

Coordination of Limb Movements: Three Types of Intersegmental Interneurons in the Swimmeret System and Their Responses to Changes in Excitation

HISAAKI NAMBA AND BRIAN MULLONEY

Neurobiology, Physiology, and Behavior, University of California, Davis, California 95616-8519

Namba, Hisaaki and Brian Mulloney. Coordination of limb movements: three types of intersegmental interneurons in the swimmeret system and their responses to changes in excitation. *J. Neurophysiol.* 81: 2437–2450, 1999. During forward locomotion, the movements of swimmerets on different segments of the crayfish abdomen are coordinated so that more posterior swimmerets lead their anterior neighbors by ~25%. This coordination is accomplished by mechanisms within the abdominal nerve cord. Here we describe three different types of intersegmental swimmeret interneurons that are necessary and sufficient to accomplish this coordination. These interneurons could be identified both by their structures within their home ganglion and by their physiological properties. These interneurons occur as bilateral pairs in each ganglion that innervates swimmerets, and their axons traverse the minuscule tract (MnT) of their home ganglion before leaving to project to neighboring ganglia. Two types, ASC_E and ASC_L, projected an axon anteriorly; the third type, DSC, projected posteriorly. Each type fires a burst of impulses starting at a different phase of the swimmeret cycle in its home ganglion. In active preparations, excitation of individual ASC_E or DSC interneurons at different phases in the cycle affected the timing of the next cycle in the interneuron's target ganglion. The axons of these interneurons that projected between two ganglia ran close together, and their firing often could be recorded by the same electrode. Experiments in which either this tract or the rest of the intersegmental connectives was cut bilaterally showed that these interneurons were both necessary and sufficient for coordination of neighboring swimmerets. When the level of excitation of the swimmeret system was increased by bath application of carbachol, the period of the system's cycle shortened, but the characteristic phase difference within and between ganglia was preserved. Each of these interneurons responded to this increase in excitation by increasing the frequency of impulses within each burst, but the phases and relative durations of their bursts did not change, and their activity remained coordinated with the cycle in their home ganglion. The decrease in duration of each burst was matched to the increase in impulse frequency within the burst so that the mean numbers of impulses per burst did not change significantly despite a threefold change in period. These three types of interneurons appear to form a concatenated intersegmental coordinating circuit that imposes a particular intersegmental phase on the local pattern generating modules innervating each swimmeret. This circuit is asymmetric, and forces posterior segments to lead each cycle of output.

INTRODUCTION

Animals that use limbs to walk or swim do so by moving those limbs periodically in a coordinated pattern. In both

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

arthropods and vertebrates, these rhythmic movements of each limb are driven by local central pattern-generating circuits. These local circuits are coordinated by interneurons the activities of which produce the particular phase differences between neighboring limbs that are characteristic of the animal's gaits. Within a local circuit, pattern-generating interneurons can be nonspiking, and their synaptic transmission can be graded (Paul and Mulloney 1985a,b; Pearson and Fourtner 1975). Communication between circuits, however, depends on impulses in axons of coordinating interneurons and on spike-mediated synaptic transmission to the targets of these interneurons. The temporal patterns of impulses in these axons contain all the information that coordinates movements of different limbs. What are these firing patterns, what mechanisms generate these patterns, and how do they change if the animal alters the period or force of its movements? We have approached these questions by investigating neurons that coordinate swimmeret movements.

The swimmerets of crayfish are a set of four paired limbs on the abdomen that beat periodically to propel the animal forward when it is swimming. The abdominal nervous system can produce the normal motor program that drives these movements even when isolated from more anterior parts of the CNS and from all sensory feedback (Hughes and Wiersma 1960; Ikeda and Wiersma 1964), so the swimmeret system provides an opportunity to investigate central neural mechanisms of intersegmental coordination. Each pair of swimmerets is innervated by an abdominal ganglion located in the same segment. Within this ganglion, each swimmeret has its own local pattern-generating module that produces alternating bursts of impulses in its own set of power-stroke (PS) and return-stroke (RS) motor neurons (Murchison et al. 1993). There is no segmental gradient of excitability in these modules (Mulloney 1997). The movements of swimmerets on each segment differ in phase from their neighbors by ~25%, independent of beat frequency (Braun and Mulloney 1993; Mulloney 1997). Any two neighboring abdominal ganglia will generate coordinated swimmeret motor output the phases of which are not significantly different from those produced by the intact nervous system (Paul and Mulloney 1986). Thus the information conducted between neighboring ganglia seems to be necessary and sufficient for coordinated swimmeret movements. What neurons conduct this information?

Swimmeret modules in different segments appear to be connected by "coordinating fibers" that project through the connectives linking neighboring ganglia (Stein 1971; Wiersma

and Hughes 1961) to form an intersegmental coordinating circuit (Paul and Mulloney 1986). These units fire bursts of impulses during either the PS phase or the RS phase of activity in their home ganglion (Stein 1971; Wiersma and Hughes 1961). These axons seem to originate in bilateral pairs in each abdominal ganglion and to project either anteriorly, in the ascending direction, or posteriorly, in the descending direction, at least to the next neighboring ganglion (Skinner and Mulloney 1998; Stein 1971). A volley of impulses in these ascending units affects the timing of swimmeret motor activity in the axon's target ganglion (Stein 1971, 1974). Although each interganglionic connective contains thousands of axons, the numbers of these coordinating fibers that have been described are small, and their locations in the connectives are the same from animal to animal. The axons of certain of these intersegmental interneurons also run in the minuscule tract (MnT) (Skinner 1985a) of the ganglion in which they originate (Mulloney et al. 1993; Paul and Mulloney 1986). To begin a cellular analysis of these intersegmental coordinating mechanisms, we have revisited these MnT interneurons.

Here we describe and name three types of coordinating interneurons that originated in each abdominal ganglion that innervates functional swimmerets. The structures and physiological properties of these interneurons were distinct from those of command neurons or local pattern-generating interneurons (Acevedo et al. 1994; Paul and Mulloney 1986). The firing of these interneurons could be recorded in MnT, and every MnT unit that fired in phase with swimmeret activity proved to be an intersegmental interneuron. These MnT interneurons sent axons unilaterally through the medial ascending and medial descending tracts of the intersegmental connectives to targets in neighboring ganglia (Stein 1971). Experimental perturbation of individual MnT interneurons by steps of depolarizing currents changed the timing of the next cycle of output from their targets modules. Severing either these intersegmental tracts or the rest of the connectives except these tracts showed that these axons were necessary and sufficient to maintain normal intersegmental coordination.

We also describe the responses of these interneurons to changes in excitation of the system. When excitation increased and the period of the motor pattern shortened, the phases and the relative durations (duration/period) of bursts of impulses in each of these interneurons did not change. As period shortened, they fired impulses at higher frequencies during each burst, but the durations of these bursts decreased proportionately, so the mean numbers of impulses per burst also did not change. Thus these three types of interneurons export to neighboring ganglia detailed information about the activity of the module in which they originate, cycle by cycle. This information is the basis of normal coordination of limb movements on different segments of the abdomen.

METHODS

Crayfish, *Pacifastacus leniusculus*, were obtained from local suppliers and kept in freshwater aquaria at 15°C. Animals were anesthetized by chilling on ice. They then were exsanguinated by perfusing physiological saline into a wound created by removing one of the claws. The posterior part of the ventral nerve cord, including thoracic ganglia 4 and 5 and all six abdominal ganglia (A1, A2, . . . , A6), was removed and pinned dorsal side up in a dish lined with silicone elastomer (Sylgard; Dow Corning, San Francisco, CA) and bathed in saline. The normal saline solutions

contained (in mM) 195 NaCl, 5.36 KCl, 2.6 MgCl₂, 13.5 CaCl₂, and 10 Tris-maleate buffer, at pH 7.4. In most experiments used to observe MnT activity, preparations spontaneously expressed coordinated swimmeret motor patterns at the outset.

Neurobiotin staining

Axons of coordinating interneurons were backfilled with Neurobiotin (Vector) through the cut ends of axon bundles stripped from the medial-dorsal region of the interganglionic connectives. Once filling was complete, ganglia were fixed >2 h in 4% paraformaldehyde in PBS (Acevedo et al. 1994; Sherff and Mulloney 1996). Fixed ganglia were washed in Dulbecco's PBS (Sigma), dehydrated to 95% ethanol to increase permeability, and then rehydrated to PBS. To visualize the Neurobiotin, ganglia were preincubated with 0.3% reduced Triton-X 100 in PBS, and incubated for 14–18 h with Cy3-streptavidin (1:200; Jackson ImmunoResearch) or horseradish peroxidase (HRP)-streptavidin (1:300; Amersham, Piscataway, NJ) in 0.3% reduced Triton X-100 in PBS. HRP preparations were developed with diaminobenzidine (DAB), using the protocol detailed in Sherff and Mulloney (1997). Finally, labeled ganglia were dehydrated, cleared, and studied as whole mounts.

Electrophysiology

Each swimmeret is innervated by one nerve, N1, which splits into an anterior and posterior branch (Sherff and Mulloney 1997). The axons of RS motor neurons exit the ganglion through the anterior branch; axons of PS motor neurons exit through the posterior branch. Action potentials of PS and RS motor neurons were recorded separately with extracellular pin electrodes on these branches (Fig. 1A).

Skinner (1985a) first described and named the MnT in these abdominal ganglia and introduced the abbreviation "MT" for it; we have substituted the abbreviation MnT here to avoid inevitable confusion with a visual area in the cortex of a minor family of chordates. To record from axons in MnT, we removed the sheath from the dorsal side of a ganglion and placed conventional suction electrodes (80–100 μm ID) on the dorsal surface of the lateral giant axon (LG) near the anterior border of the lateral neuropil, LN (Fig. 1). To confirm that these MnT units were intersegmental interneurons, another suction electrode (Fig. 1A) was placed on the medial surface of the desheathed hemiconnective at area 76 and area 78 (Wiersma and Hughes 1961).

Glass microelectrodes were filled with 2.5% Neurobiotin in 1 M KCl. Their resistances were 30–50 MΩ. Signals from intracellular recordings were amplified with an Axoclamp 2B preamplifier (Axon Instruments, Foster City, CA). Both extracellular and intracellular recordings were collected on VCR tape, using a Neuro-Corder 886 (Neurodata Instruments). Recordings were transferred to a computer for analysis with Axoscope (Axon Instruments).

Surgical interruption of regions of the interganglionic connectives

To block the flow of information between two neighboring ganglia, we desheathed and separated the two hemiconnectives as we would for suction-electrode recording but then separated the dorsal-medial region (the medial parts of area 76 and area 78) that contained axons of coordinating interneurons. Once these regions were separated from the rest of the connectives, either they or the rest of the connectives were cut with fine scissors.

At the end of each of these cutting experiments, the preparation was fixed, stained with osmium ethyl-gallate, embedded in Spurr's resin, and sectioned for light microscopy (Leise et al. 1986). The extent of each lesion was assessed by comparing transverse sections of the spared region of the connective with sections of undamaged regions of the same connective.

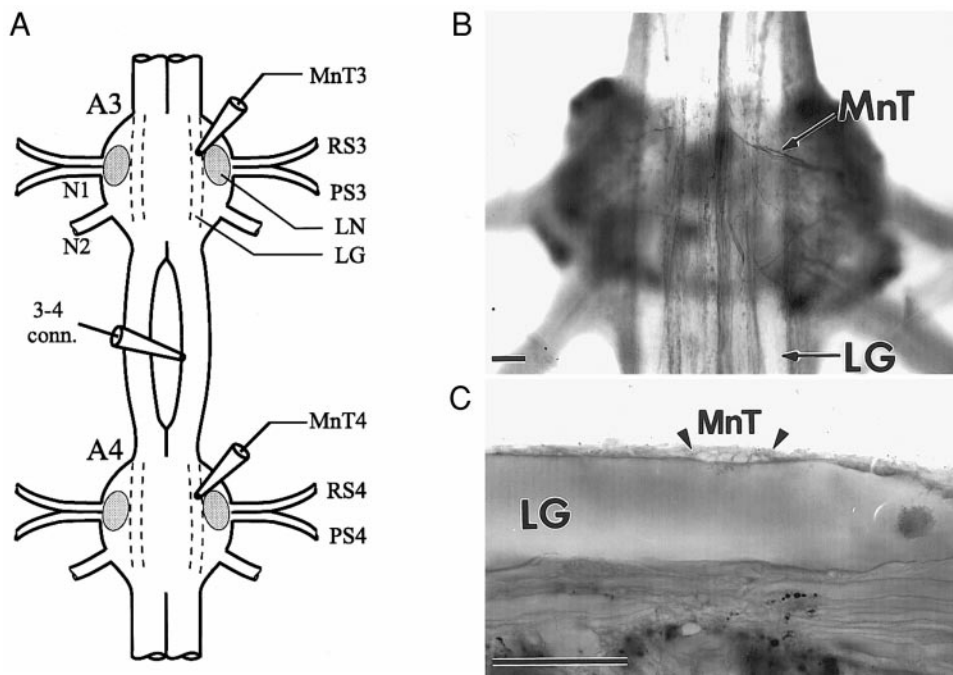


FIG. 1. *A*: diagram that shows the arrangement of suction electrodes used to record from axons in the minuscule tract (MnT) on the right sides of abdominal ganglia 3 and 4 (A3, A4). MnT crosses dorsal to the lateral giant (LG) axon before it reaches the midline, so a suction electrode can be placed on it at that point in each ganglion (MnT3, MnT4). Impulses in axons of intersegmental swimmeret interneurons also can be recorded with a third suction electrode placed on the dorsomedial surface of the connective that runs between A3 and A4 (3–4 conn.). The lateral neuropil (LN) is the anatomic site of the local module that controls each swimmeret and drives activity in power-stroke (PS) and return-stroke (RS) motor axons. *B*: frontal view of a whole mount preparation of an A4 in which MnT axons have been filled with Neurobiotin by backfilling from the 3–4 connective. Anterior is at the top of the photo. *C*: photograph of a parasagittal section through MnT at the point where it crosses dorsal to the LG axon. Arrowheads mark the boundaries of MnT. Anterior is at the left, and dorsal at the top of this photo. Scale bars in *B* and *C* mark 100 μm .

Perfusion procedures

To change the level of excitation in the swimmeret system (Mulloney 1997), we superfused the isolated abdominal nerve cord with different concentrations of carbachol (Research Biochemical International, Natick, MA). To restrict the action of carbachol to abdominal ganglia, they were separated from the thoracic ganglia with petroleum jelly (Vaseline) dams. The volume of this experimental chamber was <3 ml, and normal saline was continuously perfused through the dish at 2.5–2.8 ml/min before perfusion of carbachol solutions. The level of excitation was increased in steps: 2, 8, 14 or 15, and 20 μM carbachol in normal saline. Each test solution was perfused >15 min, and data were collected after >6 min perfusion of each concentration.

When >14 μM carbachol was used and the period was <0.4 s, the noise level often increased and bursts of MnT units were sometimes disrupted, so we analyzed only that range of periods in which individual MnT units could confidently be discriminated.

Description and analysis of recordings

To calculate parameters that describe the activity of the swimmeret system, we displayed activity recorded simultaneously by each electrode and measured the times at which each burst of impulses started or stopped, using a digitizing tablet. From these lists of times, we calculated the periods and durations of each burst and the phase at which each burst began relative to the motor output of the system (Mulloney and Hall 1987). The period of each cycle of activity was the time from the start of one burst of impulses to the start of the next burst. The duration of each burst was the time from which it started to the time at which it ended.

Phase describes the point in a cycle of activity at which some event began, for example a stimulus or a burst of impulses recorded by a second electrode on a different nerve. To measure an event's phase, we measured its latency, the difference between the time at which it began and the time at which the preceding burst in the reference recording began. We also measured the period of that cycle in the reference recording. Phase_{*i*} then was calculated as latency_{*i*}/period_{*i*}. Normally, we used PS bursts from the most posterior ganglion, A5, as the reference for each cycle (Mulloney 1997). To compare the distributions of phases recorded under different conditions and different sample sizes, we plotted their relative cumulative frequencies. This

procedure sorts the list of measured phases from smallest to largest and calculates for each phase the proportion of the sample smaller than or equal to it (Zar 1984). To calculate probabilities that phases of bursts of impulses recorded in different ganglia occurred randomly relative to one another, we used the Kolmogorov-Smirnov test (Zar 1984).

To measure the changes in period of a ganglion's output that resulted from perturbations of individual interneurons, we measured the mean period of several cycles that preceded the perturbation and the period of the cycle in which the perturbation began. We then calculated the ratio of the experimental period to the mean period; if there was no change, the ratio was 1.0.

To compare directly the durations of bursts recorded under conditions where the motor output had different periods, we calculated the relative durations of these bursts as duration/period (Skinner and Mulloney 1998).

The intervals between individual impulses recorded from single units were measured from data recorded with Axoscope (Axon Instruments) and displayed on the computer screen, using the measurement features of that program.

To calculate descriptive statistics and ANOVA, we used SigmaStat (Jandel).

RESULTS

Axons of swimmeret interneurons occur in the MnT of each abdominal segment

MnT is a small tract of axons that rises nearly vertically from each LN and then turns medially to cross dorsal to the LG axon, but ventral to the median giant axon to reach the midline of the ganglion (Skinner 1985a,b). Whenever the swimmeret system was active, a suction electrode placed on the surface of the LG axon at the level of the anterior edge of the LN (Fig. 1A) recorded bursts of impulses that occurred in phase with swimmeret motor output (Fig. 2A). These bursts of impulses in MnT occurred in each ganglion that innervated swimmerets used for locomotion, A2 . . . A5. These impulses were not synchronous with impulses in either N1 and so were not simply axon collaterals of swimmeret motor neurons.

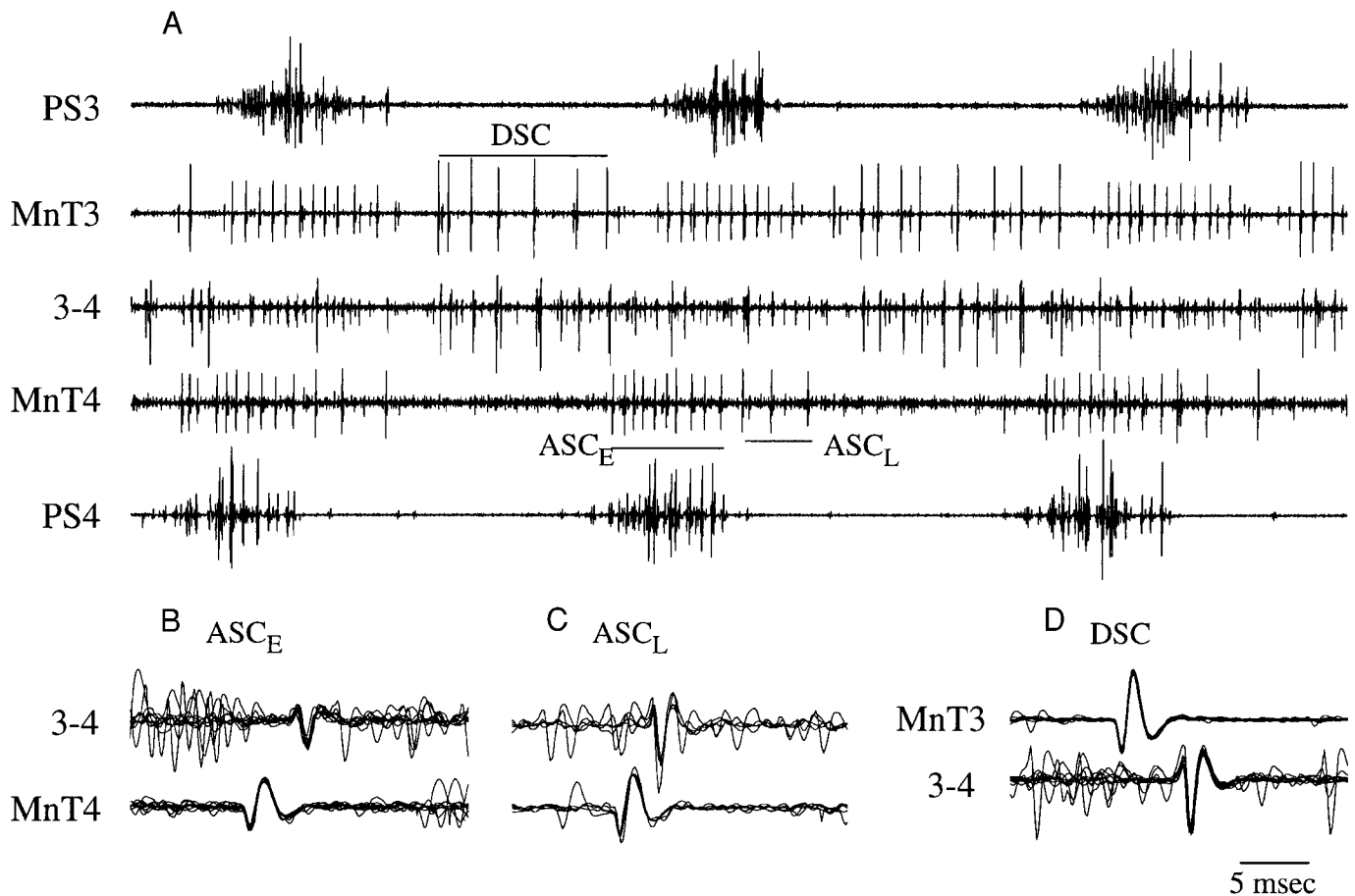


FIG. 2. *A*: simultaneous recordings from MnT3, MnT4, the 3–4 connective, and power-stroke branches of the N1s in A3 and A4 (PS3, PS4). Bursts of impulses that occurred periodically in MnT3 and MnT4 had a fixed phase relative to the swimmeret motor output in their home ganglia. *B–D*: each of these bursting units also was recorded in the 3–4 connective, so the direction in which their axons projected could be decided. MnT3 recordings included both ascending units and a descending unit (DSC) that fired when PS units in A3 were silent. MnT4 recordings included 2 different ascending units (ASC_E, ASC_L) that fired with different phases relative to PS bursts in A3. *B* and *D* show 10 superimposed traces; *C* shows 5 traces.

The MnT of each ganglion in the middle of the chain, A3 and A4, contained axons that fired bursts in phase with the swimmeret motor pattern and that projected anteriorly or posteriorly to neighboring ganglia (Fig. 2A). We recorded simultaneously with three suction electrodes, one on MnT in A3 (MnT3), one on MnT in A4 (MnT4), and one on the desheathed medial-dorsal connective between A3 and A4 (Fig. 1A). Each axon that ran anteriorly from A4 fired bursts of impulses in phase with PS bursts in its own ganglion (Fig. 2, A–C). Each axon that ran posteriorly from A3 fired bursts in phase with RS bursts in its home ganglion (Fig. 2, A and D).

Intersegmental tract that contained the axons of these MnT interneurons was necessary and sufficient for normal intersegmental coordination

The axons of these interneurons ran close together in the intersegmental connectives (Fig. 2A) in roughly the same position in each animal. This predictability allowed us to isolate the tract that contained these axons and to test the system's responses both to cutting this tract and to cutting all of the connective except this tract. In all these experiments, the preparation was superfused with 5–8 μ M carbachol to provide a

constant level of excitation to the system (Mulloney 1997). To assess the extent of the lesion, each preparation was fixed after the experiment, and transverse sections of the connective above and below the cut were compared with sections at the cut itself.

Before any parts of the connectives were cut, the phase of bursts in PS units in A3 (PS3) relative to bursts in A5 (PS5) showed little variability (Fig. 3). When all axons in the A3–A4 connectives except the tracts that contained these axons were cut ($n = 3$ experiments), the variability of intersegmental phase differences did not change (Fig. 3A). The *inset* in Fig. 3A shows the regions of the connective that were spared in this experiment. In some experiments, the mean phase shifted after the cut, but the variability of the phases around this new mean was not greater than the variability of the control in the same experiment. These results are consistent with the idea that information carried in these tracts, probably by the MnT axons, is sufficient to maintain normal intersegmental phase differences.

When only the dorsomedial parts of areas 76 and 78 in the A3–A4 connectives that contained axons of MnT interneurons were cut (Fig. 3B), the normal coordination of swimmeret activity in these ganglia collapsed ($n = 6$ experiments). After the tracts were cut, both ganglia continued to produce periodic

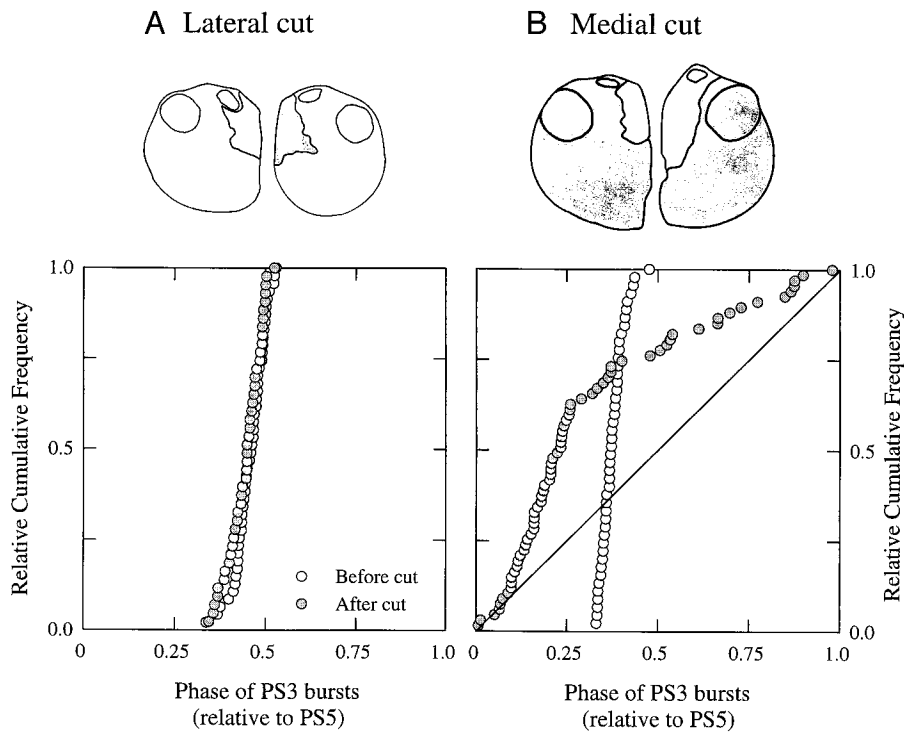


FIG. 3. *A* and *B*: relative cumulative-frequency plots of phases of bursts of impulses in PS3 motor axons recorded with the 3–4 connectives intact (before cut) and with either medial or lateral regions of these connectives cut (after cut). These phases were calculated relative to bursts in PS axons in A5. *Inset*: shaded portions of the drawings show the regions of the 3–4 connectives spared in each experiment. Two interval ovals in each hemiconnective are the MG and LG axons, included to orient the reader. Each drawing was prepared by superimposing camera lucida drawings of 2 transverse sections, 1 at the cut and 1 posterior to the cut. Diagonal line in *B* is the distribution of phases predicted if the phases of A3 were randomly distributed.

bursts of PS impulses, but the variation in the intersegmental phase differences was much greater, and PS3 bursts drifted through the subsequent PS5 periods (data not shown). The *inset* in Fig. 3*B* shows the region of the connective that was spared in this experiment. These results are consistent with the idea that information carried in these tracts, probably in the MnT axons, is necessary to maintain normal intersegmental coordination.

If the axons in these tracts were the only ones that contributed to intersegmental coordination, we would predict that once the tracts had been cut the phases of PS3 relative to PSE5 would be distributed randomly. In a cumulative frequency plot of these phases, randomly distributed phases would lie on the diagonal line from lower left to upper right (Zar 1984). This was not the case (Fig. 3*B*); a Kolmogorov-Smirnov test of the cumulative probabilities of these measured bursts gave $P < 0.001$ that the phases of PS3 bursts relative to PS5 were distributed randomly in this experiment. The classic descriptions of swimmeret coordinating units included an ascending axon located in area 81, the lateral region of the connective (Stein 1971; Wiersma and Hughes 1961). In experiments in which we cut only the medial tracts that contained axons of MnT interneurons, this other unit would have been spared and so might account for this departure from randomness. Nonetheless the information carried in the surviving regions of the connective was not enough to maintain normal coordination (Fig. 3*B*), and we conclude that axons in the severed regions, the medial parts of areas 76 and 78, are necessary for normal intersegmental coordination.

Characteristics of different types of coordinating interneurons

We have identified three types of intersegmental coordinating interneurons that have an axon in the MnT of each abdom-

inal ganglion that innervates swimmerets. They originate in the clusters of cell bodies anterior and posterior to the base of N1. Their neurites pass through the LN before they project into the MnT and exit the ganglion (Fig. 4). These interneurons occur as bilateral pairs in each ganglion and can be distinguished by their patterns of firing during swimmeret activity, by the direction of their axonal projections, by their effects on PS activity in their target ganglia, and by their structures in their home ganglia. Two types, ASC_E and ASC_L , send axons anteriorly from their home ganglia, in an ascending direction (Fig. 4, *A* and *B*). The third type, DSC, sends its axon posteriorly, in a descending direction (Fig. 4*C*). Remarkably, these are unilateral interneurons; their structures within their home ganglia are restricted to one half of the ganglion. Although other axons

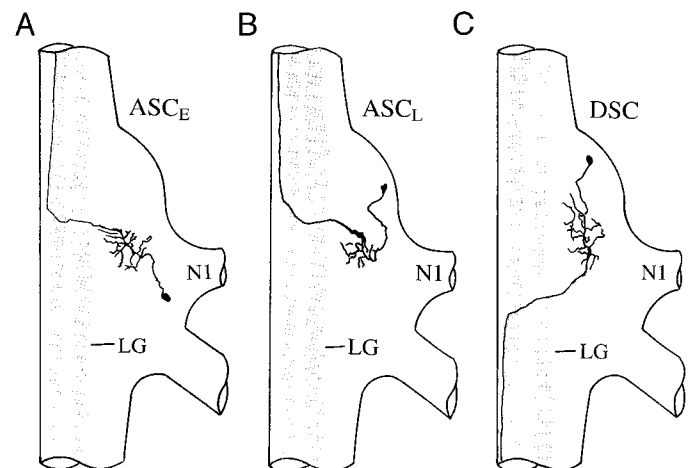


FIG. 4. Structures of 3 types of swimmeret coordinating interneurons that were filled with Neurobiotin through a microelectrode. These are camera lucid drawings of the right halves of whole mounts, viewed from the dorsal side with anterior at the top (cf. Fig. 1*B*). *A*: drawing of an ASC_E neuron. *B*: drawing of an ASC_L neuron. *C*: drawing of a DSC neuron.

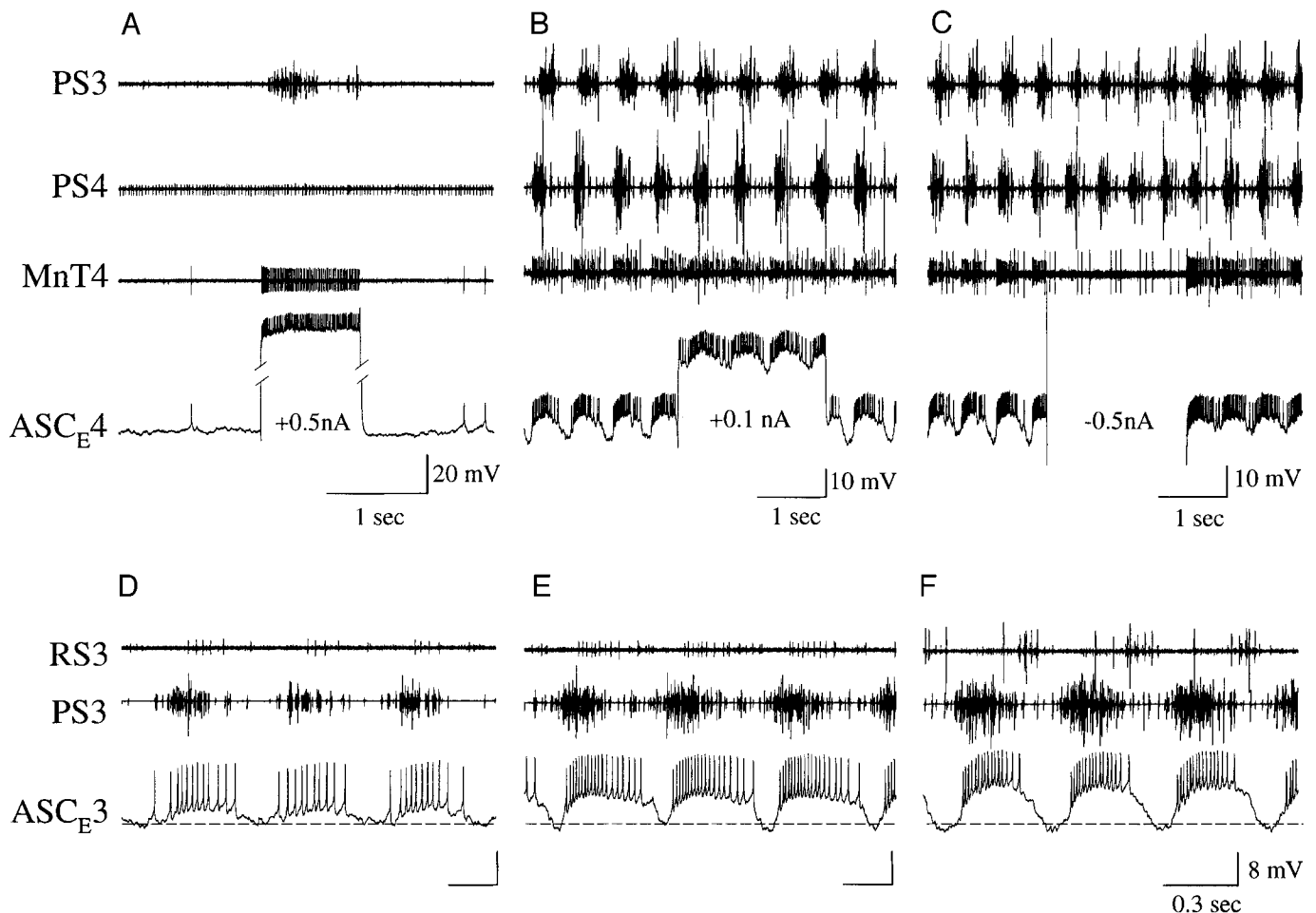


FIG. 5. Properties of the ASC_E coordinating interneurons. *A*: simultaneous intracellular recording from an ASC_E interneuron in A4 and extracellular recording from the MnT4 in which its axon ran. In this quiet preparation, driving a train of impulses in the ASC_E neuron with depolarizing current evoked a burst of impulses in PS units in A3. *B*: when later the same preparation was producing coordinated swimmeret motor activity, depolarizing current caused additional impulses in this neuron, but had little effect on the target ganglion. *C*: hyperpolarizing current stopped the firing of this ASC_E neuron (shown in extracellular trace) and decreased burst intensity in PS3. *D–F*: recordings from an ASC_E interneuron in A3 made at 3 levels of excitation of the swimmeret system that show changes in impulse frequency. *D*: spontaneous activity, period 0.70 s. *E* and *F*: in response to excitation, periods decreased to 0.66 and 0.43 s.

do cross through the MnT from one LN to the other (Paul and Mulloney 1986), as these coordinating axons approach the midline they leave MnT to enter the interganglionic connective.

Interneuron ASC_E fired bursts of impulses simultaneously with bursts in the excitatory PS motor neurons (PSE) in its home ganglion and sent an ipsilateral axon ascending to the next anterior ganglion (Fig. 2, *A* and *B*). We recorded from and filled this interneuron six times. Each of these interneurons had a small elliptical cell body, $23 \times 33 \mu\text{m}$ in diameter, located in the cluster of PSE cell bodies posterior to N1 (Sherff and Mulloney 1997). The neurite of this neuron projected anteriorly into the lateral neuropil, where it gave rise to several short branches as it rose dorsally in the MnT. The neurite crossed dorsal to LG but turned before reaching the midline to project a relatively small axon anteriorly; its structure in its home ganglion is completely unilateral (Fig. 4).

When the system was not active (Fig. 5*A*), driving one ASC_E interneuron excited PSE bursts in more anterior ganglia but had little effect on the ganglion from which it originated. This

response in the target ganglion was the same as the response reported by Stein (1969, 1974) to stimulation of bundles of axons that contained a swimmeret coordinating fiber. When the system was active, the membrane potentials of these interneurons oscillated in phase with the motor output from their home ganglion, and they fired bursts of impulses simultaneously with bursts in power-stroke excitor (PSE) motor neurons in its home ganglion (Fig. 5, *D–F*).

When the system was actively producing swimmeret motor output, depolarizing currents injected into ASC_E shortened the period of the next PS3 burst. Experimental hyperpolarization that inhibited firing of this ASC_E decreased PS activity only slightly in A4, the neurons's home ganglion, but caused a notable decrease of PS3 activity in the neurons's target ganglion (Fig. 5*C*). This hyperpolarization did not affect the period of the swimmeret motor pattern, but phasic firing of this interneuron appeared to affect the excitatory drive that underlies each PS burst in the next anterior ganglion.

In a few preparations, we recorded from and filled an ascending coordinating interneuron that fired only late in the PSE

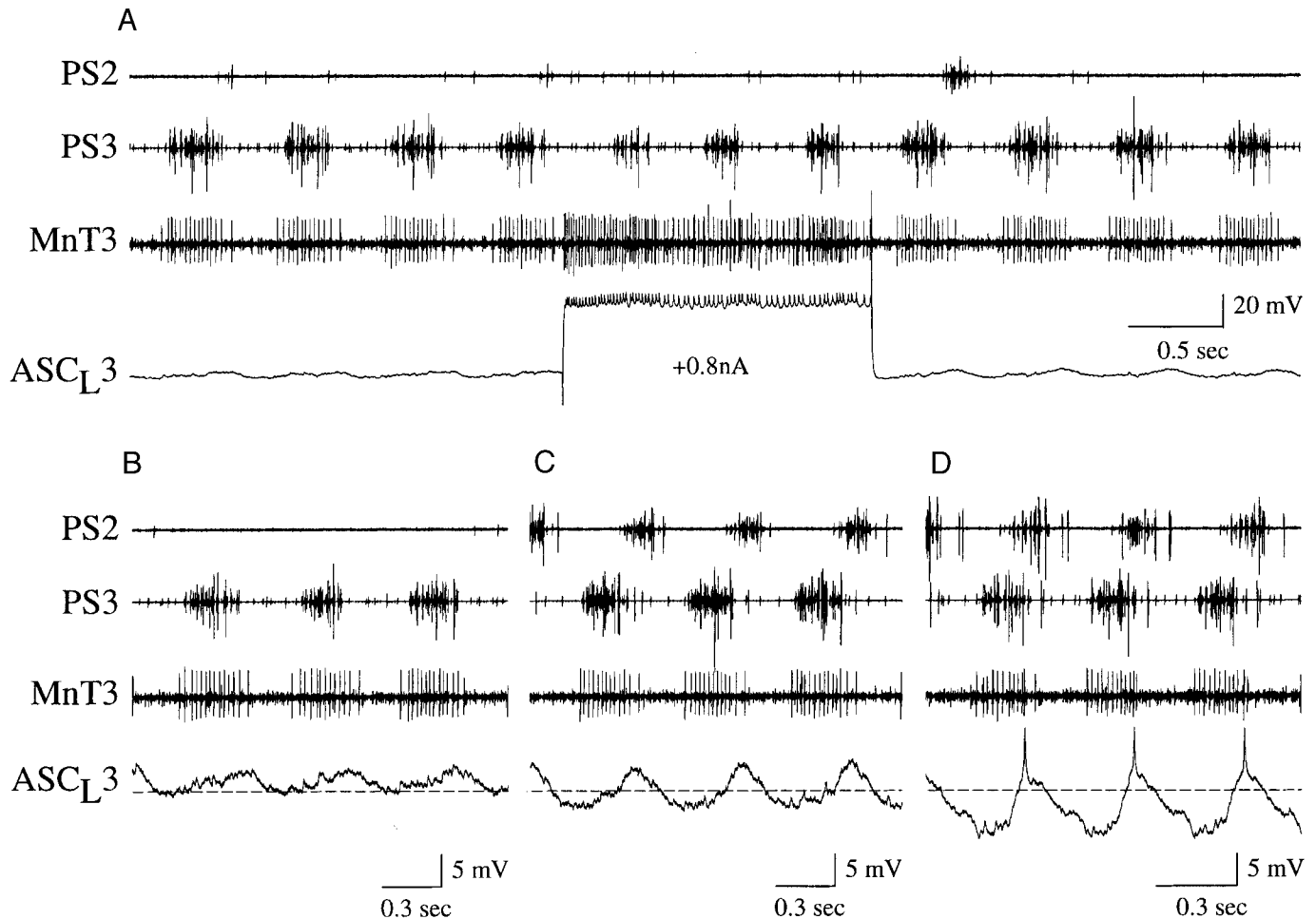


FIG. 6. Properties of the ASC_L coordinating interneurons. *A*: intracellular recording from an ASC_L neuron in A3 during spontaneous expression of normal swimmeret activity. Driving this ASC_L caused it to fire a train of impulses, and also weakened PS bursts in A3 (PS3). PS activity in A2, this neuron's target ganglion, was not excited, but a burst of PS2 impulses followed the end of the depolarization. *B–D*: recordings from an ASC_L interneuron in A3 made at 3 different levels of excitation of the swimmeret system. *B*: shows the activity of this interneuron in normal saline, when the period of the cycle was 0.58 s. Its membrane potential oscillated below threshold. *C* and *D*: increased amplitude of this neuron's oscillations and its recruitment as the period shortened to 0.52 and 0.40 s.

burst in its home ganglion but had a posterior cell body and overall structure like ASC_E neurons and had the same excitatory effect on the target ganglion that ASC_E neurons do. We therefore think that these neurons with delayed bursts of impulses were nonetheless ASC_E neurons. In a few preparations, we also saw physiological evidence of two pairs of ASC_E neurons in one ganglion, but we observed no signs of electrical coupling of these interneurons to other cells.

Interneuron ASC_L fired bursts of impulses that began late in each PSE burst in their home ganglion (Fig. 2, *A* and *C*). The numbers of impulses in these bursts were characteristically smaller than in bursts of impulses in ASC_E neurons. The conduction velocities of their impulses were higher than those of ASC_E neurons (Fig. 2, *B* and *C*), and in many recordings the amplitudes of ASC_L spikes were larger than those of ASC_E spikes. Unlike ASC_E , ASC_L neurons did not always fire during each cycle when the system was only weakly excited (Fig. 6, *B–D*).

ASC_L had an elliptical cell body $\sim 24 \times 40 \mu\text{m}$ in diameter, located in the cluster of excitatory RS cell bodies (RSE)

anterior to the base of N1 (Fig. 4). Its neurite projected posteriorly into the LN, where it joined the MnT. Its axon rose in the MnT, crossed LG, but turned anteriorly before crossing themidline. We recorded from and filled an ASC_L interneuron once.

In active preparations, the membrane potentials of ASC_L interneurons oscillated in phase with the motor output of their home ganglia (Fig. 6, *B–D*), but the trajectories of these oscillations differed from those of ASC_E neurons (Fig. 5), reaching their peaks only at the end of each PSE burst. Depolarization of a individual ASC_L neuron weakened PSE bursts in its home ganglion (Fig. 6*A*) and did not excite PSE activity in its target ganglion (Fig. 6*A*). After these depolarizations, PS activity in the target ganglion showed some rebound excitation (Fig. 6*A*).

During the experimental hyperpolarization of ASC_E shown in Fig. 5*C*, the ASC_L unit that was also active did not change its firing pattern at all. This lack of any effect on ASC_L implies that the ASC_E and ASC_L interneurons are not electrically coupled and that ASC_E is also not electrically coupled to

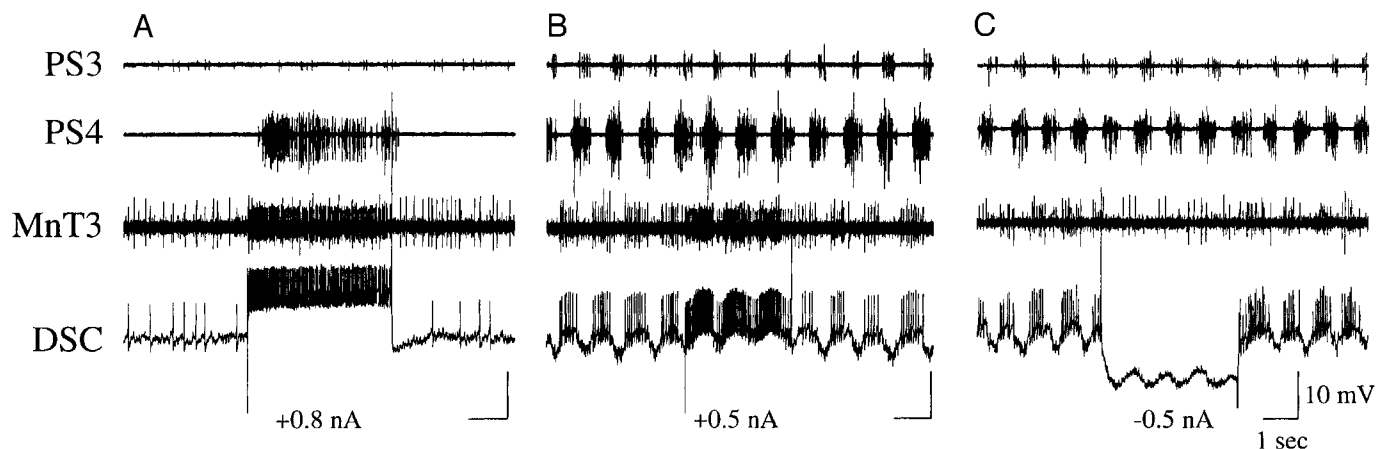


FIG. 7. Physiological properties of a DSC coordinating interneuron recorded in A3. *A*: preparation was quiet. Depolarization of this interneuron caused a monotonic train of impulses that were recorded simultaneously by the intracellular electrode (DSC) and by the extracellular electrode on MnT3. This burst of impulses caused a prolonged burst of impulses in several PS motor axons in A4, this neuron's target ganglion. *B* and *C*: during these recordings, the preparation was actively expressing the swimmeret motor pattern. Each time the PS axons in A3 fell quiet, this DSC neuron depolarized and fired a burst of impulses. *B*: further depolarization of this interneuron with a step of current caused it to fire at higher frequencies and also caused stronger, longer-lasting bursts in PS motor axons recorded in A4. *C*: hyperpolarization of this interneuron prevented firing of this neuron and revealed the underlying synaptic oscillation that drove its bursts of impulses but did not affect the output of its target ganglion.

components of the pattern-generating circuit that normally drive these two interneurons (Skinner and Mulloney 1998).

Interneuron DSC fired bursts of impulses that alternated with PSE bursts in its home ganglion (Fig. 2, *A* and *D*). This interneuron's cell body lay anterior to the base of N1, in the cluster of RSE cell bodies and near the cell body of the ASC_L neuron. Its neurite projected posteriorly to the LN, extended several short branches, and then rose in MnT to reach the midline and send an ipsilateral axon posteriorly to the next ganglion (Fig. 4).

In active preparations, the membrane potentials of DSC neurons oscillated in phase with the motor output of their home ganglia. They depolarized during each burst of impulses in RSE motor neurons and fired bursts of impulses simultaneously with these RSE bursts. Both in quiet and in active preparations, experimental excitation of individual DSC neurons increased the strength of PSE firing in the neuron's target ganglion (Fig. 7*B*), as predicted by Stein (1970). Unlike ASC_E , however, hyperpolarizing DSC in active preparations did not affect PS activity in the interneuron's target ganglion (Fig. 7*C*). Furthermore, the response of the target ganglion to depolarization of DSC in quiet preparations was relatively slow (Fig. 7*A*). These differences suggest that ASC_E and DSC have different synaptic targets in each module (cf. Skinner and Mulloney 1998).

In one preparation, we observed two sizes of DSC impulses (Fig. 7*C*), which suggests that there might be two pairs of these interneurons per ganglion, consistent with Stein's (1971) description of units in the medial descending tract.

Firing of these interneurons was coordinated with the motor output from their home ganglia

These three types usually fired bursts of impulses when the system was producing the normal swimmeret motor pattern (Fig. 2). These bursts occurred at a predictable point in each cycle of the motor pattern (Fig. 8, and probably reflect a strong synaptic drive that is common to these interneurons and to the motor neurons that fire simultaneously with them (Mulloney et al. 1997; Skinner and Mulloney 1998).

Responses of the swimmeret system to perturbation of individual coordinating interneurons

If these interneurons carry the information needed to coordinate activities of swimmeret modules in different segments, then disrupting the firing of individual interneurons should affect the intersegmental phase difference unless the system was constrained too tightly by the continued activity of other coordinating interneurons. We stimulated individual ASC_E and DSC interneurons to test the responses of their respective target

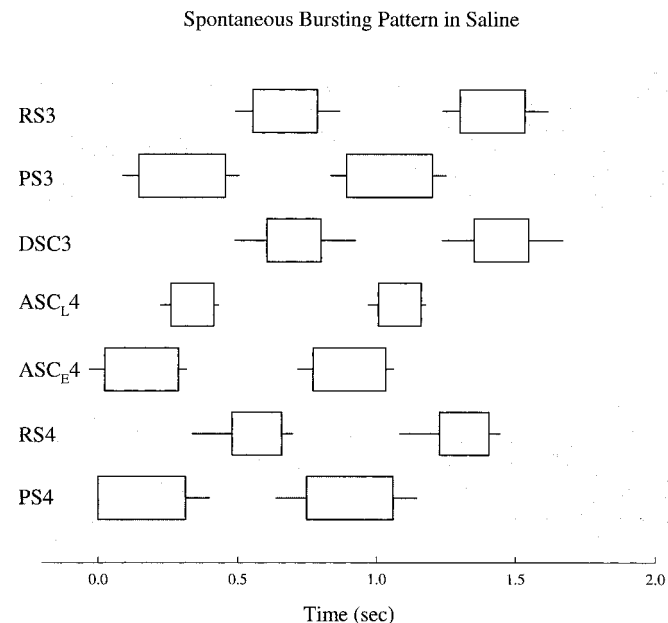


FIG. 8. Positions of bursts of impulses from each kind of coordinating interneuron within the structure of 2 cycles of swimmeret motor output from PS and RS motor neurons in ganglia A3 and A4. Each box begins at the mean latency, relative to PS4, of bursts of impulses in that type of neuron and lasts as long as the mean duration of that neuron's bursts. Error bars show the SD of duration (right) and SD of phase (on the left of each box).

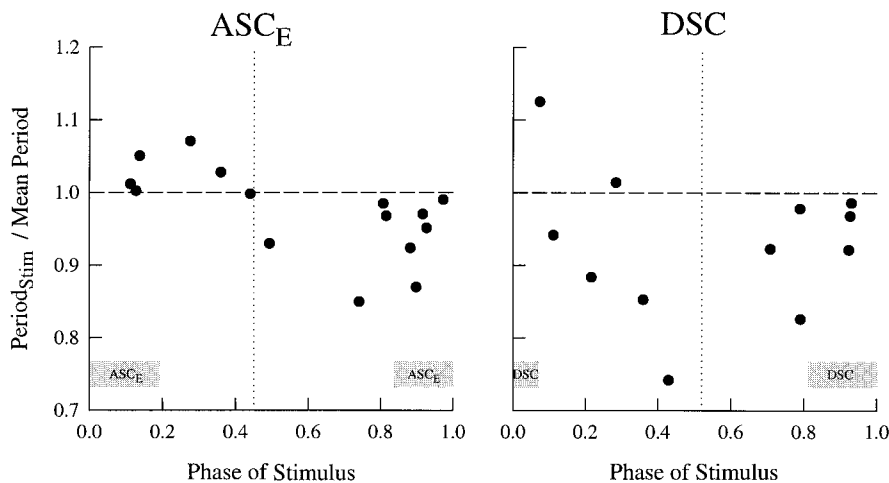


FIG. 9. Stimulation of individual coordinating interneurons affected the timing of the motor output in their target ganglia. These plots show changes in the period of PS bursts in the target ganglion caused by stimulation of an individual interneuron. For each stimulus, these changes are normalized to the mean period of a series of preceding bursts. If no change occurred, the normalized period would be 1.0 (---). Phase of each stimulus was calculated relative to the start of the associated PS burst in the target ganglion. Mean relative duration of the target's PS burst is marked (\cdots). Points to the left of \cdots mark stimuli that started while the PS motor neurons were firing; points to the right mark stimuli that started when these PS units were silent. Each point is the result of 1 stimulus. \blacksquare , those parts of each cycle in the target ganglion in which the interneuron would normally be firing (cf. Fig. 8).

modules. These stimuli began at different phases in the PS firing cycle of the target ganglion (Fig. 9).

The responses to stimulation of individual ASC_E neurons were contingent on the phase in the target ganglion's cycle at which the stimulus occurred. If the stimulus began while PSE units in the target were firing, the next PS burst in the target ganglion was delayed. If the stimulus began while PSE units in the target were silent, the next PS burst was advanced (Fig. 9).

The responses to stimulation of an individual DSC were also contingent on the phase at which the stimulus occurred. If the stimulus began during a PSE burst in the target ganglion, the response of the target was unpredictable. If the stimulus began while the target PS units were silent, the next PS burst was advanced (Fig. 9). From these results, it is evident that each swimmeret module includes both ascending and descending interneurons that carry information about activity in their homomodule and can affect the timing of activity in neighboring modules.

In recent simulations of the coordinating circuit (Skinner and Mulloney 1998), we have assumed that coordinating interneurons played no roles in generating the motor pattern within their home module. If this assumption was correct, then experimental stimulation of a coordinating neuron would have a greater effect on its target ganglion than on its home ganglion, although the existence of a polysynaptic intersegmental loop might obscure this difference. To test this assumption, we compared the responses of target modules and home modules to the same stimuli, ignoring the phase difference between the two. Changes in the periods of target modules in response to stimulation of ASC_E neurons were not significantly different from the changes in their home modules ($P = 0.44$, $n = 16$ stimuli, paired t -test). Changes in the periods of target modules in response to stimulating DSC neurons were significantly larger than the changes in their home modules ($P = 0.016$, $n = 6$ stimuli, paired t -test).

Responses of coordinating interneurons to changes in excitation

When excitation of the swimmeret system increases, the frequency of PSE and RSE bursts increase, each motor neuron fires more spikes per burst, and previously silent motor neurons are recruited (Braun and Mulloney 1993; Mulloney 1997; Mulloney et al. 1997). Each of these effects would contribute

to more frequent and more powerful swimmeret beating, more thrust, and so faster forward swimming, consistent with the behavior of intact crayfish. Nonetheless the structure of the swimmeret motor pattern is preserved; neither the phases of PSE and RSE in each segment nor the phases of bursts in neighboring segments change significantly, and the ratio of each burst's duration to the period of the pattern is preserved (Skinner and Mulloney 1998). If it is correct that MnT units are necessary components of the intersegmental coordinating circuit, then their responses to changes in excitation are key features of the system's ability to preserve the structure of the swimmeret motor pattern despite changes in period.

To study the responses of these interneurons to changes in excitation, we recorded MnT units in A3 and A4 while varying the level of excitation using different concentrations of carbachol, which excites the swimmeret system in a dose-dependent manner (Mulloney 1997). We compared the mean spike frequencies, the numbers of impulses in each burst, and the structures of individual bursts of each unit in MnT3 and MnT4 at different periods. In preparations that were spontaneously active in normal saline, the period of the swimmeret motor pattern ranged from 0.6 to 0.9 s. In response to perfusion with increasing concentrations of carbachol, the period decreased smoothly down to ~ 0.35 s.

The relative durations and phases of bursts of impulses in each MnT interneuron produced when the preparation was excited were similar to those produced spontaneously by these same preparations (Fig. 10). In this figure, we scaled the periods, latencies, and relative burst-durations of spontaneous activity to the period of the excited activity (0.4 s) so that the structures of the motor patterns could be compared directly (Mulloney 1997). In the output from excited preparations, there was a slight shift in the phases of bursts in ascending axons that correlated with a similar shift in the phases of PS3 bursts. The mean phases of ASC_E changed from 0.02 ± 0.09 to 0.11 ± 0.09 and those of ASC_L changed from 0.36 ± 0.05 to 0.40 ± 0.06 . In the preparations used for this figure, the relative durations of PS3 bursts did increase when the system was excited ($P = 0.03$).

We looked more closely at the structures of activity recorded at four different periods. An ANOVA in phase and relative duration of bursts of impulses in each type of coordinating interneuron revealed no significant differences in these param-

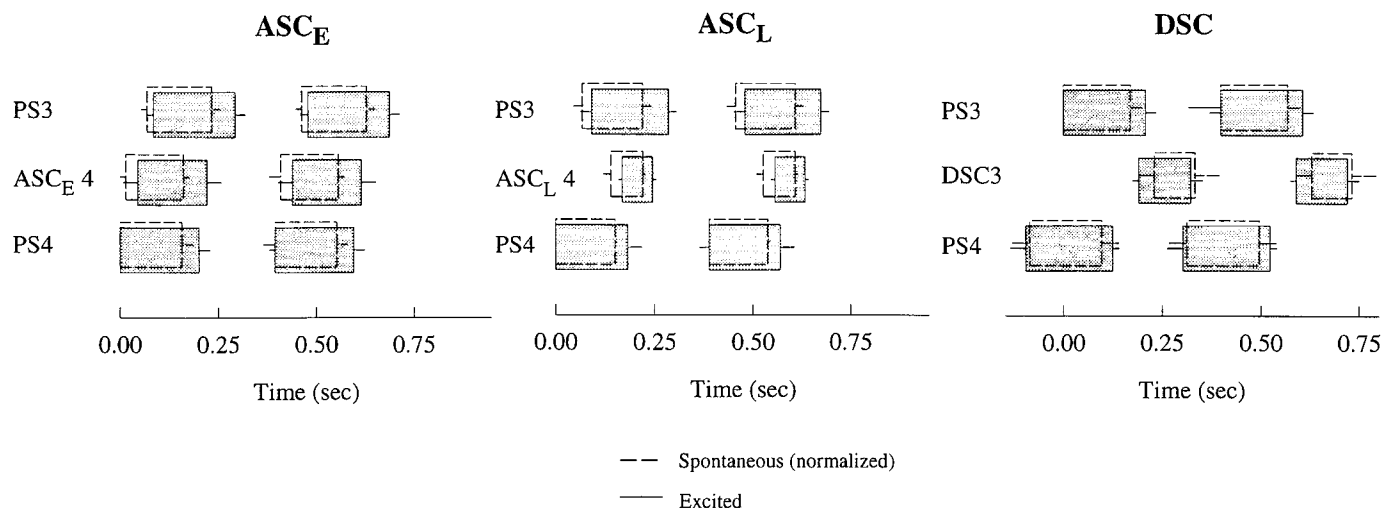


FIG. 10. When the system was excited and the period of its motor output shortened, the relative durations and phases of bursts of impulses in these interneurons did not change significantly from those produced during spontaneous activity. These boxplots show the durations and phases of bursts of impulses in coordinating interneuron that project between ganglia A3 and A4. Period, burst durations, and burst latencies recorded during spontaneous activity have been normalized here to the (shorter) period of the excited activity (Mulloney 1997). Phases of ASC_E and ASC_L bursts were calculated using the start of PS4 bursts as the reference for phase (Mulloney and Hall 1987), since these neurons originate in A4. Phases of DSC were calculated using PS3 as the reference because the DSC neurons originate in A3. Error bars are defined in the legend of Fig. 7.

eters as the period of the motor pattern ranged from 0.35 to 0.75 s (for phases and relative durations of ASC_E : $P = 0.41$, $P = 0.31$; of ASC_L : $P = 0.79$, $P = 0.60$; of DSC: $P = 0.26$, $P = 0.76$), although the increase in relative duration of the PS3 bursts was confirmed.

Firing patterns within individual bursts of impulses

To describe the dynamics of bursts of impulses in these interneurons, we measured the time intervals between impulses within bursts in a representative series of bursts for each type of coordinating interneuron. We then plotted impulse frequencies (the reciprocals of these intervals) as functions of the order in which each impulse occurred in the burst (Fig. 11).

During spontaneous swimmeret activity, impulse frequency in all three types of coordinating interneurons usually decreased monotonically as each burst of impulses progressed, as if the neurons were accommodating to a constant current (Fig. 11). Increased excitation changed the frequency and duration of these bursts (e.g., Fig. 6), but the impulse pattern within each burst did not normally change; the initial impulse frequency was higher but the monotonic accommodation of ASC_L neurons and DSC neurons did not change (Fig. 11).

ASC_E neurons responded more complexly; when the system was excited with carbachol, their impulse frequencies at first increased and then decelerated smoothly until the end of the burst (Fig. 5, C–E). Two of the six ASC_E neurons studied in A3, which we encountered in preparations that were spontaneously expressing stable swimmeret motor patterns, also fired bursts that had an initial acceleration before these preparations were exposed to carbachol.

Mean impulse frequency of each MnT coordinating interneuron increased, but the numbers of impulses per burst did not change

The mean impulse frequency of each coordinating interneuron recorded was measured for four different ranges of periods (Fig. 12A). Each point plotted in this figure is the mean impulse frequency of that MnT neuron for all experiments within that range. As period decreased, the firing frequency of each unit increased as would be expected in response to excitation.

However, because the duration of each burst decreased proportionately to the decrease in period, the numbers of impulses in each burst did not change significantly (Fig. 12B). An ANOVA found it probable that the mean numbers of

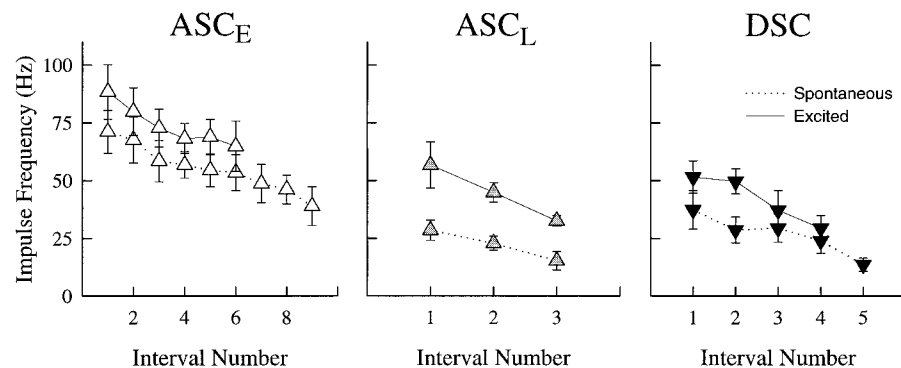


FIG. 11. Instantaneous impulse frequencies within bursts of impulses in each kind of coordinating interneuron. Instantaneous frequency is the reciprocal of the time interval between each spike. Here, instantaneous frequencies (mean \pm SD) of each impulse in a burst are plotted in the order in which each impulse occurred. Data are from a series of spontaneous bursts and from a series of bursts in the same neurons recorded when the system was excited.

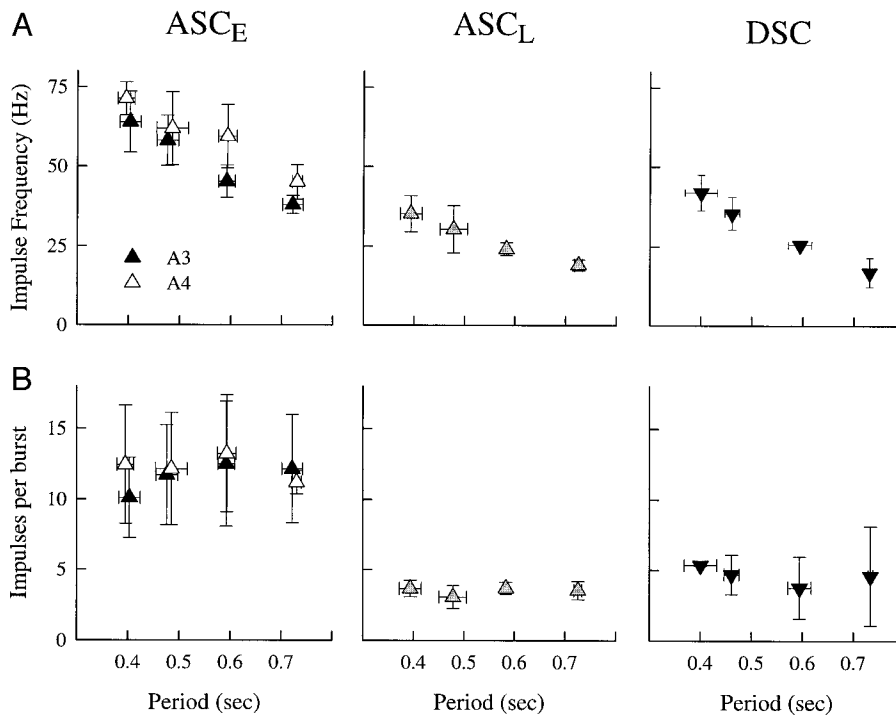


FIG. 12. Relations to the motor pattern's period of impulse frequency and of the numbers of impulses per burst in the different coordinating interneurons. *A*: impulse frequencies increased as the period shortened. Each point shows the impulse frequency (mean \pm SD) of a group of experiments with similar periods and is plotted at the period (mean \pm SD) of the group. *B*: numbers of impulses per burst did not change as period shortened. Each point shows the number of impulses (mean \pm SD) plotted at the period (mean \pm SD) of the series of bursts the impulses of which were counted.

impulses were not different in this range of periods (ASC_E: $P = 0.915$, ASC_L: $P = 0.658$, DSC: $P = 0.299$). This feature is not a necessary consequence of the preservation of relative burst duration (Fig. 10) but instead is an unexpected property of these interneurons.

From this evidence, we think that all the coordinating interneurons are affected in the same way by excitation of the swimmeret system: they receive stronger synaptic excitation and therefore fire at higher frequencies, but they do not change their firing properties. Like swimmeret motor neurons (Sherff and Mulloney 1996), the periods, durations, and phases of their bursts of impulses are determined largely by the synaptic input they get from the pattern-generating interneurons within their home ganglion.

Each abdominal ganglion had the same complement of coordinating axons in MnT

To discover the structures of these MnT neurons, we used Neurobiotin to backfill bundles of axons stripped from the dorso-medial face of each hemiconnective (areas 76 and 78; cf. insets in Fig. 3) between A3, A4, and A5 and studied the filled neurons as whole mounts (e.g., Fig. 1B). The numbers of ascending axons and descending axons filled in MnT was similar in A2, A3, A4, and A5 (Table 1). At least two interneurons the axons of which ran in MnT were filled in each preparation made from anterior to the ganglion. At least two other interneurons with axons in MnT were also visible in each preparation made from posterior to A2 and A3. Fills from the posterior direction also revealed that MnT sometimes divided laterally before crossing LG. In these preparations, ascending axons crossed LG near the anterior edge of LN but descending units crossed LG near the neuropil's posterior edge, so that anterior-projecting units were separated from posterior-projecting units in our standard recording position (Fig. 1A). We interpret this variation as individual differences in the local

position of a divergence that is present in the MnT of every ganglion.

We compared the numbers of units recorded by an extracellular MnT electrode, positioned at the anterior edge of LN (Fig. 1A), with the numbers of backfilled neurons in ganglia A2, A3, A4, and A5, the ganglia that in this species innervate functional swimmerets (Table 1). The numbers of filled axons in MnT were similar in each ganglion, but the numbers of units recorded that fired in phase with swimmeret activity were more variable. This disparity might mean that there are segmental differences in the numbers of ascending and descending coordinating axons, or it might be caused simply by mechanical damage from removing the sheath or placing the electrode because the axons in MnT are quite fine (Fig. 1C). We think there is another cause. When recording on the surface of LG at the posterior edge of LN in some preparations, we found units that fired in phase with the swimmeret system. This is the position where backfills had revealed that axons with the structure of coordinating interneurons sometimes crossed LG before joining the rest of MnT. In recordings from this more posterior position in A3, we found at least two units that fired simultaneously with RSE motor axons. Simultaneous record-

TABLE 1. *Anatomic and electrophysiological census of coordinating units in the MnT of each ganglion that innervates swimmerets*

	Numbers of Filled Axons*		Numbers of Units Recorded*	
	Descending	Ascending	Descending	Ascending
A2	2 (3)	—	2 (2)	—
A3	2 (2)	2 (3)	1 (1)	1 (2)
A4	1 (2)	2 (3)	0	2 (3)
A5	—	2 (2)	—	2 (2)

* Numbers are means (Maxima). —, not measured.

ings from the three to four connective showed that, like DSC interneurons, these axons also projected posteriorly in the dorsal-medial connective. In A4, we did not find descending units while recording from the standard MnT position, but more posterior recording positions in the same preparations found one or two descending units. These differences in recording position and routes of filled axons suggest that axons of ascending and descending interneurons do rise together toward the lateral edge of LG but can diverge even before turning toward the midline and that this divergence is more probable in posterior ganglia than in anterior ones. If this inference is correct, then the disparities in the numbers of active swimmeret units recorded in different ganglia (Table 1) might be due to this segmental difference in the course of MnT not to a segmental difference in the numbers of coordinating interneurons. Although our evidence is incomplete, we think that each ganglion that innervates swimmerets has the same complement of MnT coordinating interneurons.

DISCUSSION

The three types of MnT interneurons described here differ from other swimmeret neurons in their physiology and anatomy (Paul and Mulloney 1985b, 1986; Sherff and Mulloney 1997). Unlike command interneurons, the axons of which project the length of the abdominal nerve cord (Acevedo et al. 1994), these interneurons occur as bilateral pairs in each intermediate ganglion that innervates swimmerets and send axons into the intersegmental connective that project to neighboring ganglia (Fig. 4). Firing of these MnT units affected the timing of the next cycle of motor output from their target ganglia (Fig. 9), a property characteristic of coordinating interneurons (Paul and Mulloney 1986; Stein 1971, 1974). Each type of MnT coordinating interneuron fired bursts of impulses at a different phase of the swimmeret cycle in its home ganglion (Fig. 8), so we infer that these bursts convey different information to their targets in neighboring ganglia.

MnT interneurons are both necessary and sufficient for normal coordination of swimmeret motor patterns

Intersegmental coordination requires cycle-by-cycle information about the state of each swimmeret, and only neurons that fire impulses in phase with the local circuits driving individual swimmerets can conduct the necessary information. Each half of the bilaterally symmetrical connective that runs between neighboring abdominal ganglia contains ~2,600 axons (Sutherland and Nunnemacher 1968). Of these 2,600, only three "tracts" have been reported to contain axons that fire bursts of impulses in phase with the swimmeret motor output from their home ganglion (Skinner and Mulloney 1998; Stein 1971; Wiersma and Hughes 1961). Of these three, one ascending tract and one descending tract run in the dorsomedial region of the connective; the third runs near the lateral edge of the connective (cf. Fig. 2A).

We could sever selectively either the medial ascending and descending tracts or the rest of the connectives including the lateral ascending tract (Fig. 3). Cutting all the intersegmental axons except those in the dorsomedial tracts did not affect coordination, so we conclude that the ascending units in the lateral ascending tract are not necessary for normal coordina-

tion, and those units in the medial tracts are sufficient for normal coordination. The reciprocal experiment, cutting only the dorsomedial tracts, uncoupled activity in ganglia on opposite sides of the cut (Fig. 3B), so we conclude that the ascending axons in the lateral tract are not sufficient for normal coordination, and those units in the medial tracts are necessary for normal coordination (cf. Figs. 3, 4 of Stein 1971).

Not all three types of MnT coordinating interneuron reached threshold whenever the swimmeret system was active. ASC_E fired periodic bursts whenever the system began to oscillate, and we have argued above that the absence of DSC bursts from some MnT recordings was caused by individual differences in the courses through MnT followed by ascending and descending axons. ASC_L, however, sometimes failed to reach threshold (e.g., Fig. 6, B and C), although the intersegmental coordination of preparations in which we observed this was apparently normal. Thus although the flow of information through MnT interneurons is essential for normal coordination (Fig. 3), participation by every one of the MnT interneurons in each segment is not necessary.

These interneurons are components of a concatenated intersegmental coordinating circuit

As they project axons from their home ganglia to their targets in neighboring ganglia, they form a concatenated pathway through which information about the state of each local swimmeret module is exported to its neighbors. Although we assessed the impact of impulses in these interneurons on their targets by measuring changes in the timing of bursts in PS motor neurons, it is unlikely that the major targets of ASC_E or DSC are the PS motor neurons themselves because the responses of the target modules were contingent on the phase at which these interneurons were stimulated (Fig. 9). This phase-dependent response of the target, which we observed both for ascending and for descending MnT interneurons, means that these interneurons can effectively hold the target module to a particular phase relative to their home module's activity.

Experiments with nonuniform excitation of the system (Braun and Mulloney 1995) predict that the structure of this coordinating circuit is asymmetric. Simulations of the swimmeret system as a chain of coupled oscillators (Skinner et al. 1997a,b) showed that only a restricted set of properties of the information linking neighboring oscillators permitted the simulated system to reproduce the performance of the real system when it was not uniformly excited (Braun and Mulloney 1995). These restrictions included that the ascending and descending coupling were asymmetric and that changes in coupling did not have much effect on the period of the system's output. Those properties of MnT coordinating interneurons that we have investigated thus far conform to these specifications. ASC_E and ASC_L fire during the PS part of the cycle, but DSC fires during the RS part (Fig. 8), so there is an asymmetry in the timing of the ascending and descending signals from each ganglion.

These MnT interneurons are the classically described swimmeret coordinating fibers

The original reports of swimmeret coordinating fibers (Stein 1971) described two units in the median ascending tract (area 78) of the connectives between each ganglion. These ascending

units fired bursts of impulses that propagated anteriorly, in an "ascending" direction; a small unit that fired simultaneously with each burst of impulses in PS motor neurons and a large unit that fired late in each PS burst. Stein (1974) showed that stimulating a burst of impulses in these medial ascending units would either advance or retard the next PS burst in the target ganglion, depending on the phase at which the firing of the ascending units began. This is precisely the result shown in Fig. 9, here demonstrated by intracellular stimulation of single interneurons. Two more units in the medial descending tract (area 76, near area 78) fired bursts that propagated posteriorly, in a "descending" direction, during each burst of impulses in RS motor neurons.

The positions of these MnT units in areas 76 and 78 of the interganglionic connective (Wiersma and Hughes 1961), the phases in the swimmeret cycle at which they normally fire (Figs. 2A and 8), and their effects on the modules to which they projected (Fig. 9) match those of coordinating fibers classically described. We think that axons of ASC_E and ASC_L interneurons are homologous to Stein's coordinating fibers in the medial ascending tract, and axons of DSC interneurons are homologous to his fibers in the medial descending tract.

Dynamics of MnT coordinating interneurons

Each swimmeret module can respond to pharmacological excitation applied locally (Acevedo et al. 1994; Braun and Mulloney 1995), and each is capable of oscillating independently (Murchison et al. 1993). Changes in excitation affect the period of the swimmeret motor pattern but do not affect either the phases of PS-RS alternation within each ganglion or the intersegmental phase lags between neighboring ganglia (Braun and Mulloney 1995; Mulloney 1997; Skinner and Mulloney 1998). In these experiments as we increased excitation, the period of the motor pattern shortened, but neither the phases nor the relative durations of bursts of impulses in the MnT interneurons changed (Fig. 10). This feature of their responses to excitation resembles the responses of swimmeret motor neurons, which also preserve relative duration as period changes (Mulloney 1997; Skinner and Mulloney 1998).

Excitation increased the frequency and amplitude of synaptically driven oscillations of membrane potential in MnT interneurons (Figs. 5 and 6) (cf. Mulloney et al. 1997) and causes higher impulse frequencies within each burst (Fig. 11). The increased firing frequency would produce stronger synaptic input to the target ganglion during each cycle and increase coupling between each module. This increase may contribute to the stability of intersegmental phase differences.

One unpredicted feature these interneurons' responses to increased excitation was that the mean numbers of impulses per burst remained unchanged through a wide range of periods (Fig. 12). For each type of MnT interneuron, the increased impulse frequency was matched with the decreased burst duration so that the mean number of impulses per burst did not vary significantly in the range of periods we examined. This preservation of impulse number is not a necessary consequence of preserving relative burst duration. Instead it might reflect a feature of the nonspiking interneurons in each local pattern-generating circuit that are thought to provide the excitatory synaptic drive to the MnT interneurons (Skinner and Mulloney 1998). The postsynaptic consequences of preserving spike

number as period changes have been studied in stomatogastric muscles (Morris and Hooper 1997), but here the consequences must await the identification of neurons postsynaptic to MnT interneurons in their target ganglia.

Intersegmental coordination in other locomotor systems

Locomotion in other segmented animals also is coordinated by intersegmental interneurons. In walking insects, intersegmental interneurons fire bursts of impulses in phase with the step cycles in their home ganglion (Pearson and Iles 1973) and intersegmental interneurons that are probably active during walking coordinate limb movements during grooming (Berkowitz and Laurent 1996). We suspect that these insect interneurons will prove to be homologues of crustacean coordinating interneurons, but the evidence is too incomplete to decide the question.

In walking reptiles and mammals, propriospinal interneurons perform the same functions. In turtles, a heterogeneous population of descending propriospinal interneurons fire bursts of impulses during rhythmic scratching movements (Berkowitz and Stein 1994). Some project ipsilaterally, others contralaterally (Berkowitz and Stein 1994). These interneurons drive segmental circuits that generate motor patterns for directed scratching movements (Stein et al. 1995).

In animals that swim by undulations of a segmented trunk, there are also intersegmental coordinating interneurons essential for normal behavior. Leeches have ventrally positioned segmental ganglia that innervate their swimming musculature. The activity of the pattern-generating circuit in each ganglion is coordinated by a concatenated intersegmental circuit the component interneurons of which occur in a segmental series like the MnT interneurons, but the axons of which project beyond their neighboring ganglia (Friesen and Pearce 1993). Within their target ganglia, these coordinating interneurons synapse directly with local interneurons in the segmental pattern-generating circuit. This swimming circuit in leeches has many properties similar to the swimmeret system.

Lamprey and other fish have spinal pattern-generating circuits that are coordinated by intersegmental axons, but in these animals the roles of different interneurons is less clear. The kernel of each segmental pattern-generating circuit includes interneurons that both participate in the segmental pattern-generating circuit (Grillner and Matsushima 1991) and contribute to intersegmental coordination (Buchanan 1992; Williams 1992). Moreover in lamprey the segmental boundaries apparent in the motor output are obscure or not present in the premotor pattern-generating circuitry (Lansner et al. 1997), which makes it difficult to define the synaptic connections within and between neighboring circuits. Nonetheless coupling between nearby segments is essential for the normal phase-constant performance of the swimming circuit.

Directions for further investigation

We do not yet know the neuronal targets of these MnT interneurons in the ganglia to which they project nor the sources of the synaptic currents that make them fire when the system is active. Simulations of alternative intersegmental coordinating circuits, using a recently developed cellular model that assumes a particular organization of each local module

(Mulloney et al. 1998; Skinner and Mulloney 1998), have predicted a particular configuration of targets and synapses that are consistent with what we know thus far about the MnT interneurons. By mapping the synaptic connections of MnT interneurons in their home ganglia and their target ganglia, we hope to reveal more of the cellular basis of this complex behavior.

We thank W. M. Hall for data analysis, editorial criticism, and preparing illustrations. This work was supported by National Science Foundation Grants IBN 95-14889 and IBN 97-28791 to B. Mulloney.

Present address of H. Namba: Dept. of Molecular Neurobiology, Brain Research Institute, Niigata University, Niigata 951-8122, Japan.

Address for reprint requests: B. Mulloney, Neurobiology, Physiology, and Behavior, University of California, Davis, 1 Shields Dr., Davis, CA 95616-8519.

Received 18 December 1998; accepted in final form 1 February 1999.

REFERENCES

- ACEVEDO, L. D., HALL, W. M., AND MULLONEY, B. Proctolin and excitation of the crayfish swimmeret system. *J. Comp. Neurol.* 345: 612–627, 1994.
- BERKOWITZ, A. AND LAURENT, G. Central generation of grooming motor patterns and interlimb coordination in locusts. *J. Neurosci.* 16: 8079–8091, 1996.
- BERKOWITZ, A. AND STEIN, P.S.G. Descending propriospinal axons in the hindlimb enlargement of the red-eared turtle: cells of origin and funicular courses. *J. Comp. Neurol.* 346: 321–336, 1994.
- BRAUN, G. AND MULLONEY, B. Cholinergic modulation of the swimmeret system in crayfish. *J. Neurophysiol.* 70: 2391–2398, 1993.
- BRAUN, G. AND MULLONEY, B. Coordination in the crayfish swimmeret system: differential excitation causes changes in intersegmental phase. *J. Neurophysiol.* 73: 880–885, 1995.
- BUCHANAN, J. T. Neural network simulations of coupled locomotor oscillators in the lamprey spinal cord. *Biol. Cybern.* 66: 367–374, 1992.
- FRIESEN, W. O. AND PEARCE, R. A. Mechanisms of intersegmental coordination in leech locomotion. *Semin. Neurosci.* 5: 41–47, 1993.
- GRILLNER, S. AND MATSUSHIMA, T. The neural network underlying locomotion in lamprey-synaptic and cellular mechanisms. *Neuron* 7: 1–15, 1991.
- HUGHES, G. M. AND WIERSMA, C.A.G. Neuronal pathways and synaptic connections in the abdominal cord of the crayfish. *J. Exp. Biol.* 37: 291–307, 1960.
- IKEDA, K. AND WIERSMA, C.A.G. Autogenic rhythmicity in the abdominal ganglion of the crayfish: the control of swimmeret movements. *Comp. Biochem. Physiol.* 12: 107–115, 1964.
- LANSNER, A., EKEBERG, Ö., AND GRILLNER, S. Realistic modeling of burst generation and swimming in lamprey. In: *Neurons, Networks, and Motor Behavior*, edited by P.S.G. Stein, S. Grillner, A. I. Selverston, and D. G. Stuart. Cambridge MA: MIT Press, 1997, p. 165–171.
- LEISE, E. M., HALL, W. M., AND MULLONEY, B. Functional organization of crayfish abdominal ganglia. I. The flexor systems. *J. Comp. Neurol.* 253: 25–45, 1986.
- MORRIS, L. G. AND HOOPER, S. L. Muscle response to changing neuronal input in the lobster (*Panulirus interruptus*) stomatogastric system: spike number-versus spike frequency-dependent domains. *J. Neurosci.* 17: 5956–5971, 1997.
- MULLONEY, B. A test of the excitability-gradient hypothesis in the swimmeret system of crayfish. *J. Neurosci.* 17: 1860–1868, 1997.
- MULLONEY, B. AND HALL, W. M. The PD programs: a method for the quantitative description of motor patterns. *J. Neurosci. Methods* 19: 47–59, 1987.
- MULLONEY, B., MURCHISON, D., AND CHRACHRI, A. Modular organization of pattern-generating circuits in a segmental motor system: the swimmerets of crayfish. *Semin. Neurosci.* 5: 49–57, 1993.
- MULLONEY, B., NAMBA, H., AGRICOLA, H.-J., AND HALL, W. M. Modulation of force during locomotion: differential action of Crustacean Cardioactive Peptide on power-stroke and return-stroke motor neurons. *J. Neurosci.* 17: 6872–6883, 1997.
- MULLONEY, B., SKINNER, F. K., NAMBA, H., AND HALL, W. M. Intersegmental coordination of swimmeret movements: mathematical models and neural circuits. In: *Neuronal Mechanisms for Generating Locomotor Activity*, edited by O. Kiehn, H. Hultborn, N. Kudo, L. M. Jordan, and R. M. Harris-Warrick. New York: Ann. NY Acad. Sci., 1998, vol. 860, p. 266–280.
- MURCHISON, D., CHRACHRI, A., AND MULLONEY, B. A separate local pattern-generating circuit controls the movements of each swimmeret in crayfish. *J. Neurophysiol.* 70: 2620–2631, 1993.
- PAUL, D. H. AND MULLONEY, B. Local interneurons in the swimmeret system of the crayfish. *J. Comp. Physiol. [A]*. 156: 489–502, 1985a.
- PAUL, D. H. AND MULLONEY, B. Nonspiking local interneuron in the motor pattern generator for the crayfish swimmeret. *J. Neurophysiol.* 54: 28–39, 1985b.
- PAUL, D. H. AND MULLONEY, B. Intersegmental coordination of swimmeret rhythms in isolated nerve cords of crayfish. *J. Comp. Physiol. [A]*. 158: 215–224, 1986.
- PEARSON, K. G. AND FOURTNER, C. R. Nonspiking interneurons in walking system of the cockroach. *J. Neurophysiol.* 38: 33–52, 1975.
- PEARSON, K. G. AND ILES, J. F. Nervous mechanisms underlying intersegmental coordination of leg movements during walking in the cockroach. *J. Exp. Biol.* 58: 725–744, 1973.
- SHERFF, C. M. AND MULLONEY, B. Tests of the motor neuron model of the local pattern-generating circuits in the swimmeret system. *J. Neurosci.* 16: 2839–2859, 1996.
- SHERFF, C. M. AND MULLONEY, B. Passive properties of swimmeret motor neurons. *J. Neurophysiol.* 78: 92–102, 1997.
- SKINNER, F. K., KOPELL, N., AND MULLONEY, B. How does the crayfish swimmeret system work? Insights from nearest neighbor coupled oscillator models. *J. Comput. Neurosci.* 4: 151–160, 1997a.
- SKINNER, F. K., KOPELL, N., AND MULLONEY, B. Mathematical models of the crayfish swimmeret system. In: *Computational Neuroscience: Trends in Research*, 1997b, edited by J. H. Bower. New York: Plenum, 1997, p. 839–843.
- SKINNER, F. K. AND MULLONEY, B. Intersegmental coordination of limb movements during locomotion: mathematical models predict circuits that drive swimmeret beating. *J. Neurosci.* 18: 3831–3842, 1998.
- SKINNER, K. The structure of the fourth abdominal ganglion of the crayfish, *Procambarus clarkii*. I. Tracts in the ganglionic core. *J. Comp. Neurol.* 234: 168–181, 1985.
- SKINNER, K. The structure of the fourth abdominal ganglion of the crayfish, *Procambarus clarkii*. II. Synaptic neuropils. *J. Comp. Neurol.* 234: 182–191, 1985.
- STEIN, P.S.G. *A Neurophysiological Study of Two Systems of Coupled Oscillators in Crayfish* (PhD thesis). Stanford, CA: Stanford University, 1969.
- STEIN, P.S.G. Intersegmental coordination of swimmeret motor neuron activity in crayfish. *J. Neurophysiol.* 34: 310–318, 1971.
- STEIN, P.S.G. Neural control of interappendage phase during locomotion. *Am. Zool.* 14: 1003–1016, 1974.
- STEIN, P.S.G., VICTOR, J. C., FIELD, E. C., AND CURRIE, S. N. Bilateral control of hindlimb scratching in the spinal turtle: contralateral spinal circuitry contributes to the normal ipsilateral motor pattern of fictive rostral scratching. *J. Neurosci.* 15: 4343–4355, 1995.
- SUTHERLAND, R. M. AND NUNNEMACHER, R. F. Microanatomy of crayfish thoracic cord and roots. *J. Comp. Neurol.* 132: 499–518, 1968.
- WIERSMA, C.A.G. AND HUGHES, G. M. On the functional anatomy of neuronal units in the abdominal cord of the crayfish, *Procambarus clarkii*. *J. Comp. Neurol.* 116: 209–228, 1961.
- WILLIAMS, T. L. Phase coupling in simulated chains of coupled oscillators representing the lamprey spinal cord. *Neural Comput.* 4: 546–558, 1992.
- ZAR, J. H. *Biostatistical Analysis*. Englewood Cliffs, NJ: Prentice-Hall, 1984.