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SWIMMING IN THE PTEROPOD MOLLUSC, *CLIONE LIMACINA*
II. PHYSIOLOGY*

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SUMMARY

The central pattern generator (CPG) for swimming in *Clione limacina* was localized in cutting experiments. A separate pattern generator for each wing is located in the ipsilateral pedal ganglion. The CPGs are tightly coupled but can be isolated by severing the pedal commissure. Removal of the cerebral ganglia results in a decrease in swim frequency and regularity suggesting descending modulation of the CPGs.

Two classes of pedal neurones display firing patterns in phase with swimming movements. One class, swim motor neurones, are further divided into depressor and elevator groups. Motor neurone recordings show complex subthreshold activity consisting of alternate depolarizations and hyperpolarizations. The complex activity is in antiphase in antagonistic motor neurones. Significant motor neurone-motor neurone interactions do not occur centrally as neither electrical coupling nor chemical synaptic interactions could be demonstrated. Injected currents do not alter the motor neurone firing rhythm or the swimming rhythm.

Motor neurone cell bodies are located in the lateral region of the ipsilateral pedal ganglion, near the emergence of the wing nerve. Each motor neurone provides a single axon to the wing nerve which branches repeatedly in the wing. Each motor neurone has an extremely large innervation field, some covering up to half of the wing area.

The second class of pedal neurones that display firing patterns in phase with either wing upswing or downswing are pedal-pedal interneurones. Each swim interneurone provides axon branches in both pedal ganglia and the axon runs in the pedal commissure. Interneurone axon branches occur in the lateral neuropile of each pedal ganglion, in the region of motor neurone branching. The swim interneurones presumably play a major role in bilateral coordination of the wings and are involved in pattern generation since injected currents were found to accelerate or slow the firing rhythms of interneurones and motor neurones, and wing movements.

* A significant portion of this work was conducted at Friday Harbor Laboratories, Friday Harbor, Washington, U.S.A.

Key words: Pattern generation, swimming, motor neurone, interneurone.

INTRODUCTION

A thorough investigation of the neurobiological basis of rhythmic motor behaviour must consider four major levels of control, including (1) central pattern generation, (2) motor output, to include activity and properties of the applicable motor neurones and musculature, (3) descending control of the pattern generator by higher centres and, (4) modification of the pattern generator and motor neurone activity by afferent feedback. The generation of locomotory behaviour in invertebrate preparations and its modification has been well studied (e.g. Friesen, Poon & Stent, 1978; Getting, Lennard & Hume, 1980; Getting, 1981, 1983*a*,*b*). In these and other preparations, the pattern generator has been found to have a central origin, and operate in the absence of peripheral feedback (see Grillner, 1977; Delcomyn, 1980). In most instances, the rhythm is generated through the interaction of premotor interneurones which operate as a functional unit and impose a rhythm on appropriate groups of motor neurones (Friesen *et al.* 1978; Kaneko, Merickel & Kater, 1978; Poon, Friesen & Stent, 1978; Benjamin & Rose, 1979; Getting *et al.* 1980; Hume & Getting, 1982). The premotor elements may be non-spiking interneurones (Pearson & Fourtner, 1975; Burrows & Siegler, 1978; Burrows, 1979; Heitler & Pearson, 1980; Siegler, 1981; Simmers & Bush, 1983). Exceptions have been noted in which motor neurones show varying involvement in generation of the rhythm (e.g. Heitler, 1978; Selverston, Russell, Miller & King, 1976).

Here we make an electrophysiological study of the organization of the locomotory system of *Clione limacina*, a holoplanktonic opisthobranch mollusc. In the previous paper we described the locomotory structures, wing-like parapodia, and presented a film analysis of hovering swimming (Satterlie, La Barbera & Spencer, 1985). In a minimally dissected preparation, capable of parapodial flapping, the interneurones, motor neurones, sensory cells and muscles are simultaneously available for intracellular recording. Furthermore, the swimming system will function in a fully dissected preparation including only the central ganglia and parapodia. We believe that a complete description of swimming behaviour from biomechanical to neurophysiological levels is possible in this preparation.

MATERIALS AND METHODS

Animal collection and culture was as described previously (Satterlie *et al.* 1985). Specimens were dissected in a Sylgard (Dow Corning) coated Petri dish containing fresh sea water. The animals were first immobilized with cactus spines (*Opuntia* sp.) and an incision was made along the dorsal midline from head to tail. The central ganglia were exposed by removal of the digestive tube and reproductive organs. For semi-intact preparations, the buccal mass was left intact, and the ganglion to be investigated was elevated and pinned on a wax-coated platform. For isolated preparations, the head and buccal mass and tail were removed leaving only the central ganglia (cerebrals, pleurals, pedals and intestinals) and wings. The ganglia were

fastened to the Sylgard by pinning of nerve roots. If necessary, the wings were partially restrained with cactus spines to eliminate movement of the ganglia.

For cobalt back-fills, pedal ganglia pairs were removed from the animal, and one wing nerve was placed in a pool of 500 mmol l^{-1} $CoCl_2$ for 3–8 h. Following fixation in 10 % formalin in sea water, the ganglia were reacted in ammonium sulphide (3 drops in 25 ml sea water), dehydrated in ethanol and cleared in methyl salicylate. Ganglia were observed, drawn and photographed in whole mount preparations.

Extracellular recordings were made from nerve trunks or wing tissue with suction electrodes (tip diameters 50–250 μm). Intracellular recordings were made with 3 mol l^{-1} KCl or 2 mol l^{-1} potassium acetate filled microelectrodes with resistances of 10–70 MΩ with standard d.c. amplification and display. All data was recorded on tape for later playback and analysis. In most cases, spontaneous activity was recorded. Intracellular stimulation was *via* amplifier bridge circuits, and extracellular mechanical stimulation *via* glass probes.

Lucifer Yellow CH (4 % solution) was iontophoretically injected with 0·5–10 nA negative current pulses (500 ms duration, 1 Hz) for 5–30 min. Injected cells were usually observed live in the recording dish which was transferred to the stage of an incident light fluorescent microscope (see Stewart, 1978). It was usually necessary to anaesthetize the preparation with a 1:1 mix of 0·33 mol l^{-1} $MgCl_2$: sea water to eliminate movement during drawing and photography (this same solution was used to block synaptic transmission during electrical recordings). Some preparations were fixed, dehydrated and cleared as mentioned above prior to drawing or photography.

Horseradish peroxidase (Sigma Type VI, 4 % in 0·2 mol l^{-1} Tris and 0·5 mol l^{-1} KCl, pH 7·4) was iontophoretically injected into motor neurones with positive current pulses of 2–5 nA (500 ms duration, 1 Hz) for 20–45 min. Following fixation for 2 h in 2 % glutaraldehyde in 0·2 mol l^{-1} cacodylate buffer (adjusted to 950 mosmol l^{-1} with NaCl), tissue pieces were reacted with Hanker-Yates reagent (Polysciences, 1 mg ml^{-1} similar cacodylate buffer) for 10–15 min. If a positive reaction was indicated, the tissue was post-fixed in 1 % osmium tetroxide in cacodylate buffer for 1 h, followed by ethanol dehydration and embedding in Epon. Sections (1–2 μm thick) were cut with glass knives for light microscopical examination.

RESULTS

Organization of the central nervous system

The ganglia of *Clione* are unfused (with the exception of the intestinals) and similar in organization to those of *Aplysia* (Kandel, 1979; see Fig. 1). The paired cerebral ganglia are set back from the anterior end of the animal, approximately in line with the posterior tentacles. Each ganglion is approximately 300 μm in diameter. The two cerebrals are joined by a short cerebral commissure. Rather long anterior-running connectives join the cerebrals to the paired buccal ganglia which are situated on the posterio-ventral side of the buccal mass. Two pairs of circumoesophageal connectives run posterio-ventrally from the cerebral ganglia and connect with paired pedal and

pleural ganglia. The cerebrals also send numerous smaller nerves to the body wall of the head, to the anterior and cephalic tentacles, and to the oral structures.

The pedal ganglia are approximately 300 μm in diameter and each bears a large (100 μm diameter) statocyst. The pedals are connected by one stout commissure and a fine parapedal connective. Each pedal ganglion gives off numerous nerves which innervate the body wall of the head and trunk, and one large nerve, up to 100 μm in diameter, which innervates the ipsilateral wing.

A pleural ganglion is attached to each pedal ganglion by a very short connective. The pleurals are relatively small, approximately 150 μm in diameter, and are joined to the cerebral ganglia by long connectives. The pleurals also send connectives to form a loop with the intestinal ganglia. Of primary concern in this investigation are the cerebral and pedal ganglia, and the wing nerves.

The central pattern generator circuitry controlling swimming movements was isolated by successively removing ganglia surgically while visually observing swimming. Removal of the intestinal and pleural ganglia had no apparent affect on alternating dorsal and ventral movements of the wings. Furthermore, separation of the cerebral ganglia by a single cut through the cerebral commissure did not influence

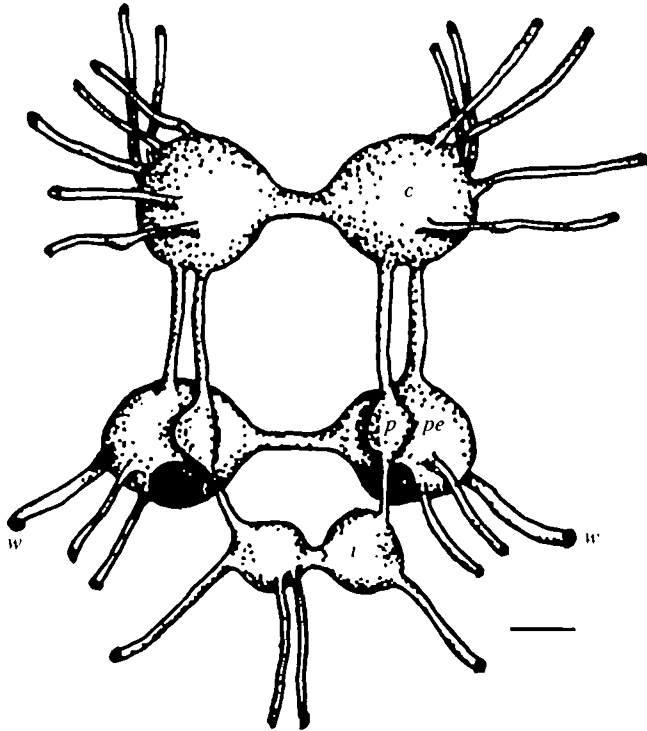

Fig. 1. Dorsal view of the central ring of ganglia of *Clione* with major nerves. *c*, cerebral ganglion; *p*, pleural ganglion; *pe*, pedal ganglion; *i*, intestinal ganglion; *w*, wing nerve. Scale bar, 100 μm.

coordination of the two wings. Severing the cerebro-pedal connectives resulted in a decrease in overall swim frequency, and frequently changed the swimming pattern from a continuous to an intermittent activity. Despite this change in overall activity, the wings were still able to perform bilaterally coordinated swimming activity consisting of alternating dorsal and ventral movements of the wings. Isolated preparations of this nature, consisting only of the two connected pedal ganglia and the two wings, would 'swim' for several hours under electrical recording conditions. A cut through the parapedal connective did not further change swimming activity; however, a similar cut through the pedal commissure destroyed bilateral coordination of the wings. Following this last cut, each wing was still able to undergo alternating upward and downward flexions. Severing of the large wing nerve left the wings motionless. Simultaneous suction electrode recordings from the dorsal wing surfaces during a sequential series of lesions, as described above, confirmed the behavioural observation that the pedal ganglia contain the neuronal circuitry capable of generating swimming movements (Fig. 2). The preparation was further reduced by removing the wings. The totally isolated pedal ganglia still generated alternate activity in up and down motor efferents, as measured from the wing nerve with suction electrodes, indicating that the central pattern generator functions in the absence of afferent sensory feedback.

Cobalt back-fills of wing nerves were used to identify the positions of cell bodies of wing efferents and afferents in the pedal ganglia. Cells were filled in the ipsilateral pedal ganglion only. In each preparation, over 70 cells were filled (Fig. 3), representing approximately 10% of pedal neurones and presumably including motor neurones innervating the swimming muscles and retractor muscles, and wing sensory neurones.

Swim motor neurones

Two classes of pedal neurones displayed complex electrical activity in phase with either wing upstroke or downstroke. One class was shown by dye fills (described below) to send axons out through the wing nerve to branch and arborize repeatedly in the wings. Intracellular depolarization of these swim neurones induces twitching movements of the wings in non-swimming preparations, hence these are motor neurones. Most motor neurone cell bodies are located in the region of the pedal ganglion near the emergence of the wing nerve, on both dorsal and ventral surfaces. Two populations of motor neurones have been identified, based on firing activity. Wing elevators fire preceding or during wing elevation (morphological upstroke) and wing depressors fire preceding or during wing depression (morphological downstroke). Thus far, we have been unable to determine the exact number of motor neurones in each population, but based on extensive microelectrode probing of the pedal ganglia, it appears that there are not more than ten in each pool. Two motor neurones have been identified, based on their consistent cell body position and firing pattern. The two cells, one elevator and one depressor (Fig. 3) are among the largest neurones in the pedal ganglia.

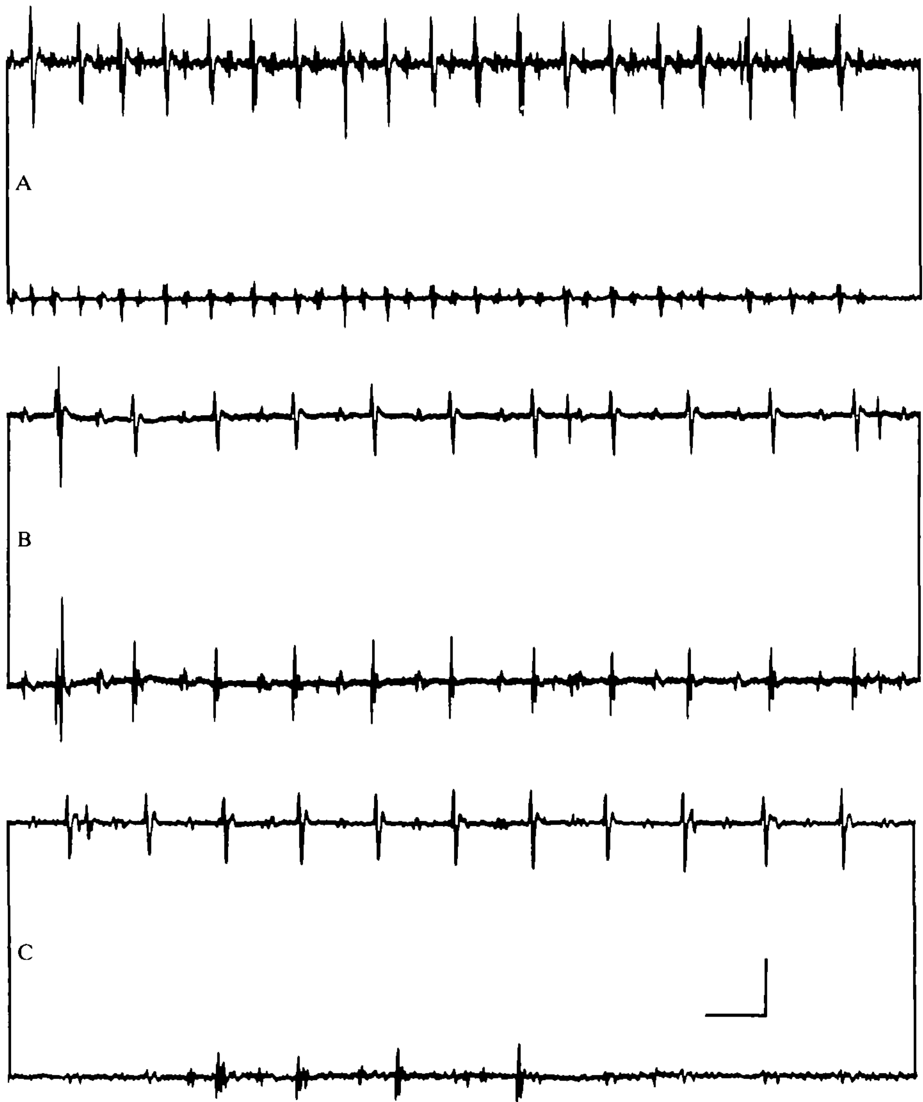

Fig. 2. Simultaneous extracellular recordings (EMGs) from the dorsal surface of each wing during a sequential lesion experiment. The large spike bursts represent activity in the dorsal wing musculature and alternate with activity in the ventral wing musculature (smaller spike bursts). (A) Activity following surgical removal of the pleural and intestinal ganglia; (B) activity following bilateral cuts through the cerebro-pedal connectives (isolated pedal ganglia with pedal commissure intact). Note the decreased swim frequency. (C) Activity following a cut through the pedal commissure. Activity in the two wings is no longer coordinated. All recordings are from the same preparation. Scale, 0·5 s, 100 μV.

Swim motor neurones displayed stable resting potentials between −40 and −68 mV. During swimming activity, complex, alternating depolarizing and hyperpolarizing synaptic inputs were recorded (Fig. 4A). All synaptic activity, and swimming, was eliminated after 2–3 min exposure to high-Mg^{2+} sea water. During

active swimming, each depolarizing or hyperpolarizing cycle was made up of at least two separate components which took the membrane potential up to 35 mV from the resting level. Individual graded potentials were occasionally recorded at the beginning or end of a swimming sequence, and in a few instances, in the middle of a swim burst (Fig. 4B,C). The potentials were up to 35 mV in amplitude and up to 130 ms in duration. During weak swimming movements, for example at the beginning or end of a swim period, only alternating synaptic potentials of this type were recorded. With stronger swimming movements, the synaptic input was complicated by additional inputs (or possibly intrinsic membrane currents of the motor neurones). Dual recordings from antagonistic motor neurones (wing elevators and depressors) revealed electrical activity exactly in antiphase (Fig. 5A). In these recordings isolated synaptic inputs were occasionally evident. When these occurred, the synaptic potentials were recorded in both neurones simultaneously, although they were of opposite sign (Fig. 5B). In all cases, the depolarizing potentials were of shorter duration (maximum, 100 ms) than the hyperpolarizing potentials (maximum, 130 ms). When sufficiently large, the depolarizing phase of the motor neurone electrical cycle induced action potentials, singly or in bursts (Figs 4, 5). Bursts included up to 11 spikes at a maximal frequency of 140 Hz. Inhibition of swimming, as occurred during mechanically stimulated wing retraction, included a total cessation of the alternating depolarizations and hyperpolarizations characteristic of swimming (Figs 4, 5). In most motor neurones swim inhibition resulted in total electrical silence, although in a few cells, a barrage of small (up to 8 mV) inhibitory postsynaptic potentials (PSPs) was evident for the duration of inhibition (Fig. 4B).

Motor neurone-motor neurone interactions were examined during simultaneous penetrations of synergistic and antagonistic cell pairs. Electrical coupling could not be demonstrated between synergistically firing motor neurones suggesting that the similar firing pattern was due primarily to common or similar input (Fig. 6). Also,

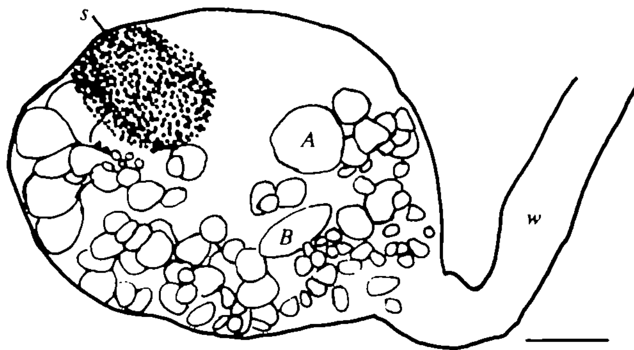

Fig. 3. Positions of cell bodies in a pedal ganglion following a cobalt back-fill of the wing nerve (*w*). *s*, statolith. Two cells have been identified thus far: *A*, large elevator motor neurone, *B*, large depressor motor neurone. Scale bar, 100 μm.

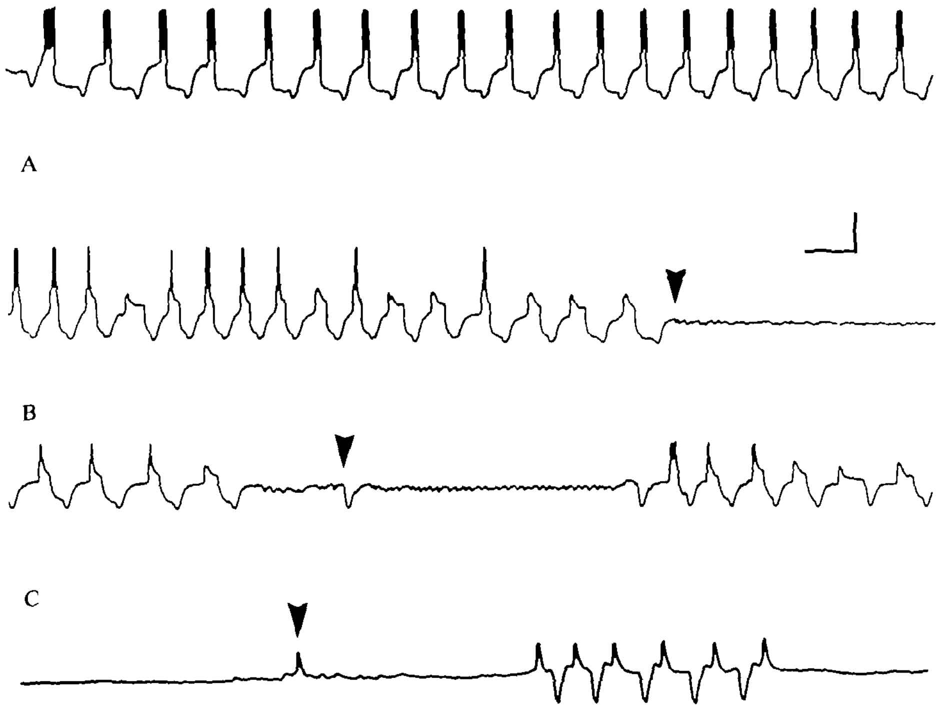

Fig. 4. Spontaneous firing activity of swim motor neurones. (A) Excerpts from a continuous record of swim activity of an elevator motor neurone showing a change from a spiking mode of firing to a non-spiking mode and, finally inhibition of swimming (arrowhead). The firing pattern consists of alternating complex depolarizations and hyperpolarizations best seen in the non-spiking mode. Note that each depolarizing and hyperpolarizing phase may be made up of more than one temporally-separated input. (B) Elevator motor neurone activity. Note the inhibitory PSP during swim inhibition (arrowhead) which is identical to the PSP that initiates the following swim burst. (C) Depressor motor neurone activity. The excitatory PSP recorded during swim inhibition (arrowhead) is again similar to the PSP occurring at the beginning of a swimming burst. Scale, 0·5 s; 40 mV (A), 50 mV (B), 33 mV (C).

electrical activity did not change in synergists or antagonists when one cell of the pair changed from a non-spiking to a spiking mode or *vice versa* (Figs 5A, 6). Finally, in quiescent preparations, in no case did induction of firing in one neurone of a pair produce any detectable response in the other cell. Analysis of current injection experiments revealed an additional significant property of the swim motor neurones. In actively swimming preparations, neither depolarizing nor hyperpolarizing currents produced a detectable resetting or alternation of motor neurone firing frequency, aside from inducement or inhibition of spiking (Fig. 6).

Motor neurone structure was determined by iontophoretic injection of Lucifer Yellow CH. The following generalizations can be made, based on over 30 successful fills. Motor neurone-motor neurone dye-coupling was not seen. In several motor neurone fills, additional cells were filled, however these occurred in preparations in

which motor neurone penetration followed rather extensive probing of the ganglion. In some preparations, weak fills were achieved in the absence of detected cell penetration or purposeful dye injection. Presumably such fills resulted from tip damage following sheath penetration with leakage of Lucifer Yellow into subsequently partially penetrated cells. In any event, we cannot rule out the possibility that some motor neurones are dye-coupled to neurones that do not fully display the background synaptic activity of the motor neurones.

As suggested by the recordings, dye-injections indicated that motor neurone cell bodies are located in the lateral half of the pedal ganglia nearest the emergence of the wing nerve. Cell bodies ranged from 10 μm to 80 μm in diameter, and each bore a single axon which provided numerous branches in the lateral neuropile near the wing nerve origin (Fig. 7). The single axon continued into the wing nerve and into the wing haemocoel. The area of neuropilar branching was quite consistent for all motor neurones, even those with cell bodies located near the wing nerve origin. The axon of these cells looped back into the neuropile before passing out of the wing nerve.

All swim motor neurone processes are restricted to the ipsilateral pedal ganglion, and all axons exit through the ipsilateral wing nerve. Axon diameters within the wing nerve have not been determined, except for one cell, the large depressor neurone

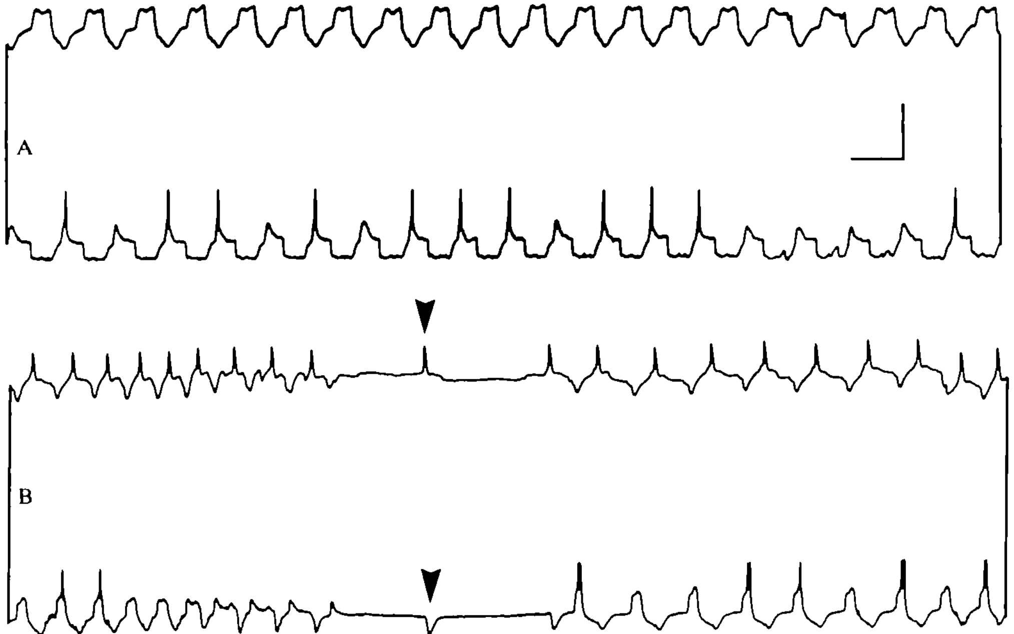

Fig. 5. (A) Dual intracellular recording from an elevator (bottom trace and cell *A* in Fig. 3) and a depressor (top trace and cell *B* in Fig. 3) motor neurone. Only the elevator fires occasional action potentials. Note that the subthreshold electrical activity is exactly in antiphase. (B) Similar dual recording from two other motor neurones (top trace, depressor; bottom trace, elevator). Note that the extra PSP initiated during swim inhibition (arrowhead) occurs in both cells and is of opposite sign. Scale, 0·5 s; 40 mV (A), 50 mV (B).

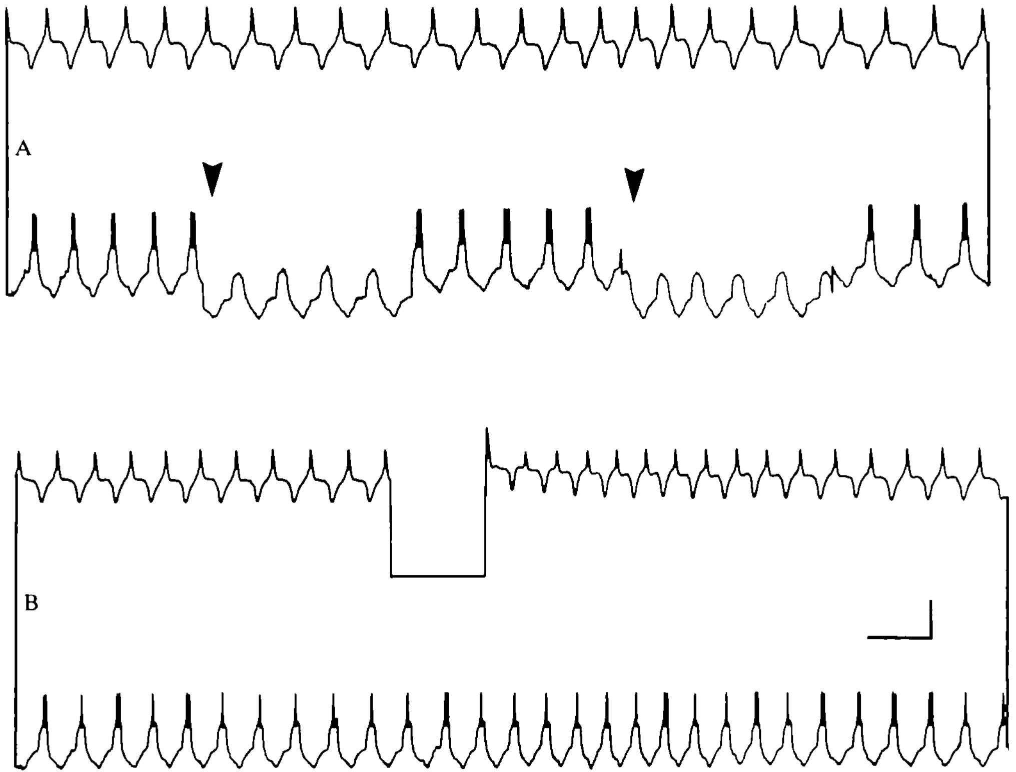

Fig. 6. Dual recording from a depressor and elevator motor neurone (same cells as in Fig. 5B). In (A) hyperpolarization of the elevator (arrows) prevents spiking, but this does not alter the frequency or quality of the depressor PSP activity. Injection of hyperpolarizing current into the depressor motor neurone (B) produces similar results. The bridge circuit was not balanced in B as the recordings were made with very high resistance electrodes. Scale, 0·5 s; 40 mV (A), 32 mV (B). Injected currents, 1·5 nA (A), 2 nA (B).

mentioned above. This cell has been filled with horseradish peroxidase in three preparations. The axon is one of four large axons (8–12 μm in diameter) running into the wings (Fig. 7B). Since two of the four ultimately run in the dorsal wing nerve branches, and two in the ventral branches, it is presumed that there are two large depressor and two large elevator motor neurones. Axons of the smaller motor neurones have not been followed into the wing nerve. All motor neurones have extensive innervation fields, some covering more than half of the wing (Fig. 7C), while others have more restricted, but still quite wide, areas of arborization (Fig. 7D).

Pedal interneurones

Pedal interneurones were identified electrically and had firing patterns in phase with either elevator or depressor motor neurone activity (Fig. 8). Thus far, four interneurones have been found, one each associated with wing upswing and

downswing in each pedal ganglion. Interneurones displayed resting potentials of between − 40 and − 65 mV and had firing patterns similar to those of motor neurones, with two differences. First, interneurone recordings showed overshooting action potentials with each depolarization in the swim sequence, and second, instead of spike bursts, the interneurones fired a single broad action potential (40–100 ms duration; Fig. 8). Each spike was followed by a hyperpolarizing PSP of up to 20 mV and 100 ms duration. There was little or no variability in the firing pattern of interneurones, exclusive of frequency. Several attempts to reset or modify interneurone firing patterns were successful in that cessation of firing or changes in firing frequency could

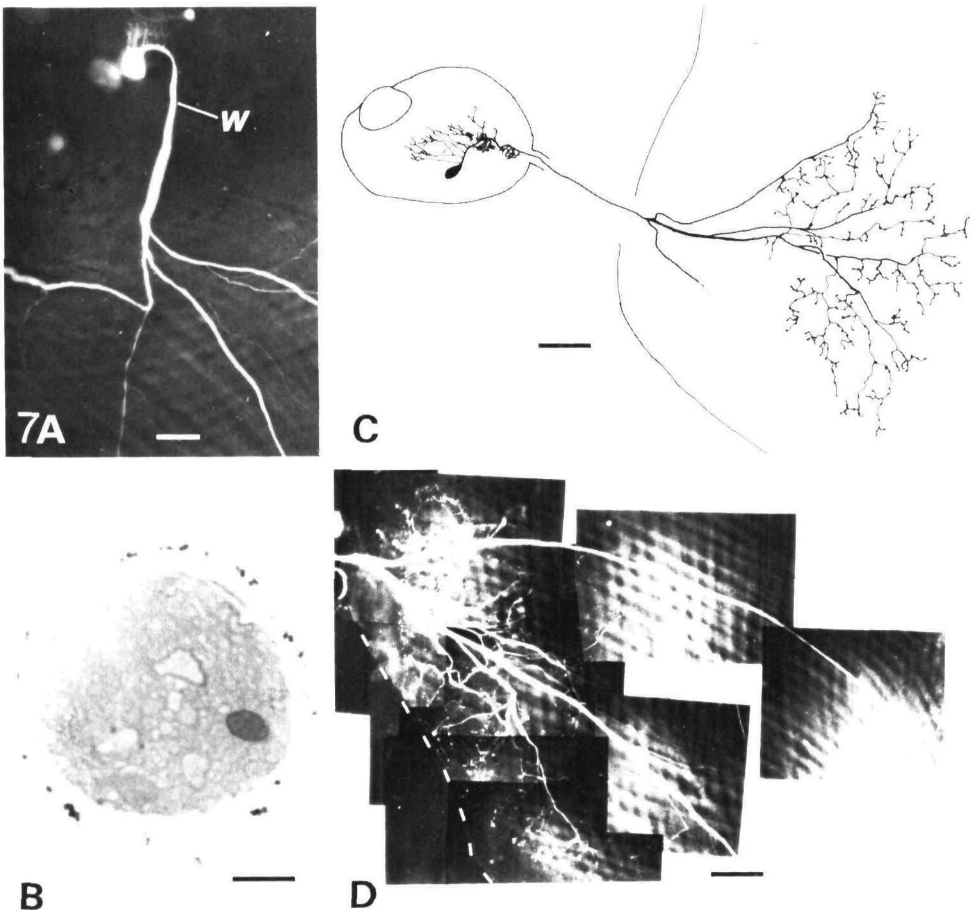

Fig. 7. Motor neurone structure. (A) Lucifer Yellow fill of an elevator motor neurone. The cell body is located in the ipsilateral pedal ganglion near the emergence of the wing nerve (*w*). The single axon branches within the lateral neuropile, exits in the wing nerve and branches in the wing. (B) Cross section of the wing nerve. In this preparation the large elevator motor neurone (cell *A* in Fig. 3) was filled with horseradish peroxidase. (C) Drawing of a depressor motor neurone. This cell exhibited the most conservative wing branching pattern of those filled. One branch could not be followed in this fill. (D) Montage of a motor neurone fill in which a portion of the wing innervation pattern is visible. Dotted line indicates the trailing edge of the wing. Scale bars, 100 μm (A, C, D); 20 μm (B).

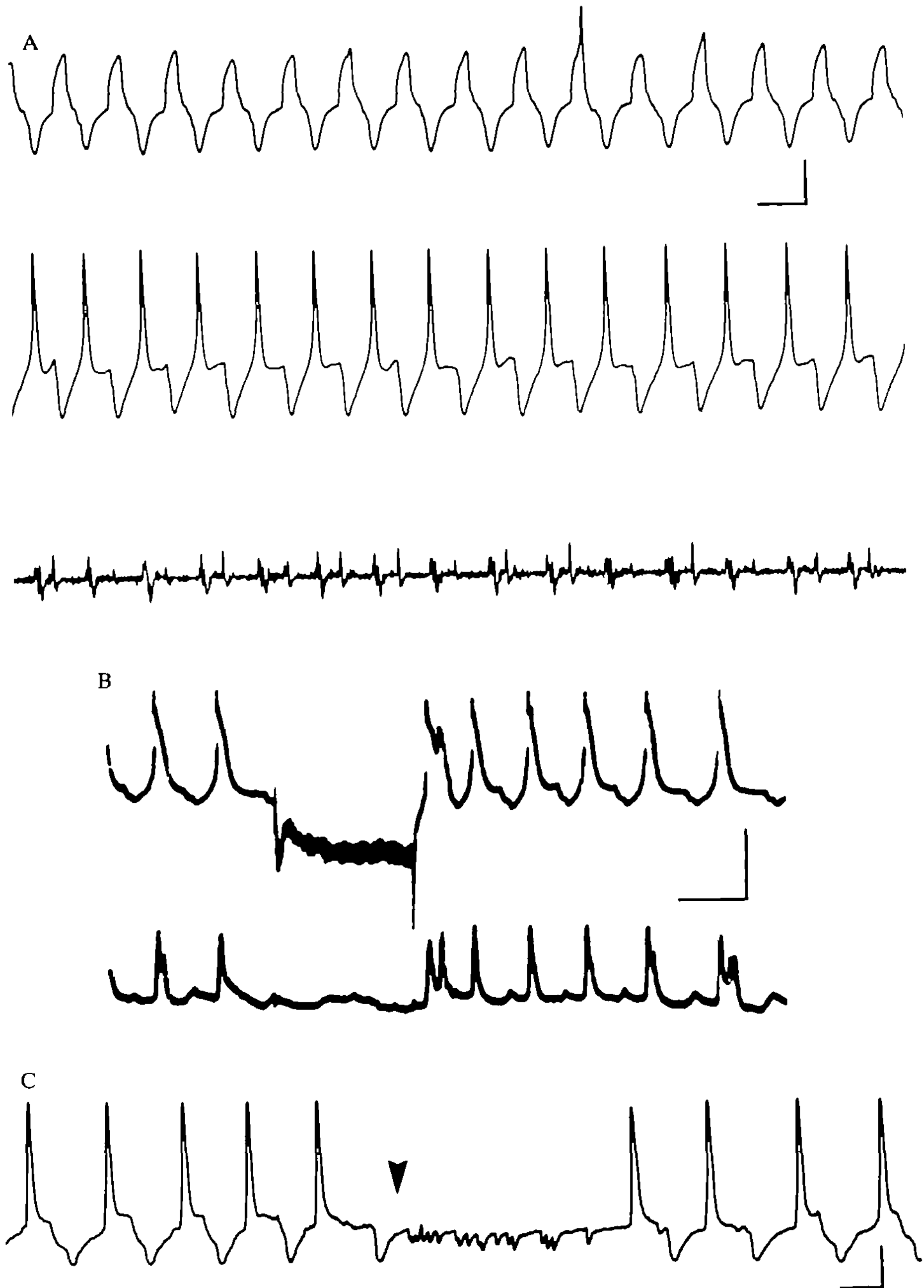

Fig. 8. Firing activity of swim interneurones. (A) Simultaneous recordings of elevator motor neurone (top trace), depressor interneurone (middle trace) and wing EMG from the dorsal wing surface (bottom trace). Note that the motor neurone spiked once in this record, but this did not alter the interneurone activity. (B) Simultaneous recording from an interneurone (top trace) and motor neurone (bottom trace). The resting potential of the motor neurone was slightly more negative than the reversal potential of the IPSPs (approx. − 66 mV). The interneurone was hyperpolarized resulting in a cessation of firing in both cells. The bridge circuit was not in balance. (C) Interneurone activity interrupted by a short swim inhibition (arrowhead). Note the hyperpolarizing PSPs during inhibition. Scales, (A) 200 ms, 20 mV; (B) 0·5 s, 40 mV; (C) 200 ms, 20 mV. Injected current in (B) 2·5 nA.

be induced by intracellular injection of depolarizing or hyperpolarizing currents (Fig. 8B).

Simultaneous recordings from an interneurone and motor neurone indicated that each interneurone fired in phase with either the depolarizing or hyperpolarizing PSP of the motor neurone (Fig. 8A,B). Monosynaptic connections from interneurones to motor neurones have been found and account for part of the complex subthreshold activity of motor neurones (R. A. Satterlie, unpublished observation), but a complete survey of interneurone-motor neurone connections has not been completed. During inhibition of swimming, each interneurone received a constant barrage of inhibitory PSPs (10 mV, 25 ms) for the duration of inhibition (Fig. 8C). In some recordings, electrical activity indicative of swimming began prior to cessation of the IPSP barrages. In these preparations, the wings were observed to begin dorsal and ventral bending movements before the wings were fully expanded.

Eight swim interneurones were filled with Lucifer Yellow. All have relatively small cell bodies (20–35 μm in diameter) and axonal branches in each pedal ganglion. Each cell body gives off a single axon that bifurcates very close to the soma (Fig. 9). The ipsilateral branch divides repeatedly in the ipsilateral pedal ganglion, while the contralateral branch runs to the other pedal ganglion through the pedal commissure. The latter branch divides repeatedly in the contralateral ganglion, and sometimes gives off branches near the cell body that ramify in the ipsilateral ganglion. Most fine branches of the swim interneurones terminate in the same region of the neuropile as the motor neurone axon branches. In dual dye-fills including a motor neurone and an interneurone, branches of the two cells were found in close proximity (observed in whole mount only; Fig. 9C).

DISCUSSION

In a number of rhythmic motor systems, the central pattern generators are centrally located, and operate in the absence of sensory feedback (see Grillner, 1977; Delcomyn, 1980). The swimming system of *Clione* is no exception as normal motor output continues when the wings are totally removed. Furthermore, the swim pattern generator has been localized in the pedal ganglia. Similar results have been obtained for swimming (von der Porten, Redman, Rothman & Pinsker, 1980) and pedal locomotion (Hening, Walters, Carew & Kandel, 1979; Fredman & Jahan-Parwar, 1980; Jahan-Parwar & Fredman, 1979, 1980) in *Aplysia*, in which the organization of central ganglia is very similar to that of *Clione*.

Two classes of neurones have been identified in the pedal ganglia of *Clione* which display firing patterns in phase with swimming movements. Motor neurones have been identified based on firing activity, dye fills and activation of wing muscle cells (R. A. Satterlie, in preparation), and comprise two antagonistic groups, elevators and depressors. As in vertebrate locomotory systems (Grillner, 1977) and the swimming system of *Tritonia* (Hume & Getting, 1982), motor neurones of *Clione* are not directly involved in pattern generation, and do not show significant direct interconnections

between synergistic or antagonistic motor neurones. In the leech swimming system, only one motor neurone has access to the central pattern generator, but motor neurone interconnections have been demonstrated (Ort, Kristan & Stent, 1974; Friesen *et al.* 1978; Poon *et al.* 1978). On the other hand, motor neurones do interact with, or are

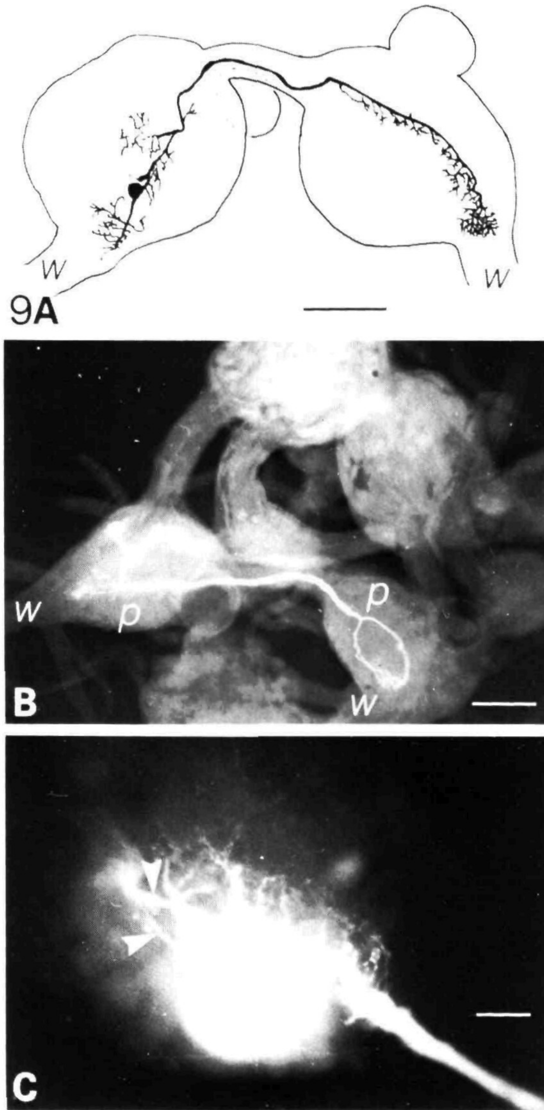

Fig. 9. Swim interneurone structure. (A), (B) Lucifer Yellow fills of swim interneurones demonstrating the degree of variability in branching structure. Note that both cells provide axon branches in the lateral neuropile of both pedal ganglia, near the emergence of the wing nerves. (C) Dual Lucifer fill of a motor neurone and a contralateral interneurone. Branches of the interneurone (arrowheads) interdigitate with those of the motor neurone axon. *p*, pedal ganglion; *w*, wing nerve. Scale bars, 100 μm (A, B); 25 μm (C).

part of, the central pattern generator in some preparations (Heitler, 1978; Selverston *et al.* 1976).

At least part of the complex subthreshold electrical activity of *Clione* swim motor neurones may be attributed to synaptic input from premotor interneurones. Evidence presented here suggests that synergistic motor neurones receive either common or simultaneous inputs. Antagonistic motor neurones also receive simultaneous PSPs, but of opposite sign. During active swimming, additional hyperpolarizations and depolarizations are recorded, suggesting additional synaptic input, or activation of intrinsic membrane currents. Preliminary experiments indicate that some motor neurones exhibit post-inhibitory rebound (Perkel & Mulloney, 1974) following non-physiological imposed currents (R. A. Satterlie, unpublished observation). A significant role of post-inhibitory rebound in pattern generation has been suggested in several invertebrate preparations (Siegler, Mpitsos & Davis, 1974; Selverston *et al.* 1976; Simmers & Bush, 1983).

Pedal interneurones comprise the second class of cells which exhibit electrical activity with a temporal relationship to swimming. As with motor neurones, there are two pools of interneurones, one which fires in phase with wing elevation and one with wing depression. Neurones of both pools have a similar morphology, with relatively small cell bodies providing major axonal branches in both the ipsilateral and contralateral pedal ganglia. The interneurones are believed to be premotor due to the exact temporal correspondence between their alternating depolarizing and hyperpolarizing electrical activity and that of motor neurones. In close examination, the two-phase interneurone firing activity can be observed to lead the suspected synaptic inputs to the motor neurones by a few milliseconds. Preliminary evidence suggests that interneurone-to-motor neurone connections are monosynaptic (R. A. Satterlie, unpublished observation). Morphological evidence indicates that terminal processes of the interneurones (both ipsilateral and contralateral) are found in the lateral neuropile of each pedal ganglion, and interdigitate with neuropilar branches of the motor neurones.

Contralateral axon branches of the swim interneurones run through the pedal commissure. Severing this connective destroys bilateral coordination of swimming activity. Identical results suggest a similar mechanism for bilateral coordination of parapodial flapping in *Aplysia* (von der Porten *et al.* 1980). Similarly, bilateral coordination at the premotor level is suggested for *Tritonia* swimming (Getting, 1983*b*). The swim interneurones of *Clione* are leading candidates for mediating coordination of contralateral motor neurones and bilateral coordination of the wings. The interneurones also contribute to the central generation of the alternating swimming rhythm as injected currents were observed to reset the interneurone rhythm. As yet we do not know the exact number of upswing or downswing interneurones, but we do know that each pedal ganglion has at least one of each. Preliminary penetrations of antagonistic interneurone pairs suggest that monosynaptic reciprocal inhibition may underlie the alternate cyclic activity in the two interneurone pools (R. A. Satterlie, in preparation).

Despite the localization of the 'basic' pattern generator (responsible for producing alternating dorsal and ventral flexions of the wings) within the pedal ganglia, the

alternating dorsal and ventral flexions of the wings) within the pedal ganglia, the cerebral ganglia maintain a descending influence on overall activity of the swimming system. Separation of the cerebral and pedal ganglia resulted in a significant reduction in swimming frequency and regularity (normally *Clione* swims continually). Despite repeated microelectrode probing of the cerebral ganglia, swim-influencing neurones have not yet been identified. In particular, cells with a strict firing relationship to swimming activity have not been found, and neurones with a 'command' function (Kupfermann & Weiss, 1978) have not been suggested. One might not expect absolute control by command neurones or higher centres (i.e. cerebral ganglia) over the central pattern generator for continuous behaviour such as swimming in *Clione*. On the other hand, induced, short-term behaviour is more apt to be initiated by input from higher centres and possibly command units. Such absolute descending control is suggested in the initiation of swimming of *Aplysia* since this behaviour is totally eliminated when the cerebro-pedal connectives are cut (von der Porten *et al.* 1980), and in swim initiation of the leech where a repeating neurone in the segmental ganglia, neurone 204, at least partially meets the functional criteria of a command neurone (Weeks & Kristan, 1978). A similar function can possibly be ascribed to certain swim interneurones in *Tritonia* (Taghert & Willows, 1978; Lennard, Getting & Hume, 1980).

The present investigation indicates that the *Clione* preparation may serve as an excellent model for the examination of locomotion from the level of pattern generation and descending control from higher centres, through motor output and muscle activation. Interneurones, motor neurones and muscle cells are simultaneously available for microelectrode recording. One important advantage of this preparation is simplicity. In many motor systems, motor neurones and locomotory muscles can be involved in a variety of different movements thereby necessitating complex premotor inputs to allow for motor variability. Furthermore, each phasic component of a locomotory cycle can involve activation of several synergistic muscle groups with slightly different activation times. Such a system requires a multi-member pattern generator which will generate a multi-phase motor rhythm (see Kristan *et al.* 1977). Swimming in *Clione* is strictly a two-phase activity, with each phase being nearly symmetrical (Satterlie *et al.* 1985). Preliminary electrophysiological evidence indicates that both motor neurone and interneurone activities are phase constant. Furthermore, swim motor neurones seem to function solely in activation of swimming musculature, with swim muscles serving this single function. Finally, in *Clione*, one would expect that behavioural variability, for example in turning, might complicate the motor output. To the contrary, turning appears to involve primarily (and possibly exclusively) bending of the rudder-like tail, associated with steady or increased swim frequency. It is too early to relate our findings to proposed pattern generator models, but certainly multiphase networks are not warranted at this time. It is tempting to speculate thatthe wiring of this system may be as simple as that of Brown's 'Half Centre' model (Brown, 1914).

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