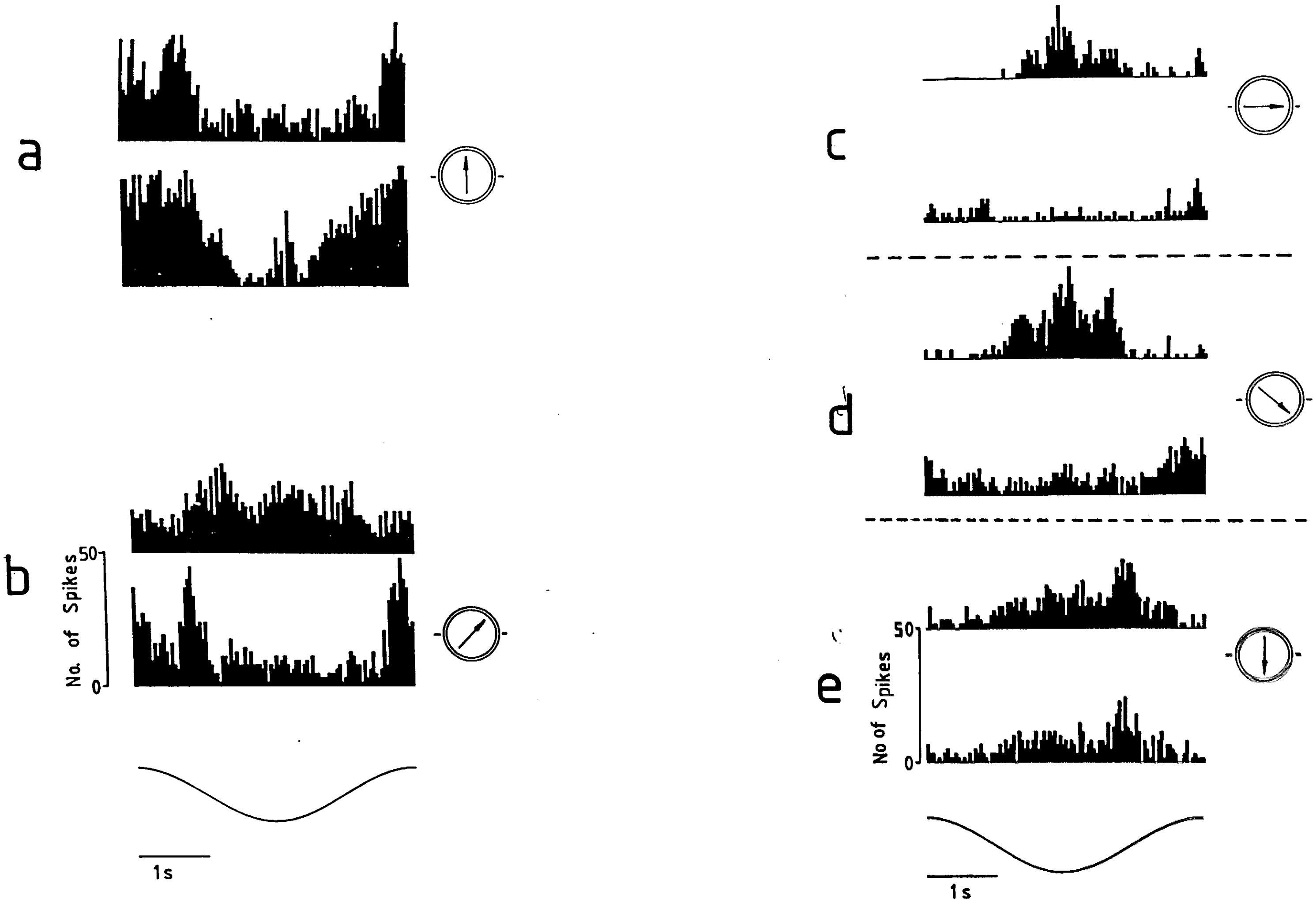

a
b
No. of Spikes
50
0
1s
c
d
e
No of Spikes
50
0
1s

Fig. 16

Cobalt backfill of motor supply to SFM's. Detailed description in text.

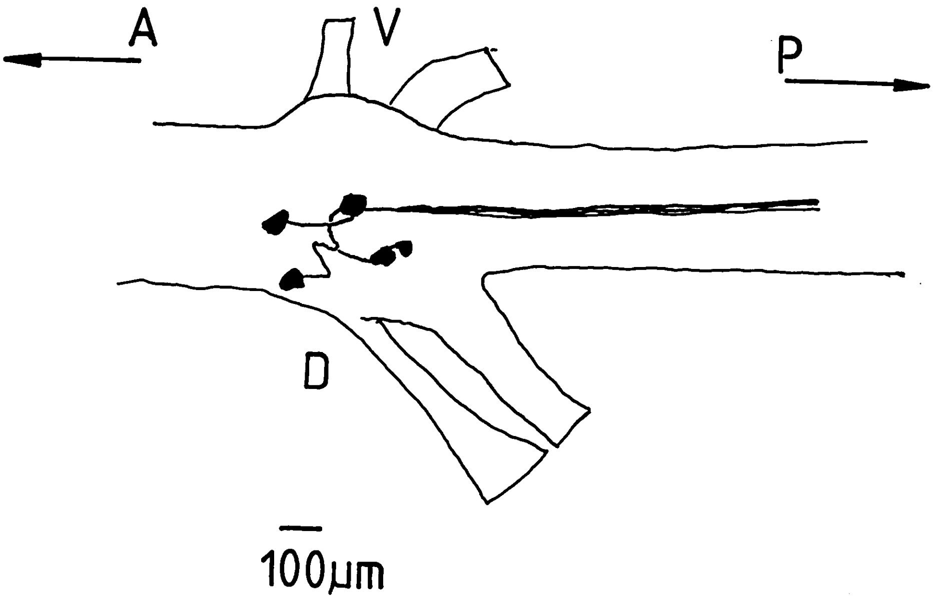

A
V
P
D
100µm

Fig. 17

Spontaneous activity recorded from 3R3s.

a. f1/f2

b. f3

c. f4

d. f5

Explanation in text.

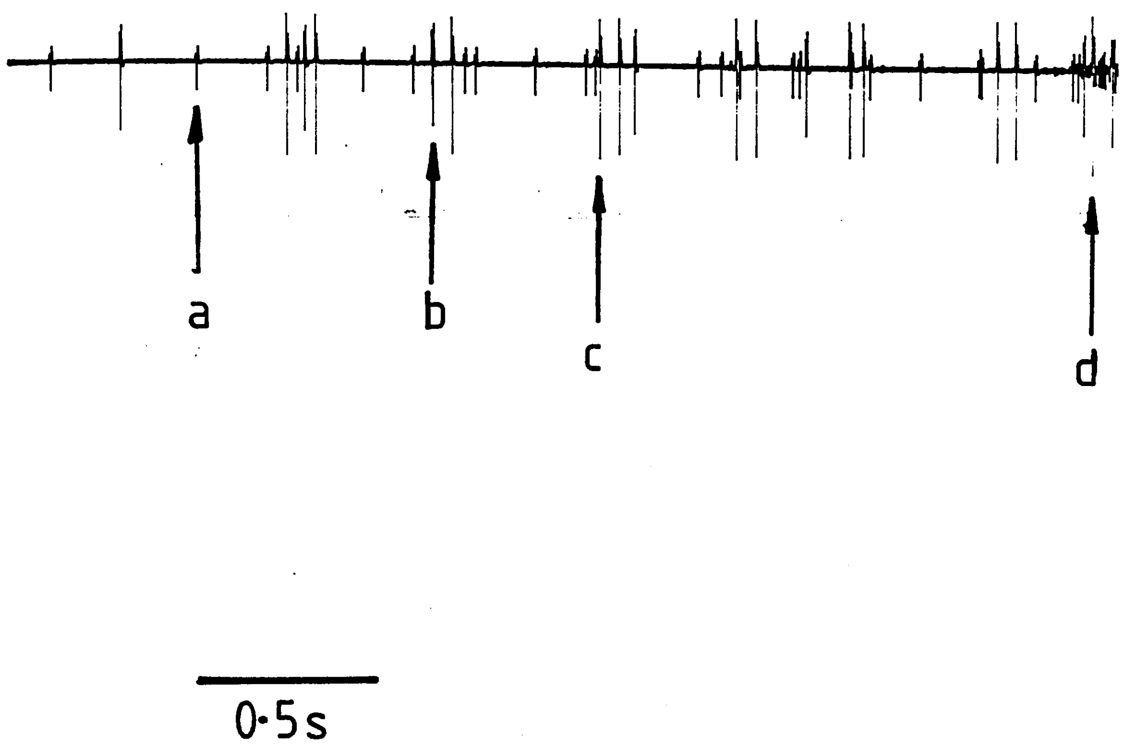

a
b
c
d
0·5s

Fig. 18

Response of SFM units to tilt in different planes.

a. 270° (Pitch; see chapter 2, Fig. 1). Upward deflection denotes head-down.

b. 315°

c. 0° (Roll).

d. 45° .

e. 90° (Pitch).

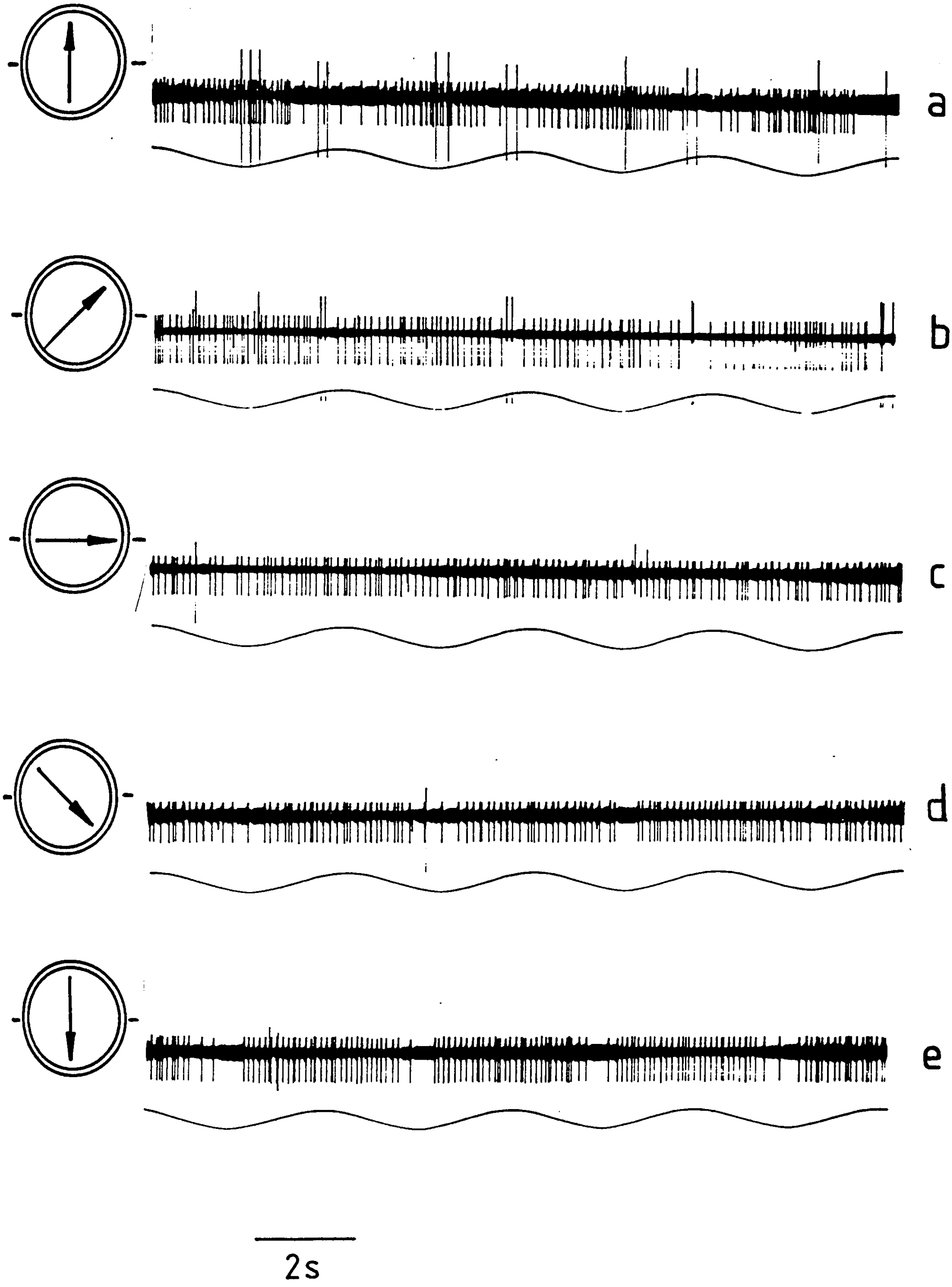

a
b
c
d
e
2s

Fig. 19

Histograms of data in Fig. 18 and two other intermediate angles.

a. 280° (Pitch; see chapter 2, Fig. 1)

b. 305°

c. 315°

d. 0° (Roll).

e. 45° .

f. 60°

g. 90° (Pitch).

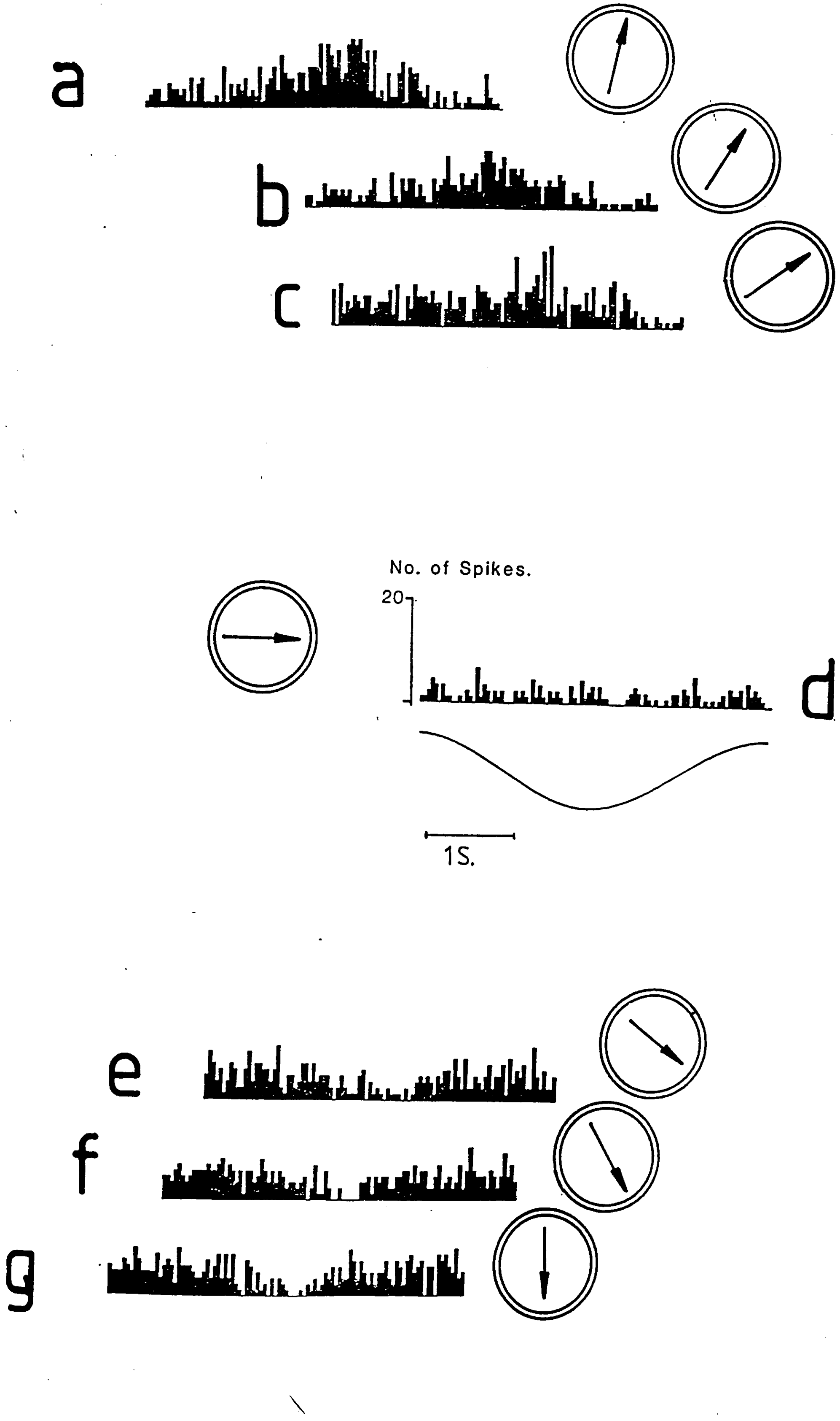

a
b
c
No. of Spikes.
20
d
1S.
e
f
g

Fig. 20

Simultaneous recording of activity of tonic returnstroke unit, (3R1a1, 1) and SFM activity (3R3s, 2) during tilt in the pitch plane over a range of velocities.

NB. Upward deflection denotes head-down.

a. 15° /s.

b. 10° /s.

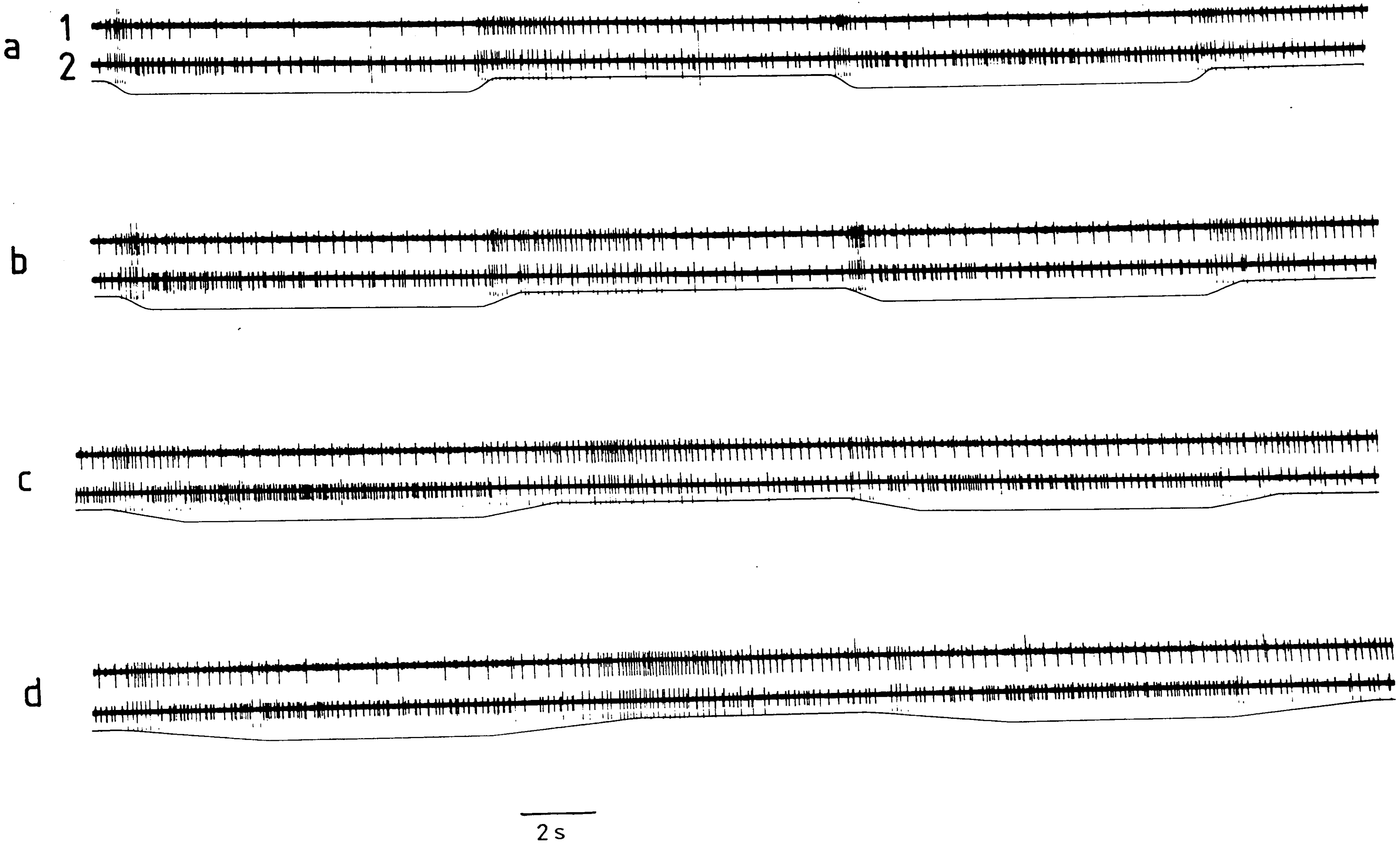
c. 5.0° /s

d. 2.5° /s.

e. 2.0° /s.

f. 1.4° /s.

g. 1.1° /s.


a
1
2
b
c
d
2 s

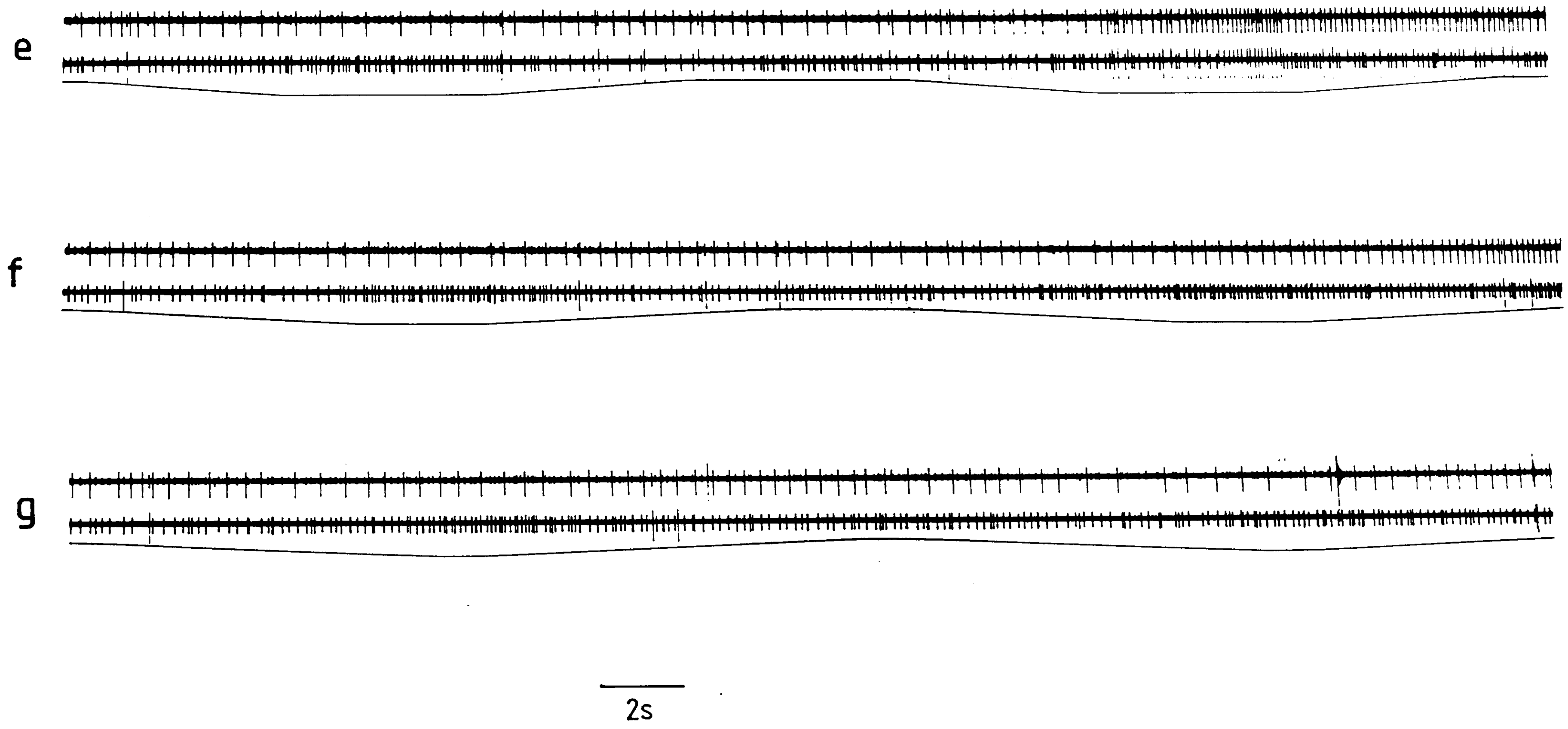

e
f
g
2s

Fig. 21

Histograms of data shown in Fig. 20. 1: tonic returnstroke motoneuron; 2: flexion ativity; 3: flexor inhibitor. Time bar, 1s. Data collected over ten cycles.

Upward deflection denotes head-up.

a. 15° /s.

b. 12.5° /s.

c. 10° /s.

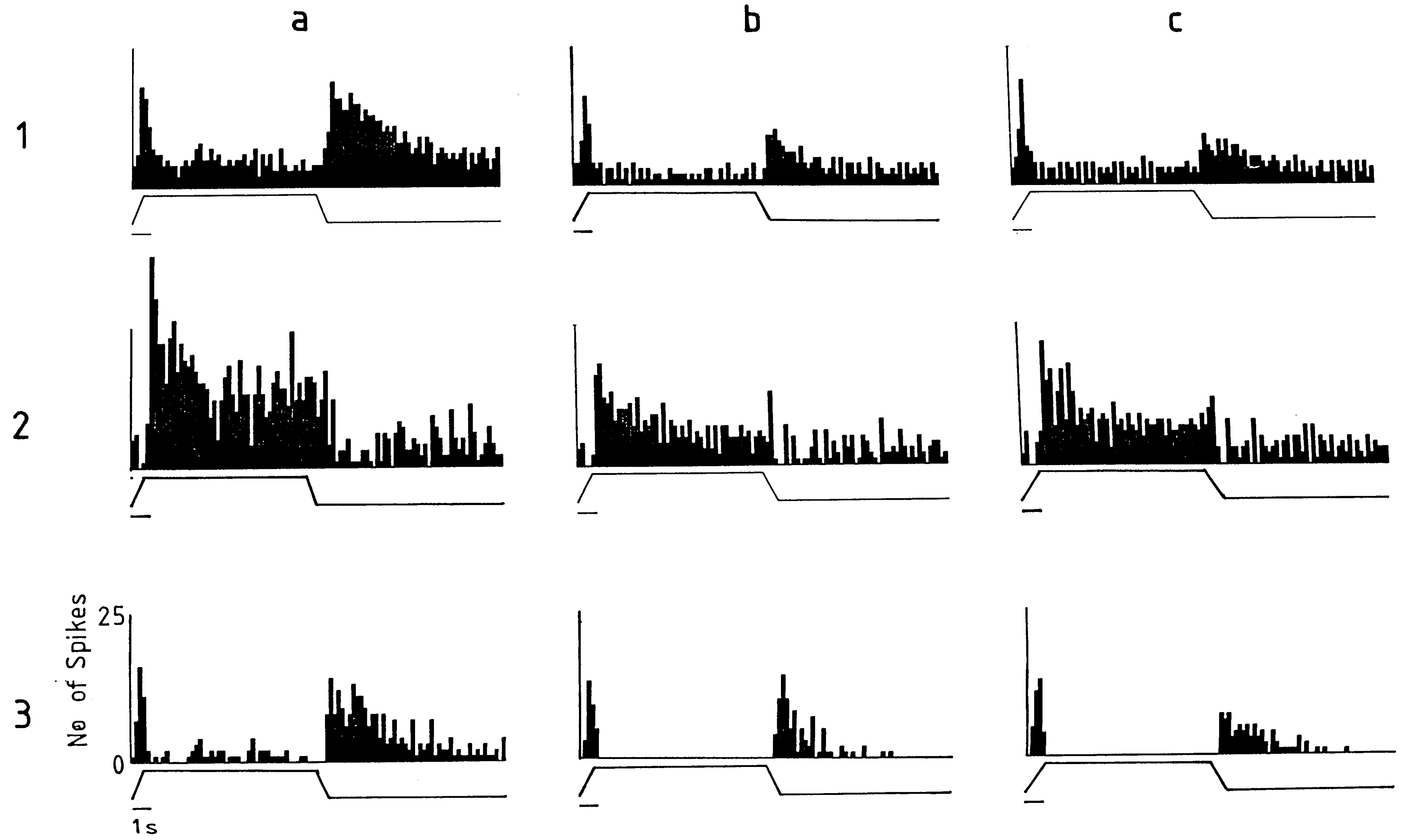
d. 5.0° /s

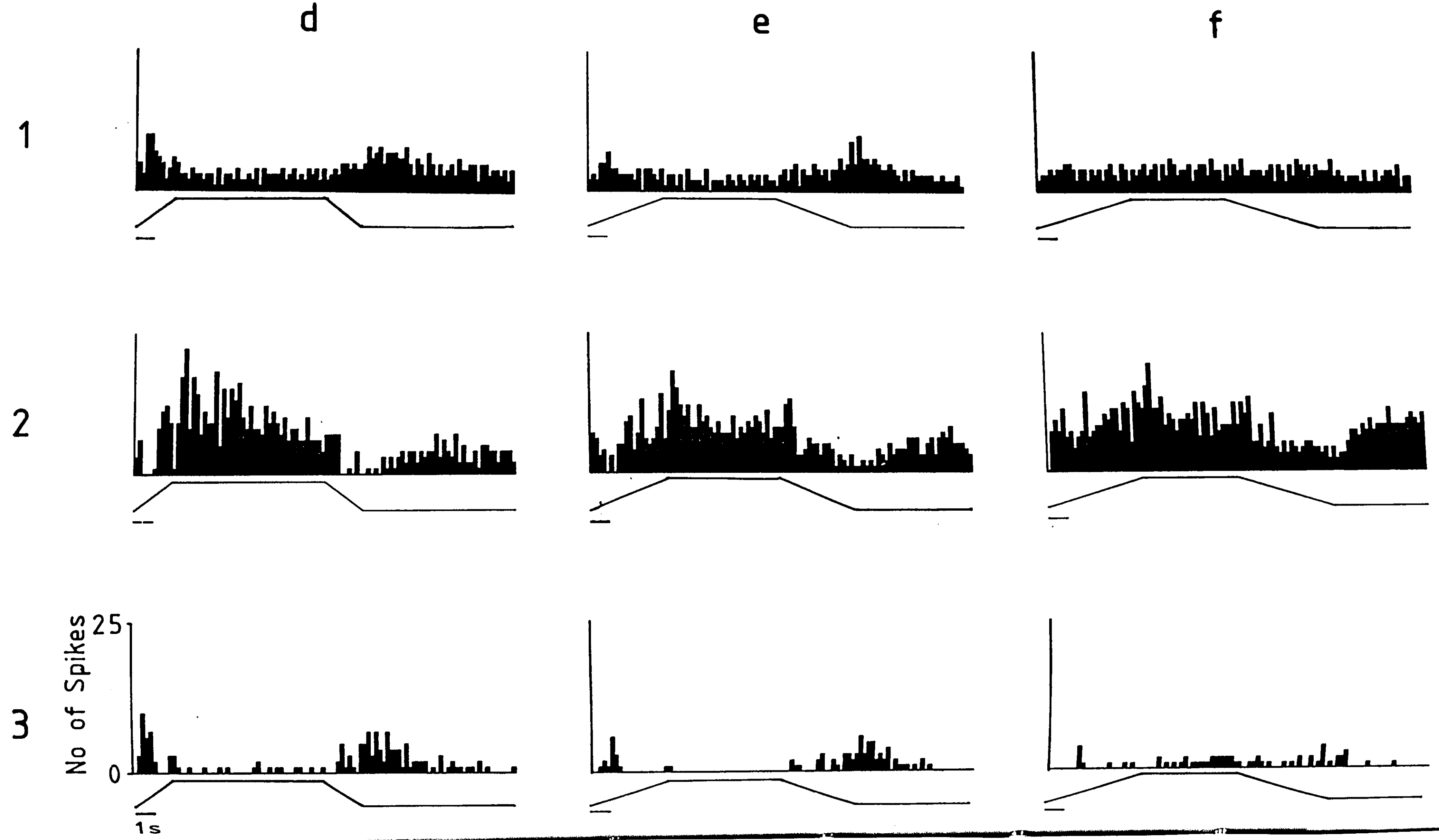
e. 2.5° /s.

f. 2.0° /s.

g. 1.4° /s.

h. 1.1° /s.


a
b
c
1
2
3
No of Spikes
25
0
1s


d
e
f
1
2
3
No of Spikes
25
0
1s

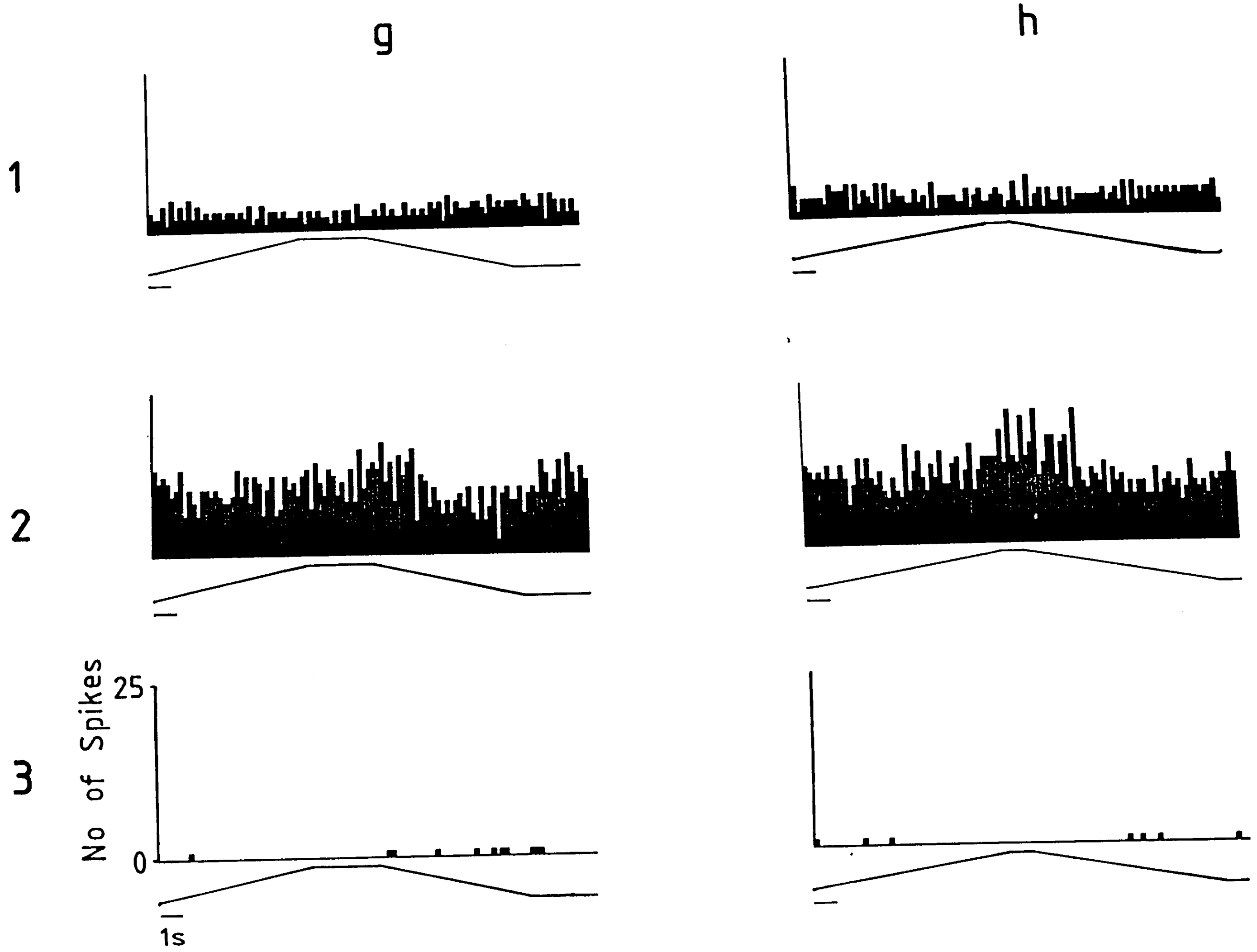

g
h
1
2
3
No of Spikes
25
0
1s

Fig. 22

Effect of pinching dactyl of L2 on both sides on activity of tonic returnstroke unit, (3R1a1, 1) and SFM activity (3R3s, 2).

A. Contralateral ie left leg.

B. Ipsilateral ie right leg.

Arrows indicate stimulus.

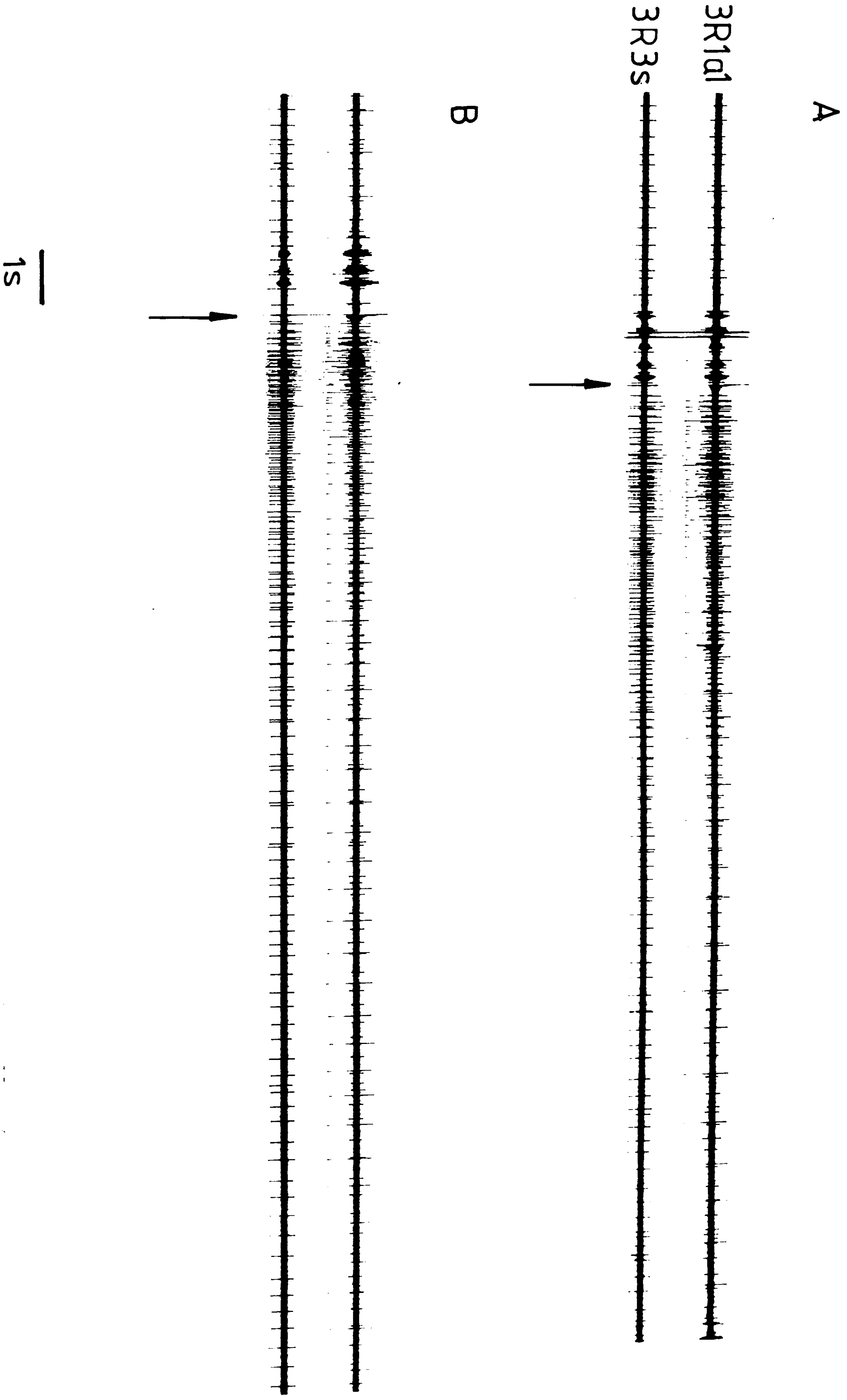
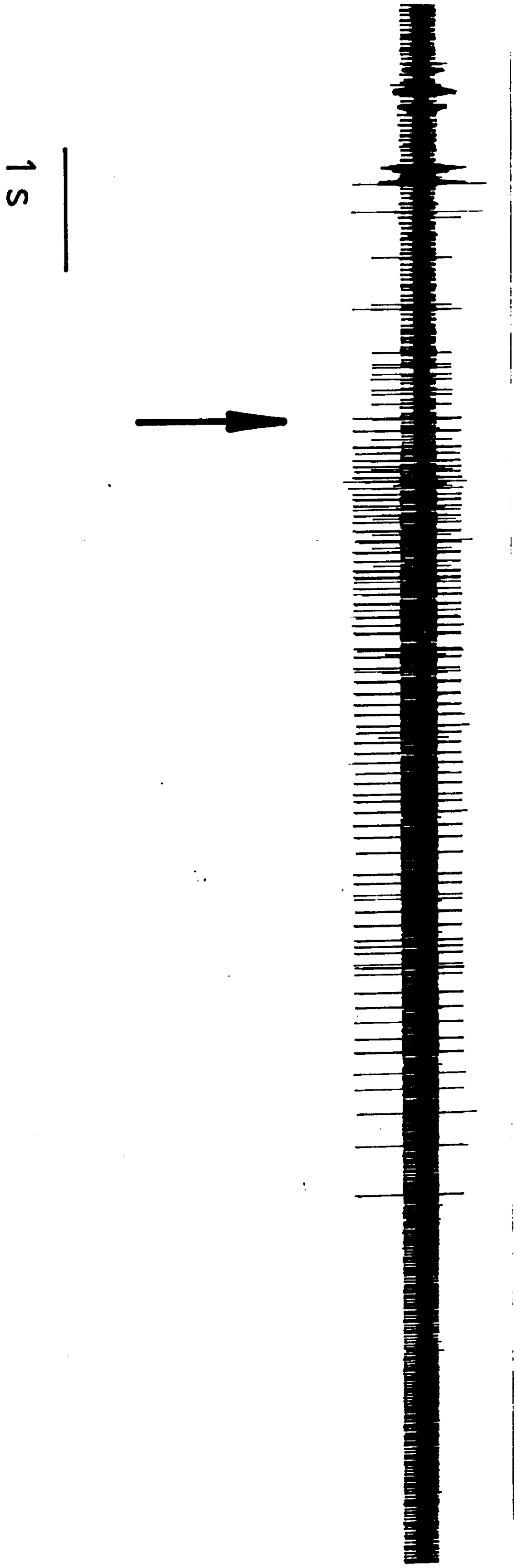

A
3R1a1
3R3s
B
1s

Fig. 23

Recording of SFM activity during pinch of ipsilateral L2. Detailed description in text.



1 s

Fig. 24

Summary diagram of responses of the two motor systems to tilt in different planes. Phase position of the circular mean (+/-circular standard deviation) plotted against angle of the plane of tilt (as defined in chapter 2).

Top.

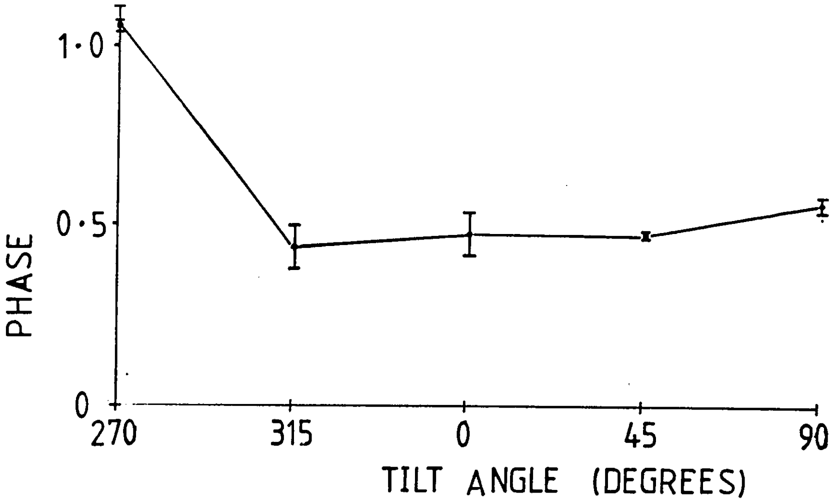
Response of tonic swimmeret returnstroke motoneuron on right side.

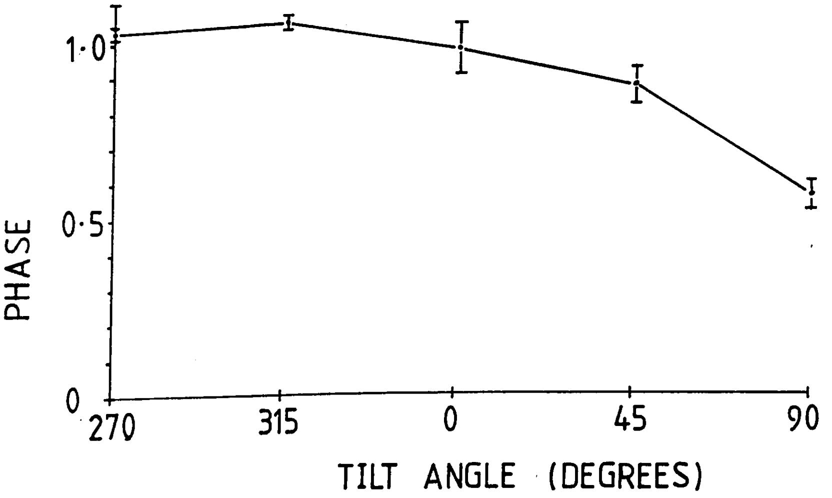
Mid.

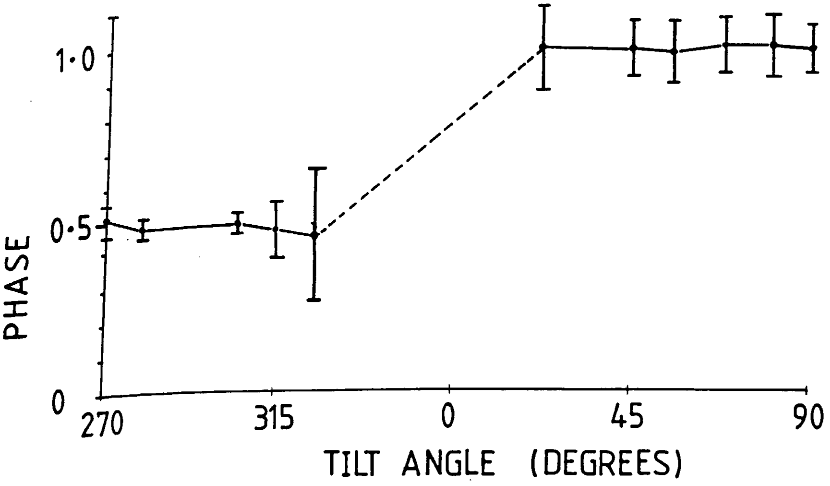
Response of tonic swimmeret returnstroke motoneuron on left side.

Bot.

Response of tonic flexor motoneuron.


1·0
0·5
0
PHASE
270
315
0
45
90
TILT ANGLE (DEGREES)


1·0
0·5
0
PHASE
270
315
0
45
90
TILT ANGLE (DEGREES)


1·0
0·5
0
PHASE
270
315
0
45
90
TILT ANGLE (DEGREES)

Chapter 7 :

Discussion.

1. Introduction.

The objective of this final chapter is to pull together the findings reported in the different chapters of this thesis and explore further some of the issues raised. In each chapter a particular aspect of the effect of tilt has been examined. Thus in chapter 2 the effect of tilt on particular statocyst interneurons was considered, and in chapters 3 and 4 the effect of this input on particular motor systems was examined. There are inherent differences between the various motor systems studied. Firstly movements of the legs depend on the coordination of muscles at several different joints (chapter 3), whereas the main movements of the swimmerets depend to a great extent on muscles acting at one joint. Secondly the motor output to swimmerets demontrates a clear temporal pattern, whereas as was argued in chapter 4, the motor output to the abdominal segments might be viewed as a spatial pattern. However, despite these differences, the responses to tilt exhibited by these various motor systems do share common features.

As well as the effects of tilt, the interactions between the descending tilt information and other inputs have been investigated. Basic questions can be asked about the organisation of these pathways and tentative answers given which will allow comparison with other species and systems, and also suggest the types of experiment which could advance our understanding.

The detailed patterns of output seen in the motor systems studied not only depend on the organisation of the motor systems themselves but also reflect the properties of the input systems, and the strategies of integration of these different inputs.

2. Experimental approach.

Before dealing in detail with the results obtained, it is worth considering the experimental approaches employed to obtain these results.

A major advantage of invertebrate preparations is that they allow studies of single identifiable neurons which have consistent effects when stimulated. This has led to considerable advances in our understanding of the operation of nervous systems (see chapter 1 for a more detailed review). However, this has had the disadvantage of overemphasising the effects of single cells in isolated preparations. The approach in the work reported in this thesis has been to utilise preparations which are as intact as possible, and use physiological stimuli where possible. This has provided new insights into the operation of well-worked systems.

A case in point is the abdominal system. The literature on the control of abdominal posture has focussed on the effects of command interneurons (eg. Kennedy et al, 1967; Evoy and Kennedy, 1967; Miall and Larimer, 1982; Jellies and Larimer, 1986). Most of these studies were carried out in isolated abdominal preparations. While this avenue of study has been most fruitful in terms of elucidating the basic components of the abdominal posture system, very little attempt has been made to relate this to the control of posture in the intact behaving animal. Only recently have questions been asked about the inputs to the command interneurons (Jellies and Larimer, 1986; Kotak and Page, 1986).

Many experiments reported in this thesis utilised the intact animal, capable of as near normal behaviour as could be arranged in an experimental situation. This animal was then subjected to a physiological stimulus eg tilt, substrate removal etc. The results of these basic behavioural experiments allowed the formulation of specific questions about the operation of the various systems under consideration. These could then be tackled by experimental manipulation of the stimuli employed or by resorting to a fixed and dissected preparation.

3. The importance of statocyst input.

Experiments on intact animals have revealed the importance of statocyst input in determining the output of several motor systems in *Nephrops*. The effects of tilt are summarised in Fig. 1. It has become clear that the type of tilt has to be specified not only in terms of the plane of tilt, but also the direction of tilt in that plane. Thus tilt in the pitch plane has distinct effects depending on whether it is head-up or head-down tilt.

As Fig. 1 illustrates, statocyst input has important effects on the legs and the abdomen. The first provides an example of the effect of a descending signal on a rhythmic system which might be analogous to the interaction between the statocyst input and the swimmerets (eg see Neil and Miyan, 1986); the second is an example of statocyst input driving a postural system which may be more analogous to the uropods (Newland, 1985; Yoshino et al, 1980).

One question of interest is why the statocysts in *Nephrops* should have such wide-ranging effects. The answer is probably related to the finding of Newland (1985) that the animal can, during the tail-flick, propel itself up into the water column. In an escape situation it would be vital not to waste the advantage this provided by a chaotic descent to the seabed. As vision is possibly of limited value as a means of detecting orientation in a relatively featureless environment, the statocysts will come into their own. Essentially they provide information regarding the animal's relationship to a constant parameter, i.e. gravity. Thus they may be the main source of information utilised during the descent to the seabed, and therefore may have wide-ranging control of many motor systems under these circumstances. These conclusions are at present speculative but suggest that comparative studies from this perspective would be of great interest.

4. Statocyst operation.

As was pointed out in chapter 2, the statocyst anatomy of *Nephrops* and *Procambarus clarkii* (in terms of the shape and number of sensory hairs in the sensory crescents) is very different. However, in general, sensory hairs detecting tilt in the pitch plane must be in the rostral and caudal regions of the crescent, and those detecting roll around the lateral edge (see Stein, 1975; Takahata and Hisada, 1982b). Note the important difference between these two: each individual statocyst will be able to detect both head-up and head-down pitch but only side-up roll. In pitch the output of the two statocysts will be essentially identical, while in roll it will be different. In *Procambarus* the pitch interneurons receive input from only one statocyst, although they subsequently cross to run in the contralateral connective. One of the roll interneurons receives input from both statocysts, while the other, I2, receives input from the ipsilateral statocyst (Takahata and Hisada, 1982b).

The crossing of statocyst information occurs in the brain. Cobalt chloride backfilling of the crayfish statocyst sensory nerve showed that sensory neurons from the posterior part of the crescent projected to the ipsilateral half of the brain while those from the anterior region projected to contralateral areas in the brain (Yoshino et al, 1983). As this information does not exist for *Nephrops*, and there are differences in the operation of the interneurons in these two species, it would be of considerable interest to compare the connections between the sensory neurons and the interneurons.

One way of doing this would be to combine extracellular methods of cobalt marking as used by Yoshino et al. (1983) with intracellular recording and dye-filling of the sensory neurons. Attempts to record from the somata of the sensory cells in *Procambarus* have met with limited success, due to the dispersion of sensory cells amongst large numbers of connective tissue cells and their relatively small size (Takahata, 1981). Another approach might be to penetrate the axons in the sensory nerve. This would entail a ventral approach, and mean that the sac could be opened allowing access to individual or groups of hairs. A wide ranging comparative study of this sensory system is needed, along with careful examination of the connections to the interneurons in the brain.

Interneurons receiving statocyst input were first described in *Procambarus* by Wiersma (1958); three interneurons were found (C4,C84 and C87) which were thought, on the basis of their location within the nerve cord, to descend to abdominal ganglia (Wiersma, 1958, Wiersma and Bush, 1963; Wiersma and Mill, 1965).

However, there are important differences between the interneurons described in Weirsma's studies on the one hand, and those found by Takahata and Hisada (1982a,b) and in the present study on the other (chapter 2). The clearest difference is in the manner of response to statocycst stimulation. The interneurons described by Wiersma (1958) responded in a phasic manner to the stimuli, whereas those decribed by Takahata and Hisada (1982a,b), and those described in this thesis responded in a phaso-tonic manner to stimuli. However, Weirsma suggested that his failure to find tonically responding fibres was due to the experimental method employed, which did not allow sustained tilting of the preparation (Wiersma, 1958).

Taylor (1968,1970) reported that two of the statocyst fibres (C87 and C4) responded to various types of vibration. C4 was studied under a variety of conditions and found to respond to vibration in air caused by tapping the table, or to waterborne vibration. However it only fired sporadically during tilt (Taylor, 1970). As pointed out by Takahata and Hisada (1982a), although this interneuron has been found in a similar area of the cord as their interneuron C1, on the basis of their very different response charateristics, the two cannot be identical. They suggest that Weirsma, in his original work, inadvertantly stimulated some of the mechanosensory hairs of the antennule (Takahata and Hisada, 1982a). The interneurons found in *Nephrops* showed very little response to vibration.

Interneurons receiving statocyst input have also been shown to play an important role in the control of equilibrium of crabs. However, these interneurons are multimodal equilibrium interneurons, receiving input not only from statocysts, but also leg proprioceptors and central neuropils (Fraser, 1974,1975). The interneurons investigated in *Nephrops* are essentially unimodal. No inputs could be found from legs, which are known to participate with the statocysts in the control of various equilibrium responses. This complements the findings of Priest (1983) that leg interneurons in the circumoesophageal connectives in *Nephrops* are not sensitive to statocyst input. Therefore an interesting comparison may exist between the channelling of sensory information in *Nephrops* and perhaps the lobsters in general, and the strategy adopted in crabs. In the former case, input from various receptor systems involved in the control of particular responses may be conducted by unimodal interneurons through the whole length of the nervous system, providing this information to the various motor systems involved. In crabs the evidence suggests that the information is fed to several pairs of equilibrium interneurons which then act as command pathways for the equilibrium responses required (Fraser, 1982).

5. Multimodality and interneurons.

Many processes rightly qualify to be called multimodal. However, two questions arise; firstly what do we mean by this term, and secondly in what situations does it apply to individual interneurons as opposed to a particular pathway?

A working definition of the term multimodal would be "involving more than one modality". However, this merely leads to the question of what constitutes modality ? Statocyst input could be defined as a single input modality only on the basis of its origin from a particular sense organ since functionally one type of statocyst input, eg roll, has very different effects from another, eg. pitch. Another intriguing possibility emerges from work already mentioned on the locust flight system (Rowell et al, 1985). Here, there is convergence from several different sources onto particular interneurons, which are thus effectively acting to detect one entity in the real world. Therefore although a thoracic interneuron in the locust may receive input from ocellar, compound eye and wind hair units, all three are stimulated in a coherent manner by the same stimulus, eg. roll to the left, and thus produce activity in the interneuron. Is this then a multimodal interneuron, or a roll-to-the-left interneuron? This sort of analysis may be relevant to the crab equilibrium interneurons. The answer is dependant on whether we choose to relate the term to the behaviour of the animal, or to our understanding of the neurophysiology which underlies the behaviour.

Situations in which multimodal interneurons have been found to occur fall into several distinct categories. There are those interneurons involved in escape responses, eg the medial giant interneurons of crayfish (Wine and Krasne, 1982), and the interneurons mediating the locust jump (M-neurons, Pearson et al, 1980). Multimodal interneurons are thought to occur in the flight system of dipterous insects as well as in the locust flight system (Strausfeld and Bacon, 1983).

In Crustacea such multimodal interneurons, with the exception of the giant fibres and crab equlibrium interneurons, seem to be relatively few. Wiersma tentatively suggested in 1958 that :

> "...integration of similar sensory impulses
> must take place much more extensively than
> integration of dissimilar ones."

In a later study the number of multimodal sensory interneurons was estimated at between 10% and 15% at each level in the nervous system (Weirsma and Mill, 1965). While in the lobsters there are many multimodal pathways (eg antennal responses, Priest, 1983), multimodal interneurons appear to be the exception, rather than the rule.

6. Strategies of integration.

Integration has to occur at many levels before a final motor output is produced. Incoming information may be divided into two categories: information coming into a given motor system from a source external to the motor system itself, e.g. the statocysts, and information from sources within the motor system monitoring the ongoing activity of the system, i.e. reafferent information. In both categories the nervous system must be able to resolve conflicts and contradictions. In the first category, conflicting information may come from two (or more) different sources, e.g. statocysts and leg proprioceptors. One approach may be to "ignore" one type of input in certain circumstances, giving preference to the other. This may be the case in various equilibrium pathways in *Nephrops* (chapter 5, Priest, 1983).

In the second category, differences between the intended effect of a motor act, the actual effect, and the mechanisms which compare the two and produce required alterations have been widely studied in many systems (eg Barnes and Neil, 1982; Barnes et al, 1972; DiCaprio and Clarac, 1981; Bush, 1962,1963,1965). The basis of these mechanisms (assistance and resistance reflexes in arthropods) has in most cases been explained in terms of premotor comparison between incoming information and an efference copy signal (von Holst and Mittelstaedt, 1950) where the difference between the two produces the corrective motor output (see Barnes, 1975; Barnes and Neil, 1982). However, in one case, it has been suggested that central mechanisms are not sufficient to explain experimental observations, and a form of peripheral mechanical integration has been proposed (Neil et al, 1982).

In the equilibrium pathways of *Nephrops* these processes must occur postsynaptically to the statocyst interneurons that have been described. One site for such processing of information is in premotor local interneurons. Spiking local interneurons have been found which integrate primary sensory input in the locust (eg Burrows and Seigler, 1982,1983,1984) and the crayfish (Reichert et al, 1982). Non-spiking local interneurons have been implicated in the control of motor output in the uropods (Takahata et al, 1981), and in the generation of rhythmic output in the swimmeret system (Heitler and Pearson, 1980).

This study has not investigated at a neurophysiological level the integration that is performed in equilibrium pathways. However the behavioural results obtained provide strong indications about the type of integration that takes place. The central interaction that has been highlighted in earlier chapters, and particularly in chapter 5, is the interaction between substrate contact and descending tilt information. Fig. 2 illustrates the type of processes which may form the basis of the observed effects. The aim is not to show connections between the various elements, but rather to suggest the way in which information may flow and decisions taken.

As has already been discussed, tilt has wide-ranging effects only in the absence of substrate contact. However, tilt will always be detected by the statocysts. As no stimulus was found which altered the effect of input from the statocysts onto the interneurons, the information from the statocysts will probably be carried by the interneurons whenever tilt is detected. Substrate effects must therefore occur post-synaptically to the interneurons. Also, as water movements were not found to enhance the response of the interneurons but fire a separate population of cells, the interaction between current detection and tilt must occur post-synaptically to the interneurons.

These two diagrams are not intended to be exclusive; indeed many connections may be made between them. They suggest that the presence or absence of substrate contact is the major factor in determining the pattern of motor output observed. The neuronal elements which subserve these interactions (with the exception of particular interneurons discussed above) may well be shared between different pathways. A similar situation arises in the spinal cord of the cat where populations of interneurons may be shared by two or more reflexes (Jankowska et al, 1981).

As it has been difficult to precisely label the leg cycling response as a compensatory, righting or substrate search response, this has been taken together with the righting responses in Fig. 2. However the effect of the cycling legs has been related to the detection of substrate, rather than the restoration of orientation.

7. Strategies of coordination.

The swimmerets and the legs are two systems which show various types of coordination. For both of these systems to work effectively, whether in locomotion, righting or detecting of substrate, precise coordination has to be achieved.

In the swimmerets, this coordination is underpinned by coordinating interneurons. The existence of such elements was inferred by Stein (1971,1974) and has lately been directly confirmed by Paul and Mulloney (1986). What is not clear in the swimmeret system is the role of sensory feedback. As pointed out in chapter 4, the swimmerets possess sensory structures which are involved in swimmeret reflexes both during rearward beating, and asymmetric beating (Miyan and Neil, 1986). However the role of the sensory information these receptors provide in the coordination between ganglia is not clear. It has been suggested that it perhaps serves to make fine adjustment in the output produced by the CPG.

Coordination of the legs in walking, in comparison to the swimmerets, is more dependent on sensory feedback from the periphery. Although the basis of the pattern may well be a centrally generated rhythm (see Sillar and Skorupski, 1986;

Clarac, 1982), contact, load, and the positions of neighbouring legs all have important effects (Klärner and Barnes, 1986; Cruse and Müller, 1986). However during cycling (as distinct from walking), the active legs are in some respects similar to the swimmerets. Most of the feedback is reafferent since the legs are moving in a relatively homogeneous medium. It is perhaps significant that it is under these circumstances that evidence of coupling between the legs and swimmerets is clear, while there is very little coupling during walking. Cycling also demonstrates that the sensory input mentioned above is not necessary for coordinated output from the legs. An interesting extension of the experiments conducted here would be to disrupt the cycling pattern by blocking legs. If sensory feedback plays the same role as in walking, there will be a disruption of the pattern, perhaps in a phase-dependent manner (Cruse and Müller, 1986; Clarac, 1985).

8. Patterns of motor output.

The patterns of motor output seen in response to tilt reflect on many of the issues raised above. Some motor systems appear to receive only one type of input from the statocysts, e.g. the legs in roll (chapter 3) and abdominal muscles in pitch (chapters 4&6). Others receive input from the statocysts in both pitch and roll, specifically the swimmerets but also the uropods (chapter 6; see also Newland, 1985). Interaction between pitch and roll input very clearly manifests itself in terms of the motor output seen. If, then, several parallel channels of statocyst input affect directly the pattern of motor output, this may suggest relatively little interference from other elements such as premotor local interneurons on the output side of the system (see Neil et al, 1982).

There is another pattern that has emerged during these experiments. Although the pitch interneurons clearly carry a good deal of information about tilt in intermediate planes, at the level of the motor outputs it is clearly roll information which dominates (Fig. 3). Thus, roll responses occur where the pitch interneurons are still strongly modulated by tilt.

Such conclusions about the pattern of motor outputs is possible because many of the responses are either present or not; the legs are either asymmetrically disposed or spread evenly, the swimmeret beat and the uropods clearly switch between different states dependent on the type of tilt, the abdomen is either flexed or extended. Such clear switching of systems is a characteristic of outputs of equilibrium pathways. This may be a reflection of relatively simple processing at the neuronal level.

9. Models of motor systems.

The swimmerets have been extensively investigated and a model proposed (Neil and Miyan, 1986). The minimum modifications to this model suggested by the results reported in this thesis involve taking into account the effects of preventing leg cycling, which abolishes beating, and substrate contact, which abolishes asymmetric responses to tilt, without necessarily preventing beating. This can be done by representing these effects as gating the statocyst input in or out at specific points (Fig. 4). Thus during leg cycling, phasic input from the legs acts on the rhythmic side of the model. It is not yet clear whether this couples the output of an already functioning CPG to the motoneurons, or is necessary to ensure the operation of the CPG itself under these circumstances (chapter 6).

Another important alteration to the model is that whereas Neil and Miyan show statocyst input acting as a gate for CPG activity, in this version it also acts directly on the CPG (see chapter 6). Tonic input from the legs, involved in detecting substrate contact, now acts as a gate between descending statocyst input and the steering muscles. Thus disruption of leg cycling might prevent the CPG coming to threshold and producing rhythmic output, whereas substrate contact might prevent statocyst information reaching the steering muscles, without necessarily affecting the CPG. There are other findings which have yet to be incorporated eg the basis of coordination between leg cycling and swimmeret beating (which may require rhythmic inputs into the CPG see chapter 3), and contralateral effects.

A simpler model has been proposed for the interaction between the abdominal posture system and the uropods in crayfish where statocyst-driven uropod responses to tilt are only expressed when a facilitatory signal from the command pathway for abdominal extension is present (Takahata and Hisada, 1985). Results obtained from *Nephrops* suggest that, at least in midwater tilt in the pitch plane, both the swimmerets and abdomen are activated by input from statocyst interneurons. The results in chapter 6 suggest a very close relation between the activity in these two systems. However, even when one, the abdomen, is held rigid, this does not prevent the other, the swimmerets, from responding. Thus the model might be modified as in Fig. 5 where, while not denying interactions between the the swimmerets and abdomen, the important factor is statocyst input. Again, however, substrate contact appears to gate out this input. These suggestions do not preclude other mechanisms dominating the interaction between abdominal posture and swimmerets in other circumstances such as walking.

10. Comparative aspects.

Clear differences have emerged between *Nephrops* and various crayfish species worked on by others. There are differences in the statocyst interneurons and differences in the outputs which tilt produces. The uropods in *Procambarus* follow a sinusoidal relation to tilt (Yoshino et al, 1980) whereas in *Nephrops* the pattern is one of switching between distinct positions (Newland, 1985). Although *Procambarus* seems to be capable of asymmetrical swimmeret beating, no reports have appeared of this occurring in response to tilt, and so it is difficult to compare this aspect of behaviour. While *Procambarus* does show abdominal extension and swimmeret beating in response to head-down tilt, no information is available on the angle of the swimmeret beat under these conditions, or quantitatively on the head-up response of the abdomen.

It has been suggested that such differences relate to the behavioural context of the animals in their natural surroundings (Page, 1975; Yoshino et al, 1982). *Nephrops* is found where there is a substrate composed of soft cohesive mud which allows burrows to be constructed (Howard, 1982). It may be that in *Nephrops* inputs from the legs other than those reporting substrate contact are relatively unimportant, whereas inputs from the statocysts are enhanced. *Procambarus* is found in habitats with a marshy bottom and rarely swim freely (Penn, 1943). The latter observation may be the most important when comparing these two species.

Contrasts have also been drawn between *Procambarus* and other crayfish species such as *Orconectes rusticus* which inhabits streams and rivers with rocky bottoms (Page, 1975). A similar comparison among lobsters would be between *Nephrops* and *Homarus gammarus* (Philips et al, 1980).

11. Conclusions.

Inputs from the statocysts are of paramount importance in the control of the orientation of *Nephrops* in midwater. The statocysts control not only the swimmerets and uropods in this situation, but also the legs and abdomen.

When the animal is in contact with the substrate the statocysts appear to be of more limited importance. Indeed, substrate contact also appears to affect other systems such as the current detecting system, changing the effects of its outputs.

There is a clear need for further comparative study of the statocysts, means of integration and behavioural contexts of a wide range of crustacean species.

Fig. 1

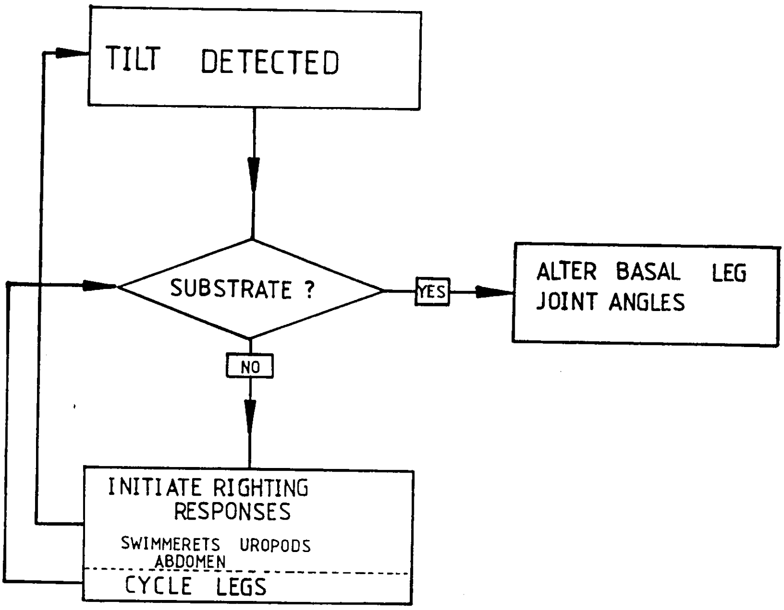
Summary table of effects of tilt on the various systems investigated.

SYSTEM	TYPE OF TILT			
	HEAD-UP	HEAD-DOWN	SIDE-UP	SIDE-DOWN
SWIMMERETS	STRAIGHT SYMMETRICAL BEAT	HALF-LATERAL SYMMETRICAL BEAT	LATERAL BEAT	STRAIGHT/ NO BEAT
ABDOMEN	FLEXION	EXTENSION	—	—
LEGS	—	—	POSTURAL RESPONSE	CYCLING RESPONSE

Fig. 2

Flow diagrams illustrating the inputs, interactions and outputs observed in these experiments.

Detailed description in text.


TILT DETECTED
SUBSTRATE ?
YES
ALTER BASAL LEG JOINT ANGLES
NO
INITIATE RIGHTING RESPONSES
SWIMMERETS UROPODS ABDOMEN
CYCLE LEGS

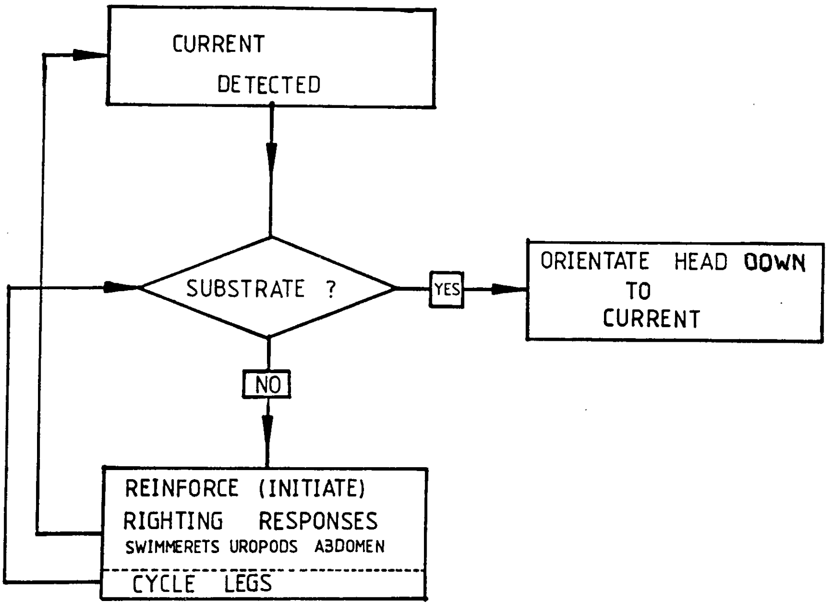

CURRENT DETECTED
SUBSTRATE ?
YES
ORIENTATE HEAD DOWN TO CURRENT
NO
REINFORCE (INITIATE) RIGHTING RESPONSES
SWIMMERETS UROPODS ABDOMEN
CYCLE LEGS

Fig. 3

Patterns of response in pitch interneurons (dotted lines) compared with output of roll responses in motor systems (dashed line).

Detailed description in text.

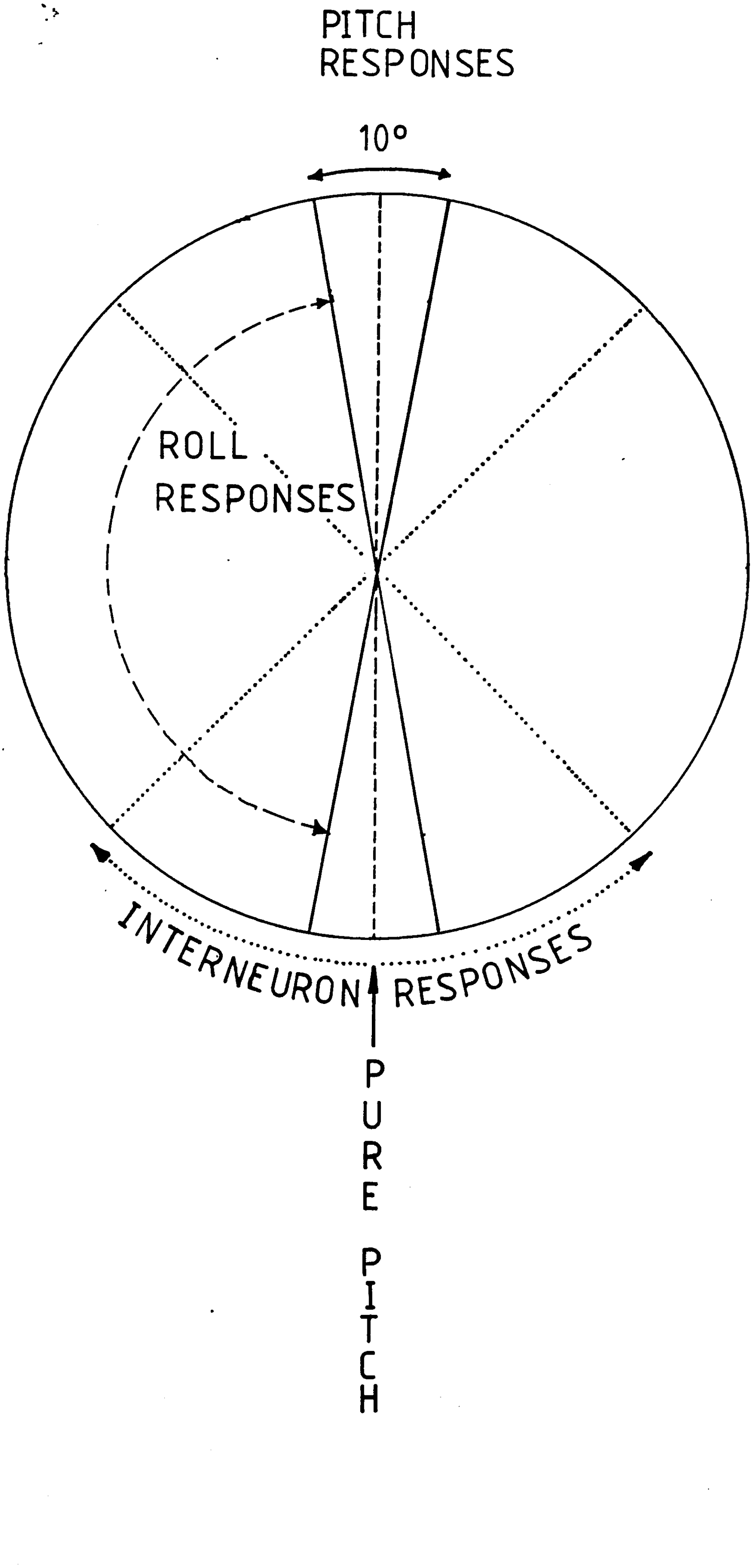

PITCH RESPONSES
10°
ROLL RESPONSES
INTERNEURON RESPONSES
PURE PITCH

Fig. 4

Possible model of the swimmeret system in the light of the results reported in this thesis.

P/S : Main powerstroke muscles.

R/S : Main returnstroke muscles.

RP/S : Rearward powerstroke muscles.

M13

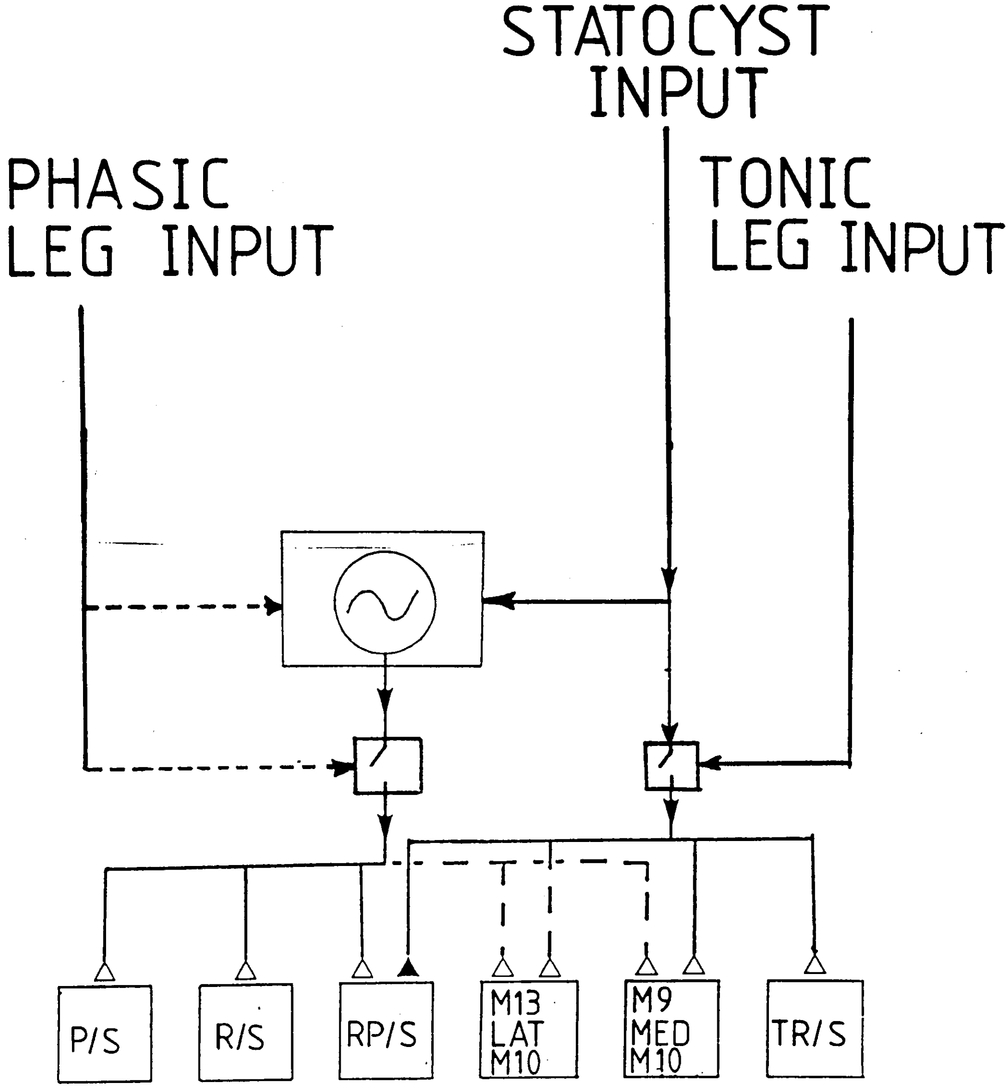
LAT M10 : Phasic steering muscles.

M9

MED M10 : Tonic steering muscles.

TR/S : Tonic returnstroke muscles.

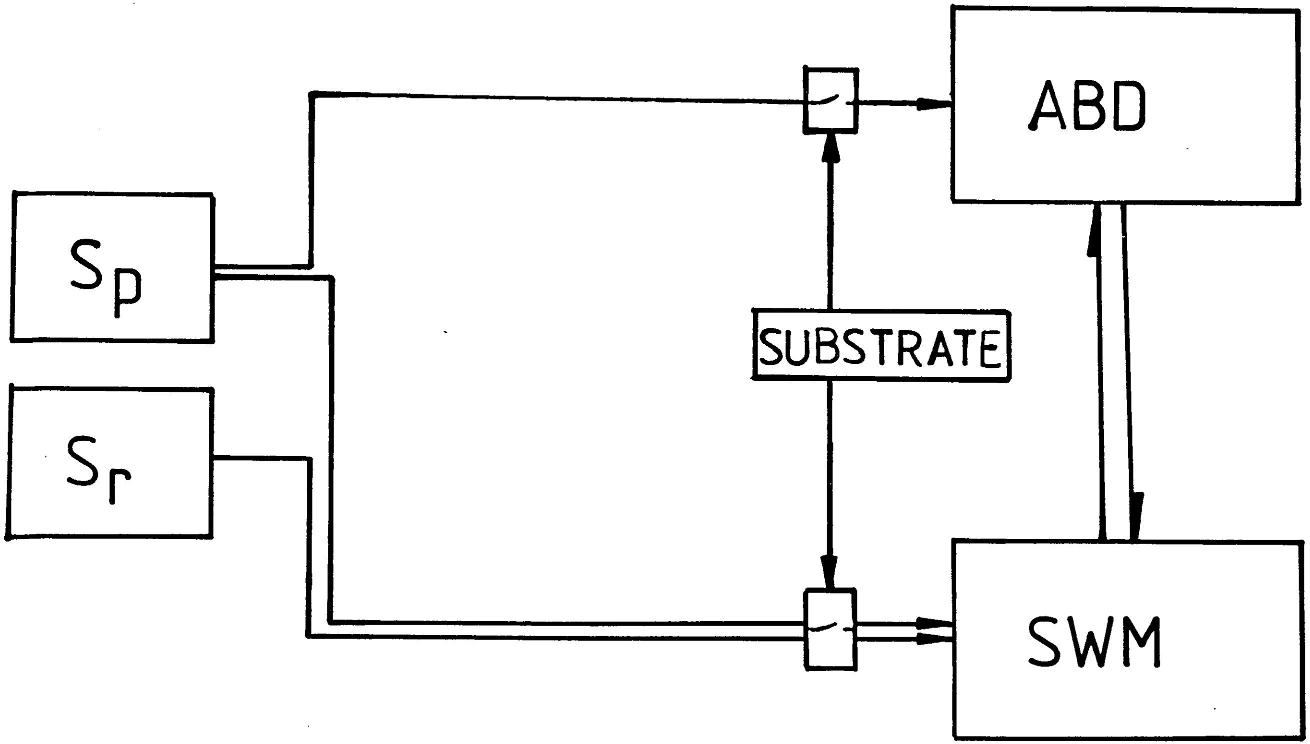
(Adapted from Neil and Miyan, 1986)



STATOCYST INPUT
PHASIC LEG INPUT
TONIC LEG INPUT
P/S
R/S
RP/S
M13 LAT M10
M9 MED M10
TR/S

Fig. 5

Model of statocyst inputs in pitch (Sp) and roll (Sr) into the abdominal posture system (ABD) and swimmerets (SWM). Substrate contact prevents the expression of tilt responses in both systems.


S_p
S_r
SUBSTRATE
ABD
SWM

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