Not by Spikes Alone: Responses of Coordinating Neurons and the Swimmeret System to Local Differences in Excitation

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INTRODUCTION

When an animal that uses limbs for locomotion changes speed, the different limbs can maintain the same phases of their movements relative to one another despite changes in the period of these movements (Orlovsky et al. 1999). In most animals, a local circuit organizes each limb’s movements by integrating proprioceptive information with its own periodic oscillation (Büschges 2005; Büschges and El Manira 1998; Marder and Bucher 1993). This integration regulates the timing and force of the motor drive to each limb’s muscles. The relative timing of movements of different limbs is controlled by a circuit of projection neurons that coordinates the local circuits driving individual limbs (Rossignol et al. 1993; Stein 2005). How these coordinating neurons respond to changes in excitation or to local perturbations is largely unknown.

Animals that swim by undulations of their segmented bodies—leeches, fish, and tadpoles—face the same problems of intersegmental coordination and also can maintain a near-constant phase, despite changes in the period of these undulations. The coordination problem in swimming lamprey (Buchanan and Kasicki 1999; Grillner 2003) and leeches (Cang and Friesen 2002; Kristan Jr et al. 2005) is solved at least in part by intersegmental axon collaterals of neurons that are components of each segmental pattern-generating circuit. These collaterals seem to make the same patterns of synapses as the neurons do in their home segment, but the strengths of these more distant synapses are weaker (Williams 1992). One intersegmental system that does separate local pattern-generating and intersegmental coordination is the leech heartbeat circuit (Kristan Jr et al. 2005; Norris et al. 2006). In the heart system, two local circuits located in adjacent ganglia determine beat frequency. These two circuits are coupled by separate coordinating neurons that originate in still other ganglia (Jezzini et al. 2004; Norris et al. 2006). The accumulated information about the neural components and synaptic organization of these lamprey and leech circuits has permitted development and testing of specific computational models that clarify the mechanisms underlying dynamic performance of these nervous systems (Hill et al. 2003; Kotaleski et al. 1999ab). We seek to bring similar progress to the problem of coordinating limbs.

The isolated crayfish CNS can express the same periodic motor pattern that drives normal swimmeret movements—a classic example of fictive locomotion (Hughes and Wiersma 1960; Ikeda and Wiersma 1964). One feature of this pattern is a posterior-to-anterior progression of power-stroke movements in different segments, with a characteristic phase lag that varies little despite large changes in the pattern’s period. In some nervous systems, the separation of local pattern-generating circuits and intersegmental coordinating circuits is far from clear because the same neurons make a series of similar synaptic connections in their home segments and in neighboring segments (Buchanan and Kasicki 1999; Cang and Friesen 2002; Cangiano and Grillner 2005). In contrast, this separation of function seems particularly clear in the swimmeret system because each of the four swimmeret-bearing segments has two local circuits that control a pair of swimmerets, eight of these modular circuits in total (Fig. 1A). The key components of each module are the motor neurons that innervate the swimmeret (Mulloney and Hall 2000), a small set of nonspiking local interneurons that form the kernel of the pattern-generating circuit (Heitler and Pearson 1980; Paul and Mulloney 1985a,b),
within each local circuit, in this case 4~, the ASCE neuron and DSC neuron that
dromic impulse conduction.
synapses, triangles symbolize excitatory synapses, the colors identify the
module’s kernel. In this diagram, solid black circles symbolize inhibitory
neighboring segments synapse onto Commissural Interneuron 1 (C1), which
originate there and project axons to other segments are driven by the pattern-
generating kernel that also controls the pools of swimmeret motor neurons
(PS4, RS4). This kernel is composed of 2 sets of reciprocally inhibitor local
neurons projecting from the system’s modular local circuits.

![Diagram of the swimmeret system and the projections of coordinating neurons from the system’s modular local circuits.](image)

and coordinating neurons that project axons to modules in other segments (Fig. 1B) (Namba and Mulloney 1999; Stein 1971; Tschuluun et al. 2001). Within each module, the den- dritic processes of the local interneurons, coordinating inter-
neurons, and motor neurons are restricted to one side of the
segmental ganglion, with almost no anatomical overlap with the
contralateral module.

When the system is active, bursts of spikes in these coordinating neurons encode information about the timing, durations, and strength of simultaneous bursts in motor neurons in their home module and conduct this information to other segments (Mulloney et al. 2006). This information is necessary and sufficient to establish and maintain the normal intersegmental phase differences in the swimmeret motor pattern (Namba and Mulloney 1999; Tschuluun et al. 2001). In their target ganglia (Fig. 1C), these coordinating axons synapse with a nonspiking commissural interneuron that integrates the information their bursts of spikes encode, transmits it to neurons in the kernel of the local module (Mulloney and Hall 2003), and thus affects the timing and strength of motor output from that module (Jones et al. 2003). If individual coordinating neurons are stimulated at different points in the swimmeret cycle, they affect the strength and timing of the output from their target modules in a phase-dependent manner (Jones et al. 2003; Namba and Mulloney 1999). The effects of these stimuli on the target are much greater than any effect on their home module. In short, these coordinating neurons appear to be essential parts of a coordinating circuit that imposes a common period and a stable phase relationship on this set of otherwise independent local pattern-generating modules. We know how each of these neurons fires when the intersegmental phases are in the normal range, and what information can be gained from their bursts of spikes (Mulloney et al. 2006). In these new experiments, we forced changes in intersegmental phase while we recorded from sets of coordinating neurons in the hope of revealing changes that could be used to explain changes in period and stabilization of phase.

The swimmeret system in an isolated nerve cord preparation can be excited both by stimulating command neurons (Acevedo et al. 1994; Stein 1971; Wiersma and Ikeda 1964) and by bath application of neurotransmitter analogues (Mulloney 1997; Mulloney et al. 1987, 1997). If the strength of excitation is increased by raising the concentration of a drug, the period of the expressed motor pattern decreases and burst strengths increase; nicotinic agonists of acetylcholine are particularly effective (Braun and Mulloney 1993; Mulloney 1997). Each of the swimmeret ganglia will respond to local application of a drug and application even to just one ganglion elicits expression of the swimmeret motor pattern from the entire system (Acevedo et al. 1994; Braun and Mulloney 1995).

If different ends of the preparation are exposed to different levels of excitation by using a “split-bath” procedure to bathe connected ganglia in different concentrations of a drug, the phase difference between PS bursts in different modules changes at the boundary between the two levels of excitation (Braun and Mulloney 1995). If anterior ganglia are more excited than posterior ganglia, the phases of their PS bursts advance compared with uniformly excited controls; if anterior ganglia are less excited, the phases of their PS bursts are delayed (Braun and Mulloney 1995). This result holds where
ever the boundary is located: between ganglia A5 and A4, A4 and A3, or A3 and A2 (Braun and Mulloney 1995). The period of the motor pattern decreases as the number of ganglia exposed to the higher level of excitation is increased and this decrease is the same whether anterior or posterior ganglia are more strongly excited (Braun and Mulloney 1995). We used this experimental design to force changes in phase while we recorded the firing of identified coordinating neurons on opposite sides of the excitation boundary.

Implications of the nonuniform excitation results reported by Braun and Mulloney (1995) are addressed in one model of the swimmeret system (Skinner et al. 1997), based on coupled-oscillator theory (Kopell and Ermentrout 1988). This model viewed the swimmeret system as a chain of four phase oscil-
lators coupled by connections in both directions between neighboring oscillators. Skinner et al. found parameters that produced phase differences between oscillators like those seen in the uniformly excited swimmeret system. We then asked what features of the model must respond to nonuniform changes in excitation if the model were to alter phase differences between oscillators in the ways Braun and Mulloney (1995) had observed. This analysis concluded that the model
could respond to nonuniform excitation like the swimmeret system if excitation affected only the intrinsic periods of each oscillator, or both intrinsic periods and the strength of coupling between oscillators. If excitation affected only the strength of coupling between oscillators, the model could not match the swimmeret system’s performance.

We previously described three types of swimmeret coordinating neurons: ASC$_E$, ASC$_L$, and DSC (Namba and Mulloney 1999). In this paper, we focus on ASC$_E$ and DSC neurons because ASC$_L$ units were often silent; we have argued elsewhere that ASC$_L$ activity cannot be necessary for normal coordination (Mulloney et al. 2006). An ASC$_E$ neuron projects from each module in ganglia A5, A4, A3, and A2 to more anterior ganglia (Fig. 1B) and fires in phase with the PS motor neurons in its home module. A DSC neuron projects from the modules in ganglia A4, A3, and A2 to more posterior ganglia (Fig. 1B), and fires in phase with the RS motor neurons of its home module. It is reasonable to think of the ascending ASC$_E$ and descending DSC axons running between modules in neighboring ganglia (Fig. 1, B and C) as neural equivalents of the abstract bidirectional coupling in Skinner et al.’s models. Our goal was to discover how the firing of these neurons differed under uniform and nonuniform conditions and to what extent these differences might predict the system’s responses to local changes in excitation.

**Methods**

To expose the CNS for recording, we first anesthetized a crayfish, *Pacifastacus leniusculus*, by chilling it on ice, and then exsanguinated it by transfusion with chilled saline. The normal saline was composed of (in mM) 5.4 KCl, 2.6 MgCl$_2$, 13.5 CaCl$_2$, and 195 NaCl, buffered with 10 mM Tris base and 4.7 mM maleic acid at pH 7.4. We removed the abdominal nerve cord, a chain of six ganglia (Huxley 1880; Mulloney et al. 2003), to a dish lined with transparent Sylgard (Dow-Corning), and pinned it out linearly dorsal-side up with stainless steel pins. To expose the tract in each ganglion that contains the axons of swimmeret coordinating neurons and to facilitate diffusion of drugs into the core of the ganglia, we used fine scissors to remove the sheath from the dorsal sides of abdominal ganglia A1 through A6.

**Excitation of the system**

We elicited stable expression of the swimmeret motor pattern by bath application of the cholinergic agonist carbachol, dissolved in normal saline (RBI, Sigma) (Braun and Mulloney 1993; Chrachri and Neil 1993). The abdominal ganglia are separated anatomically by intersegmental connectives, bundles of axons that do not contain neuronal cell bodies or synaptic processes (Mulloney et al. 2003). This separation allowed us to place a Vaseline barrier between ganglia A3 and A4 and superfuse anterior and posterior sections of the abdominal nerve cord with different concentrations of carbachol without interrupting impulse traffic across the barrier (Fig. 2). The barrier separated the solution bathing A1, A2, and A3 from the solution bathing A4, A5, and A6 (Fig. 2). By filling these compartments with the same or with different concentrations of carbachol, we could set the level of excitation for anterior and posterior parts of the system separately. The ED$_{50}$ for carbachol’s excitation of the swimmeret system is 7.8 µM (Mulloney 1997). In these experiments, we used lower concentrations than this: ≤1.5 µM for low excitation and 3–6 µM for high excitation because the motor output in this range was cleaner and more readily digitized than it was using higher concentrations.

**Electrophysiological recordings**

In this species, there are four pairs of swimmerets used for swimming, located on abdominal segments 2 through 5 (Fig. 2). Each pair of swimmerets is innervated by a pair of nerves (N1) that project from the segment’s ganglion directly to the swimmeret. Each N1 contains the motor and sensory axons that control one swimmeret. The axons of power-stroke (PS) and return-stroke (RS) motor neurons that project to each swimmeret are separated respectively into its N1’s posterior and anterior branches (Mulloney and Hall 2000). To record firing of PS and RS motor neurons from each ganglion, we separated the anterior and posterior branches of each N1 and placed extracellular stainless steel pin electrodes in contact with them. We insulated each electrode from the bathing saline with a small amount of Vaseline.

The pairs of coordinating interneurons that originate in each ganglion project their axons dorsally through the miniscule tract (MnT) (Skinner 1985) and across the lateral giant axon before entering the interganglionic connectives. We recorded their firing extracellularly with a suction electrode placed on the MnT as it crossed the lateral giant (Mulloney et al. 2003; Namba and Mulloney 1999). Spikes recorded by these electrodes could confidently be attributed to individual coordinating neurons originating in the module on the same side of the same ganglion (Mulloney and Hall 2003; Mulloney et al. 2006). We could record the activity of coordinating neurons simultaneously in ganglia on opposite sides of the barrier and selectively change the solutions bathing the different ganglia (Fig. 2). In each experiment, we recorded four bouts of steady-state activity induced by four different conditions of excitation (Fig. 3). The recording technology is described in Mulloney et al. (2006).
Period and phase under nonuniform conditions

Changes in strength of bursts of spikes

To measure the strengths of bursts of spikes in motor axons, we used a low-pass digital-filtering method that calculates the strength of each burst by dividing the area of a polygon derived from the burst’s squared voltages by the burst’s independently measured duration (Mulloney 2005). Each of these strengths ($S_i$) were then normalized to the strongest burst recorded by that electrode in the entire experiment, $S_i = x_i/s_{max}$. This yielded measures of burst strength that ranged from $0 \leq S_i \leq 1.0$ and that could be compared between different parts of the experiment.

Analysis

The swimmeret motor pattern is a periodic cycle of firing in about 600 motor neurons distributed in four ganglia (Mulloney and Hall 2000). We recorded the motor output from selected ganglia simultaneously with firing of coordinating neurons originating in these same ganglia. We digitized a continuous series of 30–50 cycles from each bout. Each cycle was defined as beginning at the start of the PS burst in the most posterior ganglion recorded, usually ganglion A5 (PS5). The start and stop of each burst of spikes in PS and RS recordings, which defined the burst’s duration, were measured using DataView 4 software (http://www.st-andrews.ac.uk/~wjh/). The period of each cycle was the interval from the start of one PS burst to the start of the next PS burst. The latencies of other events in the cycle were measured as the time interval between the start of that event and the start of the preceding PS burst that marked the start of the cycle. The phase of this event then was defined as the ratio of this latency to the cycle’s period. Phase could range from 0 to 1.0. The duty cycle of a burst was defined as the ratio of the burst’s duration to the period of the cycle in which the burst occurred.

In these same cycles, the time at which each spike in a coordinating neuron occurred was measured with the threshold-crossing algorithm of DataView. The numbers of spikes per burst and the burst’s duration were then calculated from these lists of spike times using SigmaPlot transforms and aligned with the measurements from simultaneous bursts in motor neurons. The phase of each burst of spikes in a coordinating neuron was defined as the ratio of the latency of this burst, relative to the preceding PS burst in the neuron’s home module, to the period of that cycle. We used our own software for descriptive statistics (Mulloney and Hall 1987), SigmaStat (SysStat Software, Point Richmond, CA) for Pearson correlation, linear regressions, and repeated-measures (RM) ANOVAs. Statistics that describe each parameter are given as means ± SD.

Results

In each experiment, we simultaneously recorded the system’s motor output and the activity of coordinating neurons under four conditions: uniform excitation, when all ganglia were bathed in low (LL) or high (HH) concentrations of carbachol, and nonuniform excitation, when anterior ganglia were bathed in high concentrations but posterior ganglia in low concentrations (HL), or the opposite (LH), with anterior low but posterior high. We will use these two-letter labels to identify the conditions in which particular results were obtained. In this code, the first letter describes the anterior solution, the second the posterior solution (Fig. 3A). We begin by describing how the system responded to these different patterns of excitation and then describe the responses of the individual coordinating neurons.

Uniform changes in excitation altered the period of the swimmeret motor pattern but did not affect intersegmental phase

We first confirmed that with the barrier across the 3–4 connective, changes in carbachol concentrations still caused a change in period of the motor pattern (Fig. 3B). In nine experiments, raising the concentration from low (LL) to high (HH) changed period from 0.500 ± 0.062 to 0.384 ± 0.074 s, a significant difference (RM ANOVA, $P < 0.0001$). Despite this 23% decrease in period, the phases of PS bursts in A2, A3,
TABLE 1. Phases of power-stroke bursts in ganglia A2, A3, and A4 recorded under uniform (LL, HH) and nonuniform (LH, HL) excitation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LL</th>
<th>HL</th>
<th>LH</th>
<th>HH</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS2 phase, relative to PS3</td>
<td>0.287 ± 0.094</td>
<td>0.254 ± 0.120</td>
<td>0.271 ± 0.083</td>
<td>0.290 ± 0.061</td>
</tr>
<tr>
<td>PS3 phase, relative to PS4</td>
<td>0.215 ± 0.069</td>
<td>0.142 ± 0.079</td>
<td>0.270 ± 0.072</td>
<td>0.227 ± 0.056</td>
</tr>
<tr>
<td>PS4 phase, relative to PS5</td>
<td>0.174 ± 0.059</td>
<td>0.198 ± 0.066</td>
<td>0.245 ± 0.094</td>
<td>0.201 ± 0.044</td>
</tr>
</tbody>
</table>

Values are means ± SD. For PS2 $n = 6$ experiments. For PS3 and PS4, $n = 9$ experiments. Experimental condition: LL, HH, uniform excitation of all ganglia with 1–2 μM (L) or 3–6 μM (H) carbachol; LH, anterior ganglia (A1, A2, A3) in 1–2 μM carbachol (L) and posterior ganglia (A4, A5, A6) in 3–6 μM (H) carbachol; HL, anterior ganglia in 3–6 μM (H) carbachol and posterior ganglia in 1–2 μM (L) carbachol. Values in boldface type are significantly different from each other and from the other statistics in the same row (RM ANOVA, $P < 0.0001$). †PS4 LL phase differed from PS4 LH phase, but not from other statistics in the same row (RM ANOVA, $P = 0.021$).

Changes in excitation also affected durations and strengths of PS bursts

Durations of PS3 under these four conditions scaled in proportion with periods (Table 2), so LL and HH durations also differed significantly (RM ANOVA, $P < 0.001$). This scaling is apparent in the duty cycles of PS3 bursts, which did not change ($P = 0.55$). The independent parameter here seems to be period.

The strengths of PS3 bursts followed a different pattern (Fig. 4, Table 2); whenever ganglion A3 was weakly excited (LL and HL), PS3 burst strengths were not significantly different (RM ANOVA, $P = 0.940$). When A3 was strongly excited (HL and HH), PS3 bursts were stronger than they had been in low carbachol ($P = 0.019$) and HH bursts were stronger than HL bursts ($P < 0.001$).

Durations of PS4 bursts also scaled in proportion to period (Table 2). Under these four conditions, the duty cycles of these bursts did not change significantly (RM ANOVA, $P = 0.08$). In contrast, the strengths of PS4 bursts followed a pattern like those of PS3 bursts (Fig. 4, Table 2). When ganglion A4 was strongly excited (LL and HH), PS4 burst strengths were greater than when A4 was weakly excited (HL or LL) (RM ANOVA, $P = 0.0001$). Excitation applied directly to A4 seemed to determine how strong the PS4 bursts would be; LL and HL bursts were equally strong (RM ANOVA, $P = 0.626$) and so were LH and HH bursts (RM ANOVA, $P = 0.436$). Thus the factor controlling the strengths of bursts in PS motor neurons seems to be the level of excitation applied to the ganglia in which these motor neurons are located. These results for burst strengths are novel; those for period, duration, and phase extend our earlier findings (Braun and Mulloney 1995).

In summary, the swimmeret system responded to nonuniform excitation of different ganglia in the chain by changing period and durations proportionately, by changing PS burst strengths, and by changing the intersegmental phase lag at the excitation boundary, but not elsewhere in the chain of ganglia.

Coordinating neurons in ganglia at the excitation boundary also responded to nonuniform excitation

The swimmeret system’s responses to nonuniform excitation suggested that coordinating neurons originating in ganglia closest to the A3–A4 excitation boundary might respond most strongly to disparities in excitation. There are four pairs of neurons in this position: the ASCe neurons and DSC neurons in ganglion A4 (ASCe4, DSC4) and the ASCe3 neurons and DSC neurons in ganglion A3 (ASCe3, DSC3). The axons of

Nonuniform excitation also changed period and caused a local change in intersegmental phase at the excitation boundary

Under HL and LH conditions, with different concentrations of carbachol in the solutions bathing the anterior and posterior ends of the preparation, the period of the motor pattern also changed (Fig. 3B). The HL period was 0.451 ± 0.053 and the LH period was 0.430 ± 0.068 in these nine experiments. Despite the asymmetries in excitation, these periods are not different (RM ANOVA, $P = 0.237$), shorter than LL period but longer than HH period ($P \leq 0.013$).

Significant changes in intersegmental phase occurred at the excitation boundary between ganglia A3 and A4 (Table 1; Fig. 3A). If anterior ganglia were excited more strongly than the posterior ganglia (HL), the phases of PS2 and PS3 bursts in each PS4 cycle advanced (Fig. 3C). If the anterior ganglia were less excited than the posterior ganglia (LH), the phases of PS2 and PS3 bursts in each PS4 cycle were delayed (Fig. 3C). The critical change occurred in the timing of PS3 bursts relative to the cycle of PS4 firing; PS3 phases relative to PS4 differed significantly in HL and LH conditions (RM ANOVA, $P < 0.001$) and from phases recorded under both uniform conditions ($P = 0.02$). Individual preparations responded to these conditions to different extents. The mean difference between PS3 phases relative to PS4 recorded under HL and LH conditions was 0.128 ± 0.081, but the range of these differences was 0.0 to 0.25. One of nine preparations showed a true phase reversal in the HL condition; PS3 bursts led PS4 bursts with a mean phase of $-0.035 \pm 0.054$, an unusual phenomenon illustrated in Braun and Mulloney (1995).

During these nonuniform experiments, ganglia on the same side of the barrier were always excited to the same extent (Fig. 3A). Although the period of the expressed motor pattern changed in response to these local differences in excitation, the phases of PS bursts in ganglia on the same side of the barrier relative to one another were largely unaffected (Table 1). Phases of PS2 bursts in each PS3 cycle recorded under these four conditions did not change significantly (RM ANOVA, $P = 0.88$). Similarly, phases of PS4 bursts in each PS3 cycle did not change significantly (Table 1), except that the LH phase was greater than the LL phase ($P = 0.021$).

Changes in excitation also affected durations and strengths of PS bursts

Durations of PS3 under these four conditions scaled in proportion with periods (Table 2), so LL and HH durations also differed significantly (RM ANOVA, $P < 0.001$). This scaling is apparent in the duty cycles of PS3 bursts, which did not change ($P = 0.55$). The independent parameter here seems to be period.

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Durations of PS4 bursts also scaled in proportion to period (Table 2). Under these four conditions, the duty cycles of these bursts did not change significantly (RM ANOVA, $P = 0.08$). In contrast, the strengths of PS4 bursts followed a pattern like those of PS3 bursts (Fig. 4, Table 2). When ganglion A4 was strongly excited (LL and HH), PS4 burst strengths were greater than when A4 was weakly excited (HL or LL) (RM ANOVA, $P = 0.0001$). Excitation applied directly to A4 seemed to determine how strong the PS4 bursts would be; LL and HL bursts were equally strong (RM ANOVA, $P = 0.626$) and so were LH and HH bursts (RM ANOVA, $P = 0.436$). Thus the factor controlling the strengths of bursts in PS motor neurons seems to be the level of excitation applied to the ganglia in which these motor neurons are located. These results for burst strengths are novel; those for period, duration, and phase extend our earlier findings (Braun and Mulloney 1995).

In summary, the swimmeret system responded to nonuniform excitation of different ganglia in the chain by changing period and durations proportionately, by changing PS burst strengths, and by changing the intersegmental phase lag at the excitation boundary, but not elsewhere in the chain of ganglia.
the ASC$_E$ neurons project anteriorly through A3 and A2, whereas the axons of the DSC neurons project posteriorly through A4 and A5 (Namba and Mulloney 1999; Tschuluun et al. 2001). We will begin with ASC$_E$4 and DSC3 neurons because their axons cross the excitation boundary.

ASC$_E$4 neurons were surprisingly unaffected by changes in excitation (Fig. 5). The durations of their bursts under HH conditions were significantly shorter than they were when A4 was weakly excited (HL, LL). This was not simply a matter of scaling with period; DSC3 duty cycles were also significantly different under HL and LH conditions (RM ANOVA, $P < 0.001$), although the periods were the same. The differences in a DSC3’s activity under LH and HL conditions are apparent by inspection of Fig. 5. In these same experiments, PS3 duty cycles recorded simultaneously did not change at all (Table 2).

Whenever A3 was strongly excited (HL and HH), the phases of DSC3 bursts relative to PS3 bursts advanced in the cycle compared with those when A3 was weakly excited (HL and LL) (RM ANOVA, $P < 0.03$). These phase shifts added to the phases of PS3 relative to PS4 (Table 1, Fig. 3C) to generate DSC3 phases relative to PS4 that differed significantly under nonuniform excitation (Fig. 8A; RM ANOVA, $P < 0.0001$).

The numbers of spikes in DSC3 bursts also changed significantly under LH and HL conditions (RM ANOVA, $P < 0.001$), although under both uniform conditions they were not correlated with changes in PS3 phase relative to PS4.

DSC3 neurons were more responsive to disparities in excitation (Figs. 5 and 7; Table 4). Whenever ganglion A4 was strongly excited (HL, HH), the durations of DSC3 bursts were shorter than they were when A4 was weakly excited (HL, LL). This was not simply a matter of scaling with period; DSC3 duty cycles were also significantly different under HL and LH conditions (RM ANOVA, $P < 0.001$), although the periods were the same. The differences in a DSC3’s activity under LH and HL conditions are apparent by inspection of Fig. 5. In these same experiments, PS3 duty cycles recorded simultaneously did not change at all (Table 2).

Whenever A3 was strongly excited (HL and HH), the phases of DSC3 bursts relative to PS3 bursts advanced in the cycle compared with those when A3 was weakly excited (HL and LL) (RM ANOVA, $P < 0.03$). These phase shifts added to the phases of PS3 relative to PS4 (Table 1, Fig. 3C) to generate DSC3 phases relative to PS4 that differed significantly under nonuniform excitation (Fig. 8A; RM ANOVA, $P < 0.0001$).

The numbers of spikes in DSC3 bursts also changed significantly under LH and HL conditions (RM ANOVA, $P < 0.001$), although under both uniform conditions they were not correlated with changes in PS3 phase relative to PS4.

### Table 2. Properties of power-stroke bursts in each swimmeret ganglion recorded under uniform (LL, HH) and nonuniform (LH, HL) excitation

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>LL</th>
<th>HL</th>
<th>LH</th>
<th>HH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duration, s</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS2</td>
<td>0.141 ± 0.064</td>
<td>0.153 ± 0.027</td>
<td>0.130 ± 0.030</td>
<td>0.127 ± 0.038</td>
</tr>
<tr>
<td>PS3</td>
<td>0.205 ± 0.061</td>
<td>0.206 ± 0.072</td>
<td>0.205 ± 0.072</td>
<td>0.206 ± 0.072</td>
</tr>
<tr>
<td>PS4</td>
<td>0.210 ± 0.047</td>
<td>0.166 ± 0.036</td>
<td>0.182 ± 0.064</td>
<td>0.159 ± 0.050</td>
</tr>
<tr>
<td><strong>Duty cycle</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PS2</td>
<td>0.284 ± 0.128</td>
<td>0.336 ± 0.066</td>
<td>0.310 ± 0.080</td>
<td>0.319 ± 0.069</td>
</tr>
<tr>
<td>PS3</td>
<td>0.206 ± 0.072</td>
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<td>0.159 ± 0.050</td>
</tr>
<tr>
<td><strong>Burst strength</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>PS2</td>
<td>0.276 ± 0.218</td>
<td>0.445 ± 0.128</td>
<td>0.318 ± 0.192</td>
<td>0.548 ± 0.168</td>
</tr>
<tr>
<td>PS3</td>
<td>0.502 ± 0.087</td>
<td>0.502 ± 0.087</td>
<td>0.502 ± 0.087</td>
<td>0.502 ± 0.087</td>
</tr>
<tr>
<td>PS4</td>
<td>0.309 ± 0.157</td>
<td>0.309 ± 0.157</td>
<td>0.309 ± 0.157</td>
<td>0.309 ± 0.157</td>
</tr>
</tbody>
</table>

Values are means ± SD. For PS2, $n = 5$ experiments. For PS3 and PS4, $n = 9$ experiments. Experimental conditions are defined in Table 1 and Fig. 3. Values in boldface type in each row are significantly different from the other statistics in the same row (RM ANOVA, $P < 0.001$). *PS4 LH and HH burst strengths were greater than either HL or LL strengths (RM ANOVA, $P = 0.0023$). †PS4 LH and HH burst strengths were greater than either HL or LL strengths (RM ANOVA, $P = 0.0024$).
similar (Fig. 8B; Table 4). In these bursts, spike frequencies were lowest under LL conditions, significantly lower than LH or HH frequencies (Fig. 8C).

In summary, ASC4 and DSC3 neurons responded very differently to nonuniform excitation (Fig. 7). ASC4 continued to track the timing and duration of each PS4 burst under both HL and LH conditions. The numbers of spikes in ASC4 bursts did not change significantly, although the strengths of simultaneous PS4 bursts did change (Table 2). When anterior ganglia were strongly excited (HH and HL), DSC3 bursts started earlier in each cycle and contained more spikes than when these ganglia were weakly excited (LH and LL). This phase advance and increase in numbers of spikes per DSC3 burst matches the increased strengths of PS3 bursts observed when anterior ganglia were more strongly excited (Table 2).

**TABLE 3. Properties of ASC bursts from each ganglion recorded under uniform (LL, HH) and nonuniform (LH, HL) excitation**

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>LL</th>
<th>LH</th>
<th>HH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ASC4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration, s</td>
<td>0.213 ± 0.073</td>
<td>0.105 ± 0.057</td>
<td>0.131 ± 0.074</td>
</tr>
<tr>
<td>Duty cycle</td>
<td>0.405 ± 0.143</td>
<td>0.218 ± 0.096</td>
<td>0.272 ± 0.159</td>
</tr>
<tr>
<td>Number of spikes</td>
<td>6.8 ± 2.1</td>
<td>5.2 ± 3.1</td>
<td>4.6 ± 2.2</td>
</tr>
<tr>
<td>Spike frequency*</td>
<td>34.0 ± 10.5</td>
<td>47.6 ± 4.1</td>
<td>34.7 ± 12.6</td>
</tr>
<tr>
<td>Phase, relative to PS2</td>
<td>0.106 ± 0.093</td>
<td>0.091 ± 0.031</td>
<td>0.107 ± 0.061</td>
</tr>
<tr>
<td><strong>ASC5</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration, s</td>
<td>0.221 ± 0.101</td>
<td>0.200 ± 0.053</td>
<td>0.230 ± 0.047</td>
</tr>
<tr>
<td>Duty cycle</td>
<td>0.422 ± 0.179</td>
<td>0.423 ± 0.081</td>
<td>0.476 ± 0.063</td>
</tr>
<tr>
<td>Number of spikes</td>
<td>11.4 ± 5.5</td>
<td>14.9 ± 5.0</td>
<td>13.5 ± 2.0</td>
</tr>
<tr>
<td>Spike frequency*</td>
<td>50.2 ± 4.9</td>
<td>73.9 ± 6.3</td>
<td>59.4 ± 6.8</td>
</tr>
<tr>
<td>Phase, relative to PS3</td>
<td>0.141 ± 0.018</td>
<td>0.100 ± 0.033</td>
<td>0.142 ± 0.020</td>
</tr>
</tbody>
</table>

Values are means ± SD. For ASC4, n = 8 experiments. For the rest, n = 4 experiments. Experimental conditions are defined in Table 1 and Fig. 3. Values in boldface type in each row are significantly different from each other and from other statistics in the same row (RM ANOVA, P < 0.015). *Values in italic type for ASC4 frequencies are significantly different from their immediate neighbors in the row, but not from other statistics in the same row (RM ANOVA, P = 0.009). †ASC4 HH frequencies were higher than LL frequencies (RM ANOVA, P = 0.01) and ASC5 HH frequencies were higher than LL frequencies (RM ANOVA, P = 0.026). §ASC4 LL phase differed from HL and HH phases, but not the LH phase (RM ANOVA, P = 0.014).
Coordinating neurons in ganglia farther from the boundary responded less to nonuniform excitation

ASC_E neurons originate in pairs in each ganglion from A2 to A5 (Mulloney et al. 2006; Tschuluun et al. 2001). Compared with ASC_E4, ASC_E neurons from other ganglia were even less affected by nonuniform excitation. Burst durations and numbers of spikes in ASC_E5, ASC_E3, and ASC_E2 bursts did not change significantly (Fig. 9; Table 3). These neurons continued to fire bursts simultaneously with the PS motor neurons in their home ganglia and did not change the phase of their bursts relative to these PS bursts despite changes in excitation and the disparity at the excitation boundary (Table 3). Given the responses of DSC3 to nonuniform excitation, the absence of significant changes in the parameters of ASC_E3s is particularly noticeable (Table 3). The most plastic parameter in these bursts was spike frequency. Although neither the numbers of spikes per ASC_E burst nor burst durations differed significantly under these four conditions, spike frequency tended to increase in response to increasing uniform excitation (Table 3).

DSC neurons originate in ganglia A2, A3, and A4, one pair in each ganglion (Mulloney et al. 2006). DSC4 neurons scaled their burst durations with period and their duty cycles did not change significantly (RM ANOVA, P = 0.068). Unlike DSC3, the numbers of spikes in DSC4 bursts did not change under HL or LH conditions (Table 4; RM ANOVA, P = 0.253). Because their burst durations scaled with period, spike frequency within these DSC4 bursts increased as excitation increased. The phases of DSC4 bursts relative to PS4 bursts differed under HL and LH conditions (Fig. 9; Table 4). DSC2 neurons were almost unaffected by changes in excitation (Table 4).

These results have an unexpected feature: the coordinating neurons that originate in ganglia next to the excitation boundary and whose axons project across the boundary, DSC3 and ASC_E4, react to nonuniform excitation more than the neurons from the same ganglia, DSC4 and ASC_E3, whose axons project away from the excitation boundary. We also note that the posterior-to-anterior gradients in numbers of spikes per burst that are apparent under uniform excitation (Mulloney et al. 2006) persist when the system is not uniformly excited (Tables 3 and 4).

Correlations of ASC_E and DSC firing with simultaneous PS bursts in the same ganglion

Our analysis of ASC_E firing in uniformly excited preparations showed that if the strengths of PS bursts varied spontaneously through a wide range, the numbers of spikes per ASC_E burst varied proportionately, so that the numbers of spikes per ASC_E burst were accurate reporters of PS burst strength (Mulloney et al. 2006). In these nonuniform excitation experiments, we expected to see a strong positive correlation between the strengths of PS4 bursts under different conditions and the numbers of spikes in the simultaneous ASC_E4 bursts. Instead, the mean numbers of ASC_E4 spikes changed very little despite significant changes in mean PS burst strength (Fig. 10A). In different experiments, the correlation of these parameters varied widely and in different patterns. Figure 10B shows two examples from experiments in which PS4 strength varied substantially. In both experiments (Bi, Bii), the strongest PS4 bursts occurred when ganglion A4 was bathed in a higher concentration of carbachol (LH, HH). In one experiment, these stronger bursts were accompanied by more ASC_E4 spikes, but in the other they were not. Moreover, in both experiments, ASC_E bursts with the largest numbers of spikes accompanied comparatively weak PS4 bursts during episodes when ganglion A4 was bathed in a lower concentration of carbachol.
These bursts, were at best weakly correlated with the strengths of simultaneous bursts in PS3 (Fig. 11A). Under uniform LL conditions, the durations of DSC3 bursts are positively correlated with RS durations (Mulloney et al. 2006). In most of these nonuniform excitation experiments, we did not record RS firing, and so cannot say how these correlations would hold up in the face of nonuniform excitation. In one nonuniform experiment where we did record RS firing, the durations of RS3 bursts effectively predicted the durations of DSC3 bursts; the slope of the regression equation was 0.482 (Fig. 11B).

**DISCUSSION**

The swimmeret system responded to increasing uniform excitation by increasing the strengths of bursts in PS motor neurons (Table 2) and by decreasing the period of the motor

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**TABLE 4. Properties of DSC bursts from each ganglion recorded under uniform (LL, HH) and nonuniform (LH, HL) excitation**

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>LL</th>
<th>HL</th>
<th>LH</th>
<th>HH</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSC2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration, s</td>
<td>0.142 ± 0.052</td>
<td>0.130 ± 0.017</td>
<td>0.114 ± 0.046</td>
<td>0.115 ± 0.019</td>
</tr>
<tr>
<td>Duty cycle</td>
<td>0.287 ± 0.128</td>
<td>0.282 ± 0.059</td>
<td>0.244 ± 0.105</td>
<td>0.274 ± 0.043</td>
</tr>
<tr>
<td>Number of spikes</td>
<td>4.2 ± 1.5</td>
<td>4.9 ± 1.0</td>
<td>3.8 ± 1.3</td>
<td>4.4 ± 1.4</td>
</tr>
<tr>
<td>Spike frequency</td>
<td>29.2 ± 3.6</td>
<td>37.6 ± 6.0</td>
<td>34.7 ± 8.9</td>
<td>38.0 ± 7.4</td>
</tr>
<tr>
<td>Phase, relative to PS2</td>
<td>0.493 ± 0.129</td>
<td>0.539 ± 0.094</td>
<td>0.534 ± 0.138</td>
<td>0.529 ± 0.075</td>
</tr>
<tr>
<td>DSC3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration, s</td>
<td><strong>0.170 ± 0.056</strong></td>
<td>0.207 ± 0.062</td>
<td>0.115 ± 0.042</td>
<td>0.143 ± 0.044</td>
</tr>
<tr>
<td>Duty cycle</td>
<td>0.349 ± 0.129</td>
<td>0.482 ± 0.192</td>
<td>0.276 ± 0.113</td>
<td>0.400 ± 0.175</td>
</tr>
<tr>
<td>Number of spikes</td>
<td>5.8 ± 2.4</td>
<td>8.0 ± 3.0</td>
<td>4.9 ± 2.3</td>
<td>6.1 ± 2.2</td>
</tr>
<tr>
<td>Spike frequency</td>
<td>33.6 ± 5.7</td>
<td>38.7 ± 9.2</td>
<td>41.8 ± 6.8</td>
<td>42.9 ± 8.0</td>
</tr>
<tr>
<td>Phase, relative to PS3</td>
<td><strong>0.505† ± 0.111</strong></td>
<td>0.440† ± 0.106</td>
<td>0.508† ± 0.146</td>
<td>0.451 ± 0.102</td>
</tr>
<tr>
<td>DSC4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration, s</td>
<td>0.301± ± 0.002</td>
<td>0.250 ± 0.042</td>
<td>0.238 ± 0.038</td>
<td>0.217± ± 0.041</td>
</tr>
<tr>
<td>Duty cycle</td>
<td>0.610 ± 0.147</td>
<td>0.553 ± 0.153</td>
<td>0.517 ± 0.148</td>
<td>0.513 ± 0.134</td>
</tr>
<tr>
<td>Number of spikes</td>
<td>9.4 ± 2.2</td>
<td>9.3 ± 3.1</td>
<td>10.2 ± 2.0</td>
<td>9.8 ± 2.0</td>
</tr>
<tr>
<td>Spike frequency</td>
<td>31.1 ± 7.6</td>
<td>36.4 ± 7.3</td>
<td>42.7 ± 1.8</td>
<td>45.0 ± 4.3</td>
</tr>
<tr>
<td>Phase, relative to PS4</td>
<td>0.423 ± 0.062</td>
<td>0.408± ± 0.086</td>
<td>0.493§ ± 0.067</td>
<td>0.476 ± 0.077</td>
</tr>
</tbody>
</table>

Values are means ± SD. For DSC2, n = 5 experiments. For DSC3, n = 8 experiments. For DSC4, n = 3 experiments. Experimental conditions are defined in Table 1 and Fig. 3. Values in boldface type are significantly different from each other and from other statistics in the same row (RM ANOVA, P < 0.0001). Values in italic type for DSC3 duty cycle and DSC3 number of spikes are significantly different from their immediate neighbors in the row, but not from other statistics in the same row (RM ANOVA, P = 0.0003) and DSC4 LL frequency was lower than LH and HH frequencies (RM ANOVA, P = 0.0001). †DSC3 LL phase was advanced compared with LH and HH phases (RM ANOVA, P = 0.01). §DSC3 LL durations were greater than HH durations (RM ANOVA, P = 0.015). §DSC4 HL phase was advanced compared with LH phase (RM ANOVA, P = 0.015).
burst strength, with a significant change in intersegmental
paper’s LH and HL experiments—with local increases in PS.
The system responded to local increases in excitation—this
in neighboring segments remained the same (Tables 1 and 2).

The period problem

What determines the period of the swimmeret motor pattern when
the swimmeret system is subject to a given level of excitation? We know that isolated subsets of the swimmeret
system (Mulloney 1997; Paul and Mulloney 1986) and even
individual modules (Murchison et al. 1993) can produce peri-
odic PS–RS alternations. It is natural to think of each local
module as having an intrinsic period that can be tuned by
excitation and inhibition and altered by synaptic connections
that couple these modules together so that they conform to the
same period. The pattern-generating kernel of each swimmeret
module is formed by a reciprocal circuit of inhibitory synapses
among nonspiking local interneurons that control bursting in
PS and RS motor neurons (Fig. 1C) (Mulloney 2003). Circuits
of reciprocally inhibitory neurons can produce stable oscillations because of their inhibitory interactions (Perkel and
Mulloney 1974; Wang and Rinzel 1992) and these analyses were applied to our original nonuniform experiments (Skinner et al. 1997). Skinner
et al. asked under what conditions a chain of four coupled
phase oscillators could respond to local differences in excita-
tion in the same way as did the swimmeret system. They found
that if local excitation affected only the intrinsic period of an
oscillator, or if local excitation affected both its intrinsic period
and the strength of its coupling to other oscillators in the chain,
then with appropriate coupling functions and local differences
in intrinsic periodicities the model system would change its
period and intersegmental phases as did the swimmeret system.
In contrast, if local differences in excitation affected just the
strength and timing of coupling but not the oscillators’ intrinsic
periods, the model system could not match the swimmeret
system’s performance. These analytical results provide a per-
pective in which to consider these new physiological results.

pattern, both responses consistent with the intuitive idea of
faster locomotion. Despite these quantitative changes, the
structure of the motor pattern was preserved: both the duty
cycles of PS bursts in each module and the phases of PS bursts
in neighboring segments remained the same (Tables 1 and 2).
The system responded to local increases in excitation—this
paper’s LH and HL experiments—with local increases in PS
burst strength, with a significant change in intersegmental

FIG. 9. Box plots that compare the structures of activity in 4 swimmeret
ganglia (A2, A3, A4, and A5) during 2 cycles of activity under HL (green) and
LH (gray) conditions. To factor out the normal phase progression between
ganglia (Figs. 2 and 5), the cycle for each ganglion is defined as beginning
when its own PS burst began. Each box shows the mean duty cycle of bursts
in DSC, ASCE, or PS from the specified ganglion, and begins at the mean
phase of that burst relative to PS. On each box, the right-hand error bars show
SD of duty cycle and the left-hand error bar shows SD of phase, except that the
left error bars on the PS boxes show the SD of normalized period.
neurons arise only in ganglia A2, A3, and A4 and conduct different information posteriorly (Tschuluun et al. 2001). Thus each of the four swimmeret ganglia receives a different mix of information from coordinating axons. Given these differences, it is a puzzle how the periods of the motor patterns produced under HL and LH come to be the same (Fig. 3B).

Unlike the system’s period, the strengths of PS bursts in each ganglion responded primarily to the level of excitation applied to that ganglion (Fig. 4, Table 2). This suggests a solution to this puzzle. If local excitation of each ganglion affects not only PS burst strength but also the intrinsic period of the local pattern-generating circuit that drives these PS bursts (Mulloney 1997, 2003), then under nonuniform conditions modules in ganglia subject to different levels of excitation would have different intrinsic periods. The system would still express a common period because the coordinating neurons couple the different modules together (Jones et al. 2003; Tschuluun et al. 2001), but this period emerges from the interaction of different intrinsic periods and the mechanism that generates the phase lag at the excitation boundary. Because the swimmeret motor pattern proceeds from A5 forward to A2 and then repeats, we define the latency of a PS burst relative to the just-preceding PS burst in the next posterior ganglion (e.g., Fig. 5) as the time interval between the starts of the two bursts. For a periodic motor pattern expressed in ganglia A5, A4, A3, and A2, it follows that

\[
\text{Period} = \text{Lat}_{54} + \text{Lat}_{43} + \text{Lat}_{23} + \text{Lat}_{52}
\]

where \(\text{Lat}_{ij}\) is the latency of the PS burst in ganglion \(i\) after the start of the PS burst in the next-posterior ganglion \(j\); \(\text{Lat}_{52}\) means the latency of the next PS5 burst after each PS2 burst. These definitions of latency and period lead to the definition of the phase of the PS burst in ganglion \(i\) relative to PS\(j\): \(\Phi_{ij} = \text{Lat}_{ij}/\text{Period}\). Because phase does not change despite uniform changes in excitation (Table 1), it is clear that for both HH and LL conditions this sum of latencies would equal the period.

What about periods under HL and LH conditions? In those cases, some ganglia are strongly excited and some are not. If each module has an intrinsic period that is set by the local level of excitation and integrates arriving coordinating information in the context of this intrinsic period, then the system’s period would be the sum of latencies that result from different intrin-

![FIG. 10. A: numbers of spikes per burst in ASC\(_4\) neurons and the strengths of simultaneous bursts in PS4 neurons recorded under 4 conditions (LL, HH, HL, and LH) from 7 experiments. Bii and Bi: from 2 experiments, the numbers of spikes per ASC\(_4\) bursts and the strengths of simultaneous bursts in PS4 neurons recorded under 4 conditions. In both A and B, the color of each point shows the condition under which the data were recorded. These conditions are described in Fig. 3.](image1)

![FIG. 11. A: mean numbers of spikes per burst in DSC3 neurons recorded under 4 conditions did not vary with strengths of simultaneous PS3 bursts. B: in one experiment, durations of individual DSC3 bursts increased with the durations of simultaneous RS3 bursts under 4 conditions. Color of each point shows the condition under which that burst was recorded. Regression line was calculated using the combined measurements from the 4 conditions. \(r\) is the regression coefficient.](image2)
sic periods. A model for the period under LH conditions, \(HH\text{Period}\), is

\[HH\text{Period} = HHLat_{34} + HHLat_{23} + HHLat_{15} + HHLat_{13}\]

where

\[HHLat_{ij} = H_j \phi_{ij} \cdot HH\text{Period}\]

and for the period under HL conditions, \(HL\text{Period}\), is

\[HL\text{Period} = HLLat_{34} + HLLat_{23} + HLLat_{15} + HLLat_{13}\]

where

\[HLLat_{ij} = H_j \phi_{ij} \cdot HL\text{Period}\]

In these models, \(HHLat_{34}\) and \(HLLat_{34}\) are the measured phases of PS3 in each PS4 cycle under LH and HL conditions (Table 1); \(HHLat_{ij}\) and \(HLLat_{ij}\) are the latencies under uniform HH and LL conditions. We assumed that each module’s intrinsic period was set by the level of excitation to which it was subject and the latency between ganglia was the product of this intrinsic period and the phase \(\phi_{ij}\), measured under the corresponding uniform condition. We estimated these intrinsic periods under weakly excited conditions, \(HL\text{Period}\), and strongly excited conditions, \(HH\text{Period}\), from the periods measured under uniform conditions (Table 1). Only one parameter in each model, \(HL\phi_{34}\) or \(HH\phi_{34}\), was determined by measurements made under nonuniform conditions.

Summing the latencies measured under LH or HL conditions would obviously yield LH or HL periods. It is not obvious that summing a series of latencies measured under HH and LL conditions would yield HL or LH periods. To test the plausibility of these two models for LH period and HL period, we calculated the sums of latencies for the nine experiments in Table 1 and plotted these predictions against the HL and LH periods measured in these experiments (Fig. 12). For both the LH and HL models, the resulting points are clustered about the regression line and show no systematic bias. The slope of the regression line is very close to 1.0. For the LH model, the difference between the mean of the sums of latencies and mean of measured periods was \(-0.003\, \text{s}, <1\%\). For the HL model, this difference was 0.022, \(<5\%\). These differences were smaller than those produced by alternative models with different definitions of \(HHLat_{34}\) and \(HHLat_{34}\). Therefore we find it plausible that the system’s HL and LH periods are functions of the intrinsic periods of the individual modules and these intrinsic periods are determined by the level of excitation to which each module is exposed. This is consistent with conclusions reported by Skinner et al. (1997). Because there is no evidence of a segmental gradient in excitability in the swimmeret system, at least in response to excitation by carbachol (Mulloney 1997), HL and LH periods are the same because the same number of ganglia are exposed to high and low excitation in both conditions.

How do significant differences in PS3–PS4 phases arise under HL and LH conditions when HH and LL phases are the same?

The ability of these simple models to predict the system’s period focused our attention on \(\phi_{34}\), the phase of PS bursts in ganglion A3 relative to PS bursts in A4, just across the excitation boundary. If coordinating neurons were the only neurons that responded to changing excitation, we would expect to see substantial differences in their firing when \(\phi_{34}\) changed. Instead, the differences in phase and numbers of spikes in ASC_E’s bursts are so small that it appears the system holds these parameters carefully fixed (Tables 3 and 4). Although PS burst strength responded when the concentration of carbachol changed, the numbers of spikes per ASC_E burst did not differ accordingly. DSC3’s differences are larger, but because resetting experiments showed that DSC neurons do not affect the pattern-generating circuit within their home module (Namba and Mulloney 1999), it is unclear how these changes in DSC3’s firing contribute to the changes in PS3’s phase relative to PS4. These results are consistent with the predictions drawn from the coupled-oscillator models of the swimmeret system (Skinner et al. 1997): that changes in local excitation operate on intrinsic period, or on both intrinsic period and intersegmental coupling. If coupling is affected, however, it is not the ASC_E and DSC neurons themselves that alter their firing. Instead, we propose that local excitation modulates the targets of these coordinating axons, the nonspiking neurons that decode their bursts of spikes, and so controls the context in which the information conducted by ASC_E and DSC is integrated. How might this work in cellular terms?

Axons of ASC_E neurons project anteriorly through the ventral nerve cord and synapse in each more anterior ganglion with a nonspiking commissural neuron called ComInt 1 (Fig. 13) (Mulloney and Hall 2003). DSC axons projecting posteriorly from more anterior ganglia also synapse with the same ComInt 1 (Fig. 1C). Because of the 0.25 difference in phase between modules in neighboring segments and the 0.5 phase difference between ASC_E and DSC neurons in each module, the bursts of spikes in ASC_E and DSC axons from neighboring ganglia arrive simultaneously in ComInt 1 (Mulloney and Hall 2003). Each burst of spikes in ASC_E and DSC causes a cluster of excitatory postsynaptic potentials (EPSPs) in ComInt 1 that sum to depolarize it and affect transmitter release onto ComInt 1’s targets. When the system is actively expressing the swim-
period, local excitation acted directly on ComInt 1 (Fig. 13 with a period identical to that of the motor pattern. Meret motor pattern, ComInt 1’s membrane potential oscillates out requiring significant changes in firing by ASC4 or DSC3.

Changes in cellular properties of neurons in oscillatory local circuits were modified, the intersegmental phase correlation with the periods expressed by segmental circuits demonstrated elegantly in the leech heartbeat system. There, variation in intersegmental phase observed in different individuals correlate with the periods expressed by segmental circuits when these circuits are uncoupled (Masino and Calabrese 2002). Moreover, when the intrinsic periods of individual segmental circuits were modified, the intersegmental phase differences between neurons in the stomatogastric ganglion involves differential effects of two transmitters, dopamine and histamine, on transmitter release from two different presynaptic neurons (Claiborne and Selverston 1984; Eisen and Marder 1984; Mulloney and Hall 1991). In that system, the phase shift arises from the different kinetics of the receptors for these two transmitters.

The importance of intrinsic periods for setting the phase differences between sets of coupled neural circuits has been demonstrated elegantly in the leech heartbeat system. There, variation in intersegmental phase observed in different individuals correlate with the periods expressed by segmental circuits when these circuits are uncoupled (Masino and Calabrese 2002). Moreover, when the intrinsic periods of individual segmental circuits were modified, the intersegmental phase differences between neurons in the stomatogastric ganglion involves differential effects of two transmitters, dopamine and histamine, on transmitter release from two different presynaptic neurons (Claiborne and Selverston 1984; Eisen and Marder 1984; Mulloney and Hall 1991). In that system, the phase shift arises from the different kinetics of the receptors for these two transmitters.

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The importance of intrinsic periods for setting the phase differences between sets of coupled neural circuits has been demonstrated elegantly in the leech heartbeat system. There, variation in intersegmental phase observed in different individuals correlate with the periods expressed by segmental circuits when these circuits are uncoupled (Masino and Calabrese 2002). Moreover, when the intrinsic periods of individual segmental circuits were modified, the intersegmental phase differences between neurons in the stomatogastric ganglion involves differential effects of two transmitters, dopamine and histamine, on transmitter release from two different presynaptic neurons (Claiborne and Selverston 1984; Eisen and Marder 1984; Mulloney and Hall 1991). In that system, the phase shift arises from the different kinetics of the receptors for these two transmitters.
also changed in predictable ways (Masino and Calabrese 2002).

In lamprey, the normal anterior-to-posterior progression of the swimming motor pattern was attributed to two factors: segmental differences in excitation and asymmetries in intersegmental projections of axon collaterals from neurons in segmental pattern-generating circuits (Kotaleski et al. 1999b; Matsushima and Grillner 1992). The intersegmental phase lags in the lamprey cord are more flexible than those in the swimmeret system. Nonuniform excitation will effectively reverse the normal phase progression, something we have never seen in the isolated swimmeret preparation. This flexibility may reflect an operating range of periods in these lamprey circuits that is twice as wide as the operating range we know in the swimmeret system (Matsushima and Grillner 1992; Mulloney 1997; Sigvardt and Williams 1996).

In two other well-studied systems, walking in insects and crustaceans (Büschges 2005) and swimming in leeches (Kristan Jr et al. 2005), cycle-by-cycle proprioceptive feedback seems to be required if the CNS is to express a coordinated intersegmental motor pattern with period and phasing in the normal behavioral range. In these walking systems, the concept of the intrinsic periods or even the definition of segmental oscillators is uncertain (Büschges 2005). The isolated leech nervous system will produce coordinated fictive swimming motor patterns, but the periods and intersegmental phase lags of these patterns are abnormally long. If the leech’s body wall remains connected to the CNS so that proprioceptive stretch receptors can affect the central circuits, both deficits in the motor pattern are corrected (Cang and Friesen 2000, 2002).

Starting with the observation that the strengths of PS bursts in each swimmeret module are determined by the excitation to which the module is subject, we have extended the effects of this local excitation to tuning the intrinsic period of the module’s pattern-generating circuit. The additional hypothesis about the mechanism that regulates intersegmental phase says that local modulation of the target module’s intrinsic period changes the context in which arriving coordinating information is integrated. If this hypothesis is correct, adjustment of each ComInt 1 neuron’s membrane potential about the threshold for transmitter release determines the phase differences of PS bursts in neighboring ganglia, but the bursts of EPSPs arriving in ComInt 1 entrain the local pattern-generating circuit and keep the period of all four ganglia the same.

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References

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