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THE NEURAL BASIS OF ESCAPE SWIMMING BEHAVIOUR IN THE SQUAT LOBSTER *GALATHEA STRIGOSA*

I. ABSENCE OF CORD GIANT AXONS AND ANATOMY OF MOTOR NEURONES INVOLVED IN SWIMMING

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SUMMARY

1. The anatomy and physiology of neurones underlying escape swimming behaviour in the squat lobster, *Galathea strigosa*, have been investigated, and the results are discussed in the context of the evolution of decapod escape behaviour.

2. In contrast to crayfish, hermit crabs and a number of other related decapods, *Galathea* does not possess a giant fibre system for escape.

3. Fast flexor motor neurones (FFs) and fast extensor motor neurones (FEs) have been shown, by cobalt backfilling, to be homologous with crayfish FFs and FEs in number, size and distribution of somata. A small degree of intersegmental and interspecific variation is noted.

4. The flexor inhibitor (FI) neurone is described in terms of its central anatomy, peripheral function and peripheral branching pattern. In each of these respects the neurone is found to be homologous with the crayfish FI.

5. The neurone homologous with the crayfish motor giant (MoG) in its soma size and position is found to be a typical FF in *Galathea*. This 'MoGH' contrasts with the crayfish MoG in having central neuropilar arborization and in lacking axonal branches in the connectives. These differences can be accounted for by the absence of cord giant axons.

INTRODUCTION

Closely related species may display patterns of behaviour which are basically similar, but differ in detail between species either qualitatively or quantitatively (Tinbergen, 1958, 1960; Berg 1974; Blest, 1960). Such patterns, which have diverged from a common ancestor through different selection pressures in their respective habitats (Baerends, 1958; Wickler, 1961), are termed homologous. In multisegmented animals, basically similar structures may be differentiated and specialized in successive segments, and the types of behaviour in which these structures are used can also differ either qualitatively or quantitatively. Interspecific and intersegmental

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differences in homologous patterns of behaviour are reflected in the structure and function of neurones and neural circuits. A determination of the extent and nature of neuronal homology and variation through the comparative study of related segments and species should facilitate an understanding of the processes which underly the development and evolution of neural circuits and of behaviour.

Many decapod crustaceans escape from threats by means of a rapid abdominal flexion, or tailflip. In crayfish, this behaviour is mediated by two pairs of interneurones with giant axons (for recent review see Wine & Krasne, 1982). These make direct electrical connections with a pair of specialized giant motor neurones (the Motor Giants, MoGs) in the connectives between each abdominal ganglion, and indirect weaker connections with a pool of fast flexor motor neurones in each ganglion. During escape the anterior and posterior segments behave differently, depending on the direction of the initiating stimulus. Mittenthal & Wine (1973, 1978) have found that the connections between the giant axons and MoGs are present in some ganglia, but absent in others. In the lobster, escape behaviour does not differ qualitatively from the crayfish, and the giant interneurones and fast flexor motor neurones show apparent strict homology with those of the crayfish (Otzuka, Kravitz & Potter, 1967; Mittenthal & Wine, 1978). In hermit crabs (Crustacea, Anomura) escape behaviour consists of a withdrawal reflex during which they rapidly retract into their shells (Wiersma, 1961; Chapple, 1966). The behaviour is mediated by only one pair of giant axons, thought to be homologous with the crayfish MG axons (Umbach & Lang, 1981); the LG axons appear to be absent in this species. The giant motor neurones (GMs) of the hermit crab are functional homologues of the crayfish MoGs, but their anatomy is significantly different since they synapse with the giant axons close to the ganglionic neuropile. In contrast, the homologous connection in crayfish is located in the connectives near to the exit of the third root (Furshpan & Potter, 1959).

The squat lobster, *Galathea strigosa* (Crustacea, Anomura), differs markedly from both crayfish and hermit crabs. When threatened or startled, it escapes with a series of extension-flexion movements of the abdomen which resemble crayfish tailflips. In contrast to crayfish, however, the abdomen is held flexed beneath the cephalothorax in the stationary animal (Sillar & Heitler, 1982). Thus escape must begin with extension and not flexion. In this paper we examine the anatomy of neurones underlying the escape. The behaviour is not mediated by giant interneurones since these are absent in the abdominal nerve cord. The fast flexor and extensor motor neurones appear homologous with related species. However the neurone homologous with the crayfish MoG is an unspecialized fast flexor. The results, which suggest that escape behaviour is homologous with non-giant swimming in crayfish (Schrameck, 1970), are discussed in the context of the evolution of giant-fibre mediated escape circuits. In the two papers which follow (Sillar & Heitler 1985*a,b*) we have analysed the motor programme for the behaviour, the role of sensory feedback and the mechanisms for burst production in flexor and extensor motor neurones during swimming.

METHODS

Squat lobsters, *Galathea strigosa*, were collected from lobster creels in St Andrews Bay and maintained in circulating sea water aquaria at temperatures ranging from 3

to 15 °C. Mature, healthy adults of either sex, measuring 6–9 cm from tip of rostrum to caudal edge of telson, were used in all experiments.

Anatomy

Relevant parts of the abdominal nervous system were dissected, fixed for 3 h in 3 % phosphate buffered gluteraldehyde and post-fixed in 1 % osmium tetroxide for 30 min. 1-μm sections were stained with Toluidine Blue. The peripheral nervous system of the abdomen was stained with 1 % Methylene Blue (BDH 154490). To examine the central anatomy of extensor and flexor motor neurones, respectively, cobalt chloride (Pitman, Tweedle & Cohen, 1972) was introduced into the cut ends of axons in abdominal 2nd and 3rd roots. On occasions where staining was light, silver intensification was used to enhance cobalt-sulphide stains, using the method of Davis (1982). The following results are based on over 100 such preparations.

Physiology

Conventional methods of extracellular and intracellular recording and display were used throughout. Fast flexor muscle fibres were penetrated using glass microelectrodes filled with 3 mmol l^{-1} potassium acetate and having resistances of 20–40 MΩ. The somata of motor neurones were penetrated in an isolated nerve cord preparation. The nerve cord was pinned ventral side up in a Sylgard-lined Petri-dish and immersed in chilled oxygenated saline (Mulloney & Selverston, 1974, with Tris substituted by TES and buffered to pH 7·2–7·4). The ganglion under study was mechanically desheathed and motor neurone somata were located visually on the basis of soma size and position. Somata were penetrated with glass microelectrodes filled with 5 % Lucifer Yellow (Stewart, 1978) in 1 mol l^{-1} LiCl (40–60 MΩ). Motor neurones were filled using 0·5-s, 10-nA pulses of negative current every second for 20–60 min.

RESULTS

Gross anatomy

The abdominal nervous system in *Galathea* consists of five free abdominal ganglia (G1–G5) linked by paired connectives (Fig. 1C). In crayfish there are six abdominal ganglia. In *Galathea* the first true abdominal ganglion (TAG) has become fused to the posterior end of the thoracic ganglionic mass (TGM). Therefore reference to G1 in *Galathea* corresponds to G2 in crayfish, and so on. Three paired roots arise from each ganglion. As in crayfish and hermit crabs, abdominal third roots (r3) appear to be purely motor and exclusively innervate the flexor muscles. The point of exit of r3 from left and right connectives is asymmetrical; r3 on one side leaves the connective up to 1 mm more rostrally than the other. In addition the third roots of G3 leave the connectives from its dorsal aspect while other abdominal roots arise more laterally (Fig. 1C). These features of abdominal asymmetry and torsion may reflect the close taxonomic relationship between the Galatheid and Pagurid anomurans. However the abdomen of *Galathea* appears to be externally symmetrical (Fig. 1A), and no asymmetries have been observed in either the motor neurone pools or the abdominal musculature.

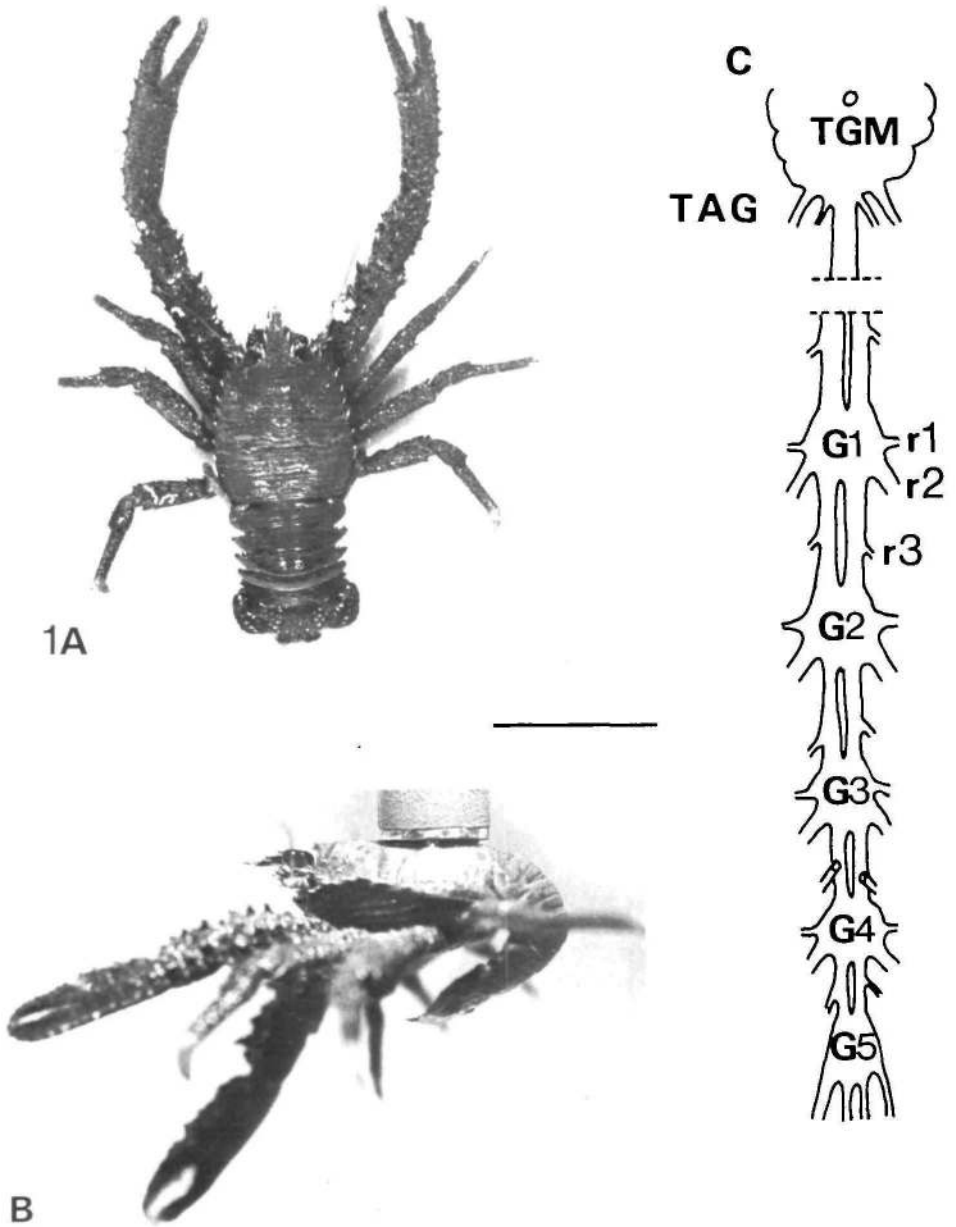


Fig. 1. Anatomy of *Galathea* and its abdominal nerve cord. (A) Dorsal view of *Galathea strigosa* with the abdomen manually extended. Note that the abdomen is externally symmetrical. (B) Lateral view of *Galathea strigosa*. The abdomen is tonically flexed in the resting animal. The animal is suspended from a magnet *via* a small metal plate glued to the dorsal cephalothorax. (C) Schematic representation of the abdominal nerve cord. The five abdominal ganglia (G1–G5) are linked by paired connectives. Above G1 these fuse to form a single nerve cord which enters the first true abdominal ganglion (TAG). TAG is attached to the posterior end of the thoracic ganglionic mass (TGM). The distance between TGM and G1 is up to 20 mm in large specimens. r1, r2, r3 are first, second and third roots, respectively. Note the alternating asymmetry in r3 exit. Scale bar, 4 cm (A, B); 2 mm (C).

Absence of giant axons in the connectives

A striking feature of the abdominal connectives in *Galathea* is an absence of large diameter axons. Cross sections cut at all levels of the abdomen reveal no axon profiles of similar relative diameter to the giant fibres of crayfish or hermit crabs (Fig. 2). In the dorsal region of the connectives just posterior to each ganglion is a group of seven or eight axons which are relatively large in diameter (approx. 20–30 μm) compared with other profiles. In serial sections of the nerve cord these axons leave the connective

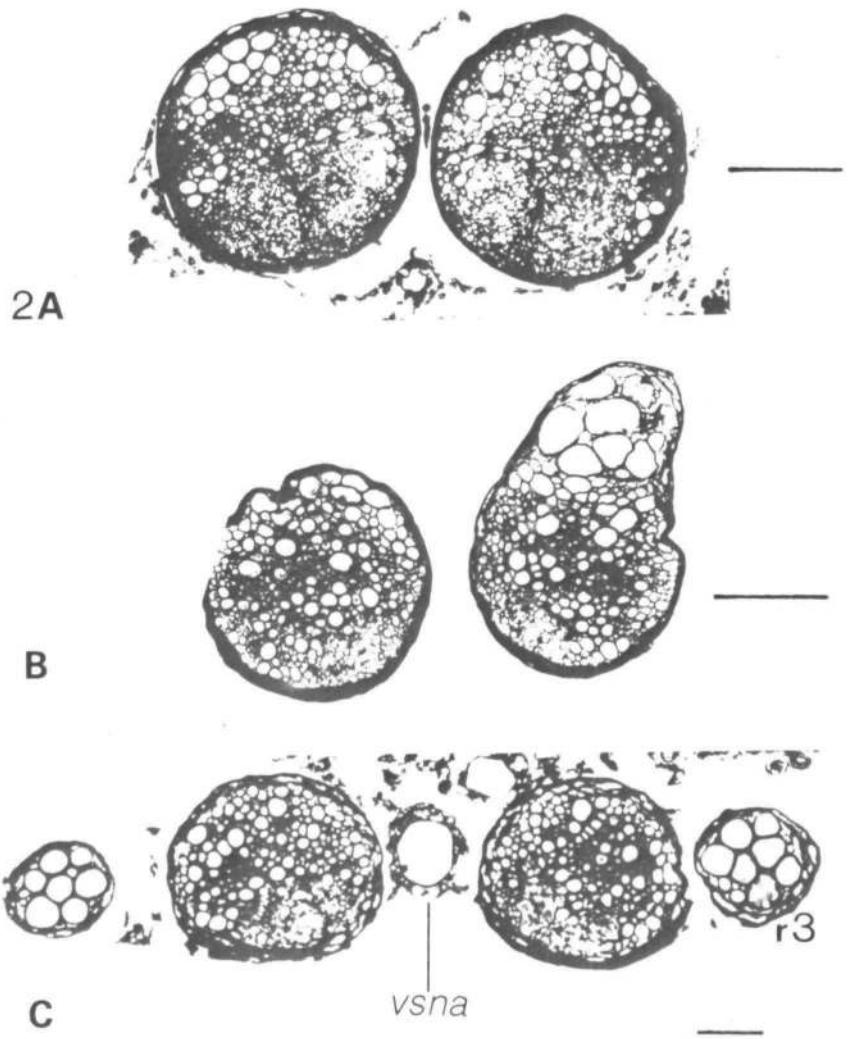


Fig. 2. Cross-sections of the connectives cut at a variety of levels in the abdomen show no giant fibres. (A) Just posterior to G1; (B) between ganglia G3 and G4; (C) just anterior to G5. In (B) r3, containing the FFs, is budding off the right connective, dorsally. In (C) both r3ms, containing the FF axons, lie laterally. *vsna*, ventral subneural artery. Scale bars: A, 100μm; B, 70μm; C, 60μm.

via r3 and are absent in sections cut more posteriorly. Thus, these are the axons of fast flexor motor neurones (FFs). With the exception of these, the dorsal region of the connectives contains a homogeneous population of axon profiles which rarely exceed the diameters of the FFs. In addition, FF axons all have similar diameters, both in the connectives and in r3. This feature also contrasts with crayfish and hermit crabs, in which the axon of the giant motor neurone is significantly larger than other FFs. These results suggest that *Galathea* lacks both giant interneurones and the giant motor neurone although it is likely that homologues of the crayfish neurones are present in reduced form.

Flexor motor neurones

1 mm after leaving the connective, r3 bifurcates into a small superficial branch (r3s) which extends laterally to innervate a thin sheet of ventral tonic flexor muscles, and a larger main branch (r3m) which ascends dorsally and rostrally to innervate the fast flexor muscles. The central anatomy of fast motor neurones has been visualized by selective cobalt backfilling of r3m. Backfilling r3m G2 stains two soma clusters in G2 and one in G3 (Fig. 3). In describing these clusters the terminology of Mittenthal & Wine (1978) for crayfish FFs will be used for ease of comparison. Anterior and contralateral to the filled root is a medial cluster of cells (flexor medial contralateral cluster: FMC) which normally contains four somata. The two largest (approx. 90–110 μm diameter) are homologous with the crayfish F1 and MoG neurones (see below). A second group of neurones have somata located ipsilateral to the filled root in G2 (flexor posterior ipsilateral cluster: FPI). This cluster also contains four somata in G2 with diameters in the range 70–90 μm. Often the FPI neurones form two pairs and the neurites of each pair follow similar paths into the neuropile. A third group (flexor anterior contralateral cluster: FAC), comprising two cells, has somata in the anterior portion of G3 contralateral to the axons.

In terms of the number, size and distribution of somata, FFs in G2 show almost strict homology with crayfish FFs in G3. An exception to this is the occurrence of three FAC FFs in crayfish compared with only two in the homologous cluster in *Galathea*. A further difference is that no branches have been observed arising from any FF axons in the connectives (e.g. Fig. 3A). This contrasts with the crayfish in which the MoG neurone sprouts featherlike processes from its axon near r3, and provides further evidence that *Galathea* may lack a specialized giant motorneurone.

Segmental homology and variation among FFs

The three FF soma clusters have been visualized in G1 to G5 by backfilling r3m of these ganglia (Fig. 4). Attempts to fill r3m to the fused thoracic-abdominal ganglion (TAG) have failed to stain FMC and FPI clusters successfully, probably due to the large distances involved (up to 20 mm in large specimens). However two FAC neurones stain in G1 and eight large axons ascend the connectives towards TAG, suggesting strict homology with G2.

The FAC cluster contains two somata in G1 to G4, but no FAC neurones are present in G5. Each FAC neurone has almost identical anatomy. The axons and neurites of each pair follow similar paths and the two somata are always closely apposed in the anterior contralateral portion of the ganglion.

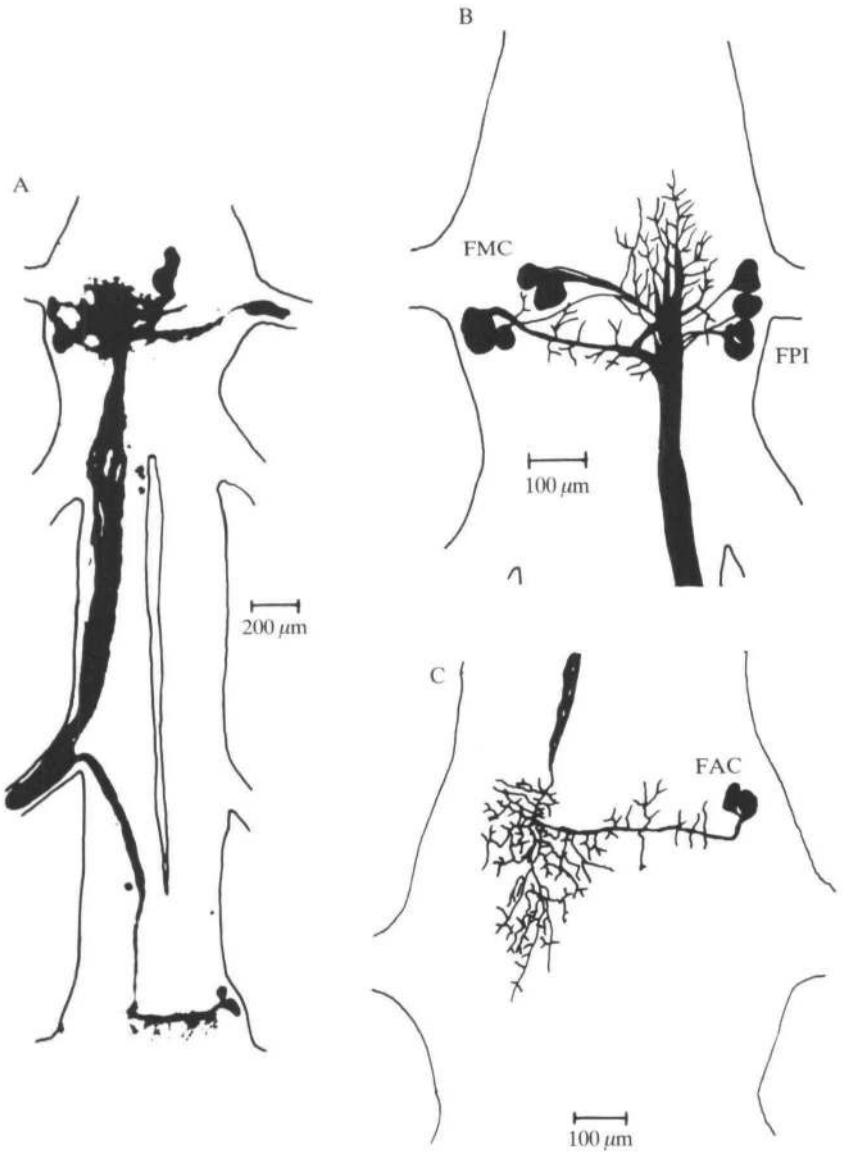
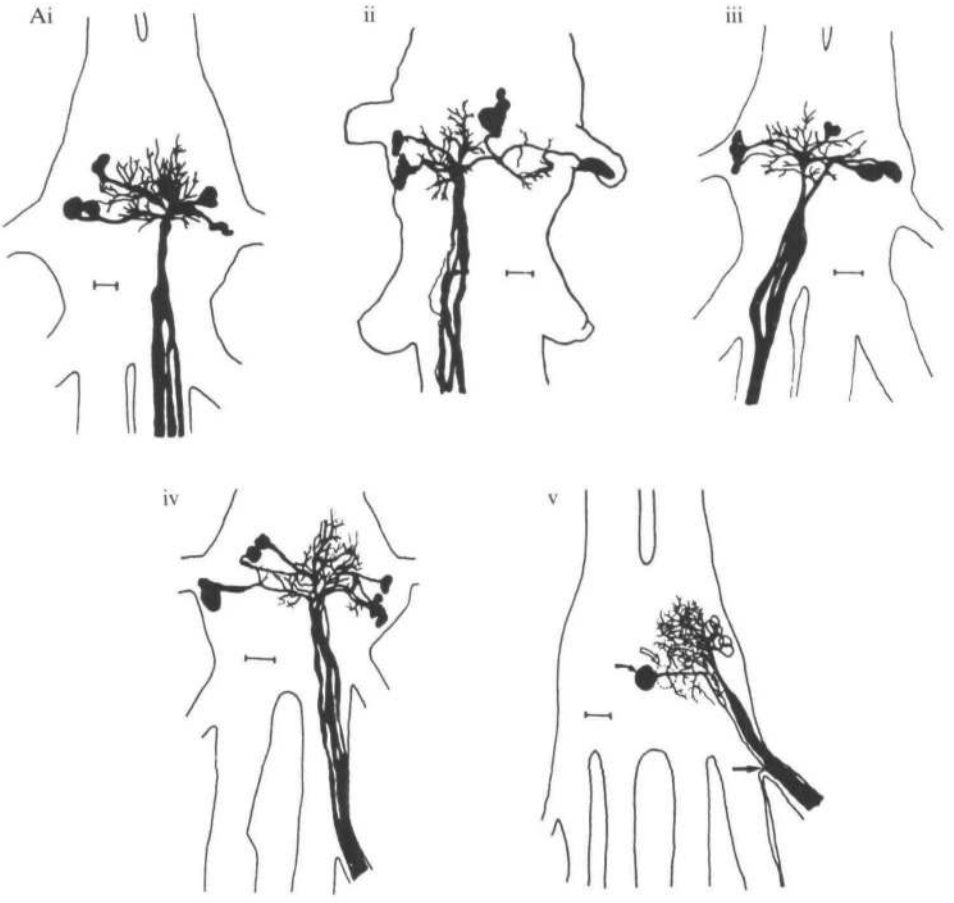


Fig. 3. The central anatomy of fast flexors (FFs) in G2. Backfilling r3m G2 stains three soma clusters. Two are located in G2 and one in G3 (A). In G2 (B) one soma cluster lies contralateral to the filled root (FMC) and comprises four somata. A second soma cluster is located ipsilateral (FPI) and also contains four cells. A third cluster (FAC) comprises two somata which lie in the anterior contralateral portion of G3 (C). (A) is a photograph of a wholemount unintensified backfill. The outline of the nervous system has been added for clarity. B and C are *camera lucida* drawings of wholemount preparations. C is silver-intensified. A, B and C are from different preparations.

The FMC cluster contains four somata in G1 to G3, three in G4 and one in G5. Thus G1, G2 and G3 show strict serial homology in the number, size and distribution of FMC somata. Of the three somata in G4, one is large (approx. 100 μm diameter) and extremely lateral in its location, and is therefore likely to be a homologue of the



B

	Crayfish			*Galathea*			
	FAC	FMC	FPI	FAC	FMC	FPI	
G1	1	4	4	–	(4)	(4)	TAG
G2	3	4	4	2	4	4	G1
G3	3	4	4	2	4	4	G2
G4	2	4	4	2	4	4	G3
G5	0	4	3	2	3	4	G4
G6	–	–	–	0	1	6	G5

Fig. 4.(A) Segmental homology and variation among FPI and FMC fast flexor soma clusters in G1 (i), G2 (ii), G3 (iii), G4 (iv) and G5 (v). G1, G2 and G3 show strict homology. In G4 only three FMC somata stain and no FAC axons descend to G5. G5 deviates most from serial homology with six FPI somata and only one FMC soma. In (v), closed arrows indicate the soma and axon bifurcation of the FMC cell in G5. The open arrow indicates a large unstained soma which may be the contralateral homologue of this cell. Scale bars, 100 μm. (B) Comparison of number and distribution of FFs in abdominal ganglia of crayfish, *Procambarus clarkii* (after Mittenthal & Wine, 1978) and *Galathea*. Dashes indicate lack of data. Numbers in brackets are predicted on the basis of eight ascending axons from fills of r3 TAG.

crayfish FI neurone (see below). The remaining two are smaller and anterior in location. Therefore the missing neurone in this cluster may be the homologue of the crayfish MoG neurone. The single FMC neurone in G5 is large (approx. 100 μm diameter) and located near the midline of the ganglion (Fig. 4Av). It is difficult to establish homology with anterior ganglia on the basis of soma position. However the soma can be traced to an axon which bifurcates before exiting *via* r3. This may indicate that the neurone is an FI homologue (see next section).

The FPI cluster contains four somata in G1 to G4 and shows strict serial homology in these ganglia. G5 deviates from strict homology by having six FPI somata. The similarities with crayfish are striking (Fig. 4B). In both species there is a small anterior-posterior reduction in the total number of FFs. In crayfish this has been correlated with a reduction in the flexor muscle mass in caudal segments.

The flexor inhibitor (FI)

In each of G1, G2 and G3 the FMC cluster is strictly homologous with the crayfish in number, size and distribution of somata. The largest and most lateral of these is homologous with the crayfish FI neurone, identified by Wine & Mistick (1977). Cobalt backfilling either r3m or r3s stains the FI soma and an axon which projects

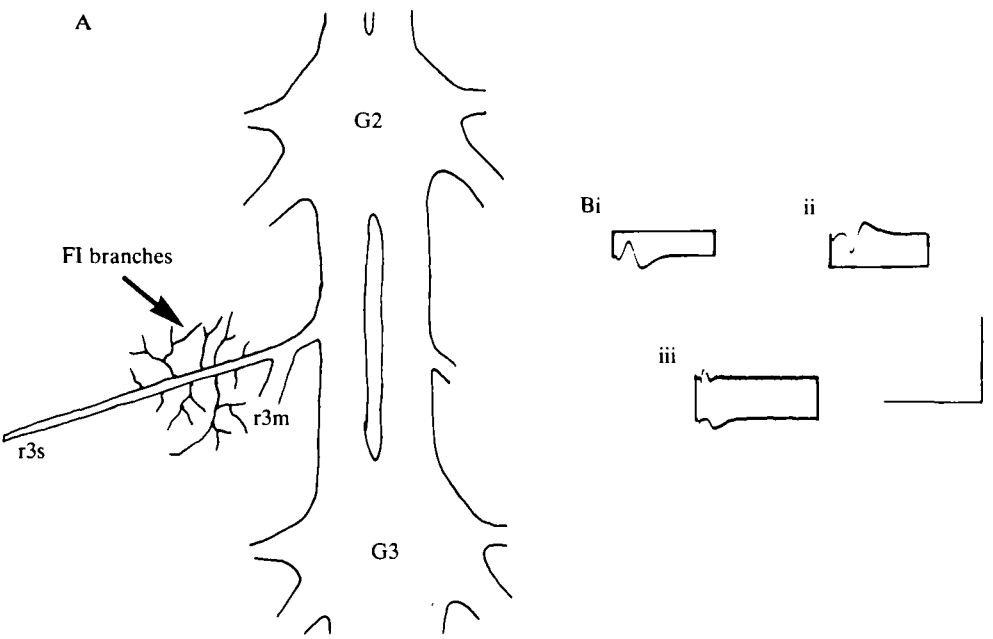


Fig. 5. Anatomical and physiological characterization of FI. (A) Methylene blue stains reveal numerous fine processes arising from the medial portion of r3s and ramifying over the ventral FF muscle fibres. Since FI is the only FF which has an axon branch in r3s, these processes are presumed to belong to FI. (B) Physiological identification of the FI branch point. (i) Stimulating r3s (top trace) results in a short latency spike in r3m (bottom trace) (ii) Stimulating r3m causes a spike in r3s. (iii) Subsequent penetration of ventral FF muscle fibres (in the region depicted in A) results in records showing an IJP (bottom trace, intracellular) correlated 1 : 1 with the spike recorded in r3s (top trace). In this record the FF muscle fibre was depolarized with a holding current of 10 nA. Arrow in A represents region of penetration. Sweeps triggered by stimulus artefact. Scale bars: horizontal, 10 ms (i, ii), 20 ms (iii); vertical, 50 mV (iii).

down the remaining unstained branch of r3. Thus this neurone is the only member of the FF pool whose axon branches at the first bifurcation of r3. Its unique peripheral branching pattern has allowed the following unambiguous identification of the neurone's function and the establishment of homology with the crayfish FI.

Physiological identification of the FI branch point has been obtained as follows (Fig. 5). Extracellular stimulation of r3s or r3m G2 results in a short latency spike recorded in the remaining r3 branch (Fig. 5Bi, ii). This potential is elicited at a set threshold of stimulation and further increases in stimulus intensity (up to 100 V) do not alter its amplitude or waveform. Subsequent intracellular penetration of ventral FF muscle fibres in the region depicted in Fig. 5A, give records showing an inhibitory junctional potential (IJP, Fig. 5Biii) which is tightly coupled to the extracellular spike in threshold and latency. The FI IJP is not always visible in intracellular recordings, presumably because its reversal potential lies close to the resting potential of FF muscle fibres (approx. −70 mV). It was found difficult to alter the membrane potential of FF muscle fibres by injection of up to 10 nA current, probably because of their large volume. On several occasions, however, sufficient depolarization of the muscle membrane was achieved to enhance the amplitude of the FI IJP (Fig. 5Biii). 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 IJPs were hyperpolarizing at resting potential. This may have been due to artificial membrane depolarization resulting from the penetration process.

The central branching of FI has been revealed by injection of Lucifer Yellow into its soma in each of G1, G2 and G3 (Fig. 6). The FI soma was located visually on the basis of its size and position. The dendritic field and major dendritic branches of FI are similar in each ganglion. In addition, the central branching of FI closely resembles that of the crayfish homologue (see Fig. 7 in Mittenthal & Wine, 1978), and no major

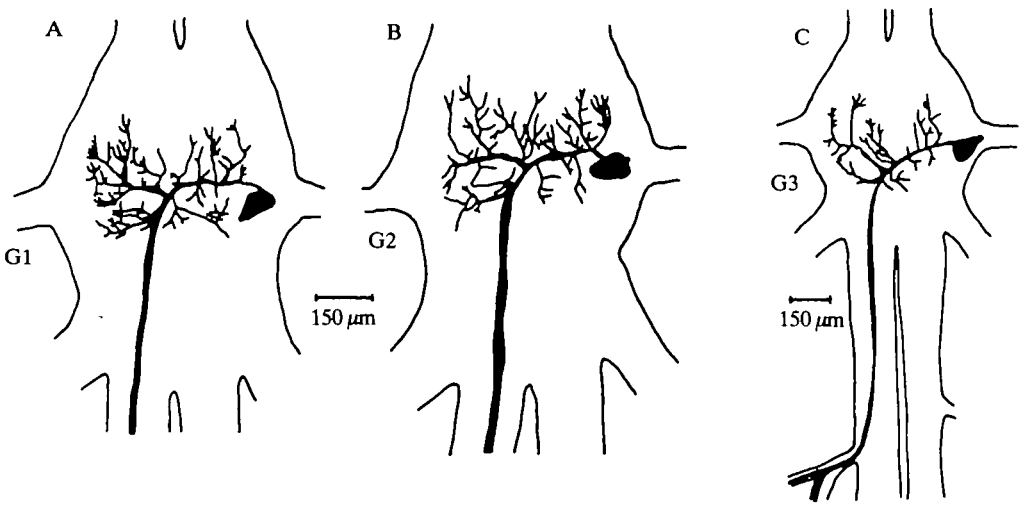


Fig. 6. The central branching of FI in G1 (A), G2 (B) and G3 (C) revealed by injection of Lucifer Yellow. FI has a constant soma position and characteristic central branching pattern. Note in C the FI axon branches at the first bifurcation of r3.

differences exist between the two species. The FI soma is always large (approx. 110 μm diameter) and located at the extreme lateral edge of the ganglion, near the origin of r1. The FI soma often appears ovoid in shape and on some occasions projects partially into the base of the 1st root. This may be due to tissue distortion during processing.

The motor giant homologue (MoGH)

The more medial of the two large FMC somata in G1, G2 and G3 is homologous with the crayfish MoG neurone in terms of its size and position. In contrast to the crayfish MoG, however, this neurone has a complex dendritic tree in the neuropile (Fig. 7A-C) and in this respect appears to be a typical FF. The crayfish MoG has no central dendrites but sprouts featherlike processes in the connectives in the region of the giant fibres (Mittenthal & Wine, 1978). No processes have been observed arising from the MoGH axon in the connectives of *Galathea*. The crayfish MoG branches extensively in the periphery and bifurcates at the first branch point of the third root (Furshpan & Potter, 1959). The MoGH axon in *Galathea* does not branch down r3s but its axon often branches at each bifurcation of r3m suggesting a wide distribution in the periphery.

The crayfish MoG is specialized with respect to the central giants, and receives excitatory input only from them (Wine & Krasne, 1982). In contrast, intrasomatic recordings from the MoGH in *Galathea* reveal a low level of spontaneous synaptic input from a number of unidentified neurones. Single electrical shocks to the TAG–G1 connective elicit a complex compound EPSP in the MoGH soma in G2 (Fig. 8A) in addition to suprathreshold activation of other r3m units. We were unable to observe soma spikes in response to cord stimulation. However, injection of 10 nA

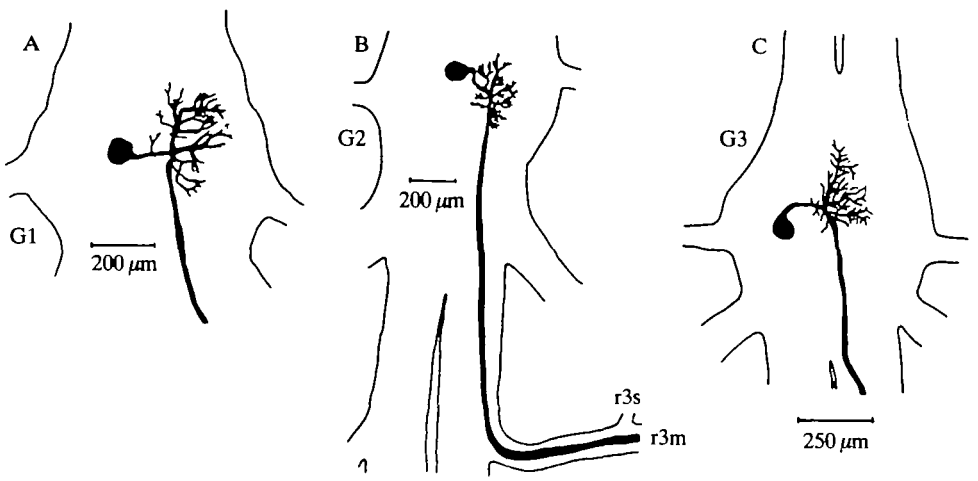


Fig. 7. The central anatomy of MoGH in G1 (A), G2 (B) and G3 (C) following injection of Lucifer Yellow. MoGH arborizes in the main region of flexor neuropile. The MoGH axon has no processes arising in the connectives. It exits *via* r3 and projects down r3m but not r3s (B). In B the MoGH axon branched at the next bifurcation of r3m (not shown).

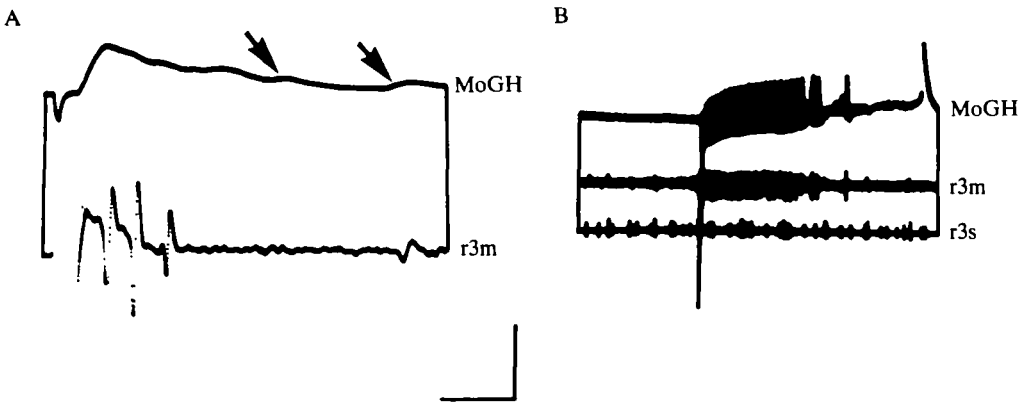


Fig. 8. Physiology of MoGH. The MoGH soma was penetrated in G2. (A) Single electrical shocks (0·5 ms duration) to the TAG–G1 connective elicited a compound EPSP in MoGH (top trace) as well as spiking in a number of other ipsilateral FFs (bottom trace, r3m). Arrows indicate apparent unitary EPSPs. (B) Current injection (+ 10 nA, not monitored) into the MoGH produced a train of soma spikes (top trace) correlated 1 : 1 with spikes recorded extracellularly in r3m (middle trace) but not in r3s (bottom trace). Scale bars: horizontal, 5 ms (A), 250 ms (B); vertical, 5 mV (A), 10 mV (B).

depolarizing current into the soma resulted in a train of spikes which correlated 1 : 1 with spikes recorded extracellularly in r3m (Fig. 8B).

Extensor motor neurones

As in crayfish and lobsters, the axons of efferent and afferent neurones involved in abdominal extension enter the CNS *via* the second root (r2) of abdominal ganglia. Cobalt backfills of r2 G2 stain up to 12 somata in G2 which are distributed among three clusters. Identification of these extensor efferents is based on homologies with neurones in crayfish (Treistman & Remler, 1975; Wine & Hagiwara, 1977) and lobster (Otsuka *et al.* 1967) abdominal ganglia.

As shown in Fig. 9, two clusters of somata are located ipsilateral and one contralateral to the filled root. The somata of four or five fast extensor motor neurones (FEs) form a tight cluster at the ipsilateral edge of the ganglion between the bases of r1 and r2. The diameters of FE somata show considerable variability with a mean of 64 μm. A second cluster lies anterior to the FEs, approximately at the origin of r1, and comprises the somata of four smaller, slow extensor motor neurones (approx. 20–30 μm diameter). In most preparations the neurites of SEs enter the neuropile along a tight tract, distinct from the neurite path of FEs.

In both crayfish (Wine & Hagiwara, 1977) and lobsters (Otsuka *et al.* 1967) the soma of the peripheral inhibitor to the fast extensor muscles (EI) is large (approx. 110 μm diameter in crayfish) and located contralateral to the filled root. Located contralateral to r2 in *Galathea* are two somata (Fig. 9). Of these the largest has a soma diameter of approximately 95 μm; and is therefore labelled the phasic extensor inhibitor (EI). The remaining cell in this cluster has a much smaller soma (approx. 30 μm diameter). In view of its close proximity to EI and its similarity to a small contralateral

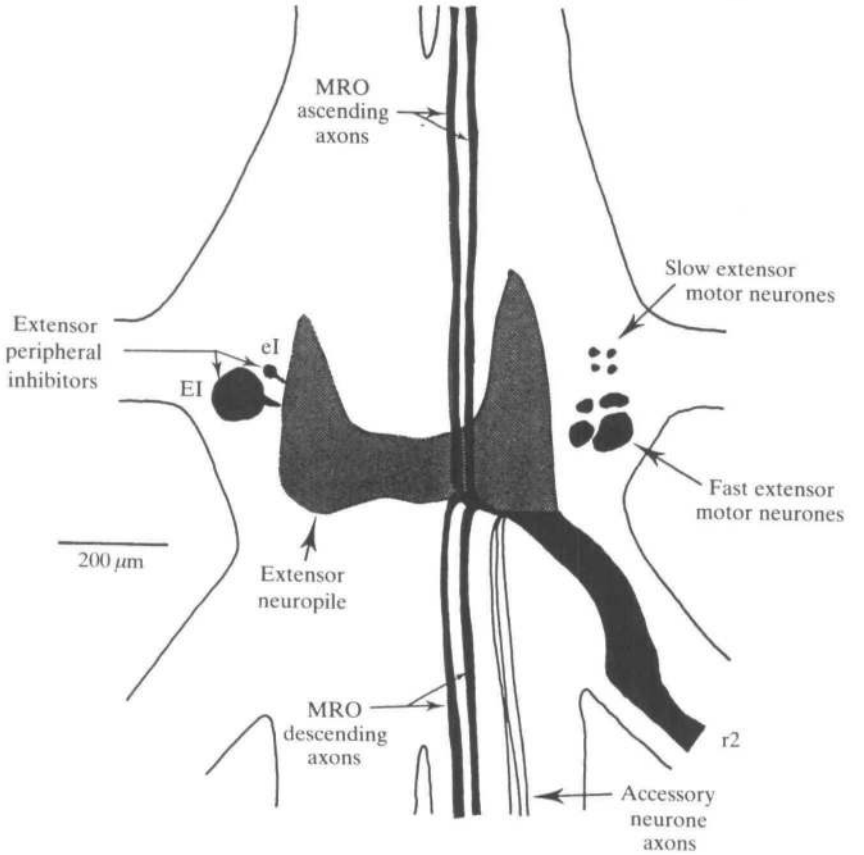


Fig. 9. Overview of extensor efferent somata and MRO axons after a cobalt fill of r2 G2. Stippled area represents the approximate extent of extensor neuropile. Identification of somata is based on a similar overview of crayfish r2 fills (Wine & Hagiwara, 1977).

soma in crayfish this cell is labelled the tonic extensor inhibitor (eI). In crayfish ganglia (Wine & Hagiwara, 1977) a third smaller contralateral soma (approx. 15 μm diameter) of unknown function stains in about 25 % of preparations. In contrast only two contralateral somata are observed in r2 G2 fills in *Galathea*.

The only consistent differences between these results and those of Wine & Hagiwara (1977) for crayfish are the absence of a third contralateral soma and a small reduction in the total number of ipsilateral cells. There are four or five SEs and five or six FEs in crayfish G2. In all essential respects the two pools are homologous in soma size and distribution.

Segmental homologies

Homologous extensor efferents have been stained in each abdominal ganglion except the 5th (last ganglion, not studied) and the fused last thoracic – first abdominal ganglion (Fig. 10). G1, G2 and G3 show strict serial homology in number size and distribution of somata (Fig. 10A–C). In G4, however, there are only three SEs, three FEs and the two contralateral inhibitors (Fig. 10D). This caudal decline in the

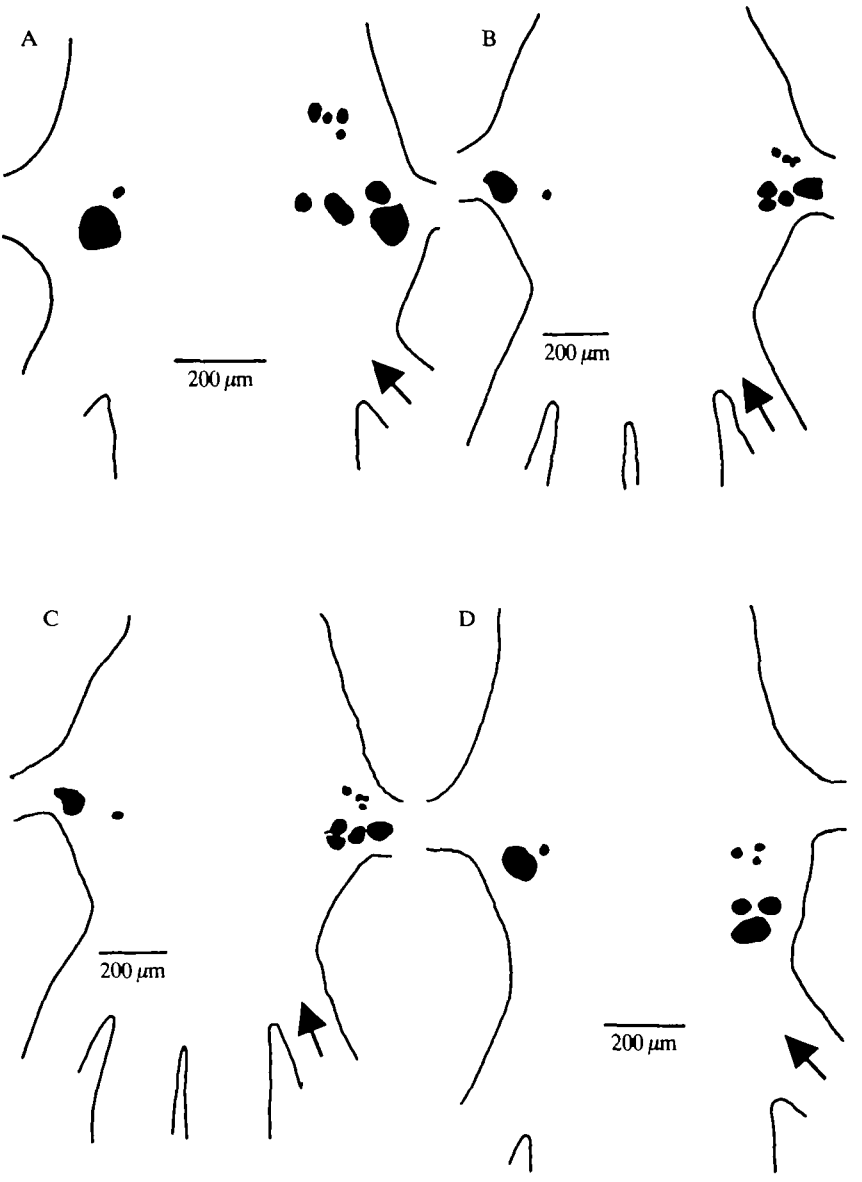


Fig. 10. Segmental homology and variation among extensor efferents in abdominal ganglia G1 (A), G2 (B) and G3 (C) show strict homology in the number, size and distribution of efferent somata, with four FEs, four SEs and two contralateral inhibitors. G4 (D) deviates from strict homology in having only three FEs and three SEs. Arrows indicate roots containing extensor efferent axons. Anterior is at the top.

number of efferent somata is consistent with the result of Wine & Hagiwara (1977) for the penultimate abdominal ganglion in crayfish. TAG also departs slightly from strict serial homology in that only seven ipsilateral and two contralateral somata are stained. Of the ipsilateral cells there are four FEs and three SEs.

Extensor neuropile

The major dendritic domain of the extensor efferents forms a well defined U-shaped region of dorsal neuropile, (Fig. 11), with the densest accumulation of

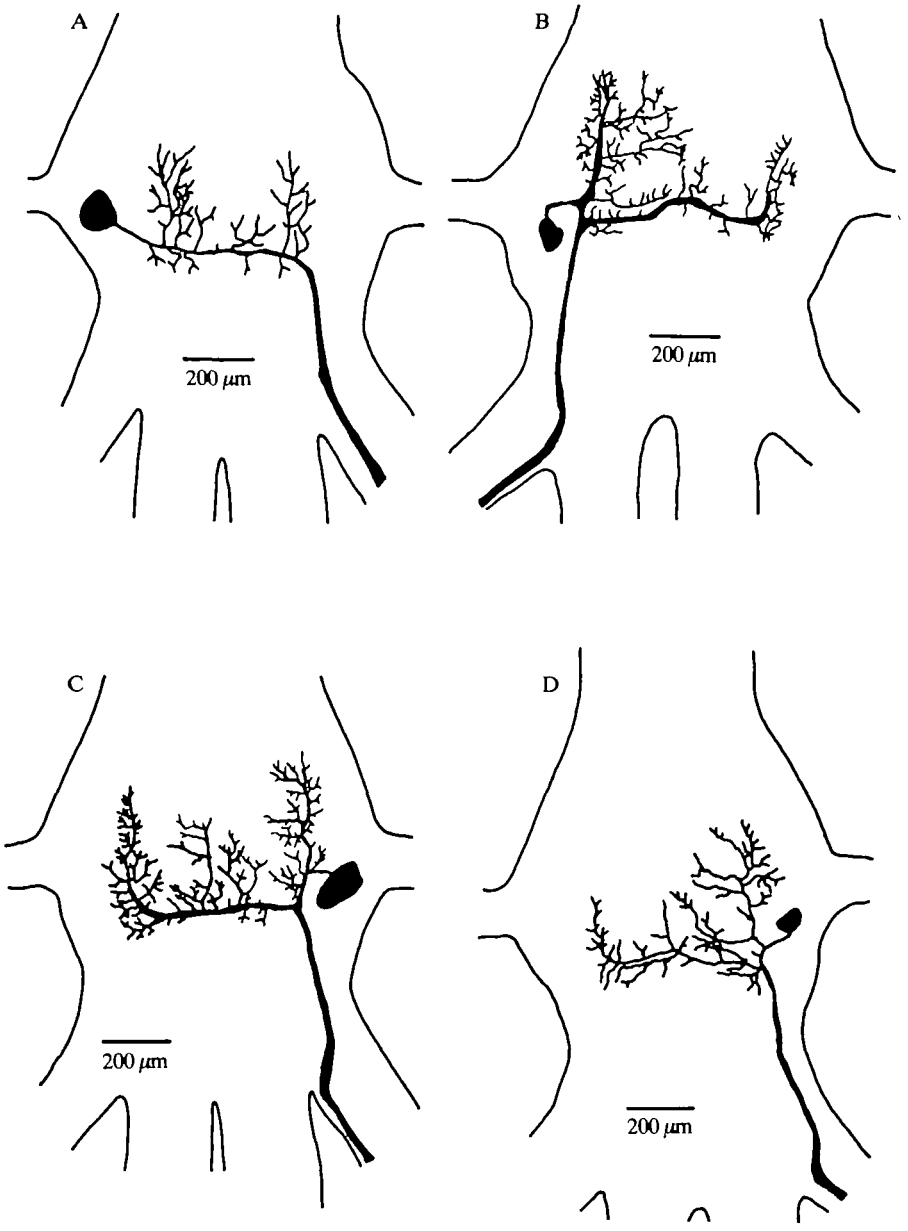


Fig. 11. Central dendritic domains of extensor motor neurones in G2 after intrasomatic injection of Lucifer Yellow. (A) EI has a large contralateral soma and two major regions of dendritic arborization which lie in parallel with the connectives and project rostrally from the neurite. (B)–(D) The FEs all show a similar basic plan in having bilateral dendritic domains.

processes lying on the ipsilateral side of the ganglion. Some of the contralateral neuropilar arborizations can be attributed to EI. When EI is stained selectively by injection of Lucifer Yellow into its soma it is seen to have two major dendritic loci which project anteriorly from the neurite, in line with each contralateral hemiganglion (Fig. 11A). In favourable cobalt fills a tight tract of several large neurites courses horizontally across the ganglion, suggesting that other extensors arborize contralaterally. This has been confirmed by intrasomatic injection of Lucifer Yellow into a number of FEs (Fig. 11B–D). They have complex bilateral branching patterns. The major dendritic branching occurs ipsilateral to the soma but in each fill a large neurite courses laterally across the ganglion to an area of often dense arborization. This feature of FE anatomy contrasts directly with homologous neurones in crayfish (Wine & Hagiwara, 1977), where only a few branches of the extensor excitors may cross the midline of the ganglion.

DISCUSSION

Bullock & Horridge (1965) state that 'Crustacea with a rapid tailflip can be expected to have giant fibres running the length of the cord'. Indeed, among the Crustacea giant fibre systems for escape appear to be widespread, especially amongst those with elongated, flexible abdomens. There are two pairs in *Homarus* (Allen, 1894), *Cambarus* and *Palaemonetes* (Johnson, 1924, 1926) and *Leander* (Holmes, 1942). The stomatopod, *Squilla* has one pair only (Bullock & Horridge, 1965), as do the Anomurans, *Callianassa* (Turner, 1950) and *Pagurus* (Umbach & Lang, 1981). The squat lobster, *Galathea strigosa* (Anomura) has a tonically flexed abdomen at rest (Fig. 1A) which can be repeatedly extended and flexed during escape but it does not possess giant fibres.

In crayfish the giant fibre circuit is now well understood and involves three key neural components: the giant fibres themselves; the MoGs and the Segmental Giants (SGs; Roberts *et al.* 1982). Each of these is thought to be derived from primitive elements in an ancestral non-giant swimming circuit (Wine & Krasne, 1982). Thus it is thought that the giant fibres evolved from smaller premotor interneurones, the MoGs from smaller unspecialized FFs and the SGs from conventional limb motor neurones. The swimming circuit, still present in crayfish, is under the control of a central pattern generator (CPG) which drives extension first with flexion following at short and near constant latency (Reichert & Wine, 1982). Thus the species from which crayfish evolved must have lacked both giant axons in the cord and a pool of MoGs in abdominal ganglia. Moreover escape must have involved backwards swimming by repeated extension-flexion cycles of the abdomen which began with extension. On the basis of the present data, we hypothesize that *Galathea* has features of such an ancestor. If this is so then, in theory at least, each of the three key elements of the crayfish giant fibre circuit will be present in unspecialized or reduced form.

Since the premotor interneurones of the crayfish swimming circuit are numerous, small and largely unidentified (Wine & Krasne, 1982), it may be impossible to identify primitive homologues of the giants in *Galathea* on an anatomical basis. The complete absence of giant fibres in the connectives provides evidence only that escape in *Galathea* involves non-giant circuits. Similarly the SGs probably evolved from

conventional limb motor neurones. The remaining 50 or so swimmeret motor neurones in each hemiganglion are very similar anatomically and hence the search for a primitive SG homologue in *Galathea* is also likely to be unsuccessful.

In contrast, the crayfish MoG was recruited from a relatively small population of large, uniquely identifiable FFs in each ganglion. The MoG differs from other FFs in its soma size and position, location of dendrites and extent of peripheral distribution. Therefore, the primitive homologue of the MoG is identifiable in a non-giant circuit (MoGH).

In terms of the number, size and distribution of FF somata, the homologies that exist between crayfish and *Galathea* are striking (Fig. 4B). In both species anterior ganglia show strict serial and close interspecific homology. In posterior ganglia a decline in the number of FFs occurs and this is correlated in crayfish with an observable reduction in the volume of muscle to be innervated in these segments (Mittenthal & Wine, 1978). The most convincing evidence for homology among FFs in the two species is the FI. This neurone is identifiable on the basis of soma size and position, central branching and peripheral function in *Galathea* and crayfish (Wine & Mistick, 1977). These data imply that the two species derive from a common ancestor and that the FI neurone has changed little since they diverged.

In contrast to FI, the MoGH differs considerably from the crayfish MoG (despite the fact that the two neurones are homologous in soma size and position), having a complex dendritic tree in the neuropile and no axonal branches in the connectives. The major selection pressure on the evolution of the crayfish MoG must have been to ensure a rapid and complete tail flexion in response to a single impulse in the giant fibres. It would seem logical, then, for crayfish to have recruited the largest FF from the available pool (perhaps with the widest peripheral distribution) in order to minimize the amount of modification required in its structure. MoGH in *Galathea* happens to be the largest flexor excitor motor neurone, but other important factors such as axon course and established functional connections with particular premotor elements may well have been important. The possibility that another different FF has assumed the role of MoG in *Galathea* can be ruled out since no axonal specializations have been seen in any FF from cobalt backfills. The FF pools in *Galathea* consist of a homogeneous population of neurones, each with similar anatomy.

The only notable difference that we have observed among FEs in the two species is the presence of strongly bilateral dendritic domains among the fast extensors in *Galathea*. The homologous neurones in crayfish have mainly ipsilateral dendrites with few processes ever crossing the midline of the ganglion (Wine & Hagiwara, 1977).

In crayfish, the presence of ipsilateral FE dendrites has been associated with the retention of a system suitable for independent lateral activity whereas the flexors, with bilateral dendrites (Wine & Hagiwara, 1977), appear specialized to operate bilaterally. But clearly, if *Galathea* does represent the primitive crayfish condition as we have suggested, then the demonstration of bilateral dendrites in this species suggests that crayfish FEs may have secondarily lost their contralateral processes. Crayfish may have evolved lateralized FE dendrites as a concomitant of the giant-fibre system. Post-giant extension in crayfish differs from non-giant extension in being a chain reflex induced by the initial flexion and which is dependent upon patterned sensory feedback

(Reichert, Wine & Hagiwara, 1981). Perhaps it is crucial that post-giant extension is lateralized with respect to this afferent input which may be non-uniform in a changing environment.

Thus several features of escape in *Galathea* resemble the predicted ancestral crayfish form. (1) There are no giant axons in the connectives. (2) The FF and FE pools are homologous with crayfish. (3) The MoGH is an unspecialized FF. (4) Escape involves repeated extension-flexion cycles of the abdomen which begin with extension.

Recent evidence on the escape withdrawal reflex of the hermit crab shows that there is a single pair of giant axons in the connectives which may be homologues of the crayfish medial giant axons. Each central giant axon makes direct electrical connections with a pool of specialized giant motor neurones in the abdomen. These GMs, homologues of the crayfish MoGs, possess most of the anatomical specializations of the crayfish neurone. However, the axonal dendrites are located at the extreme caudal edge of the neuropile and not in the mid-connectives (Umbach & Lang, 1981). Thus the hermit crab lies somewhere between *Galathea* and crayfish in terms of the anatomy of its escape circuitry. The homologies that exist between the FF pools in these three species imply that they all 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 the crayfish are a late development (Wine & Krasne, 1982) and thus unless *Galathea* has secondarily lost the giant fibre-MoG system it must represent an ancestral form in terms of the neural control of abdominal flexion. This means that either the escape system of crayfish and hermit crabs evolved independently but convergently after the divergence of the Macrura and Anomura, or perhaps hermit crabs are more closely related to crayfish than they are to *Galathea*. In either case the non-giant, backward swimming of *Galathea* may represent the primitive form of escape behaviour from which the others evolved.

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