Bursts of Information: Coordinating Interneurons Encode Multiple Parameters of a Periodic Motor Pattern

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Mulloney, Brian, Patricia I. Harness, and Wendy M. Hall. Bursts of information: coordinating interneurons encode multiple parameters of a periodic motor pattern. J Neurophysiol 95: 850–861, 2006; doi:10.1152/jn.00939.2005. The limbs on different segments of the crayfish abdomen that drive forward swimming are directly controlled by modular pattern-generating circuits. These circuits are linked together by axons of identified coordinating interneurons. We described the distributions of these neurons in each abdominal ganglion and monitored their firing during expression of the swimming motor pattern. We analyzed the timing, the numbers of spikes, and the duration of each burst of spikes in these coordinating neurons. To see what information these neurons encoded, we correlated these parameters with the timing, durations, and strengths of bursts of spikes in motor axons from the same modules. During the power-stroke phase of each output cycle, the anterior-projecting neurons fired bursts of spikes that encoded information about the start-time, duration, and strength of each burst of spikes in power-stroke motor neurons from the same module. When the period and intensity of the motor output fluctuated, the bursts of spikes in these neurons tracked these fluctuations accurately. Each additional spike in these neurons signified an increase in the strength of the power-stroke burst. The posterior-projecting neurons that fired during the return-stroke phase encoded similar information about the return-stroke motor neurons. Although homologous neurons from different ganglia were qualitatively similar, neurons from posterior ganglia fired significantly more spikes per burst than those from more anterior ganglia, a segmental gradient that correlates with the normal posterior-to-anterior phase progression of limb movements. We propose that this gradient and a similar gradient in the durations of bursts in power-stroke motor neurons might reflect a hitherto-undetected difference in the excitation or intrinsic excitability of swimmeret modules in different segments.

INTRODUCTION

Effective behaviors demand that sequences of movements by different parts of the body be coordinated so that the mechanical forces that are the immediate causes of these behaviors come into play at optimal times. It is remarkable that for many behaviors, the CNS can achieve the core features of this coordination without peripheral feedback (Hughes and Wiersma 1960; Marder and Bucher 2003). In few cases, however, do we have any idea how the CNS does this, or even what components of the CNS are essential for this coordination. We recorded impulse traffic in coordinating neurons in the crayfish CNS during expression of the fictive swimming motor pattern that drives forward swimming. These neurons are necessary and sufficient for normal intersegmental coordination (Namba and Mulloney 1999). Our goal was to observe what the essential neurons do under steady-state conditions and when the system is perturbed, and to analyze how much of the system’s integrated performance can be explained by this impulse traffic.

Each of four abdominal segments—segments 2 through 5—in the crayfish, Pacifastacus leniusculus, bears a pair of limbs called swimmerets, eight limbs in all. During forward swimming, swimmerets beat in a coordinated cycle of movements that produce forward thrust. The normal sequence of swimmeret movements is driven by a motor pattern that starts with the most posterior pair and progresses anteriorly, with an intersegmental phase difference of about 0.25 that is unaffected by changes in the cycle’s period. The isolated ventral nerve cord can express the same motor pattern (Hughes and Wiersma 1960; Ikeda and Wiersma 1964). When the period of this fictive motor pattern changes in response to experimental changes in excitation, the isolated cord also maintains a stable intersegmental phase (Braun and Mulloney 1995; Mulloney 1997). How does the isolated CNS achieve this stable and predictable intersegmental coordination?

The swimmeret system is modular. These modules are anatomically separated in different ganglia and even on opposite sides of the same ganglion (Mulloney and Hall 2000; Paul and Mulloney 1985a,b). When impulse transmission is blocked, the local pattern-generating circuits in these modules can continue to oscillate, but with different periods and independent phases (Murchison et al. 1993). Each limb is innervated by its own set of motor neurons whose axons project directly from the ganglion to the limb (Mulloney and Hall 2000). Each module also has three kinds of coordinating interneurons that project to other ganglia in more anterior or more posterior segments (Stein 1971). These coordinating neurons occur as bilateral pairs: one on the left and one on the right side of each ganglion that innervates a functional swimmeret (Fig. 1A). During each cycle of motor output, these neurons export information from their home modules as bursts of spikes that occur at particular phases in the cycle (Fig. 1B). From ablation experiments (Namba and Mulloney 1999; Tschulun et al. 2001), we know that the information conducted by these coordinating axons is both necessary and sufficient to lock modules in different ganglia to the same period and to establish the characteristic posterior-to-anterior progression of power-stroke (PS) bursts that are the basis for the phase difference between movements of swimmerets on different segments.

In isolated CNS preparations subject to uniform pharmacological excitation (Braun and Mulloney 1995; Mulloney 1997),...
we compared firing of homologous neurons from left and right sides of the same ganglion and from different ganglia. Firing of homologous neurons from the same segment did not differ systematically in any parameter we measured. In contrast and to our surprise, neurons from more posterior ganglia fired significantly more spikes per cycle than did their anterior homologs in the same preparations. These intersegmental differences are the first known cellular differences in swimmeret modules that correlate with the posterior-to-anterior progression of the coordinated cycle of movements. We propose that these gradients in firing might reflect an underlying difference in either excitability or excitation that affects the pattern-generating circuits in swimmeret modules in different segments.

To discover what information coordinating neurons encode, we correlated the timing, durations, and numbers of spikes per burst in a coordinating neuron with parameters of the simultaneous firing of PS and return-stroke (RS) motor neurons and coordinating neurons from 1 module during 2 cycles of swimmeret activity. These statistics were calculated from measurements of 7 spontaneously active preparations bathed in normal saline. Each cycle is defined as beginning when the PS burst began. Each blue box begins at the mean phase at which bursts in PS or RS motor neurons began and ends at the mean relative duration of that burst. Right error bars show SD of durations. Left error bars of all but PS show SD of mean phases; left error bars for PS show SD of normalized period. Each red box shows these same parameters for ASC_E, ASC_L, and DSC.

FIG. 1. A: cartoon of locations of pairs of coordinating neurons (ASC_E, ASC_L, DSC) in a canonical abdominal ganglion, viewed from the dorsal side. Gray ovals show positions of the lateral neuropils (LN). PS, RS: power-stroke and return-stroke branches of paired swimmeret nerves. N1, N2: 1st and 2nd paired segmental nerve. A, anterior; P, posterior; L, left; R, right. B: box plots show phases and durations of bursts of spikes in the PS and RS motor neurons and coordinating neurons from 1 module during 2 cycles of swimmeret activity. These statistics were calculated from measurements of 7 spontaneously active preparations bathed in normal saline. Each cycle is defined as beginning when the PS burst began. Each blue box begins at the mean phase at which bursts in PS or RS motor neurons began and ends at the mean relative duration of that burst. Right error bars show SD of durations. Left error bars of all but PS show SD of mean phases; left error bars for PS show SD of normalized period. Each red box shows these same parameters for ASC_E, ASC_L, and DSC.

C: when PS motor neurons fired bursts of spikes, the ASC_E neuron in their module also fired, and the DSC fired bursts that alternated with simultaneous PS and ASC_E firing. This recording shows 2 short episodes of swimmeret activity recorded from an isolated nerve cord. Inset: normalized strengths of each PS burst on the same time axis as the recording.
METHODS

To expose the ventral nerve cord for recording, we first anesthetized a crayfish, *P. leniusculus*, by chilling on ice and exsanguinated it by transfusion with chilled saline. The normal saline was composed of (in mM) 5.4 KCl, 2.6 MgCl₂, 13.5 CaCl₂, and 195 NaCl buffered with 10 mM Tris base and 4.7 mM maleic acid at pH 7.4. The thorax was separated from the abdomen anterior to the fifth pair of walking legs. In each segment from thoracic segment 5 to abdominal segment 6, the ventral exoskeleton was cut with a scissors laterally at the boundary between the sternal ribs and pleural plates. The ventral exoskeleton with the ventral nerve cord and the superficial flexor muscles attached was lifted to expose the third paired segmental nerves (N3) in each segment (Mulloney et al. 2003). Each N3 was cut, and the exoskeleton was removed and pinned ventral side down in a Sylgard-lined dish. The first segmental nerves (N1) that innervate each pair of swimmerets run from the ganglion in that segment beneath the superficial flexor muscles to reach the swimmeret socket (Davis 1968). By severing the superficial muscles anterior to N1, we freed a long length of this nerve. The remaining segmental nerves were cut closer to each ganglion, between the sternal ribs and pleural plates. The ventral exoskeleton was cut with a scissors laterally at the boundary between the sternal ribs and pleural plates. The ventral exoskeleton with the ventral nerve cord and the superficial flexor muscles attached was lifted to expose the third paired segmental nerves (N3) in each segment (Mulloney et al. 2003). Each N3 was cut, and the exoskeleton was removed and pinned ventral side down in a Sylgard-lined dish. The first segmental nerves (N1) that innervate each pair of swimmerets run from the ganglion in that segment beneath the superficial flexor muscles to reach the swimmeret socket (Davis 1968). By severing the superficial muscles anterior to N1, we freed a long length of this nerve. The remaining segmental nerves were cut closer to each ganglion, freeing the ventral cord from the rest of the abdomen. We removed the cord to a smaller dish lined with transparent Sylgard and pinned it out in an orderly way using fine stainless steel pins. The anterior and posterior branches of each N1 were separated and pinned separately under slight tension. 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–A6.

Excitation of the system

Isolated ventral nerve cord preparations like these sometimes express the normal swimmeret motor pattern spontaneously (Ikeda and Wiersma 1964; Sherff and Mulloney 1996), but more often are silent. We elicited stable expression of the swimmeret motor pattern using cholinergic drugs, carbachol (RBL, Sigma) or pilocarpine (Sigma) (Braun and Mulloney 1993; Chrachri and Neil 1993); or the neuropeptide CCAP (Peninsula Laboratories, San Carlos, CA) (Mulloney et al. 1997). Drugs were dissolved in normal saline and bath-applied.

Electrophysiological recordings

In this species, the axons of PS and RS motor neurons that project from the CNS through N1 to one swimmeret are separated respectively into the posterior and anterior branches of N1 (Mulloney and Hall 2000). To record the firing of these motor neurons from each ganglion, we placed an extracellular stainless steel pin-electrode in contact with the appropriate branch and insulated it from the bathing saline with a small amount of petroleum jelly. Commonly, we recorded only from the PS branch because bursts of spikes in PS neurons provide a well-defined marker of the start of a new cycle of activity (Fig. 1B). In experiments where we intended to examine RS activity directly, we placed electrodes on the RS branches too.

The pairs of coordinating interneurons that originate in each ganglion A2–A5 (Namba and Mulloney 1999) project their axons from the CNS through N1 to one swimmeret are separated respectively into the posterior and anterior branches of N1 (Mulloney and Hall 2000). To record the firing of these motor neurons from each ganglion, we placed an extracellular stainless steel pin-electrode in contact with the appropriate branch and insulated it from the bathing saline with a small amount of petroleum jelly. Commonly, we recorded only from the PS branch because bursts of spikes in PS neurons provide a well-defined marker of the start of a new cycle of activity (Fig. 1B). In experiments where we intended to examine RS activity directly, we placed electrodes on the RS branches too.

The pairs of coordinating interneurons that originate in each ganglion A2–A5 (Namba and Mulloney 1999) project their axons from the lateral neuropils (Skinner 1985b) dorsally through the minuscule tract (MnT) (Skinner 1985a) toward the midline before entering the interganglionic connectives. We recorded action potentials in these axons extracellularly with a suction electrode placed on the MnT as it crossed dorsal to the lateral giant axon (Mulloney et al. 2003). All extracellular electrodes were connected to high-gain band-pass amplifiers. The output of these amplifiers was recorded simultaneously with a Neurodata 890 VCR recorder (Cygnus Technologies, Delaware Water Gap, PA) and a PC using a Digidata 1200B and Axoscope (Axon Instruments, Foster City, CA).

Because of the details of the anatomy of these coordinating neurons and the swimmeret system (Mulloney and Hall 2003; Namba and Mulloney 1999; Skinner 1985a,b), spikes recorded with suction electrodes positioned carefully on a left or right MnT could confidently be attributed to individual coordinating neurons originating in the module just below that MnT. In our prior papers (Mulloney and Hall 2003; Namba and Mulloney 1999; Tschulun et al. 2001), these attributions have repeatedly been confirmed by simultaneous microelectrode recordings from and dye-fills of the coordinating neurons.

Analysis

For each experiment, we digitized a continuous series of cycles of swimmeret activity in which the motor output from selected ganglia was recorded simultaneously with the firing of coordinating neurons originating in these same ganglia. In recordings from just one ganglion, each cycle was defined as beginning at the start of the PS burst from that ganglion. When we recorded from more than one ganglion, each cycle was defined as beginning at the start of the PS burst in the most posterior ganglion recorded, usually A5 (PS5). The start and stop of each burst of spikes in PS and RS recordings, which defined the burst duration, were measured using a digitizing tablet and our own analysis software (Mulloney and Hall 1987), or using Dataview 4 (http://www.st-andrews.ac.uk/~wjl/Dataview.html). The cycle period was the interval from the start of one PS burst to the start of the next PS burst. The phase of a burst in other neurons was defined as the time difference between the start of this burst and the preceding PS burst, divided by the period of that cycle. 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 the same series of cycles, the time of occurrence of each spike in a coordinating neuron was measured using either Clampfit (Axon Instruments) or the threshold-crossing algorithm of Dataview. For each neuron, the numbers of spikes per burst, the instantaneous spike frequencies, and the burst duration were calculated from these lists of spike times.

The descriptive statistics for the motor patterns themselves were calculated with our own software (Mulloney and Hall 1987). ANOVAs, Pearson correlation coefficients, and linear regressions were calculated using SigmaStat (SysStat Software, Point Richmond, CA).

Changes in strength of bursts of spikes

To measure differences in the strengths of a series of bursts, we used a low-pass digital-filtering method that measures the strength of a burst of spikes by calculating the area of a polygon derived from the squared voltages of each recorded burst and dividing this area by the independently measured burst duration (Mulloney 2005). To compare bursts recorded with different electrodes or in different preparations, the calculated strength of each burst, ₙ, was normalized to the strongest burst recorded by the same electrode in that experiment, ₙ = ₙ/ₙ. This yielded measures of strength that ranged from 0 ≤ ₙ ≤ 1.0. This method effectively expressed these changes as single numbers that were well correlated with the perceived differences in the original recording (Fig. 1C).

To compare variations in burst strength between different preparations, the range of each experiment’s measured strengths was normalized as (ₙ − ₙ)/ₙ and expressed as a number from 0 to 1. As variation increased in an experiment, this number approached 1.

RESULTS

Précis

Within each ganglion that innervates a pair of swimmerets, coordinating interneurons arise as bilateral, mirror-image pairs from a bilateral pair of neural modules (Fig. 1A). In each
module, the axons of two of these neurons, ASC_E and ASC_L, project to targets in more anterior ganglia. The third neuron in each module, DSC, projects to targets in more posterior ganglia (Mulloney and Hall 2003; Namba and Mulloney 1999). When an isolated CNS preparation expressed the normal swimmeret motor pattern, ASC_E, DSC, and sometimes ASC_L neurons fired bursts of spikes that began at characteristic phases in each cycle of motor output in their home ganglion (Fig. 1B). The information conducted by these axons as bursts of spikes is necessary to establish and maintain the phase difference between modules in neighboring ganglia (Namba and Mulloney 1999; Tschuluun et al. 2001). When periodic bursts of spikes in PS and RS motor neurons stopped or became disorganized, coordinating neurons also fell silent or fired regularly. The blue box-plots in Fig. 1B show the mean phases and relative durations, or duty-cycles, of the bursts of spikes in PS and RS motor neurons in one module of ganglion A4 during two cycles of the swimmeret motor pattern. The red box-plots show the mean phases and relative durations, or duty-cycles, of bursts of impulses in the same module’s ASC_E, ASC_L, and DSC neurons. Each coordinating neuron began to fire at a different phase in the cycle of activity. In some preparations, ASC_L neurons did not reach threshold in every cycle.

We described more precisely the distributions of these neurons in abdominal ganglia. We compared the performance of homologous neurons from different segments during active expression of the coordinated swimmeret motor pattern. Finally, we described how their firing changes with cycle-by-cycle variation in the motor output from their home modules.

Only ganglia that innervate functional swimmerets have operational coordinating neurons

In a chain of ganglia like the crayfish CNS, ganglia at the ends of the chain necessarily receive different information than do those in the middle. Only four of the six abdominal ganglia—A2, A3, A4, and A5—innervate swimmerets that are used for locomotion, but A2 and A5 are not at the ends of the chain. Axons of coordinating neurons from these four ganglia project anteriorly beyond A1 and posteriorly as far as A6 (Tschuluun et al. 2001). We considered the possibility that serial homologs of these coordinating neurons occurred in A1 and A6 and were active contributors to the system’s performance. We searched in the MnT of A1 and A6 for units that fired bursts of spikes with the same period as the ongoing swimmeret activity in intermediate ganglia.

We first located and recorded from coordinating neurons in one or more of the intermediate ganglia—A2…A5—in preparations that were expressing the normal swimmeret motor pattern. Then we searched systematically for equivalent activity in the MnT of A1 or A6, using the same suction electrode and procedure that had succeeded in the intermediate ganglia of the same preparation. In five experiments during which we identified one or more ASC neuron in more anterior ganglia, we found no ASC-like units in A6 (0/5 experiments). In four experiments during which we identified a DSC axon in A2 or A3, we found no DSC-like units in A1 (0/4 experiments; 3 female, 1 male crayfish). If serial homologs of the swimmeret coordinating neurons do exist in A1 and A6, they were not firing during these experiments and therefore are not necessary for the normal coordination of the system.

We also searched for DSC axons originating in A5, which we had not observed during the many experiments in which we had recorded ASC_E and ASC_L neurons originating in that ganglion (e.g., Fig. 3). In three experiments during which we first identified ASC units in A5 and DSC units in more anterior ganglia, we found no DSC-like units in A5 (0/3 experiments). Until now, we thought that modules in different ganglia had the same components, including the same complement of one ASC_E, one ASC_L, and one DSC neuron. Our failures to find active DSC neurons in A5 despite determined searches mean that this idea was wrong; A5 has no functional DSC neurons.

From this evidence, the intersegmental components of this coordinating circuit consists of four pairs of ASC_E neurons, four pairs of ASC_L neurons, and three pairs of DSC neurons. If this is correct, each swimmeret module is a target of three coordinating axons from neighboring modules, but which axons they are depends on the ganglion in which the module lies. In particular, the modules in A2 receive no input from DSC neurons, and the modules in A5 receive no input from ASC_E or ASC_L neurons.

Within each ganglion, homologous coordinating neurons fire similar bursts of spikes

In principle, ASC_E, ASC_L, and DSC neurons from opposite sides of the same ganglion are independent channels because the two swimmeret modules within each ganglion are anatomically separate (Mulloney and Hall 2000; Murchison et al. 1993) and neither the branches of these coordinating neurons nor their axons cross the midline in their home ganglion (Mulloney and Hall 2003; Namba and Mulloney 1999). We examined variability in firing of homologous neurons from the same and different ganglia. In six experiments, we recorded firing of ASC_E, ASC_L, and DSC neurons simultaneously from both sides of A2, A3, A4, or A5, in addition to the firing of PS motor neurons on both sides of the same ganglion (Fig. 2). The recordings in Fig. 2 are from an A4 ganglion, but the results of all six experiments were similar to this example. Comparing pairs of homologous neurons from the same ganglion, the numbers of spikes per burst did sometimes differ, but these differences were neither robust between preparations nor between different ganglia in the same preparation.

Posterior-to-anterior gradients in the numbers of spikes per burst

To explore the performance of the coordinating circuit under steady-state conditions, we recorded the firing of coordinating axons and PS bursts simultaneously from different ganglia in preparations exposed to uniform cholinergic excitation.

ASC_E neurons from A2, A3, A4, and A5 each fired bursts of spikes simultaneously with the PS bursts in their home ganglion (Fig. 3). In these simultaneous recordings, the periods of the cycles in all these ganglia were the same, but the ASC_E bursts from each ganglion differed quantitatively from those from neighboring ganglia (Table 1). The largest numbers of spikes per burst occurred in ASC_E units from A5. In each cycle, more anterior units fired progressively fewer spikes (ANOVA, P < 0.001 that they were the same). The durations of bursts in the more posterior ganglia were also longer (ANOVA, P < 0.001) than those of their more-anterior homologs (Table 1).
DSC neurons from A2, A3, and A4 fired bursts simultaneously with bursts in RS motor neurons in these ganglia; these bursts alternated with the PS bursts and ASC_E bursts in their home ganglion (Figs. 2 and 4). Despite the difference in their preferred phases and in the direction of their impulse traffic, these DSC bursts showed the same posterior-to-anterior gradients in numbers of spikes and durations (Table 2). The most posterior DSC, from A4, fired more spikes per burst than its more-anterior homologs, and these bursts had longer durations (ANOVA, \( P < 0.001 \) that they were the same).

Because mean burst durations increased with mean number of spikes, it seemed possible that these differences in numbers of spikes per burst would be accounted for simply by the longer burst durations, but this was not so. Using the mean numbers of spikes per burst and the mean durations of these bursts to calculate the mean spike frequencies within these bursts revealed that ASC_E bursts from A2 had a mean frequency of 34 Hz, whereas bursts from A5 had a mean of 51 Hz (Table 1). DSC bursts from A2 had a mean frequency of 31 Hz, whereas bursts from A4 had a mean of 40 Hz (Table 2).

To compare graphically the temporal structures of the output from local modules in different ganglia, we plotted the duty cycles of bursts in PS motor neurons and coordinating neurons and mean numbers of spikes per burst against the phase of PS firing in each ganglion (Fig. 5). The data used to construct this figure were simultaneous recordings of PS firing and ASC_E firing from four ganglia and simultaneous recordings of PS firing and DSC firing from A2, A3, and A4. They are a subset of that collected in Tables 1 and 2, selected because the periods of the expressed motor patterns were similar; these periods ranged from 0.485 to 0.545 s. Except for the absence of DSC from A5, the output of modules in different ganglia are qualitatively similar, but the posterior-to-anterior gradients in burst durations and numbers of spikes remain.

These segmental gradients persist when the period of the motor pattern changes

The results reported above were obtained from preparations that were uniformly excited with low levels (\( \leq 1.5 \mu M \)) of bath-applied carbachol (see Methods). Because it seemed possible that under a different regime these differences in numbers of spikes and durations might disappear, we raised the concentration of carbachol (\( \geq 3 \mu M \)) to change the period of the motor output (Mulloney 1997). In response, the periods expressed by each preparation shortened significantly. In six ASC_E experiments, the mean period during low-level excitation was 0.602 ± 0.015 s, whereas the mean period during high-level excitation was 0.448 ± 0.024 s. ASC_E neurons responded to this decrease in period by shortening their burst durations and increasing their firing frequencies (Namba and Mulloney 1999), but these changes had no effect on the relative segmental differences in numbers of spikes or durations of ASC_E bursts, which continued to differ as they had when periods were longer. A one-way ANOVA for spikes per burst gave \( F = 1.346 \) and for durations gave \( F = 261 \) \( (P < 0.001, \text{ Holm-Sidak } t \geq 3.488) \).

Changing the periods of the DSC preparations also did not affect the segmental gradients of DSC firing. In five preparations, the mean period during low-level excitation was 0.519 ±
spikes, durations, and phases of bursts of spikes in ASCE and Mulloney 1997). We analyzed the covariation of numbers of fire can vary in response to changes in excitation (Davis 1971; threshold and the frequencies with which individual neurons 2000), but both the numbers of motor neurons that reach by about 30 PS and 30 RS motor neurons (Mulloney and Hall system can vary from Burst of spikes in coordinating neurons encode details of the motor output from each module

The period of the motor pattern produced by the swimmeret system can vary from <0.5 to >6 Hz. Each limb is innervated by about 30 PS and 30 RS motor neurons (Mulloney and Hall 2000), but both the numbers of motor neurons that reach threshold and the frequencies with which individual neurons fire can vary in response to changes in excitation (Davis 1971; Mulloney 1997). We analyzed the covariation of numbers of spikes, durations, and phases of bursts of spikes in ASCE and DSC neurons with changes in simultaneous PS and RS bursts originating in the same modules.

ASCE. What information do ASCE neurons encode? Each burst of spikes in an ASCE neuron began simultaneously with a PS burst in the same module (Figs. 1C and 2). In preparations that were active only intermittently (Fig. 1C), ASCE neurons fell silent simultaneously with the PS units in their home module. Different preparations varied in the precise phase at which the ASCE bursts began, but phase did not vary with period (Fig. 6), and each preparation was less variable than the population as a whole. Therefore the start of each ASCE burst signaled the start of another PS burst.

Each ASCE burst lasted about as long as the simultaneous PS burst (Fig. 1B). In preparations in which the levels of expression of the motor pattern fluctuated, ASCE burst durations tracked changes in PS durations (Fig. 7). Their correlation coefficients, \( r \), ranged as high as 0.9, and the slopes of the regression of PS durations on ASCE durations approached 1.

If PS bursts also varied widely in the numbers of units firing and the numbers of spikes per unit (e.g., Fig. 1C), the numbers of spikes in the ASCE burst were highly correlated with these variations in PS strength (Fig. 7). This figure shows the relation of the numbers of spikes per burst in ASCE neurons from A2 and A3, recorded from two preparations in which PS burst strengths varied widely. Under these conditions, the correlation coefficients of the number of spikes and burst strength were high, and individual spikes in each neuron were meaningful. The 95% confidence limits (CLs) about the mean burst strengths show that each additional spike in this ASCE2 signaled about a 10% increase in strength. Similarly, each addition of two spikes to an ASCE3 burst signaled a 10% increase.

In those stable preparations where strengths of PS bursts varied little, the correlation of numbers of ASCE spikes and PS strength was not as strong (Fig. 8). This figure shows Pearson correlation coefficients of the numbers of spikes per ASCE burst with strengths of simultaneous PS bursts in 20 experiments, plotted as functions of the range of PS burst strengths. These correlation coefficients ranged from 0 to >0.9 and increased significantly as the range of PS strength increased. Strong correlations were restricted to modules whose motor output was varying widely. In the same preparations, the numbers of spikes per ASCE burst recorded simultaneously from other modules whose PS firing was less variable were less highly correlated.

In summary, each burst of spikes in an ASCE neuron encoded three parameters of the simultaneous multiunit PS burst in its home module: the beginning of a new PS burst, the duration, and the strength of that burst.

DSC. What do DSC neurons encode? In preparations that were expressing the coordinated swimmeret motor pattern (Figs. 2

<table>
<thead>
<tr>
<th>Parameter</th>
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<tr>
<td>Spikes per burst*</td>
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<td></td>
<td>A3</td>
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<td></td>
<td>A4</td>
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<tr>
<td>8.2 ± 0.90</td>
<td>14.8 ± 0.98</td>
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<tr>
<td>16.3 ± 0.70</td>
<td>17.4 ± 1.1</td>
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<tr>
<td>0.242 ± 0.035</td>
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<td>0.320 ± 0.024</td>
<td>0.340 ± 0.023</td>
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<tr>
<td>34.0</td>
<td>51.6</td>
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<td>6 Hz</td>
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<td>51.2</td>
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Results are means ± SD. \( N = 145 \) cycles from six experiments, whose mean period was 0.602 ± 0.015 s. *Each ganglion’s numbers were significantly different from the others: One-way ANOVA, \( P < 0.001 \), Holm-Sidak \( t > 6.25 \). For spikes per burst, \( F = 2.875 \). For durations, \( F = 364 \).
For spikes, preparations was 0.519 strength of that RS burst. (Fig. 8). In summary, each burst of spikes in a DSC neuron showed this difference (Fig. 7). These ASCE3 data and DSC3 experiment that also had a wide range of PS burst strengths numbers of spikes per burst in ASCE tracked changes in PS data were recorded simultaneously from the same module. The PS durations, not with RS durations (Fig. 9). In four experiments during which we recorded PS and RS firing simultaneously with DSC firing from the same module in A3 or A4, correlations of RS durations and DSC durations ranged from 0.175 (P = 0.03) to 0.535 (P < 0.001). Correlations of RS phases and DSC phases ranged from 0.476 to 0.878 (P < 0.001). These high correlations mean that each DSC burst reflects a systematic difference in the strengths of bursts in homologous motor pools in different ganglia. This suggestion is consistent with anecdotal observations that isolated nerve cords like those used here sometimes express the swimmeret motor pattern only in ganglia A3, A4, and A5, and that motor output from A2 is often intermittent and relatively weak. We do not have a quantitative method to compare the absolute strengths of multiunit bursts recorded by different electrodes, but comparisons of durations of PS bursts recorded simultaneously from different segments provides some insight into this question.

When the crayfish CNS is expressing the swimmeret motor pattern, all the active ganglia produce their motor output with the same period (e.g., Figs. 3 and 4). If the swimmeret modules in different segments were subject to the same levels of excitation and were otherwise identical, we would predict that the mean durations of PS or RS bursts recorded simultaneously in different segments would be the same. They were not (Fig. 5). PS2 duty cycles were significantly smaller than the rest: 0.368 versus 0.428 – 0.455 (ANOVA, F = 241, P < 0.001, Holm-Sidak t > 7.31). From this evidence, it is possible that segmental differences in the numbers of spikes per burst in homologous ASCE and DSC neurons reflect either a difference in excitation impinging on modules in different segments or an intrinsic difference in excitability of these different modules.

Do differences in numbers of spikes per burst in coordinating neurons from different ganglia correlate with strengths of bursts in motor neurons from these ganglia?

We found that the numbers of spikes in homologous coordinating neurons from different segments differed systematically (Tables 1 and 2) and that the numbers of spikes per burst in ASCE or DSC neurons were correlated with strengths of bursts in PS or RS motor neurons (Figs. 7 and 9; Table 3). These findings suggest that these differences in spike numbers reflect a systematic difference in the strengths of bursts in homologous motor pools in different ganglia. The durations and phases of DSC bursts were correlated with the durations and phases of bursts of spikes in RS motor neurons. In some preparations, the intensity with which RS motor neurons fired fluctuated. We observed two patterns of variation in RS firing: a simultaneous waxing and waning of burst intensity in both PS and RS neurons, as if the general level of excitation was being modulated, and a slow increase in intensity of firing in one pool while the intensity of the other pool decreased. If fluctuations in PS and RS firing were not themselves positively correlated, DSC durations increased with RS durations, not with PS durations (Fig. 9). In four experiments during which we recorded PS and RS firing simultaneously with DSC firing from the same module in A3 or A4, correlations of RS durations and DSC durations ranged from 0.175 (P = 0.03) to 0.535 (P < 0.001). Correlations of RS phases and DSC phases ranged from 0.476 to 0.878 (P < 0.001). These high correlations mean that each DSC burst could serve as an indicator of when the RS burst began and how long it continued.

In the same four experiments, correlations of the numbers of DSC spikes per burst with RS burst strength ranged from 0.384 to 0.700 (P < 0.002). However, when strengths of PS and RS changed in opposite ways, DSC firing was correlated with RS activity, not with PS activity (Fig. 9). Plots of PS burst strength versus numbers of spikes in ASCE and DSC from another experiment that also had a wide range of PS burst strengths show this difference (Fig. 7). These ASCE3 data and DSC3 data were recorded simultaneously from the same module. The numbers of spikes per burst in ASCE tracked changes in PS burst strength accurately, but DSC spike numbers did not. Furthermore, Pearson correlation coefficients for the numbers of DSC spikes per burst with PS strength ranged from ~0.6 to 0.8 and were unrelated to the range of variation in PS strength (Fig. 8). In summary, each burst of spikes in a DSC neuron signaled the beginning of a new RS burst and the duration and strength of that RS burst.

This interpretation is borne out by comparisons of regression coefficients for the numbers of ASCE or DSC spikes per burst on strengths of PS and RS bursts (Table 3). ASCE fired more spikes per burst as PS strength increased but DSC bursts often did not. On the other hand, DSC fired more spikes per burst as RS strength increased but ASCE did not always do so. These results mean that the number of spikes in a DSC bursts signals the strength of the simultaneous RS bursts, independent of the PS activity in the same module.
ASC<sub>L</sub>. The other anterior-projecting coordinating neuron in each module, ASC<sub>L</sub>, fires later in each cycle than do ASC<sub>E</sub> neurons (Fig. 1B), and ASC<sub>L</sub> bursts are briefer and have fewer spikes than ASC<sub>E</sub> bursts (Namba and Mulloney 1999). When the system is actively expressing a motor pattern with normal intersegmental coordination, ASC<sub>L</sub> neurons are often silent. In preparations where this is true, intracellular recordings from ASC<sub>L</sub> neurons in their home module reveal that their membrane potentials oscillate in phase with the motor pattern but do not reach threshold (Mulloney and Hall, personal communication). From this observation, ASC<sub>L</sub> cannot be necessary for normal coordination. Nonetheless, ASC<sub>L</sub> axons do conduct information about activities in their home modules to other segments. In six experiments, we correlated ASC<sub>L</sub> firing with parameters of simultaneous PS bursts. In three of these experiments, the expressed motor pattern was quite stable, and the resulting correlations of spike numbers, durations, and phases of ASC<sub>L</sub> bursts were both weak and variable. In the other three experiments, the motor pattern was more variable, and a stronger correlation of numbers of spikes per ASC<sub>L</sub> burst with strength of the simultaneous PS burst emerged. These correlations ranged from 0.200 to 0.613 (P ≤ 0.001 that ASC<sub>L</sub> and PS were uncorrelated). In these less stable preparations, each additional spike in an ASC<sub>L</sub> burst could be interpreted as signaling a significant increase in the strength of the PS burst (Fig. 10). However in these same preparations, correlations of numbers of ASC<sub>L</sub> spikes with PS durations remained weak, and correlations of ASC<sub>L</sub> phases with PS durations were not significant.

**DISCUSSION**

What information is encoded by a neuron’s train of action potentials, and how accurately does the neuron encode this information? These questions have been addressed repeatedly in sensory systems by recording responses either to repeated presentations of the same well-defined stimulus or to long runs of “white” random stimuli (de Ruyter van Steveninck et al. 1997; Meister and Berry 1999; Rieke et al. 1997; Warland et al. 1997). Deeper within the CNS, less is known about the performance of neurons that conduct information as trains of spikes, in part because the stimulus that drives a bout of firing is less well-defined. The problem of knowing what aspect of the stimulus is encoded can be side-stepped by repeatedly injecting well-defined currents through a microelectrode and recording the series of spikes that result (Beierholm et al. 2001; Mainen and Sejnowski 1995). We have taken an alternative...
approach: to record firing of identifiable interneurons during expression of behaviorally significant fictive locomotion and to correlate changes in the neurons’ firing with changes in that motor output.

Coordinating neurons from each module collectively encode a detailed cycle-by-cycle report on different phases of the module’s motor output that in an intact crayfish would drive swimmeret movements. Consider what can be learned about activities in a distant swimmeret module by listening to the train of spikes in the ASCE axon. Each burst of spikes in an ASCE neuron can inform an intelligent (matched) decoder about three features of PS activity in its home module: 1) when a new PS burst has begun; 2) how long this PS burst lasts; and 3) how strong this PS burst is. In a system where coordinated changes in force and timing are required for effective behavior, these details might be sufficient to permit accurate adjustment of the output from the target module in the same cycle. From a functional perspective, ASCE neurons can be viewed as reporters that encode information about the immediate state of PS motor neurons.

DSC neurons have a different task; they appear to be reporters that encode information about RS motor neurons. The tendency of DSC neurons to fire steadily at low frequencies when the system stops expressing the swimming motor pattern (Fig. 1C) is what we would predict if some RS neurons were also firing steadily, holding the swimmerets in a fully protracted position. Although in some experiments DSC firing was correlated with parameters of PS bursts, this was not always the case (Table 3). If we look at the ensemble of experiments (Fig. 8), DSC parameters were poor predictors of PS activity.

There is another sense in which ASCE and DSC neurons seem to encode different information. ASCE neurons are precise reporters of PS burst strengths and durations (Fig. 7), but we have not seen DSC neurons achieve similar fidelity for RS bursts (Fig. 9).

The structure of a train of spikes can encode more than a simple running average of the driving stimulus, and bursts of spikes have been suggested to constitute units of information that can be tuned to the resonance properties of the postsynaptic target (Izhikevich et al. 2003). Whether such tuning occurs in this system remains to be studied. However, the immediate results of this analysis show that the duration of an ASCE burst encodes a different parameter (PS duration) than the number of spikes in the burst (PS strength). In this sense,

![Figure 7](image-url) Numbers of spikes in an ASCE burst varied with strength of the simultaneous PS burst, but numbers of spikes in DSC neurons did not (for definition of burst strength, see METHODS). ASCE and DSC data were recorded simultaneously from the same preparation. r is the Pearson correlation coefficient; β is slope of the regression line; n is the number of data points. Mean points lacking 95% CL bars had just 1 or 2 measurements. CL, confidence limits.

| TABLE 3. Relations of numbers of spikes per burst in ASCE and DSC neurons to the strengths of simultaneous bursts in PS and RS motor neuron pools |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Slope of Regression, r | ASC E Spikes vs. PS Strengths | ASC E Spikes vs. RS Strengths | DSC Spikes vs. PS Strengths | DSC Spikes vs. RS Strengths |
| Positive | P < 0.05 | 11 | 1 | 6 | 4 |
| Zero | P ≥ 0.05 | 3 | 1 | 5 | 1 |
| Negative | P < 0.05 | 0 | 1 | 3 | 0 |

P, probability that slope of the regression equation equals zero; n, number of experiments: 14, 3, 14, 5, respectively. ASC E and DSC are coordinating neurons; PS, power stroke; RS, return stroke.

J Neurophysiol • VOL 95 • FEBRUARY 2006 • www.jn.org
each burst of spikes in an ASC
E or DSC neuron is not a unit of information.

When the swimmeret system responds to increased excitation, the firing rates of motor neurons and coordinating neurons increase but their burst durations and the cycle periods decrease. In our initial description of these coordinating neurons (Namba and Mulloney 1999), we noted that the increasing spike rate and the decreasing durations seemed to balance, so that the numbers of spikes per burst fired by a particular coordinating neuron did not change even though period changed substantially. In these recent experiments, this was true on average across numerous experiments, but not necessarily true in every experiment. Sometimes the numbers of spikes per burst increased as period shortened, sometimes it decreased, and sometimes it did not change. The differences averaged out.

In thinking about the organization of each modular pattern-generating circuit, we have distinguished the synaptic connections that drive PS motor neurons from those that drive coordinating interneurons (Jones et al. 2003; Skinner and Mulloney 1998). This distinction might be incorrect. The significant correlations between parameters of ASC
E and PS bursts (Table 3) might occur because within each module the PS motor neurons and the ASC
E neuron share the same synaptic input. Similarly, the DSC neuron and the RS motor neurons within each module might share common synaptic input that is different from that which drives PS and ASC
E neurons. Much of this input comes from nonspiking local interneurons whose

FIG. 8. Correlation, \( r \), between numbers of spikes in an ASC
E burst and strength of the simultaneous PS burst increased as range of strengths of PS bursts increased. This was not so for numbers of DSC spikes per burst. For each experiment, ranges of measured strengths of PS bursts, \( x_i \), were normalized to maximum strength in each recording, \( \text{Range} = (x_{\text{max}} - x_{\text{min}})/x_{\text{max}} \), and could vary from 0 to 1. \( r \) is the regression coefficient; \( \beta \) is slope of the regression line; \( P \) is probability that these parameters are uncorrelated. \( n = 20 \) experiments.

FIG. 9. Numbers of spikes in a DSC burst and burst duration varied with strength and duration of the simultaneous RS burst, not with the PS burst in the same cycle. \( r \) is the regression coefficient; \( \beta \) is slope of the regression line; \( P \) is the probability that these parameters are uncorrelated; \( n \) is number of data points.
processes are confined within the lateral neuropil, where both coordinating neurons and swimmeret motor neurons have most of their dendrites (Mulloney 2003; Paul and Mulloney 1985a; Skinner 1985b). If the presynaptic local neurons that drive PS bursts provide the same information to the ASCe neuron, the ASCe encoder would have only to match the responses of the PS motor neurons to this common information. This sharing would be achieved if the presynaptic neurons released transmitter from neighboring sites onto closely packed processes of both motor neurons and coordinating neurons, a synaptic arrangement that would be permitted by the cellular anatomy of these neurons in each module (Mulloney and Hall 2000; Namba and Mulloney 1999).

Differences in the strength of these correlations depended on the stability of the motor patterns expressed in different preparations (Fig. 8). In cases where there was little cycle-by-cycle variation, correlations were weaker because the relative influence of factors other than changes in PS or RS firing grew, but as the ranges of burst strength grew these correlations increased too.

The process of encoding information about the states of local swimmeret modules is restricted to ganglia that innervate limbs used for forward swimming

Ganglia A1 and A6 in the crayfish CNS are serial homologs of the intervening ganglia (Mulloney et al. 2003). In male crayfish of this species, A1 innervates a pair of modified swimmerets, both ASCe and DSC axons from other segments converge onto an identified local commissural neuron, ComInt 1, in each of their target ganglia (Mulloney and Hall 2003). Each ComInt 1 integrates the burst of excitatory postsynaptic potentials (EPSPs) caused by each burst of spikes in these axons and conducts that information to targets in one of the ganglion’s swimmeret modules. Small depolarizations of a ComInt 1 neuron, within the range of depolarizations caused by these bursts of EPSPs, excite PS units and inhibit RS units in the target module (Mulloney and Hall 2003), the same responses that stimulating the presynaptic axons elicit from the target module (Jones et al. 2003). We suggest that the coordinating neuron–ComInt 1 connections function as matched encoder–decoder pairs that can accurately transmit several features of each cycle of motor output.

Two features of the encoding system, the distribution of each type of coordinating neuron and the segmental differences in numbers of spikes per burst, mean that ComInt 1 neurons in different ganglia receive different ensembles of synaptic input. ComInt 1 neurons in A5 receive DSC2, DSC3, and DSC4; in A4 receive ASCe5, DSC2, and DSC3; in A3 receive ASCe5, ASCe4, and DSC2; and in A2 receive ASCe5, ASCe4, and ASCe3 (Fig. 5).

The timing and structure of bursts in individual coordinating neurons matters. The high correlation between numbers of spikes in an ASCe burst and the strength of the simultaneous PS burst implies that whenever a local module alters the strength of PS firing, other modules are immediately informed. In animals with a complex CNS like crayfish and mammals, it is conventional to think that single interneurons make at most tiny contributions to the integrated performance of the whole, and from this perspective, the strong influence of each ASCe neuron might seem exceptional. However, it is not unique. Stimulation of individual pyramidal neurons in rat motor cortex can cause discrete movements (Brecht et al. 2004), and individual propriospinal interneurons in turtle spinal cord encode specific details of the motor system’s activity during fictive scratching (Berkowitz 2001; Stein and Daniels-McQueen 2002). Whether the propriospinal neurons also can affect the system’s output is yet to be learned.

Sources of individual variation in swimmeret motor patterns

In the absence of sensory feedback, motor output from the isolated CNS can be bilaterally asymmetrical to an extent that would produce bizarre behaviors in an intact animal. Under these open-loop conditions, individual animals and preparations may produce characteristically asymmetric output that is promptly corrected if sensory feedback is restored (Wilson 1968). From this perspective, the left-right differences in numbers of spikes per burst, etc. that we observed in bilateral recordings from homologous neurons (Fig. 2) are not surprising and perhaps not important. These differences are signs of the intrinsic individual variation in performance of these local pattern-generating circuits, apparent here because proprioceptive feedback was eliminated (Bucher et al. 2005; Wilson 1968).
Significance of segmental differences in numbers of spikes per burst

One unanswered question about the performance of the swimmeret system is why does the most posterior pair of swimmerets always begin each cycle? There is no unique Zeitgeber in A5 (Ikeda and Wiersma 1964), and experiments that attempted to show differences in excitability between anterior and posterior ganglia failed to do so (Mulloney 1997). Therefore we were surprised to discover systematic differences in the numbers of spikes per burst in ASC and DSC neurons that originate in different ganglia. We attempted to excite all modules uniformly by desheathing the ganglia and exposing them to the same concentrations of drugs, but we do not know the drugs’ sites of action. Differences in numbers of spikes can be interpreted as a posterior-to-anterior gradient of burst strength in coordinating neurons that parallels the posterior-to-anterior phase progression in PS bursts. Because both ASC and DSC neurons conform to this pattern, although they differ in many other ways, we think this gradient is unlikely to be a quirk of a particular type of neuron. These quantitative differences might be caused either by an uncontrolled but systematic difference in excitation impinging on modules in different segments or by an intrinsic difference in the excitability of these modules that does not affect the motor pattern’s period (Mulloney 1997). Experimental tests of these possibilities may lead to an answer to the question.

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