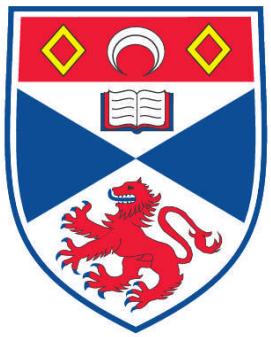
NEURAL EVENTS UNDERLYING ESCAPE SWIMMING BEHAVIOUR IN THE SQUAT LOBSTER 'GALATHEA STRIGOSA' (CRUSTACEA, ANOMURA)

Keith T. Sillar

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



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A thesis presented for the degree of Doctor of Philosophy

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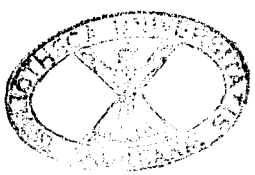
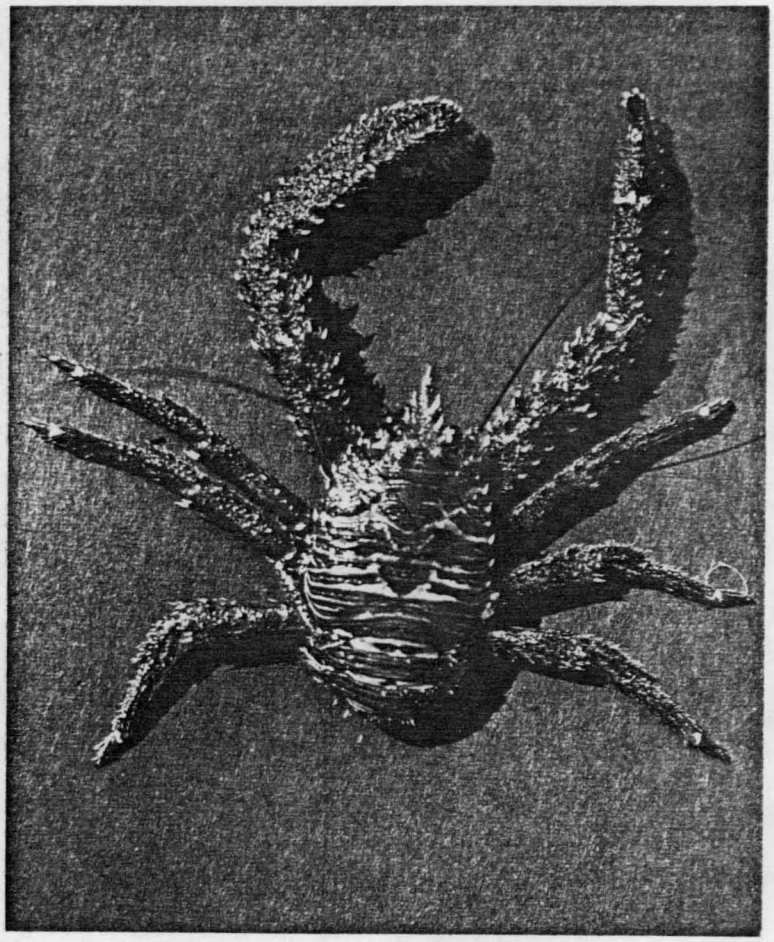
To Lorna

Gatty Marine Laboratory

University of St. Andrews

September 1983





Galathea strigosa

"Whoso will question the validity of the conclusions of sound science, must be prepared to carry his scepticism a long way; for it may safely be affirmed that there is hardly any of those decisions of common sense upon which men stake their all in practical life, which can justify itself so thoroughly on common sense principles, as the broad truths of science can be justified."

T.H. Huxley (1880)

DECLARATION

I declare that the work reported in this thesis is my own and has not been submitted for any other degree.

KEITH T. SILLAR

Curriculum Vitae

I graduated from the University of Glasgow in 1980 with an honours degree in Zoology. The work described in this thesis was carried out between October 1980 and September 1983 in the Gatty Marine Laboratory, the University of St. Andrews.

KEITH T. SILLAR.

SUPERVISORS CERTIFICATE

I certify that Keith T. Sillar has fulfilled the conditions laid down under Ordinance General No. 12 and Resolution of the University Court 1967, No. 1, of the University of St. Andrews and is accordingly qualified to submit this thesis for the degree of Doctor of Philosophy.

DR. William J. Heitler,
B.A., D. Phil (Oxon)

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SUMMARY

1. The anatomy and physiology of escape swimming behaviour in the squat lobster, Galathea strigosa, have been investigated and the results discussed in the context of comparative mechanisms of escape in related species.

2. In contrast to many other decapods, G. strigosa, does not possess a giant-fibre system which underlies escape.

3. In terms of the number, size and position of neuronal somata, the fast flexor motorneuron pools in Galathea and crayfish are homologous.

4. A neuron, homologous with the crayfish MoG, has been studied. Unlike the crayfish neuron, MoGH is a typical, unspecialized fast flexor motorneuron.

5. The anatomy of afferent and efferent neurons involved in abdominal extension has been investigated. The extensor motorneuron and accessory neuron pools in crayfish and Galathea are largely homologous.

6. A small degree of intersegmental and interspecific variation in abdominal flexor and extensor motorneuron pools is reported.

7. The anatomy and physiology of the abdominal MRO's has been examined. These are found to be homologous in structure and function with other decapod MRO's.

8. The considerable differences between the phasic and tonic MRO sensory dendrites may account for their different response characteristics.

9. The MRO's excite both extensor motorneurons and the flexor inhibitor motorneuron via an apparently monosynaptic pathway. Similar input properties have been described for the crayfish MRO's.

10. The MRO's, which are shown to fire in response to abdominal flexion, produce EPSP's in extensor motorneurons which both summate and facilitate. This feature has not been decribed previously and may be important in the reflex function of the MRO's during escape swimming behaviour.

11. The relative roles of proprioceptive and exteroceptive feedback on the generation of the swimming rhythm have been studied using a variety of preparations involving restraint and deafferentation.

12. Sensory feedback both excites and inhibits swimming. It is deduced that proprioceptive feedback has excitatory effects and exteroceptive feedback inhibits swimming behaviour.

13. It is suggested that the MRO's may play a role in exciting the neural circuits underlying swimming behaviour via both direct connections with the thoracic nervous system and a restricted portion of the abdominal motorneuron pool.

14. A deafferented preparation has been used to analyse the motor programme underlying swimming behaviour. The ability to record swimming activity, identical with that recorded in the intact animal , in the absence of sensory feedback from the abdomen, suggests that swimming behaviour is controlled by a central pattern generator (CPG).

15. A method of inducing swimming activity by high frequency electrical-stimulation of abdominal sensori-motor roots is described.

16. The CPG for swimming behaviour is shown to be most likely to reside in the suboesophageal or thoracic ganglion.

17. The activity of flexor and extensor motorneurons in abdominal ganglia has been analysed at the cellular level using both extra-cellular and intracellular recording techniques.

18. Fast flexor motorneurons are driven by a combination of brief unitary synaptic potentials and a large underlying oscillatory slow wave depolarization.

19. Current injection into the somata of fast flexor motorneurons during swimming has dramatic effects on slow wave amplitude and suggests that motorneuron drive results from powerful periodic excitation via chemical synapses.

20. In contrast to the fast flexor motorneurons, fast extensor motorneurons are driven by only brief unitary synaptic potentials and not by an underlying slow wave depolarization. The contrasting mechanisms for excitation in antagonistic sets of motorneurons is documented and a possible explanation presented.

21. Among the fast extensor motorneurons there is an apparent gradation in spike thresholds which can be correlated with a gradation in soma diameter. The largest of the available pool of extensor motorneurons has the highest spike threshold.

22. The activity of the phasic inhibitors of the extensor and flexor muscles has been analysed. The extensor inhibitor, which fires in antiphase with other extensor motorneurons during the flexion phase of the swim cycle, appears to receive the same slow wave depolarization as fast flexor motorneurons. The extensor inibitor motorneuron burst is terminated by a high frequency barrage of IPSP's superimposed upon the membrane slow wave. The flexor inhibitor motorneuron receives complex excitation and inhibition during swimming, involving both unitary events and membrane waves.

23. The coordination of segmented limb structures during swimming has been investigated. The walking legs are physically protracted during flexion while the unmodified male swimmerets are flicked posteriorly.

24. Swimmeret retraction during swimming is controlled by the activity of a single swimmeret motorneuron which appears to be part of the swimming circuit and which may also be a primitive homologue of the Segmental Giant neuron in crayfish.

25. It is concluded that escape swimming behaviour is homologous with non-giant backwards swimming in crayfish and may also be homologous with swimming in certain sand crab species. The evolutionary relationships of a number of decapods is discussed on the basis of escape circuitry and it is suggested that Galathea may represent an ancestral type of swimming decapod.

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GENERAL INTRODUCTION

The squat lobster, Galathea strigosa (Crustacea, Anomura) is commonly found around the British coast, normally beneath stones and rocks on the lower shore and down to about 35 m. When threatened or startled in aquarium tanks, Galathea normally responds with a series of extension-flexion cycles of the abdomen which propel the animal away from the stimulus. This rapid escape response which occurs at about 10 Hz and may last for several seconds, begins with abdominal extension since the abdomen is normally held flexed beneath the cephalothorax in the stationary animal. This thesis reports an analysis of the neural mechanisms underlying escape swiming behaviour in Galathea.

Mechanisms of Escape

The ability to escape successfully from predators is crucial to the survival of most animals and consequently the selection pressures on escape behaviour are inherently high. There are two major focal points for the evolution of escape behaviour - the skeletal components which effect escape and the neural circuits which control the movements of those components. Natural selection has capitalized on variations in mechanical and neural organization to maximize the survival value of escape behaviour. This has resulted in many elaborate and ingenious mechanisms for escape. Considering that only one goal has been sought - that of effective escape - the diversity of solutions, particularly amongst invertebrates, is astonishing. The arthropods have evolved many types of escape behaviour in which the neural and mechanical systems act in concert but have been developed to different extents. The relationship between neural and mechanical design is exemplified by the macruran tailflip escape response and escape jumping in certain

orthopterous insects. The differences between the two types of escape have arisen because of different environmental constraints on ballistic movement. The force of gravity in water is considerably less than in air but water offers a greater resistance to movement than air. Consequently the distance achieved by a ballistic movement in water is less than the same movement in air. However the power required to initiate a ballistic movement in air is normally much larger than that required for an object of similar mass under water, because of the differential forces of gravity acting against the movement. Therefore when an insect jumps it must generate more power to become air-borne than a crayfish of similar mass must generate to begin swimming. Once the movement is initiated, however, the insect is likely to travel farther than the crayfish.

In the desert locust, Schistocerca gregaria, escape involve a rapid extension of the greatly enlarged metathoracic (hind) limbs. Here the rapidity of escape is largely due to a highly specialized mechanical system (Heitler, 1974; 1977). The neuromuscular system, though intricate in its design, is insufficient on its own to produce the necessary power for an effective jump (Hoyle, 1955). Prior to a jump the limbs flex and there follows a period of co-contraction in the extensor and flexor muscles (Godden, 1969) which lasts for about 300-600 ms (Heitler and Burrows, 1977a). During this time the limb is locked in a flexed position by a connective tissue pocket on the flexor tendon which fits over a specialized cuticular invagination of the femoral-tibial joint. The extensor muscle develops tension isometrically to the level required for an effective jump and when this level is reached the jump is triggered by an inhibition of the flexor muscle which allows release from the lock and rapid tibial extension. Clearly the inadequacies in the power

output of the extensor tibia muscle have been overcome by storing energy over a period of time in specializations of the mechanical design of the leg. This design has occurred at the expense of a short response latency since the co-contraction phase must last at least 300 ms in order to generate sufficient power for jumping.

Crayfish escape behaviour, in contrast to the locust, utilizes a highly specialized neural system which causes a rapid flexion of the large and powerful tail (the tailflip). The abdominal flexor muscles are extremely large and when contracted rapidly produce sufficient power to propel the animal away from threats at great speed, without requiring any specialized energy storage system. Crayfish have opted to minimize the reponse latency of their escape behaviour through specializations of the neural system. In fact, crayfish have a minimum response latency of about 7 ms during escape (Reichert and Wine, 1982). This has been achieved by minimizing the neural pathway between stimulus and response, maximizing the conduction velocity of the neurons comprising the pathway and minimizing the conduction delay between those neurons. Thus the pathway involves a minimum of five neurons,most of which have large axon diameters and are electrically coupled to each other (Wine and Krasne, 1982). The key components of the escape circuit are the paired lateral and medial giant fibres (LG, MG) whose axons travel the length of the central nervous system (CNS) and which conduct neuronal impulses at about 10 m. sec^{-1} (one of the highest conduction velocities recorded in invertebrate neurons). The LG axon network, normally considered as a single pair of neurons, is actually a network of at least 12 pairs of neurons, electrically coupled via junctions that transmit impulses 1:1. Longitudinal non-rectifying coupling among segments causes them

to conduct as a single neurone (Wiersma, 1947; Kao, 1960; Watanabe and Grundfest, 1961), while extensive non-rectifying cross coupling insures that both neurons always fire (Watanabe and Grundfest, 1961; Kusano and Grundfest, 1965). These features confer a high safety factor for transmission within the network. The large diameter, and therefore high current density, of these axons also imparts a high safety factor for transmission between the giants and the pool of abdominal flexor motorneurons. The LG's are fired by mechanical stimuli to the abdomen and produce a tailflip which causes the animal to somersault in the opposite direction to the original stimulus. The MG's are also electrically coupled, within the brain, and function as a single neuron. Mechanical or visual stimuli to the head end of the animal fire the MG's and they produce, with a single impulse a tailflip which projects the animal directly backwards. The two types of tailflip are mediated by electrical connections between the giant axons and a pool of specialized giant motoneurons (the Motor Giants; MoG's) in each abdominal ganglion (Furshpan and Potter, 1959). The LG's excite the MoG's only in anterior ganglia while the MG's excite the entire pool of MoG's. This pattern of connectivity results in the two types of tailflip (Mittenthal and Wine, 1973; Wine and Krasne, 1982). The neuromuscular system is also specialized for speed of transmission. The MoG's, in addition to causing rapid contraction of the fast flexor muscles also have widespread peripheral efects. All muscle fibres except the transverse muscles receive input from the MoG's (Selverston and Remler, 1972) and hence the MoG's cause a complete and powerful flexion when activated.

Thus the success of crayfish escape behaviour is largely due to specializations of the nervous system. The giant fibres have evolved to reduce the response latency of escape, but this has occurred at the

expense of directionality since the giant-fibre mediated tailflips are essentially stereotyped in form. Crayfish compensate for this stereotypy by possessing a directionally versatile backwards swimming response (Schrameck, 1970). This behaviour is activated at the same time as the giant circuit by the same initiating stimulus but has a much longer response latency (about 240 ms; Reichert and Wine, 1982). The important characteristics of non-giant swimming are that it is generated centrally by a central pattern generator (CPG) which drives extension before flexion. The non-giant circuit is more complex in terms of the number of neurons involved but imparts a high degree of directional sensitivity on escape behaviour following the stereotyped tailflip. In addition, the swimming behaviour may help to overcome the problems inherent in movement through a highly resistive medium compared to air.

Giant fibre systems are widespread throughout the Animal Kingdom and are normally associated with fast start or escape behaviours. Although giant fibres occur most frequently in invertebrate phyla they also occur in vertebrates (e.g. fish Mauthner cells; Diamond, 1971). The presence of giant fibre systems in invertebrates may occur because of a lack of myelination since in unmyelinated axons the conduction velocity is more dependent on axon diameter (Rushton, 1951). Among the Crustacea, giant fibre systems appear to be widespread, particularly amongst those with elongated abdomens. There are two pairs in Homarus (Allen, 1983a), Cambarus and Palaemonetes (Johnson, 1924, 1926), and Leander (Holmes, 1942). Callianassa (Anomura) has one pair only (Turner, 1950) as do hermit crabs (Umbach and Lang, 1981) and Squilla (Bullock and Horridge, 1965). Bullock and Horridge (1965) state that "Crustacea with a rapid tail flip can be expected to have giant fibers running the length of the cord".

The Field of Study

It is almost certain that crayfish and squat lobsters are derived from a common ancestor early in the Jurassic (Glaessner, 1969) and that different selection pressures in their respective habitats have led to the evolution of contrasting mechanisms of escape. When startled, squat lobsters frequently escape with a series of rapid extension-flexion cycles of the abdomen which may last for several seconds and which project the animal backwards through the water. The behaviour apparently always begins with extension (since the abdomen is tonically flexed beneath the cephalothorax in the stationary animal) and therefore contrasts with the giant fibre mediated crayfish tailflip. This feature provided the main motive for studying escape in Galathea - in what way does the neural circuitry underlying the two escape responses differ? Despite the initial phase of escape being different, backwards swimming in Galathea closely resembles the non-giant repetitive swimming which often follows giant-fibre mediated tailflips in crayfish since in this behaviour extension also precedes flexion (Schrameck, 1970). Little is known, so far, about non-giant swimming in crayfish, partly because the giant fibres share some of the motor circuitry of the non-giant system and it is therefore difficult to separate the two experimentally. Galathea has provided an opportunity to study the neural control of a fast and episodic rhythmic behaviour. Such data is likely to be of general interest since most of our knowledge on the cellular generation of rhythmic behaviour is derived from slower, continuous activities such as heart beat, mastication and ventilation (Delcomyn, 1980).

An examination of the neural circuits and neuronal anatomy underlying escape through comparative study should aid our understanding of the processes which generate differences in behaviour. This thesis provides a detailed account of the neural correlates of escape swimming in Galathea with which to compare the vast amount of data on the crayfish tailflip.

With the exception of an account of the general anatomy of Galathea by Pike (1947) no previous research into the neural generation of escape behaviour of this species (or any other squat lobster) has been documented. Recent evidence has shown that the squat lobster tailfan is largely homologous with that of certain sand crab species (Maitland et al., 1982). The sand crabs, Emerita and Blepharipoda (Anomura) are taxonomically close to Galathea and display rhythmical swimming movements during escape (Paul 1981a, b). Although the tailfan neuromuscular system is homologous in the three species, Emerita swims with its uropods while Galathea and Blepharipoda perform repeated tailflips. The possible interrelationships of the sand crabs, squat lobsters, hermit crabs and crayfish warrant comparison and are discussed at the end of this thesis.

The key questions that have been addressed in this thesis are as follows:

1. Does Galathea possess a giant fibre system and if so, how does it compare with the crayfish system? (Chapter 1).

2. To what extent are the motorneuron pools homologous in the two species and what differences exist between them? (Chapter 1).

3. What mechanisms account for extension as the leading phase of escape? (Chapter 3).

4. How do backwards swimming in Galathea and non-giant swimming in crayfish compare? (Chapter 3).

5. Is the rhythm centrally generated? (Chapter 3).

6. What role does sensory feedback play in the generation and control of backwards swimming? (Chapter 3).

7. What is the structure of the MRO's and how do they function in escape? (Chapter 2).

C H A P T E R 1.

NEURAL ANATOMY UNDERLYING ESCAPE SWIMMING BEHAVIOUR

CHAPTER 1.

INTRODUCTION

Structure and function are inseparable and entirely compatible facets of all biological systems. Within nervous systems this is evident at every level of investigation, from gross anatomy where, for example, cephalization has accumulated the neural networks controlling a variety of sensory structures and motor acts into an integrated mass or brain, down to the molecular level where the microanatomy of the synapse clearly reflects its function in the transmission of chemical substances between neurons. At an intermediate level, that of the single neuron, the concept still holds. One obvious example, appropriate to this thesis, is that of the specialized structure of the crayfish MoG neurons and their functional relationship with the LG and MG axons (Wine and Krasne, 1982). Such aesthetically pleasing phenomena are now common observations in the study of single identified neurons and support the idea that the anatomy of every neuron reflects its function. Since behaviour is generated, at least in part, by the connectivity of a pool of neurons, it follows that the structure of neural circuits also reflects the behaviour they generate.

Closely related species display behaviours which may differ either qualitatively or quantitatively (Tinbergen 1958, 1960; Blest 1960; Berg 1974). Such behaviours, which must have evolved from a common ancestor through different selection pressures in their specific habitats, (Baerends 1958; Wickler 1961) are termed homologous. Interspecific differences in homologous behaviours must be reflected in differences in the structure of neurons and the function of neural circuits. For example the escape responses of crayfish and hermit crabs are homologous

behaviours with clear interspecific differences. The extreme modifications of the hermit crab abdomen for life in empty Gastropod mollusc shells is associated with the presence of a single pair of giant interneurons (GI) which trigger a rapid escape withdrawal reflex (Umbach and Lang, 1981). In contrast, the crayfish is equipped with two sets of giant axons: one set, the MG's (homologous to the hermit crab GI's) mediate escape tail flexions in response to rostral stimuli; a second set, the LG's, mediate escape behaviour in response to caudal stimuli. Clearly the hermit crab does not require such a facility and this is mirrored by an absence of LG neurons. That the two behaviours are homologous is supported by the presence of MG escape circuitry.

In segmented species the concept of neuronal homology is more powerful since it can be assumed that serially repeated structures are controlled by serially repeated pools of motorneurons which participate in homologous circuits. For example, the 300 or so pairs of legs in Juliform millipedes are essentially identical in structure and function and each segmental pair is a serial repeat of its neighbours. In cases where the structure of adjacent segments is different it is possible to compare the structure of the motorneurons underlying their activity and to determine a structural basis for differences in function. Again the escape circuit of the crayfish serves as a good example. Here the subtle differences in the behaviour of abdominal segments during two forms of giant-fibre mediated tailflip can be accounted for by differences in the functional connectivity between the MoG's and the LG and MG neurons in anterior and posterior ganglia. This can be correlated with differences in the anatomical connections between the two sets of neurons in these ganglia (Mittenthal and Wine, 1973; 1978).

It is clear that neuronal homology does exist but it is difficult to arrive at a clear operational definition of the term. When do two neurons cease to be homologous? Two neurons, apparently homologous in terms of their structure and function may not derive from the same ancestral neuron but may be arrived at by convergent evolutionary pathways. Conversely, homologous neurons derive from common precursors during develoment but may assume different structures and physiological properties in the adult so that individual neurons may no longer be recognizable as homologues of their developmental counterparts (Goodman et al., 1980, 1981).

The "functional switch" hypothesis of Wilson and Hoyle (1978) underlines a further problem in the concept of homology. The extensor tibia muscle of the pro-, meso- and metathoracic legs of locusts is controlled by both fast and slow motorneurons (FETi and SETi respectively). The serial homologues of the metathoracic FETi's are the pro- and mesothoracic SETi's and the serial homologues of the metathoracic SETi's are the pro- and mesothoracic FETi's. This "functional switch" is associated with the specialization of the metathoracic legs for jumping. In this example the metathoracic FETi's are homologous in terms of their soma position with pro- and mesothoracic SETi's but have assumed quite different functional roles.

Proving homology between identified neurons is complicated by the fact that the term itself is not rigidly defined and is open to interpretation. Clearly, if two neurons are to be labelled homologous then the criteria that have been used for the comparison must be stated.

The abdominal ganglia of crayfish and related species offer a favourable preparation for studying patterns of variation among segmentally repeated neuron pools since the general functional roles of these ganglia and the morphology of the segments they innervate are similar but not identical. In the crayfish, small segmental variations in the structure of motorneurons innervating fast flexor muscles account for functional differences in the behaviour of segments during escape (Mittenthal and Wine, 1978). Comparisons of fast flexor soma maps in crayfish, lobster (Otsuka et al., 1967) and hermit crabs (Marrelli, 1975) show striking similarities. The lobster and crayfish fast flexor somata show complete homology. In the hermit crab, extreme modifications in abdominal architecture may account for small variations in the fast flexor soma pool compared with the crayfish. In their general morphology, number and distribution however the two pools are homologous.

Homologies have also been suggested between Crustacea and insects (Mittenthal and Wine, 1978; Hoyle, 1976) but the risk of erroneously ascribing homology to analogous neurons is high.

The squat lobster, Galathea strigosa is an Anomuran and is therefore more closely related to the hermit crabs than to the crayfish. Since homologies between hermit crab and crayfish abdominal motorneuron pools have been demonstrated, similar homologies between these two species and the squat lobster might be predicted. The occurrence of a normally flexed abdomen in Galathea which is extended during escape contrasts with both crayfish and hermit crabs. This animal has therefore provided an opportunity to examine differences in behavioural output in terms of cross-specific variation in segmentally repeated motorneuron pools. In addition, the presence of highly modified giant fibre systems in crayfish and hermit crabs has provided a second locus for comparative study.

METHODS

Animals

Squat lobsers, Galathea strigosa, were collected in lobster creels from St. Andrews Bay and maintained in circulating aerated sea water aquaria at temperatures ranging from 3 to 15°C. Mature, healthy adults of both sexes measuring 6 to 9 cm from tip of rostrum to caudal edge of telson were used in all experiments.

Anatomy

a. Semi-thin sectioning.

Relevant parts of the abdominal nervous system were dissected, fixed for 3 hours in 3% phosphate buffered glutaraldehyde, post-fixed in osmium tetroxide for 30 minutes, dehydrated in an acetone series and vacuum embedded in Durcupan resin in a conventional way. Semi-thin (about 1 μm) sections were cut with a Cambridge Huxley ultramicrotome and stained with Toluidine Blue. Sections were examined, drawn and photographed using a Zeiss photomicroscope.

b. Methylene Blue Staining.

The peripheral nervous system of the abdomen was examined by staining with 1% Methylene Blue (BDH 154490). The ventral cuticle of the isolated abdomen was removed to reveal the underlying nervous system. A few drops of stock solution were added to the sea water of the preparation bath until it turned light blue and the preparation was incubated at 4°C. Staining was normally complete after 2 to 4 hrs but faded rapidly if the Methylene Blue solution was removed.

c. Cobalt Backfilling.

Cobalt chloride "dye" (Pitman et al., 1972) was introduced to the cut ends of axons in abdominal nerve roots to examine the central anatomy of neurons involved in backward swimming.

Isolated abdomens or intact animals were pinned to the sylgard bottom of a perspex chamber filled with chilled sea water. After removal of the thick ventral abdominal cuticle and underlying layer of chromatophores, the abdominal roots were cut as far from the CNS as possible to facilitate cobalt backfilling. In most cases the abdominal nerve cord was left attached by 5th ganglion nerves to the uropods and telson and placed in sea water in a separate sylgard-lined petri dish. Where necessary the ventral thorax was removed to expose the thoracic ganglionic mass and the CNS from the suboesophageal ganglion to the last abdominal ganglion (G5) was removed. The nervous system was then pinned to the sylgard bottom of a second preparation dish and immersed in chilled sea water.

After removal of most of the sea water a well was constructed with a vaseline/paraffin oil mixture from a hypodermic syringe around the cut end of the root or roots to be stained. Prior to introducing cobalt chloride, the wells were checked for leakage by filling them with a small drop of distilled water and repaired as necessary. This procedure may have the advantage of facilitating cobalt chloride transport by causing the axons to swell. A small drop of cobalt chloride was introduced into the well from the end of a glass pipette drawn to a fine tip and the well completed by adding more vaseline/paraffin oil mixture. The consistency of this mixture proved to be important in the prevention of leakage. Vaseline was diluted with paraffin oil until the right

consistency was achieved. The sea water was then replaced and the preparation incubated at 4°C for 18-36 hours. After removal of the vaseline well a few drops of ammonium sulphide solution were added to the preparation in a fume cupboard. The reaction with cobalt chloride occurred over a period of 1-5 minutes to produce a black cobalt sulphide precipitate in filled neurons. The preparation was washed several times in sea water and fixed for 15 minutes in 5% Formalin. The preparation was then dehydrated in an alcohol series (70%, 90% and 2 x 100% ethanol for 10 minutes each) cleared and mounted in methyl salicylate on a cavity slide. Whole mounts of successfully stained ganglia were observed with a Zeiss microscope, drawn using a Zeiss camera lucida, and variable intensity light source, and photographed using FP4 film with an Olympus OM2 camera mounted on the microscope. Preparations were stored in methyl salicylate at 4°C but usually faded considerably after as little as 1 week.

d. Silver Intensification.

On occasions where staining was light or where poor filling was expected due to large distances or small neurons, silver intensification was used to enhance cobalt sulphide stains. The method of Bacon and Altman (1977) was found to be largely unsuccessful with this preparation. The technique of Davis (1982) was found to be quicker, easier and gave superior results in 100% of attempts. A major advantage of the technique is that the intensification stage can proceed in the light whilst the preparation is viewed with a binocular microscope. This allows intensification to be carried out without undue darkening of the ganglionic sheath.

Intensification involved treating the preparation as described for cobalt backfilling until the fixation stage. Ganglia were fixed for 15 minutes each. The preparation was then placed in distilled water for 10 minutes and pretreated for intensification in 2% sodium tungstate for 10 minutes. Intensification took place in a freshly made 8:1:1 mixture of solutions A, B and C. Solution A comprised 355 ml distilled H_2O, 15 mls 1% Triton X-100, 1.5 g sodium acetate $3H_2O$, 30 mls glacial acetic acid and 0.5 g silver nitrate. Solutions B and C were 5% sodium tungstate and 0.25% ascorbic acid, respectively. Intensification normally took 5 to 10 minutes but with thicker preparations up to 30 minutes was required. Intensification was stopped either when portions of neurons close to the surface of the ganglion were observed to go black or when the ganglionic sheath began to darken. Intensification was stopped by rinsing the preparation in several changes of distilled water for about 5 minutes. Ganglia were partially cleared in 0.1 M NaOH to check that intensification had occurred satisfactorily. Following this step ganglia were again washed for 5 minutes in distilled water, dehydrated in an ethanol series and cleared in methyl salicylate for photomicroscopy as described above (c. cobalt backfilling). Silver intensified cobalt stains were found not to fade significantly over several months. In some cases permanent preparations were made by mounting the preparation on a cavity slide in a solution made by dissolving polystyrene balls in xylene. Some shrinkage of the mounting medium occurred during hardening and if necessary more solution was added. No adverse reaction was found to occur between the preparation and the mounting medium, and after a few days at room temperature a permanent slide was made. Most of these have retained their original intensity of staining over several months.

Physiology

a. Isolated preparation

In physiological studies of neurons in abdominal ganglia the isolated nerve cord, left attached by G5 nerves to the uropods and telson, was pinned to the sylgard bottom of a petri-dish and immersed in cooled, oxygenated saline. The saline used was a modification of the Mulloney and Selverston (1972) lobster saline with Tris substituted by TES. The ventral or dorsal sheath of the ganglion under study was mechanically desheathed to facilitate microelectrode penetration of neuronal somata and neuropilar segments, respectively.

b. Abdominal preparation

In studies of peripheral branching and innervation, the isolated abdomen was placed in cooled saline, as above. The ventral cuticle was removed to expose the abdominal nervous system for stimulation and recording.

c. Recording and Intracellular Staining

For full details of extracellular and intracellular recording and intracellular staining techniques refer to the Methods section of Chapter 3.

RESULTS

A. GROSS ANATOMY OF THE ABDOMINAL NERVOUS SYSTEM AND THE ABSENCE OF CORD GIANT FIBRES

1. Gross Anatomy

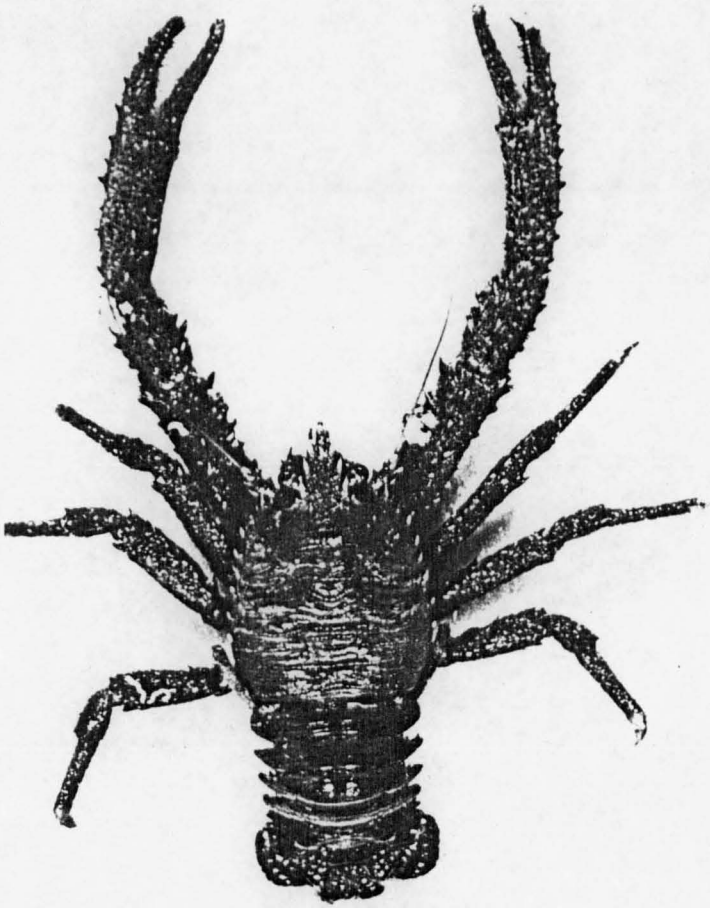
The Anomura is a diverse group which includes a range of forms intermediate between the Macrura (lobsters and shrimps) and the Brachyura (true crabs). The diversity of the Anomura is most obvious in the structure of the abdomen. The porcelain crabs (Porcellanidae) have reduced abdomens and resemble the true crabs, while the mud shrimps (Thalassinoidea) have extended abdomens and resemble most Macrurans. The Galatheidae have an intermediate form of abdomen (Fig. 1). This range in abdominal structure is paralleled by the gross anatomy of the central nervous system. The CNS of the Anomura forms a spectrum between the extended ganglionic chain of Macrurans and the compacted thoracic nervous system of Brachyurans (Fig. 2a). In G.strigosa the thoracic ganglia make up a concentrated mass and the chain of abdominal ganglia is somehwat shortened such that the "first" abdominal ganglion (Th. Ab. G) is fused to the posterior end of the thoracic ganglionic mass (TGM). The structure of each free abdominal ganglion is qualitatively similar and only small differences exist between segmentally repeated ganglia. In general, the absolute size of abdominal ganglia decreases caudally. The gross morphology of each ganglion is similar to other Anomuran and Macruran ganglia. Three paired roots arise from each (Fig. 2b). The most anterior of these, the first root (r1) arises from the lateral edge of the ganglion about half way down its length. r1 is about 150-200μ in diameter and extends laterally to innervate the swimmeret of that hemisegment.

FIG. 1 a, b.

Dorsal (a) and lateral (b) views of the male squat lobster, Galathea strigosa. The abdomen was manually extended in (a). Note the tonically flexed position of the abdomen in (b).

Magnification = x1 approximately.

a



b

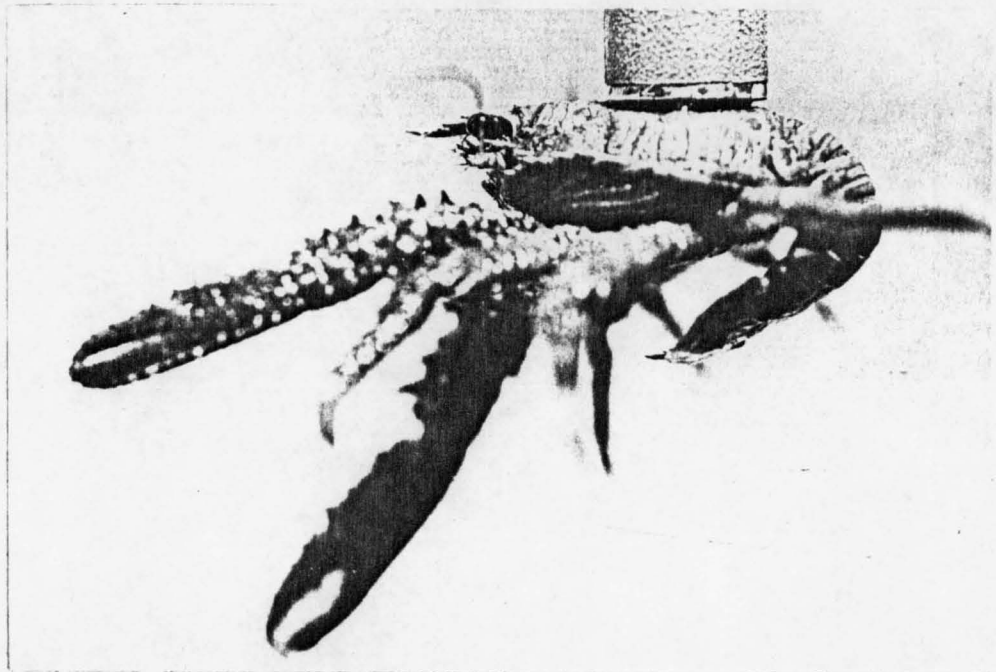
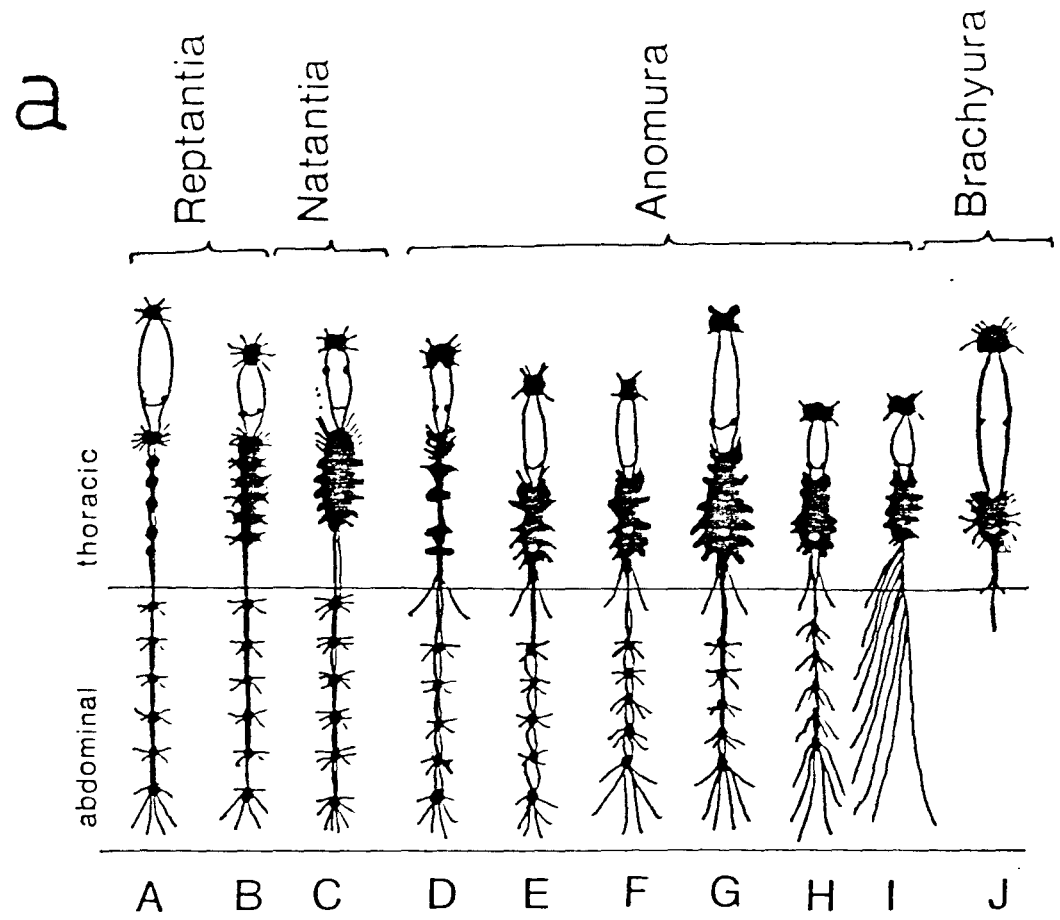
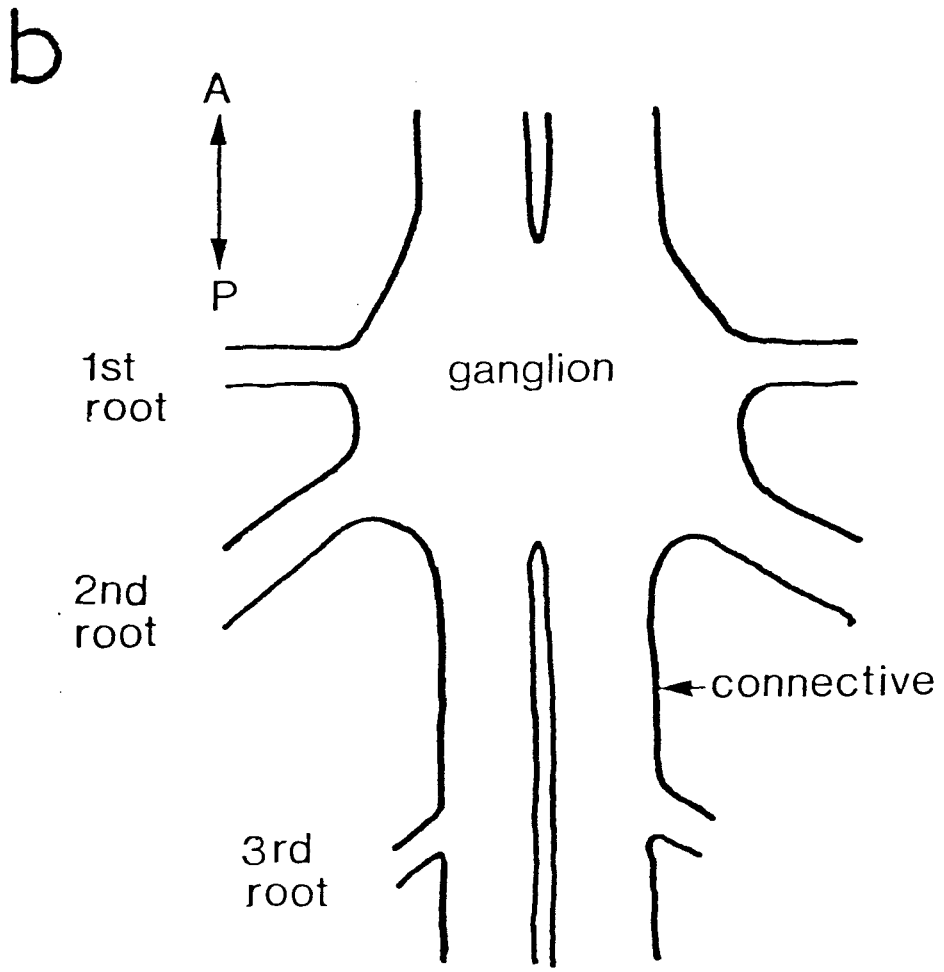


Fig. 2 a, b

(a) Comparative anatomy of crustacean nervous systems: A - Homarus; B - Scyllarus; C - Palaemon; D - Upogebia; E - Eupagurus; F - Galathea; G - Munida; H - P. longicornis; I - P. platycheles; J - Cancer. (Adapted from Pike, 1974).

(b) Schematic representation of a typical abdominal ganglion in Galathea strigosa (not to scale).


a
Reptantia
Natantia
Anomura
Brachyura
thoracic
abdominal
A
B
C
D
E
F
G
H
I
J


b
A
P
1st root
ganglion
2nd root
connective
3rd root

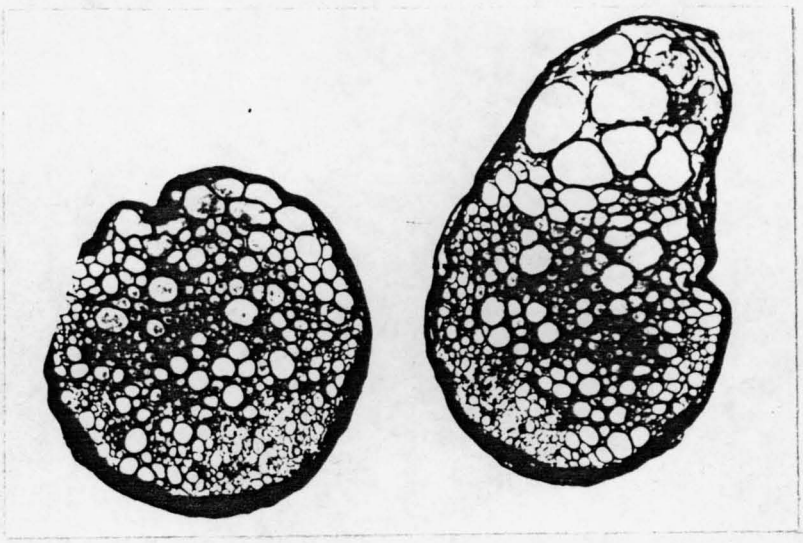
In contrast to the crayfish this root does not bifurcate into posterior and anterior branches. A much thicker 2nd root (r2) arises posteriorly to r1, projecting laterally and slightly posteriorly. About two thirds across the ventral surface, r2 passes dorsal to the thin sheet of superficial flexor muscles and ascends to innervate the extensor muscles, the M.R.O.'s and a number of sensory structures on the dorsal carapace. The most posterior root, and third root (r3) arises from the connective midway between adjacent ganglia and innervates the fast and slow flexor muscles.

The ventral nervous system consists of five free abdominal ganglia (G1 to G5) linked by paired connectives. Within the thorax the connectives fuse to form a single nerve cord which enters the T.G.M. The gross morphology of the abdominal connectives and the size and distribution of axon profiles has been examined by semi-thin sectioning and staining with Toluidene Blue (see Methods). The CNS is surrounded by a fibrous connective tissue sheath approximately 20-40μ thick (e.g. Fig. 3). Running along the external ventral surface of the entire CNS is a large artery, the ventral sub-neural artery (v.s.n.a.). The v.s.n.a. is only loosely associated with the abdominal connectives. As it passes over each abdominal ganglion the v.s.n.a. ramifies over the ventral surface and embeds in the connective tissue sheath.

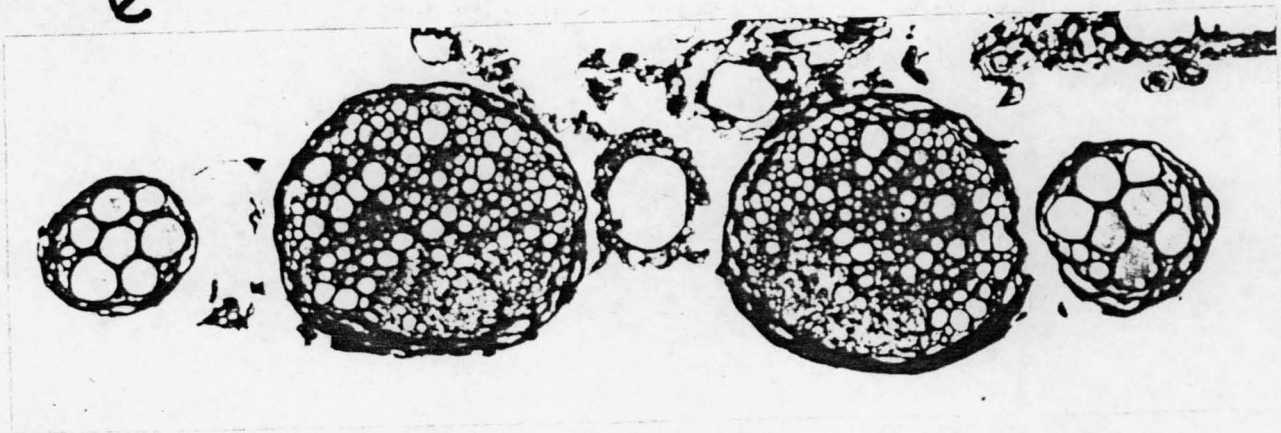
FIG. 3 a - e.

Cross-sections of the nerve cord at various levels in the abdomen: a - above G1; b - G1-G2; c - G2-G3; d - G3-G4; e - G4-G5. v.s.n.a. = ventral sub-neural artery. Closed arrow in (a) indicates the two large diameter axons which may correspond to MRO axons (see text). These axons occur at approximately the same place in (b). Note in (d) that the 3rd root axons enter the cord from its dorsal aspect. In (e), cut posterior to the 3rd root, each r3 is seen in cross-section beside the connective. In each of a - e the dorsal aspect of the connective is uppermost. For approximate dimensions of the connectives, see text.

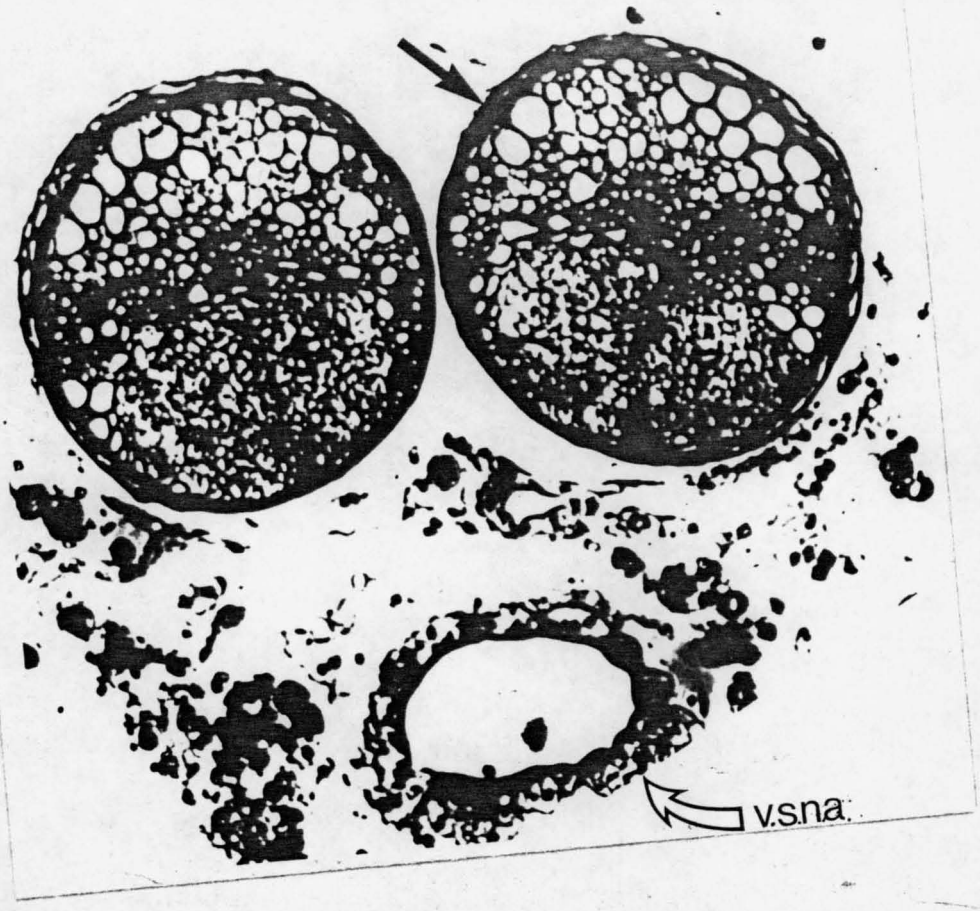
d



e



a



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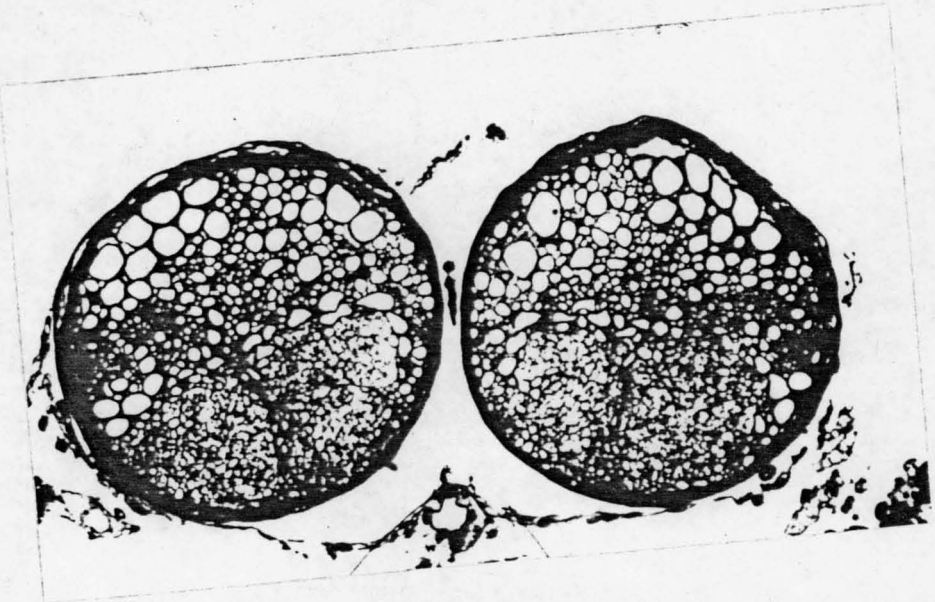


c

2. Are there giant axons in the connectives?

The most striking anatomical feature of the crayfish nerve cord is the presence of two pairs of interneurons with giant axons. The LG and MG axons are about 100μ in diameter and run the entire length of the CNS. In cross section the LG and MG axons are located in the dorsal region of the nerve cord and are an order of magnitude larger than other axon profiles. In the hermit crab (Umbach and Lang, 1981) a single pair of giant axons homologous with the crayfish MG's is found in cord sections. The most striking feature of the abdominal connectives in Galathea is an absence of cord giant axons. Sections cut at all levels of the abdomen have failed to reveal any axon profiles of similar relative diameter to the LG and MG axons of the crayfish. (Fig. 3 a-e).

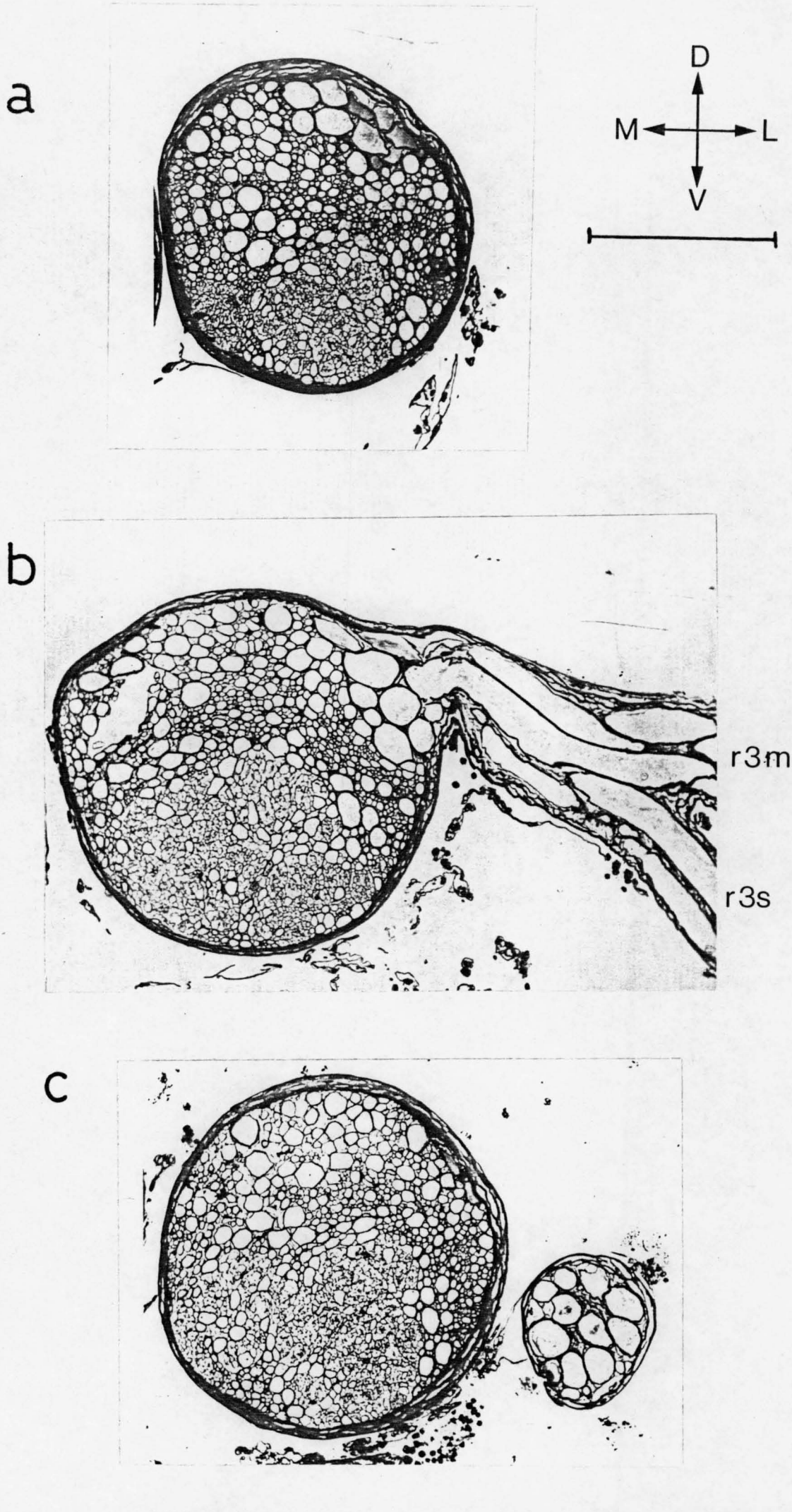
In the dorsal region of Galathea connectives posterior to each ganglion is a pool of 7 or 8 axons of relatively large diameter (c. 30-40μ) compared with other profiles. In serial sections of the nerve cord cut between two ganglia these axons exit the connectives via the 3rd root and are absent in sections cut more posteriorly (Fig. 4, a-c). These are the axons of fast flexor motorneurons.

A further specialization of the crayfish and hermit crab giant fibre system is the presence of a giant motorneuron (MoG:GM respectively) with which the giant axons synapse (Furshpan and Potter, 1959; Umbach and Lang, 1981). In addition to their close association with the giant axons in the connective the axons of MoG and GM are much larger than other FF axons in sections of the 3rd root. In contrast the FF axons in abdominal 3rd roots of Galathea are all of similar diameter (Fig. 4c). This evidence suggests that the specialized giant motorneuron may be absent in Galathea.

FIG. 4 a - c.

Toluidine Blue-stained sections of the right hemi-connective cut between G1 and G2. b and c are from the same animal, a is from a different animal. The group of large diameter axons in the dorso-lateral portion of a, cut anterior to r3, are the axons of flexor motor-neurons which descend the connective and exit via r3 in b. Sections cut posterior to the 3rd root show an absence of large diameter axons c. Note the slight tissue distortion in b. About 300μ after leaving the connective r3 bifurcates into a main branch (r3m) and a thinner, superficial branch (r3s).

Scale bar = 200μ.


a
D
M
L
V
b
r3m
r3s
c

With the exception of the FF axons, the dorsal aspect of the connectives is comprised of a relatively homogeneous distribution of axon diameters up to about 20μ. In sections of anterior connectives, above G1 and between G1 and G2, occur two pairs of axons of similar diameter to FF axons (Fig. 3 a, b) which do not leave the CNS via the 3rd root (Fig. 4 a-c). These are located medial to FF's near the dorsal surface of the connectives and it was considered possible that they may represent reduced forms of the crayfish giant axons. However, attempts to demonstrate physiological connections between these axons and abdominal motorneurons have been unsuccessful. When they are teased free from the connectives and stimulated with discrete electrical shocks there is no evidence for suprathreshold flexor motor output. In the light of evidence presented in section C of this chapter it seems probable that these are MRO axons.

3. Segmental Gradients

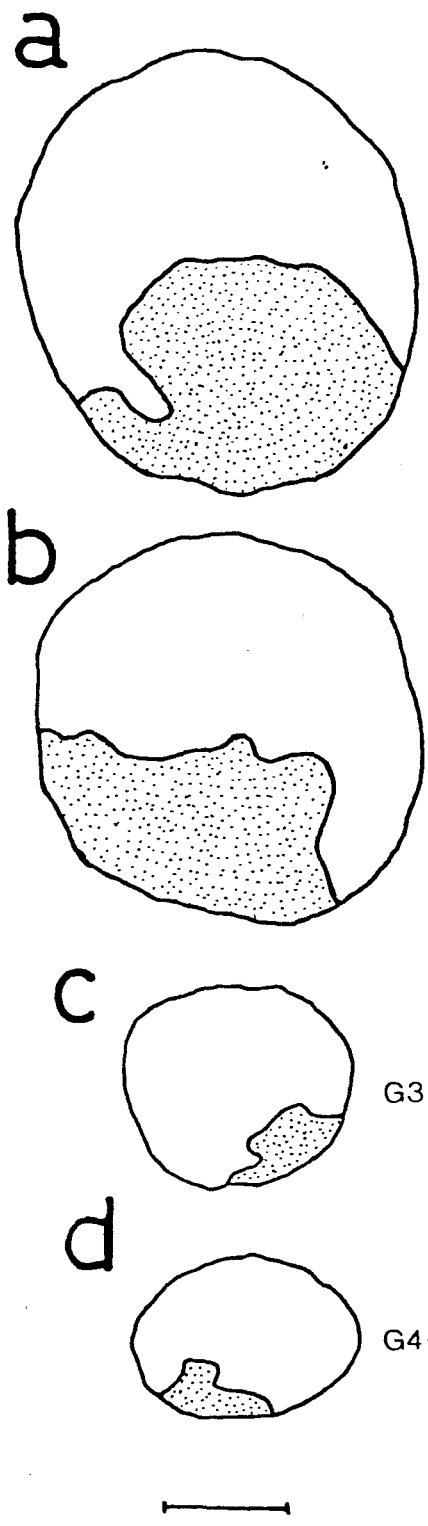
The diameter of abdominal ganglia and connectives decreases in caudal segments. In fixed wholemounts of the chain of abdominal ganglia, the diameter of the connectives decreases in a stepwise fashion. In a medium sized animal (8 cm from tip of rostrum to caudal edge of telson) connective diameter decreased from approximately 315μ above G1, to 300μ between G1 and G2, 280μ between G2 and G3; 230μ between G3 and G4 and 180μ between G4 and G5. The rate of decrease in connective diameter was largest between G2 and G4.

Each connective can be divided into two regions in terms of the diameter of axon profiles. The ventral region is composed almost entirely of extremely small diameter axons. This area may therefore be predominantly sensory in function (Bullock and Horridge, 1965). Axons in the

dorsal region of the connective are generally larger. Although the separation between these two regions is not absolute it is possible to delimit the two regions with a best-fit line. There is a clear segmental gradient in the division of the connectives into small diameter (SD) and large diameter (LD) axons (Fig. 5). Sections of the connectives from various regions of the abdomen were projected with the aid of a camera lucida attachment. The outline of one connective and a best-fit line delimiting the two areas were drawn on sectional graph paper and the areas measured. In anterior sections, (G1-G2), the area occupied by SD axons is large (37%). Between G2 and G3 the total cross-sectional area of the connective decreased as did the relative area of SD axons ($\sim$ 32%). The results for G3-G4 and G4-G5 were taken from a different, smaller animal so that the total cross sectional area of each connective is much reduced. However, there was a substantial reduction in the relative area of SD axons ($\sim$ 14% in G3-4; $\sim$ 11% in G4-5). The relative areas of SD and LD axons are likely to be similar in the same region of the connectives in different animals. Therefore, it can be concluded that in addition to an absolute reduction in connective diameter there is a relative decrease in the area occupied by SD axons. A possible explanation for this phenomenon is that ascending sensory interneurons relay information, coded in fibres which enter abdominal roots, rostrally towards the thoracic nervous system. The anterior connectives contain sensory interneurons relaying input from the entire chain of abdominal roots.

FIG. 5 a - d.

Segmental gradients in connective and axon diameters. Semi-thin sections of the abdominal connectives were drawn with the aid of a camera lucida. Each connective was divided with a best-fit line into regions of large diameter (LD) and small diameter (SD; stippled) axons. The absolute and relative areas of each region was measured (right-hand columns). Data pertains to the right connective just posterior to G1 (a), G2 (b), G3 (c) and G4 (d). Note the decline in connective area and in the relative area of SD axons in caudal sections. a and b are from one animal, c and d are from a different, smaller animal.



		AREAS		
		LD(μ^2)	SD(μ^2)	%SD.
a	G1 – G2	6128	3628	37
b	G2 – G3	6067	2917	32
c	G3 – G4	1925	324	14
d	G4 – G5	1619	206	11

B. THE ANATOMY OF EFFERENT NEURONS INVOLVED IN ABDOMINAL FLEXION

As in crayfish and lobsters abdominal third roots are purely motor. Within 1 mm of leaving the connective r3 bifurcates; a thin superficial branch (r3s) extends laterally, innervating a thin ventral sheet of tonic flexor muscles; a thicker main branch (r3m) ascends dorsally and rostrally, innervating the massive fast flexor muscles of that segment. The superficial tonic flexor muscle is situated at the extreme lateral edge of the abdomen and may be only a few fibres thick. A more medial slow flexor muscle, present in lobsters and crayfish is absent in Galathea.

The point of exit of r3 from left and right abdominal connectives is asymmetrical: from any given abdominal ganglion (G1 to G4) r3 on one side leaves the connective up to 1 mm more rostrally than the contralateral root. In two successive ganglia this asymmetry alternates. In addition, r3 G3 leaves the connective from its dorsal aspect while r3 G1 2, and 4 exit the connective more laterally. These features of asymmetry and torsion may reflect the relatively close taxonomic relationship between squat lobsters and hermit crabs (Anomura) in which the abdomen is spiralled to fit into empty Gastropod shells and r3 exit is also asymmetric. However, no functional asymmetries have been observed in Galathea and the central anatomy of the fast flexor motorneurons appears to be symmetrical.

The slow flexor system

Physiological experiments on r3s have shown that this nerve contains several tonically active motorneurons. Impulses recorded in r3s with en passant hook electrodes can be correlated 1:1 with junctional potentials recorded intracellularly in slow flexor muscle fibres (Fig. 6a, b). As in crayfish (Kuwada and Wine, 1979) each slow flexor muscle fibre is innervated by a subpopulation of slow flexor motorneurons (sF's). This pattern of innervation has not, however, been quantified. At least one of the units recorded in r3s produces IJP's in slow flexor muscle fibres (Fig. 6d). This unit has a large extracellularly recorded potential compared with other tonically active units. Stimulation of r3s results in an IJP which has a low threshold compared with EJP's, consistent with observations on the crayfish tonic inhibitor. Every slow flexor muscle fibre tested received innervation from the inhibitory motorneuron. The large amplitude of the extracellular potential and the low threshold for stimulation suggest that this neuron has a relatively large axon diameter and this would be consistent with a wide distribution among slow flexor muscle fibres.

The central anatomy of sF motorneurons has been visualized by cobalt backfilling of r3s G2. Six somata are stained: five in G2 and one in G3 (Fig. 7). Within G2, three somata are located ipsilateral to the filled root. Contralaterally, three somata are stained of which two are sF's. In addition, the large contralateral fast flexor inhibitor (FI) soma is stained due to its unique peripheral branching pattern (see following section on FF's). The diameter of sF somata in G2 is 20-50μ. Each is located laterally in the ganglion. A single sF descends to G3. (Fig. 7b). Its thin (c. 5μ) axon enters the neuropile at the

FIG. 6 a - d.

Activity of slow flexor motorneurons recorded in r3s G2 (top traces) and their effects in slow flexor muscle fibres (bottom traces, intracellular).

a. Spontaneous activity in a number of r3s units, one of which innervates the slow flexor muscle fibre recorded below.

b. During high frequency firing, EJP's summated and correlate 1:1 with extracellular spikes at a constant latency of about 3 ms.

c. Spontaneous activity in a unit with a large extracellular spike height produces small IJP's (arrowed). These occur at constant latency (d).

Scale bars: horizontal - a, c = 50 ms; b = 2 ms, c = 5 ms; vertical - a, c, d = 10 mV; b = 5 mV.

a
c
b
d

FIG. 7.

Central anatomy of slow flexor motorneurons.

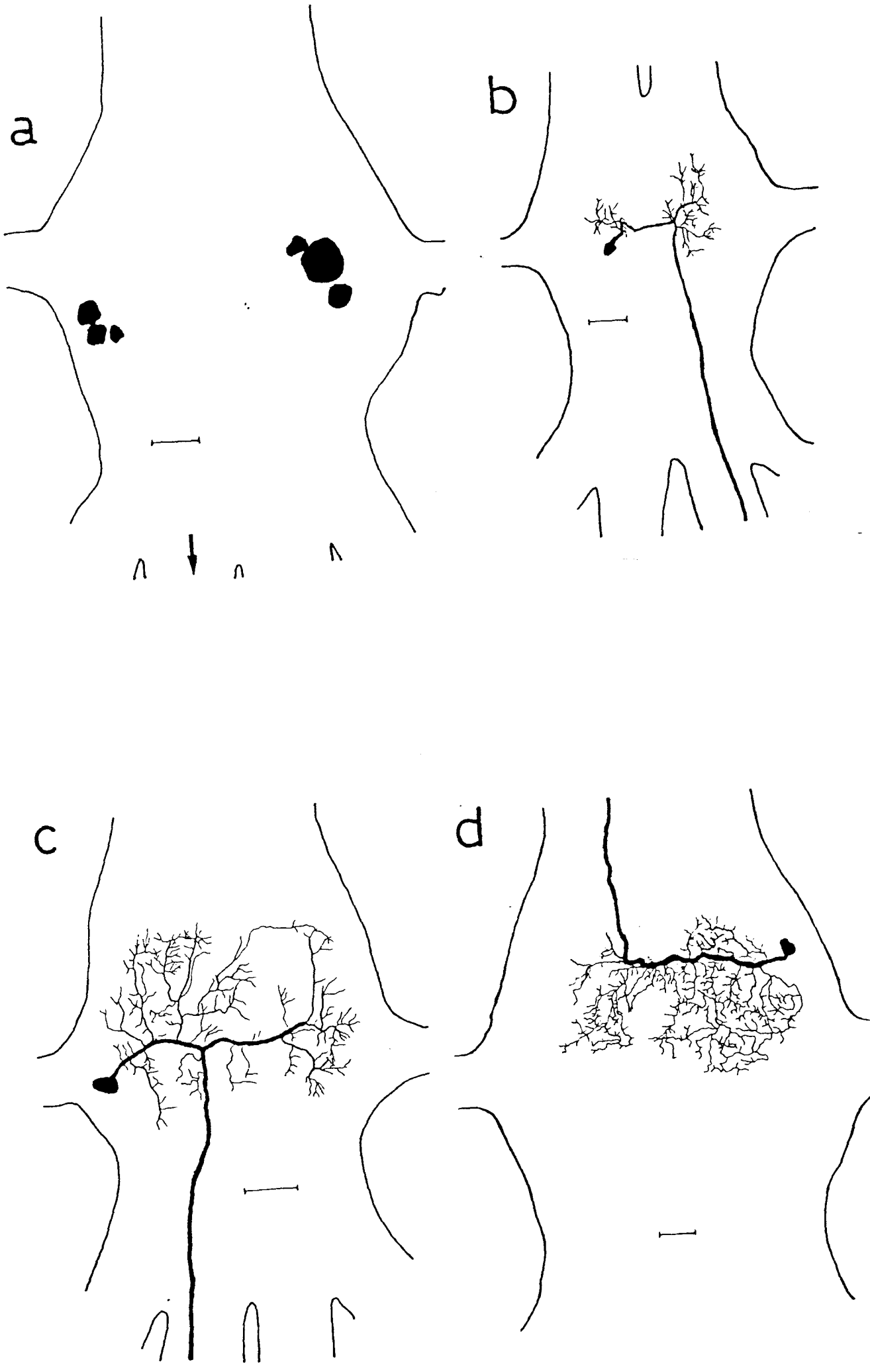
a. Soma map of sF's in G2 following cobalt backfilling of r3s. Arrow indicates direction of axons and side of cord stained. The largest contralateral soma is FI.

b, c. Camera lucida drawings of Lucifer Yellow stained sF's in G2.

d. In backfills of r3s G2 a single sF stains in G3.

d was drawn from a silver intensified wholemount.

Scale: 100μ a, b, c; 50μ d.


a
b
c
d

level of the 1st roots, and expands into a large (c. 15μ) neurite which courses laterally across the ganglion to a 30μ cell body at the rostro-lateral edge of the ganglion. A dense and complex dendritic tree arises from the neurite. Most processes project caudally in the ganglion with few arising rostrally.

Lucifer Yellow staining of sF somata in G2 reveals that these have complex branching patterns with arborizations reaching every quadrant of the ganglion (Fig. 7c, d).

Fast flexor motorneurons

The central anatomy of fast flexor motorneurons (FF's) has been studied by cobalt backfilling r3m of abdominal ganglia. As in crayfish, FF somata are distributed among three clusters. Backfilling r3m G2 stains two soma clusters in G2 and one in G3. (Fig. 8 a, b). I will use the same terminology as Mittenthal and Wine (1978) for crayfish in describing FF soma clusters, for ease of comparison. Situated contralateral to the filled root is a medial group of cells (FMC) containing four somata. A posterior group (FPI) consisting of four somata lies ipsilateral to the filled root in G2. A third group (FAC) comprising two cells have somata in the anterior portion of G3 contralateral to their axons. Each of the three clusters will be described in turn for each abdominal ganglion. The dense array of FF processes in the neuropile (Fig. 8) has precluded a characterization of the branching pattern of individual FF's from cobalt backfilled preparations. Where available, data is presented from Lucifer Yellow stained preparations.

FIG. 8 a - c.

Central anatomy of FF's in G2 after cobalt-filling r3m.

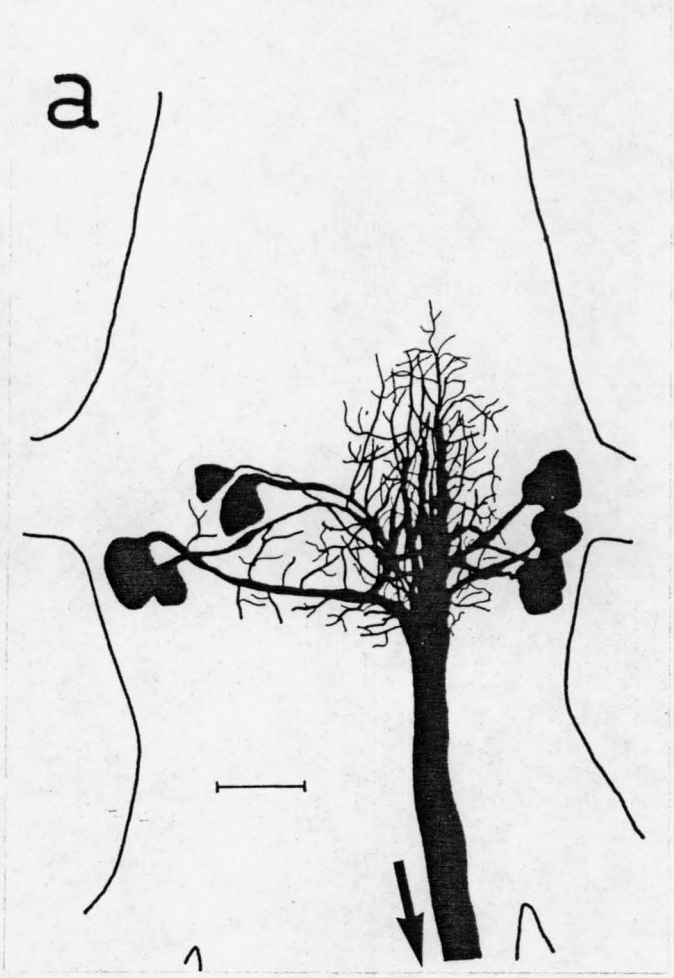
a. FMC (contralateral to axons) and FPI (ipsilateral to axons) soma clusters in G2.

b. FAC neurons in G3.

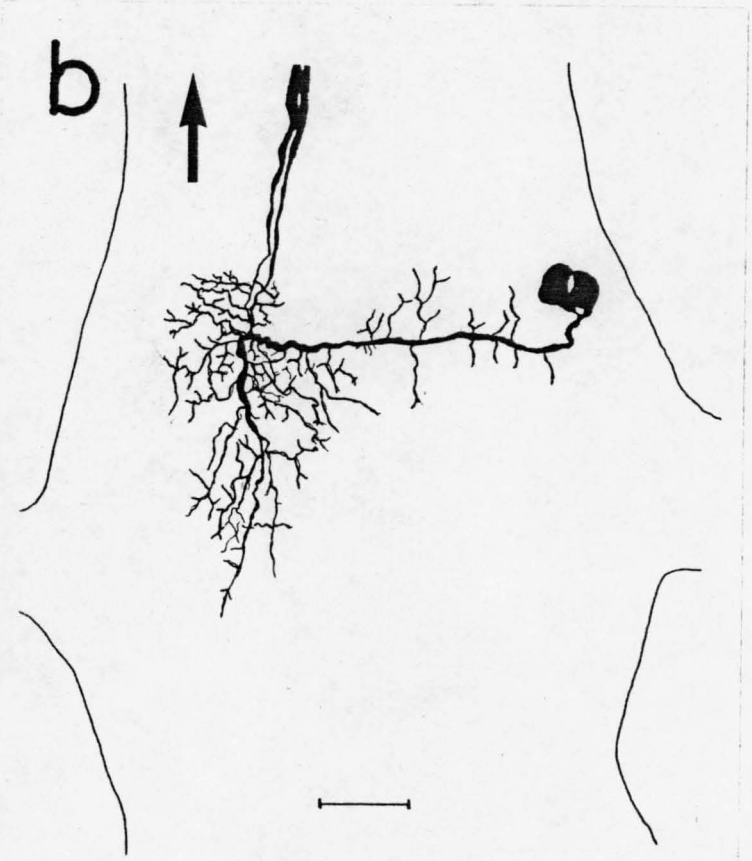
c. Entire hemi-segmental FF pool in G2 and G3.

a, b and c are from different preparations. a and b are camera lucida drawings from wholemounts of intensified cobalt staine. c. is a photograph of an unintensified wholemount.

Scale bars: a, b, 100µ.


a


c


b

Anterior group

Two classes of neurons have somata in the ganglion posterior to the third root from which their axon exists - slow flexor motoneurons and FAC fast flexor motorneurons. These can be distinguished on the basis of morphological criteria. Backfilling the whole third root in G2 stains three somata in G3. One of these is a sF (Fig. 7d) with a soma of c. 30μ diameter. The remaining two are FAC FF's with much larger (c. 40-50μ) somata. (Fig. 8b). The two FF neurites follow almost identical paths in the ganglion, distinct from the neuritic path of the sF. Selective backfilling of r3m and r3s illustrates the different dentritic domains of the sF (Fig. 7d) and the FAC FF's (Fig. 8b). Whereas the sF has a complex bilateral branching pattern, FAC FF's have dendritic domains restricted mainly to the ipsilateral hemiganglion.

Of the three clusters of FF's, the FAC group was found to vary most in crayfish abdominal ganglia (Mittenthal and Wine, 1978) with one soma in G1, three in G2 and G3, two in G4 and none in G5. In contrast I find little variation in the number of FAC somata in abdominal ganglia of Galathea. Homologous FAC neurons are present in G1 to G4 with each cluster containing two somata of 40-50μ (Fig. 9 a-c). There are no FAC somata present in the terminal 5th abdominal ganglion.

Medial group

The FMC cluster contains 4 somata in G1 to G3, three in G4 and one in G5 (Fig. 10 a-e). The number of FF somata in Th.Ab.G has not been successfully resolved but up to 8 axons have been seen to ascend the G1-Th.Ab.G connective following backfilling of the appropriate third root branch.

FIG. 9 a - d.

Soma positions of FAC FF's in G1 (a), G2 (b), G3 (c), G4 (d), drawn from wholemounts of cobalt- filled r3m branches (arrowed). Two somata contralateral the filled root stain in each ganglion. Note the almost identical path of the axons and neurites of the two neurons in each case.

Scale bars = 250µ.

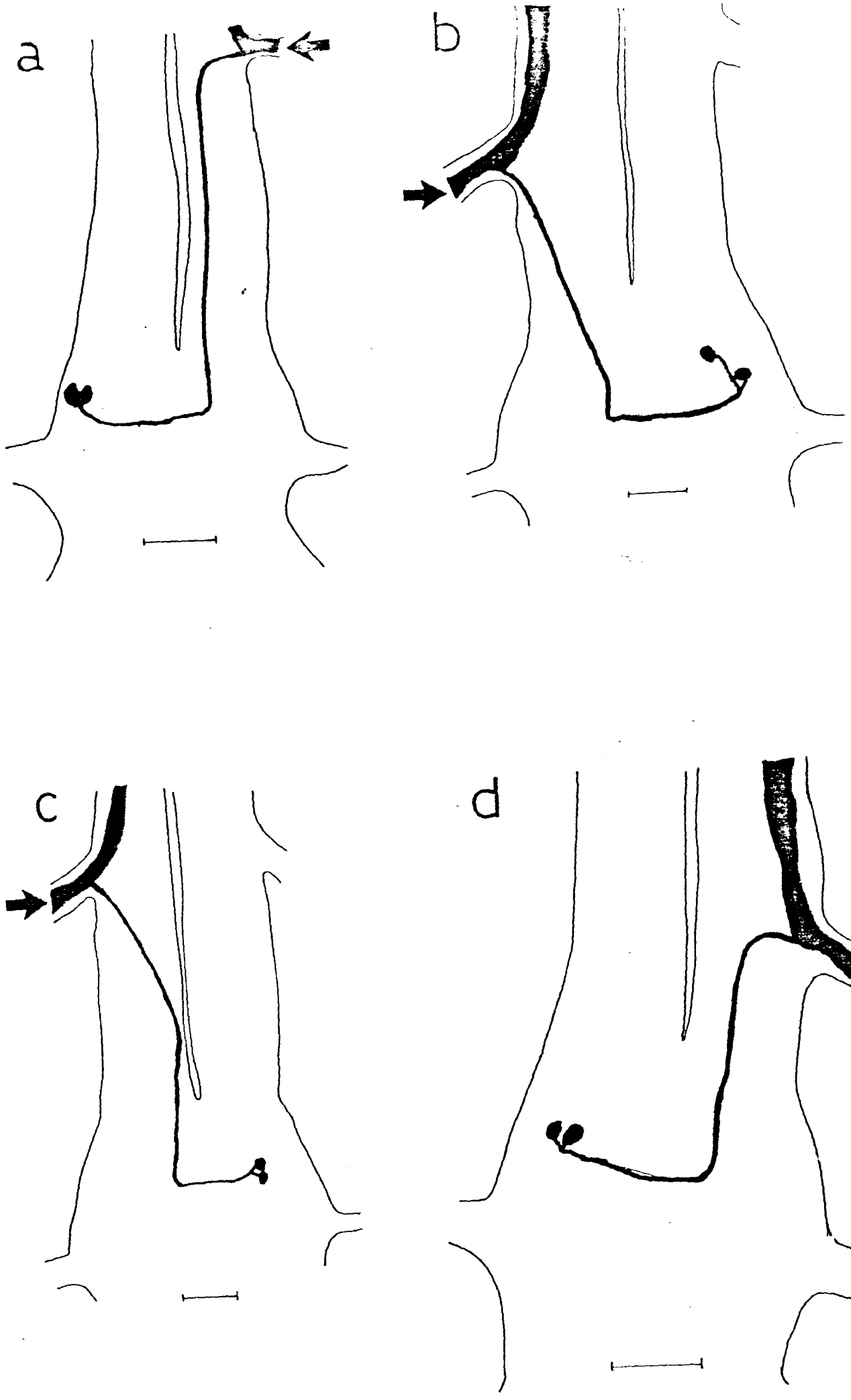
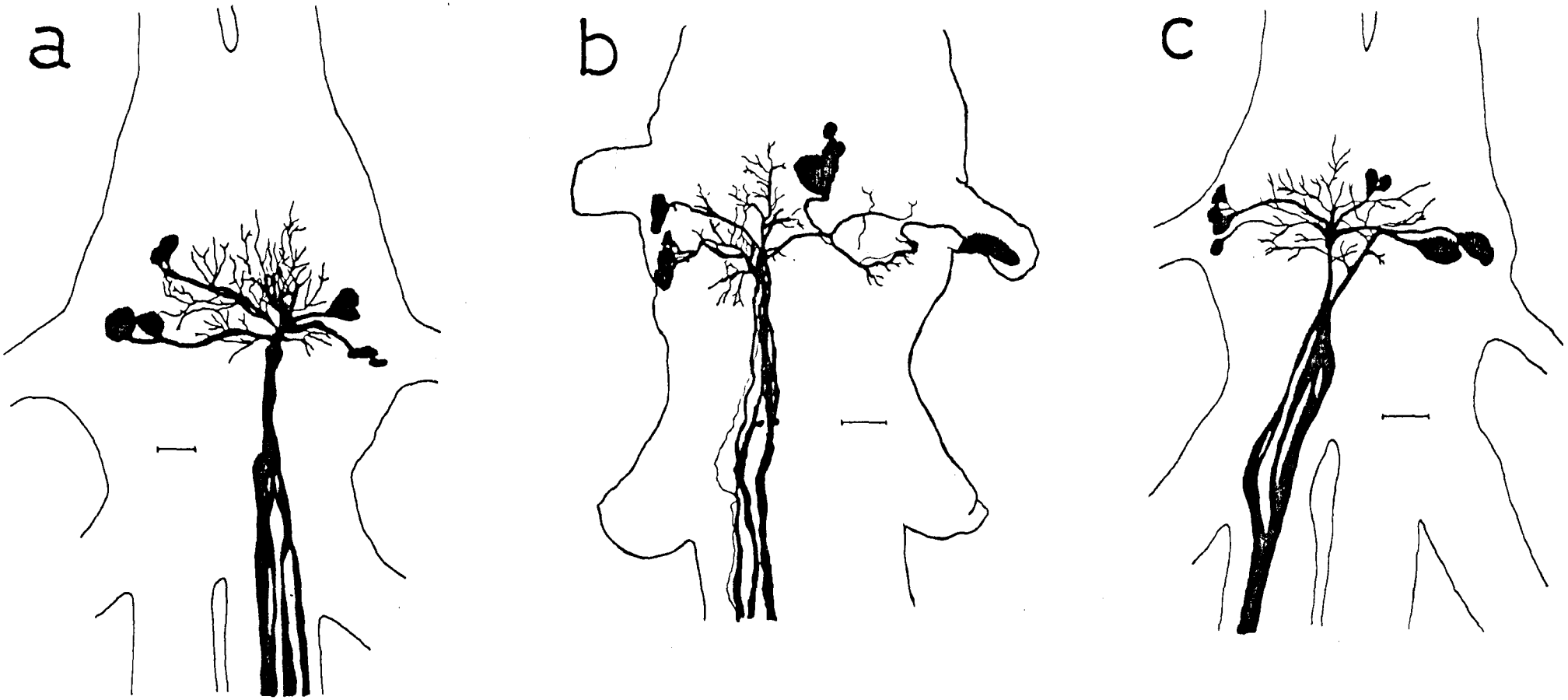
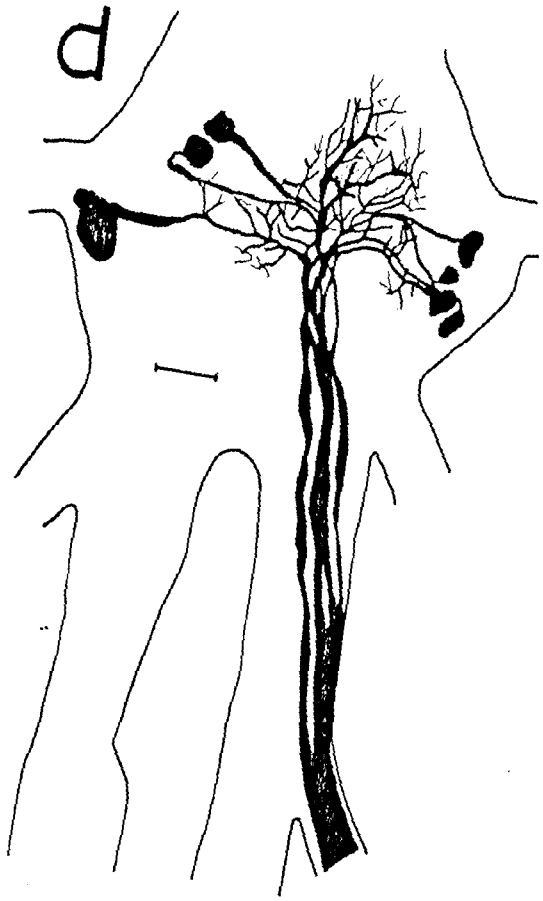

a
b
c
d

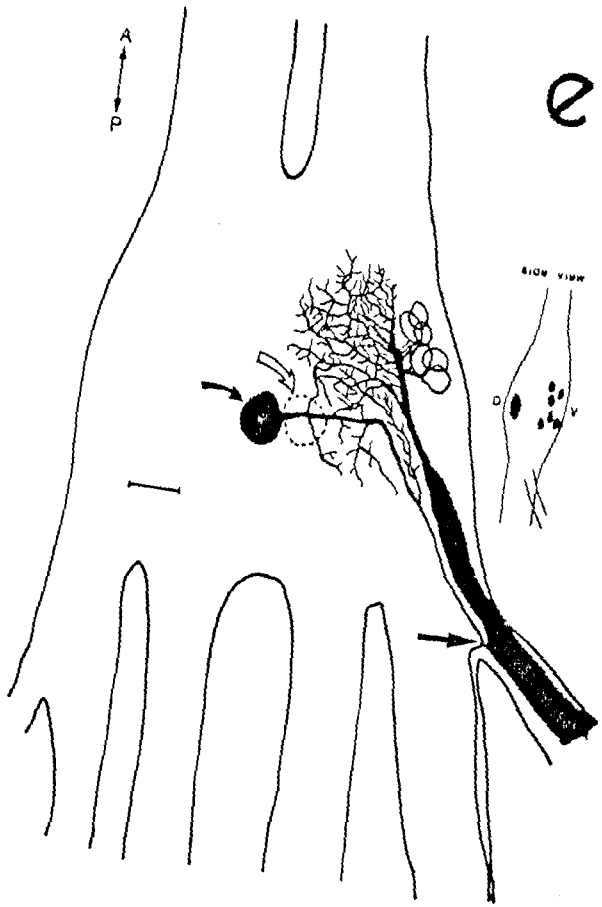
FIG. 10 a - e.

Segmental homology and variation among FPI and FMC FF soma clusters in G1 (a), G2 (b), G3 (c), G4 (d) and G5 (e). Note the complete homology in G1, G2 and G3. In G4 there are only 3 FMC somata and no FAC neurons descend to G5. In G5 there are 6 FPI somata and one FMC soma. This may be FI. In e, closed arrows indicate the soma and axon bifurcation of the single FMC neuron. The open arrow points to a large unstained soma which may be a contralateral homologue of the single stained FMC soma. Insert in e represents a side view of G5 showing the extreme dorsal location of the FMC soma compared with FPI somata. Some of the FPI cluster may be sF's since this root does not bifurcate into r3s and r3m as do anterior 3rd roots.

Scale bars = 100μ.


a
b
c


d


A
P
e
SIDE VIEW
D
V

In G1 to G3 the FMC cluster contains 4 somata as in crayfish (Mittenthal and Wine, 1978). Two of these are large (c. 100μ) and in terms of their size and position and axon course they are homologous with the MoG and FI somata of the crayfish. The remaining two somata are smaller (c. 50-70μ).

The Motor Giant homologue (MoGH)

The more medial of the two large FMC somata is homologous with the crayfish MoG in terms of the size and position of its soma. The anatomy and physiology of this neuron have been studied in more detail by recording from the soma in isolated abdominal nerve cords and iontophoretic injection of the fluroescent dye Lucifer Yellow (see Methods).

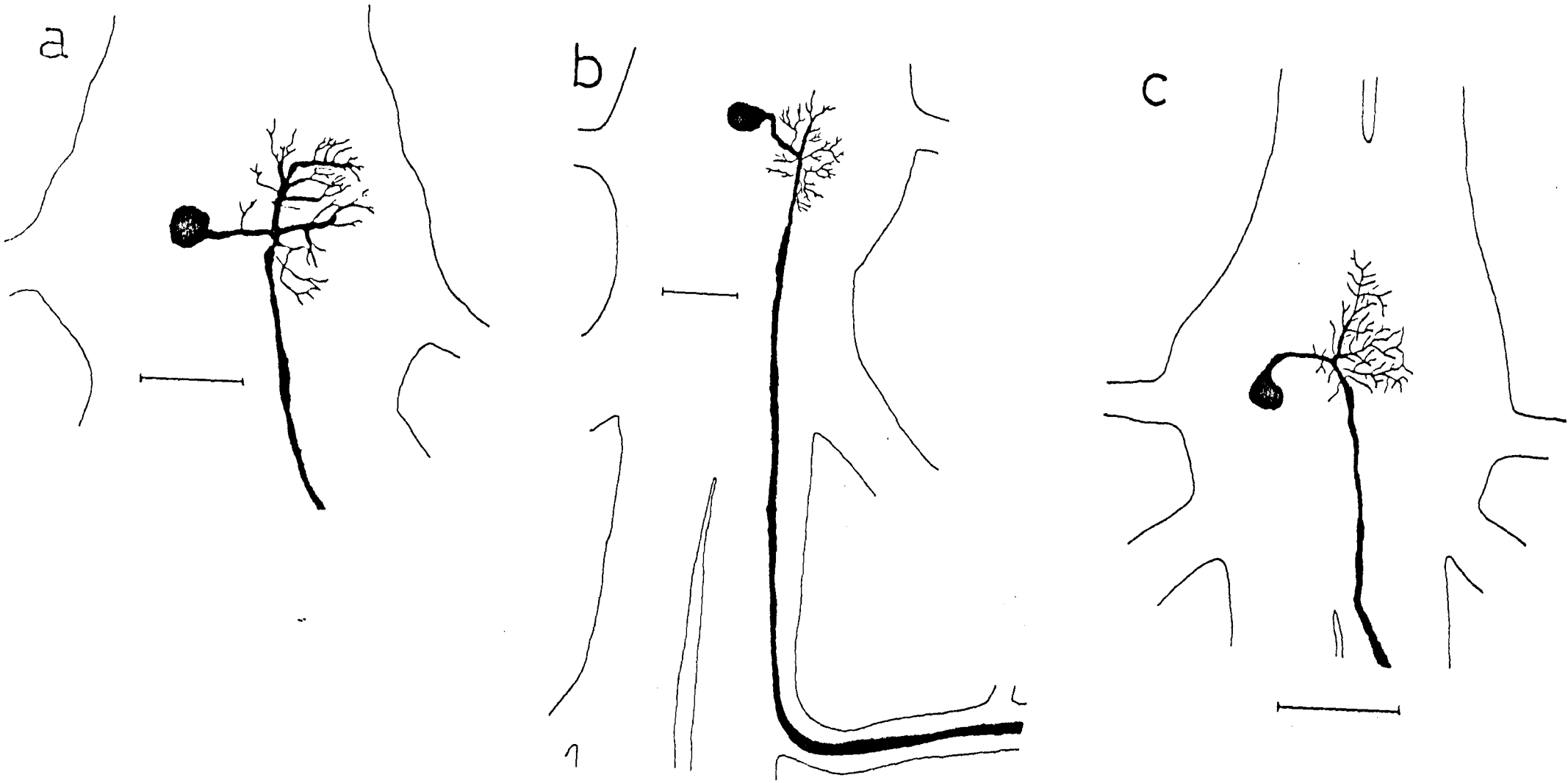
In contrast to the crayfish MoG, this neuron has a complex branching pattern in the neuropile of the ganglion (Fig. 11) and in this respect appears to be a typical FF. Crayfish MoG neurons have no arborizations in the neuropile of the ganglion but sprout featherlike processes in the connectives in the region of the giant fibres (Mittenthal and Wine, 1978). From the large (c. 90-100μ) MoGH soma a thick neurite projects dorsally to a large (c. 40μ) axon which descends the medial portion of the contralateral connective and exits r3m. No processes have been observed arising from the axon of MoGH in the connective. In the crayfish, MoG axon branches down both r3s and r3m. MoGH does not branch down r3s but in favourable preparations its axon branches at each bifurcation of r3m suggesting that this neuron has a wide distribution in the periphery. This would be consistent with the large diameter of its soma.

The crayfish MoG is specialized with respect to and receives input only from the cord giant fibres (Wine and Krasne, 1982). In contrast,

FIG. 11 a - c.

The central anatomy of MoGH in G1 (a), G2 (b) and G3 (c) after injection of Lucifer Yellow. In each fill the large soma was connected by a thick neurite to an area of neuropilar arborization. The large diameter MoGH axon leaves the connectives via the posterior 3rd root and projects down r3m. In b, the axon was observed to branch at each bifurcation of r3m.

Scale bars: 200μ a, b; 250μ c.


a
b
c

intrasomatic recordings from MoGH in Galathea reveals a low level of spontaneous synapic potentials from unidentified neurons. Single electrical shocks to the G1-Th.Ab.G connective elicits a complex compound EPSP in the MoGH soma in G2 (Fig. 12a) in addition to suprathreshold activation of other r3m units. I was unable to observe soma spikes in response to cord stimulation. However injection of 10nA depolarising current into the soma resulted in a train of attenuated soma spikes which could be correlated 1:1 with spikes recorded extracellularly in r3m (Fig. 12b).

Thus, the MoGH is anatomically similar to other FF's and the ability to record a variety of subthreshold post-synaptic responses in the soma correlated with suprathreshold drive of other FF's suggests that MoGH receives similar inputs but may have a higher threshold for spike initiation.

The Flexor Inhibitor (FI)

The FMC neuron with the most lateral soma in the crayfish FF pool has been shown by Wine and Mistick (1977) to be the peripheral inhibitor of phasic flexor muscles, FI. The unique identifiable soma of FI is large (c. 175 μm) and second only in size to the MoG soma. A neuron homologous with the crayfish FI in terms of the size and position of its soma stains in G1 to G4 of Galathea (Figs. 8, 10). This anatomical homology is substantiated by the physiological evidence presented below and thus the neuron is positively identified as the inhibitor of the phasic flexor muscles, FI.

FI has a unique peripheral branching pattern which has allowed an unambiguous identification of its peripheral function. FI is the only flexor motorneuron which bifurcates down both r3m and r3s.

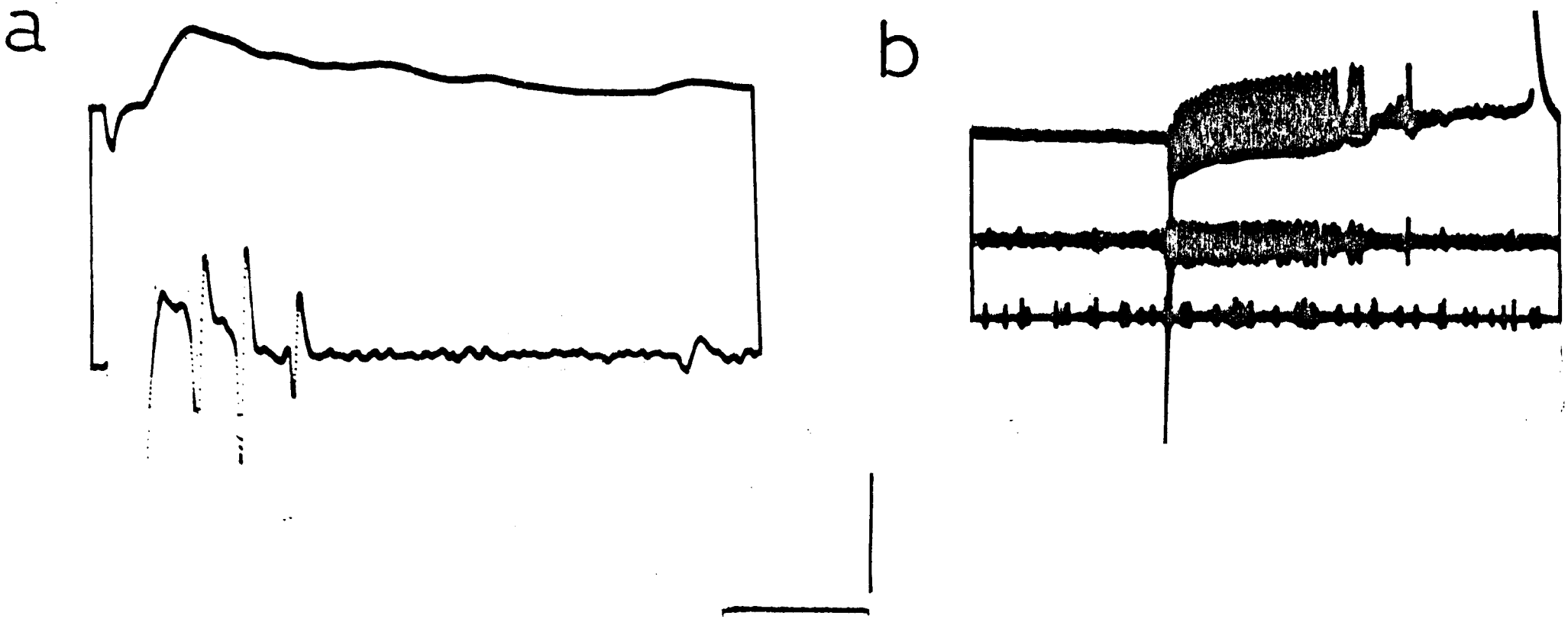
FIG. 12 a - b.

Physiology of MoGH. MoGH soma was penetrated in G2 of an isolated nerve cord preparation.

a. Single electrical shocks delivered to the anterior cord (above G1, elicited a compound PSP in MoGH (top trace) as well as suprathreshold activity in a number of other ipsilateral FF's (bottom trace, r3m).

b. Current injection (10 nA, not monitored) into MoGH produced a train of spikes (top trace) which could be correlated 1:1 with spikes recorded extracellularly on r3m (2nd trace, ipsilateral to MoGH) but not in r3s (bottom trace).

Scale bars: horizontal - 5 ms a; 250 ms b. vertical - 5 mV a; 10 mV b.


a
b

Cobalt backfilling of either r3s or r3m stains the FI soma and an axon which projects down the remaining branch of the third root (Fig. 13). Methylene Blue staining of the isolated abdomen reveals a number of fine branches which arise from the medial section of r3s, as it projects laterally to the slow flexor musculature, and ramify over the ventral surface of the fast flexor muscles. These processes are presumed to belong to FI. (Fig. 14a).

Physiological identification of the FI branch point has been obtained as follows (Fig. 14 a, b). Extracellular stimulation of either r3s or r3m results in a short latency spike recorded in the other third root branch (Fig. 14 b). This potential is elicited at a set threshold of stimulation and further increases in stimulus intensity (up to 100v) do not alter its amplitude or waveform. Subsequent intracellular penetration of ventral fast flexor muscle fibres records an IJP which is coupled to the extracellularly recorded potential in threshold and latency (Fig. 14 b, iii). The FI IJP is often not visible in intracellular recordings, presumably because its reversal potential lies close to the resting potential of FF muscle fibres (c. -50 to -70 mV). Probably due to the large volume of individual FF muscle fibres it was often impossible to alter the membrane potential significantly by injection of up to 10nA depolarizing current. On several occasions however sufficient depolarization of the muscle membrane enhanced the amplitude of the FI IJP (Fig. 14 b, iii). It is not clear whether this was possible because of lower electrode resistance or because penetrations were made closer to FI endplates. On a number of other occasions FI IJP's were hyperpolarizing at normal resting potentials. This may have been due to artificial membrane depolarization caused by the penetration process. In the majority of penetrations FI IJP's were either invisible or were slightly depolarising.

FIG. 13 a, b.

The peripheral branch point of FI revealed by selective backfilling of r3s and r3m in G2.

a. Backfilling r3m (arrowed) stains FI, the FF pool (somata circled) and an axon which projects down r3s.

b. Backfilling r3s (arrowed) stains FI, the sF pool (somata circled) and an axon which projects down r3m.

Thus the FI axon branches down r3s and r3m.

In these sketches of cobalt filled wholemounts, the FI soma andaxon course is filled-in black. The FI soma is approximately 100μ in diameter.

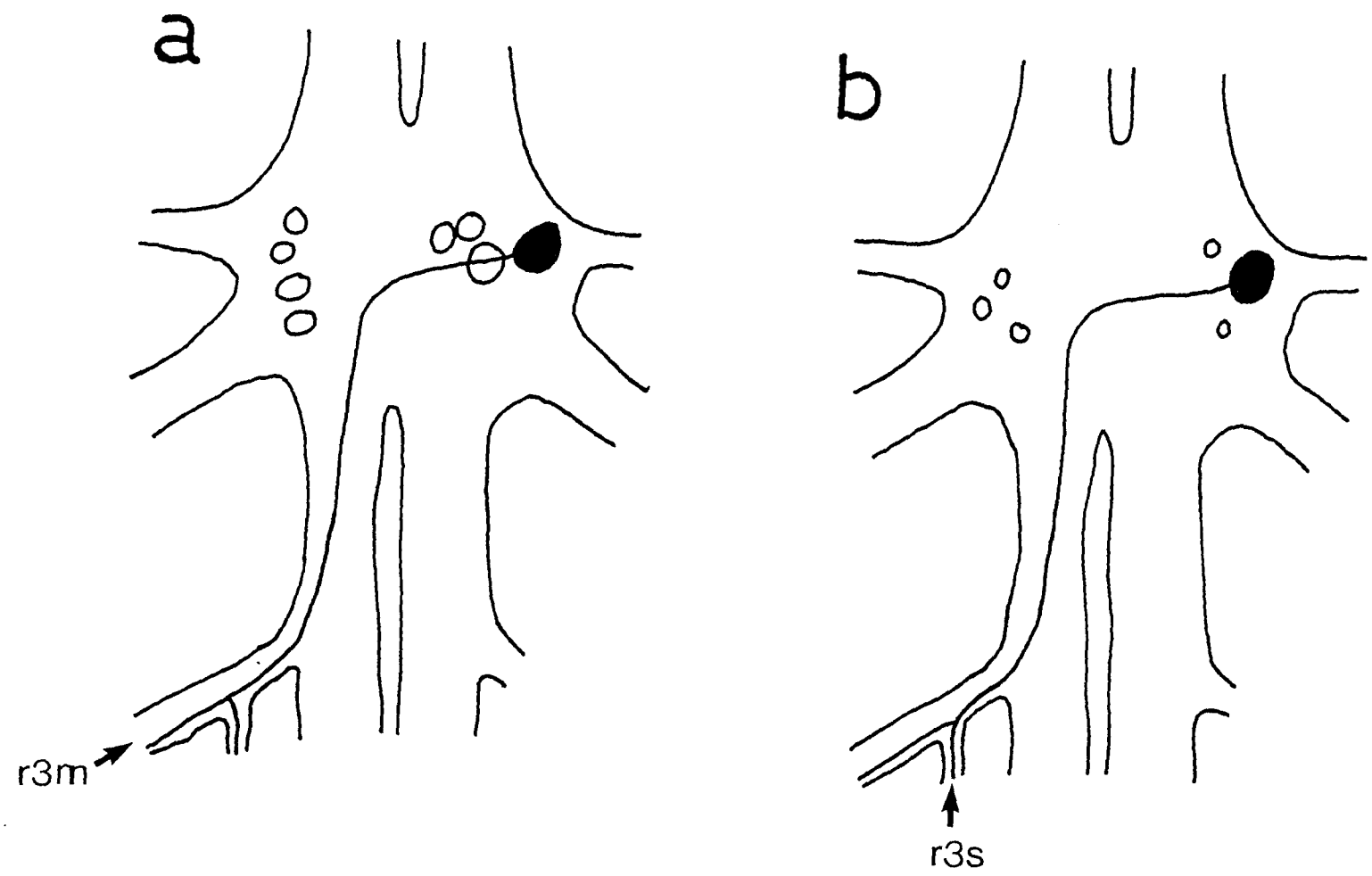

a
b
r3m
r3s

FIG. 14 a, b.

Anatomical and physiological characterization of FI.

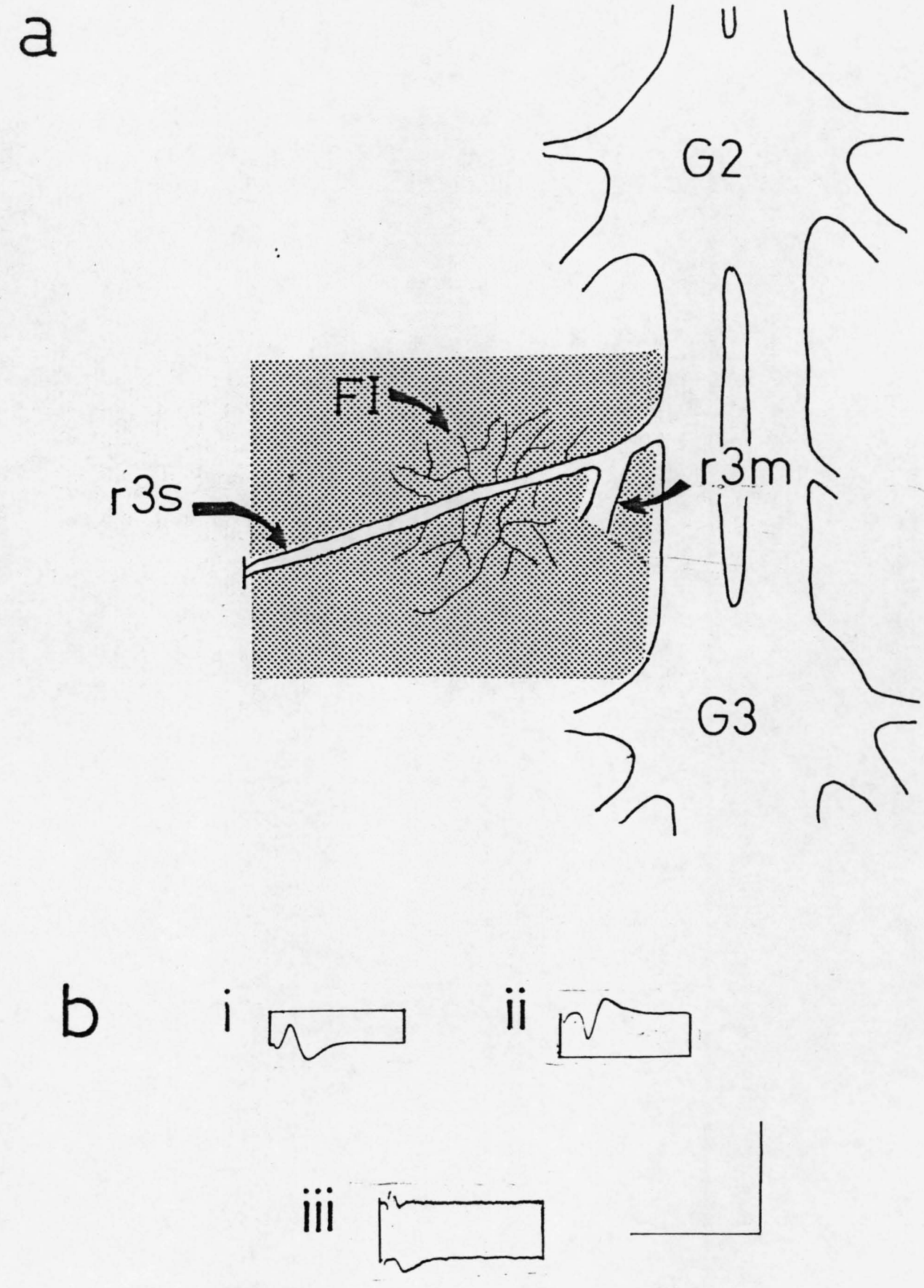
a. Methylene Blue staining reveals numerous fine branches which arise from the medial portion of r3s and ramify over the ventral surface of FF muscle fibres (stippled). Since FI is the only member of the FF pool which has an axon branch in r3s, these processes are presumed to belong to FI.

b. Physiological identification of FI branch point.

i. Stimulating r3s results in a short latency spike in r3m. ii. Stimulating r3m results in a short latency spike in r3s. iii. Subsequent penetration of FF muscle fibres in the region of presumed FI branches records an IJP (bottom trace) correlated 1:1 with the spike recorded in r3s (top trace).

Scale bars: horizontal - 10 ms i, ii; 20 ms iii.

Vertical - 5 mV b, iii.


a
u
G2
FI
r3s
r3m
G3
b
i
ii
iii

The anatomy of FI has been revealed selectively by injection of the fluroescent dye Lucifer Yellow (Fig. 15 a, b). The soma is always large (c. 100-110μ) and located at the extreme lateral edge of the ganglion at the origin of the first root. On some occasions FI soma projects into the first root and appears ovoid in shape. A large 25μ neurite arises from the soma and courses dorsally and laterally towards the midline of the ganglion. From this primary neurite another secondary neurite projects to the contralateral edge of the neuropile and arborizes. Where these two neurites meet in the middle of the ganglion a large 40μ axon courses caudally in the connective contralateral to soma and exits r3 whereupon it invariably branches down both r3m and r3s. FI dendrites form two major loci in line with each connective. Smaller branches arise both rostrally and caudally from the two major dendrites.

The remaining somata in the FMC cluster are smaller and are generally located more medially and anteriorly to MoGH and FI. They are presumed to be homologous with the two smallest FMC neurons in crayfish. Only one neuron other than FI occur in G4 of Galathea. In all other respects the FMC clusters in G1 to G3 of crayfish and Galathea are homologous in terms of their size, number and distribution. In G5 the FMC cluster contains only one soma. It is not clear whether this is a FF or a FI.

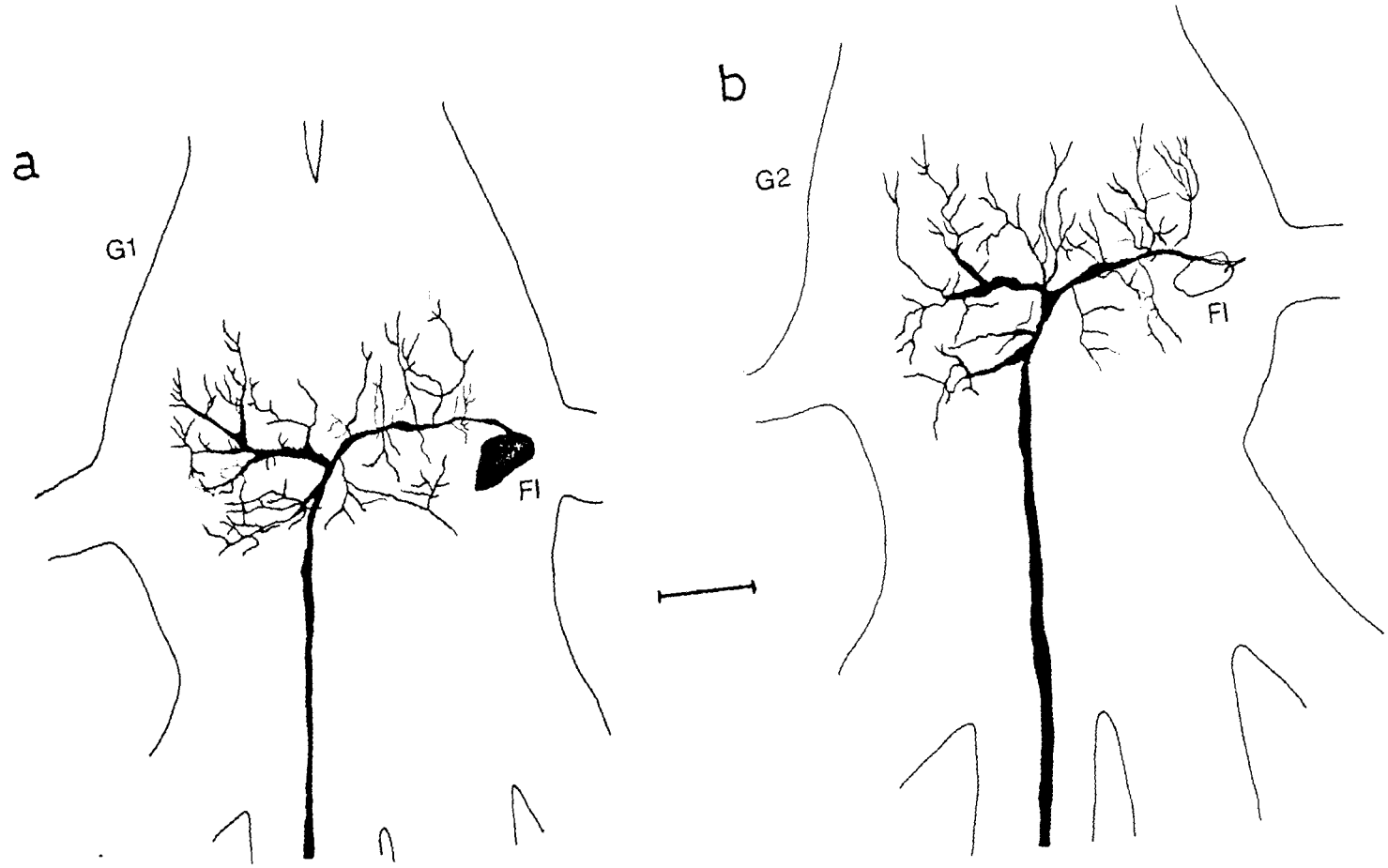
Posterior group

In crayfish the posterior group (FPI) consists of 4 somata in G1-G4 and 3 or 4 in G5 (G6 not studied). I find FPI cluster contains 4 somata in G1 to G4 and 6 in G5 (Fig. 10). G5 is the terminal ganglion in Galathea and since no study was made of the last 6th ganglion in crayfish, no comparisons can be made. In anterior ganglia the only difference between crayfish and Galathea FPI neurons is that Mittenthal and Wine (1978) normally found only 3 FPI neurons in the penultimate

FIG. 15 a, b.

The central anatomy of FI in G1 (a) and G2 (b) following injection of Lucifer Yellow. Note the extensive bilateral branching of FI.

Scale bar: 200µ.


a
G1
F1
b
G2
F1

5th ganglion. The penultimate 4th ganglion in Galathea consistently features 4 FPI neurons.

Processes of FPI neurons intermingle and the somata are closely clustered forming an approximate line which follows the lateral edge of the ganglion from the posterior edge of the 1st root (Fig. 6a) towards the second root. Often these neurons form two pairs with each pair having neurites which are closely apposed in the ascent from the soma to dorsal neuropile (Fig. 16a). In general it is difficult to identify the dendritic processes of individual FPI neurons from cobalt backfilled preparations.

Lucifer Yellow injection has revealed that FPI neurons have complex dendritic branching patterns located mainly ipsilateral to the soma and axon (Fig. 16 b, c). FPI somata are normally 70-80μ in diameter. A neurite courses medially and dorsally towards the midline of the ganglion. Before reaching the midline the neurite bifurcates into an axon which courses caudally in the ipsilateral connective and a neurite which projects anteriorly. Many fine processes arise from the neurites but few major branches ever cross the midline of the ganglion.

In G5, the FPI cluster contains six somata which form a rind around the lateral edge of the ganglion. It is not clear whether each of these neurons is phasic or whether some are tonic. The larger number of somata may be because, as in crayfish, the terminal abdominal ganglion is a fusion product of more than one ancestral ganglion. (J.J. Wine, pers. comm.).

FIG. 16 a - c.

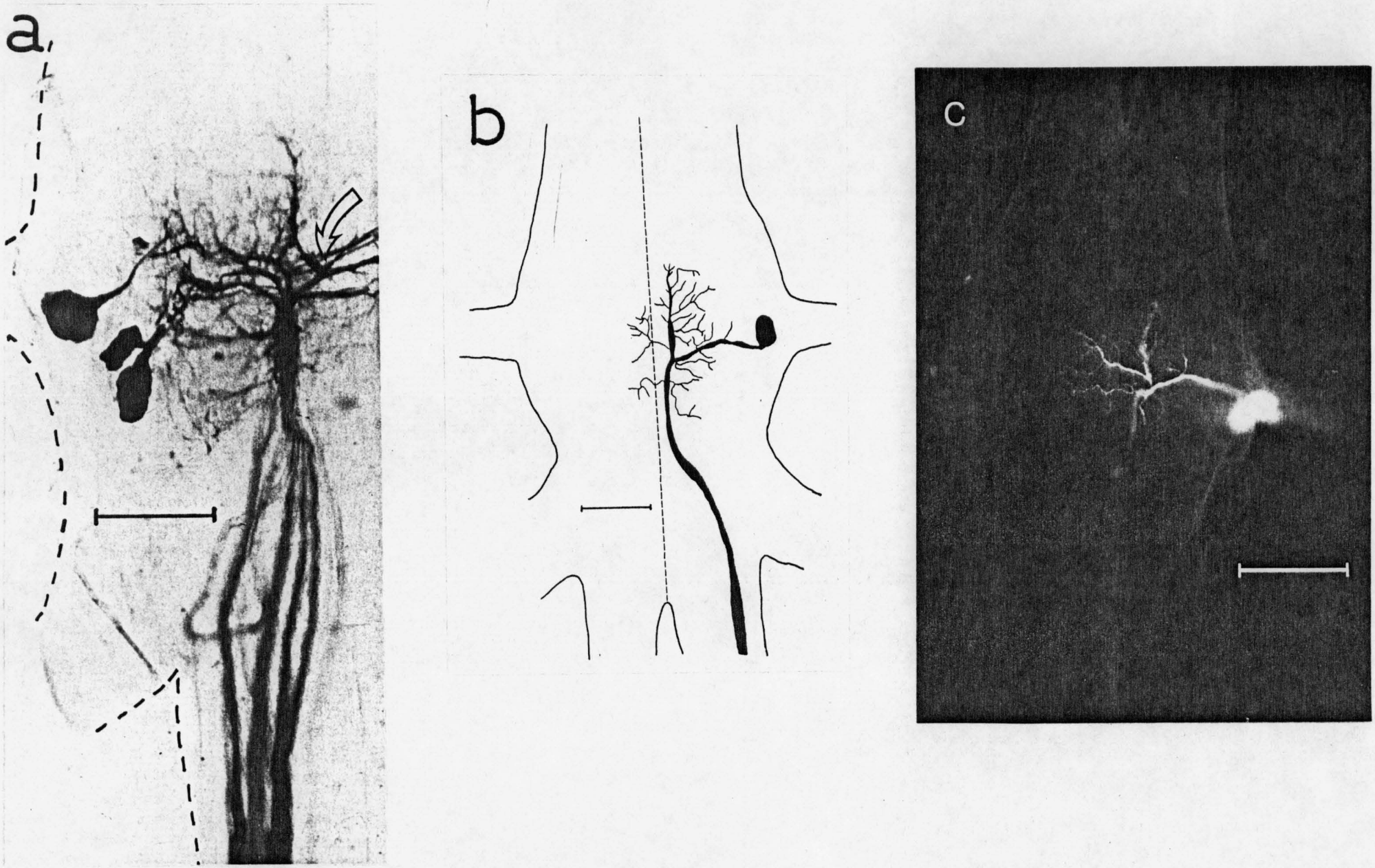
Dendritic branching pattern of FPI FF's in G2.

a. The density of FF processes in the neuropile prevents a characterization of the individual branching patterns of the four FPI neurons. Note that FPI neurites form two pairs. Open arrow indicates the FI neurite at its bifurcation in the neuropile.

b. Camera lucida drawing of a FPI neuron stained with Lucifer Yellow. Note that most neuropilar processes are ipsilateral and few cross the midline (dashed line).

c. A photograph of a Lucifer Yellow stained FPI neuron in G2.

Scale bars = 200μ.


a
b
c

C. THE ANATOMY OF AFFERENT AND EFFERENT NEURONS INVOLVED IN ABDOMINAL EXTENSION

Cobalt backfilling of r2 G2 stains 10 to 12 somata in G2 distributed among three clusters within the ganglion. Identification of these extensor efferents is based on homologies with neurons in crayfish (Treisman and Remler, 1975; Wine and Hagiwara, 1977) and lobster (Otsuka et al., 1967) abdominal ganglia, and is substantiated by physiological data presented in Chapter 3. Soma size is based on measurements from cleared wholemounts of cobalt stains and does not take into account distortion due to tissue processing.

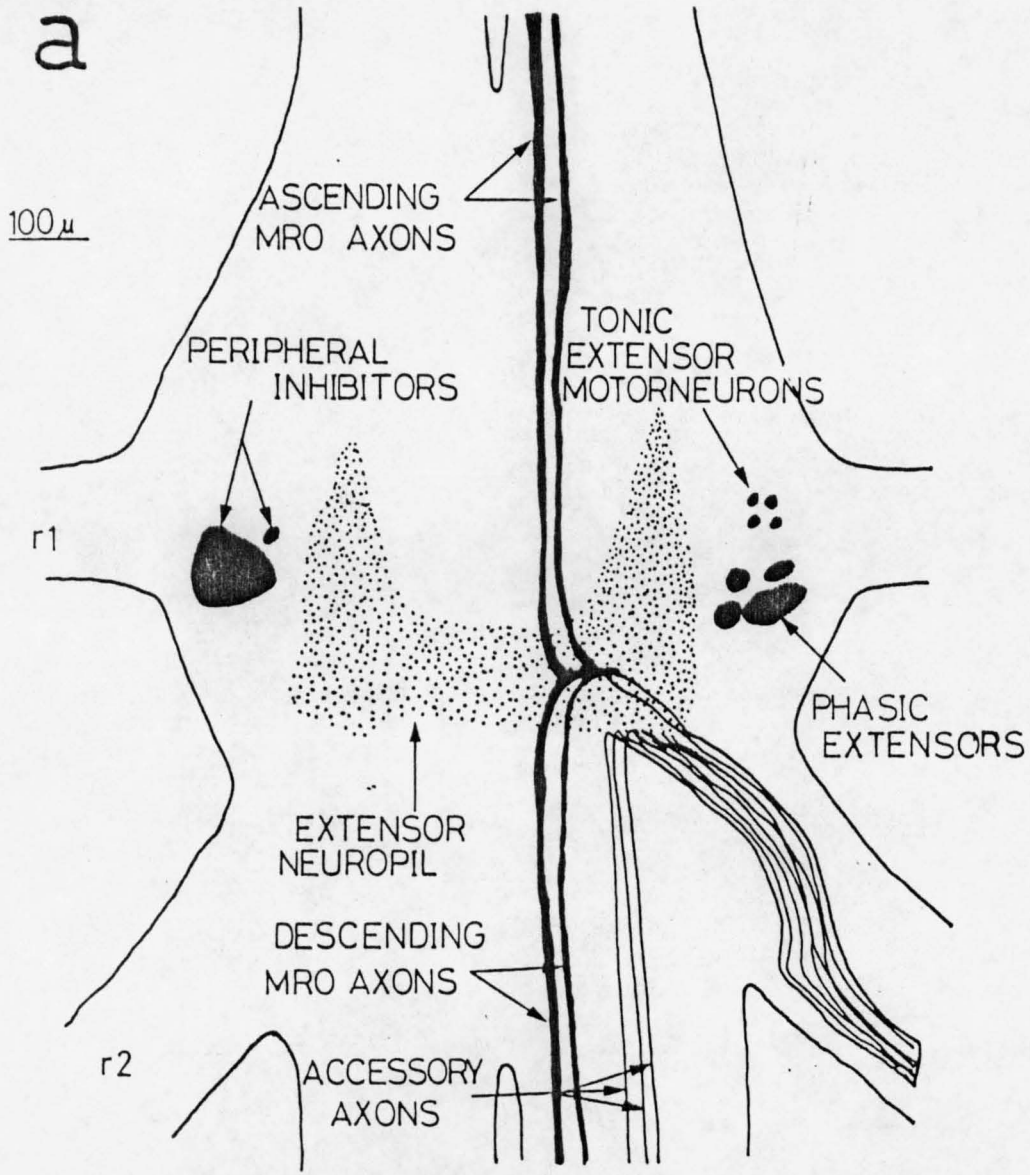
An overview of the extensor system in G2, compared with homologous somata in crayfish (after Wine and Hagiwara, 1977) is depicted in Fig. 17. Two clusters of somata are located ipsilateral and one contralateral to the filled root. The structure of the neurons comprising each group will be described in turn.

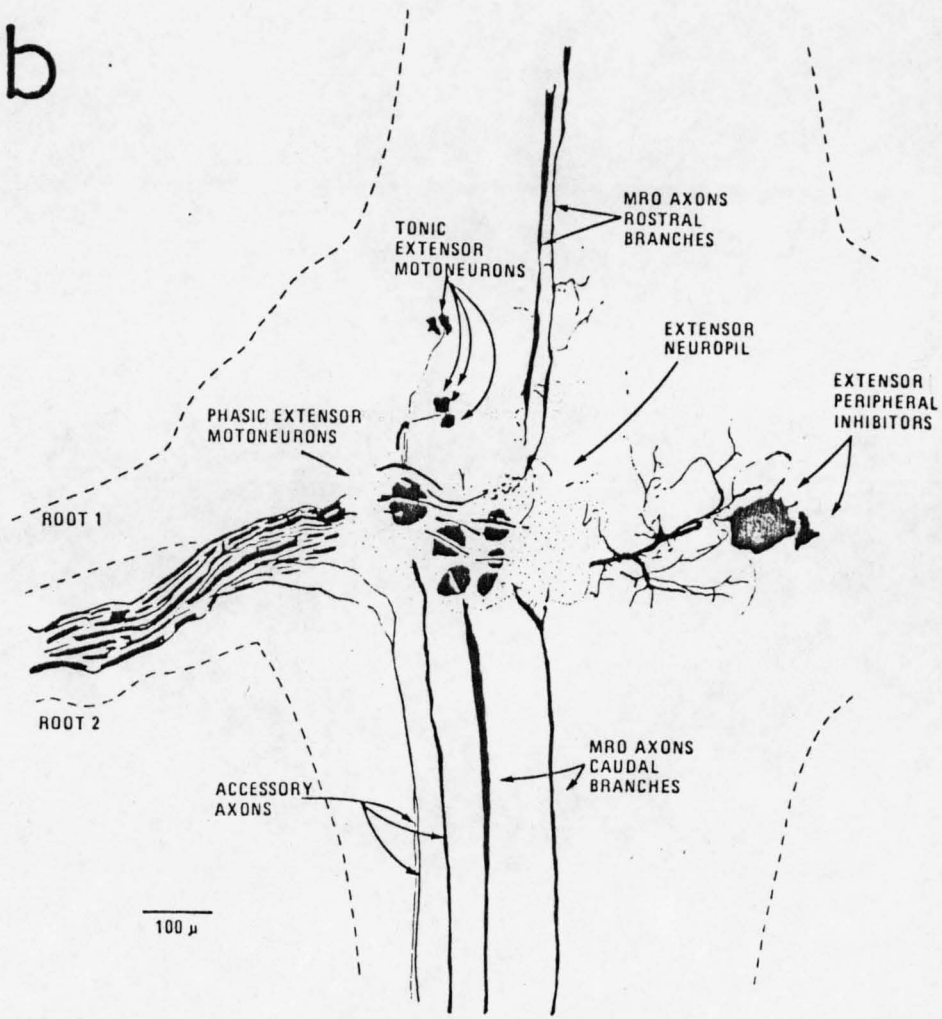
The somata of fast (phasic) extensor motorneurons (FE's) form a tight cluster located between r1 and r2 at the extreme lateral edge of the ganglion. In the majority of fills four somata are stained but on one occasion a fifth was observed. The diameter of FE somata shows considerable variability with a mean of 64μ (s.d. = 21.8μ; n = 33). Crayfish FE's have soma diameters of 50 to 100μ (Wine and Hagiwara, 1977).

A second cluster lies anteriorly at the approximate origin of the first root and comprises the somata of four or five slow (tonic) extensor

FIG. 17 a, b.

An overview of efferent neurons with axons in r2 G2 in Galathea (a) and A2 of crayfish (b) [reproduced from Wine and Hagiwara (1977)]. The two pools show almost strict homology except that there are only 4 FE's and 4 sE's in Galathea compared with 5 somata in each of the two crayfish pools. An additional difference is occurrence of a dense bilateral area of arborization in the neuropile of Galathea.


a
100 μ
ASCENDING
MRO AXONS
PERIPHERAL
INHIBITORS
TONIC
EXTENSOR
MOTORNEURONS
r1
PHASIC
EXTENSORS
EXTENSOR
NEUROPIL
DESCENDING
MRO AXONS
r2
ACCESSORY
AXONS


b
MRO AXONS
ROSTRAL
BRANCHES
TONIC
EXTENSOR
MOTONEURONS
EXTENSOR
NEUROPIL
EXTENSOR
PERIPHERAL
INHIBITORS
PHASIC EXTENSOR
MOTONEURONS
ROOT 1
ROOT 2
MRO AXONS
CAUDAL
BRANCHES
ACCESSORY
AXONS
100 μ

(mean sE diameter = 23.7μ (sd = 11.3μ; n = 32). In addition, in favourable preparations the neurites of sE's enter the neuropile along a tight tract which is distinct from the neuritic path of FE's.

In both the crayfish (Wine and Hagiwara, 1977) and the lobster (Otsuka et al., 1967) the soma of the peripheral inhibitor to the phasic extensor muscles (EI) is large (c. 110μ diameter in crayfish) and located contralateral to the filled root. Located contralateral to the second root in Galathea are two somata (Fig.16). The largest of these has a soma diameter of 97μ (s.d. = 12μ; n = 8) and is labelled the phasic extensor inhibitor (EI). The remaining cell in this cluster has a much smaller soma (mean = 28μ; s.d. = 7.7μ; n = 8). In view of its close proximity to EI and its similarity to a soma in the crayfish this soma is labelled the tonic extensor inhibitor (eI). In crayfish fills (Wine and Hagiwara, 1977) a third smaller contralateral soma (c. 15μ diameter) of unknown function is stained in approximately 25% of preparations. In contrast I have never observed more than two contralateral somata in r2G2 fills in Galathea.

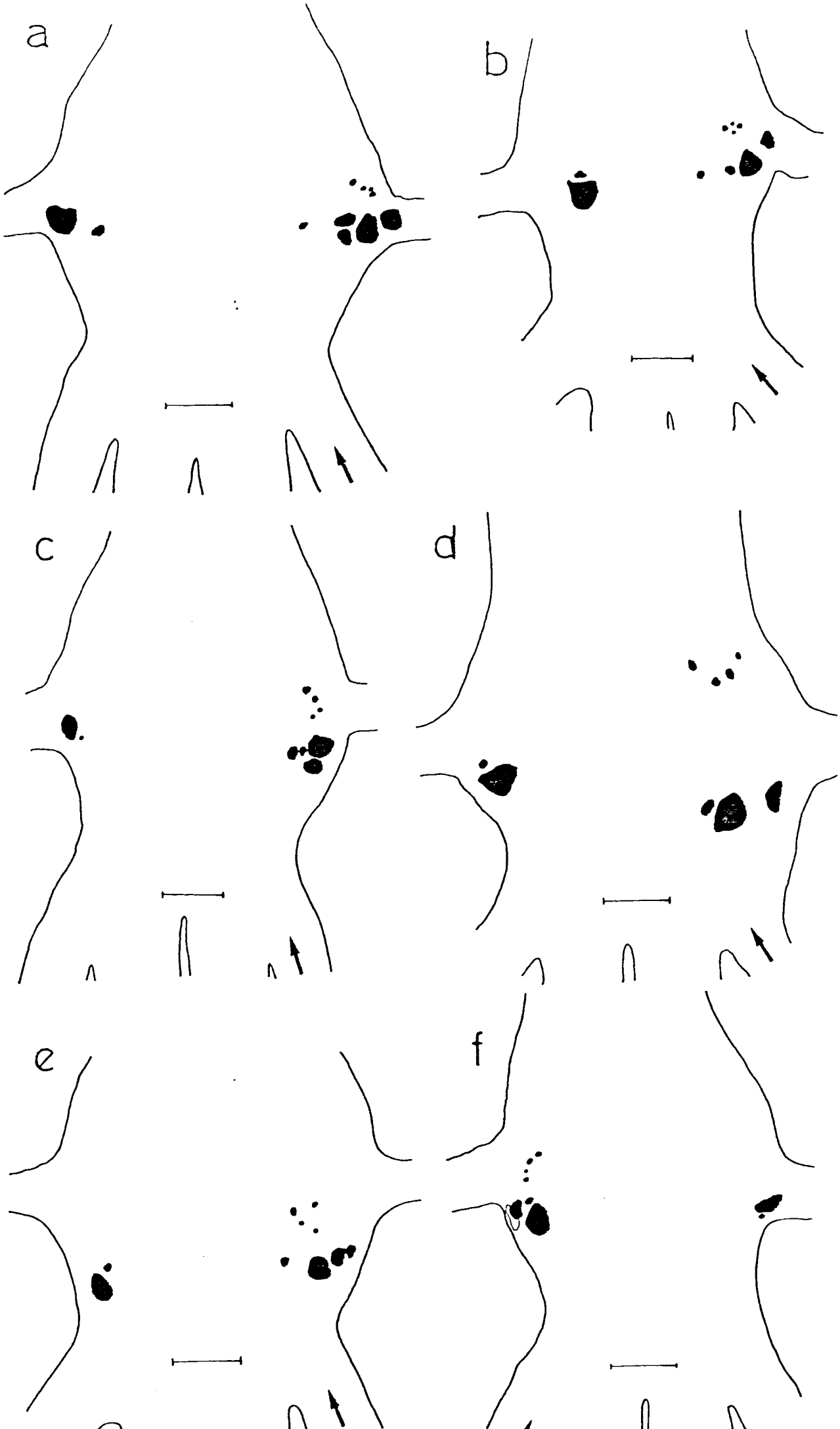
Interanimal homologies

Variations in the size number and distribution of efferent somata of G2 in different animals were small. Within the contralateral cluster, on one occasion eI was missing but in all other fills eI and EI were present. The two ipsilateral clusters always comprised 4 or 5 somata. Comparisons of soma positions for the three clusters is shown in Fig. 18 for six fills of r2 G2. The general features are consistent in each case. Occasionally the geographical separation of sE's and FE's was not clear but the two pools were easily distingished on the basis of soma diameter. The only consistent differences between these results and those of Wine and Hagiwara (1977) for the crayfish are the absence of

FIG. 18 a - f.

Interanimal homologies among extensor efferents. r2G2 was cobalt filled in six different animals. In each fill there are normally 4 FE's, 4 sE's and two contralateral inhibitors. However in (a) and (d) there were 5 and 3 FE's respectively. Note the gradation in soma size among FE's. Arrows indicate direction and root filled.

Scale bars = 200μ.


a
b
c
d
e
f

a third contralateral soma and a small reduction in the number of cells in the two ipsilateral clusters. In all essential respects the two pools are homologous.

Intersegmental homologies

Homologous extensor efferents have been stained in each abdominal ganglion, except the 5th (terminal ganglion, not studied), and in the fused last thoracic - first abdominal ganglion (Th.Ab.G). As little variation was found between ganglia as was found between animals. G1 to G4 show strict serial homology in terms of the size and distribution of efferents (Fig. 19). In G1 and G3 four tonic and four phasic efferents are normally stained as well as eI and EI. In G4 however there appear to be only three FE's, three sE's and the two contralateral inhibitors. This result is consistent with that of Wine and Hagiwara (1977) for the penultimate fifth abdominal ganglion of the crayfish. Th.Ab.G also departs slightly from strict serial homology. Only seven ipsilateral and two contralateral somata are stained. Of the ipsilateral cells, there are four FE's and three sE's (Fig. 20).

Extensor neuropile

The major dendritic domain of the extensor efferents forms a well defined U-shaped region of dorsal neuropile, with the densest accumulation of processes lying at the ipsilateral edge of the ganglion parallel with the connective. Some of the contralateral neuropilar arborizations can be attributed to EI. When EI is stained selectively with Lucifer Yellow it is seen to have two major dendritic loci which project anteriorly in line with each connective (Fig. 21a) as well as a number of smaller processes arising rostrally from the neurite. Few, if any, processes are directed caudally, presumably because the neurite of EI courses

FIG. 19 a - d.

Segmental homology and variation in extensor efferents of G1 (a), G2 (b) G3 (c) and G4 (d). G1, G2 and G3 show strict serial homology. In G4 there are only 3 FE's and 3 sE's, and no descending accessory neuron or MRO axons. Each soma map was constructed from whole mounts of cobalt fills to r2 (arrowed).

Scale bars: a, d - 200μ; b, c - 250μ.

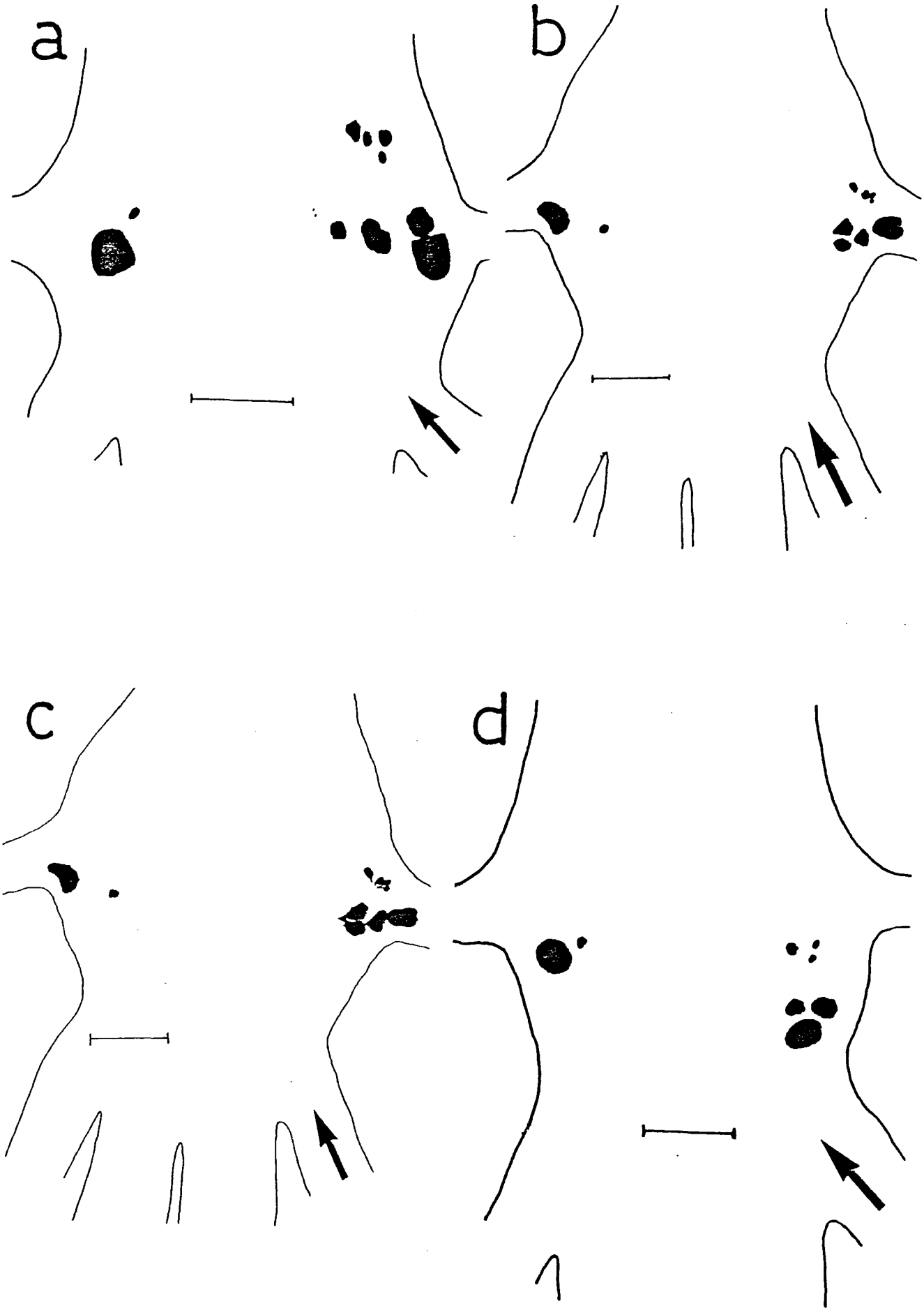

a
b
c
d

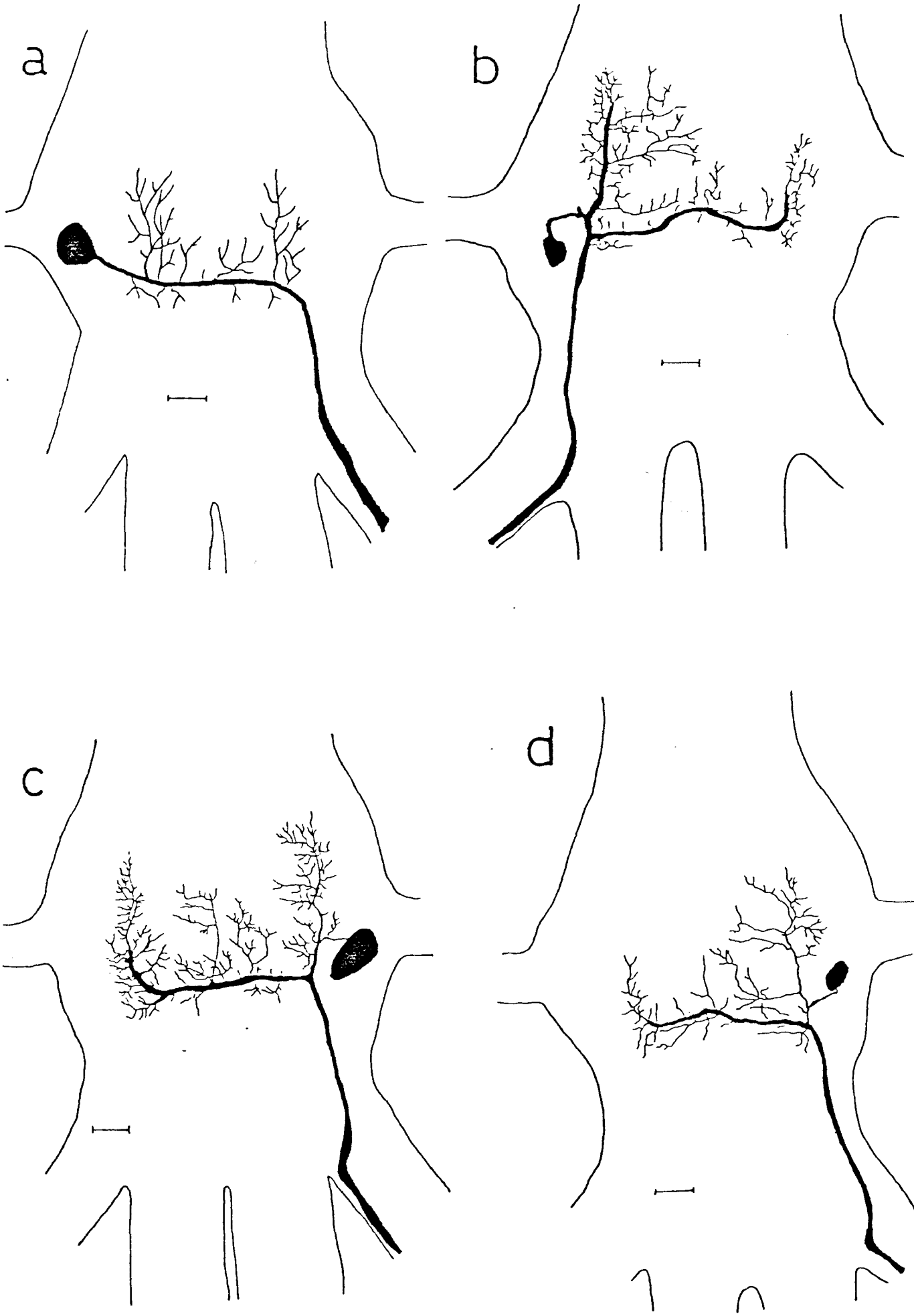
FIG. 20.

Anatomy of extensor efferents in the fused last thoracic - "first" abdominal ganglion following backfilling of the appropriate 2nd root (r2, arrowed). 7 ipsilateral and 2 contralateral somata are stained. Two bifurcating MRO axons are also present. The anterior branches of these could be traced anteriorly through the fused thoracic ganglia to the base of the suboesophageal ganglion where they faded, but apparently did not terminate. Two smaller axons descended the lateral edge of the connectives. These are presumed to belong to accessory neurons but their terminations in G1 were not revealed.
Scale bar = 250μ.


MRO
axons
EI
r2
A
P
MRO
axons

FIG. 21 a - d.

The dendritic domains of extensor efferents revealed by intrasomatic injection of Lucifer Yellow in G2. a, EI; b - d, FE's. Note the bilateral domain of neuropilar segments of each neuron. Among FE's, the densest accumulation of processes occurs ipsilateral to the soma. Scale bars = 100μ.


a
b
c
d

horizontally across the extreme lateral edge of the ganglion.

The morphology of FE's has also been examined by intrasomatic injection of Lucifer Yellow. FE's have complex dendritic trees with two major areas of projection, one ipsilateral and one contralateral to the axon. In each case the large ipsilateral soma is linked by a thin neurite to an axon of 20-40μ diameter. A second expanded neurite courses laterally across the ganglion from the anterior end of the axon to an area of often dense arborization. This feature of FE anatomy contrasts with observations on crayfish where only a few branches of the extensor excitors may cross the midline of the ganglion.

The anatomy of sE's was not examined with Lucifer Yellow.

Accessory neurons

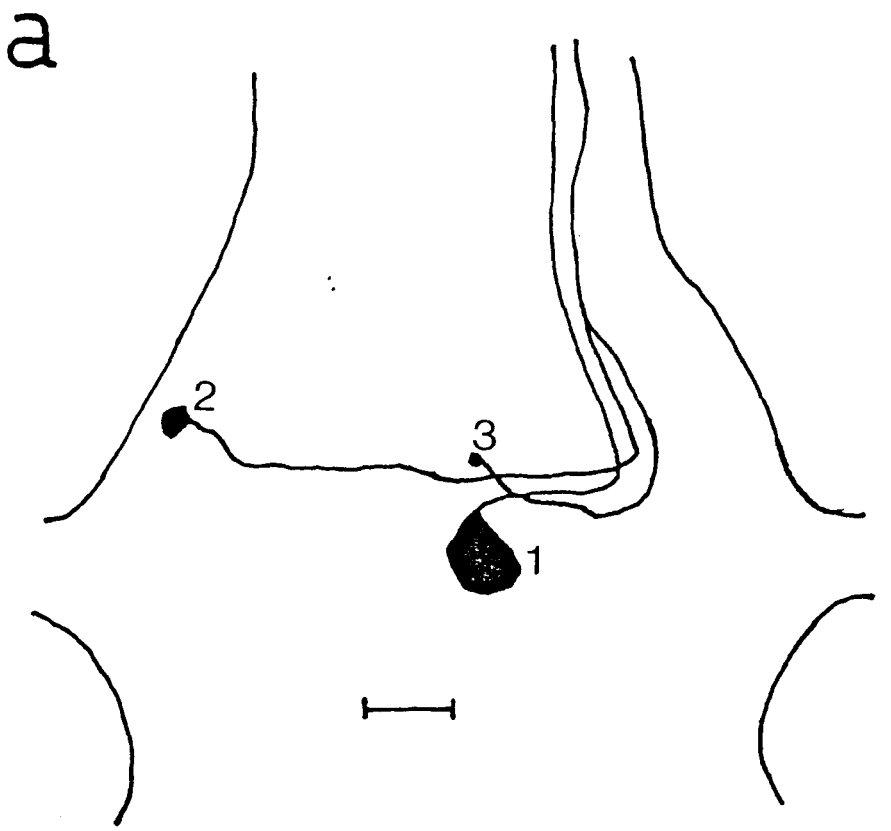
In the original description of the muscle receptor organs (MRO's) in the lobster, Alexandrowicz (1951) noted that the sensory neurons as well as the muscles of the MRO receive efferent innervation from what he called accessory nerves. The thick accessory nerve was shown to be an inhibitor (Kuffler and Eyzaguirre, 1955). Jansen et al. (1971) obtained physiological evidence for three separate inhibitory inputs to the crayfish MRO. Wine and Hagiwara (1977) presented evidence that each accessory neuron has a central cell body in the ganglion posterior to the root from which the axon exits.

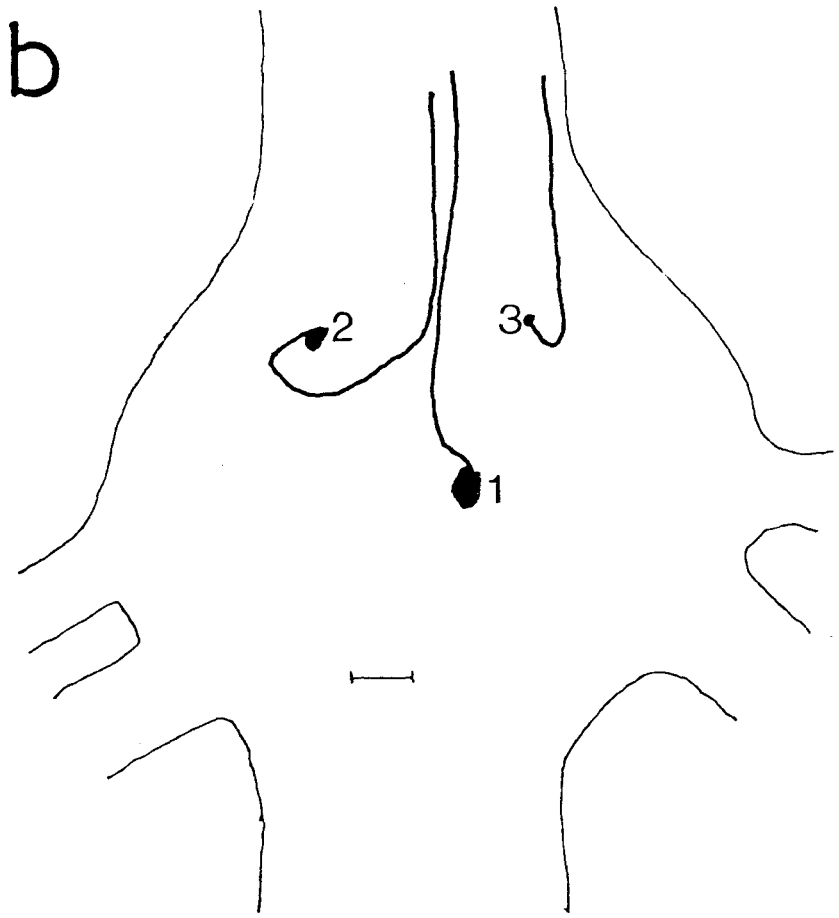
In addition to the three clusters of extensor efferents described above a fourth group comprising three somata is consistently stained in the next caudal ganglion, G3 (Fig. 22a). These have axons which run anteriorly in the connective ipsilateral to the root of exit. On the basis of strong homologies with somata in crayfish ganglia, I label these putative accessory neurons to the MROs. In crayfish this cluster

Fig. 22 a, b.

Soma locations of putative accessory neurons to the MRO's in the 3rd abdominal ganglion of Galathea (a) and crayfish (b), following cobalt backfilling of the anterior right 2nd root. b. re-drawn from Wine and Hagiwara (1977). Accessory neurons 1. 2 and 3 have similar soma diameters and relative positions in each species.

Scale bars = 100μ.


a
2
3
1


b
2
3
1

contains four somata but the strikingly similar anatomy of the remaining three leaves little doubt as to their homologous relationship (Fig. 22, b). I present no physiological evidence for the function of these neurons and rely heavily on their homologies with crayfish somata and the conclusions of Wine and Hagiwara (1977). In order of decreasing soma size I describe the central anatomy of each accessory neuron in G3 based on silver intensified preparations of cobalt fills to r2 G2.

Accessory 1.

Accessory 1 has a large (c. 100μ) soma located near the ventral midline ipsilateral to the axon (Fig. 22a). Wine and Hagiwara (1977) noted that a surprising feature of Acc. 1 was a prolific branching in G2 but relatively few dendrites in the ganglion of origin. In contrast, I have been unable to observe the detailed branching of Acc. 1 in G2 (though branching does occur) because of unselective staining and the density of other extensor elements in that ganglion. However Acc. 1 has a complex dendritic tree in G3. This density of arborizations was revealed only after silver intensification. Many fine branches arise from a single large posteriorly directed dendrite (Fig. 23). In addition several smaller processes arise independently, from the neurite. Some are closely apposed to the ipsilateral MRO axons as they course rostrally through the ganglion (Fig. 23). Medially directed branches of the major Acc. 1 dendrite rarely, if ever, cross the midline of the ganglion. Acc. 1 has a large (c. 35μ) axon which runs with other accessory axons rostrally in the ipsilateral mid-connective.

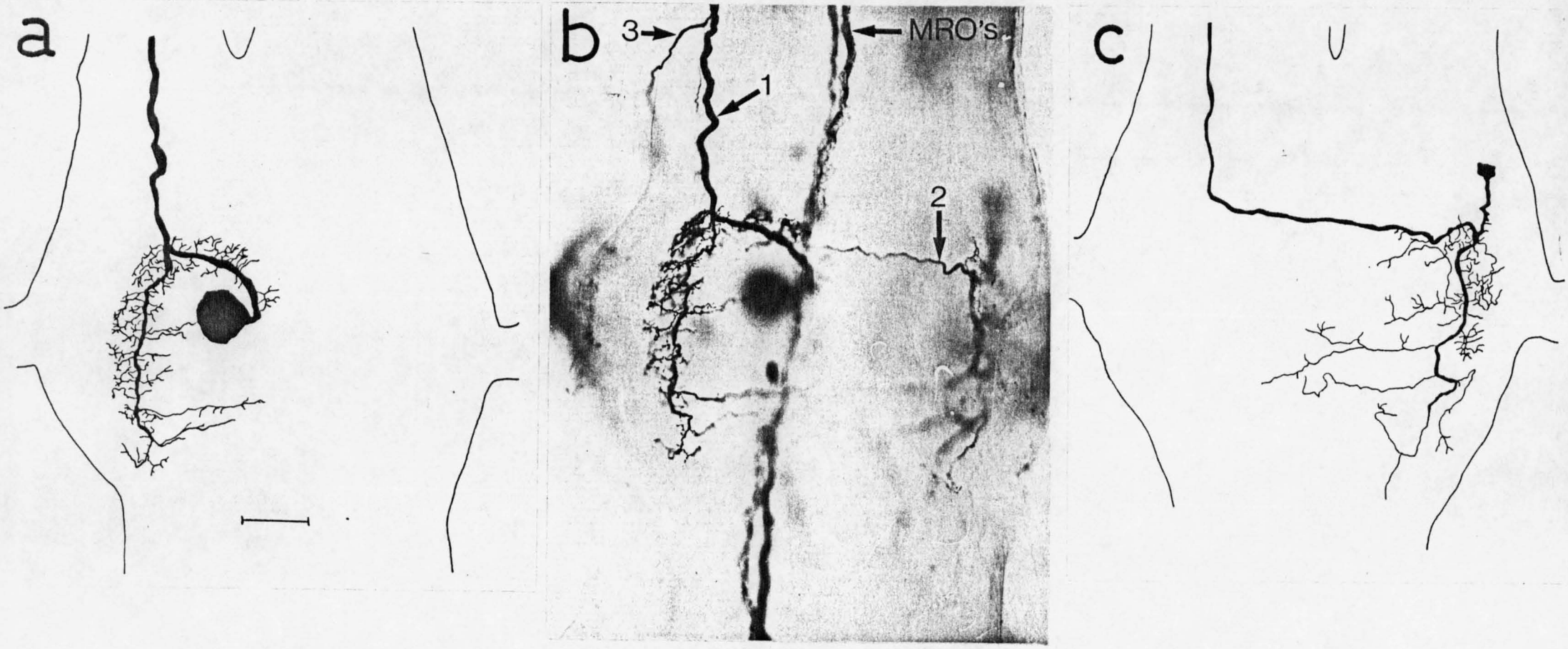
FIG. 23 a - c.

Central anatomy of putative accessory neurons to the MRO's.

a, c. Camera lucida drawings of Acc. 1 and Acc. 2 in G3 following silver intensification a cobalt fill to the left anterior r2 G2.

b. Photograph of the same stain showing the course of the descending MRO axons in relation to the dendrites of Acc 1.

Scale bar in a = 100μ and applies to b and c.


a
b
3
1
MRO's
2
c

Accessory 2.

Accessory 2 has a 30-50μ soma, located, contralateral to its axon, at the rostrolateral edge of G3 (Fig. 23c). Acc. 2 dendrites are complex, being located almost entirely contralateral to the axon with only a few processes crossing the midline of the ganglion. Unlike Acc. 1, no processes are found in close association with the ipsilateral MRO axon. However, in bilateral fills of r2, G2, the processes of Acc. 2 which extend towards the midline were seen to run close to and make contact with the MRO axon ipsilateral to the soma and contralateral to the axon. In contrast to Acc. 2 in crayfish (Wine and Hagiwara, 1977) no processes have been seen to arise from the neurite and ascend the connective ipsilateral to the soma.

Accessory 3.

The smallest of the accessory neurons, Acc. 3 has a 15-30μ soma located in the rostral ipsilateral quadrant of G3. The soma was most often found close to Acc. 1 soma but on one occasion it was seen at the extreme rostrolateral edge of the ganglion. Few branches arise from Acc. 3 in G3 and its thin (c. 3μ) axon ascends the ipsilateral connective close to Acc. 1 and Acc. 2 axons and exits r2 G2.

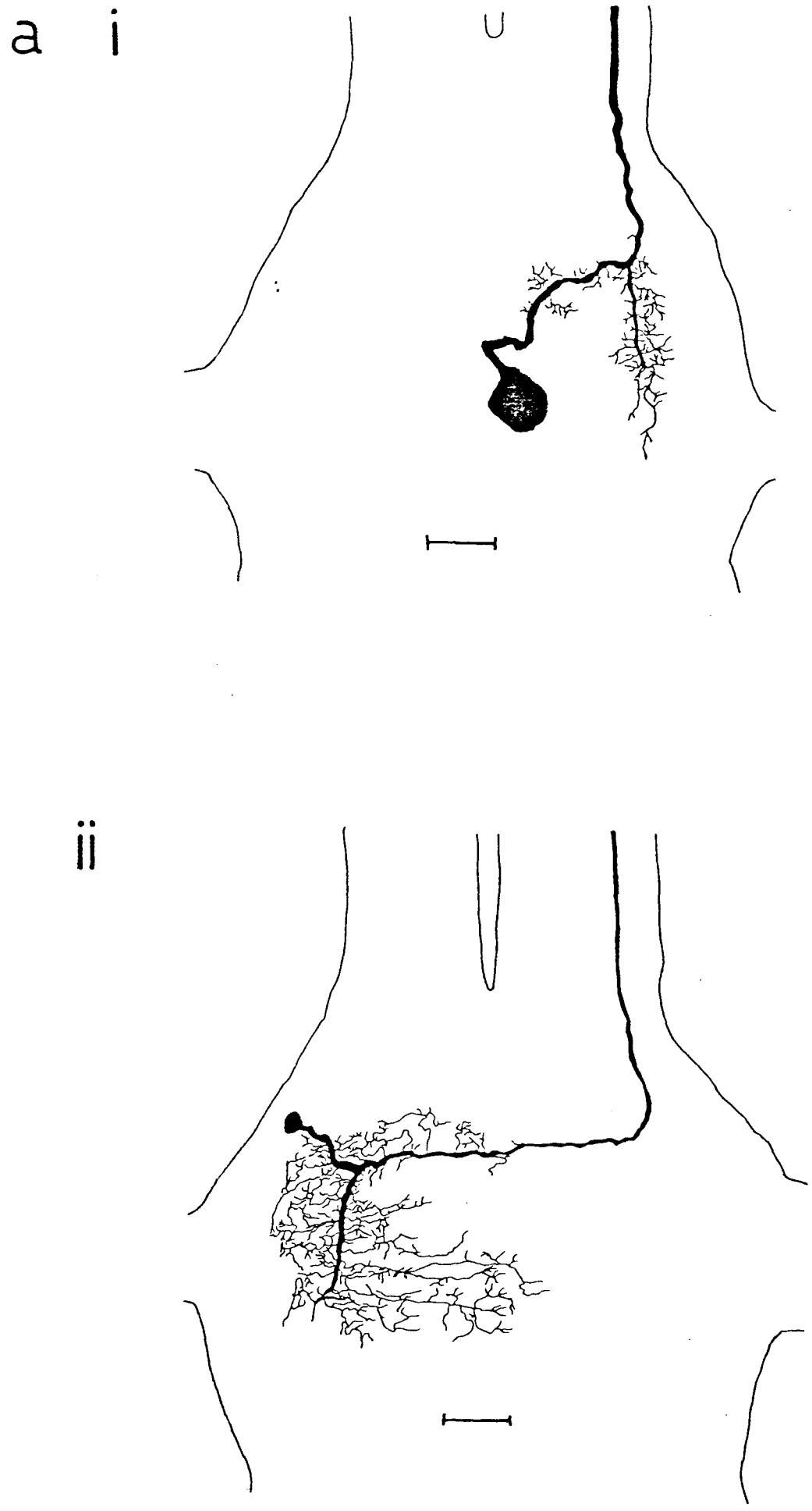
Intersegmental homologous accessory neurons

Homologous accessory neurons have been observed in the 2nd and 4th abdominal ganglia (Fig. 24).

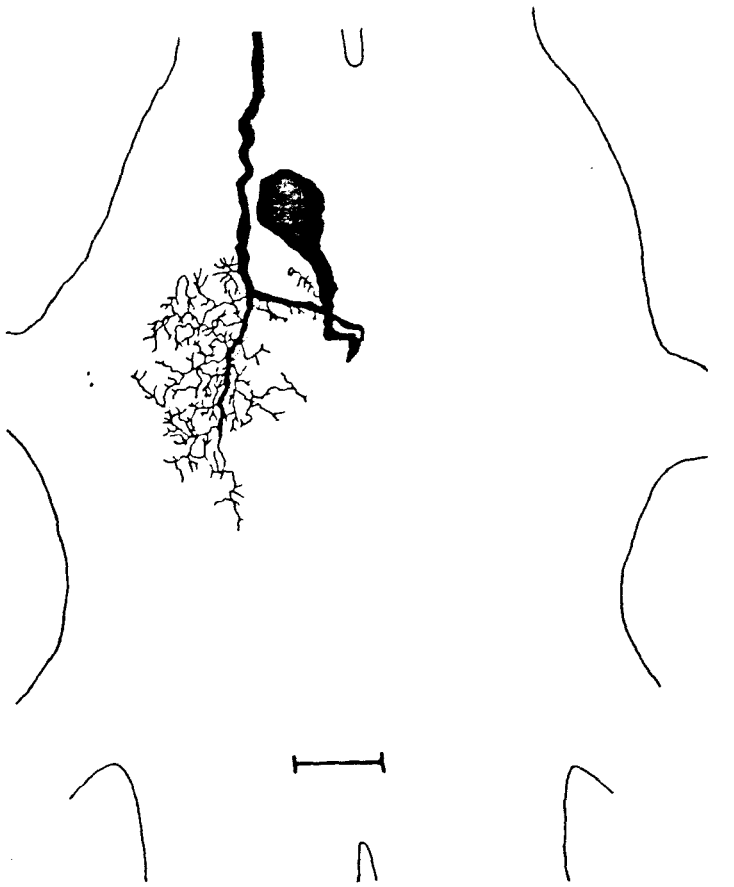
No descending accessory axons have been observed in fills of r2 G4. This may be due to pool filling: r2 G4 is thinner and more fragile than homologous roots in anterior ganglia. Shortly after leaving the

FIG. 24 a, b.

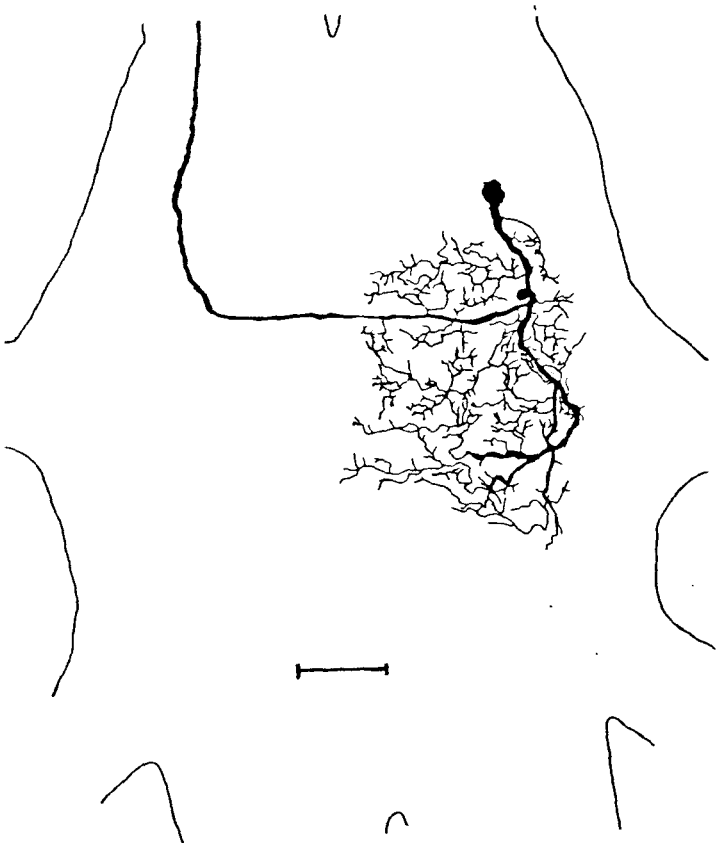
Homologous accessory neurons in G2 (a) and G4 (b) after cobalt backfilling r2 G1 (a) and r2 G3 (b). i, ii are camera lucida drawings of Acc 1 and Acc 2, respectively, from silver intensified wholemounts. In G2 and G4 the soma position and diameter, axon course and location of major dendrites are very similar. (Compare with Fig. 23 for homologous neurons in G3). Scale bars = 100μ.


a i
ii

b i



ii



ganglion r2 G4 embeds in a connective tissue network and is consequently more difficult to dissect without damage. However the successful filling of extensor motorneurons in G4 suggests that accessory neurons may be absent from the terminal 5th ganglion. In support of this view, there are no accessory neurons in the last ganglion of crayfish (Wine and Hagiwara, 1977).

Fills of r2 to Th.Ab.G normally show four posteriorly directed axons in the connective. Two large medial axons are those of the MROs. The remaining two are probably accessory neurons. Due to the large distance between Th.Ab.G and G1 (up to 2 cm) the termination of these axons have not been successfully stained even after silver intensification. However in view of the homologous relationship of these axons with axons in abdominal ganglia it seems very likely that they belong to accessory neurons with somata in G1.

Central projections of the MRO's

The axons of the paired MRO's in each hemisegment enter the CNS via abdominal 2nd roots into the ipsilateral extensor neuropile where they bifurcate and project rostrally and caudally in the ipsilateral connective (Figs. 17, 25). Fills of r2 G2 allow MRO axons to be traced rostrally through G1 and towards Th.Ab.G. Within G1 these axons sprout small knoblike arborizations (Fig. 25). Axons can normally be traced posteriorly through G3 and towards G4 but in general fade either within G4 or shortly thereafter. Fills to r1 G2 have allowed the terminations of both MROs to be visualized in G5. Each axon enters G5 along an identical path and occupies a very similar region of neuropile. The arborizations of the MROs in G5 have a beaded appearance after silver intensification. G5 is the only ganglion in which the terminations of the

FIG. 25 i - iii.

Central projections of the MRO axons in abdominal ganglia.

i. The axons of the paired MRO's enter G1, G2 and G3 via the 2nd root whereupon they bifurcate and project anteriorly and posteriorly in the ipsilateral connectives.

ii. In fills of r2 G2 the MRO axons can be traced through G1 where they sprout numerous small dendrites in the neuropile of the ganglion.

iii. In fills of r2 G3 the MRO axons could be traced through G4 and into G5. Both axons followed a similar path into the neuropile of the ganglion where they terminated in a diffuse network of arborizations.

i. is a sketch based on fills of r2 G1, G2 and G3.

ii and iii are camera lucida drawings from intensified wholemounts of r2 fills.

Scale = 200μ.

A
P
I
II
III
G1
G5

receptors have been observed. Fills of r2 Th.Ab.G stain ascending MRO axons which project to the base of the suboesophageal ganglion where they fade but do not terminate. The destination of these axons must either be the suboesophageal ganglion or brain.

This evidence suggests that the axons of the MRO's of each hemisegment may project the entire length of the CNS (up to 8 cm in large specimens) in addition to projecting up to 2 cm more to their peripheral destination.

DISCUSSION

Deviations from intersegmental and interspecific homology

Flexor system

G1, G2 and G3 in Galathea show strict serial homology in terms of the number size and distribution of FF motorneuron somata. G4 and G5 deviate from strict homology only to a small extent. In G4, the FMC cluster has only 3 somata (not 4) and there are no FAC FF's descending to G5. G5, the terminal ganglion shows the largest amount of variation with no FAC FF's, only one FMC soma (probably FI) and up to six FPI neurons (some of which may be slow flexor motorneurons).

With few exceptions this schema parallels the segmental variations among FF's in crayfish ganglia (Mittenthal and Wine, 1978) in which there is also a caudal decline in the number of FF's in each cluster. It has been hypothesized that in the crayfish system, intersegmental variations in the numbers of FF's parallels intersegmental differences in the volume of the muscles which these motorneurons innervate. Similarly in Galathea, the reduction in the number of FF somata roughly

matches an observable reduction in the mass of FF muscle in caudal segments. In all essential respects the FF motorneurons in crayfish and Galathea are homologous. The chief exception to this is the absence of a specialized MoG neuron (discussed below).

Extensor system

A small degree of intersegmental variation was also noted in the extensor motorneuron pools of abdominal ganglia. G1-G3 had identical numbers of somata with similar distributions. In G4 there was a small reduction in the number of somata in each cluster.

Wine and Hagiwara (1977) documented similar variations in the extensor system, though the absolute number of fast and slow extensor motorneurons was slightly larger in crayfish. This may reflect a reduction in total extensor muscle mass in Galathea compared with crayfish. A notable difference in the fine structure of FE's in the two species is the presence in Galathea of complex dendritic arborizations contralateral to the soma. Wine and Hagiwara (1977) found that FE's had mainly ipsilateral processes though, ". . . in more caudal ganglia some branches of the excitors may extend across the midline." It seems likely that this difference is not attributable to different staining techniques since crayfish FE's were also observed in cobalt stained and intensified wholemounts. The functional significance of prolific contralateral branching of FE's in Galathea is not clear but may reflect the existence of strong bilateral inputs to these neurons.

The accessory neurons to the MROs, where present, provide some of the strongest evidence for homology between crayfish and Galathea abdominal motorneurons. The smallest of the four crayfish accessory

neurons is absent in Galathea but the position and relative soma diameter of the remaining three is strikingly similar in the two species. Even the fine structure of these neurons is remarkably alike. In particular the dendritic domains of Acc. 1 and Acc. 2 are almost identical. The specific location of the major dendrites reflects the functional relationship of the two accessory neurons with ipsilateral and contralateral MRO axons in the crayfish. Similarly in Galathea, Acc. 1 dendrites are closely apposed to the ipsilateral MRO axon while Acc. 2 dendrites seem to contact contralateral MRO axons. This would appear to be an aesthetic example of structure relating to function, although the details of that function have not yet been elucidated in Galathea.

In sum, the abdominal extensor and flexor motorneurons in Galathea and crayfish are essentially homologous. The most significant difference between the two is the absence of a specialized MoG neuron in Galathea.

On the absence of giant fibres in Galathea and the evolution of the crayfish giant fibre system

The most significant findings to emerge from this study of the abdominal nervous system are the absence of giant axons in the connectives and the concomitant effects of this absence on serially repeated members of the FF motorneuron pool. Comparative studies have shown that many related species possess one or more pairs of giant axons which travel the length of the CNS and which mediate fast tail flexions via connections with specialized flexor motorneurons.

In the crayfish, the giant fibre circuit involves three key neural components: the giant fibres themselves; the MoG's; and the Segmental

Giants (SG's; Roberts et al., 1982). Each of these is believed to be derived from primitive elements in a non-giant escape circuit (Wine and Krasne, 1982). Thus it is thought that the giant fibres evolved from smaller premotor interneurons; the MoG's from smaller unspecialized FF's and the SG's from conventional limb motorneurons. By deduction therefore the species from which crayfish evolved must have lacked giant axons in the connectives and a pool of MoG's. The FF's must have consisted of a relatively homogeneous population of structurally similar motorneurons. Four features of escape in Galathea suggest that this species resembles the ancestral crayfish form: 1. Galathea and crayfish are relatively close, taxonomically; 2. Backwards swimming in Galathea and non-giant swimming in crayfish are very similar with extension preceding flexion; 3. There are no giant axons in the nerve cord of Galathea; 4. The serially repeated pools of motorneurons whose activity underlies escape, are homologous in the two species.

If Galathea does represent a primitive crayfish then, in theory at least, each of the three elements of the crayfish escape circuit will be present in Galathea in an unspecialized or reduced form. The premotor interneurons of the crayfish non-giant circuit are numerous, small and largely unidentified. The same is true in Galathea and hence it may be impossible to predict which one of these is a primitive giant fibre homologue. Similarly the SG probably evolved from an unspecialized limb motorneuron. The remaining 50 or so motorneurons in the hemisegmental pool innervating each swimmeret are very similar, anatomically. Thus the search for a primitive anatomical homologue of the SG in Galathea is also likely to be unsuccessful. The MoG neuron, in contrast, was recruited from a relatively small population of large, uniquely identifiable FF's in each hemi-ganglion. The anatomy of the MoG's differs from

other FF's in soma size, dendritic domain and extent of peripheral distribution. However the repeatable soma position in relation to other FF's has probably not changed as the MoG evolved. In terms of the number size and distribution of neuronal somata, the FF pools in Galathea and crayfish are homologous. Each FF in Galathea is qualitatively similar in its course of axon and dendritic domain in the ganglionic neuropile. Hence the soma homologous to the crayfish MoG in terms of its size and position in relation to other FF's has typical FF anatomy. The only differences in its morphology compared with other members of the pool are a slightly larger soma and axon diameter.

The major selection pressure on the evolution of the crayfish MoG must have been to provide a means of ensuring a rapid and complete tail flexion in response to a single impulse in the giant fibres. It would seem logical then to recruit the largest of the available pool of FF's (which is likely to have the widest distribution in the periphery) in order to minimize the amount of modifications to its structure. MoGH in Galathea happens to be the largest flexor excitor motorneuron but it is difficult to infer that this particular FF was selected purely for its larger soma diameter (and peripheral distribution). Other important factors such as axon course in relation to the giant fibres and functional connections with particular premotor elements may well have been important.

Trends in the evolution of giant fibre systems in crustacean escape

The absence of a specilized MoG in Galathea supports the idea that in crayfish this neuron evolved from a typical unspecialized FF. Recent evidence from work on the hermit crab giant fibre system provides further support for this notion (Umbach and Lang, 1981). In this Anomuran there occurs a single pair of GI's in abdominal connectives which are homologues of the crayfish MG's. The LG's may have been lost as hermit crabs took up life in empty Gastropod mollusc shells. Each GI makes direct electrical connections with a pool of GM's in abdominal ganglia. The GM, a homologue of the MoG, possesses most of the anatomical specializations of the crayfish neuron. However the axonal dendrites of the GM are located at the extreme posterior edge of the ganglionic neuropile and not in the mid-connectives (Chapple, pers. comm.).

The hermit crab would appear to fit conveniently between the crayfish and Galathea in terms of the anatomy of its escape circuitry and the structure of the GM is certainly intermediate between the MoG of crayfish and the MoGH of Galathea. However it is difficult to assess the precise evolutionary relationship between the three species. The homologies that exist between the motorneuron pools suggest that crayfish, hermit crabs and Galathea derive from a common ancestor and that the differences in their escape circuits have resulted from different selection pressures in their respective habitats. The giant fibres of crayfish are a late development (Wine and Krasne, 1982) and presumably the same applies to hermit crabs. If this is the case then Galathea must represent some form of decapod ancestral to both hermit crabs and crayfish, and non-giant swimming may be the primitive escape behaviour. Why did crayfish and hermit crabs evolve giant fibres? In crayfish, the giant fibres may

have arisen at the same time as a gradual extension of the abdomen. If Galathea represents the crayfish prior to the evolution of the giant fibres then the abdomen may also have been tonically flexed. Abdominal extension must have been important in the development of an efficient forward walking behaviour which would undoubtedly have allowed crayfish to extend their range. As crayfish ventured onto the sea bed from the relative safety of rocks and stones an efficient and rapid bidirectional escape system may have been crucial to their survival.

The MG-MoG (GI-GM) circuitry in hermit crabs is used effectively in escape withdrawals into their shells. LG-MoG circuitry is clearly redundant in this species. The necessity to change shells as the animal grows must have been an important selection pressure on the evolution of giant fibres in hermit crabs.

If it is true that hermit crabs and crayfish evolved from a Galathea-like ancestor then the important question arises of which is more advanced - crayfish or hermit crabs. Several possiblities exist and it is impossible to decide, on the existing data, which is correct. For example, hermit crabs may first have evolved two sets of giant fibres, like the crayfish and then secondarily lost the LG circuit when taking up life in empty mollusc shells. Alternatively they may never have evolved a LG circuit but might have gone directly from a non-giant escape system to a MG-MoG system. By a similar argument crayfish forms might have evolved via the hermit crab. One could envisage a Galathea-like ancestor venturing from safety beneath a rock in a mollusc shell, developing a MG-MoG for rapid withdrawal and eventually further extending its range by leaving the shell completely and evolving a LG-circuit for bidirectional escape.

The hypotheses presented above are based on evidence from only three inter-related Decapods. The results of Silvey and Wilson (1979) are contradictory. The primitive syncarid crustacean Anaspides tasmaniae belongs to a family which arose more than 200 million years ago in the late Palaeozoic, about 100 million years earlier than the now dominant advanced eucarid crustaceans, the Decapoda (cf. Brooks, 1962a; Glaessner, 1969). This animal possesses two pairs of giant axons which are thought to be homologues of the crayfish LG and MG axons and which mediate rapid tail flexions during escape. There are close morphological and physiological similarities between the LG's in crayfish and Anaspides, suggesting that the LG system arose in the common ancestor to syncarids and eucarids, prior to the Carboniferous. However this suggestion conflicts with the opinion of Wine and Krasne (1982) that the giant fibres are a later acquisition in the crayfish escape circuit. The LG systems in Anaspides and crayfish are not identical (for example the LG's in Anaspides are not electrically coupled) and while these differences could be explained in terms of adaptation to particular niches, they might also be explained in terms of convergent evolution and not homology.

The above discussion is based on morphological evidence from only a few decapod escape systems. Clearly further comparative evidence from related species will aid our understanding of how natural selection has adapted neural networks to suit particular functional roles. Developmental evidence may also be important in this regard. It is interesting for example, that the early larval stages of hermit crabs, crayfish and squat lobsters all have symmetrical abdomens which are normally extended. Investigations into the morphology of the larval nervous system may provide clues as to the precise taxonomic relationship of Anomuran and Macruran species.

C H A P T E R 2.

ANATOMY AND PHYSIOLOGY OF THE MRO's

CHAPTER 2.

INTRODUCTION

Muscle receptor organs (MRO's) are the paired stretch-sensitive proprioceptors spanning each hemisegment of the jointed decapod abdomen. Originally described by Alexandrowicz (1951) in the lobster, Homarus, they have since been observed in most other decapods (Pilgrim, 1960) and in the Stomatopoda (Alexandrowicz, 1954). Comparable structures are present in the insect abdomen (Finlayson and Lowenstein, 1958) and in vertebrates (muscle spindles; Merton, 1972). Within the Anomura, homologous receptor organs have been documented in a number of species including Munida quadrispina, Benedict (Galatheidae; Pilgrim, 1960), Callianasa gigas, Dana (Thalassinidae; Pilgrim, 1960) and Eupagurus pridauxi, Leach (Paguridae; Alexandrowicz, 1952a). Although MRO's have not been described previously in Galathea strigosa, they are known to be present (M.S. Laverack, personal communication). MRO's also occur in thoracic segments (Alexandrowicz, 1952b, 1956) with interesting variations in morphology. In the stomatopod, Squilla mantis, the fourth thoracic segment has a single receptor muscle in which are embedded two sensory neurons.

The crayfish MRO's are among the most extensively studied sensory receptor systems. Each MRO comprises a modified receptor muscle (RM) strand lying in parallel with the extensor muscles and longitudinal body axis on the dorsal surface of the abdomen. Embedded in each receptor muscle are the dendrites of a sensory neuron (SN) which has a peripheral soma and an efferent axon arising from a branch of the second root. The receptor muscles are stretched by abdominal flexion and deformation of the sensory dendrites gives rise to a depolarizing receptor potential

and spiking in each MRO (Eyzaguirre and Kuffler, 1955). One MRO, consisting of receptor muscle 1 (RM1) and sensory neuron 1 (SN1) has a low threshold for spike initiation and fires tonically to maintained stretch, adapting slowly. The other (RM2; SN2) fires phasically only near maximal flexion (Wiersma et al., 1953).

The activity of the MRO's is modulated by the CNS in two ways. Firstly, the phasic and tonic receptor muscles share innervation with the phasic and tonic extensor muscles, respectively (Fields and Kennedy, 1965). Thus, extensor motorneuron activity contracts the extensor muscles and extends the abdomen, so reducing tension on the receptor muscles. The same activity also contracts the receptor muscles themselves so maintaining tension and receptor sensitivity. Secondly, the receptor muscles and sensory neurons receive efferent inhibitory innervation from a pool of accessory neurons (Kuffler and Eyzaguirre, 1955). The role of the accessory neurons in escape behaviour is obscure although some reflex pathways have been found which activate them (Eckert, 1961). Thus the MRO's are immensely complex structures and their detailed functional role in behaviour is not completely understood. Hirosawa et al. (1981) in freeze fracture and EM studies have identified at least six different types of synaptic structure in the MRO system of the crayfish, further emphasizing their complexity.

The axons of the sensory neurons enter the CNS via abdominal 2nd roots. Here they bifurcate and project a large distance both anteriorly and posteriorly in the nerve cord. It has been demonstrated physiologically that they travel the entire length of the CNS (Hughes and Wiersma, 1960). Anatomical evidence has shown that the rostral branches of the MRO's give off numerous fine branches in their ganglion of origin (Wine

and Hagiwara, 1977). Here the MRO's make a number of functional connections which provide a basis for their segmental reflex functioning. Both the tonic and phasic MRO's produce unitary EPSP's in phasic extensor motorneurons and appear to be the exclusive source of monosynaptic sensory input to these cells (Wine, 1977c). The MRO's also converge on the tonic extensory system (Barker et al., 1972). Moreover, MRO input is distributed directly to anterior and posterior ganglia although EPSP amplitude decreases in distant ganglia (Wine, 1977c). In addition, FI receives large EPSP's from both MRO's which can summate with central excitation to fire FI. Interestingly RM1 input to FI is larger than RM2 input. Such highly specific, direct connections with phasic motorneurons strongly suggest that the MRO's may play a crucial role in moulding the centrally generated motor programme underlying escape swimming behaviour. That the MRO axons travel the length of the CNS suggests that they may interact in some way with higher centres.

In the preceding chapter anatomical evidence was presented which indicates that the extensor and flexor motorneuron pools, the accessory neurons and the MRO axons are essentially homologous in crayfish and Galathea. One implication of this finding is that the circuits in which these neurons are involved may also be homologous. However the abdomen of Galathea is significantly different to that of the crayfish, being normally flexed beneath the cephalothorax in the stationary animal. Hence escape begins with extension. In view of the important connections made by the MRO's with phasic swim motorneurons in crayfish, it was considered important to investigate homologous structures in Galathea. It was possible that either the central connectivity of the MRO's or the geometry of the receptor muscles themselves might differ from the crayfish and provide a basis for extension and not flexion as the initial stage of escape.

MATERIALS AND METHODS

Anatomical

The MRO's were revealed by a similar dissection technique to that used in Cambarus (Wiersma et al., 1953). The anatomy of the MRO's was investigated with Methylene Blue staining and cobalt backfilling of second roots (see Chapter 1, Methods). With the latter technique receptor anatomy was only revealed after silver intensification had been applied.

Physiological

Isolated preparation

To analyse the basic sensory responses of the MRO's a similar dissection to that above was used. Silver-wire hook electrodes placed beneath the appropriate 2nd root monitored sensory activity in the receptors. The receptor muscles are easily visible in these preparations. A bent micropin attached to a servomex electromechanical transducer was placed beneath the posterior ends of the receptor muscles. In this way the receptors could be stretched repeatedly in a controlled manner.

Semi-isolated preparation

The MRO's were left attached via 2nd roots to the intact chain of abdominal ganglia. The dissection was as follows. The isolated abdomen was secured in an experimental chamber filled with cooled aerated saline and the ventral abdominal cuticle removed. All nerve roots were cut except the 2nd roots to the anterior 3 ganglia on one side. The chain of ganglia was then placed to one side and the attached 2nd roots dissected back to the lateral edge of the animal, past the major flexor muscle bundles. The flexors and underlying gut were removed and the

dorsal carapace bisected along its midline and one side discarded. The lateral spines were cut and the abdominal nerve chain secured dorsal side up on a sylgard platform. The MRO's were revealed by the sequential removal of the extensor muscles and stretched as described above. MRO activity was monitored with hook electrodes placed on 2nd roots. The ganglionic sheath was removed to facilitate microelectrode penetration of neuropilar segments. In some experiments the chain of ganglia was twisted through 180° to allow penetration of neuronal somata. In most cases 3rd root activity was monitored with polythene tipped suction electrodes. Penetrated neurons in the CNS were routinely stained with the fluorescent dye, Lucifer Yellow.

RESULTS

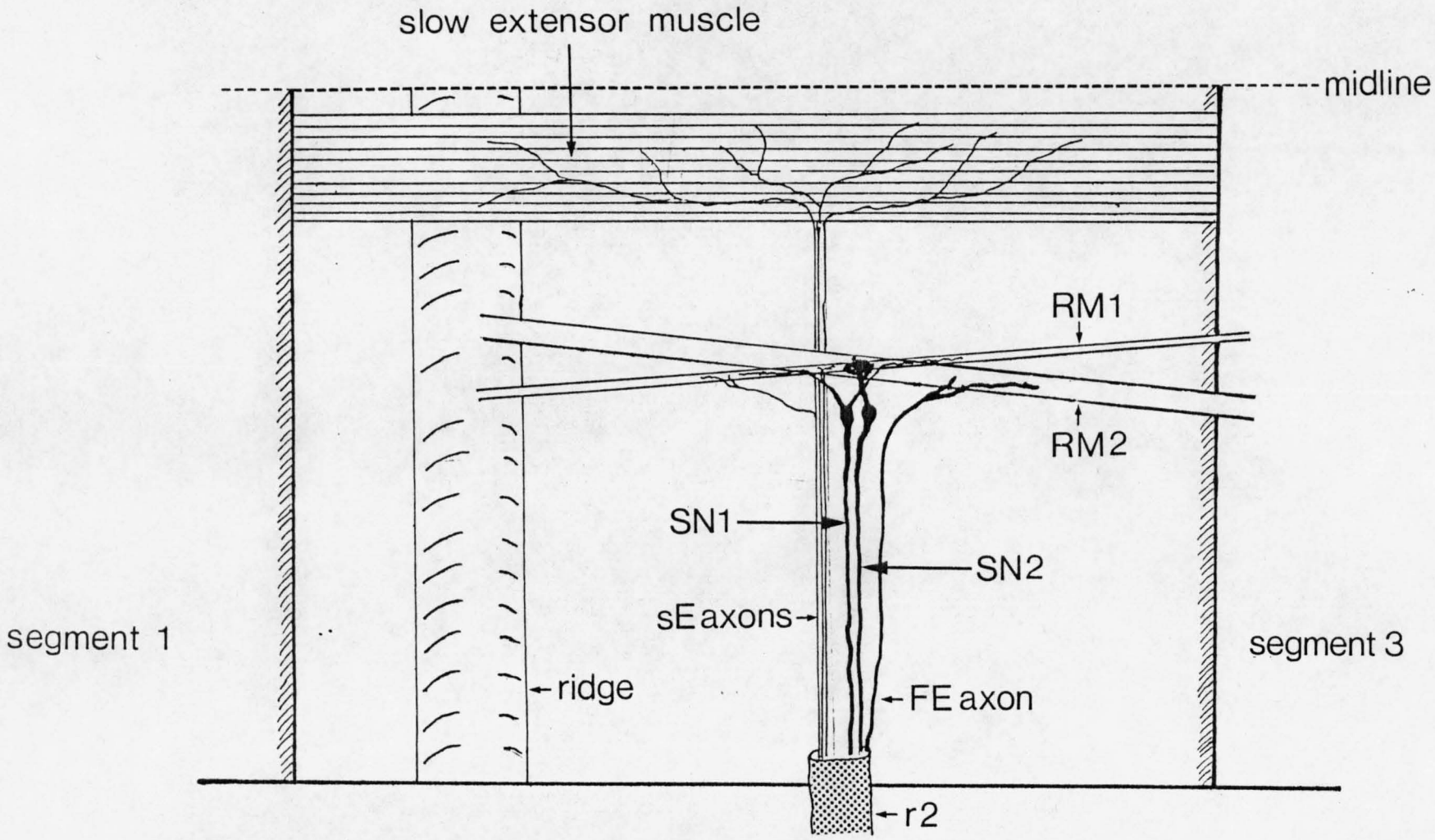
A. Anatomy

Paired MRO's are present in each of the anterior four abdominal segments, but not in the 5th. Consistent with this observation is the finding that there are no central MRO axons or descending accessory neuron axons in the 4th ganglion (see Chapter 1). The 2nd root of G4 innervates abdominal segment 5. Homologous MRO's have not been investigated in thoracic segments.

The location of each MRO is illustrated in schematic form in Fig. 26 for segment 2. This schema is similar for each of 8 pairs of MRO's in the anterior four abdominal segments. The two receptor muscles, RM1 and RM2 lie approximately in parallel with the main longitudinal body axis on the dorsal surface of the abdomen. Approximately half-way along their length the two muscles cross over so that the thick RM2

FIG. 26.

A schematic representation of the location of the MRO's in the 2nd abdominal segment, based on Methylene Blue stains. Two receptor muscles (RM1, RM2) lie in parallel with the main longitudinal body axis. Two sensory neurons, SN1 and SN2 embed in RM1 and RM2, respectively. These have peripherally located somata and axons which enter the CNS via r2. RM1 and RM2 are also innervated by a number of FE's and sE's. RM1 and RM2 insert anteriorly on a skeletal ridge and posteriorly on the edge of segment 3. Not to scale.


slow extensor muscle
midline
RM1
RM2
SN1
SN2
sE axons
segment 1
segment 3
ridge
FE axon
r2

ends medial to RM1 at its anterior insertion and lateral to RM1, at its posterior insertion. Posteriorly the two receptor muscles insert on the anterior edge of segment 3. Hence it is possible to slide segment 2 beneath segment 3 and reduce tension on both receptor muscles. At the anterior end each muscle inserts on a skeletal ridge or swelling on the internal dorsal surface of segment 2. Manual flexion of a strip of dorsal abdominal cuticle causes segments 2 and 3 to partially separate, thus increasing tension on the receptor muscles. This anatomical relationship of RM1 and RM2 with the dorsal carapace is similar to that found in crayfish and implies that the receptors fire when the abdomen is flexed. At the point where they cross over the two receptor muscles are partially fused. In this region two sensory receptor neurons, SN1 and SN2 embed in RM1 and RM2 respectively (Figs. 26, 27).

The anatomy of SN1 and SN2 has been investigated by cobalt backfilling r2G2. However, only after silver intensification was their anatomy revealed (Fig. 27). Each sensory neuron has a peripheral soma (c. 35-40μ diameter) and an axon which runs laterally with a number of motor axons in a branch of the 2nd root. Projecting medially from the two sensory somata are complex dendrites which ramify in the central region of the appropriate receptor muscles. The fine structure of the dendrites of the two sensory neurons differs in two main respects. First, the dendrites of SN1 which embed in RM1 form two major branches which arise from the soma. Hence, unlike crayfish, SN1 is a tripolar neuron. In contrast SN2 is bipolar with a single large dendritic arborization arising from its soma and embedding in RM2. A second difference is that the dendrites of SN1 terminate over a wide area of RM1 while SN2 dendrites embed in a restricted central portion of RM2 and are dense and compacted by comparison (Fig. 27). It is possible that this clear

FIG. 27 a, b.

The peripheral anatomy of SN1 and SN2 dendrites at their insertion into RM1 and RM2, respectively.

a. From the large peripheral somata (arrowed) arise SN1 and SN2 dendrites which embed in their respective receptor muscle strands. SN1 has two major dendritic loci, while SN2 has only one. The open arrow points to the axons of FE's which embed in RM2.

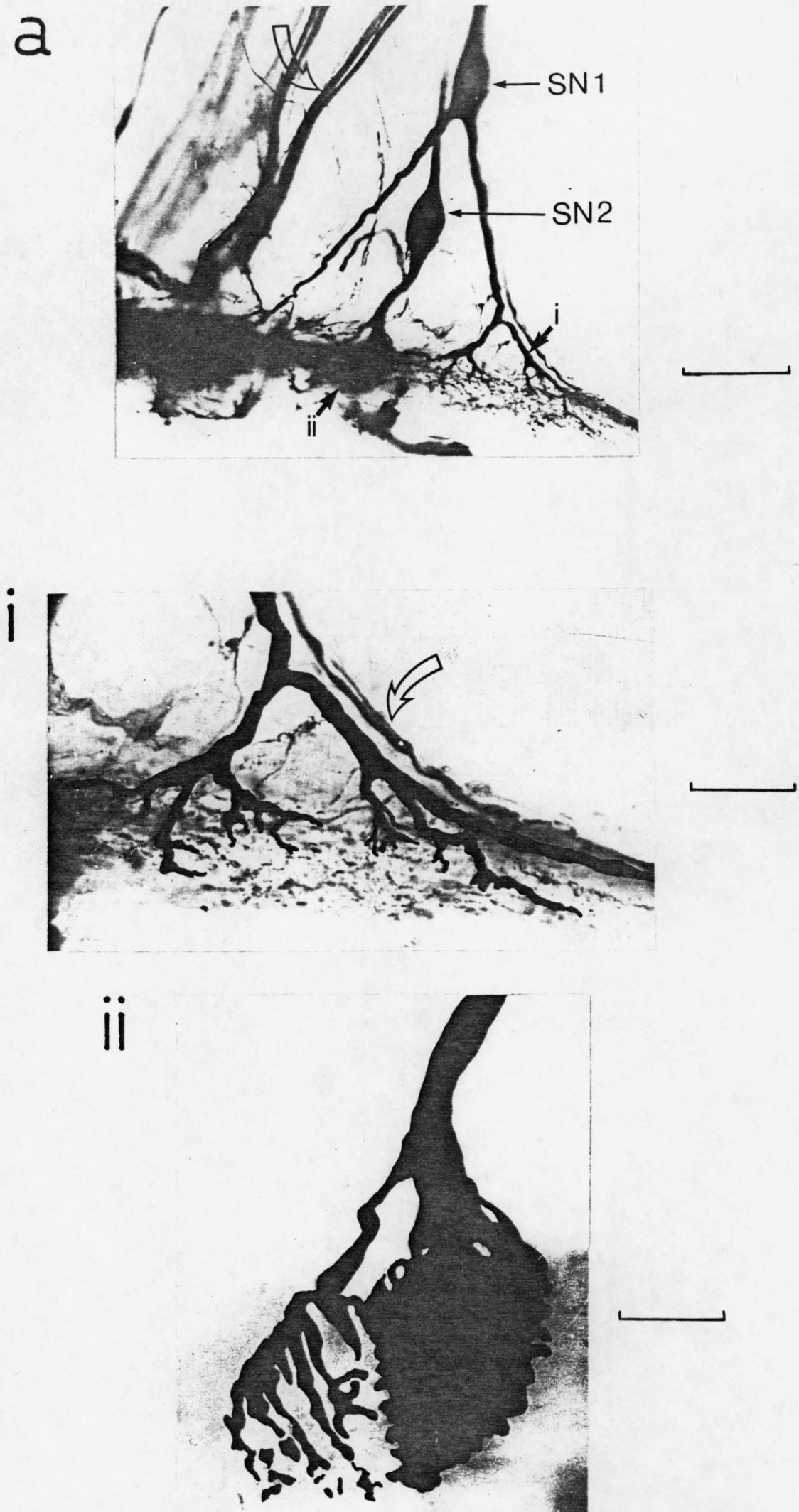
i and ii refer to b i and b ii, respectively.

b i. The dendrites of SN1 embed over a wide area of RM1. Open arrow indicates the axons of sF's which also embed in RM1.

b ii. The dendrites of SN2 embed in a restricted area of RM2 compared with those of SN1 and are dense and compacted in appearance.

b i and b ii are retouched.

Scale bars = 100μ a; 50μ b i; 25μ b ii.


a
SN1
SN2
i
ii
bi
ii

anatomical distinction between the dendritic arrays of the two neurons provides a basis for the equally clear physiological differences in SN1 and SN2 responses to stretch (see below).

In addition to SN1 and SN2, RM1 and RM2 receive innervation from a number of efferent axons (Fig. 26; open arrows, Fig. 27). The extent and nature of this innervation have not been quantified. However, in view of the homologous relationship between both the MRO's and extensor efferents in crayfish and Galathea it seems likely that the MRO's are innervated by both extensor efferents and accessory neurons.

B. Physiology

(i) Basic sensory responses

To analyse the basic sensory physiology of the MRO's, extracellular recordings have been made of 2nd root activity in response to electro-mechanically induced stretch of the receptor muscles and manual flexions of the abdomen. In terms of their output properties, the receptors function in a similar way to the crayfish MRO's (Fig. 28-31).

Identification of RM1 and RM2: RM1 and RM2, the fast and slow adapting stretch receptors have been identified using a combination of anatomical and physiological criteria. In Homarus the two receptor muscles are of unequal dimensions with RM1 slightly thinner than RM2 (Alexandrowicz, 1967). The dendrites of the slow receptor neuron branch over a wider region of RM1 while those of the fast adapting organ are more compact. These anatomical features are also present in Galathea, in a more exaggerated form. During dissection the thick RM2 often displays twitch contractions, typical of fast muscle. At rest, RM2 is under less tension than RM1. Similar observations have been made in lobster and crayfish MRO's.

FIG. 28 a, b.

Identification of RM1 and RM2.

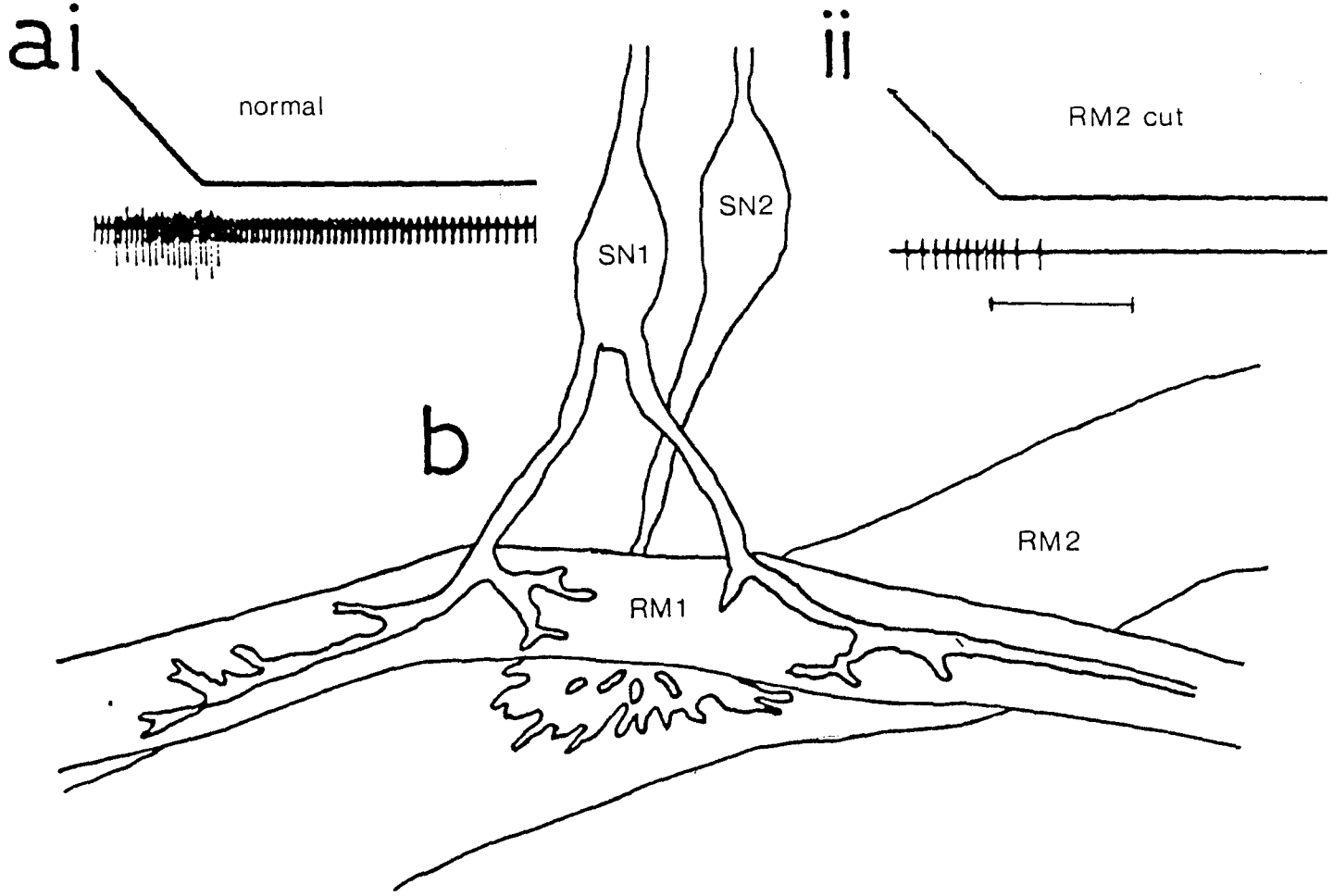
a i, ii. a i represents the normal response of SN1 and SN2 (large and small spikes, respectively, bottom trace, r2) to phasic ramp pulls on the receptor muscles.

a ii shows that after cutting RM2 at its posterior insertion the large spike is removed. Top traces in a i are monitors of receptor stretch (stretch-downwards deflection). Note the reduced SN1 response after cutting RM2. This may be due to elastic recoil of RM2.

b. A camera laucida drawing of the sensory neurons and receptor muscles from a silver intensified wholemount preparation.

Data in a and were obtained from the second abdominal segment of different preparations.

Time bar in a = 200ms.


ai
normal
ii
RM2 cut
SN1
SN2
b
RM2
RM1

In isolated preparations the responses of RM1 and RM2 can be distinguished by selectively cutting either receptor muscle. Cutting the thick RM2 for example removes the fast adapting unit in physiological responses to stretch (Fig. 28). Similarly, cutting RM1 removes the slow adapting unit. In Fig. 28, RM1 response following removal of RM2, is somewhat different to normal. Presumably either the surgery itself or the recoil of the cut end of RM2 is responsible for this change.

The above criteria are considered sufficient to characterize the thin receptor muscle as the slow adapting RM1 and the thick as the fast adapting RM2.

Response characteristics: SN1 has a low threshold for spike initiation and often discharges in response to small vibrations of the base plate. SN1 response is slow adapting and the neuron fires tonically for the duration of maintained stretch. In response to a phasic pull of the receptor muscles SN1 discharges at initial high frequency and adapts approximately logarithmically over the duration of maintained stretch (Fig. 29). SN1 discharge frequency increases with the rate and amplitude of stretch (Fig. 30a, b). At high rates and amplitudes of stretch, a second, normally much larger unit, SN2, is activated (Fig. 31). SN2 has a high threshold for activation and normally fires a maximum of 5 spikes when the receptor muscles are stretched maximally. Both SN1 and SN2 show some sensory adaptation (Fig. 31b). When a large amplitude and high rate of stretch is applied at 10 second intervals, adaptation in SN1 and SN2 is evident as a progressive reduction in the spike frequency of both sensory neurons.

Thus SN1 and SN2 have essentially identical response characteristics to the crayfish MRO's, and those of other decapods.

FIG. 29.

The tonic receptor output adapts slowly during maintained stretch. Spike frequency in impulses per second is plotted against time from the point of peak frequency following the stimulus. The frequency response curve was constructed from physiological records similar to the one shown in the inset.

Time bar in inset = 200 ms.

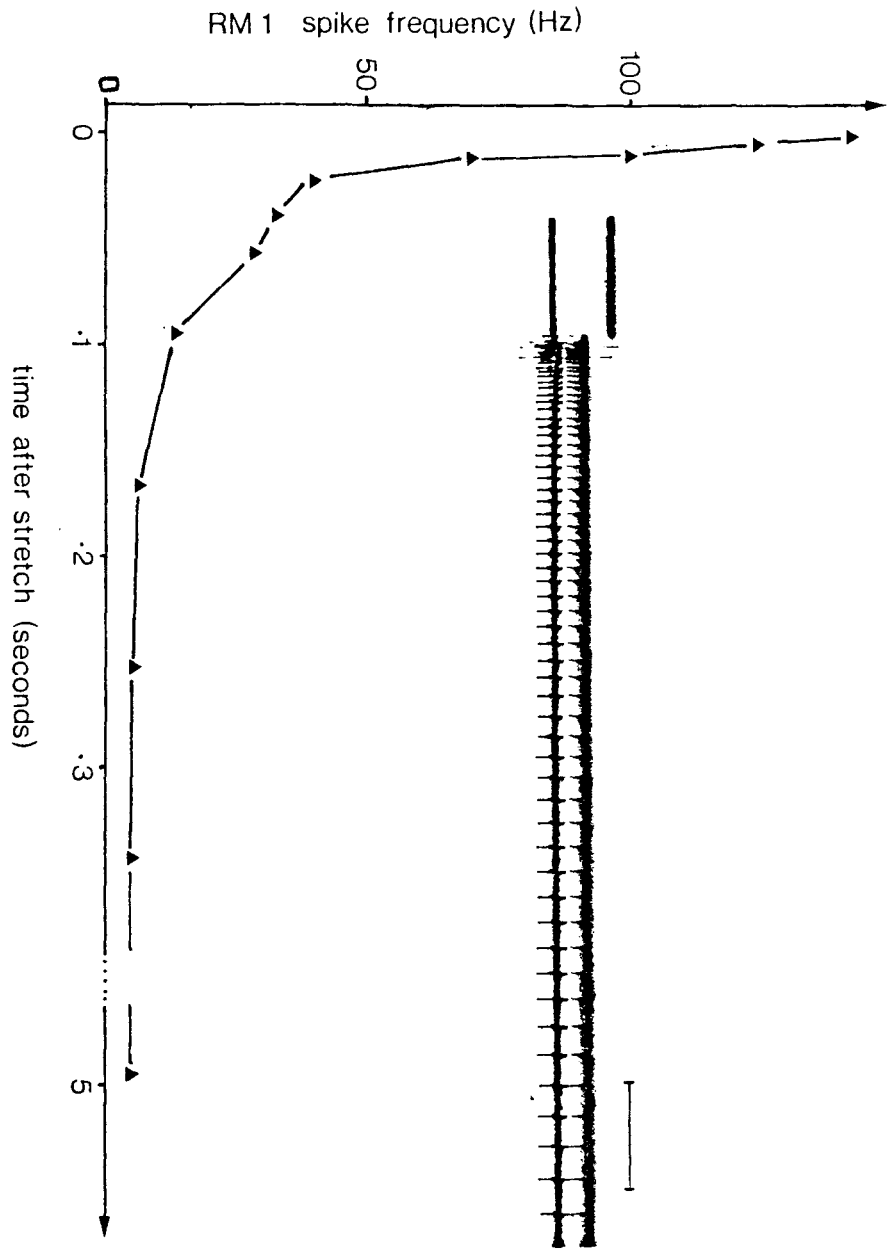

RM 1 spike frequency (Hz)
0
50
100
time after stretch (seconds)
0
.1
.2
.3
5

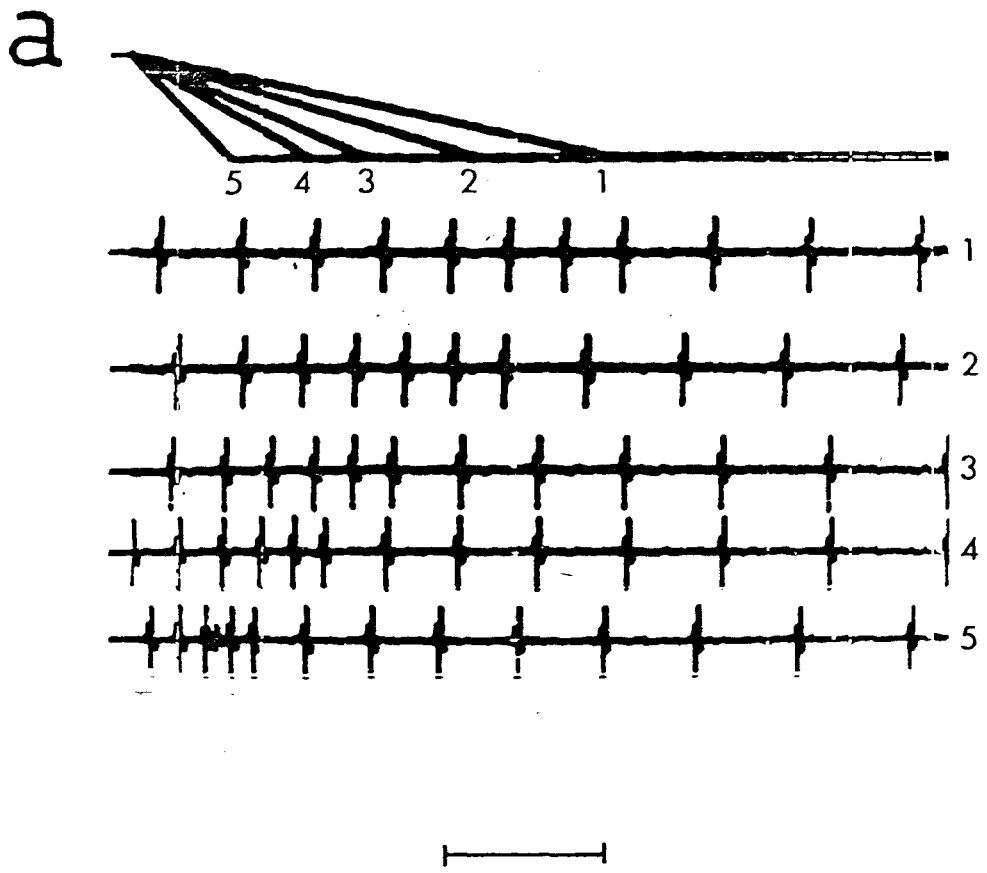
FIG. 30 a, b.

Sensory responses in SN1 in response to electromechanically induced stretch of the receptor muscles.

a. Responses of SN1 (1 - 5, lower traces) to increasing rates of stretch of the receptor muscles (1 - 5, top traces).

b. Effects of increasing amplitudes of stretch (1 - 3, top traces) and correspoding SN1 responses (1 - 3, bottom traces). In a and b successive traces represent repeated stretches of the receptor muscles at approximately 5 second intervals. In stretch monitor traces a downwards deflection of the oscilloscope beam represents stretch of the receptor muscles. Stretch monitor calibration is different in a and b.

Scale bar = 1 second a; 400 ms b.


a
5
4
3
2
1
1
2
3
4
5

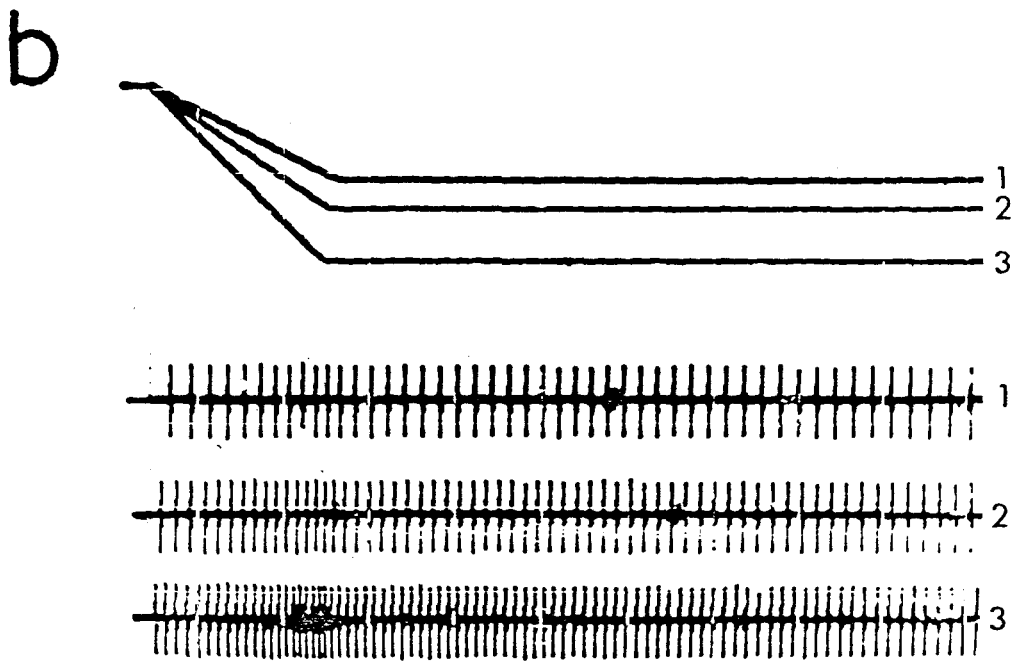

b
1
2
3
1
2
3

FIG. 31 a, b.

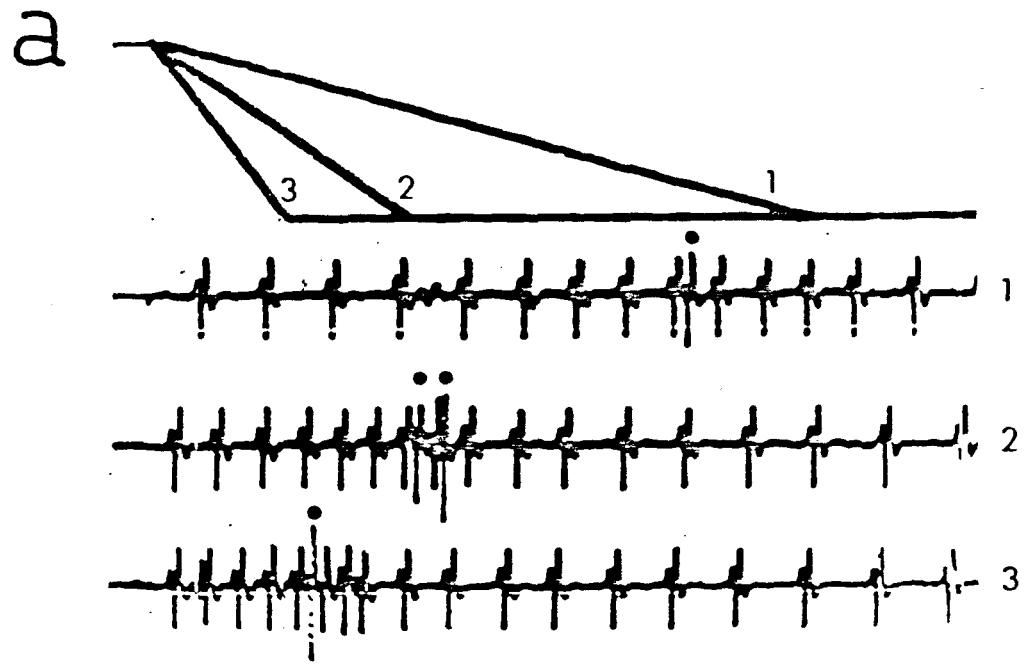
Basic sensory responses of SN1 and SN2 to electromechanically induced stretch of the receptor muscles.

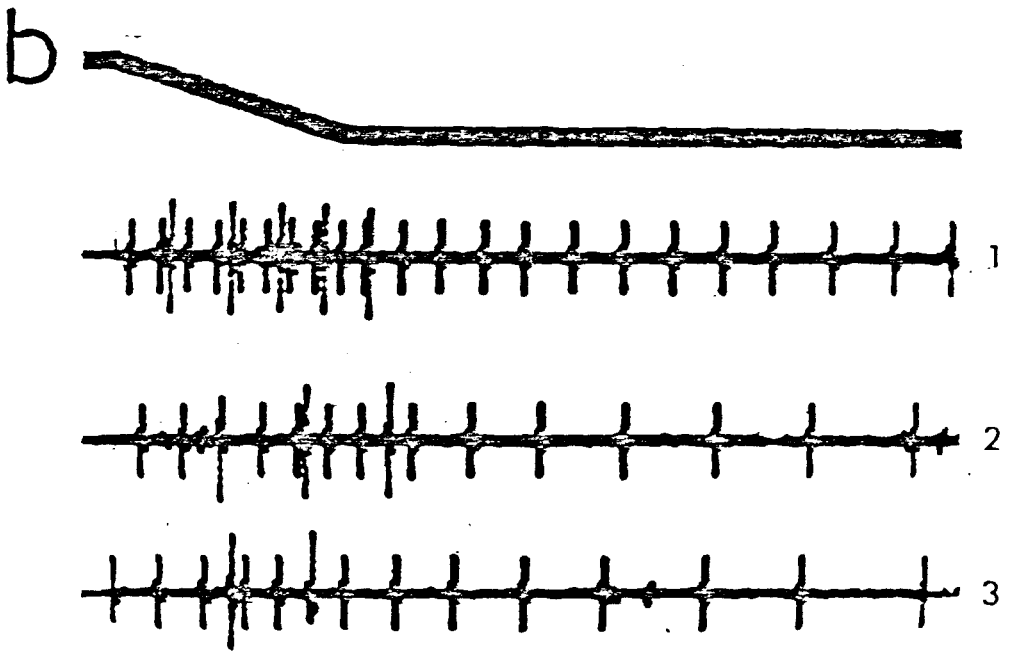
a. High rates of stretch (1 - 3, top traces) activate SN1 and SN2 (dotted, large spikes; 1 - 3, bottom traces).

b. SN1 and SN2 show some sensory adaptation. A large amplitude ramp stretch (top trace) was applied successively at 10 second intervals (1 - 3 bottom traces).

In stretch monitor traces, stretch is represented by a downwards deflection of the oscilloscope beam. Stretch monitor calibration is different in a and b.

Scale bars: 200 ms a; 400 ms b.


a
3
2
1
1
2
3


b
1
2
3

MRO's are activated by abdominal flexion: Of fundamental importance in this analysis of receptor function was a determination of which abdominal movements are sufficient to activate the MRO's. The isolated abdomen was pinned ventral side up at its rostral end. After removal of the ventral cuticle hook electrodes were placed under r2G2. The root was cut medially and sensory discharges were monitored in response to manual movements of the abdomen (Fig. 32). Neither RM1 nor RM2 fires when the abdomen is manually extended or when the abdomen is held in an extended position. Most flexions of the abdomen were sufficient to fire RM1, while more powerful flexions fired both RM1 and RM2 (Fig. 32). When the abdomen was manually flexed and held in a semi-flexed position RM1 fired at initially high frequency ($\sim$ 125 Hz) and adapted slowly. RM1 fired for periods of over 1 minute under these conditions and was always active for as long as the abdomen could be held flexed. In Fig. 32a the abdomen was flexed for about 45 seconds during which time RM1 frequency fell from an initial 125 Hz to 5 Hz. More rapid abdominal flexions recruited the larger RM2 unit (Fig. 32 b-d).

RM2 response adapted rapidly and the number of RM2 spikes depended, qualitatively, on the rate and amplitude of flexion. In addition to RM1 and RM2 a number of other sensory neurons were activated during rapid flexions. In sum, the MRO's of Galathea, as in other decapods, respond to abdominal flexion.

FIG. 32 a - d.

Activation of the MRO's by abdomional flexion.

a - d are sensory responses recorded in r2 G2 to manual tail flexions. Arrows indicate the approximate onset (up) and offset (down) of flexion.

a. Flex and hold response in SN1.

b, c, d. Increasing rates of tail flexion activate both SN1 and SN2. Dots indicate the occurrence of spikes in SN2. In d, the 3rd and 4th SN2 spikes are larger, presumably because they coincide with spikes in SN1 or in other sensory neurons.

Time bars: 1 second a; 50 ms b; 100 ms c, d.

a
c
b
d

(ii) MRO input to the CNS

Fast extensor motorneurons

In three separate preparations neuropilar penetrations have been made of FE's in G2 while the intact MRO's were activated. These penetrations were, however unstable, lasting for less than one minute. In each case these recordings showed that FE's receive apparently monosynaptic EPSP's from the fast and slow receptors. In order to gain more stable penetrations the chain of abdominal ganglia was twisted through 180° and recordings made from the somata of FE's. Again, it was found difficult to record from these neurons but on several occasions stable penetrations were achieved. In the majority of these, MRO evoked EPSP's were minute and often fell below the noise level of the recording (~ 1/4 mV). On two occasions, however, these EPSP's were larger (~ 1/2 mV) (Fig. 33). Unfortunately during the experiment in which these recordings were made, a fault in the servomex transducer device prevented electromechanical stretch of the receptor muscles. The receptors were stretched manually and only the tonic receptor could be activated in this way. In each of the two examples shown in Fig. 33 a, b, FE's received EPSP's from the tonic MRO. These small, long duration EPSP's summated during high frequency MRO firing. The neuron recorded in Fig. 33a was identified by Lucifer Yellow staining as the largest of the available pool of FE's. The neuron recorded in Fig. 33, from the same hemiganglion, was identified by an antidromic spike following ipsilateral r2 stimulation and by deduction must correspond to another smaller FE. This evidence suggests that RM1 input to the FE pool is widespread but it is uncertain whether every FE gets input from the MRO of its own hemisegment. An interesting and potentially important feature of these MRO-evoked EPSP's is that they facilitated following

FIG. 33 a, b.

SN1 input to two FE motorneurons in G2.

In each recording the top trace is an intrasomatic recording of an FE, 2nd trace is an extracellular recording of r2 G2. Manual stretch of the receptor muscles gives rise to trains of spikes in SN1 (and in some other neurons) followed at short latency by EPSP's in FE's. The EPSP's both facilitate and summate.

Scale bars: horizontal - 400 ms; vertical - 5 mV.

I
II
III
IV
1a
1b

high frequency firing of RM1. It has been shown previously (see Fig. 32) that rapid tail flexions, similar to those occurring during swimming fire RM1 at initial high frequency. Towards the end of flexion it can be predicted that tonic MRO frequency will be much reduced and EPSP's in extensor motorneurons will have facilitated. Thus at the moment when extensors are about to be activated MRO evoked EPSP's will be large and potentially more effective in contributing to FE excitation during swimming.

Flexor Inhibitor

FI also received powerful input from the tonic MRO in the form of EPSP's (Fig. 34 a, c). In addition FI receives EPSP's from the phasic MRO (Fig. 34 b, c). Both types of EPSP are capable of summation following high frequency firing of the MRO's. Interestingly RM1- and RM2- EPSP's are of similar amplitude, as recorded in the neuropilar segments of FI (Fig. 34 a, b). In contrast Wine (1977c) has demonstrated that the crayfish FI receives larger EPSP's from RM1 than from RM2. This may be a function of recording sites. Wine recorded FI in the soma, and it is possible that RM1 and RM2 EPSP's are of similar amplitude at or close to the synaptic sites. If SN2-FI synapses are further from the soma than SN1-FI synapses then this may account for the difference in amplitude as recorded in the soma. In support of this idea, EPSP's recorded in the soma of FI in Galathea differ in amplitude (Fig. 34c), with SN1-EPSP's approximately twice the size of SN2-EPSP's. EPSP's recorded in the neuropile were about 2 mV in amplitude. Recorded in the soma however their amplitude was much reduced (< 1 mV).

FIG. 34 a - c.

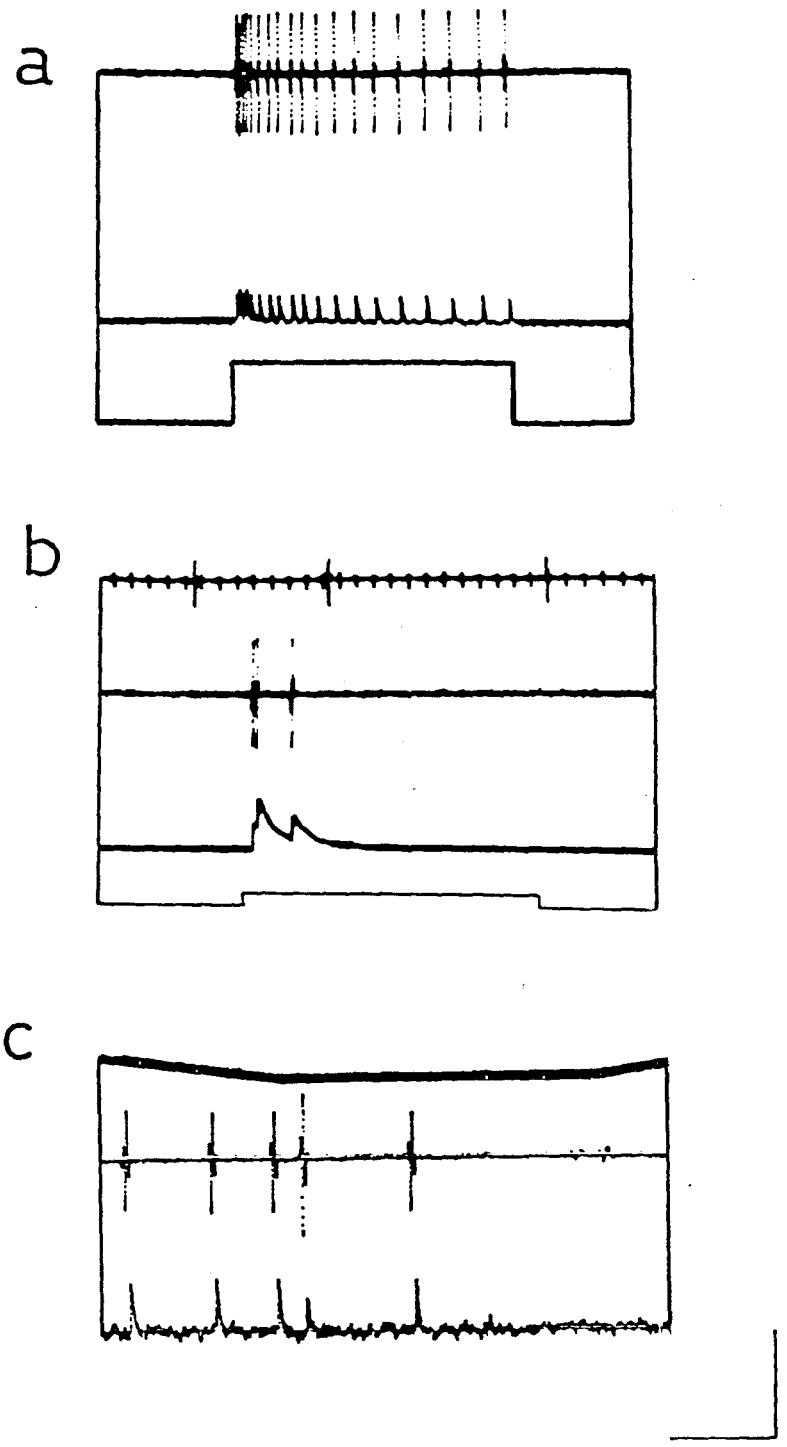
MRO input to FI.

a. RM1 (top trace, r2) elicits EPSP's in the neuropilar segments of FI in G2 (2nd trace) which show a small degree of summation. Bottom trace is a monitor RM stretch (stretch upwards).

b. RM2 (2nd trace) also elicits EPSP's in the neuropilar segments of FI in G2 (3rd trace) which summate and are of similar amplitude to RM1 - EPSP's. Top trace is r3 ipsilateral, bottom is monitor of stretch.

c. Intrasomatic recordings from FI (bottom trace) reveal that RM1 and RM2 EPSP's are of different amplitudes. Each is smaller than neuropilar recordings (a, b). Top trace is a monitor of receptor stretch (stretch downwards) 2nd trace is r2.

Scale bars: horizontal - 400 ms a, c; 200 ms b;
vertical - 10 mV a, b; 2 mV c.


a
b
c

FF's and EI

There is a reciprocal arrangement between the MRO's and the phasic extensor and flexor motorneurons. Complete reciprocity would entail inhibition of the fast flexors and of the peripheral inhibitor to the extensors by the MRO's. Such connections are conspicuous in their complete absence. In several recordings of FF's and EI neither phasic nor tonic MRO's had any discernible input, either excitatory or inhibitory. It is possible that other polysynaptic pathways exist to inhibit FF's and EI but that activation of the MRO's in only one hemisegment in these experiments was insufficient to activate them. It must be borne in mind that 4 MRO's in each segment converge on the CNS and may distribute their input over more than one ganglion. In crayfish it has been shown that the MRO's excite FE's and FI in at least three adjacent ganglia. Polysynaptic pathways for inhibition seem intuitively unlikely however. The FF's are normally only active during backward swimming in Galathea and tailflipping in crayfish. Other central pathways, not involving the MRO's are active during backwards swimming which powerfully inhibit the FF's during interburst intervals (see next chapter). In the absence of any peripheral feedback these mechanisms are sufficient to ensure proper phasing of FF activity and complete repolarization of the membrane potential following FF bursting.

Tonic motorneurons

Tonic motorneurons are normally characterised by a low-spike threshold, tonic spike activity and a high level of synaptic activity. It is likely therefore that any MRO input to tonic motorneurons would be apparent as a change in their ongoing spike frequency. In many preparations one or more tonic motorneurons have been spontaneously active

as recorded extracellularly on r2 and r3. However activation of the MRO's in such preparations had no discernible effects on spontaneous discharge frequency. Small changes in spike frequency did occur in these motorneurons and it is possible that polysynaptic input from the MRO's was involved. However at the level of extracellular activity no direct correlation could be observed. In conclusion it seems likely that the MRO's have no significant effects on tonic motorneuron activity.

Intracellular recordings from tonic flexor motorneurons reveals a lack of monosynaptic connections with the MRO's (Fig. 35). However in this preparation, there was an increase in synaptic activity associated with high frequency firing of the MRO's. Although these potentials were depolarizing I was unable to determine whether they were excitatory or inhibitory.

Central projections of the MRO's

Among the easiest neurons to penetrate in the extensor neuropile area are the two sensory receptor neurons (Fig. 36). Lucifer Yellow staining has confirmed that the axons of each receptor bifurcate and project rostrally and caudally in the ipsilateral connective. In addition, intracellular staining has revealed numerous fine branches which arise from the initial portion of the rostral axon and project into the ipsilateral extensor neuropile area. Each sensory neuron had a resting potential of about -75 mV but spike amplitude varied considerably between 15 and 50 mV.

FIG. 35 a, b.

Lack of monosynaptic input from the MRO's to a tonic flexor motorneuron recorded in the soma in G2.

a. On initial penetration sF spiked tonically (arrowed, top trace). sF spikes are also monitored on the ipsilateral r3 (2nd trace; arrowed). A phasic stretch of the MRO's (bottom trace) fired SN1 and SN2 (3rd trace) but did not result in monosynaptic input to sF.

b i. Later in the same experiment, sF stopped firing but could be induced to spike by the injection of small amounts of depolarizing current ($\sim$ 2 nA, not monitored, bridge partially balanced). Spikes recorded intracellularly in sF (bottom trace) could be correlated 1:1 with spikes recorded extracellularly on r3 (top trace).

b ii. Subsequent stretch of the receptors at low amplitude (2nd trace) did not affect the frequency of synaptic potentials in sF (bottom trace).

b iii. Phasic stretches of the receptors resulted in an increase in synaptic activity in sF.

Scale bars: horizontal - 400 ms a, 800 ms bi - iii; vertical- 20 mV a, 5 mV b i, 2 mV bii, iii.

a
bi.
ii:
iii:

Fig. 36 a, b.

Confirmation of MRO axon bifurcation in the CNS.

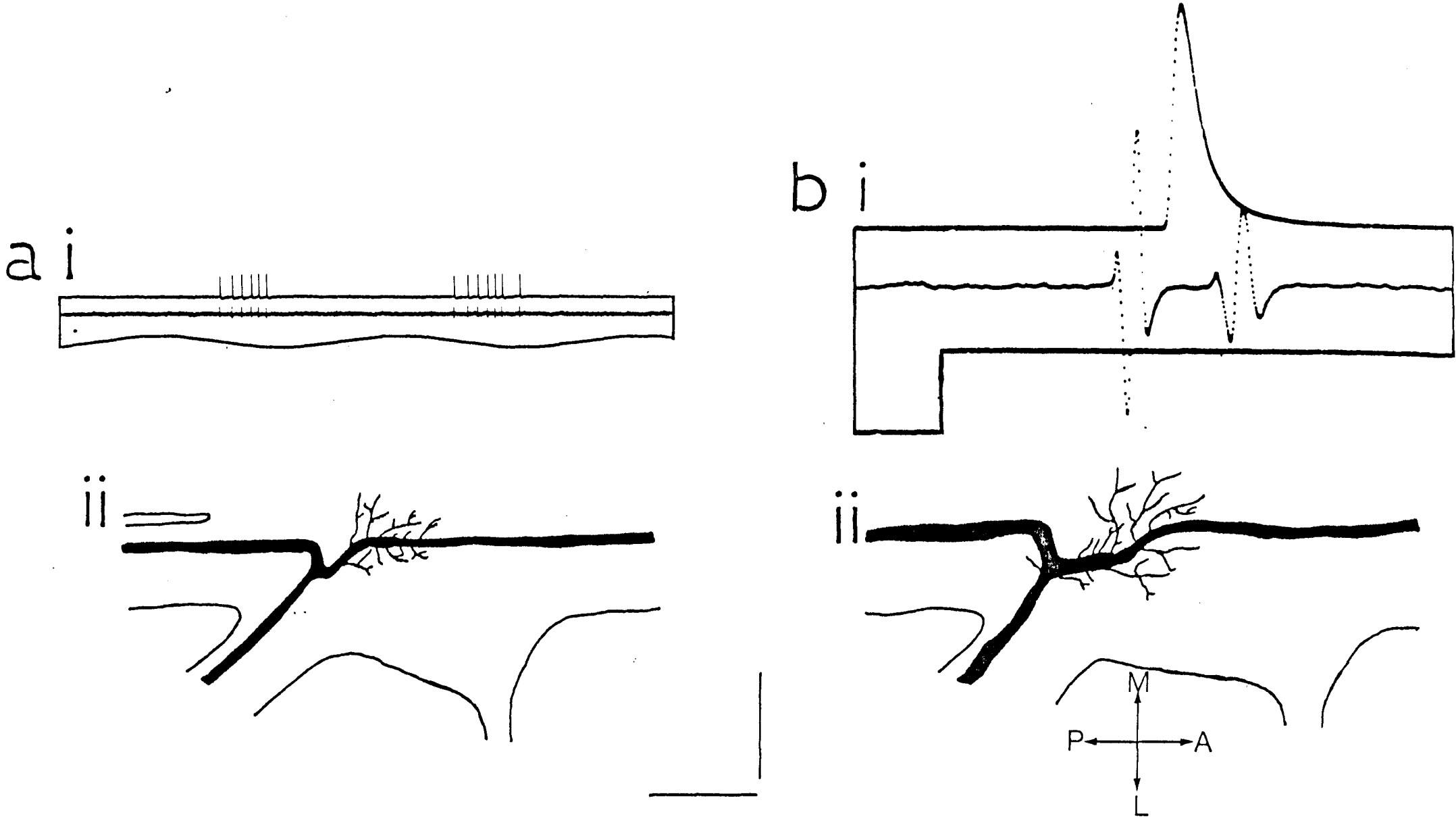
a i. SN1, penetrated intracellularly in the neuropile of G2 (top trace) fired in response to stretch of the receptors (bottom trace). Spikes recorded in SN1 could be correlated 1:1 with spikes recorded on r2 (2nd trace).

ii. The neuron recorded in i was subsequently stained with Lucifer Yellow. The axon of SN1 enters the CNS via r2 and bifurcates, projecting anteriorly (to the right) and posteriorly in the ipsilateral connective.

b i. SN2, penetrated in the neuropile of G2 (top trace), fired in response to a phasic stretch of the receptors (bottom trace). The SN2 spike recorded intracellularly could be correlated 1:1 with the larger of the two spikes recorded in r2 (2nd trace).

ii. The neuron recorded in i was subsequently stained with Lucifer Yellow and also had a bifurcating axon which projected down r2. Note the small number of dendrites in a ii and b ii, arising from the anterior portion of the MRO axon shortly after bifurcating.

Scale bars: a i - 200 ms; 200 mV b i - 10 ms, 20 mV, a ii, b ii - 250μ.


a i
b i
ii
ii
M
P
A
L

DISCUSSION

The preceding results confirm that the MRO's as segmental proprioceptors are widespread among the Decapoda and that they function in an almost identical way in diverse species within the group. In addition, the results provide a second comparative study of the central connectivity of the MRO's. The synaptic basis for reflex functioning of the receptors was first determined for the crayfish by Wine (1977c). With respect to the abdominal motorneuron pool the MRO's make a limited number of excitatory central connections with the fast extensors and FI. Despite considerable differences in abdominal structure in crayfish and Galathea the MRO's make identical connections in the CNS in both species. This finding suggests that the MRO's may be an important feature of the non-giant escape circuit and hence may have undergone little or no transformation as the giant fibre system evolved in crayfish.

The inherently high selection pressure on crayfish escape behaviour posed serious problems for the evolution of giant and non-giant escape systems which share the same motor circuitry since mutual interference must have been avoided as one system evolved on top of another. This must also have applied to sensory structures with feedback effects on the motor circuitry. However the only swim motorneurons that were altered during the evolution of the crayfish giant fibres were the MoG's. Since the FF portion of the motor circuit does not receive input from the MRO's (Wine, 1977c) there would be no a priori reason for modification of the MRO's, nor would there be any mutual interference. Post-giant extension is a chain reflex which is wholly dependent upon patterned sensory feedback evoked by the preceding flexion movement. Thus it appears that as the giant fibres evolved in crayfish the powerful central

connections of the MRO's were incorporated into the circuit. In semi-isolated preparations, the firing of single receptors is not sufficient to cause spiking in the motorneurons, but input from the MRO's in at least three segments, polysynaptic sensory input, and perhaps rebound from inhibition all contribute to post-giant excitation of the extensors.

The structure and function of the MRO's in Galathea does not in itself provide a basis for escape which relies on initial extension. Neither is it an important factor in the initial fast flexion phase of the crayfish tailflip. Rather it appears that during the evolution of the crayfish giant fibre system the central connections of the MRO's, which were already established components of the non-giant circuit were retained. The end result is two integrated escape systems which are serially ordered by virtue of their different response latencies.

Any differences between the MRO's in Galathea and crayfish are minor and may reflect different requirements in the fine tuning of sensory responses, commensurate with differences in abdominal joint geometry in the resting state. Anatomically the MRO's are very similar though in Galathea, RM2 is relatively much thinner than RM1 and SN1 has two major branches compared with only one in SN1 of the crayfish. In all major respects the MRO's in Galathea and crayfish are homologous structures.

A possible role for the MRO's during escape swimming in Galathea is discussed on the basis of results presented in the next chapter.

C H A P T E R 3

THE PHYSIOLOGY OF ESCAPE SWIMMING

CHAPTER 3.

INTRODUCTION

Since 1947 (Wiersma) research into the role of the giant-fibre system in macuran escape behaviour has been extensive (for recent review see Wine and Krasne, 1982). Yet even now, after almost 40 years of intensive study our knowledge of their precise role in behaviour is incomplete. At the level of cell to cell connections and circuitry there is a wealth of evidence for their powerful effects on flexor motorneurons, especially the Motor Giants. As these connections were revealed, so a quantum leap was attempted towards an explanation of the role of the giant fibres in behaviour. The lateral and medial giants were assigned the role of command neurons for escape since they produce, with a single impulse a stereotyped, coordinated tailflip response (Kupfermann and Weiss, 1978). However, escape behaviour is not always a stereotyped response. Schrameck (1970) has shown previously in a series of elegant experiments on intact crayfish, that the giant fibres may or may not fire prior to the first or to subsequent flexions in a bout of swimming. She also demonstrated that giant fibre spikes interpolated into the swim cycle produce little or no flexor muscle activity unless they occur up to 20 ms before an expected flexion. Hence under certain conditions the giant fibres are neither sufficient nor necessary for escape in freely behaving animals. Despite this the LG's and MG's possess most of the operational features of command neurons (Kupfermann and Weiss, 1978; Olson and Krasne, 1981). The low behavioural response latency of the giants coupled with their powerful excitation of the MoG's suggests that they are responsible for many initial fast flexions. As for their role in subsequent swim cycles it has been suggested that they may act to syncronize flexor motorneuron activity or to amplify power output, or both (Schrameck, 1970).

Crayfish escape behaviour utilizes a combination of giant and non-giant circuits. The giant and non-giant systems are coupled in parallel by the stimulus which initiates escape (Reichert and Wine, 1982). A single, transient sensory stimulus activates both systems. The giant fibres, which have a low behavioural response latency ($\sim$ 7 ms) trigger a stereotyped flexion and re-extension which is completed in approximately 190 ms. In contrast, the mean modal latency for non-giant responses to the same stimulus is about 240 ms. This latency is long enough to allow the giant fibre mediated tailflip to occur in the interim and in this way the two escape systems are activated in parallel to produce serial ordering of escape behaviour. The adaptive significance of this parallel processing strategy is that it takes advantage of a fast but bilaterally symmetrical giant-fibre response and a slower but laterally asymmetric non-giant response. The former ensures fast start while the latter can steer the animal away from threat.

Non-giant escape circuitry is poorly understood. Non-giant swimming is thought to be under the control of a CPG or oscillator (Schrameck, 1970). Brainless animals are capable of swimming while those with transected abdominal nerve cords are not, suggesting that the neural circuitry comprising the CPG is located either in the suboesophageal or thoracic ganglion. In contrast to the giant circuitry, the non-giant CPG drives extension before flexion and elicits rhythmical alternating bursts of activity in the two antagonistic pools of motorneurons. The premotor interneurons responsible for FF excitation have only begun to be identified. A large population of axons in the dorsal nerve cord fires in phase with depolarizations of the FF's seen during non-giant swimming. The two largest axons in this population have been identified as corrolory discharge interneurons, CDI 2 and CDI 3 (Kramer et al., 1981). These

interneurons are located entirely within the abdominal nerve cord and are recruited during giant and non-giant tailflips. Both provide weak excitation to the FF's (compared to that from the giants) and this input requires summation with other premotor elements to fire the FF's. Their role in escape is thought to be primarily in the coordination of non-giant flexions (J.J. Wine, pers. comm.).

The basic escape system, which is thought to have evolved first, is the non-giant one with the fast flexor motorneurons as its efferent elements (Wine and Krasne, 1982). The giant fibre system probably evolved later through three basic changes. (1) The giant fibres arose from smaller flexor premotor elements. (2) The MoG's evolved, presumably promoted from the FF motorneuron pool, as the efferents of the giant system. (3) Primary 'central driver' neurons, the SG's (Roberts et al., 1982) evolved, probably from limb motorneurons.

Backwards swimming in Galathea appears to be very similar to non-giant swimming in crayfish in that it involves rapid abdominal cycling which propels the animal backwards, away from threatening stimuli. The striking homologies between the extensor and flexor motorneuron pools in the two animals suggests that backwards swimming and non-giant swimming are homologous behaviours. The absence of a giant fibre system in Galathea suggests that this animal may represent a form comparable to crayfish prior to the evolution of the giant fibres. In support of this notion, a neuron homologous with the crayfish MoG in terms of the size and position of its soma has been identified as a typical, but large FF in Galathea (see Chapter 1). Crayfish swimming, the ancestral escape behaviour drives extension before flexion, as in Galathea. One might hypothesize then that prior to the evolution of the giant fibres, crayfish, like Galathea had abdomens which were tonically flexed

in the resting animal. The giant fibres may have evolved as the crayfish abdomen extended, to provide for fast start tail flexions during escape. The present chapter is aimed at investigating the neural control of backwards swimming in Galathea.

The chapter is divided into four sections. In the first, the behaviour has been studied under various conditions of restraint and deafferentation to determine the normal sequences of contraction of abdominal muscles during escape and to investigate the role of sensory feedback in swimming. In the second, a deafferented preparation has been used to analyse the structure of the motor programme for swimming. In the third a cellular analysis of the activity of the motorneurons during swimming is presented. Finally, the coordinated activity of the limbs during swimming has been studied.

METHODS

Electromyography

Extracellular recordings of muscle potentials were made using pairs of 100μ or 200μ copper wires, insulated except at their tips. These were implanted either directly into exposed muscles or through small holes made in the carapace. In the latter case EMG electrodes were secured in place with superglue or wax. Muscle potentials recorded in this way were amplified differentially using RP2 pre-amplifiers manufactured in the Gatty Marine Laboratory (GML).

Extracellular recording and stimulation

A variety of extracellular recording techniques were used. In most cases connective, 1st root, 2nd root and superficial 3rd root recordings were made with silver wire hook electrodes. In some instances where multiple recordings were necessary, pin electrodes were used to monitor activity in 1st, 2nd and superficial 3rd roots. Recordings of main 3rd root activity were always made with polythene tipped suction electrodes. Hook and suction electrodes were mounted in Prior micromanipulators. The output from each electrode was fed through a junction box (built personally in the GML) into a high input impedance differential amplifier with a maximum gain of x1000 (manufactured in the GML: type R P2). The output of each amplifier was displayed on one channel of a 4-channel Textronic 5103N storage oscilloscope. For filming of data, activity was slaved onto a 4-channel Textronix 561A oscilloscope equipped with a Nihon Kohden film unit. Some data was passed directly into a RACAL 7-channel DC tape recorder for future analysis and filming. Still photographs were taken of recorded data onto black and white 35 mm FP4

film using a Canon AE1 SLR camera adapted with a x2 close-up lens for oscilloscope photography. Nerve activity was also monitored with an audio amplifier (built in the GML) connected in parallel with the CRO.

A Textronix 160A power supply was used to drive a 162 waveform generator and a 161 pulse generator. The square wave output of 15v was used to drive an isolated stimulator (Digitimer, Type 656) whose output (0 - ± 100v) was directed into the junction box and fed directly to the appropriate electrode. This allowed stimulation or recording through any electrode.

Intracellular recording and stimulation

Micropipettes were pulled from thin walled fibre-filled electrode glass (Clark Electromedical Instruments GC 150 tF-10 on an Industrial Associates Electrode Puller (Type M1). Microelectrodes were made by back-filling with the appropriate electrolyte from a hypodermic needle. Electrode resistance varied according to the solution used. In general 10 - 80 mΩ (with 2M potassium acetate) and 20 - 50 MΩ (with 5% Lucifer Yellow in 1M lithium chloride) were found to be suitable. Electrode resistance was determined with a series voltage divider in the intracellular amplifier circuit (see below). Microelectrodes were mounted on a Carl Zeiss Jena sliding plate micromanipulator. A silver-chlorided silver wire inserted into the electrode barrel led to the head stage of a high impedance amplifier with a gain of x10 and a wheatstone bridge circuit for balancing injected DC current. A switch mechanism on the amplifier allowed injection of up to ± 10nA DC current into impaled cells to alter their membrane potential. For dye injection current was passed directly from the output of the Digitimer Isolated stimulator via the amplifier into the microelectrode (see below). The output of

the amplifier was displayed on one channel of a Textronix 5103N storage CRO on DC mode and recorded as for extracellular activity.

Lucifer Yellow staining

Intracellular dye injection was used to determine the anatomy of impaled neurons. Where anatomical data was required, microelectrodes were routinely filled with 5% Lucifer Yellow CH (Stewart, 1978) in 1M lithium chloride. Dye was iontophoresed with 500 ms negative-going square wave pulses of 5 - 100 nA at a frequency of 1 Hz for up to 40 minutes. Current was usually monitored with a current monitor manufactured in the GML. Electrode blockage occurred in some preparations and was manifest as a decrease in injected current and an increase in electrode resistance. The electrode could usually be unblocked using a brief high voltage positive-going pulse to the electrode. In some cases impaled somata were seen to turn pale yellow in colour indicating that successful filling had been achieved.

Following injection, stained ganglia were prepared for photomicroscopy as were cobalt sulphide stained preparations (see Chapter 1, methods). Cleared wholemounts in cavity slides were examined with a Zeiss transmission fluorescence microscope equipped with a Zeiss mercury lamp, a BG12 excitation filter and Zeiss 44 and 53 barrier filters. Preparations were drawn using a Zeiss camera lucida and a variable intensity light source.

A. MOTOR PROGRAMME AND SENSORY FEEDBACK INTERACTIONS IN THE GENERATION OF THE SWIMMING RHYTHM

Background

It is now widely accepted that rhythmic behaviours are generated by the interplay between central motor programmes and peripheral feedback (Delcomyn, 1980). The essential neural activity underlying each rhythmic behaviour (the motor programme) is produced by a CPG comprising neurons located entirely within the CNS. Motor programmes are moulded by sensory feedback to create behaviourally appropriate motor acts. The relative role of sensory feedback divides rhythmic behaviours into four categories.

1. Rhythms which reflect the fine structure, though not necessarily the frequency, of central motor programmes in the complete absence of sensory feedback (e.g. Dorsett et al., 1969; Hartline and Maynard, 1975; Ikeda and Wiersma, 1964; Kovac, 1974; Maynard, 1972; Wilson, 1961; Young, 1975). Sensory feedback which affects the motor programme must, nevertheless, be considered an integral part of such motor systems (e.g. Burrows, 1975; Davis, 1969b; Kater and Rowell, 1975; Paul; 1971c).

2. Rhythms in which the central programme generates a basic framework upon which sensory feedback acts (e.g. Evoy and Fourtner, 1972; Grillner, 1975; Pearson et al., 1975; Pearson and Fourtner, 1975.

3. Rhythms in which one phase is centrally generated but sensory feedback is required to complete the cycle (Snow, 1975; Paul, 1975; Paul, 1976; Reichert et al., 1981).

4. Rhythms which depend upon sensory feedback for their generation and constitute a chain of reflexes (the only known example is swimming in the scallop (Mellon, 1969)

Rhythmic behaviours form a spectrum between those which are continuous (e.g. ventilation) and those which are episodic (e.g. walking, swimming, flight). Motor systems which are subject to abrupt changes in the environment are often episodic and here sensory input can function to compensate to some extent for unexpected environmental perturbations (Forssberg, 1979; Goodwin et al., 1978; Bizzi et al., 1978). Sensory input is also involved in the modulation of central programmes to meet prolonged changes in the internal or external environment (Polit and Bizzi, 1979; Robinson, 1976). The role of sensory input in motor control is, however, poorly understood (Pearson, 1981).

Locomotory behaviours in Crustacea are clearly episodic. Sensory feedback effects on locomotory programmes derive from two known sources in these animals. First, a diverse array of proprioceptors monitor changes in joint angle, joint position, muscle tension and cuticle distortion induced by muscular contraction during locomotion. Second, a large number of exteroceptors monitor environmental stimuli including those induced by locomotory movements. The mechanisms whereby central effects are induced by these receptors are diverse. In the swimmeret system, non-spiking stretch receptor (NSSR) neurons conduct graded electrical information into the CNS and synapse directly with motorneurons (Heitler 1982). In the locust flight and crayfish escape systems, spiking stretch receptors are monosynaptically coupled to the motorneurons (Burrows, 1975; Wine, 1977c). The relative roles of proprioceptive and exteroceptive feedback on locomotor programmes are poorly understood, although both are known to have effects (Pearson, 1981).

Experimental approaches

Backwards swimming in Galathea is a fast, rhythmic and highly episodic form of locomotion. I have studied this behaviour using a number of different experimental approaches to analyse the relative roles of proprioceptive and exteroceptive feedback on the generation of the rhythm. Using a variety of extracellular recording techniques, backwards swimming has been investigated in four types of preparation. In each of these proprioceptive and exteroceptive feedback states are different.

1. Free swimming preparation

EMG electrodes inserted into phasic extensor and flexor muscles were used to monitor swimming activity in animals swimming freely in a perspex chamber. Proprioceptive and exteroceptive feedback systems were both intact.

2. Semi-restrained preparation

Animals with inserted EMG electrodes were held in one position and allowed to flip their abdomens freely without mechanical interference. Here proprioceptive feedback is normal but exteroceptive feedback is disrupted.

3. Fully restrained preparation

Intact animals were fully restrained with their abdomens extended. Exteroceptive and proprioceptive feedback were thus both disrupted. With intact motor roots, abdominal extensor and flexor muscles contracted phasically. Any residual mechanical slack is therefore assumed to generate some sensory reafference.

4. Deafferented preparation

Abdominal nerve roots were surgically cut resulting in the total removal of exteroceptive and proprioceptive feedback from the abdomen.

The results, documented in the following pages have permitted an assessment of the relative roles of proprioceptive and exteroceptive feedback on the centrally generated swimming rhythm.

RESULTS

1. Free swimming preparation

The contraction sequences of abdominal muscles have been examined by recording EMG activity in phasic extensor and flexor muscles during periods of swimming in unrestrained animals. Following electrode placement animals were placed in a large perspex chamber filled with sea water and induced to swim by tactile stimulation of the head or thorax. Swimming was always highly episodic and a single tactile stimulus rarely resulted in more than three consecutive cycles of activity (Fig. 37b). Extensor activity always preceded flexion with flexion following at a short and near constant latency of 20-40 ms. Swims were normally terminated through mechanical interference when, for example, the side of the experimental chamber was encountered. When faced with a continuous threat stimulus animals responded by prolonged periods of swimming usually with long and variable cycle periods, despite encountering the side of the chamber repeatedly (Fig. 37a).

When this occurred the approximate durations of extension and flexion were always the same and the increase in cycle period involved a long interburst interval between the end of flexion in one cycle and the onset of extension in the next. One interpretation of this data is

FIG. 37 a - c.

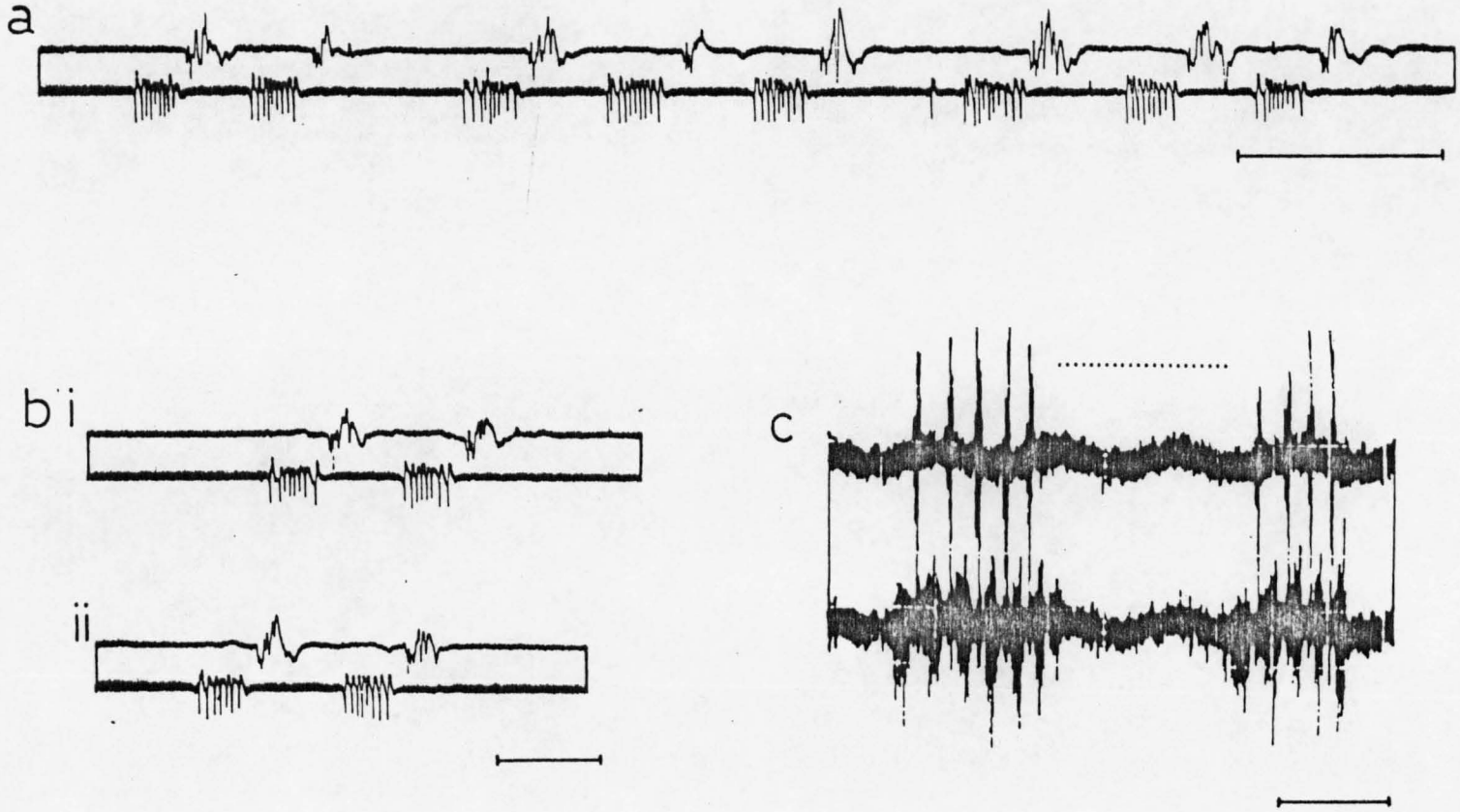
EMG activity recorded in phasic flexor (top traces) and extensor (bottom traces) muscles in the 2nd abdominal segment of freely swimming animals.

a. A constant threat stimulus resulted in a series of extension-flexor cycles of the abdomen which began with extension. Note that frequency is highly variable.

b i, ii. Responses to single tactile stimuli. Bouts of swimming were short and were normally terminated when the animal contacted the experimental chamber.

c. Semi-restrained animal. A pencil was placed at the base of the thorax for the approximate duration of the dotted line (see text).

Scale bars: 200 ms a; 100 ms b, 1 second c.


a
b i
ii
c

that backward swimming is highly susceptible to exteroceptive feedback. Once swimming has been initiated external perturbations are capable of switching the behaviour off. This is shown in Fig. 37c. The animal was held in one position and induced to swim. After 5 consecutive swim cycles a pencil was placed at the base of the thorax. The animal stopped swimming and resumed immediately the pencil was withdrawn. The details of how this sensory interaction is achieved have not been investigated, but the data show that Galathea is capable of adjusting its swimming behaviour in a changing environment.

2. Semi-restrained preparation

To investigate the role of proprioceptive feedback on the generation of the swimming rhythm, animals were secured ventral side up with their abdomens free to move. This effectively removed most exteroceptive feedback but left proprioceptive feedback normal. EMG electrodes were inserted into phasic extensor and flexor muscles to monitor swimming activity in reponse to tactile stimulation of the thorax.

The most consistent feature of bouts of swimming recorded in this preparation was that the number of consecutive cycles was much greater than in freely swimming animals (Fig. 38). This occurred when the animal was either fully submerged under sea water or when all sea water was removed. For convenience, recordings were made in the latter conditions. Up to 10 consecutive cycles have been recorded in response to a single tactile stimulus to the thorax, although many more cycles have been observed in unoperated animals. Frequency remained relatively constant for the duration of each bout (Fig. 38a). An additional feature of swims recorded in this preparation was a large variability in the phasing of the rhythm. Normally swims began with extension, closely followed by flexion and then repeated extension-flexion cycling. However, on

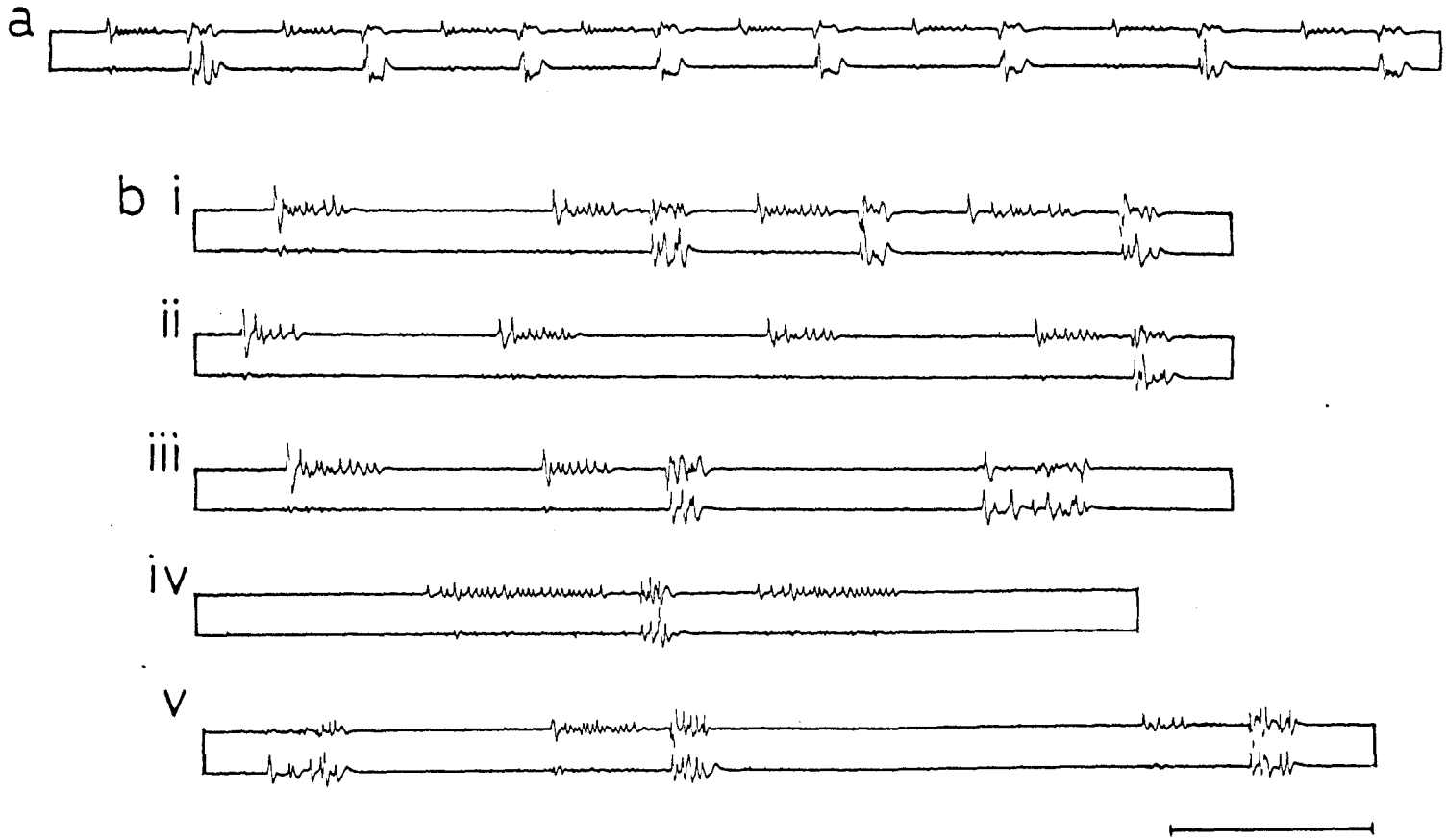
FIG. 38 a, b.

EMG activity recorded in phasic extensor (top trace) and flexor muscles (bottom traces) in the 2nd abdominal segment in a semi-restrained preparation.

a. A typical high frequency, systained swimming response in a rested animal, to tactile stimulation of the ventral thorax. Note the almost constant frequency of swimming.

b i - v. A variety of responses recorded in the same preparation after frequent stimulation. There is a small amount of extensor crosstalk on the flexor EMG and a large amount of flexor crosstalk on the extensor EMG.

Scale bar: 200 ms a, b.


a
b i
ii
iii
iv
v

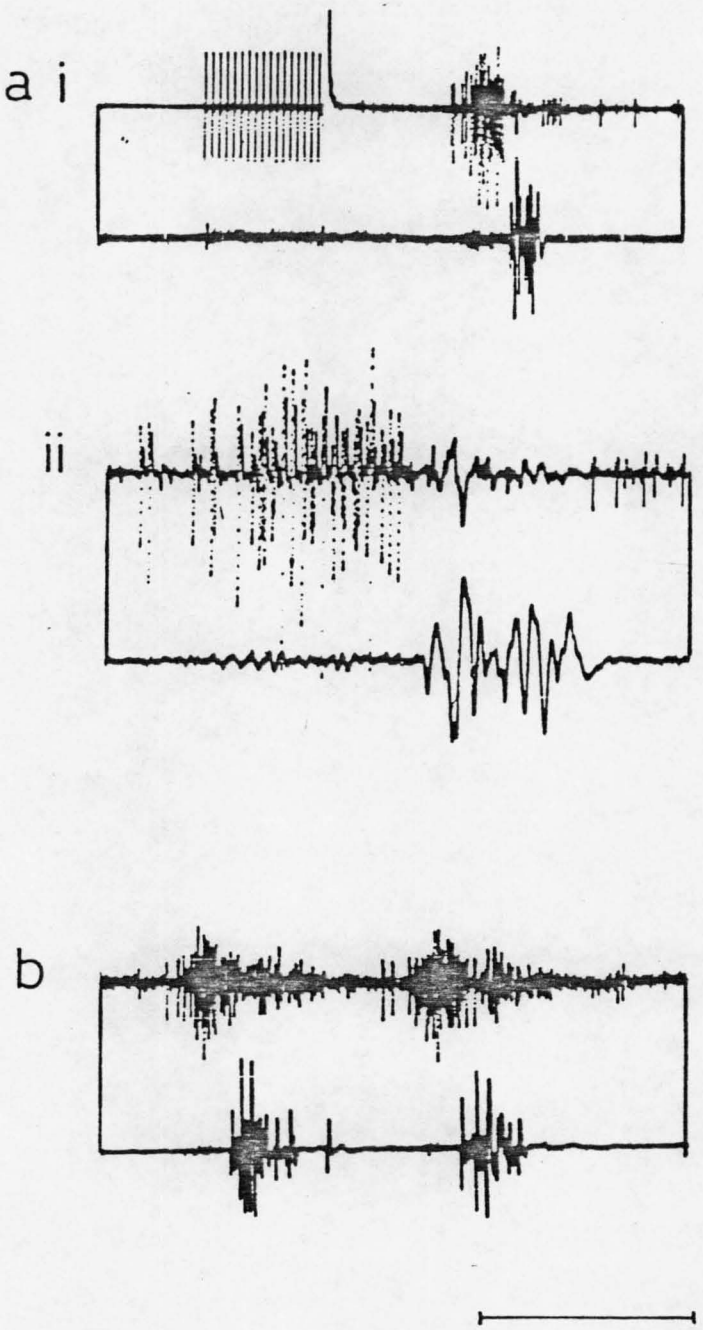
several occasions, particularly in the latter stages of an experiment after frequent stimulation several variations were observed. Swims began with up to four bouts of extensor muscle activity without accompanying flexion (Fig. 38 b i, ii): ended in flexion without prior extension (Fig. 38 b, iii); ended in extension without subsequent flexion (Fig. 38 b, iv); or began with flexion (Fig. 38 b, v). Thus under these conditions extension and flexion were not always coupled.

3. Fully restrained preparation

The aim of this section is to examine backwards swimming under conditions in which normal exteroceptive and proprioceptive feedback are both disrupted. Animals were secured to the sylgard bottom of a petri dish with the abdomen pinned in an extended position. All nerve roots were left intact and the animal induced to swim by tactile stimulation of the ventral thorax. EMG electrodes inserted into phasic flexor muscles and hook electrodes placed on 2nd roots monitored the occurrence of swimming activity (Fig. 39). Swimming consisted of short bouts of extensor and flexor activity similar in phase to those recorded in free swimming and semi-restrained preparations. However, the normal response consisted of only one or two cycles of activity. In each case extension preceded flexion with flexion following at short latency. 2nd root activity sometimes continued for a significant portion of flexion phase (Fig. 39, b). Extensor efferent discharge in deafferented preparations (see below) ceases abruptly at or prior to the onset of flexion. Consequently the extra activity recorded here is either sensory reafference induced by extension or motor discharge induced by reafference (or both). This activity may be generated either by active muscular contractions in the abdomen or by slight joint movement induced by those contractions.

FIG. 39 a, b.

Examples of swimming activity recorded in the 2nd abdominal segment of fully restrained animals in response to r2 stimulation (a) and tactile stimulation of the ventral thorax (b). In each example the top trace is r2 activity and the bottom trace is an ipsilateral flexor EMG.
a ii is an expanded version of a i. Note the r2 activity following the main extensor burst (see text).
Scale bar: 200 ms a i, b; 80 ms b.


a i
ii
b

Hence it is difficult to deduce whether this information is proprioceptive or exteroceptive. In some preparations one or more r2 units fired a burst of action potentials following the end of flexion. The absence of such activity in deafferented preparations suggests that the neurons involved are afferents. Their firing pattern in relation to flexion indicates that they may be MRO spikes. However I have been unable to demonstrate this conclusively.

4. Deafferented preparation

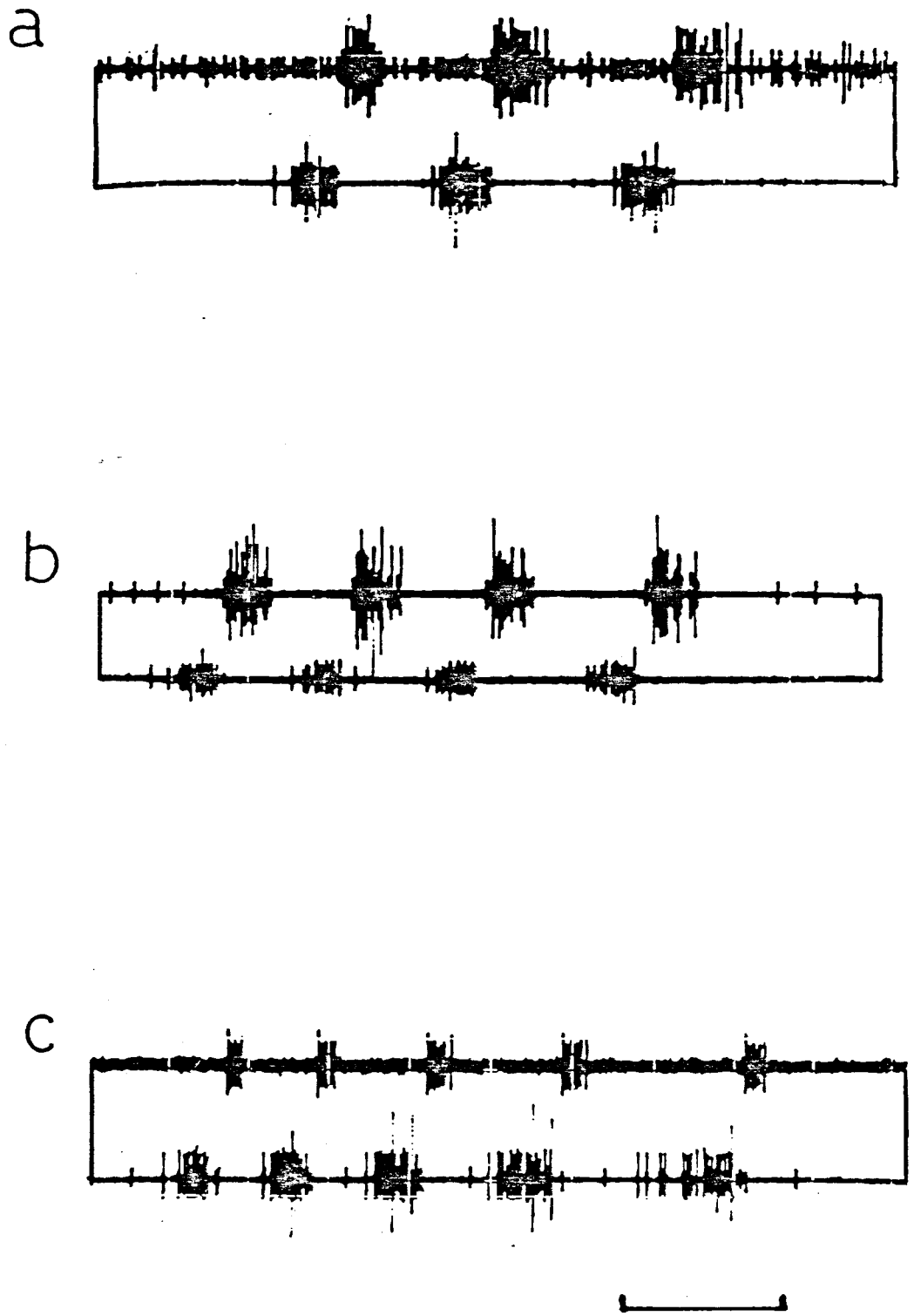
The motor programme underlying rhythmical abdominal cycling can be recorded in preparations in which all abdominal roots have been surgically cut (Fig. 40). Thus the fundamental motor programme for swimming is probably generated centrally. Swimming continues after removal of the brain but not after transection of the nerve cord above G1 (as in crayfish), suggesting that the CPG is located either in the suboesophageal or thoracic ganglion.

In the absence of any sensory feedback from the abdomen up to 7 cycles of swimming activity have been recorded from severed abdominal roots, although two or three cycles is more common (Fig. 40). In each case frequency gradually declined over a bout of swimming. Extension always preceded flexion with flexion following at short latency and hence the internal phase relationships were qualitatively similar in this preparation to the previous three. A more detailed analysis of the components of the motor programme for swimming is presented in section B of this chapter based on experiments in deafferented preparations.

FIG. 40 a - c.

Swimming activity recorded from ipsilateral 2nd (bottom traces) and 3rd roots (top traces) of G2 in a deafferented preparation. a, b and c are examples from three different prparations. Note the gradual decline in swim frequency in each example.

Scale bars: 200 ms a, b; 400 ms c.


a
b
c

Comparison of 4 preparations

Swimming activity recorded in each of the four preparations differed quantitatively in frequency and duration (Fig. 41). Qualitatively, however, the phasing of the rhythm was identical in each case, with extension normally preceding flexion. Deprived of all sensory feedback from the abdomen swim frequency declined gradually in each bout and bouts normally lasted for 3 or 4 cycles (Fig 41c). The presence of sensory feedback in a variety of forms had both excitatory and inhibitory effects on the motor programme for swimming (Fig. 41 a, b, d).

Excitatory effects on the CPG.

When proprioceptive feedback is intact, but exteroceptive feedback disrupted, swim frequency is maintained relatively constant and the duration of each bout is longer than in other preparations. Proprioceptive feedback, therefore, has excitatory effects on the CPG for swimming. The particular proprioceptors responsible for this phenomenon have not been identified nor their mode of action investigated. However in the light of previous evidence the MRO's appear possible candidates for three reasons.

1. The MRO's make powerful direct connections with abdominal swim motorneurons and hence are capable of increasing the firing frequency of these cells during swimming. This pathway need not however increase the overall frequency of the rhythm and could only do so if the motorneurons themselves had access to the central pattern generating circuitry. There is, as yet, no evidence for this in Galathea (or in crayfish).

FIG. 41 a - d.

Comparison of the frequency and duration of swimming activity recorded in the four preparations described in the text. Each graph includes data from two examples of the same preparation in different animals (circles and triangles). Data is plotted as cycle period against cycle in bout.

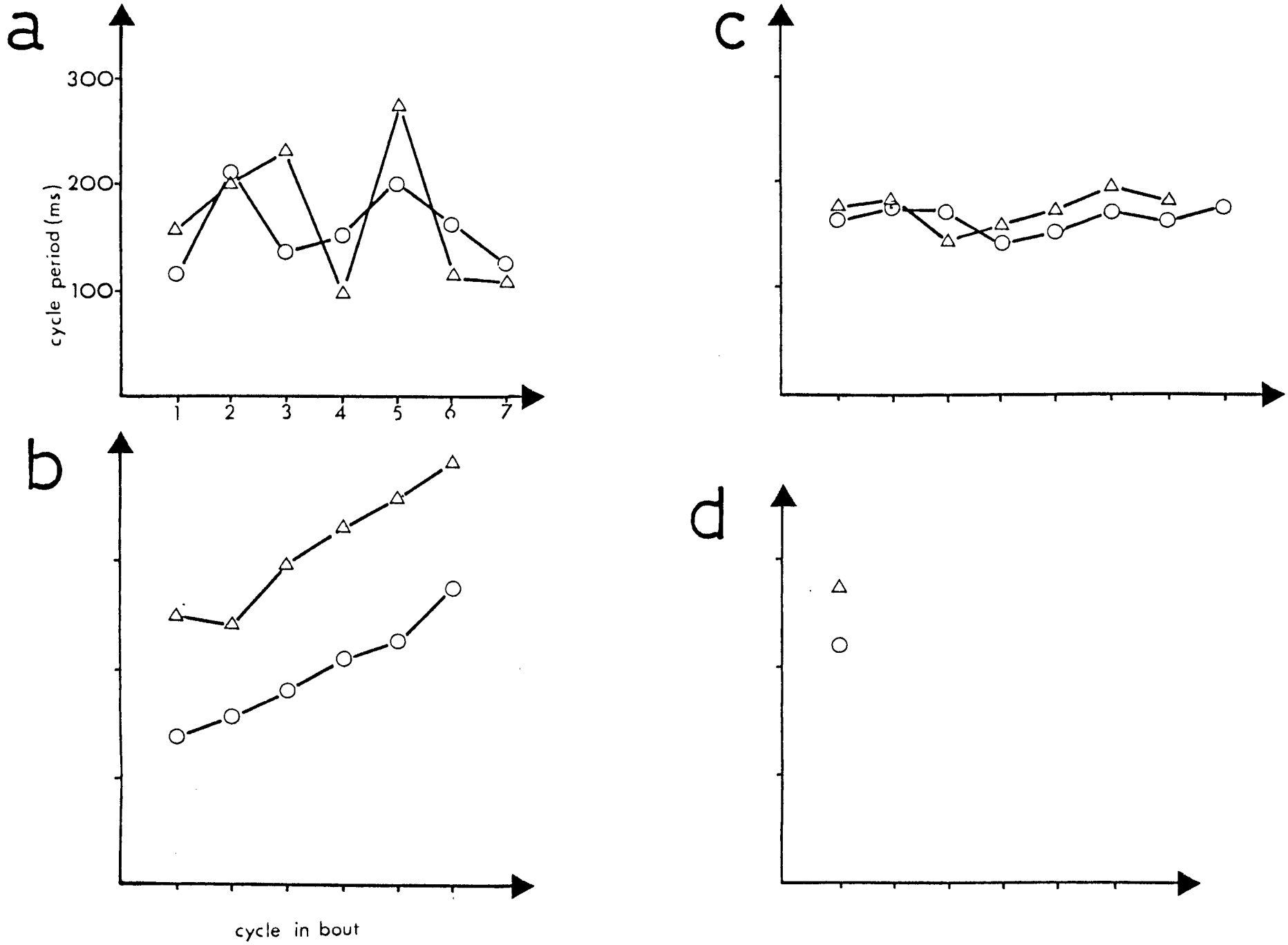
a. Freely swimming animals in response to constant threat. Note the highly variable frequency.

b. Deafferented preparation. Note the gradual increase in cycle period.

c. Semi-restrained preparation. Note the relatively constant swim frequency.

d. Fully-restrained preparation. Swimming activity never lasted more than two cycles.

See text for full description of each preparation.


a
300
200
100
cycle period (ms)
1
2
3
4
5
6
7
b
cycle in bout
c
d

2. The MRO's have axons which travel apparently the entire length of the CNS and therefore project into the region where the CPG is thought to be located

3. The MRO's are the major identified proprioceptors in the abdomen and are known to respond phasically to tail flexions.

At least 8 pairs of MRO's converge on the CNS in Galathea and each projects a large distance in the cord. Thus the powerful central effects of the single MRO demonstrated in the previous chapter are amplified considerably during swimming. On the basis of the foregoing evidence an hypothesis for MRO function during swimming is that ascending activity induced by tail flexion interacts with the CPG to set the overall frequency of the rhythm. If this is the case then the intraganglionic excitatory effects may ensure that the motorneurons fire at a frequency appropriate to the output frequency of the CPG.

Inhibitory effects on the CPG.

In the fully restrained preparation, the CPG is clearly inhibited. This inhibition must derive from sensory feedback. The MRO's have only known excitatory effects on the CNS, but it cannot be ruled out completely that these or other proprioceptors have inhibitory effects on the CPG when their normal firing pattern is disrupted. It seems more likely however that CPG inhibition is mediated by exteroceptive feedback. In freely swimming animals for example, exteroceptive feedback generated by contact with enviromental obstacles was sufficient to terminate swimming (Fig. 37 c). Under constant threat animals swam despite repeated contact

with the experimental chamber, but at low and highly variable frequency (Fig. 41a). This can be attributed to exteroceptive feedback inhibition of the CPG, the behaviour was repeatedly switched on by constant threat and off by mechanical interference. In the fully restrained preparation the CPG is constantly inhibited by exteroceptive feedback through prolonged contact with the substrate. However a powerful tactile stimulus can override this inhibition to produce one or two cycles. Interestingly, exteroceptive feedback can either switch on or switch off swimming behaviour. The loci for these different effects and their complex interactions have not been investigated.

B. THE MOTOR PROGRAMME

1. Induction of fictive swimming

The motor programme underlying backward swimming was recorded via *en passant* extracellular electrodes from the nerve roots of deafferented abdominal ganglia in restrained animals (as in preparation 4 of preceding section). Swimming behaviour was induced in one of two ways:

(i) Tactile stiulmation

A tactile stimulus to the ventral thoracic carapace, head or leg stumps was often sufficient to elicit swimming activity. Gentle stroking with the blunt end of a glass pipette (or similar blunt object) was found to be the most successful method. Up to seven consecutive swim cycles have been recorded in this way. Occasionally a similar stimulus delivered to the uropods or telson was also successful, but less frequently. Swims recorded in this way were identical in all essential respects to those evoked by thoracic stimulation.

(ii) Electrical stimulation

A high frequency electrical pulse train delivered to sensorimotor 2nd roots of abdominal ganglia via extracellular electrodes was also successful in eliciting swimming behaviour in many preparations. Normally, 0.5 ms square wave pulses at 50-100 Hz for 100-200 ms were applied. The stimulus intensity required to elicit swimming was highly variable, though 2 to 3 volts via hook electrodes was sufficient initially. The threshold stimulus tended to rise during the course of each experiment. Trains of stimuli delivered more frequently than once every two minutes resulted in a rapid decline in the probability of a response and normally

caused an increase in the threshold stimulus intensity required to induce swimming activity. It is assumed that this technique mimics a strong sensory stimulus to the appropriate abdominal segment. However the technique is very unspecific and probably involves the activation of mechanosensory units, joint receptors, the MRO's and antidromic stimulation of fast and slow extensor motorneurons since all of these have axons in the second root. The resulting motor programme for swimming is indistinguishable from that elicited in response to tactile stimulation, however the number of swim cycles was rarely more than three. The delay between the onset of stimulation and evoked swimming activity was also highly variable and ranged between 100 ms and 1 second, approximately.

2. Characteristics of the motor programme

The motor programme for backward swimming is a fast, rhythmic, biphasic response, consisting of an initial extension phase followed at short latency by flexion (Fig. 42). These fundamental components underlying rhythmic abdominal cycling can be recorded from r2 and r3 in preparations in which all abdominal roots have been cut. In the majority of experiments however, and unless otherwise stated, nerves arising from the terminal G5 innervating the uropods and telson were left intact to increase the overall excitability of the preparation. When these nerves were cut recordings of the motor programme were identical in all essential respects to those recorded in the normal preparation

Cord activity during 'swimming'

During 'swimming' there is a general increase in dorsal cord activity (DCA) which involves a high amplitude burst in phase with flexion (Fig. 42c). Since at all points in the dorsal cord between Th.Ab.G and G5 there occur the axons of FF's it is assumed that the ascending activity of these

FIG. 42 a - c.

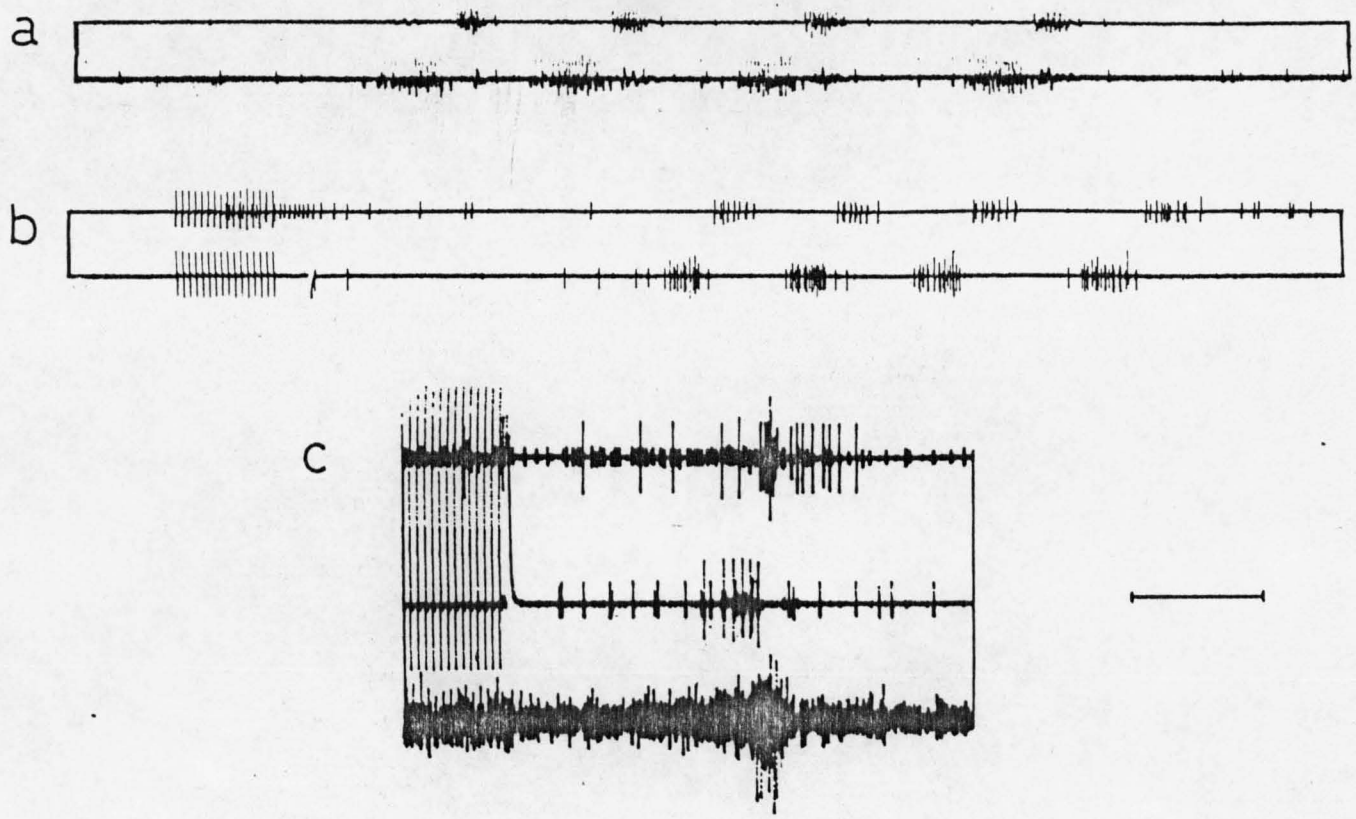
The motor programme underlying swimming, as recorded in r3 (top traces) and r2 (2nd traces) of G2 in deafferented preparations.

a. 4 cycles of swimming activity induced by tactile stimulation of the ventral thorax.

b. Swimming activity induced by high frequency electrical stimulation of r2.

c. A single cycle of swimming activity during which neural activity in the dorsal cord above G1 was monitored (bottom trace). Top trace = r2; 2nd trace = r3.

Scale bar: 200 ms.


a
b
c

units contributes to DCA in phase with flexion. With the exception of these there are no giant fibre spikes in DCA. In its overall appearance DCA is very similar to DCA in crayfish during non-giant swimming (Schrameck, 1970). It was impossible to determine the number of cord axons active during swimming. The response is undoubtedly complex and involves many units.

Cycle period

Cycle period was measured as the interval between the onset of phasic extension in one cycle to the onset of phasic extension in the next. Cycle period varied between a minimum of about 100 ms (10 Hz) and a maximum of about 600 ms. In a bout of swimming, cycle period was short initially and increased in later cycles, almost doubling over six cycles in most cases (Fig. 43a) (mean increase 74%; n = 4). However this increase did not occur between the first and second cycles in most bouts of swimming. The cycle period of the 1st cycle in each bout was often longer than that of the second cycle. The reason for this is that extensor burst duration is significantly longer relative to cycle period in the first cycle than in subsequent cycles (Fig. 43b). Thus, in cases where cycle period of the first cycle in a bout was shorter than in the second, extensor burst duration always occupied a greater proportion of cycle period in the first cycle.

Extension phase.

Both fast and slow extensor efferents have axons which exit r2. In many recordings of extensor discharge during swimming there is a background level of activity in tonically active motorneurons. This activity is recorded normally with a poor signal to noise ratio compared with activity in phasic extensor efferents. Presumably, this feature reflects

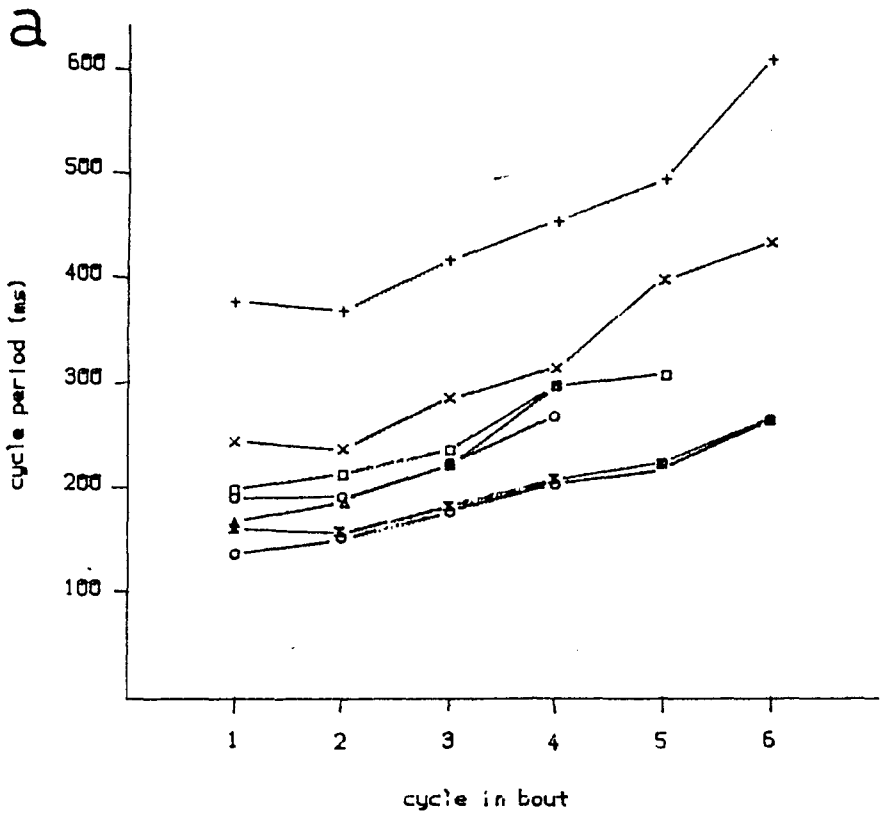
FIG. 43 a, b.

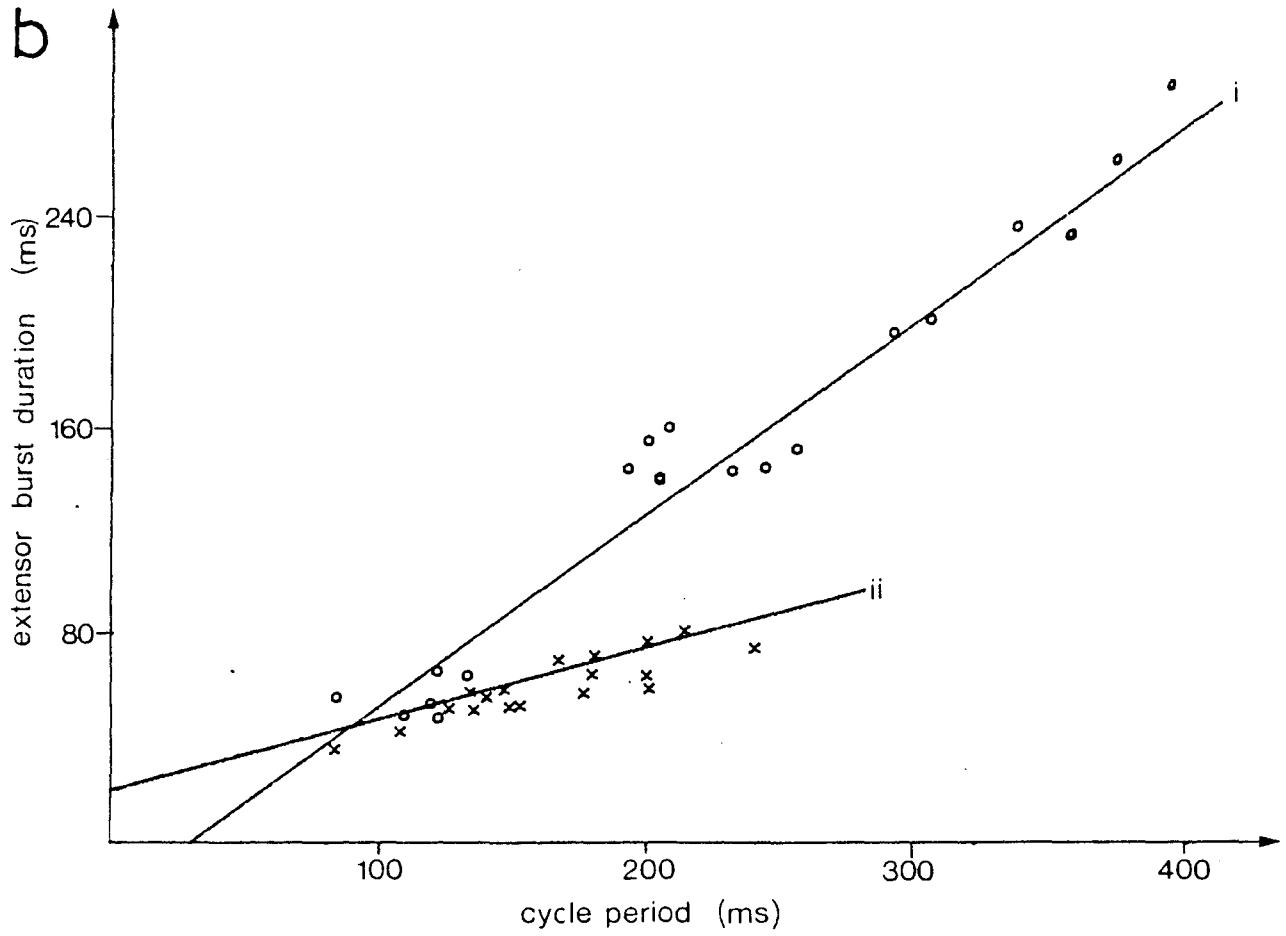
a. Plots of cycle period against cycle in a bout for 7 different bouts of swimming in deafferented preparations. Note the gradual decline in swim frequency. Note also that in three examples, cycle period is less in the 2nd cycle than in the 1st (see text for explanation).

b. Graphs of extensor burst duration versus cycle period for the 1st cycle in a bout of swimming (i) and for subsequent cycles (ii). The extensor burst in the first cycle is normally longer than subsequent extensor bursts (see text).

i. $R = 0.982$; $y = 0.65\,x - 21$; $n = 19$;

ii. $R = 0.956$; $y = 0.27\,x + 20$; $n = 18$.


a
600
500
400
300
200
100
cycle period (ms)
1
2
3
4
5
6
cycle in bout


b
extensor burst duration (ms)
240
160
80
100
200
300
400
cycle period (ms)
i
ii

the relative diameters of the axons involved and was not a problem when measuring the onset or offset of phasic extension during swimming. The difficulties involved in discriminating between the activity of different neurons on the basis of their extracellular spike height precluded an accurate determination of the number of units firing during phasic extension. The approximate number varied between experiments, between different bouts of swimming in the same preparation and between successive cycles in the same bout. In many cases the number of units involved in phasic extension declined towards the end of each experiment and occasionally towards the end of each bout.

In many recordings larger units are recruited in the latter half of each extensor burst (Fig. 42). One interpretation of this phenomenon is that larger units have a higher threshold for spike initiation than do smaller units. This interpretation assumes that the available pool of extensor efferents receives common input during swimming and that extracellular spike height is a reflection of neuron size. There remains the possibility that larger r2 units have similar spike thresholds but that their driving input is delayed with respect to smaller r2 units. Only intracellular recordings from these neurons can decide which, if either, of the two hypotheses is correct. The large amplitude of spikes occurring later in extension may be due to the collision of two smaller spikes since several extensor efferents fire at high frequency during extension.

The duration of extension varies directly with cycle period (Fig. 43b). The duration of the 1st cycle extension in a bout is significantly longer than subsequent extensor burst durations relative to cycle period. Initial extension occupies a mean 0.58 of cycle period while subsequent extensions comprise only 0.35 of cycle period. Over a wide range of swim frequencies

of about 2-10 Hz both initial extensions and subsequent extensions increased linearly with cycle period. The two regression lines cross over at a cycle period of 90 ms which approaches the maximum swim frequency recorded. A single FE motorneuron appears to be responsible for this increase in the duration of extension in the first cycle. It fires earlier in the first cycle than in subsequent cycles, usually at initial low frequency.

Flexion phase

Flexor burst duration also increases linearly with cycle period but is much shorter than extensor burst duration (Fig. 44) occupying, on average, 0.22 of cycle period. Consequently flexor burst duration is difficult to measure accurately when cycle period is short. Unlike extension, the first flexion in a bout of swimming is not longer than subsequent flexions relative to cycle period. Each flexor burst occupies a relatively constant proportion of each cycle. Similar problems were encountered for flexion when attempting to analyse the number of FF's firing in each cycle. It appears that several FF's discharge at high frequency during flexion and the activity patterns of individual FF's were impossible to determine due to the intensity of each burst.

Extension-flexion coupling

Previous reports on backwards swimming in crayfish (Wine and Krasne, 1982) have shown that during non-giant escape, extension leads flexion with flexion following at near constant latency. I find that over a range of cycle periods the latency between extension and flexion increases linearly with cycle period (Fig. 45). The correlation coefficient for the relationship between the two is extremely high and indicates that the onset of flexion relative to extension is highly dependent on cycle period.

FIG. 44.

Graph of flexor burst duration against cycle period over a range of swim frequencies. The linear relationship is highly significant.

$R = 0.955$; $y = 0.23x - 4$; $n = 30$.

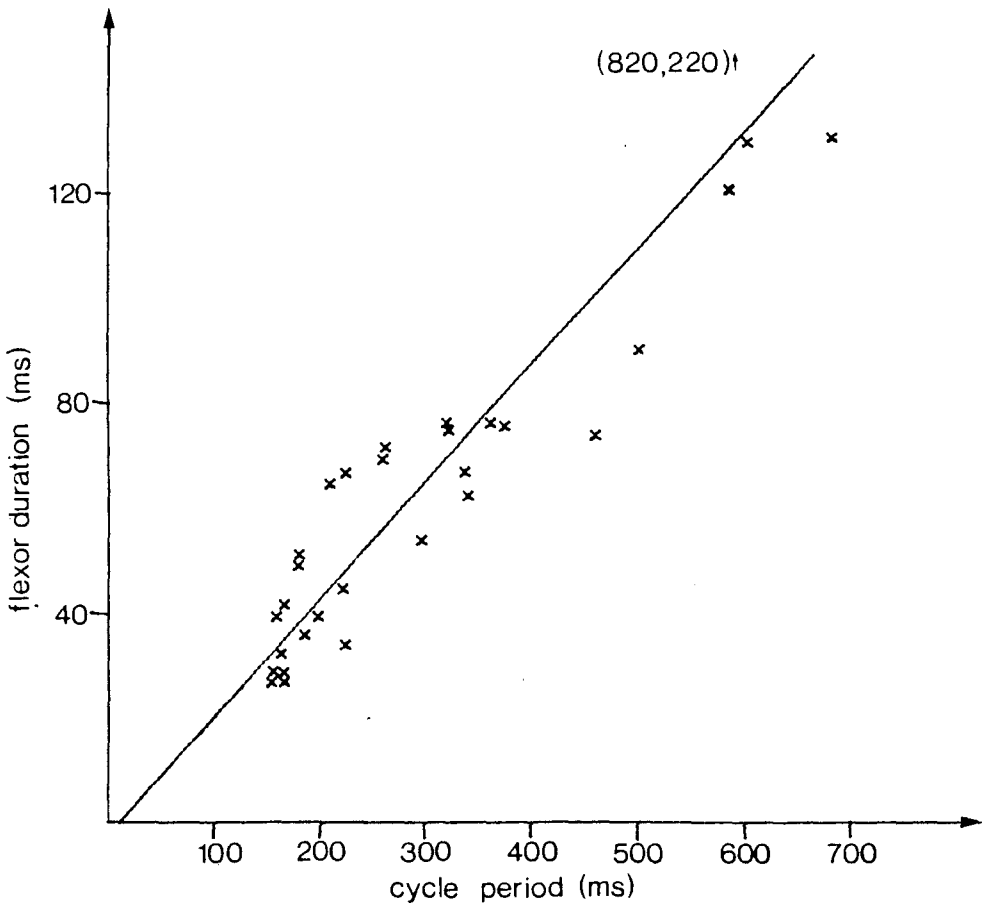
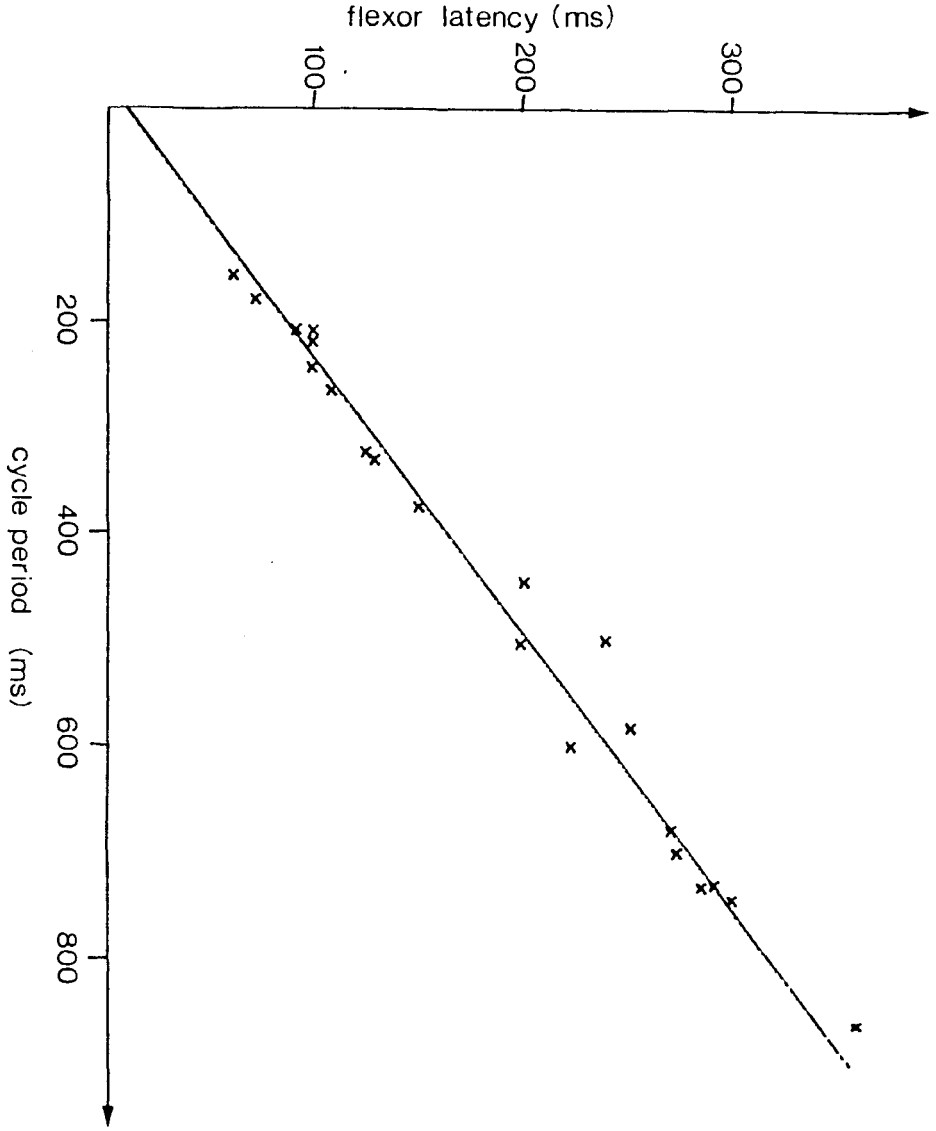

(820,220)
120
80
40
flexor duration (ms)
100
200
300
400
500
600
700
cycle period (ms)

FIG. 45.

Extension-flexion coupling. The graph depicts flexor latency from the onset of extension, against cycle period over a range of swim frequencies. The correlation coefficient, R = 0.99, is highly significant.

$y = 0.38\ x + 9.$ $n = 21.$


flexor latency (ms)
100
200
300
cycle period (ms)
200
400
600
800

Bilateral coupling

The motor programme for swimming in G1-G4 is bilaterally symmetrical. This is particularly evident in recordings of second root activity from the left and right sides of one abdominal ganglion (Fig. 46a). The extensor motorneurons discharge at almost exactly the same time and in most cases, particularly during the prolonged initial extension phase, it is possible to identify individual units which have very similar firing patterns. These neurons are presumably left/right homologues. The high degree of synchrony may reflect common inputs driving these neurons during swimming. The high level of bilaterally synchronous discharge is evident in swims evoked by tactile stimulation. High frequency r2 stimulation also produces highly synchronous extensor motorneuron activity during swimming, regardless of whether the right or left r2 is stimulated (Fig. 46 b, c). In fact under no experimental conditions have asynchronous motorneuron discharges been observed from the roots of G1-G4. Thus it is possible that the facility for directional sensitivity observed in the intact, freely swimming animal, does not occur at the level of the contraction of abdominal muscles. This facility must reside at some other locus such as asynchronous flaring of the uropods or movements of the legs. Flexor motorneuron discharge also shows a high degree of bilateral synchrony (Fig. 46d).

Intersegmental metachrony

A motor output pattern, identical in its internal phase relationships can be recorded from the cut roots of each of G1 to G4 during swimming. Multiple recordings from homologous roots of successive ganglia reveal a metachronal wave of excitation which passes in an anterior to posterior direction. In Fig. 47a extracellular recordings from ipsilateral 2nd

FIG. 46 a - d.

Bilateral symmetry of the swimming rhythm.

a - d are extracellular recordings of swimming activity in deafferented roots of G2 from four animals.

a - c; top trace = r3 G2; 2nd trace = r2 G2 ipsilateral; 3rd trace = r2 G2 contralateral.

d: top trace = r3 G2 ipsilateral; 2nd trace = r3 G2 contralateral. 3rd trace = r2 G2

a, d. Swimming activity in response to tactile stimulation.

b. Swimming activity evoked by r2 G2 ipsilateral stimulation.

c. Swimming in response to r2 G2 contralateral stimulation.

Note the highly symmetrical responses from homologous roots.

Scale bar: 200 ms a; 400 ms b - d.

a
b
c
d

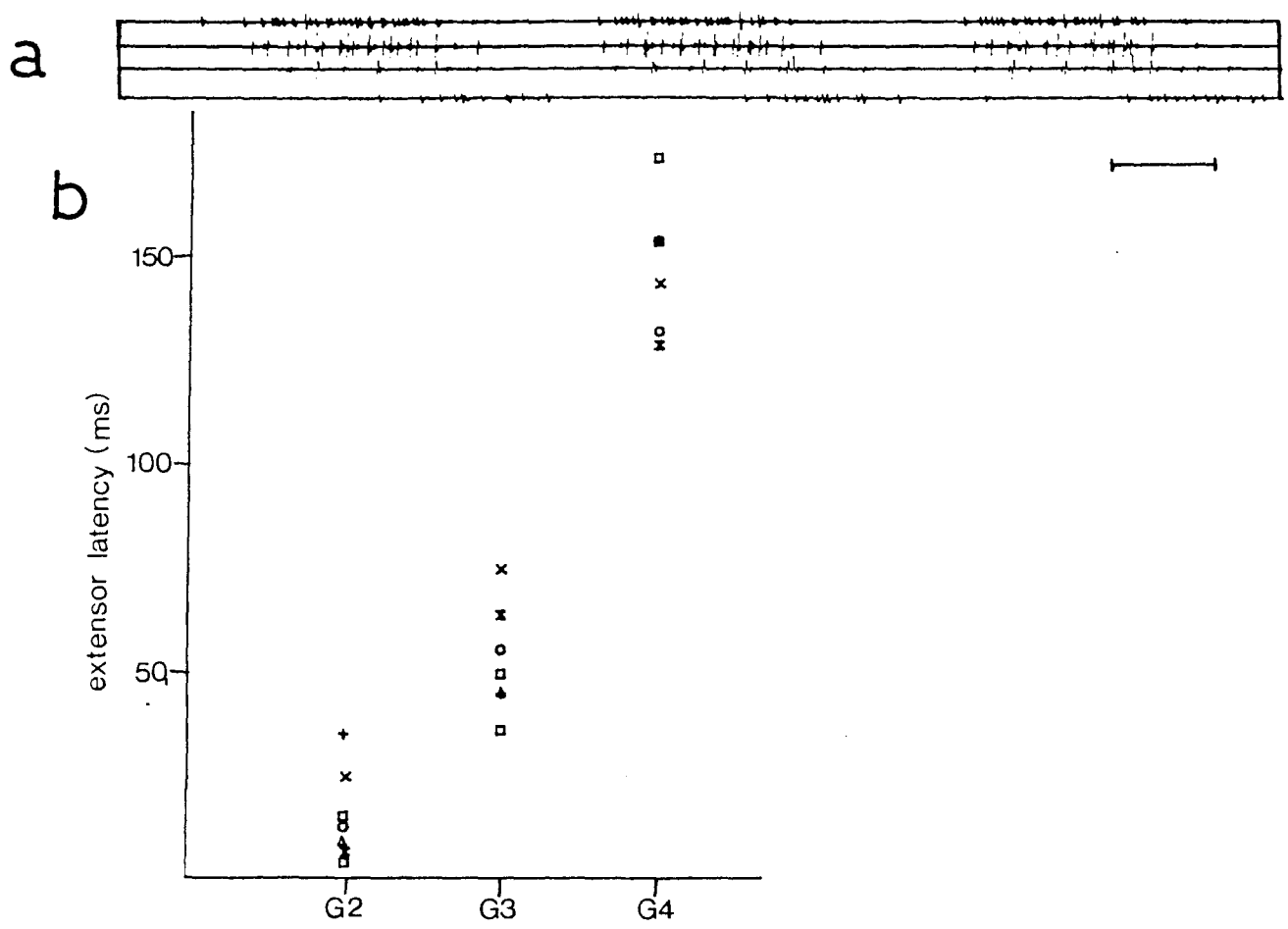
FIG. 47 a, b.

Intersegmental metachrony of phasic extension.

a. Physiological record of r2 activity during swimming in G1 (top trace), G2 (2nd trace), G3 (3rd trace) and G4 (bottom trace). Note the intersegmental latency increases in caudal segments.

b. Plot of latency of extension in G2, G3 and G4 relative to extension in G1 for 7 bouts of swimming. Measurements were made from records similar to that shown in a.

Scale bar in a = 100 ms.


a
b
150
100
50
extensor latency (ms)
G2
G3
G4

roots of G1 to G4 have been made. The precise timing of extension in two successive ganglia was found to be difficult to measure and rather variable. The latency between extension in one ganglion and extension in the next posterior ganglion increases in a caudal direction (Fig. 47b). Thus the latency between G1 and G2 extension is less than between G2 and G3 which is less than between G3 and G4.

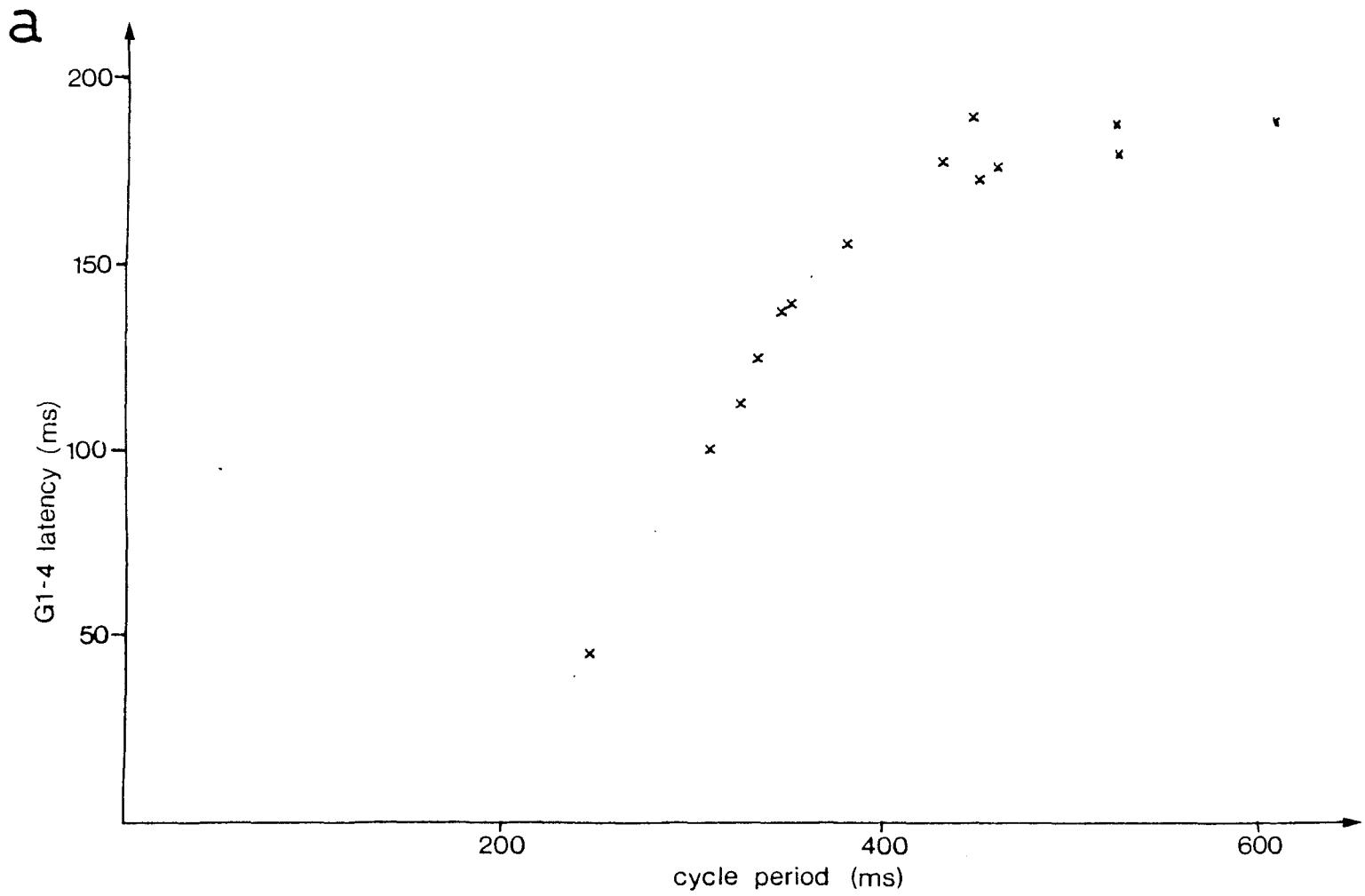
The latency between the onset of extension in G1 and the onset of extension in G4 increases with cycle period (Fig. 48a). For most of the range of cycle periods analysed the relationship is linear. When cycle period is long, however, the G4 extension latency relative to G1 reaches an apparent maximum and over the available range of cycle periods never exceeded 190 ms. The latency between extension in two adjacent ganglia also increases with cycle period, as shown for G1-G2 extension latency in Fig. 48b.

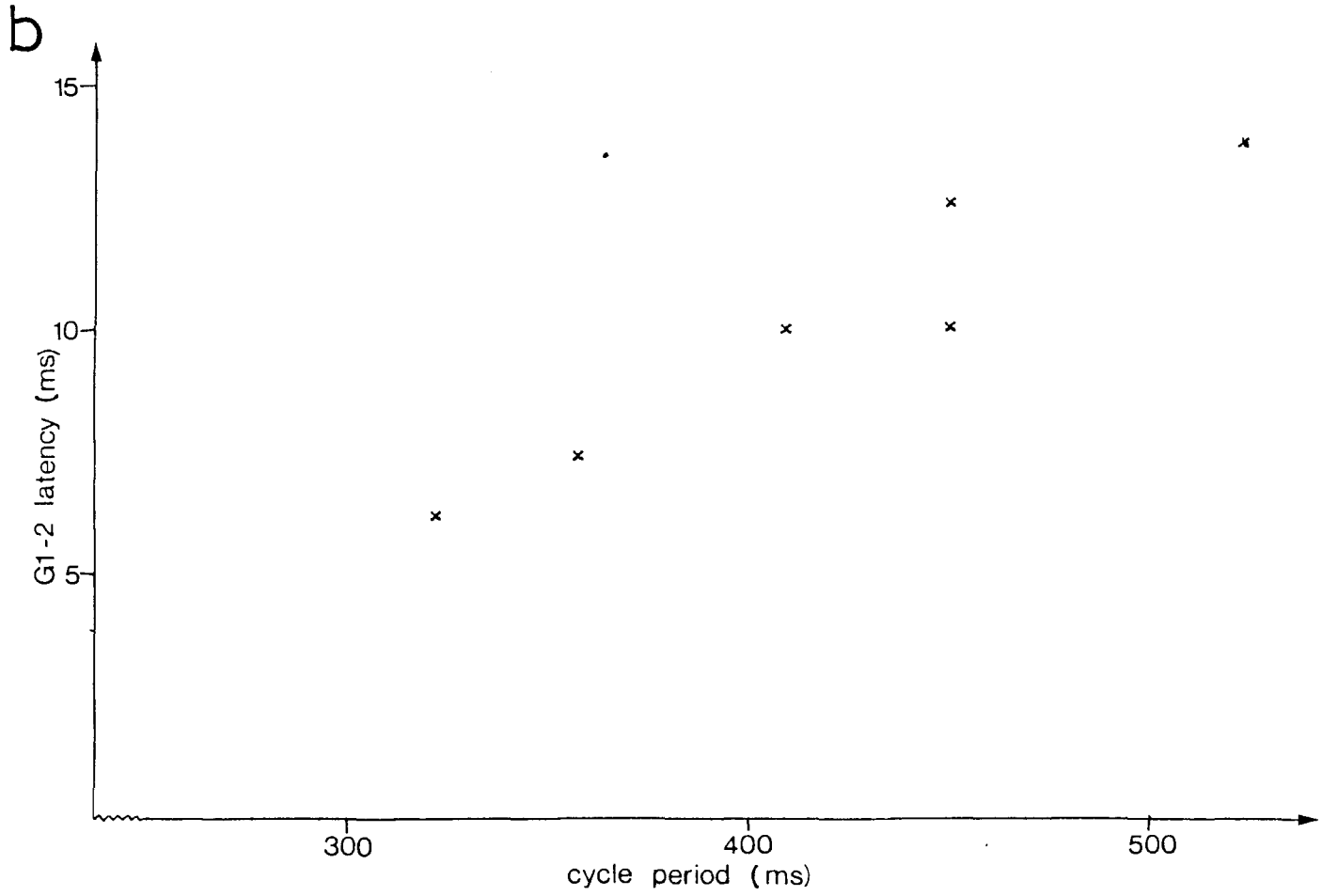
FIG. 48 a, b.

Dependence of intersegmental extensor latency of cycle period.

a. Graph of the delay between the onset of extension in G1 and G4 against cycle period. Note the apparently linear relationship at low cycle periods. At cycle periods in excess of about 400 ms, G1 - G4 latency reaches an apparent maximum of about 190 ms. Data points are taken from physiological records of a number of different bouts of swimming in the same preparations.

b. Graph of the delay between the onset of extension in G1 and G2 against cycle period for one bout of swimming. Note the gradual increase in cycle period and approximately linear increase in G1 - G2 extensor latency.


a
200
150
100
50
G1-4 latency (ms)
200
400
600
cycle period (ms)


b
15
10
5
G1-2 latency (ms)
300
400
500
cycle period (ms)

C. ACTIVITY OF THE MOTORNEURONS

The purpose of this results section is to examine the activity of swim motorneurons during swimming as recorded intracellularly from their somata. The preparation used was similar to that used in the previous section (B. The Motor Programme). The deafferented chain of abdominal ganglia was pinned securely to a wax platform (14 mm x 8 mm approximately) to stabilize the preparation for intracellular recording. Extracellular electrodes placed on 2nd and 3rd roots of abdominal ganglia monitored the occurrence of swimming activitity. Lucifer Yellow filled micro-electrodes were used to penetrate the somata of motorneurons in the ventral regions of the 1st, 2nd or 3rd abdominal ganglion. Penetrations were made through the ganglionic sheath.

Identification of swim motorneurons

Penetrated motorneuron somata were identified using a number of electrophysiological and anatomical criteria:

1. Presence of an antidromic spike with short and constant latency on electrical stimulation of the appropriate nerve root.

2. A 1:1 correspondence of spikes recorded intracellularly with spikes recorded extracellularly on the appropriate nerve root. This was not always possible either because the particular nerve root was not being monitored or because spikes occurred during high intensity firing of other motorneurons in the same nerve.

3. The firing pattern of each neuron during swimming when compared with the extracellularly recorded rhythm was normally an accurate indication of the neurons type.

4. Neurons were routinely injected with Lucifer Yellow. This aided the identification of motorneurons belonging to a functionally homologous group and in some experiments clarified cases where a neurons firing pattern was not an accurate indication of its type.

Fulfillment of each of the above criteria was considered sufficient to identify motorneurons in all intracellular recordings from abdominal ganglia. Since most swim motorneurons form a cluster around the base of the 1st root it was often difficult to locate a particular motorneuron on the basis of its soma position.

Properties of swim motorneurons

Somata penetrated by microelectrodes usually had resting potentials of -45 mV or more, up to a maximum of -70 mV on emergence at the end of a recording. Phasic motorneurons did not spike spontaneously or show any sign of injury discharge upon initial penetration. Normally, spikes could not be elicited in response to depolarizing current pulses injected through the recording electrode (up to 10 nA). Injection of up to 100 nA depolarizing current could usually induce spiking in motorneurons but this was avoided due to its deleterious effects on the cells normal activity. Such large amounts of injected current frequently reduced the probability of firing of a motorneuron during swimming. Some phasic motorneurons showed a low level of spontaneous synaptic activity in quiescent ganglia, particularly FI. Episodes of swimming activity normally resulted in spiking in these motorneurons. Soma spikes reached an amplitude of 2-12 mV and never became positive at peak potential. As with most other arthropod neurons, therefore, the somata of swim motorneurons appear incapable of generating overshooting action potentials (See Kennedy and Davis, 1977 for references). The attenuated spikes recorded intrasomatically

are the result of passive electrotonic conduction from a spike initiating zone located more distally, presumably near the point at which the axon leaves the neuropile. Soma spikes had durations (at half amplitude) of about 2-3 ms.

Fast flexor motorneurons

During periods of induced swimming activity, fast flexor motorneurons (FF's) display large (5-15 mV) oscillations in membrane potential, phase locked to the peripherally recorded rhythm with bursts of attenuated soma spikes superimposed upon depolarized peaks (e.g. Figs. 49, 50). The rhythmic responses of each FF during swimming were qualitatively similar regardless of whether the motorneuron belonged to the FMC or FPI cluster. Most recordings were made from FPI FF's since these were found to be easier to penetrate. MoGH, the neuron homologous with the crayfish MoG (see Chapter 1), has been recorded from and stained intracellularly only once. In accordance with its anatomy, MoGH's firing pattern during swimming was typical of other FF's (Fig. 50). Thus each member of the FMC and FPI FF pool, with the exception of FI (see later) has a qualitatively similar firing pattern and in this respect the FF's are a functionally homologous population.

A characteristic feature of all intracellular recordings from FF's during swimming was an apparent combination of brief unitary synaptic potentials and a large slow wave depolarization underlying FF firing. There is no evidence to suggest that the slow wave does not result from many summed synaptic inputs. However the slow wave is temporally separate from other unitary synaptic potentials and for the purposes of this section the two will be regarded as different phenomena.

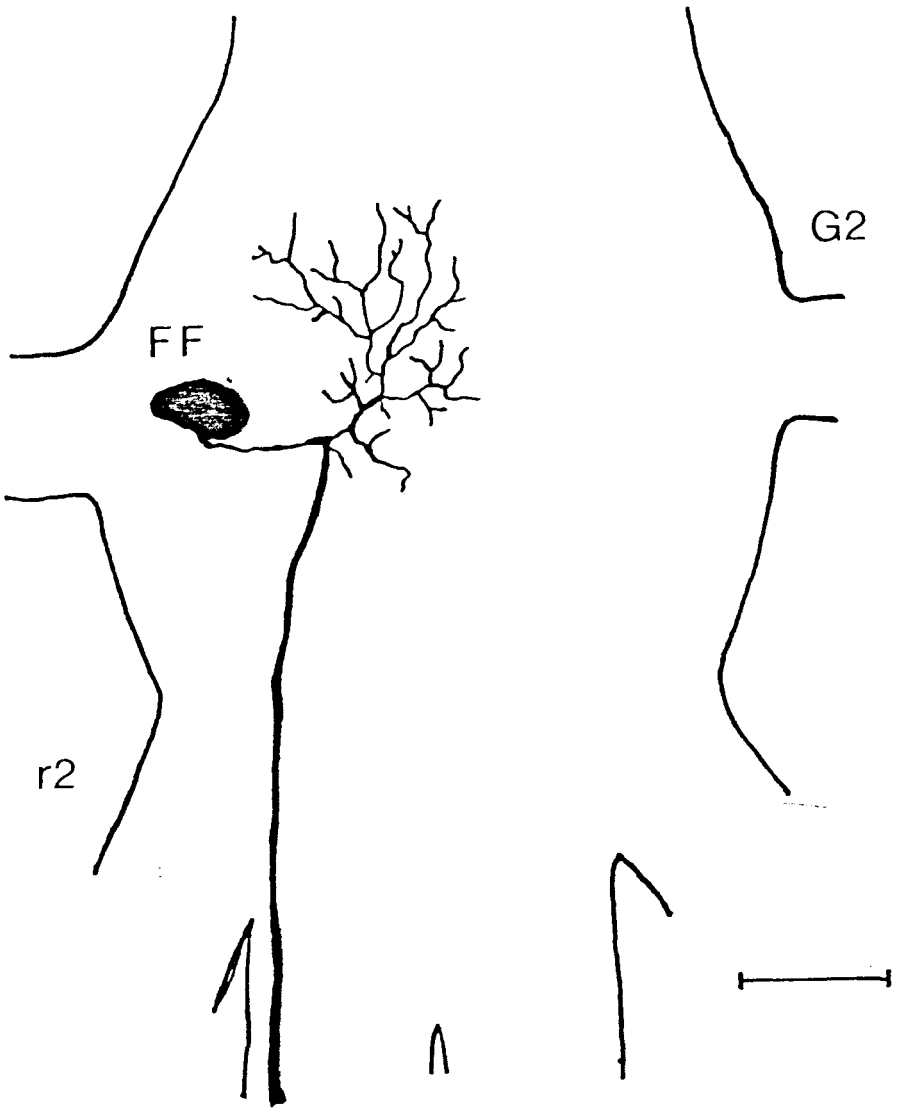
FIG. 49 a, b.

a. Anatomy of a FPI FF stained with Lucifer Yellow.

b. Activity of the neuron drawn in a. during swimming. FF activity (top trace, intrasomatic) displays rhythmical oscillations in membrane potential and bursts of spikes phase-locked to the peripherally recorded rhythm. 2nd trace = r2, 3rd trace = r3. Note the gradual decline inslow wave amplitude and FF spike frequency as cycle period increases.

Scale bars: 200μ a. 100 ms; 4 mV b.

a



b

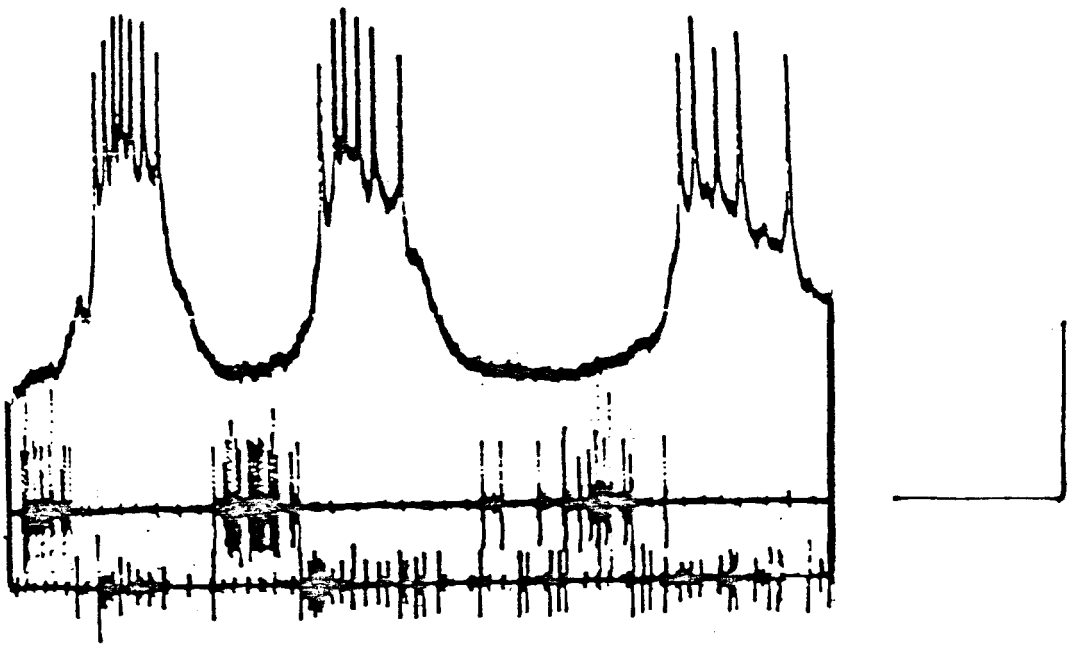


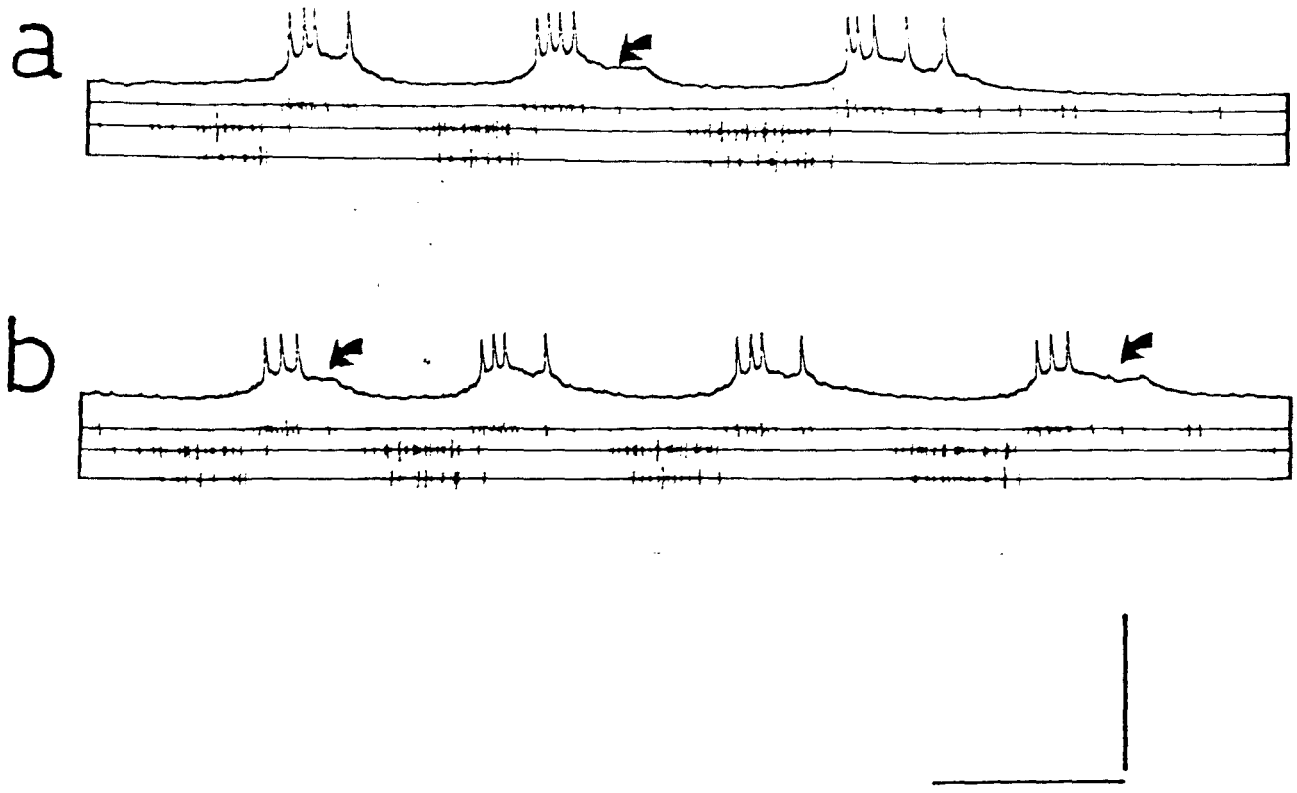
FIG. 50 a - c.

MoGH activity during swimming activity.

a, b. Two examples, from the same preparation, of activity recorded in the soma of MoGH (top traces) 2nd trace = r3 (ipsilateral to axon); 3rd trace = r2 ipsilateral, 4th trace = r2 contralateral. Arrows indicate the occurrence of a ramp-like slow wave potential (see text).

c. The neuron record in a and b, in G2 was filled with Lucifer Yellow and confirmed as MoGH. The activity of MoGH during swimming is qualitatively similar and FPI FF's.

Scale bar: a, b, horizontal 400 ms; vertical 20 mV. c. 100μ.


a
b
c

Slow wave depolarization

The most significant event in the generation of rhythmic FF bursts is the occurrence of a large slow wave depolarization. Slow wave amplitude varied from one preparation to the next. For any one recording there was a clear relationship between the amplitude of the slow wave and cycle period. In bouts of swimming consisting of two or more cycles, an increase in cycle period was accompanied by a decrease in slow wave amplitude and a decrease in instantaneous FF spike frequency (Fig. 49b). The duration of the slow wave also increased as cycle period increased, so that FF's fired for longer and at lower frequency as cycle period increased. In principal at least, pattern generation could be accounted for in terms of the amplitude and duration of the slow wave depolarization. In cases where slow wave amplitude failed to reach or only just reached FF spike threshold the wave form emerged as a smooth potential with little evidence of superimposed discrete synaptic potentials. (Fig. 50 a, b).

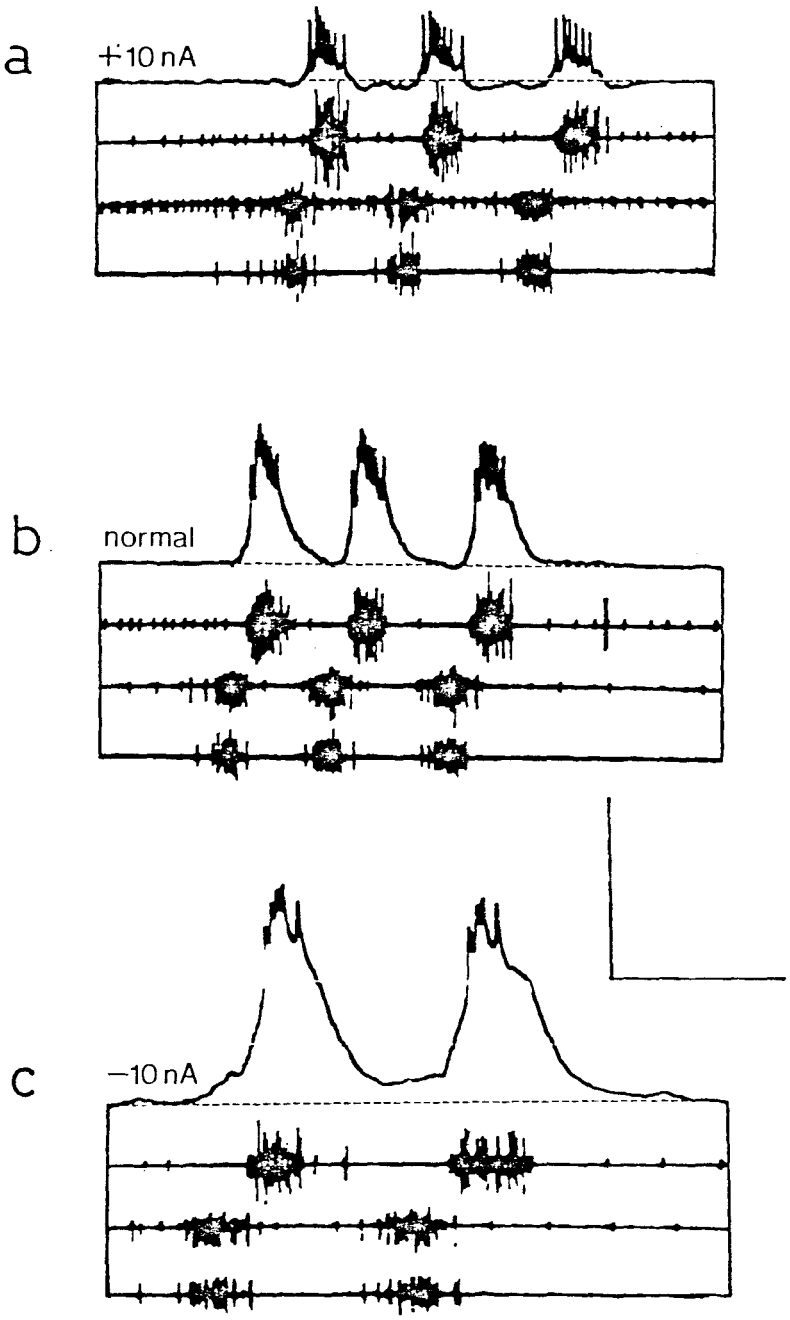
There is direct evidence however that the smooth oscillations result from strong synaptic input to the motorneurons (Fig. 51). Injection of current via the microelectrode for the duration of a bout of swimming had strong effects on slow wave amplitude in FF's. The magnitude of these effects varied considerably. In most cases a 5-fold change was observed for ± 10 nA current injection but in favourable recordings up to a 10-fold increase was observed (Fig. 51). 10 nA hyperpolarizing current injected into the FF soma of Fig. 51 greatly increased the amplitude of membrane oscillation but did not block spike discharge. With 10 nA depolarizing current, slow wave amplitude was greatly reduced but did not disappear. These current induced effects on the amplitude of the oscillatory waveform imply that FF's receive periodic excitation which is mediated by chemical

FIG. 51 a - c.

Effects of injected current (± 10 nA, not monitored) on FF activity during swimming.

In a, b and c, swimming activity was induced by tactile stimulation of the ventral thorax. FF activity was recorded intrasomatically in G2 (top traces) and extracellular activity in r3 (2nd trace), r2 ipsilateral (3rd trace) and r2 contralateral (4th trace) monitored the occurrence of swimming activity. Prior to swimming, the FF was injected with +10 nA current (a), no current (b) or -10 nA (c) for the duration of swimming. Dotted lines indicate the approximate resting potential of the FF in each case.

Scale bars vertical - 20 mV; horizontal - 400ms a, b; 200 ms c.


a
+10 nA
b
normal
c
−10 nA

synaptic transmission and not by electrical junctions. In Fig. 51c it can be seen that following injection of 10 nA hyperpolarizing current the membrane potential did not return to rest following the FF burst but remained relatively depolarized in the interburst interval. When 10 nA depolarizing current was injected into this cell (Fig. 51a) the membrane potential became slightly hyperpolarized in the interburst interval. Thus, in addition to slow wave excitation, FF's are synaptically inhibited following each flexion phase. This inhibition follows the repolarization phase of the slow wave to ensure that FF's are effectively clamped near resting potential while the extensors are active (Fig. 51b). The inhibitory component is not evident at resting potential but can be phase inverted by current injection. In contrast,the excitatory slow wave depolarization cannot be phase-inverted because its reversal potential probably lies close to spike threshold.

Unitary synaptic input

In favourable preparations a variety of post-synaptic potentials are recorded in FF's prior to, during and following episodes of swimming. Their amplitude at the cells resting potential is invariably small and approaches the noise level of the recording. This is presumably because the somata of the motorneurons are some distance from the synaptic sites in the neuropile and PSP's, like spikes, become attenuated as they are passively propagated back to the recording site. On occasions IPSP's were apparent prior to and following slow wave excitation of FF's (Fig. 52 a, b i, arrowed). Current injected into this FF soma enhanced the amplitude and altered the polarity of unitary synaptic potentials. In Fig. 52 b ii, 10 nA hyperpolarizing current reversed the polarity of these IPSP's while in Fig. 52 b iii 10 nA depolarizing current enhanced the amplitude of mid-cycle inhibition. In the 20 ms or so prior to slow wave

FIG. 52 a, b.

Evidence for unitary synaptic activity in FF's during swimming.

a. Camera lucida drawing of a Lucifer Yellow stain of a FF in G2 (inset). Dotted line indicates approximate midline of the ganglion.

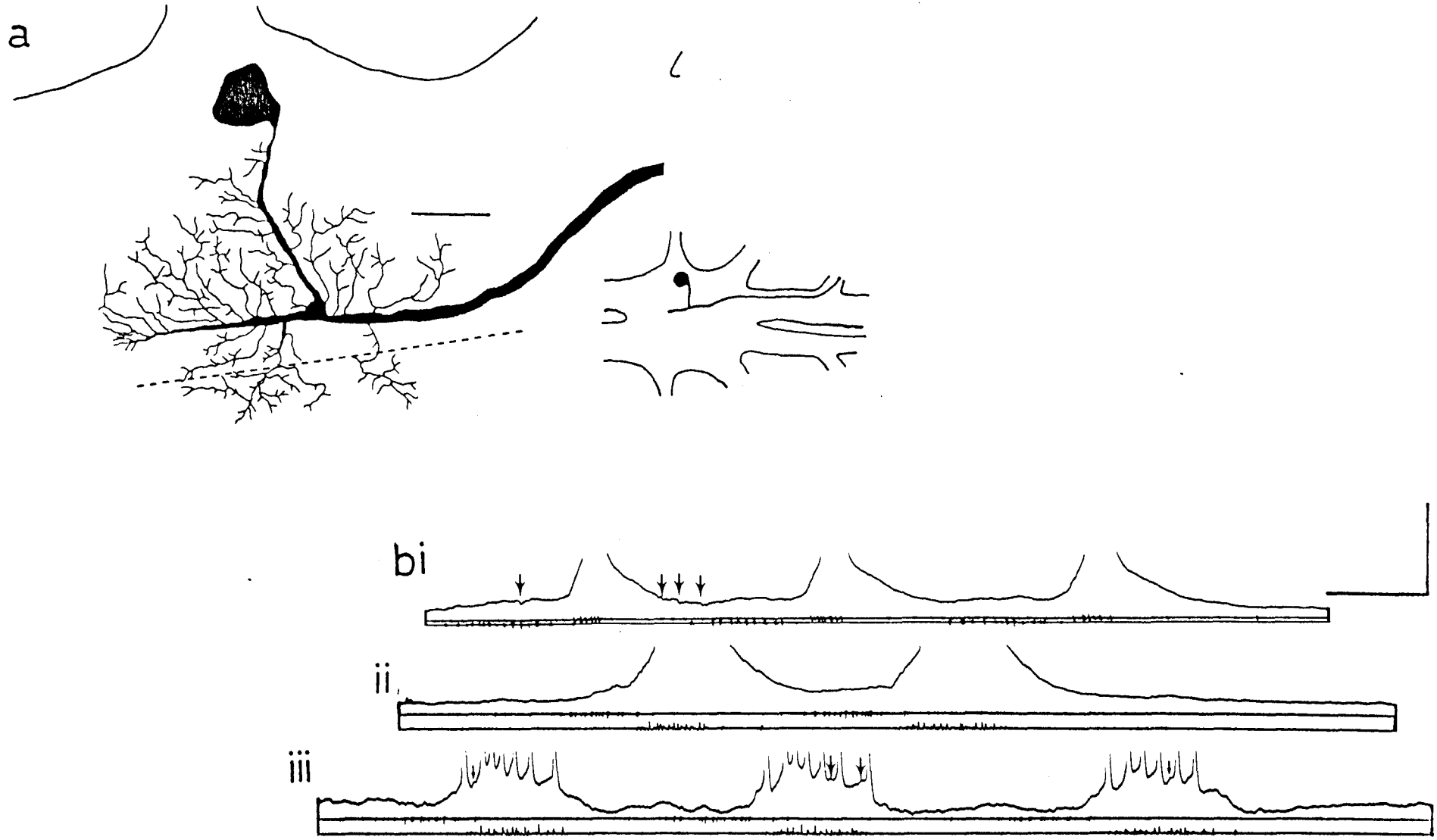
b i - iii. Synaptic potentials and slow wave oscillations recorded in the somata of FF's (ii and iii are recordings from the neuron drawn in a.).

b i. Top trace - FF, 2nd trace - r3, 3rd trace - r2. Arrows indicate unitary IPSP's. The gain required to reveal these IPSP's caused FF spikes to go off screen.

ii. 10 nA hyperpolarizing curring reverses the IPSP's arrowed in i.

iii. 10 nA depolarizing current injected into the FF soma reveals mid-cycle, interburst inhibition. Note the mid-burst EPSP's (arrowed).

Scale bars: a. 100μ. b. horizontal, 250 ms; vertical, 10 mV i, ii; 5 mV iii.


a
bi
ii
iii

excitation of this FF, the frequency of synaptic input increased and it became difficult to pick out unitary potentials. Some EPSP's were evident but the overriding influence of many more IPSP's produced an overall inhibition and hyperpolarization. Similarly in the interburst interval the membrane is effectively clamped near resting potential by synaptic inhibition. At the end of a bout the membrane gradually returned to rest but was tonically inhibited for about 1 second thereafter (not shown in 52 b iii). Interestingly, in this preparation there was direct evidence for mid-burst unitary EPSP's (arrowed in 52 b iii).

Thus the pattern of synaptic events in FF's during a bout of swimming consists of at least two significant phases:

1. Slow wave excitation driving the motorneurons above spike threshold.

2. Phasic inhibition through unitary IPSP's prior to the first burst and in interburst interval.

In addition there is a low frequency of IPSP's for up to 1 second following each bout of swimming.

Possible sources of synaptic input to FF's

The source of both unitary synaptic potentials and slow wave oscillations underlying the rhythmical firing of FF's during swimming is uncertain. Four sources of synaptic input to FF's have been encountered in this study (Fig. 53).

1. Most FF's received a constant low frequency barrage of spontaneous synaptic potentials in quiescent preparations.

FIG. 53 a - d.

Possible sources of synaptic input to FF's.

a. Intrasomatic response of an FPI FF (bottom trace) to tactile stimulation of the ventral thorax. Synaptic potentials could be correlated (but not 1:1) with spiking in another 3rd root unit (top trace).

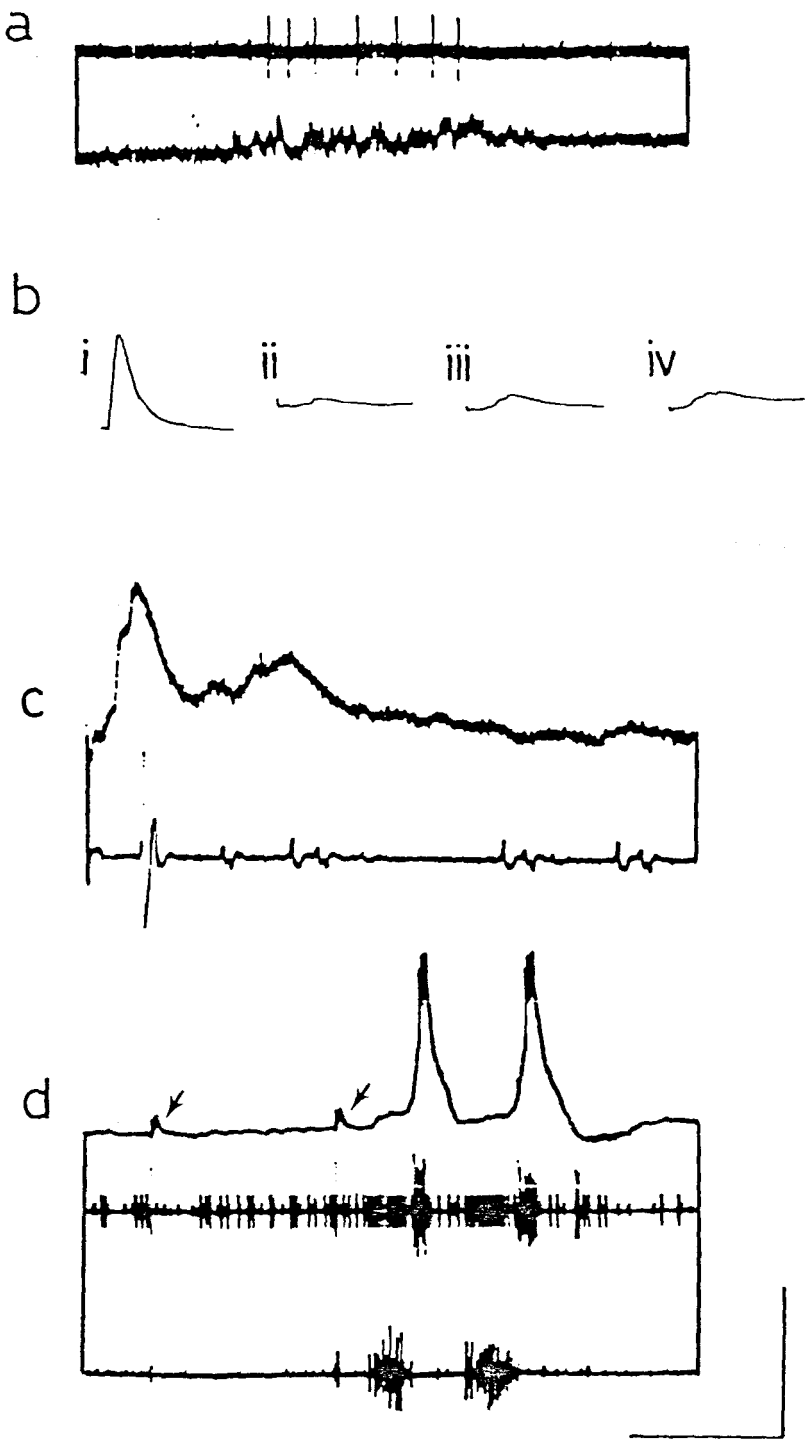
b i - iv. 1st root input to a FF. i. Antidromic spike in FF. (r1 stimulated at 1 Hz)

ii - iv. Synaptic potentials in response to stimulation of the ipsilateral 1st root at increasing intensities.

c. 1st root stimulation in another preparation. Synaptic potentials recorded in an FF (top trace) occurred at the same time as 3rd root motor output (bottom trace).

d. A train of single shocks to an ipsilateral 1st root elicited synaptic potentials in an FF (arrowed, top trace) and gave rise to a bout of swimming. 2nd trace - ipsilateral r3; 3rd trace - r2. Each FF soma was penetrated in G2.

Scale bars: horizontal - 250 ms a, 50 ms b, 25 ms C, 500 ms d; vertical - 1.25 mV a c, 10 mV b, 25 mV d.


a
b
i
ii
iii
iv
c
d

2. Stroking the ventral thorax, head or uropods and telson resulted in a flurry of synaptic potentials in most FF'S. In the example shown in Fig. 53a these synaptic potentials were correlated with spiking in another main 3rd root units (but not a 1:1 correspondence). This implies that FF's receive some common synaptic input, presumably, in this example, from sensory interneurons.

3. Single electrical shocks to the ipsilateral 1st root at increasing intensities produced a variety of post synaptic responses in FF's which summated (Fig. 53 b c, d). On most occasions these EPSPs could be correlated with ipsilateral 3rd root motor output (Fig. 53c) and on one occasion gave rise to a bout of swimming lasting two cycles (Fig. 53d).

4. 2nd root stimulation elicits a complex response in FF's which consists of an initial IPSP followed at longer latency by a compound EPSP. When the ipsilateral r2 is stimulated at high frequency the overall FF response is weak and was never sufficient to elicit spiking.

Extensor motorneurons

It was found more difficult to obtain stable recordings from fast extensor motorneurons (FE's) and to manipulate their membrane potentials by current injection. During episodes of swimming the majority of FE's fired high frequency bursts of attenuated soma spikes phase locked to the peripherally recorded rhythm. In contrast to FF's, however, FE's did not undergo large slow depolarizations although a smaller membrane oscillation was apparent. The primary driving force underlying rhythmical firing of FE's appeared as a high frequency barrage of EPSP's. I encountered two distinct types of FE in terms of their firing pattern during swimming - those that fire high frequency bursts (Type 1) and those that fire infrequently (Type 2). The latter may be a single member of the FE pool in each hemiganglion.

Type 1 FE's

The majority of FE's encountered in this study fired high frequency bursts of spikes during the extension phase of the swim cycle. Spikes recorded in the somata of these neurons were similar in amplitude and time course to those recorded in FF somata. The primary driving force underlying rhythmical firing, however, appeared as a barrage of unitary EPSP's (Fig. 54). Spike frequency reached a maximum at approximately mid-extension of greater than 50 Hz, and decreased slightly towards the end of each bout. Bursts terminated with an abrupt repolarization phase driving the membrane potential towards and occasionally below resting level. In the interburst interval during flexion phase, unitary synaptic potentials were often evident. I was unable to determine the nature of these potentials but two possibilities exist: either they are IPSP's whose effects are partially cancelled by excitation prior to the next cycle; or they are EPSP's whose effects are damped by some other source of inhibition. In either case there is strong support for the existence of mid-cycle inhibition. Towards the end of flexion phase the membrane potential is again driven towards threshold. However in the second cycle of Fig. 54a the neuron reached threshold far quicker than in the first or than in the single cycle of Fig 54b. This may account for the longer burst duration of the first extension phase of each bout in this and other records of the swim motor programme. There are two non-mutually exclusive explanations to account for the increase in the rate of depolarization in subsequent cycles: either the frequency of impinging EPSP's is higher, or there is some degree of rebound from interburst inhibition in type 1 FE's (or both).

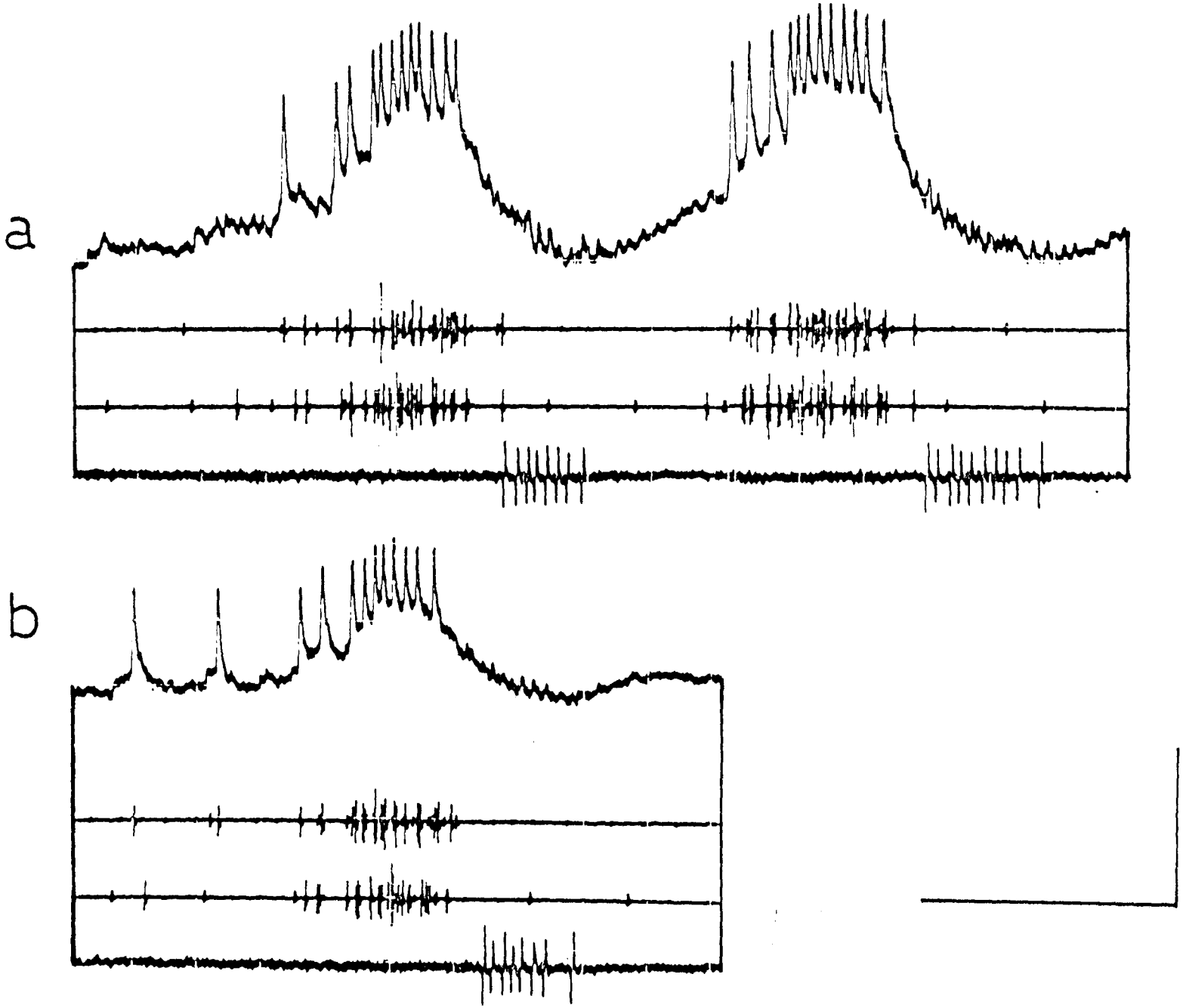
FIG. 54 a, b.

Type 1 FE activity during swimming.

a. Two cycles of activity in response to tactile stimulation of the ventral thorax. The FE (top trace) is depolarized by unitary EPSP's and fires a burst of spikes which increase in frequency towards mid-extension phase. FE spikes are also recorded extracellularly on the ipsilateral r2 (2nd trace). 3rd trace = r2 contralateral, 4th trace = r3 ipsilateral.

b. A single cycle of swimming from the same preparation. Note the extended extensor burst duration caused by low frequency firing of this FE. Note also the tight coupling between ipsi- and contralateral r2 activity.

Scale bars: horizontal - 200 ms; vertical - 5 mV.


a
b

Attempts to alter the amplitude of synaptic events in FE's were largely unsuccessful. Any effects caused by the injection of large amounts of hyperpolarizing or depolarizing current were always small. For example in Fig. 55 a-c, peak membrane potential oscillation was 3.5 mV with +10 nA current injected, 4 mV in the absence of injected current and 4.5 mV with -10 nA. Clearly this result implies that type 1 FE's are driven by periodic chemical synaptic excitation but the magnitude of current induced effects is considerably smaller than those observed in FF's. In each case when this experiment was repeated in good penetrations of FE's with healthy resting potentials, spike amplitude was comparable with FF's. Thus these effects are probably not the result of poor pentrations with blunt electrodes but may result from properties intrinsic to the membranes of FE's (e.g. cable properties).

Type 2 FE's

On three occasions penetrations were made of a FE which rarely spiked during episodes of swimming (Fig. 56). This neuron received a barrage of EPSP's which drove it towards threshold during extension phase. However only rarely did the neuron spike (e.g. in the third cycle of Fig. 56c). The extracellular spike, monitored on the ipsilateral 2nd root occurred approximately at mid-extension phase when type 1 FE's were firing near maximum frequency. The occurrence of a high frequency of EPSP's of varying amplitude phase-locked to the peripherally recorded extensor burst implies that during swimming type 2 FE's receive input from a number of premotor elements which discharge almost synchronously. As cycle period increases the initial rate of EPSP's declines (compare Fig 56a, 4th cycle with Fig. 56b 4th cycle). This may reflect a decline in the frequency of spiking in premotor neurons. Thus, there is an apparent threshold effect controlling the spiking of FE's. This effect may be dependent

FIG. 55 a - c.

Effects of injecting ± 10 nA current on type 1 FE activity during swimming. Top trace - FE soma, G2; 2nd trace - r3 ipsilateral; 3rd trace - r2 ipsilateral; 4th trace - r2 contralateral.

a. Swimming activity induced by tactile stimulation with - 10nA injected into the FE soma.

b. No current.

c. +10 nA current.

Current induced effects are negligible: peak membrane oscillations are 4.5 mV (a), 4 mV (b), 3.5 mV (c).

Scale bars: 250 ms, 10 mV.

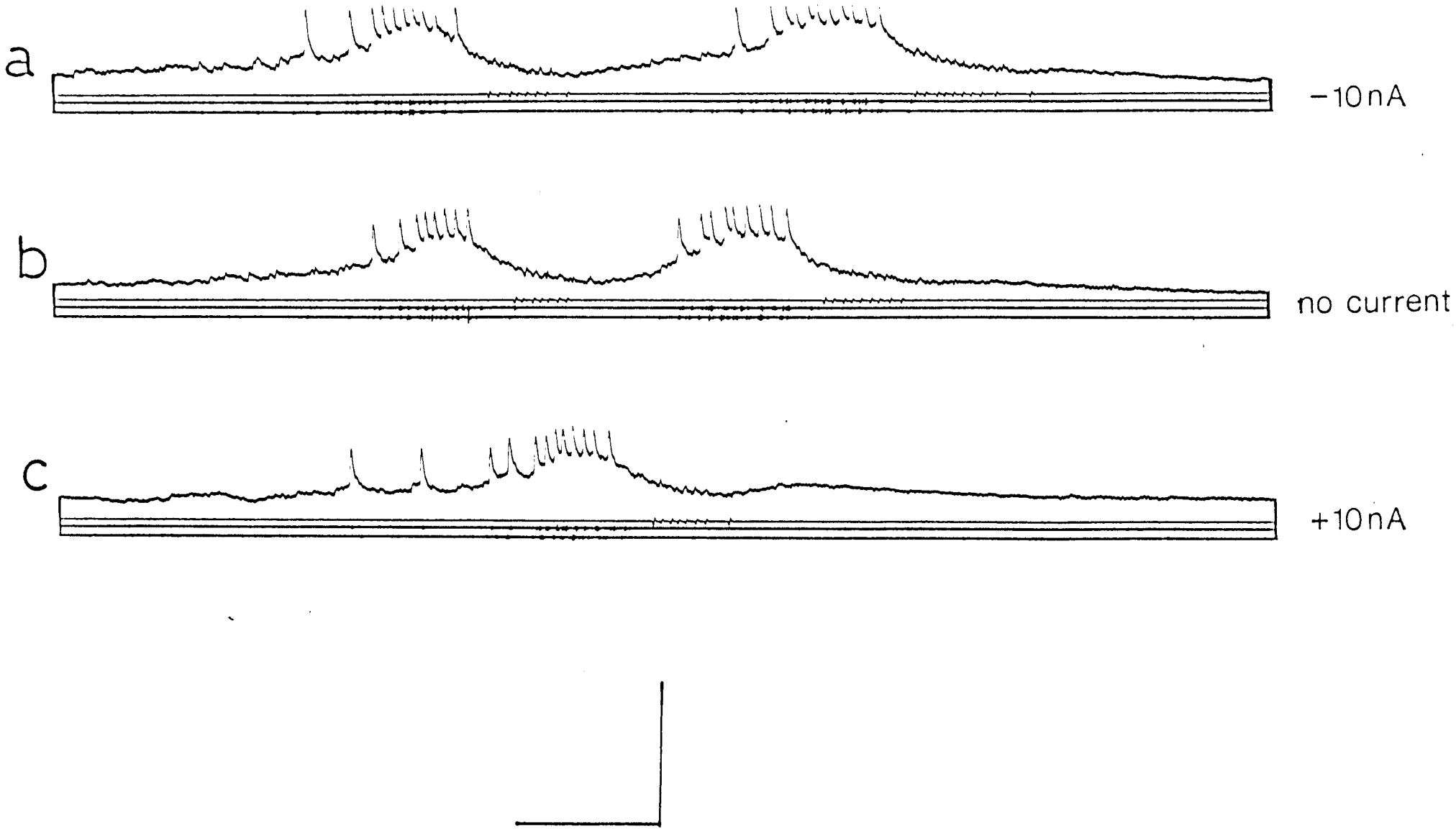
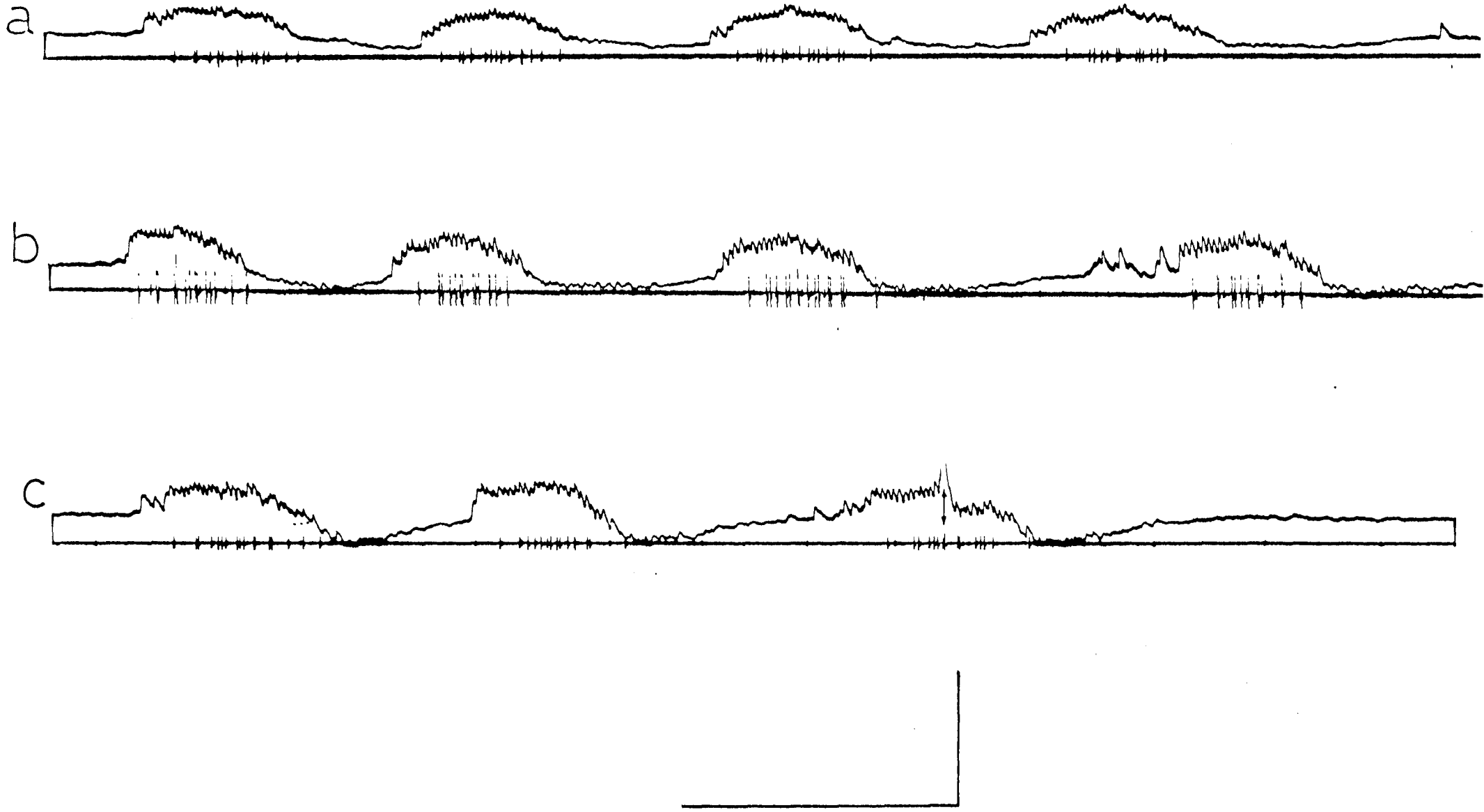

a
−10nA
b
no current
c
+10nA

FIG. 56 a - c.

Type 2 FE activity during swimming.

a, b and c represent three examples, from the same preparation of activity recorded intracellularly in the soma of a type 2 FE in G2 during swimming (top traces). During extension phase (r2, bottom traces) this neuron was depolarized by a high frequency barrage of EPSP's of varying amplitude. The FE spiked once only in these records (arrowed in c.). The large extracellular spike occurred at approximately mid-extension when other FE's are normally firing maximally. As cycle period increased the initial frequency of EPSP's declined (e.g. 4th cycle of b and 3rd cycle of c.). Note the powerful hyperpolarization of the neuron immediately, following extension and during flexion phase, effectively clamping the membrane potential below rest while FF's are active.

Scale bars: horizontal - 200 ms; vertical - 10 mV.


a
b
c

on factors intrinsic to type 1 and type 2 FE's since there is a clear anatomical difference between the two (Fig. 57). On the three occasions that type 2 FE's were successfully stained they were found to have very large (up to 110μ) somata. By comparison, the diameters of type 1 FE somata were consistently smaller (50-70μ). Thus it appears that within the available pool of FE's, those with larger somata (type 2) have higher spike thresholds than those with smaller somata (type 1). The difficulties encountered in recording successfully from FE's have prevented a more quantitative analysis of FE spike thresholds. The evidence presented above demonstrates that the largest of the available FE pool has a higher spike threshold than the remainder. Whether or not there is a further gradation in spike thresholds among these neurons is unclear but is a possibility. With the exception of soma diameter the FE's have very similar anatomy, particularly in the distribution of their major dendrites. An additional feature of the activity of type 2 FE's is that mid-cycle inhibition is more pronounced (Fig. 56) and consistently and effectively held the membrane potential below rest during FF activity.

The phasic inhibitors

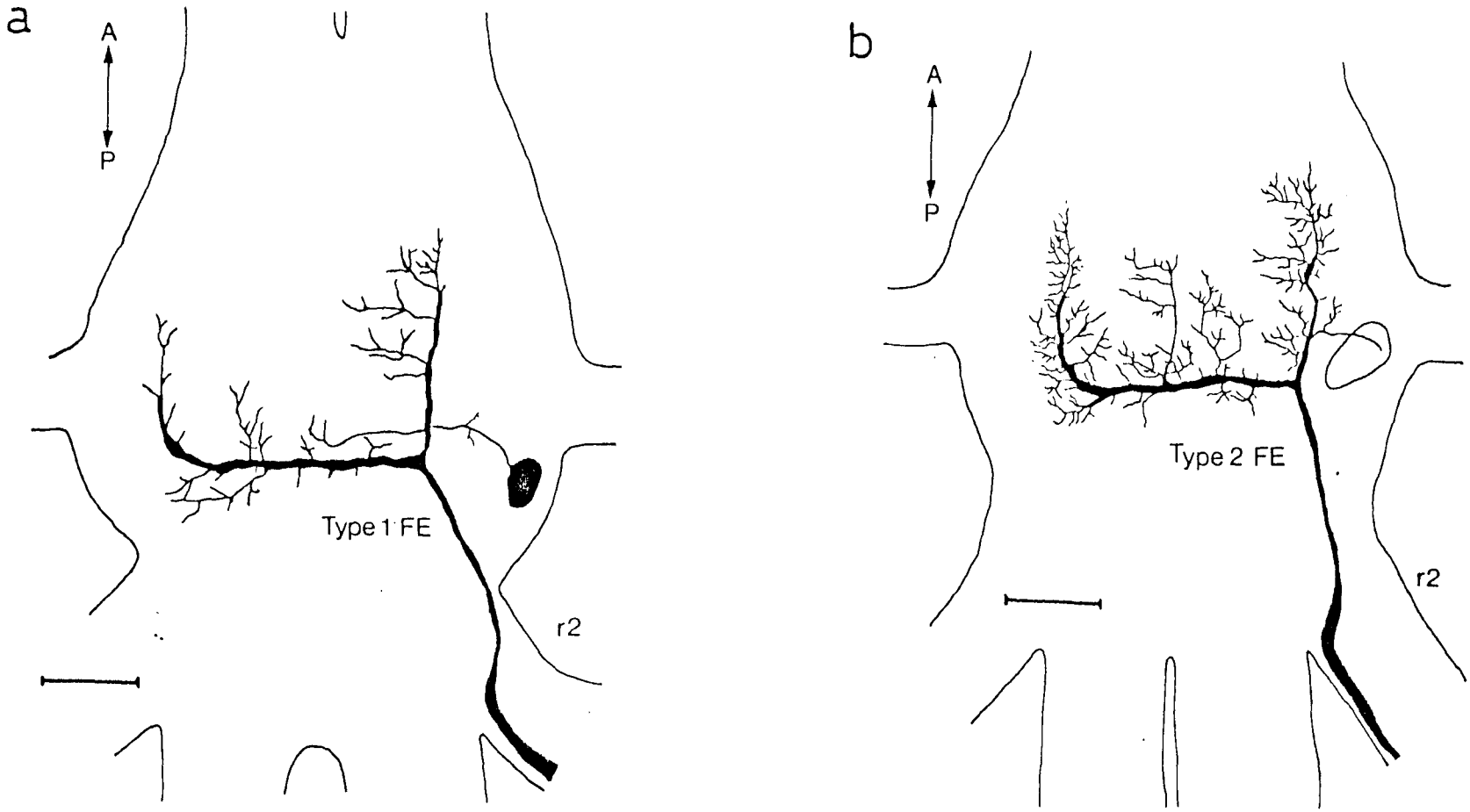
FI

FI is one of the largest and easiest to penetrate of the phasic swim motorneurons. The dorsal position of its soma at the extreme lateral edge of the ganglion near the base of the 1st root greatly facilitated microelectrode penetration. A characteristic feature of FI activity during swimming, in contrast to the FF's, is that it spiked a maximum of once in each cycle in these preparations (Fig. 58). FI frequently failed to fire, particularly in later cycles of swimming. The sequence of events underlying FI spiking resulted in a complex waveform in favourable preparations.

FIG. 57 a, b.

The anatomy of type 1 (a) and type 2 (b) FE's revealed by injection of Lucifer Yellow in G2. Each has an extensive bilateral neuropilar branching pattern and an axon whih exits the 2nd root (r2) ipsilateral to the soma. Note the type 2 soma is considerably larger in diameter.

Scale bar: 100μ.


a
A
P
Type 1 FE
r2
b
A
P
Type 2 FE
r2

At the onset of the first extension phase FI was depolarized. At approximately mid-extension FI became gradually repolarized and was clamped on, or just below, the cells resting potential. From about two thirds through flexion phase the membrane was rapidly depolarized, driving the FI towards threshold. This delayed excitation of FI ensured that when it fired it did so shortly after the FF's had stopped firing. From this point until mid-way through extension phase of the next cycle FI was maintained in a depolarised state. The sources of excitation responsible for this ramp effect are two fold. The first involves the sequence of events which drive FI to spike, the second is the excitation which depolarised FI at mid-extension. Hence as cycle period increased (see 2nd cycle of Fig. 58 a iii) this ramp potential became loop shaped. The function of this effect is not clear. Presumably when FI is depolarised (from mid-flexion to mid-extension) it is more likely to fire in response to other (e.g. sensory) synaptic input. For the remainder of each cycle (most of flexion phase) FI is clamped near resting potential and thus prevented from firing while FF's are active.

On one occasion ipsi-and contralateral FI's in G2 were penetrated simultaneously (Fig. 58b i, ii). Both neurons received rather weaker input during swimming than in more favourable recordings but they were confirmed anatomically as FI's. Only one of the two FI's spiked in this preparation (see Fig. 58 b i, 1st cycle). However, many of the synaptic potentials were common to both recordings implying that during swimming at least some input to FI's comes from common presynaptic elements. Consistent with this implication is the observation that, in contrast to FF's, the dendritic domain of each FI is strongly bilateral with major processes extending into each half of the ganglion. Thus the contralateral

FIG. 58 a - c.

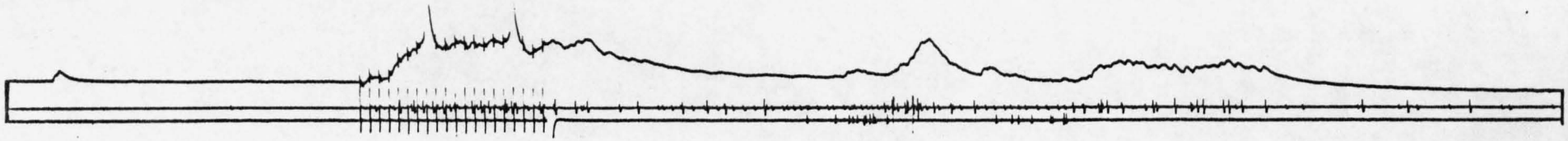
Activity of FI during swimming.

a i - iii. Typical activity recorded in the FI soma during swimming. ii and iii are from the same preparation. i. is from a different preparation. The FI in i. did not spike during swimming (top trace) but was driven to spike by r2 (bottom trace) stimulation. 2nd trace = r3. In more favourable recordings FI spiked during swimming (ii, iii) but usually only once per cycle (top traces). 2nd trace = r3. 3rd, 4th traces - ipsi- and contralateral r2, respectively.

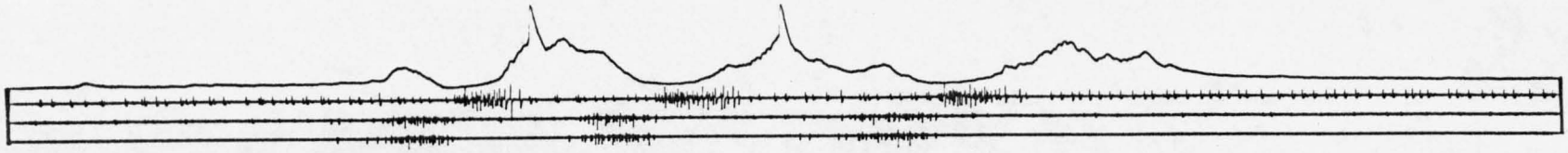
b i, ii. Recordings from a preparation in which both FI's in G2 had been penetrated (3rd and 4th traces). Many, though not all, synaptic potentials are common to both neurons. Top trace - r2; 2nd trace - r3. c. A recording from FI (top trace) during a bout of swimming which began with flexion (2nd trace) and not extension (3rd trace).

Scale bars: horizontal - 200 ms a, c; 100 ms b.
vertical - 5 mV a, 10 mV b, c.

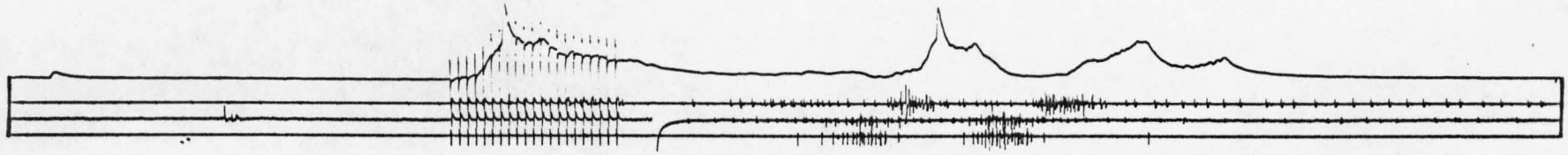
a i

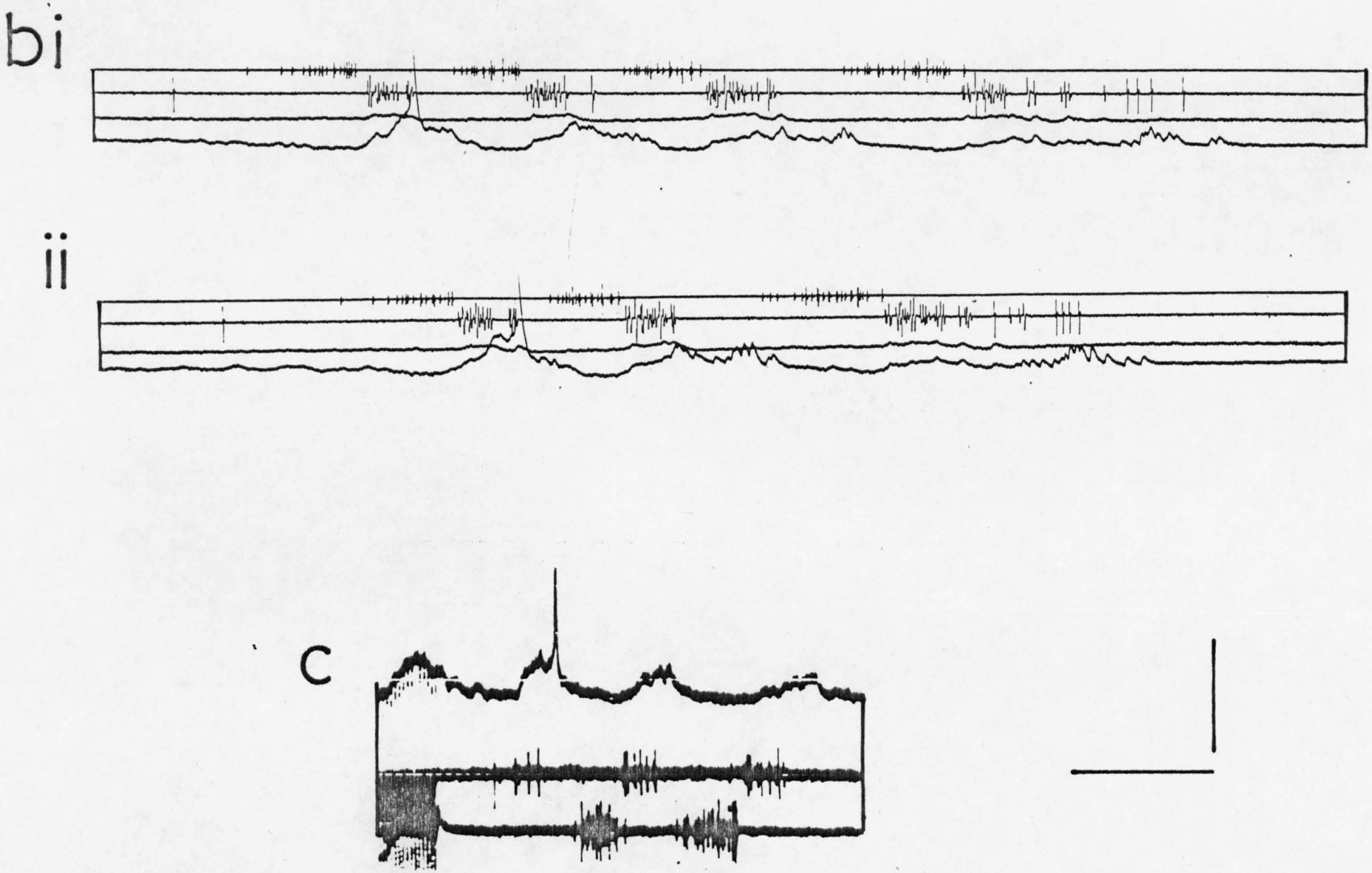


ii



iii




bi
ii
c

processes of one FI occupy similar regions of neuropile to the ipsilateral processes of its contralateral homologue. This matching of dendritic domains cannot however be identical since many of the inputs of one FI are not common to the other (Fig. 58b).

In one experiment when FI had been penetrated the first flexion of a bout of swimming occurred without prior extension (Fig. 58c). FI received similar input to that observed during normal swimming activity. The implications of this result are four fold:

1. The flexor burst is not dependent upon firing of extensor motorneurons.

2. FI spiking and excitation is independent of extensor motorneuron discharge.

3. FE activity does not contribute significantly to FI excitation.

4. The mechanisms driving FF's and FI are dissimilar: the most powerful excitation of FI occurred in a cycle when FF discharge was weak.

In addition, FI is not depolarized by a slow wave oscillation but by discrete synaptic potentials as is evident in cycles when FI did not fire.

EI.

The phasic inhibitor of the extensor muscles (EI; see Chapter 1), like FI, has a large, laterally located soma which is relatively easy to penetrate. However, like FE's, it was found difficult to obtain stable recordings from EI.

EI fired a maximum of two spikes in each cycle of swimming and displayed oscillations in membrane potential phase locked to the peripherally recorded rhythm. These oscillations occured in phase with flexion and

resembled the slow wave depolarizations seen in FF's (Fig. 59). Injection of current into the soma of EI induced similar changes in the amplitude of this oscillation as in FF's (Fig. 60). The flurry of synaptic potentials which occurred in EI following the last cycle of Fig. 60a became reversed after injection of 10 nA depolarizing current implying that EI is synaptically inhibited following slow wave excitation (Fig. 60b). In addition to the interburst and post-burst inhibition evident in Fig. 59, EI spiking was terminated by a barrage of IPSP's superimposed upon the depolarized peak of the slow wave oscillation (Fig. 59c). Thus it would appear that EI receives a similar slow wave oscillation to FF's.

Dual intracellular recordings from EI and an ipsilateral FAC FF Fig 59, d) support this idea since many inputs to the two neurons are common. EI always crossed threshold in the early part of the flexion phase, sometimes prior to the onset of flexor motor discharge, but always after the end of the main extensor burst. The number of EI spikes occurring in each cycle of swimming appears to be limited by mid-burst IPSP's which may originate in the activity of FF's. The inability to drive the FF in Fig. 59d with injected current prevented an investigation of synaptic connections between the two neurons.

FIG. 59 a - d.

Activity of EI during swimming.

a - c. Three examples, from the same preparation, of synaptic drive to EI (top traces) during swimming. 2nd trace = r3 ipsilateral, 3rd trace = r2 ipsilateral to EI axon, 4th trace = r2 contralateral to EI axon. Note the apparent IPSP's which follow E1 spikes in c (labelled).

d. A preparation in which both an FPI FF (top trace) and EI (2nd trace) were penetrated simultaneously and the animal induced to swim. Note the common input to the two prior to and following the slow wave in FF.

Scale bars: horizontal - 400 ms a, b; 100 ms, c; 200 ms d. vertical - a 20 mV, b 20 mV, c 2.5 mV, 5 mV EI, FF.

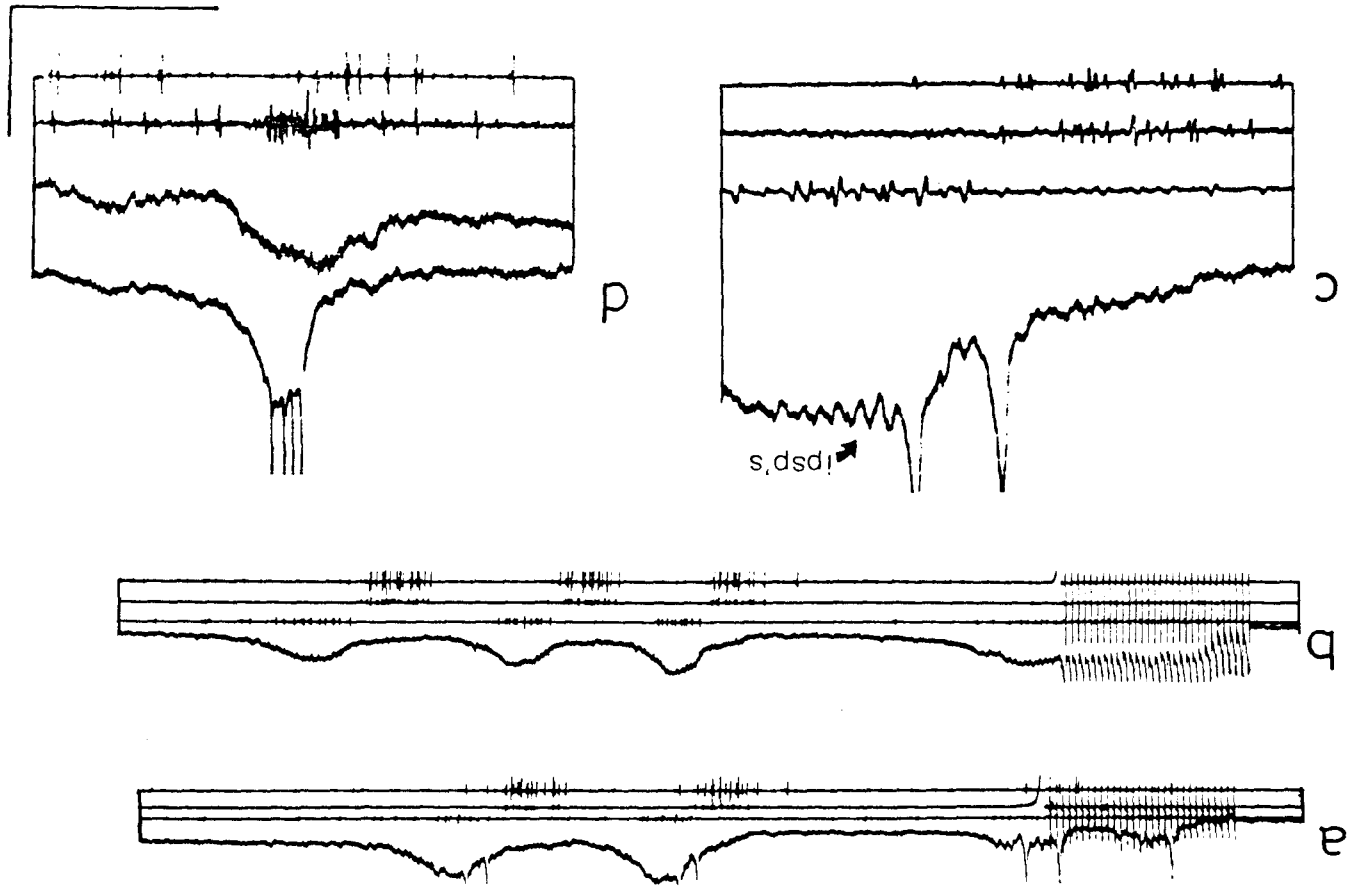

p
c
s,dsd
q
e

FIG. 60 a, b.

The effects of current injection on the activity of EI during swimming.

a. Two cycles of activity in which EI (top trace) spikes once on the 1st cycle of swimming. Note the apparent IPSP's after the second burst (arrowed). 2nd trace - r3; 3rd trace - r2.

b. EI was injected with -10 nA current prior to swimming activity. Note the increase in slow wave amplitude and reversal of post-burst IPSP's.

Scale bars: 200 ms, 5 mV.

a
b

D. THE COORDINATION OF SEGMENTAL LIMB STRUCTURES DURING ESCAPE SWIMMING

Background

Segmental limb structures in decapods are used in a variety of rhythmic behaviours. In the head region most limbs are used in activities such as feeding, chemosensation and ventilation and each of these can be rhythmic (e.g. chewing, antennal flicking, scaphognathite beating). The five pairs of thoracic limbs which characterize the group are the walking legs. The first pair are normally enlarged as chelipeds and the last pair may be reduced as cleaning appendages (as in Galathea). The remaining three pairs of unmodified walking legs play a major role in locomotion and are often used in a number of complexly coordinated rhythmic behaviours. The segmental appendages of the abdomen, with the exception of the terminal uropods, are often modified as copulatory appendages in the male, or reduced in the female. The remaining unmodified swimmerets can beat in a metachronal rhythm. In some smaller species they are actively used in swimming while in others they can be used in righting responses, burrow ventilation and may assist the walking legs in locomotion.

The behaviour of the walking legs and swimmerets during escape behaviour has not been described previously. However the escape system of the crayfish does interact with limb motor circuitry since the central actions of the LG axons include firing of swimmeret motorneurons (Wiersma, 1947; Roberts, 1968). During tailflipping and backwards swimming the walking legs and swimmerets may acquire a different behavioural role associated with lack of substrate contact and rapid abdominal cycling. It was considered important to investigate this role in the escape swimming

response of Galathea. The present section, therefore is aimed at determining the behaviour of limb structures and their coordination during swimming. In the first part the activity of the legs and in the second part the activity of the swimmerets has been investigated.

RESULTS

Leg movements

Observations on unrestrained animals reveal that during episodes of swimming the anterior three pairs of walking legs and the chelae are thrown forwards (protracted). Presumably this functions to increase streamlining and reduce drag as the animal is propelled backwards. In animals which have autotomized their legs and chelae and are pinned ventral side up in a sylgard dish this forward thrusting of the limbs appears as a protraction of the remaining leg stumps. Two questions arise: 1. are the legs protracted phasically with each flexion? and 2. are legs protracted synchronously or metachronously?

To answer these questions I have recorded EMG activity in the main leg protractor muscles situated in the ventral thorax at the base of the leg stumps. Animals were pinned ventral side up with their abdomens free to move and small holes were made in the ventral thorax to allow access to leg protractor muscles with EMG wires. My observations are summarized in Fig. 61.

Recordings from the protractor muscles of a single leg show that the legs are pulsed forward with each cycle, in phase with flexion (Fig. 61a). The phasic protraction lasts about 20-40 ms and occurs with each cycle of the abdomen, terminating with the last flexion of a bout of swimming.

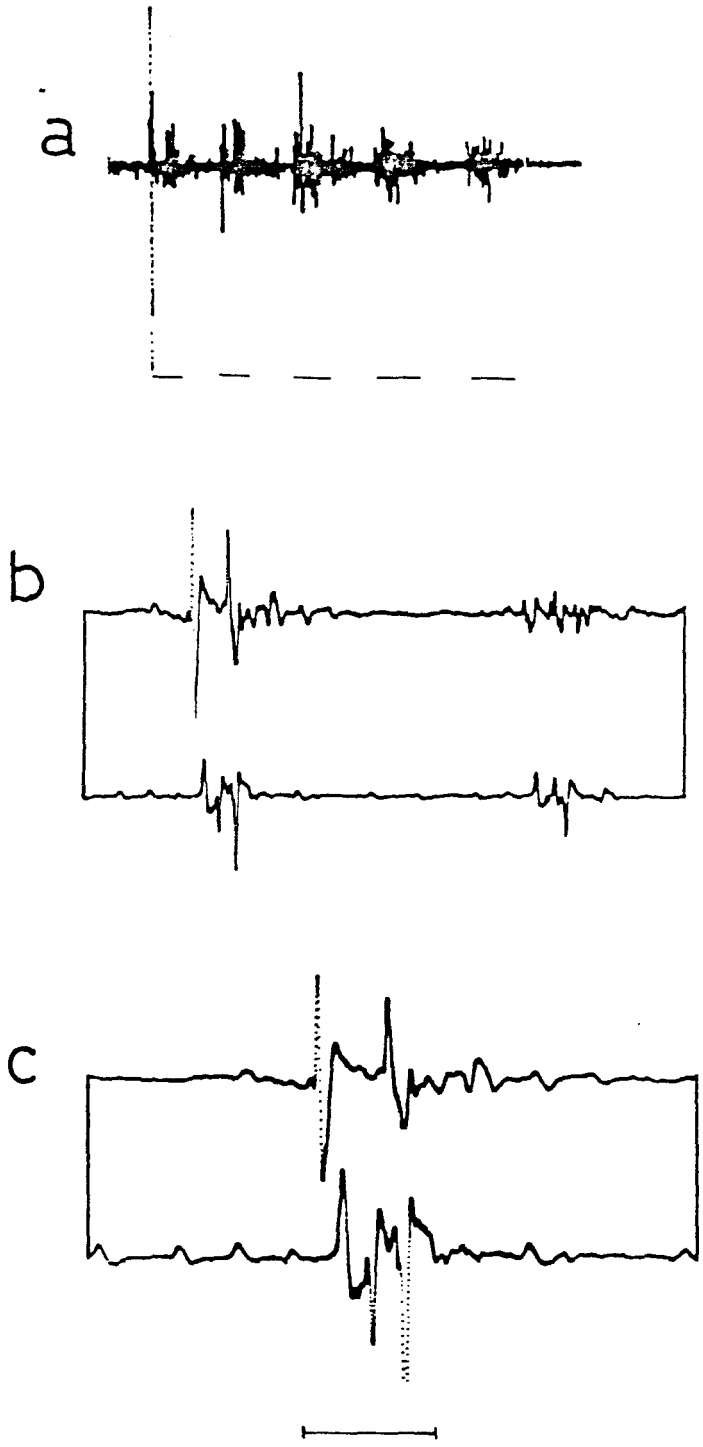
FIG. 61 a - c.

EMG activity recorded in leg protractor muscles during swimming.

a. Activity in leg 2 during swimming. Bars indicate duration of activity and approximate occurrence of abdominal flexion. Note the larger amplitude burst in the 1st cycle.

b, c. Simultaneous EMG recordings from the 2nd (top traces) and 3rd (bottom traces) leg protractor muscles during swimming. c. is an expanded version of the 1st cycle in b.

Scale bar: 200 ms a; 20 ms b: 10 ms c.


a
b
c

Simultaneous recordings from successive leg protractor muscles reveals that leg protraction during swimming occurs as a metachronous wave which moves in an anterior to posterior direction. In the example shown in Fig. 61b recordings were made from the 2nd and 3rd walking legs respectively and the anterior leg protraction phases leads the posterior by 5-10 ms. Recordings from other pairs of legs show a similar anterior-posterior phase lead. Thus the legs are sequentially thrown forwards during swimming starting with the chelae. This anterior-posterior metachronicity may ensure that neighbouring legs do not collide as they are thrown forwards.

The amplitude of the first protraction cycle was sometimes larger than in subsequent cycles. This may ensure that the legs are effectively protracted at the onset of swimming regardless of their position prior to escape. In subsequent cycles the legs are in a relatively protracted state and lower amplitude protractor activity may be sufficient to maintain the legs in a forwards position.

It seems likely that the major function of leg protraction is to reduce drag during backwards swimming. However the fact that leg protraction occurs in phase with flexion (the power stroke) suggests that the legs may also contribute to the backwards thrust of the animal. Limb structures such as the swimmerets do act as oars or paddles in some species. The coupling between adjacent leg protraction and flexor muscle activity during swimming suggests that leg protractor and abdominal flexor motorneurons may share the same or similar premotor circuitry. It is possible that the same CPG driving abdominal cycling also activates the leg motorneuron pool during swimming and overrides other local or segmental oscillators controlling rhythmic behaviours such as walking. The cellular mechanisms underlying rhythmical limb movements is outside the scope of this thesis but may provide a favourable locus for future experiments on motor programming and motor programme switching.

Activity of the swimmerets

In Galathea there are five pairs of swimmerets in the male and four in the female. The swimmerets of the female are fragile feather-like structures, designed primarily for egg bearing. In the male the anterior two pairs of swimmerets are also modified for reproductive purposes (sperm transfer) while the posterior three pairs are paddle shaped. During episodes of swimming the modified reproductive swimmerets of both the male and female are tonically protracted. The unmodified male swimmerets are "flicked" posteriorly in phase with each abdominal flexion. This rapid retraction is preceded by a slower and less powerful forwards movement occurring in phase with extension. Clearly the power produced by swimmeret movements is insignificant compared with the massive abdominal flexion seen during swimming. This flicking movement of the unmodified swimmerets may act to streamline them against the abdomen during flexion.

Extracellular recordings of activity in abdominal 1st roots innervating the swimmerets normally reveals a low level of discharge from tonically active motorneurons. Occasionally this activity is patterned into discrete rhythmic bursts (swimmeret beating) involving many motorneurons. Bursting activity is rare, however, occurring in less than 10% of preparations and cycling at about 1 Hz, often intermittently. On many occasions, no spontaneous discharge of swimmeret motorneurons was observed.

During episodes of swimming activity it is possible to record neural correlates of the swimmeret movements observed in intact animals. G1 1st roots innervating the modified male swimmerets and 1st roots innervating female swimmerets show a gradual increase in activity which is not rhythmic and does not follow the swimming rhythm (Fig. 62a, b).

FIG. 62.

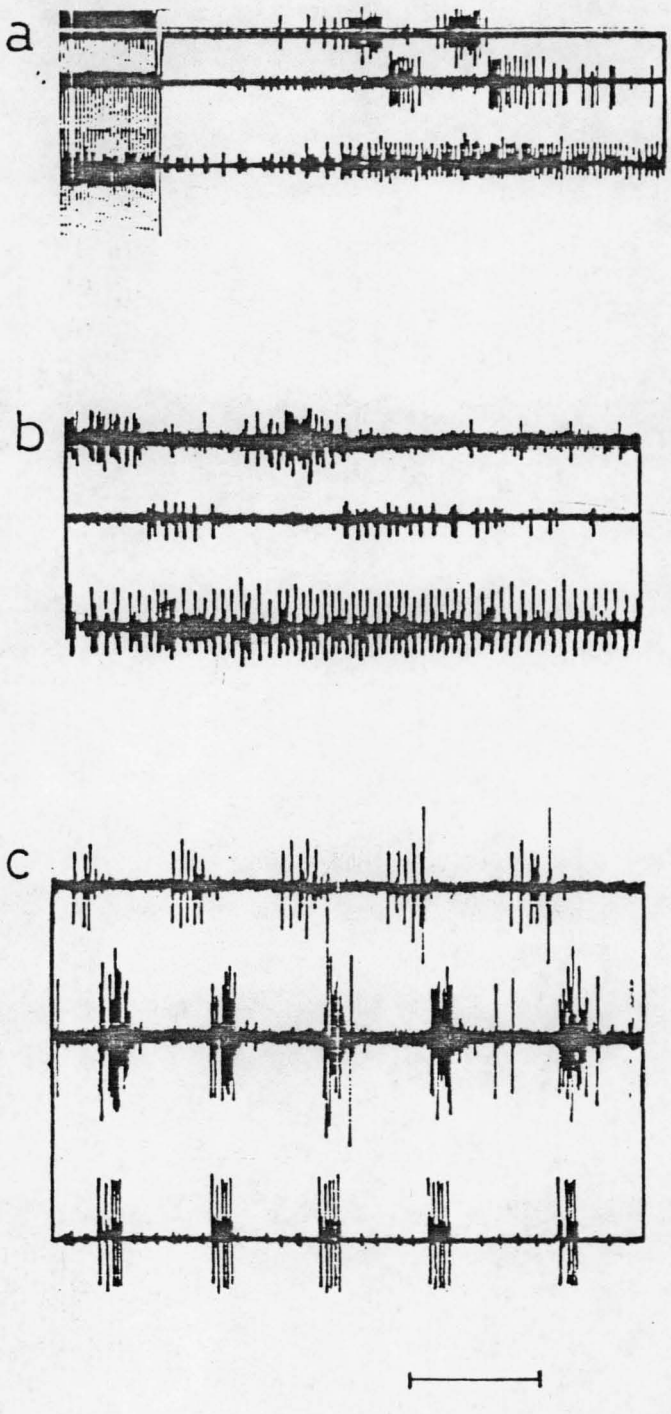
Neural activity underlying swimmeret movements during swimming. Top traces - r2, 2nd traces - r3, 3rd traces - r1.

a. During swimming, r1 G1 in females shows a gradual non-rhythmic increase in motor activity.

b. The same occurs in r1 G1 in males.

c. The unmodified male swimmerets are rhythmically retracted during the flexion phase of the swim cycle. In r1 G2 there is a phasic burst of spikes in a single unit. This burst is phase-locked to flexion.

Scale bars: 500 ms a, b; 1 second c.


a
b
c

The 1st roots from G1 to G4 innevating the unmodified male swimmerets display oscillatory motor activity phase-locked to the swim motor programme (Fig 62c). This activity consists of two phases: phase 1 and phase 2. During the extensor burst of each swim cycle, one or more 1st root units fires a phasic burst of spikes (phase 1, fig. 63d). In phase with flexion a single 1st root neuron discharges at high frequency. This neuron has a large extracellularly recorded potential, double that of other 1st root units in most cases. Phase 1 is less consistently recorded than phase 2; while phase 1 is either absent or comprises activity in a subpopulation of swimmeret neurons, phase 2 is always present in healthy preparations and involves the same large unit. Stimulation of intact 1st roots to unmodified male swimmerets resulted in a rapid flicking similar to that observed during swimming, which could be correlated with a single evoked potential recorded with a second electrode at a point more distal on the root. Dual first root recordings during swimming have allowed this unit to be identified as a motorneuron with a conduction velocity of 5-7 m/sec. The number of spikes recorded in phase 2 varied from preparation to preparation, from zero (on rare occasions) up to 11 at up to 100 Hz (Fig. 63). The frequency and duration of spiking in phase 2 varied with the intensity and duration of the flexor burst (Fig. 63 a-d). The onset of activity in phase 2 is coupled to the onset of flexion but may either lead or follow flexion by up to 30 ms.

Stimulating r1 G2 in males activates some but not all fast flexor motorneurons in the ipsilateral 3rd root (Fig. 53c and 64). Further evidence for 1st root flexor drive derives from high frequency stimulation of the first root in deafferented preparations. This is capable of switching on the swim motor programme, but less consistently than high frequency r2 stimulation . When the swim oscillator is "running" r1 stimulation

/

FIG. 63 a - d.

Correlation between phase 2 activity and flexor motor output. Top traces - r1 G2, 2nd traces r2 G2, 3rd traces - r3 G2. As r3 burst intensity increases so does the frequency and duration of spiking in phase 2. Only in the most intense bouts of swimming does rhythmical phase 1 activity occur (see top trace in d). Scale bar: 50 ms.

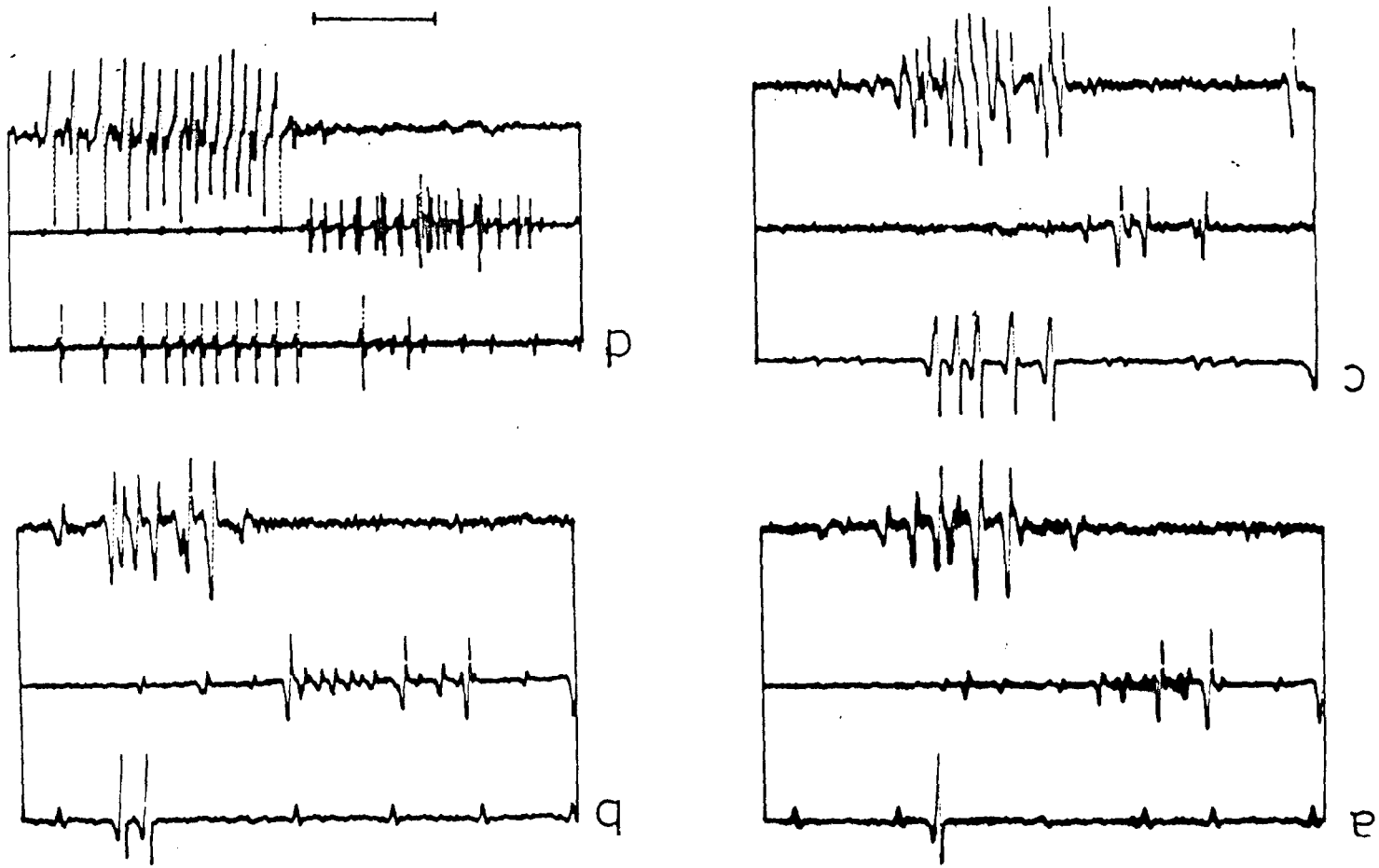

p
c
q
e

FIG. 64a, b.

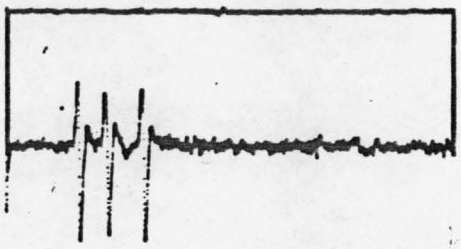
r1 stimulation drives flexor motorneurons (a) and affects the swimming rhythm (b).

a. Single electrical shocks to r1 G2 at 1 Hz(top trace) causes suprathreshold activation of some, but not all, ipsilateral r3 units (bottom trace).

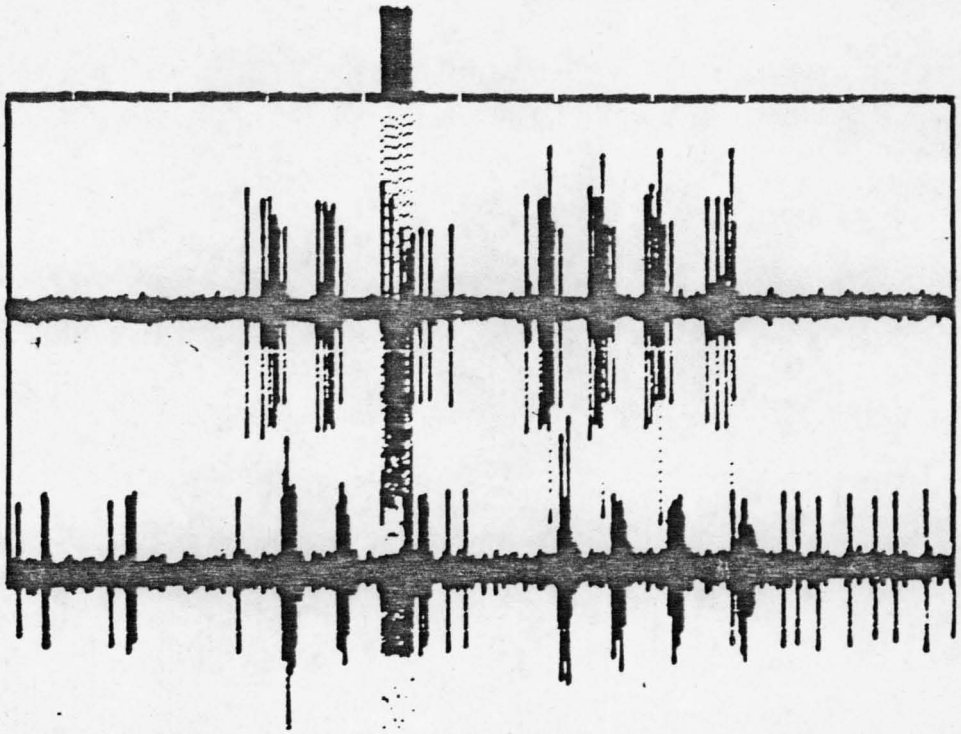
b. High frequency r1 stimulation (top trace) affects the motor programme for swimming. 2nd trace - r2, 3rd trace - r3. Swimming was induced by tactile stimulation. At the onset of extension in the 3rd cycle r1 was stimulated. Swimming ceased but was resumed less than 1 second later and continued for a further 4 cycles. It is impossible to determine whether or not r1 stimulation resets the CPG since swim frequency is variable.

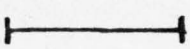
Scale bar: 10 ms a; 1 second b.

a



b





can alter the rhythm. In most cases the effect is to cause the rhythm to continue for longer than might normally be expected (Fig. 64b). However it is difficult to claim resetting effects since it is impossible to predict with accuracy the occurrence of a motor programme which is episodic and variable in frequency.

The neuron active during phase 2 is a possible candidate to produce this flexor motor drive, circumstantially. However I have been unable to perform the most convincing experiment of stimulating the 1st root at one point and correlating flexor discharge with an evoked potential at a second point on the root. The 1st root is a fragile featherlike structure which is easily damaged and moreover it is exceedingly difficult to isolate extracellular electrodes such that the stimulus pulse artifact delivered at one point does not interfere with the recording electrode at a second point. The first root is a mixed sensori-motor nerve and therefore it is also possible that stimulation of sensory neurons is responsible for the observed flexor drive.

A swimmeret motorneuron

A neuron has been penetrated intracellularly in the 2nd ganglion which fired rhythmically in phase with the peripherally recorded flexor burst and which had an axon in the first root. Although this neuron was penetrated successfully only once, its characteristic firing pattern, coupled with an axon in the 1st root is strong evidence that it corresponds to the phase 2 unit described above (Fig. 65a). During flexion the neuron displayed a large (c. 10 mV) oscillation in membrane potential (similar to FF's) superimposed upon which was a phasic burst of attenuated soma spikes. Stimulation of the ipsilateral 1st root resulted in an initial hyperpolarizing potential, followed at slightly higher stimulus intensity by a short latency antidromic spike (Fig. 65 b, i). Unfortunately the

FIG. 65 a - c.

Anatomy and physiology of a specialized swimmeret neuron. The neuron, penetrated in G2, fired bursts of spikes (top trace) whose frequency and duration correlated with the intensity of flexor motor output during swimming (2nd trace).

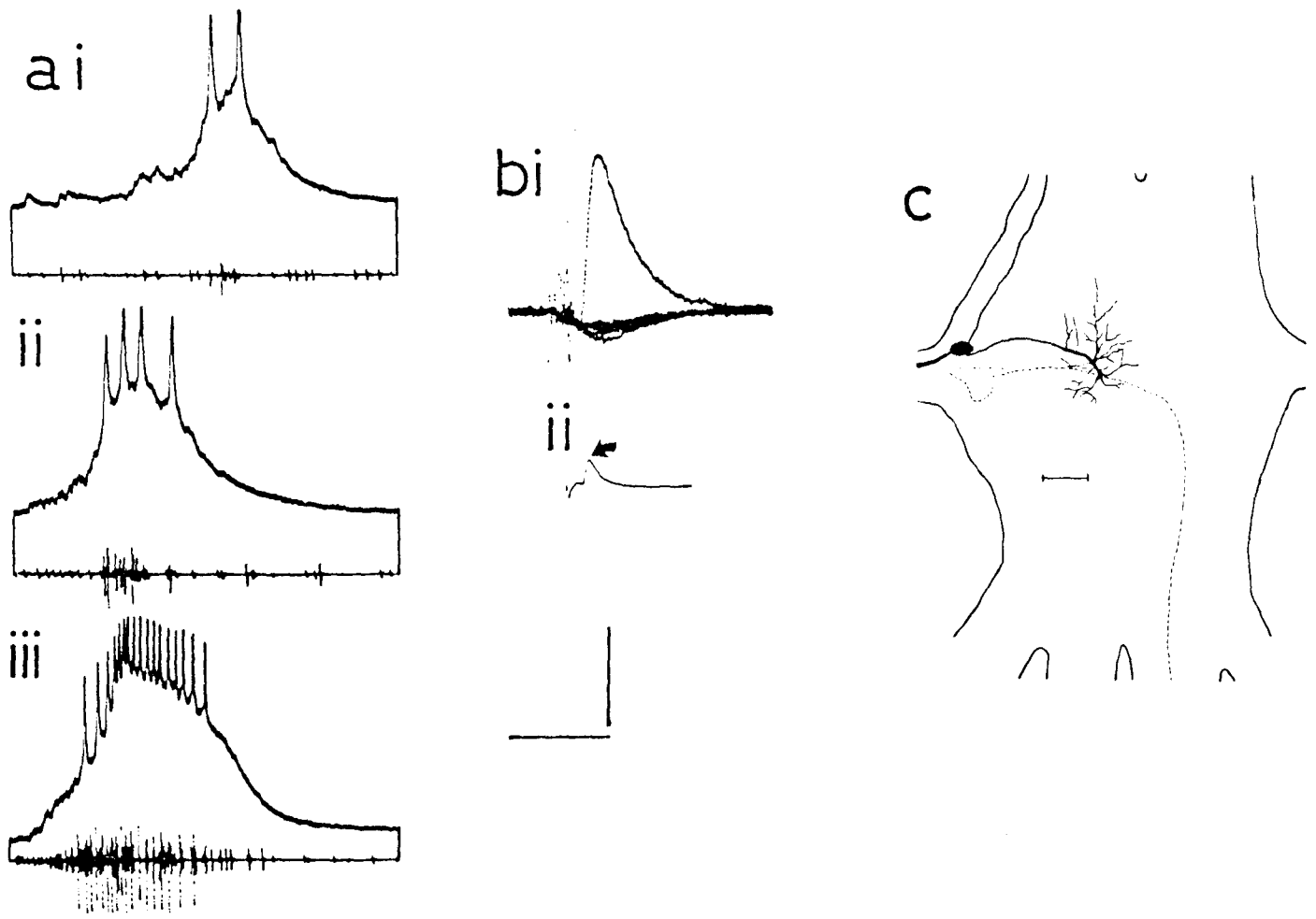
b i. Stimulating r1 at 1 Hz resulted in a small hyperpolarizing potential at low intensity, followed at higher intensity by a short latency antidromic spike (several sweeps superimposed at increasing intensities of stimulation).

ii. Stimulating the ipsilateral r3 caused an initial hyperpolarization followed by an EPSP which frequently triggered a spike. The arrow indicates the spike which masks the underlying EPSP.

c. Subsequent injection of Lucifer Yellow revealed a neuron with a 1st root axon, a small soma, an axon branch which ascended the ipsilateral connective and large neuropilar processes in a similar region to that occupied by FF dendrites. Some dye-coupling occurred between this neuron and FI (outline dotted).

The activity of this neuron during swimming corresponds to the phase 2 unit in 1st root recordings (see text).

Scale bars: horizonal - 50 ms a; 10 ms i; 40 ms b ii; vertical - 5 mV a; 10 mV b i; 70 mV b ii. c - 100μ.


a i
ii
iii
bi
ii
c

1st root recording in this preparation was poor and, despite being able to evoke an antidromic spike, intracellular activity could not be correlated with peripheral activity. This neuron received powerful input from the third root. r3 stimulation produced a short latency hyperpolarizing potential followed by an EPSP which frequently triggered a spike (Fig. 65 b, ii). It would appear that this 1st root unit is driven by a synaptic source common to FF's and is simultaneously driven by 3rd root units. I was unable to drive this cell with injected current and so was unable to determine whether or not it was responsible for the flexor motor drive seen on stimulation of the 1st root. On the basis of its firing pattern and 1st root axon, this neuron corresponds to the phase 2 unit. Its anatomy, revealed by Lucifer Yellow injection was substantially different from the majority of other 1st root motorneurons (as revealed by cobalt backfilling) on two accounts (Fig. 65c). Firstly, in addition to the peripheral axon, a thin axon ascended the lateral edge of the ipsilateral connective towards G1. Secondly the neuropilar segments of this neuron were large and projected into a region also occupied by FF dendrites. In this second respect the neuron is analagous (and possibly homologous) to the specialized SG neuron of the crayfish.

DISCUSSION

Central pattern generation and homologous behaviour

The generation of rhythmical alternating bursts of activity in extensor and flexor motorneurons appears to reside in a CPG located either in the thoracic or the suboesophageal ganglion. The most convincing evidence for central pattern generation of the swimming rhythm is the ability to evoke activity, identical in all essential respects to that recorded in the intact swimming animal, in the total absence of sensory feedback from the abdomen (Fig. 40). That brainless animals can swim, apparently normally, rules out the supraoesophageal ganglion as a locus for central pattern generation. Transection of the nerve cord between the thorax and abdomen terminates all swimming activity and therefore dismisses the possibility that the rhythm originates in the abdominal ganglia. It remains feasible, however, that subordinate neural oscillators reside in each abdominal ganglion which require input from a higher order thoracic CPG to produce patterned motor output. There is a small chance that sensory input from the thorax is involved in pattern generation since in most experiments the thoracic nervous system was left intact. However, cutting the motor nerves to the legs does not abolish swimming activity and therefore this possibility seems unlikely. Tactile stimulation of the thorax is capable of switching on the rhythm. However on most occasions a single stimulus resulted in up to seven consecutive cycles of activity. The stimulus must account for the first, but probably not subsequent cycles. Hence thoracic sensory feedback is probably not crucial to the generation of the swimming rhythm.

These results are compatible with what is known about non-giant swimming in the crayfish (Schrameck, 1970; Wine and Krasne, 1982). The

homologies indicated by the comparative anatomy of swim motorneurons in crayfish and Galathea (Chapter 1) are therefore substantiated by the present physiological data and in conclusion the two behaviours are homologous.

Two species of sand crab, Emerita and Blepharipoda (Anomura) provide further examples of contrasting decapod escape behaviour from which possible homologies can be drawn (Paul, 1981a, b). These species belong to two closely related sand crab families and yet their escape behaviour is very different. The abdomen of Emerita remains folded upon itself during swimming while the uropods of the 6th abdominal segment perform rapid sculling movements that propel the animal backwards (Paul, 1971a). Blepharipoda does not beat its uropods but performs a rapid abdominal cycling behaviour that, ". . . resembles macruran tailflipping". (Paul, 1981a). In fact the behaviour resembles non-giant crayfish swimming and backwards swimming in Galathea, since the abdomen is tonically flexed in the resting animal and escape begins with extension. Strong homologies exist between the tailfan neuromuscular systems of Emerita and Blepharipoda (Paul, 1981b) and Galathea (Maitland et al., 1982), and the three species probably derive from a common ancestor. Emerita's novel use of the uropods appears to be a development of the Blepharipoda tailflip, presumably in response to pressures to move intertidally and and to become filter feeders (Paul, 1981a). The existing data on the Blepharipoda tailflip and the tailfan neuromuscular system of Galathea point to strong similarities in the escape of the two species. The present data would support the idea that tailflipping in Blepharipoda and backwards swimming in Galathea are homologous behaviours but until more information on the abdominal system in Blepharipoda is gained this support can only be tentative.

Sensory feedback interactions

In the absence of sensory feedback from the abdomen, CPG output is normally short in terms of the number of cycles in each bout and always shows a characteristic decline in frequency. The presence of proprioceptive feedback affects both the frequency and duration of the swimming rhythm (Fig. 41). The results indicate that proprioceptive feedback, presumably from the MRO's, has excitatory effects on the CPG and tends to maintain swim frequency at a constant level. Although the MRO's have been shown to make direct excitatory connections with certain swim motorneurons their possible effects in setting swim frequency are probably the result of direct interactions with the CPG itself. The MRO's are the only identified proprioceptors in the abdomen which are capable of such a role since they have axons which project the length of the CNS and therefore into the region where the CPG is thought to be located. The synaptic connections with the motorneurons are probably secondary with respect to their effects on the motor programme but may be important in firing the motorneurons at a frequency which is appropriate to the output frequency of the CPG. Sensory feedback also has demonstrable inhibitory effects on the swimming rhythm (Figs. 39, 41). Exteroceptive feedback is capable of switching on, terminating and interupting swimming. The receptors involved in producing these effects have not been investigated and it has not been resolved whether inhibition occurs at the level of the motorneurons or by interactions with the CPG. Of these, the latter possibility is more likely since stimulation of abdominal second roots does not produce signficant inhibition of the motorneurons.

It has now been established that sensory feedback plays an important role in patterning motor activity in a number of other rhythmic systems. However very few underlying principles have emerged so far from studies in a variety of vertebrate and invertebrate species. This may be due to the fact that central pattern generation itself is substantially different in many rhythmic behaviours. In some, for example the swimmeret system (Heiter, 1978), the motorneurons themselves are an integral part of the CPG and therefore sensory receptors with synaptic connections on the motorneurons can have direct effects on the frequency and timing of the rhythm. In the cockroach, specific receptors have been described at a leg joint which inhibit the production of bursts in leg flexor motorneurons when they are stimulated (Wong and Pearson, 1976). There is no evidence that the motorneurons are an integral part of the CPG in Galathea. If this is the case then sensory feedback interactions must affect the thoracic CPG to modulate swim frequency.

Clearly there is a dual function for sensory input in the swimming rhythm. The inhibitory effects of exteroceptive feedback appear to compensate for perturbations in the external enviroment while proprioceptive feedback excitation is obviously involved in the normal patterning of activity. In some respects this phenomenn is perplexing. If peripheral elements were constant there would be little or no need for proprioceptive feedback to operate in such a way. The motorpattern could be generated entirely centrally and the system function in an open loop manner. But biological structures are not constant. They change as the animal grows and their properties are altered by frequent use. The latter is certainly true of the MRO's. Thus a continuous monitor of the internal environment is necessary for any motor system to function effectively throughout life.

The motor programme

Each of the two components of the motor programme (extension and flexion) is phase and latency constant over a wide range of swim frequencies, typical of centrally generated rhythms. The output of the CPG for swimming decreases in frequency and amplitude in the absence of sensory feedback from the abdomen. As this occurs the duration of extensor and flexor bursts and the interval between the two increases as a constant function of cycle period. This finding suggests that the mechanisms which drive the two antagonistic sets of motorneurons are coupled. On some occasions however, extension or flexion could occur in isolation, particularly in intact animals. Hence the two phases are not mutually dependent on each other. The absence of one phase does not result in the absence of activity in antagonistic motorneurons. It is difficult to assess from intact preparations whether or not the absence of one phase resulted from inhibition of the motorneurons or the part of the CPG controlling them.

Each of the anterior four abdominal ganglia elicits rhythmical alternating bursts of activity in extensor and flexor motorneurons during swimming. The neural mechanisms which produce these bursts are likely to be similar for each ganglion. The chain of ganglia is serially linked and during swimming a metachronal wave of excitation passes in an anterior to posterior direction. The delay between successive ganglia is not constant, however, and increases with cycle period. It seems likely therefore that there is a built in interganglionic delay line which determines the latency between successive ganglia and which is dependent on cycle period. This might consist of a series of coordinating interneurons. The latency between G1 and G4 reaches an apparent maximum of 190 ms regardless of further increases in cycle period. Clearly a 190 ms delay means that G4 FE's will be active when G1 FF's are firing. Perhaps further increases in

delay would result in interference between closely located antagonistic muscles, a result which naturally must be avoided. Anterior flexion can occur at the same time as posterior extension so long as the two do not overlap at any time.

In the course of this study I have never observed any reversal in the phasing of the rhythm. Flexion never preceded extension at short latency and in this respect swimming is an essentially stereotyped motor programme. In a number of other crustacean rhythmic motor systems the normal motor programme is periodically interupted and the CPG can spontaneously switch into a different mode in which the coordination of the entire rhythm is completely reversed. This has been observed in the rhythmical beating of the scaphognathites or gill bailers of Carcinus (Arudpragasm and Naylor, 1964) and, later, in the swimmeret system of the crayfish, Pacifastacus (Heitler, 1981). The role of these reversals in power stroke and return stroke phases is thought to be in cleaning the respective appendages of debris. The inability of the swimming rhythm in Galathea to phase-reverse may be due to the fact that there can only be one power stroke in this behaviour (flexion). The extension phase of the swim cycle has evolved in such a way that abdominal extension occurs with the minimum of counter thrust and as a consequence of design is ineffective in producing power.

A hierarchy of linked oscillators?

The motor programme for swimming is not restricted to the phasic extensor and flexor muscles. There is a simultaneous activation of portions of the segmental limb motor circuitry which is sufficiently powerful to override any ongoing rhythmic activity in the walking legs and swimmerets.

During swimming the walking legs are thrown forwards (protracted) in an anterior to posterior metachronal wave which occurs approximately

in phase with each abdominal flexion. This activity presumably functions in streamlining the body as the animal is propelled backwards during swimming, and the metachronal wave ensures that successive limbs do not collide as they are thrown forwards.

Concurrent with leg protraction during swimming the swimmerets are flicked posteriorly (retracted) by the action of a single swimmeret motorneuron. This neuron was recorded intracellularly only once during swimming but on that occasion it appeared to receive similar input to the FF's. The frequency and duration of spiking of the neuron could be correlated with the intensity of flexor motor output and this also suggests similar inputs to the two. The dendrites of this neuron projected into the major ipsilateral flexor neuropile area and therefore it may share some of the premotor FF circuitry. These dendrites may also be a locus for 3rd root drive of the neuron and 1st root excitation of FF's. It is not clear whether this neuron functions only during swimming or whether it is also involved in swimmeret beating. Some features of this neuron resemble the SG of the crayfish giant fibre escape system. In particular the neuron, which has powerful peripheral effects compared with other swimmeret neurons, appears to be the only one of the available pool that is specialized with respect to the swimming circuit. On the basis of this fact alone the phase 2 unit is clearly a good candidate for the primitive homologue of the SG, assuming that such a neuron exists. Unfortunately the substantial differences between the two make it impossible to claim that they are homologous. The crayfish SG has no known peripheral effects despite having a large diameter axon in the first root. The phase 2 unit in Galathea produces phasic twitches of the swimmeret but these powerful peripheral effects may not be crucial to escape behaviour since the powerful thrust of the abdomen during flexion is likely to force the swimmerets into a posterior position anyway.

The CPG for swimming resides in the thoracic nervous system but its effects are clearly distributed throughout most of the animal. During swimming the activity of the segmental limbs is dominated by a CPG which is not involved in other rhythmic behaviours. Clearly this situation constitutes a hierarchy of linked oscillators in which the CPG for swimming, when active, is dominant and the segmental limb CPG's are subordinate. Within the swimmeret system, the limb CPG has been shown to have two output modes (MPI and MPII; Heitler, 1981). During swimming in Galathea a different rhythmical output mode is activated while all others are inhibited. In the walking legs the situation is more complex since, as yet, there is no clear evidence that their rhythmic movements are generated entirely within the CNS. In addition the legs display a wider range of complexly coordinated rhythms. The neural mechanisms responsible for these changes in output mode may be complex but the present results emphasize the occurrence of tight coordination between anatomically distinct sets of rhythmic structures which are not normally coordinated.

Different mechanisms drive antagonistic sets of motorneurons

A significant finding to emerge from this study is the existence of two apparently different neural mechanisms driving antagonistic FF's and FE's during swimming. Unitary synaptic potentials contribute to the rhythmical firing of both types of motorneurons but the FF's, in addition, undergo large slow wave oscillations in membrane potential of up to 15 mV in amplitude. Current injection experiments have shown that FF depolarizations result from periodic cyclical excitation probably via chemical synapses. In contrast FE's are driven by a barrage of high frequency EPSP's and there is no slow wave oscillation in membrane potential comparable in magnitude to that seen in FF's. FE's often spike on the rising phase of their depolarization while FF spikes always sit on top of the membrane slow wave.

Based on the present data it is only possible to speculate as to the possible reason for these apparent differences in excitation. The activity of FE's and FF's produce substantially different motor responses: FF's are responsible for the power stroke phase of swimming while FE's produce the return stroke. Perhaps this is the key to their different modes of activation. In order to produce an effective and powerful power stroke movement, FF's must fire at high frequency for a limited duration. The occurrence of a large slow wave oscillation in FF's may ensure that the entire pool fires more or less synchronously and at high frequency. It may be less important for FE's to discharge with such a high degree of synchrony. A mechanism which allows FE's to cross threshold in a graded manner may allow for a greater degree of control over the magnitude and direction of the return stroke. Such a gradation in FE spike thresholds does exist (type 1 and type 2 FE's). Cobalt backfills of FE's (Chapter 1) shows a distinct gradation in soma diameters between about 40 μm and 100 μm. Type 2 FE's which have very large somata correspond to the largest of the available pool. Type 2 FE's also have high thresholds and often do not spike during swimming. Further gradations in spike threshold among the remaining type 1 FE's may exist but these have not been investigated. It is recognised that in many invertebrate motor systems, soma diameter is a reflection of the extent of peripheral distribution of motorneurons (e.g. crayfish MoG, Wine and Hagiwara, 1978). It is probable then that type 2 FE's have a larger peripheral distribution than type 1 FE's. The relatively high threshold of type 2 FE's may ensure that the neurons with the most powerful effects in the periphery are only recruited during intense bouts of swimming when CPG amplitude is higher.

Comparisons with other crustacean motor systems

The slow wave excitation of FF's closely resembles the oscillatory potentials seen in scaphognathite motorneurons (Simmers and Bush, 1983). Interestingly however, the synaptic mechanisms underlying motorneuron burst production in the two systems are completely different. In the scaphognathite system, motorneuron bursts are generated by a periodic release from rhythmic chemical synaptic inhibition and no evidence exists to suggest any synaptic excitation of scaphognathite motorneurons. In contrast, FF's in Galathea are driven by synaptic excitation during bursts and receive periodic synaptic inhibition while antagonists are firing. Clearly these differences are likely to be generated at the premotor level since in neither system does current injection reveal an endogeneous bursting capability similar to, for example, pyloric motorneurons in the stomatogastric system (Selverston et al., 1976). In the scaphognathite system two types of non-spiking premotor interneurons have been identified which depolarize rhythmically in phase with activity in power and return stroke motorneurons (Simmers and Bush, 1980). These excite agonists and inhibit antagonists and may account for the smooth membrane potential oscillations and lack of discrete synaptic potentials in scaphognathite motorneurons. Non- spiking transmission is feasible in this system because the scaphognathite CPG's are small localized networks. This is not the case in Galathea. The swim CPG is located several centimetres away from the abdominal motorneuron pools and CPG output involves digitized spiking in a pool of descending premotor interneurons. This is substantiated by the ability to record discrete synaptic potentials in favourable preparations. The often smooth slow wave depolarization may result from many summated pre-synaptic inputs occurring some distance from the soma which consequently become smooth and attenuated as they are passively propagated

back to the recording site. It is possible that spiking information may be gated via a pool of local non-spiking interneurons in each abdominal ganglion. Clearly, future experiments must be aimed at recording from the premotor circuits to investigate this possibility.

A second interesting difference between the two systems is that both levator and depressor motorneuron pools innervating the scaphognathites receive similar rhythmical oscillations in membrane potential. This occurs only in FF's and not FE's in Galathea. The previous speculation that slow wave excitation of FF's occurs because flexion is the power stroke of the behaviour could also be applied to these interspecific differences. In the scaphognathite system either phase of the rhythm can adopt the role of power stroke depending on whether the CPG is in forward or reversed mode. Clearly the contrasting mechanisms for rhythmic burst generation in these motorneurons is an important unresolved issue and further experiments on different motor systems will aid our understanding of why these differences occur.

Central mechanisms for peripheral inhibition

The phasic flexor and extensor muscles of Galathea are innervated by two large inhibitory motorneurons in each hemiganglion, FI and EI, respectively. Although direct evidence for peripheral inhibition has only been obtained for FI in this study, the strong case for homology with the crayfish documented in chapter 1 leaves little doubt that the large contralateral soma seen in r2 backfills inhibits the phasic extensor muscles. Prior work on the crayfish has shown that the giant axons reliably excite EI and FI with an appropriate delay (summarized in Wine and Krasne, 1982) but their activation during non-giant tailflips has not yet been documented. The present study has permitted an analysis

of the firing patterns and modes of activation of EI and FI during backwards swimming in Galathea.

Each neuron fires a limited number of spikes per cycle, normally at the onset of activity in the pool of antagonist excitors. However the synaptic mechanisms underlying their activity appear to be substantially different. The first of the two neurons to fire in a bout of swimming is EI. Interestingly the mechanisms driving EI to spike are very similar to those driving FF's but dissimilar to those driving FE's. EI appears to receive the same underlying slow wave as FF's and dual recordings from EI and an FF (Fig. 59d) reveal that many synaptic inputs to the two neurons are common. In contrast to the FF's, however, EI spiking is terminated abruptly by high frequency IPSP's superimposed upon the slow wave. Thus EI fired a maximum of two spikes per cycle at the onset of flexion. The advantages of driving FF's and EI with the same mechanism are two fold. Firstly, EI must fire immediately following the major burst of extensor excitor activity. Since flexion is tightly coupled to the offset of extension at short latency, EI should fire at the onset of flexion. Secondly, it is crucial that neither EI nor FF's fires during extension and therefore a powerful mechanism for post-burst inhibition is required for both neuron types. The synaptic drive to EI and FF's differs only in the powerful mid-burst inhibition of EI which limits the number of spikes in this neuron to a maximum of two per cycle.

The generation of rhythmical activity in FI differed from all other swim motorneurons. FI fired a maximum of one spike per cycle which always occurred after the end of the main flexor excitor burst. In favourable preparations the mechanisms driving FI to spike appeared as a complex waveform with both excitatory and inhibitory components. The major source of excitation to FI began towards the end of flexion but did not resemble that seen in either FF's or FE's.

The limited number of spikes occurring in EI and FI may, in part, be due to surgical deafferentation. For example, it has been shown in chapter 2 that the MRO's monosynaptically excite FI and clearly this source of excitation is removed following deafferentation. Since the MRO's are normally active during abdominal flexion the EPSP's could conceivably fire FI for longer and at higher frequency during swimming. EI, which does not receive input from the MRO's is nevertheless driven powerfully by r2 stimulation and perhaps other sensory inputs arising from abdominal extension excite EI during swimming. It is clearly essential that during swimming one set of muscles is prevented from contracting while antagonistic muscles are active. But it may not be necessary in this system for the inhibitors to fire multiple spikes. EI and FI are two of the largest somata in each ganglion and presumably, therefore, they have extensive peripheral distributions. Hence a single spike, or pair of spikes in either neuron may be sufficient to cause total inhibition of the relevent segmental musculature. Antagonistic contractions of the phasic abdominal muscles are further prevented by central mechanisms which inhibit the excitor motorneurons in the interburst interval and hence peripheral inhibition need not be long lasting.

CONCLUDING REMARKS

Evolutionary considerations

The comparative study of escape mechanisms among decapod crustaceans is yielding important insights into the processes which generate different behaviours in related species. So far, information is available on escape behaviour in a limited number of decapods but the clear establishment of neuronal and behavioural homology facilitates comparisons of escape in these species. Evidence from macruran and anomuran species indicates that certain portions of the escape circuits in diverse forms are homologous. A paired giant fibre escape system is common to every macruran thus far studied. No anomuran has so far been found to possess two pairs of giant fibres but some species (e.g. hermit crabs and Callianassa have MG-MoG circuitry while others (e.g. Galathea) have only non-giant escape circuits.

An important deduction to arise from these comparative studies is that the giant fibre system of crayfish is a relatively late development in the escape circuit (Wine and Krasne, 1982). If this is true then a brief survey of the existence of giant fibre escape systems in decapods should reveal which species are primitive and which are advanced in their escape behaviour. On this basis, Galathea is primitive compared with crayfish, hermit crabs and Callianassa. The strong homologies that exist in the motor circuitry of crayfish, hermit crabs and Galathea suggest that all three derive from a common ancestor, resembling Galathea. The close taxonomic relationship between the squat lobsters and the sand crabs (Hippoidea) is reflected in the similarity in their escape responses and the homologies in their tailfan neuromuscular system. Galathea is probably closer to Blepharipoda than to Emerita in its escape system. Blepharipoda performs repeated tailflips which begin with extension while Emerita

performs rapid sculling movements with its uropods. It is thought that uropod beating in Emerita is a late development of Blepharipoda's tailflip (Paul 1981b). Taken together, these homologies indicate that the Galatheids (and perhaps Blepharipoda) are a primitive stock from which the more advanced forms developed and that non-giant escape swimming driving extension before flexion is the primitive escape system. Perhaps Emerita is an evolutionary stage in tail reduction which ultimately became the Brachyurans.

It has long been known that the Anomura is a division of convenience comprising a range of forms intermediate between the Macrura and the Brachyura. Clearly the hypotheses presented above suggest that the division requires revision. I would not suggest that decapod classification be modified purely on the basis of interspecific differences in escape circuitry since other features are likely to be important and perhaps conflicting. For example the decapods are alone among the Crustacea in possessing square-faceted compound eyes, and these only occur in long bodied forms. The crabs (Brachyura) and hermit crabs, but interestingly not the squat-lobsters, have hexagonally faceted eyes (Fincham, 1980). Thus, in contrast to the escape circuitry, eye structure would link the squat lobsters with the Macrura and the hermit crabs with the Brachyura. However the larval stages of all these forms have hexagonally faceted eyes, and symmetrical extended abdomens. The taxonomy of the decapods is clearly complex and must remain enigmatic at present.

Unresolved issues

A compelling feature of scientific research is that for every problem solved, many more are created. Consequently research into the "simpler" neural networks underlying behaviour in invertebrates has revealed unexpected levels of complexity. Within escape systems, where survival depends upon milliseconds and millimetres the existence of subtle and sophisticated networks and pathways have been documented (Wine and Krasne, 1982). Such is the nature of this type of research that any project is likely to end with the investigator more inquisitive (and perplexed) than he was to begin with. In the project reported in this thesis, the technical difficulties of recording intracellularly from neurons in semi-intact preparations have been compounded by the problems involved in keeping Galathea alive following surgery. The development of a successful preparation for intracellular recording during fictive swimming occurred only recently and consequently the data reported on motorneuron activity is in some ways preliminary. An important unresolved question is whether or not the motorneurons in abdominal ganglia are coupled. Future experiments on swimming in Galathea should be directed at dual intracellular recordings from pairs of motorneurons to answer this question. Intrasomatic recordings from the motorneurons reveal small attenuated synaptic potentials which are clearly visible only in the most favourable penetrations. The development of techniques for recording from neuropilar segments of swim motorneurons would further aid these investigations. Clearly the mechanisms responsible for firing the motorneurons rhythmically reside in the activity of premotor interneurons of the CPG and these must be an important locus for future research into the central pattern generation of the swimming rhythm. The premotor circuits are complex and since the intersegmental

latency is not fixed, coordinating interneurons must exist between two adjacent ganglia. It would be feasible in future experiments to search for these in the abdominal connectives.

The role of sensory input in the generation of rhythmic behaviour is an important unresolved issue not only in this species but in virtually every other neurobehavioural preparation. The investigation presented in chapter 3 of this thesis indicates that the MRO's may play an important role in setting swim frequency via direct connections with the CPG for swimming. This topic requires further investigation. The ease with which one can penetrate the central axons of the receptors and drive them with injected current could be used successfully in the semi-intact preparation to look more closely at CPG-MRO interactions.

Glossary of abbreviations

Acc. 1, 2, 3	- accessory neurons of the MRO's.
A1 - A6	- 1st - last abdominal ganglion in crayfish.
CDI	- corrolary discharge interneuron.
CNS	- central nervous system
CPG	- central pattern generator (oscillator).
DCA	- dorsal cord activity.
eI	- tonic inhibitor of the slow extensor muscles.
EI	- phasic inhibitor of the fast extensor muscles.
EJP	- excitatory junctional potential in muscle.
EPSP	- excitatory post-synaptic potential.
EMG	- electromyogram.
FAC	- anterior cluster of FF's.
FE	- fast extensor motorneuron.
FETi	- fast extensor tibia motorneuron in the locust.
FF	- fast flexor motorneuron.
FI	- phasic inhibitor of the fast flexor muscles.
FMC	- medial cluster of FF's.
FPI	- posterior cluster of FF's.
GI	- giant interneuron in hermit crabs.
GML	- Gatty Marine Laboratory.
G1 - G5	- chain of free abdominal ganglia in Galathea. G5 is caudal.
IJP	- inhibitory junctional potential in muscle.
IPSP	- inhibitory post-synaptic potential.
LG	- lateral giant fibre in crayfish.

Mg	- medial giant fibre in crayfish.
MoG	- motor giant neuron of crayfish.
MoGH	- a soma homologous with the crayfish MoG in Galathea.
MRO	- muscle receptor organ.
NSSR	- non-spiking stretch receptor.
r3s	- superficial branch of the 3rd root.
r3m	- main (or deep) branch of the 3rd root.
r1, r2, r3	- 1st, 2nd and 3rd roots of abdominal ganglia.
RM1, RM2	- receptor muscles of the MRO.
sE	- slow extensor motorneuron.
SETi	- slow extensor tibia motorneuron in the locust.
sF	- slow flexor motorneuron.
SG	- segmental giant neuron.
SN1, SN2	- sensory neurons of the MRO embedding in RM1 and RM2 respectively.
Th.Ab.G.	- fused thoracic-abdominal ganglion.
TGM	- thoracic ganglionic mass.
v.s.n.a.	- ventral sub-neural artery.

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