J. exp. Biol. 159, 285–301 (1991)
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ELECTROPHYSIOLOGY OF SWIM MUSCULATURE IN THE PTEROPOD MOLLUSC *CLIONE LIMACINA*

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Accepted 12 April 1991

Summary

Intracellular recordings from the swim musculature of the pteropod mollusc *Clione limacina* revealed two types of activity during spontaneous swimming movements. One group of muscle cells produced either excitatory junctional potentials (EJPs) or spike-like responses during both slow and fast swimming. The second group of muscle cells were either silent or produced extremely small EJPs during slow swimming, and EJPs or spike-like responses during fast swimming. Injections of horseradish peroxidase (HRP) into the two types of muscle cells indicated that the former are slow-twitch fibers while the latter are fast-twitch fibers. A comparison of the two muscle cell types indicated that the slow-twitch fibers are larger and have less negative resting potentials than the fast-twitch cells. Electrical coupling was detected between pairs of slow-twitch cells, but could not be demonstrated between pairs of fast-twitch cells or between mixed pairs of cells. Spike-like responses in both cell types exhibited non-synaptic facilitation in response to direct depolarizing stimulation. Both the maximum size and the initial rate of facilitation were frequency-dependent. Spike-like responses were followed by a depolarizing afterpotential that apparently summed to produce a baseline depolarization that may be involved in non-synaptic facilitation.

Introduction

In the pteropod mollusc *Clione limacina* (Phipps), swimming is a virtually continuous activity usually only interrupted for brief periods following mechanical stimulation of certain parts of the body (e.g. wing retraction reflex; Arshavsky *et al.* 1985*a*; Satterlie *et al.* 1985; Huang and Satterlie, 1990), although longer periods of quiescence have been described (Kabotyanski and Sakharov, 1988). Swimming activity takes three forms; slow, fast and escape (Satterlie *et al.* 1990). Despite these three distinct forms of swimming, central pattern generator activity and neuromuscular organization suggest that the swimming system is a 'two-geared' system (slow and fast). For example, the swim pattern generator is reconfigured during the change from slow to fast swimming (Arshavsky *et al.*

Key words: mollusc, *Clione limacina*, muscle electrophysiology, facilitation, locomotion, swimming.

1985*d*, 1989). This 'change-of-gears' induces recruitment of motor neurons that are active only during fast swimming (Satterlie, 1989; Satterlie *et al.* 1990). In this context, a change-of-gears involves a unique change in pattern generator activity and recruitment (or dropping out) of motor neurons that were not (or were) previously active. The primary thrust of our work is to describe how a two-geared locomotory system can produce a three-phase behavioral repertoire. Towards this end, this paper provides electrophysiological evidence suggesting that the swim musculature includes two muscle fiber types whose activity can be correlated with slow and fast swimming activity.

The swim muscles of *Clione* include four layers of oblique muscle bundles (Satterlie *et al.* 1985). Two layers are associated with the dorsal side of the wing and co-contract to produce a dorsal bend of the wing. Similarly, two layers of muscle bundles are found on the ventral side of the wing and co-contract to produce a ventral wing bend. Individual muscle cells are cross-striated (Neal, 1988) and produce relatively fast, phasic contractions (Satterlie *et al.* 1990). Two types of swim muscle cells have been histochemically identified in the *Clione* wing (slow-twitch fatigue-resistant and fast-twitch fatigable; Satterlie *et al.* 1990). Since *Clione* has two distinct swimming speeds, slow and fast, it is assumed that the slow-twitch muscles are active during slow swimming whereas the fast-twitch fibers are recruited during fast swimming, an assumption supported by twitch-time recordings following stimulation of slow and fast motor neurons (Satterlie *et al.* 1990).

Although a great deal is known about the central generation of rhythmic activity and the gross organization of wing musculature in *Clione* (Arshavsky *et al.* 1985*a*,*b*,*c*,*d*, 1986, 1989; Satterlie, 1985, 1989; Satterlie and Spencer, 1985; Satterlie *et al.* 1985), information on the morphology and electrophysiology of individual swim muscle cells is lacking. This report contains a morphological and electrophysiological description of the swim musculature of *Clione* including a comparison of the electrical activities of the slow- and fast-twitch fibers. In addition, a unique property of these muscles, non-synaptic facilitation of spike-like responses, is described.

Materials and methods

Animals were collected from the breakwater at Friday Harbor Laboratories, Friday Harbor, Washington, and held in 3.79-l jars partially submerged in sea tables. Specimens were dissected in Sylgard (Dow Corning) coated Petri dishes containing sea water. Animals were immobilized with cactus spines (from the fruit of the prickly pear, *Opuntia* sp.). Wing movements were prevented or minimized in the recording area by placing a tight circle of spines in a restricted area of the wing. Care was taken to avoid pinning through the main branches of the wing nerve. The cactus spines allowed the tissue to be stretched taut owing to the many small barbs on the spine shafts. Reduced preparations and isolated wings were used for recording. The reduced preparation consisted of the central ganglia and attached wings, and was produced by making a dorsal incision in the body wall,

removing the digestive tube, reproductive organs and buccal mass, removing the head, and carefully trimming away the body wall of the body and tail. This preparation can produce swimming movements of the wings for up to 2 days.

Intracellular recordings were made with microelectrodes filled with 3 mol l^{-1} KCl or 2 mol l^{-1} potassium acetate with resistances of 10–50 MΩ. Currents were injected through recording electrodes *via* amplifier bridge circuits. All experiments were conducted at room temperature (16–18 °C). Standard d.c. amplification, display and storage were used. Lucifer Yellow CH, carboxyfluorescein or horseradish peroxidase (Sigma type VI) was ionophoretically or pressure injected (Picospritzer II) into cells through a recording electrode. For the former two dyes, injected cells were viewed live in the recording dish with a fluorescence microscope fitted with FITC filters. To prevent movement during photography and drawing, the preparations were anesthetized in a 1:3 mixture of 0.33 mol l^{-1} $MgCl_2$:sea water. HRP-filled cells were fixed overnight in 4 % paraformaldehyde in 2 mol l^{-1} phosphate buffer. The tissue was dehydrated in graded ethanols and embedded in plastic (EMBED 812, Electron Microscopy Sciences). 2–3 μm sections were cut with glass knives and observed either unstained or with a 1 % Methylene Blue/1 % Toluidine Blue stain.

Results

Organization of swim musculature

The organization of swim musculature in the *Clione* wing has been described in detail (Satterlie *et al.* 1985, 1990). Briefly, the swim musculature consists of four layers of oblique muscle bundles (Fig. 1) with a pair of layers found immediately under each epithelial surface of the wing. Each layer is made up of a single row of parallel muscle bundles that form a complete layer extending across the entire wing. The bundles in adjacent layers run almost at right angles to one another (Fig. 1E). Both layers are oblique to the longitudinal axis of the body (see Satterlie *et al.* 1985). The pair of dorsal muscle layers co-contract during dorsal bending of the wing while the ventral layers co-contract during ventral bending.

Muscle bundles are up to 90 μm in diameter and, when cut in cross section, can include more than 30 muscle fiber profiles (Satterlie *et al.* 1990). Two types of fiber can be recognized: a small number of larger-diameter fibers making up the superficial one-third of each bundle, and a larger number of smaller-diameter fibers making up the deeper two-thirds of each bundle (Neal, 1988). Recent evidence suggests that the larger fibers are slow-twitch, fatigue-resistant fibers while the smaller ones are fast-twitch fatigable fibers (Satterlie *et al.* 1990). Both fiber types are striated and can be easily recognized in histological sections without special staining techniques (Fig. 1). Throughout this paper, the two fiber types will be referred to as 'slow-twitch' and 'fast-twitch' fibers.

Identification of muscle fiber types

Early in this study it was noted that there were two kinds of patterned electrical

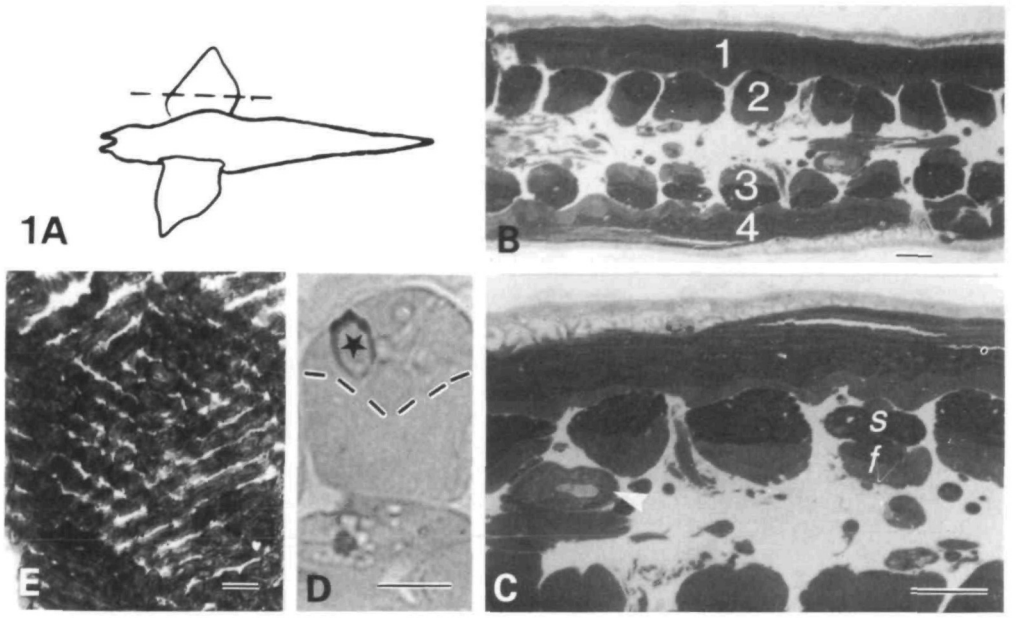

Fig. 1. Organization of swim musculature in the wing of *Clione limacina*. (A) Plane of section (wing cross section) for B, C and D. (B) Four layers of swim muscle bundles are separated by a central hemocoelic space. Layers 1 and 2 co-contract to produce dorsal bending of the wing while layers 3 and 4 co-contract to produce ventral bending. Note that the outermost portion of each muscle bundle contains cells that stain more darkly than the more medial cells of the bundle (plastic thick section stained with Methylene Blue/Toluidine Blue). (C) Enlargement of a portion of layers 3 and 4 from B shows the different staining patterns of the two types of muscle cells in each muscle bundle. Note that the two layers of bundles are oriented almost at right angles to one another. *s*, slow-twitch fibers; *f*, fast-twitch fibers; arrowhead, branch of wing nerve. (D) Phase-contrast photograph of an unstained section showing one muscle bundle in which a single muscle cell was injected with horseradish peroxidase following electrophysiological 'identification' (star, slow-twitch fiber). The dashed line separates the two different types of fibers in this bundle. (E) A glancing section of the dorsal surface of a wing shows the nearly perpendicular arrangement of muscle bundles in the two dorsal layers of swim muscles. Anterior is to the top of the figure, medial is to the left. Scale bars, B,C, 50 μm; D, 25 μm; E, 100 μm.

activity in oblique muscle cells during spontaneous swimming movements of the wings. One group of muscle cells exhibited junctional potentials or spike-like responses during all forms of swimming, including both slow and fast activity. The second group was electrically silent or produced very small (1–3 mV) junctional potentials during slow swimming and produced much larger junctional potentials or spike-like responses only during fast swimming (Fig. 2). In the light of the recent histochemical, morphological and physiological description of muscle fiber types (Satterlie *et al.* 1990), it became necessary to confirm that the electrophysiological differences could be correlated with the known muscle fiber types. To do

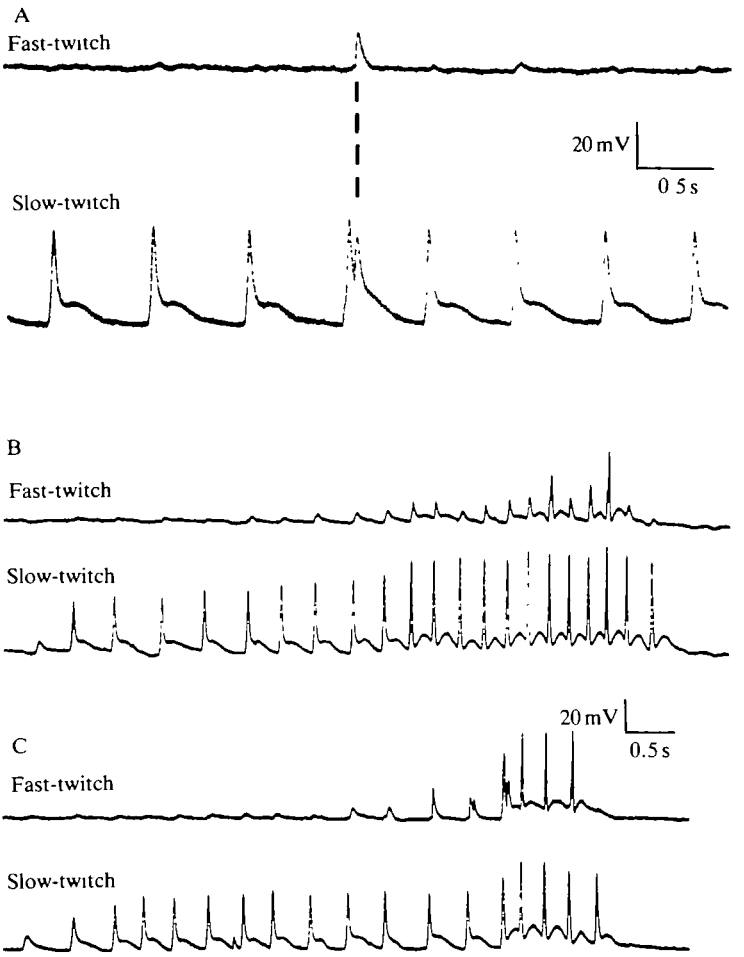

Fig. 2. (A) Dual recording from a fast-twitch muscle cell (top trace) and a slow-twitch muscle cell (bottom trace). Note that the slow-twitch cell produces a spike-like response in every swim cycle. The fast-twitch cell is either silent or receives extremely small excitatory junctional potentials (EJPs) for all but one of the swim cycles. Production of a large EJP in the fast-twitch cell occurs in conjunction with a doublet spike in the slow-twitch cell and simultaneously with the second impulse of the doublet. Note the depolarizing afterpotential that follows each spike-like response. (B) Dual recording from fast-twitch (top trace) and slow-twitch (bottom trace) muscle cells, showing a spontaneous increase in cycle frequency accompanied by an increase in the size of spike-like responses in the slow-twitch cell and an apparent recruitment of activity in the fast-twitch cell. Note the baseline shift in the slow-twitch cell. (C) Dual recording from fast-twitch and slow-twitch muscle cells when a stimulus was delivered to the tail (intact preparation) at the filled circle. Note the sudden change in cycle frequency and the production of spike-like responses in the fast-twitch cell.

this, eight muscle cells were penetrated with HRP-filled microelectrodes, electrophysiologically 'identified' as described above (four suspected fast-twitch and four suspected slow-twitch fibers), filled with HRP and histologically prepared. Light microscope cross sections of these HRP-filled cells confirmed that the fibers that were active during both forms of swimming are found in the upper one-third of a bundle and are of relatively large diameter (Fig. 1D). The location of these cells is, therefore, within the region of the bundles histochemically identified as slow-twitch, fatigue-resistant fibers (Satterlie *et al.* 1990). Fibers that were active only during fast swimming are found in the lower two-thirds of a bundle and have relatively small diameters. These cells were all found in the regions of muscle bundles histochemically identified as fast-twitch, fatigable fibers (Satterlie *et al.* 1990).

Morphology of individual muscle cells

The morphology of individual muscle cells was determined by intracellular injection of one of three markers: Lucifer Yellow, carboxyfluorescein or HRP (Fig. 3). Fills with all three markers provided similar results. Individual muscle cells are long and spindle-shaped with occasional simple branching involving a bifurcation of one end of the cell (branching occurred in only 6 of 31 fills: fills included 19 slow-twitch and 12 fast-twitch cells). The longest cell filled was 1.3 mm in length, indicating that the cells do not extend across the width of the wing. Despite the use of fluorescent dyes of small molecular size, no dye-coupling was found between adjacent cells.

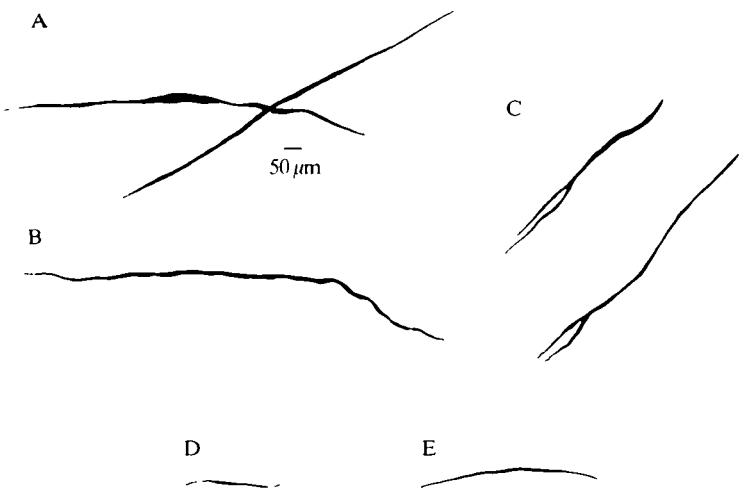

Fig. 3. Drawings from Lucifer Yellow or HRP fills of wing muscle cells. In A, two cells were filled, one from each of the two layers of bundles that co-contract to produce a dorsal bend of the wing. In C, two cells were filled from the same layer of bundles. The cells were three bundles apart. The cells in A, B and C are slow-twitch fibers. The cells in D and E are fast-twitch fibers. Note the relative size differences of the two types of cells.

A comparison of slow- and fast-twitch fibers indicated that the slow fibers are longer and have greater diameters than the fast-twitch ones. Slow-twitch cells injected with HRP were between 0.8 and 1.1 mm in length, with a maximal diameter of 35 μm. The maximum diameter of all filled cells, including those filled with fluorescent dyes, was 55 μm. It should be noted that the cells labelled with the fluorescent dyes were photographed or drawn from live, anesthetized preparations. Reflection from the brightly lit cells as well as swelling in the area of dye-injection probably gave an overestimate of the actual diameter of these cells. Fast-twitch cells, injected with HRP, were between 0.28 and 0.54 mm in length, with a maximal diameter of 15 μm. All six of the cells exhibiting a terminal branch were slow-twitch cells.

Electrophysiology of muscle cells

Muscle cells were routinely impaled in tightly pinned, unanesthetized wing preparations. Dual recordings, in which one electrode was placed in a slow-twitch cell and the other in a fast-twitch cell, confirmed that the fast-twitch fibers are inactive during slow swimming while the slow-twitch fibers are active during both forms of swimming (Fig. 2). In dual recordings, activity in the fast-twitch cells was delayed with respect to that of the slow-twitch fibers. This is particularly evident in Fig. 2A, where a single EJP occurred simultaneously with the second peak of a spike-like doublet response in a neighboring slow-twitch cell. Slow-twitch cells had a mean resting potential of -65.1 mV (range$=-56$ to -75; $N=43$) whereas fast-twitch cells had a mean resting potential of -69.3 mV (range$=-62$ to -77 mV; $N=39$).

Electrical coupling was tested with paired recordings from cells within the same muscle bundle. To ensure that the two electrodes were not placed in the same muscle cell, they were always placed perpendicular to the long axis of the muscle bundle and at a distance greater than the greatest measured diameter of the muscle cells. Reliable coupling ratios could not be determined since currents were injected into the cells through one of the recording electrodes. No electrical coupling was detected between pairs of fast-twitch fibers or between mixed pairs of fast- and slow-twitch fibers, but coupling was detected between pairs of slow-twitch fibers (Fig. 4).

Apart from the differences in timing of activation, resting potentials and electrical coupling, the electrophysiological properties of the two muscle cell types were similar, and will be described together. In all cases, the following observations were made independently for both slow-twitch and fast-twitch muscle cells. Muscle cells exhibited two kinds of electrical activity in phase with appropriate swimming movements. Following a period of quiescence (slow-twitch cells), or recruitment of activity (fast-twitch cells), the initial swimming movements were accompanied by graded excitatory junctional potentials (EJPs) in the muscle cells. EJPs were either single or compound. Individual EJPs were up to 25 mV in amplitude and 120ms in duration (Fig. 5A). Compound EJPs typically exceeded the size of individual EJPs. No inhibitory junctional potentials were

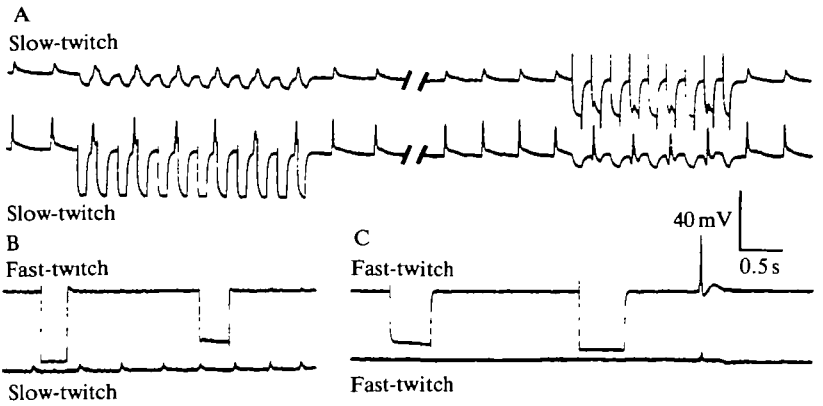

Fig. 4. Examination of electrical coupling between swim muscle cells. Pairs of slow-twitch muscle cells from the same bundle (A) show electrical coupling, while mixed pairs of slow-twitch and fast-twitch cells (B) and pairs of fast-twitch cells (C) do not.

recorded from any of the swim muscle cells, even during wing retraction (which centrally inhibits swimming; Fig. 5B,C). EJPs showed amplitude facilitation, eventually producing the second type of electrical event, spike-like responses (see Fig. 7). Spike-like responses were variable in size, with a maximum amplitude of 85 mV (overshooting zero potential by up to 15 mV). Spike duration varied over a wide range, 40–100ms, but was relatively consistent in individual muscle cell penetrations. Typically, spike-like responses occurred singly; however, doublets and triplets were sometimes recorded.

Muscle cells of both types occasionally slipped into a period of repetitive firing lasting from a few seconds to a minute or so (Fig. 6). Within such a bout, spike-like responses were produced at a rate of up to 11 Hz, with a mean maximal firing rate of 9.6 Hz (N=19). During repetitive firing, spike activity was independent of motor neuron input as the firing rate was always well above the frequency of existing swimming activity. During these bouts, occasional enhanced spike-like responses were noted. Each of these occurred when an input from the swimming system arrived at the moment of spike generation (Fig. 6). Repetitive firing was observed in both slow- and fast-twitch cells, and could sometimes be induced by direct depolarization of the cells.

Spike-like responses of both muscle cell types displayed amplitude facilitation during the first few cycles of a swimming bout (Fig. 7A). Similar facilitation was observed in response to direct depolarization of the muscle cells (non-synaptic facilitation). Increases in spike-like response amplitude could be induced with individual, brief depolarizations (Fig. 7D) and were also evident in the first three or four impulses of induced bursts (Fig. 7B,C). When individual spikes were repetitively initiated, the rate and degree of facilitation were dependent on the frequency of stimulation. Fig. 8 shows the facilitation profile for a slow-twitch fiber whose penetration was held long enough to allow testing at five different frequencies, each within the normal physiological range for *C. limacina* and with

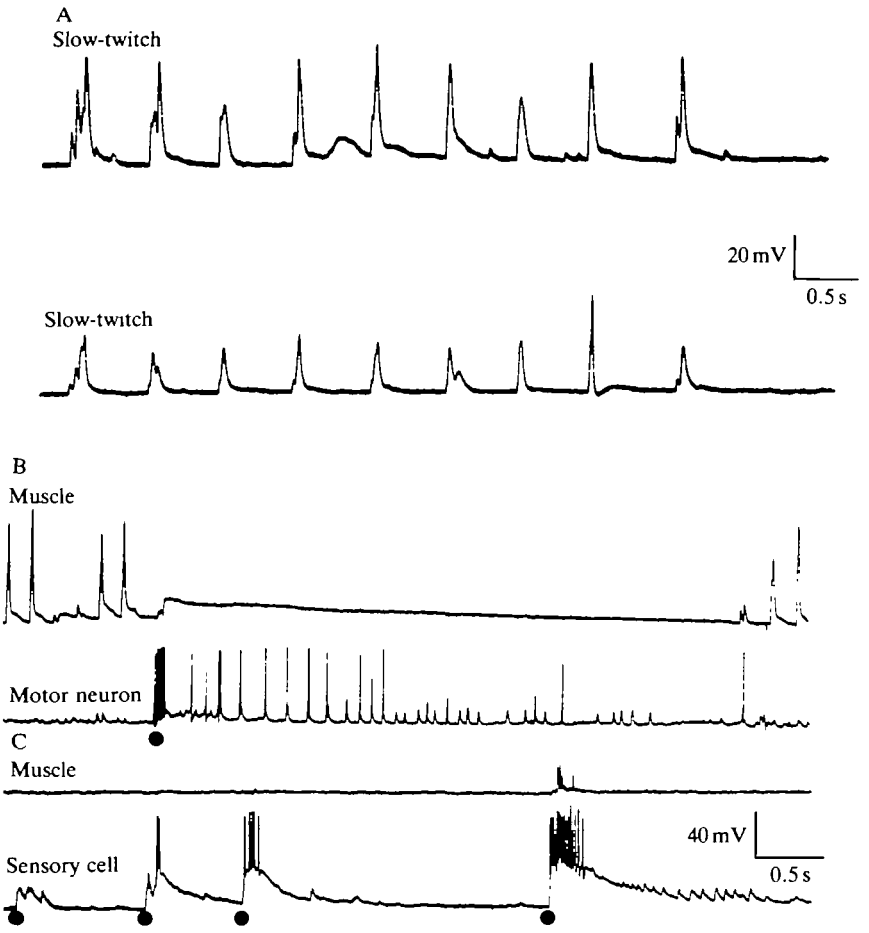

Fig. 5. (A) Dual recording from two slow-twitch fibers located three muscle bundles apart. Note the multiple nature of some of the spike-like potentials, and the similarity of inputs to the two cells. Activity is spontaneous. The nature of the 'hump' in the middle of the top trace is unknown. (B,C) Dual recordings from wing muscle cells and cells of the wing retraction system. Wing retraction induces inhibition of swimming. In B, existing swim activity in the muscle cell (top trace) is halted at the time of initiation of the burst of spikes in a wing retraction motor neuron. The long-lasting upward shift in the muscle cell record is believed to be a movement artifact produced by the wing retraction movement. A mechanical stimulus was delivered to the wing tip at the filled circle. In C, several mechanical stimuli were delivered to the wing tip (filled circles) while recordings were made from a swim muscle cell and a wing sensory neuron (sensory component of the wing retraction reflex). This is one of only two cases in which recorded muscle activity coincided with wing retraction. Note that in neither of the recordings were inhibitory inputs recorded from the muscle cells.

2 min rest periods between trials. These results were representative of data obtained with shorter, less complete trials. Three results are immediately apparent from Fig. 8. First, the size of spike-like responses can vary by more than a factor of

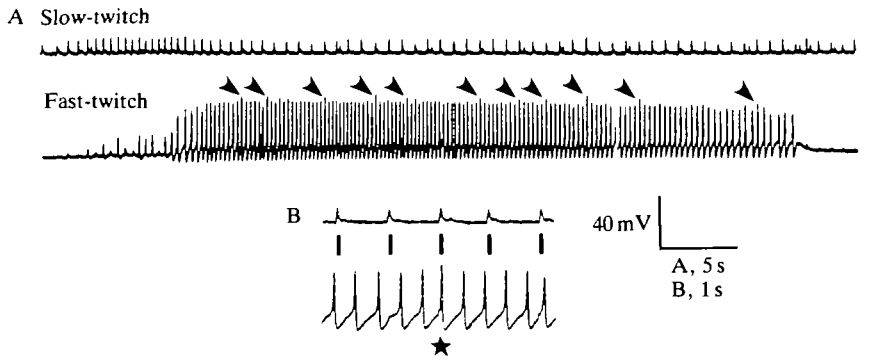

Fig. 6. Dual recording from a slow-twitch muscle cell (top trace) and fast-twitch muscle cell (bottom trace). At the beginning of A, an increase in cycle frequency is accompanied by the appearance of EJPs in the fast-twitch cell. Soon after this speed increase, the fast-twitch cell begins a prolonged period of repetitive firing. Note that the spikes are independent of normal swimming activity (shown by EJP activity in the slow-twitch cell). Occasional enhanced spikes are apparent (arrowheads). In B, an expanded portion of A shows that enhanced spikes are recorded when a spike occurs at the same time as an EJP in the slow-twitch cell (star), suggesting a common input to the two types of cells.

two. Second, the maximal size of spike-like responses is dependent upon frequency of activity, at least for the first 15 impulses. The trials were limited to fifteen stimuli since further stimulation at all frequencies produced no further changes in impulse amplitude (initial trials included 30–40 stimuli). Third, the initial rate of amplitude increase was greater at higher frequencies. This is particularly apparent when comparing the 1 Hz and 7 Hz trials in Fig. 8. These frequencies represent wingbeat frequencies for hovering swimming (1 Hz) and near-maximal fast swimming (7 Hz). Similar results were obtained for both cell types, with the exception of a few cells exhibiting extremely high thresholds for spike initiation upon which this type of experiment could not be completed.

The mechanism of spike facilitation has not been examined, but it may be related to the presence of a distinct depolarizing afterpotential (DAP) that follows each spike-like response (Figs 2, 7, 9). DAPs were not observed following EJPs (Fig. 9). Although the shape of the DAPs varied among cells, and they were sometimes preceded by a hyperpolarizing afterpotential, their amplitude (4–12 mV) and their duration (280–360ms) were relatively constant. DAPs are not believed to be movement artifacts for the following reasons. (1) DAPs were evident following spike-like responses only, even though EJPs produced contractions. (2) DAPs of similar size were recorded from a single cell following spontaneous, whole-wing contractions and spike-like responses induced by intracellular depolarization (without whole-wing contraction), as well as in preparations in which synaptic activity was chemically blocked. (3) The shape of DAPs could be altered by changing the membrane potential of the cell. (4) A change in swimming speed from slow to fast, which involves a significant increase

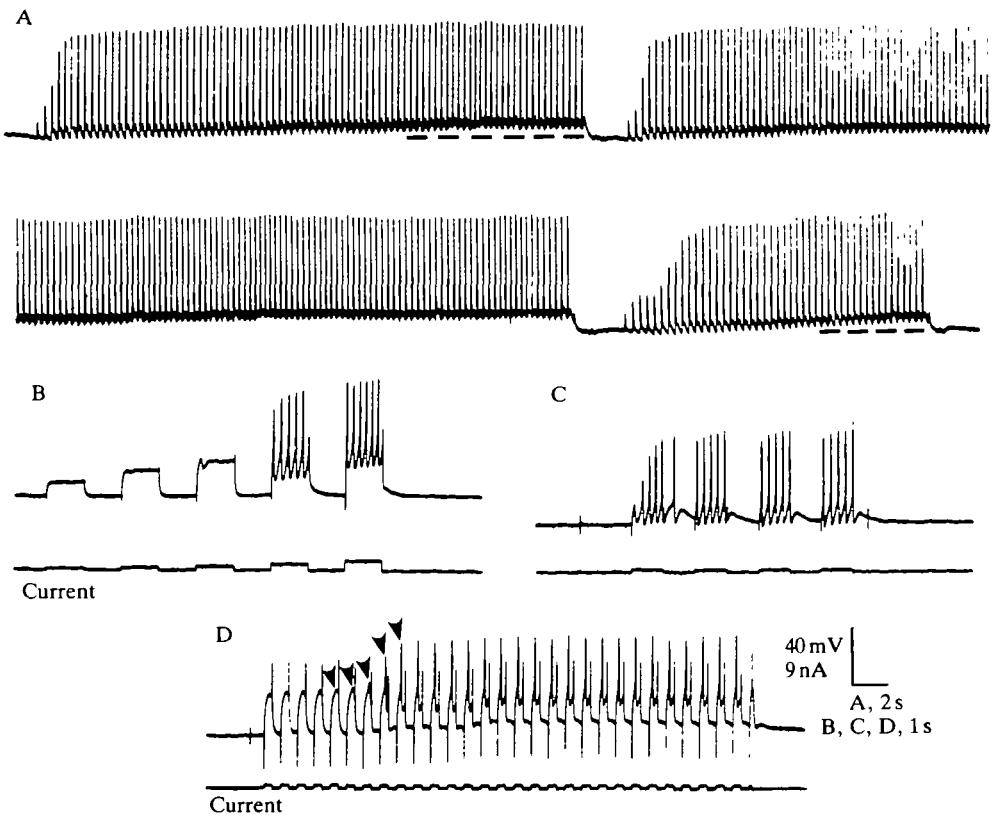

Fig. 7. Intracellular recordings from slow-twitch muscle cells showing amplitude facilitation of EJPs and spike-like responses. A is a continuous record from a preparation that was swimming intermittently. At the beginning of each swimming bout, there is an increase in the size of EJPs and spike-like responses. Note that depolarizing afterpotentials only occur following spike-like responses, and that with prolonged activity there is a tonic depolarization of the muscle cell (broken lines). In B, C and D, currents were injected into three different slow-twitch cells through the recording electrodes in an isolated wing preparation (no input from swim motor neurons). In B, successively increasing currents were used, whereas in C, identical currents were injected. Note that the muscle cells respond with bursts of spike-like responses that show amplitude facilitation (non-synaptic) both within a burst and between bursts. In D, brief currents were injected to show amplitude facilitation with single spikes (arrowheads). Note that depolarizing afterpotentials occur following active membrane responses, and appear to produce a baseline shift in the depolarizing direction.

in wing contractile vigor, did not produce a significant increase in the size of DAPs. This last observation was based on both spontaneous and induced changes in swimming speed (Fig. 2).

With repetitive swimming activity, muscle cells exhibited tonic depolarization, provided the swim frequency was above approximately 2 Hz (Figs 2, 7). Baseline

shifts of up to 15 mV were recorded from representatives of both muscle cell types (see Fig. 2). Similar baseline shifts were noted in isolated wing preparations in response to direct depolarization of muscle cells (Fig. 7), suggesting that the tonic

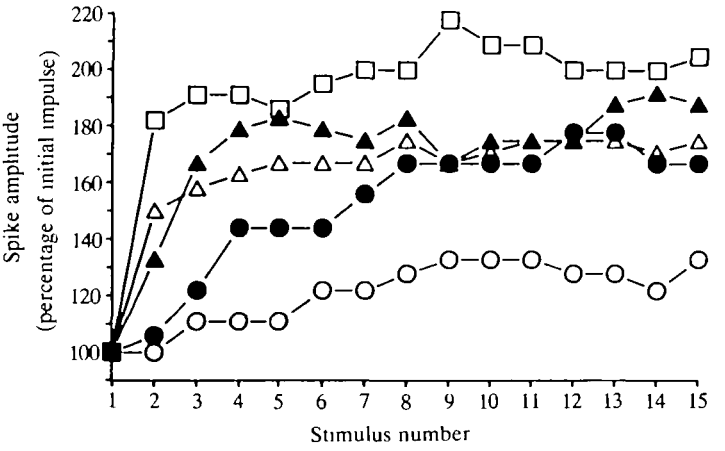

Fig. 8. Facilitation profile for a slow-twitch muscle cell stimulated intracellularly (isolated wing preparation) with brief stimuli delivered at five different frequencies: 1 Hz, ○; 2 Hz, ●; 3 Hz, △; 5 Hz, ▲; 7 Hz, □. Results from 16 similar but incomplete series, including both muscle cell types, gave similar results.

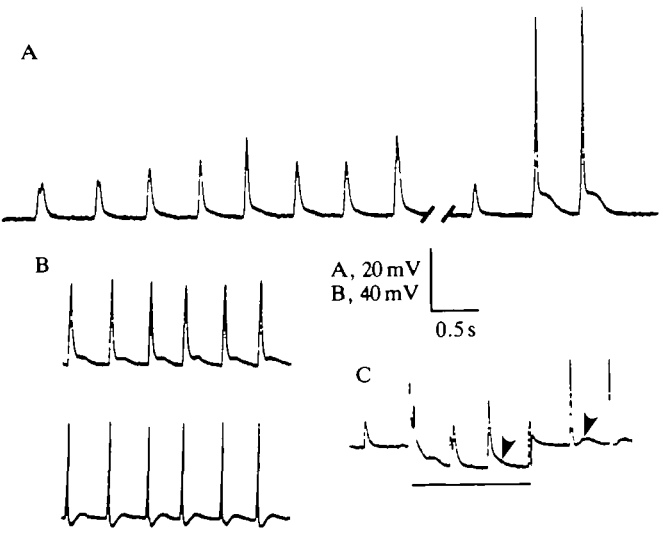

Fig. 9. Intracellular recordings from slow-twitch muscle cells showing the depolarizing afterpotential that follows spike-like responses. (A) DAPs follow spikes, but are not seen following EJPs. (B) Dual recording from slow-twitch muscle cells in adjacent muscle bundles showing that DAP shape can be different in neighboring cells. (C) The shape of DAPs can be changed by changing membrane potential. Bar, 1 nA hyperpolarization. Resting potentials for cells in B are −62 mV (top trace) and −65 mV (bottom trace).

depolarization may be a consequence of endogenous muscle cell properties rather than synaptic or modulatory input from central neurons.

Discussion

Previous histochemical and physiological evidence suggests that the swimming musculature of *Clione* includes two distinct types of muscle cells, referred to in relative terms as slow-twitch fatigue-resistant and fast-twitch fatigable (Satterlie *et al.* 1990). This dichotomy can be extended to include some electrophysiological and structural properties of the muscle cells. Size differences (length and diameter) are evident: slow-twitch fibers are generally larger than fast-twitch fibers. The two types of fibers show slight differences in resting potentials, which are within the range of values obtained from intracellular recordings from other molluscan muscle preparations (e.g. Twarog, 1967; Wilkens, 1972; Brezden and Gardner, 1984; Tattersall and Brace, 1987; Dorsett and Evans, 1989).

The principal means of identifying muscle fiber type during intracellular recordings involved observing electrical activity during slow swimming. As verified by histological examination of identified, HRP-injected fibers, only slow-twitch fibers exhibit significant motor neuron input during slow swimming. Both fiber types are active during fast swimming. This information, together with histochemical and twitch-time data (Satterlie *et al.* 1990), indicates that slow swimming involves activation of the slow-twitch fibers, while fast swimming results from recruitment of fast-twitch fibers.

The distribution of electrical activity throughout a muscle field is dependent upon the organization of the innervation fields of motor neurons, the presence or absence of a peripheral nerve net, and the presence or absence of electrical coupling between muscle cells. Only the last possibility is addressed in this report. Electrical coupling between muscle cells has been found in several molluscan preparations (Florey and Kriebel, 1969; Twarog *et al.* 1973; Orkand and Orkand, 1975; Gilloteaux, 1976; Tareskevich *et al.* 1977; Cohen *et al.* 1978; Peters and Altrup, 1984). Coupling could not be demonstrated between pairs of fast-twitch fibers or between slow- and fast-twitch fibers. Relatively strong coupling was found between pairs of slow-twitch fibers. The significance of this organization is not immediately apparent, but may be related to the arrangement of peripheral modulatory inputs to the swimming system. For example, serotonin has an excitatory influence on swimming in *Clione*, initiating fast swimming by reconfiguring the central pattern generator (Arshavsky *et al.* 1985*a*,*d*, 1986; Satterlie, 1989). Serotonin also acts peripherally by enhancing the contractile activity of muscle cells (Satterlie, 1990). Serotonin-immunoreactive processes are only found adjacent to slow-twitch fibers of the swimming system (R. A. Satterlie, unpublished observation). The selective coupling of slow-twitch fibers may thus aid in intercellular distribution of modulatory information within a restricted portion of the swim musculature.

One additional difference between the two fiber types concerns the organization

of motor neuron innervation. The delay between slow-twitch EJPs and fast-twitch EJPs in dual recordings, as well as the correspondence between fast-twitch EJPs and the second spike in slow-twitch spike doublets (e.g. Fig. 2), suggests that slow-twitch fibers are polyneurally innervated and that one input may also innervate the fast-twitch fibers. Several molluscan muscles have been found to be polyneurally innervated (Carew *et al.* 1974; Cohen *et al.* 1978; Kobayashi, 1972; Orkand and Orkand, 1975; Peters, 1979; Peters and Altrup, 1984; Tattersall and Brace, 1987). Polyneural innervation of *Clione* swim muscles is supported by ultrastructural evidence; two types of synaptic terminals are found adjacent to slow-twitch fibers, while three types of terminals abut fast-twitch fibers (Neal, 1988; T. L. Neal and R. A. Satterlie, in preparation).

Slow- and fast-twitch fibers are very similar in terms of their electrical activities. Both produce graded EJPs and variable spike-like responses that can overshoot zero potential. Several molluscan muscles exhibit overshooting action potentials (e.g. Twarog, 1967; Prior, 1975; Peters and Altrup, 1984). Inhibitory junctional potentials (IJPs) were not recorded in either muscle cell type in *Clione*, even during induced wing retractions (which inhibit swimming). IJPs have been described in other molluscan muscle preparations (Mayeri *et al.* 1974; Banks, 1975, 1978; Rock *et al.* 1977; Sawada *et al.* 1981; Kurokawa and Kuwasawa, 1988).

The spike-like responses of *Clione* show significant amplitude facilitation that is frequency-dependent in terms of both overall impulse amplitude and facilitation rate. This facilitation is not of synaptic origin, but appears to result from endogenous properties of the muscle cells themselves. Similar activity is observed in both muscle cell types. One endogenous property of the muscle cells that may be involved in this non-synaptic facilitation is the depolarizing afterpotential (DAP) that follows each spike-like response. DAPs have been described in several molluscan neurons, where they are believed to be involved in generation of repetitive firing activity (Thompson and Smith, 1976; Bulloch and Willows, 1981; Lewis, 1984). At least some of the muscle cells of *Clione* do exhibit repetitive firing capabilities, but it is not known if this is physiologically significant or is an artifact caused by microelectrode penetration or non-physiological depolarizations. Firing rate during repetitive firing exceeds the maximum swim cycle frequency and is independent of motor neuron input. If repetitive firing does occur during normal swimming activity, it may be involved in increasing wing stiffness during fast swimming. Wing stiffness appears to increase dramatically during fast swimming (the degree of wing bending is greatly decreased; R. A. Satterlie, personal observation). Muscle cells show a significant baseline depolarization when swim cycle frequency exceeds approximately 2 Hz. These baseline shifts could also contribute to increases in wing stiffness, provided the level of depolarization produces development of tension in the muscle cells. The exact role of DAPs and of the baseline depolarization in non-synaptic facilitation is not known.

The frequency profile of non-synaptic facilitation is interesting when placed in a behavioral context. Changes in swimming speed in *Clione* involve not only a change in cycle frequency but also a change in contractile force in the wing

musculature. Slow, hovering swimming typically involves a wingbeat frequency of around 1 Hz, while maximal swimming occurs at 7–8 Hz. Fig. 8 indicates that muscle spike amplitude reaches a relatively stable value at around the tenth impulse in a series, and that the maximal spike amplitude is frequency-dependent and dramatically different in the 1 Hz and 7 Hz trials. Thus, the endogenous properties of the muscle cells are such that increases in contractile force may occur with simple changes in the frequency of the motor drive. This could, at least in part, help to explain how the system changes swimming speed within 'gears' (fast or slow). Add to this the possibility of recruitment of an additional muscle cell type (change-of-gears), and of neuro- or hormonal modulation of central and peripheral activity, and this relatively simple neuromuscular system is capable of a wide range of subtle and dramatic changes in locomotory speed.

I thank Dr A. O. D. Willows, Director of Friday Harbor Laboratories, for providing space and generous assistance, Drs Ann Kammer and Ted Goslow for critically reading the manuscript, and Lou and Alison Satterlie for help in collecting animals. This study was supported by NSF grant BNS-8511692 and a research grant from the Whitehall Foundation.

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