

Quantification of Cardiac Sac Network Effects on a Movement-Related Parameter of Pyloric Network Output in the Lobster

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Thuma, Jeff B. and Scott L. Hooper. Quantification of cardiac sac network effects on a movement-related parameter of pyloric network output in the lobster. *J Neurophysiol* 89: 745–753, 2003; 10.1152/jn.00631.2002. Cardiac sac network activity (cycle period tens of seconds to minutes) has long been known to alter pyloric network activity (cycle period approximately 1 s), but these effects have not been quantified. Some pyloric muscles extract cardiac sac timed variations in pyloric motor neuron firing, and consequently produce cardiac sac timed movements even though no cardiac sac neurons innervate them. Determining pyloric behavior therefore requires detailed description of cardiac sac effects on pyloric neural output. Pyloric muscle activity correlates well with motor neuron overall spike frequency (OSF, number of spikes per burst divided by cycle period). We therefore quantified the effects of cardiac sac activity on the OSF of all pyloric neurons in the lobster, *Panulirus interruptus*. The ventricular dilator (VD) neuron had a biphasic response, with its OSF first increasing and then decreasing during cardiac sac bursts. Lateral pyloric (LP) neuron OSF decreased during cardiac sac activity. The pyloric (PY) neurons had two responses, with OSF either decreasing or increasing just after the beginning of cardiac sac activity. The pyloric dilator (PD) neurons had a triphasic response, with OSF increasing slightly at the beginning of cardiac sac activity, decreasing during the cardiac sac burst, and strongly increasing after cardiac sac activity ended. The inferior cardiac (IC) neuron had a biphasic response, with OSF decreasing at the beginning of cardiac sac activity and strongly increasing when cardiac sac activity ceased. These data provide the quantitative description of cardiac sac effects on pyloric activity necessary to predict pyloric movement from pyloric neural output.

INTRODUCTION

The pyloric network of the stomatogastric nervous system of decapod crustacea is a well-established model system for studying neural network activity (Harris-Warrick et al. 1992). The neural pattern the network produces, and many of the cellular and network properties underlying its activity, are well described. However, the movements the network produces remain unknown due to the internal position and small size of the pylorus. In the absence of other information, it was long assumed that the rapid (approximately 1 Hz), triphasic pyloric neural pattern resulted in a similarly rapid, triphasic movement pattern (Kennedy and Marder 1992; Maynard and Selverston 1975; Selverston et al. 1976; Turrigiano and Heinzel 1992).

However, recent work has shown that many pyloric muscles relax much too slowly to accurately follow the pyloric neural

pattern (Ellis et al. 1996; Harness et al. 1998; Koehnle et al. 1997; Morris and Hooper 1998a), and that some of these muscles extract low-frequency changes in pyloric motor neuron firing that occur in-phase with another stomatogastric neural network, the cardiac sac network (Morris et al. 1999, 2000). These data indicate that for some pyloric muscles, cardiac sac timed contractions are a large component of muscle output and thus add a previously unrecognized importance to these inter-network interactions.

Russell and Hartline (1981) first described the effects of an input to the pyloric network (the inferior ventricular nerve through fibers, *ivnTF*) that alters pyloric cycle period and pyloric dilator (PD) neuron spike frequency. These *ivnTF* are part of the cardiac sac network (Dickinson and Marder 1989) and make a variety of synapses onto pyloric network neurons (Claiborne and Selverston 1984; Sigvardt and Mulloney 1982). Although this work showed that cardiac sac activity profoundly alters pyloric network activity, these changes were not quantitatively described. We have quantitatively analyzed the effects of cardiac sac activity on one measure of neuronal firing—overall spike frequency (OSF, burst spike number divided by cycle period)—that strongly correlates with muscle activity (Morris and Hooper 1998a,b). This analysis shows that cardiac sac activity alters the activity of all pyloric neurons and provides the quantitative data necessary for predicting pyloric movement from pyloric neural activity.

A preliminary report of these data has appeared in abstract form (Thuma and Hooper 1999).

METHODS

Panulirus interruptus (500–1,000 g) were obtained from Don Tomlinson Commercial Fishing (San Diego, CA) and maintained in aquaria with chilled (13–15°C), circulating, artificial seawater. Stomachs were dissected in the standard manner (Selverston et al. 1976), and preparations were continuously superfused with 13–15°C *Panulirus* saline. Extracellular nerve recordings of pyloric network activity were made using stainless steel pin electrodes and an A-M Systems amplifier. Intracellular neuronal recordings were made with glass microelectrodes (filled with 0.55 M K₂SO₄, 0.02 M KCl, resistance 10–20 MΩ) and an Axoclamp 2A or 2B. Data were recorded on a MicroData Instruments DT-800 digital data recorder and transferred to computer using a Cambridge Electronics Design 1401plus interface. Data were analyzed using scripts written in Spike II (Cambridge Electronics Design) and plotted using Kaleidagraph (Synergy Soft-

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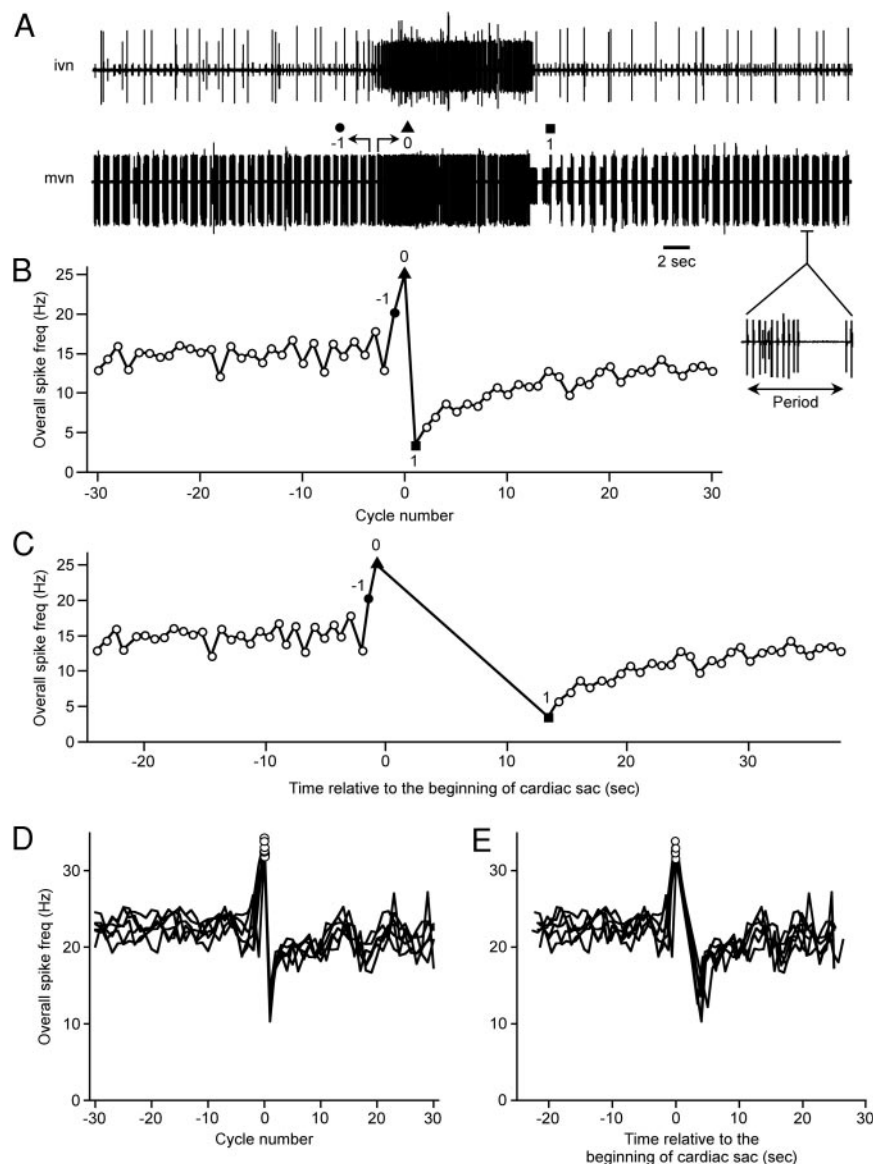


FIG. 2. Analysis of cardiac sac effects on VD neuron activity. *A*: extracellular recordings of ivnTF (medium-sized spikes) and VD neuron (large spikes) from the ivn and mvn, respectively. Cardiac sac activity was defined as beginning when the VD neuron began to fire tonically (*cycle 0*, triangle here and in *B* and *C*). Pyloric cycles were numbered sequentially (-1 , circle; 1 , square) relative to *cycle 0*. *Inset*: overall spike frequency (OSF) calculation. For each cycle period (as defined by the neuron's own activity), OSF equals burst spike number divided by cycle period. *B*: OSF vs. cycle number. Note that, since the varying durations of the various cycles are not being accounted for, this plot is not equivalent to OSF vs. time relative to cardiac sac beginning. *C*: OSF vs. time relative to cardiac sac beginning. *D* and *E*: plots of VD neuron OSF in 6 cardiac sac bursts vs. cycle number (*D*) and time relative to cardiac sac beginning (*E*). In both plots the *cycle 0* data points are marked with open circles. See METHODS for explanation of averaging in and across experiments.

occur 14 s after cardiac sac beginning). To obtain a complete set of data points at each time to average (that is, to have data from all experiments at *time 0*, 0.1, 0.2 s, etc.), we linearly interpolated (step size 0.1 s) between each experiment's data points.

Third, in different experiments the most salient response of the neuron might occur at different times after the beginning of cardiac sac activity (except for the VD neuron because its activity was used to define cardiac sac activity). For instance, for the PY neurons that responded to cardiac sac activity with excitation (Fig. 5*A*), in one experiment the increase might occur 0 s after the beginning of cardiac sac activity, but in another at 0.5 s after the beginning. Given the steepness of the responses of many of the pyloric neurons, this would have resulted in peak values in one experiment being averaged with baseline values of another. To overcome this difficulty, the appropriate value was added to all the time points of each experiment to make the most salient feature of their response occur at *time 0* (for the PD, IC, and excited PY neurons, the maximum of the excitation; for the LP and inhibited PY neurons, the minimum of the inhibition). This procedure resulted in the OSF plot of each experiment being put into register such that the most salient feature of the response occurred at the same time. The interpolated OSF values at each time point were then averaged. The average was then placed relative to cardiac sac

activity by averaging the values that had been added to each experiment's time axis to make the time of the most salient feature zero. For example, the four experiments that comprise the LP overall average had to be adjusted -0.54 , $+2.45$, $+0.66$, and -0.89 s to make the minima of the LP neuron response in each experiment's average occur at *time 0*. The mean of those times is 0.42 ± 1.5 s. Therefore the minimum of the LP inhibition occurred at an average time of 0.42 s after the beginning of cardiac sac activity. The horizontal error bar present in each neuron's average plot is the SD of these times and shows the variation with which the neuron response occurred relative to the beginning of cardiac sac activity. This procedure is the equivalent of the pattern-based analysis performed in Thuma and Hooper (2002). However, because the delay to most salient response feature was constant inside each individual experiment, it did not need to be performed on each cardiac sac burst, but only to average the data across experiments.

Fourth, due to the different VD neuron burst lengths during the cardiac sac bursts and the different pyloric cycle periods present in the various experiments, ± 30 cycles correspond to different duration ranges in different experiments. For instance, in *experiment A* ± 30 cycles might correspond to a duration range of -19 s to $+20$ s, while in *experiment B* ± 30 cycles might correspond to a duration range of

-25 s to +25 s. *Experiment A* thus has no data to average with the data from time -25 to -19 s, and time +20 to +25 s, in *experiment B*. Therefore in the across experiment averages, OSFs and relative times were averaged only for interpolation points that had data from at least four of the six experiments. The average baselines of all experiments were then averaged and this value was added to the normalized average OSF to give the true average OSF.

The statistical significance of pyloric neuron responses to cardiac sac activity were assessed by Student's *t*-test (paired data) comparison performed in Kaleidagraph between the data point at the time of interest (e.g., the peak VD neuron OSF in Fig. 3*B*) and an average of several seconds of control data before the cardiac sac burst.

RESULTS

We measured the OSF of all pyloric neurons for 30 pyloric cycles before cardiac sac activity, during the cardiac sac burst, and for 30 pyloric cycles after the burst, using the long, high-frequency burst induced in the VD neuron by *ivnTF* firing as a monitor of cardiac sac activity. The VD neuron had a biphasic response, with its OSF first increasing and then decreasing during cardiac sac bursts. Figure 3*A* shows an extracellular recording from the ventricular dilator nerve (*vdn*), a branch of the *mvn* containing only the VD neuron's axon; Fig. 3*B* shows the VD neuron across experiment OSF average ($n = 5$, 27 cardiac sac bursts). VD neuron OSF significantly increased from a baseline value of 16 to 25 Hz at the beginning of cardiac sac activity ($P < 0.004$).

VD neuron OSF decreased to 10 Hz at the end of cardiac sac activity, but this decrease was not significant when the OSF values of the individual experiments at the time of the minimum in the averaged data were compared with control values. However, in one of these experiments the cardiac sac burst was longer than in the others. As a consequence, the trough of this experiment was at a later time than those of the other experiments, and, the trough shown thus underestimates the true magnitude of the decrease (note large error bars on the trough

data points). For the statistical comparison of trough and control values, when the true minimum OSF of the experiment with the long cardiac sac burst was used, the trough differed from control ($P < 0.006$). VD neuron OSF did not return to baseline for 20–150 s (mean, 53 ± 33 s).

LP neuron OSF decreased during cardiac sac activity. Figure 4*A* shows an extracellular *lvn* recording of LP neuron activity (all non-LP neuron spikes have been removed for clarity). The bar above the trace represents cardiac sac activity. For the LP neuron, as well as the PY, PD, and IC neurons, *cycle 0* (asterisk) was defined as the first cycle after the beginning of cardiac sac activity. Figure 4*B* shows the LP neuron across experiment OSF average ($n = 4$, 22 cardiac sac bursts). LP neuron OSF sharply and significantly ($P < 0.006$) decreased from 12 to 7 Hz at the beginning of cardiac sac activity and returned to baseline values within 10 s after the end of cardiac sac activity.

Two PY neuron responses to cardiac sac activity (Fig. 5*A*, bars) were observed. The first was to fire a long, high-frequency burst during the cardiac sac burst (Fig. 5*A*, left); the other was to be inhibited during cardiac sac activity (Fig. 5*A*, right). Figure 5*B* shows the across experiment OSF averages for both types of PY neurons ($n = 5$, 8 cardiac sac bursts for the excited PY; $n = 4$, 22 cardiac sac bursts for the inhibited PY); the excited PY neuron's OSF increased from 8 to 12 Hz and the inhibited PY neuron's OSF decreased from 7 to 2 Hz during cardiac sac activity. Both changes were significant ($P < 0.007$ and $P < 0.03$, respectively).

The PD neurons also had two responses to cardiac sac activity (bars, Fig. 6, *A* and *B*). In the first, PD neuron cycle period and intra-burst spiking were altered during the cardiac sac burst but the pyloric rhythm nonetheless continued (Fig. 6*A*); OSF decreased from 11 to 4 Hz at the onset of cardiac sac activity and strongly increased to 22 Hz immediately after it (Fig. 6*A*, plot). Figure 6*B* shows the second response, in which the PD neurons stopped firing during cardiac sac activity and then fired a large rebound burst on cardiac sac cessation; OSF decreased from 10 to 3 Hz at the onset of cardiac sac activity and strongly increased to 20 Hz immediately after (Fig. 6*B*, plot). Thus, despite the different visual appearances of these two responses, with respect to the OSF changes at the start and end of cardiac sac activity, they were very similar.

VD, LP, and PY neuron cardiac sac responses correlated well with the beginning of cardiac sac activity. However, the responses of the PD and IC neurons did not. Figure 7*A* shows the responses of a PD neuron for all cardiac sac bursts in one experiment (left) and the average across the experiment (right) when plotted against time relative to the beginning of cardiac sac activity. The peaks and troughs for each trace are not well aligned. Consequently, averaging the data in this reference frame averaged peaks with troughs, which resulted in an underrepresentation of the individual responses and large SD (Fig. 7*A*, right). This was true of the IC neuron as well (data not shown). Using the end of cardiac sac activity as a reference point resulted in the peaks and troughs of the data aligning very well (Fig. 7*B*, left), and an average that better reflected the individual responses and had much smaller SD (Fig. 7*B*, right).

Figure 7*C* shows the PD neuron across experiment OSF average ($n = 6$, 32 cardiac sac bursts); both PD neuron response types (Fig. 6) were included in this average because of their similar OSF changes. PD neuron OSF in-

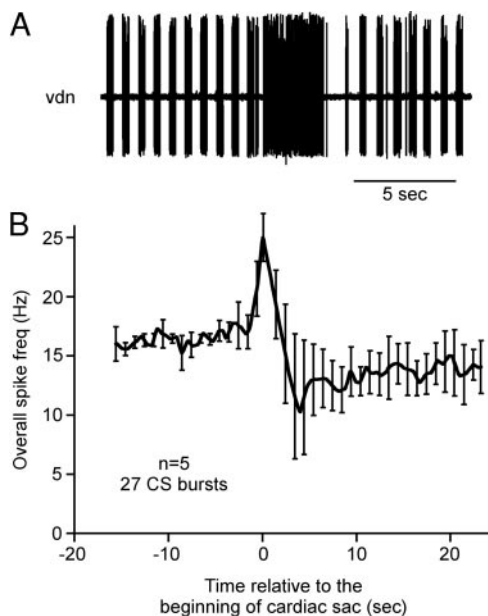


FIG. 3. VD neuron response to cardiac sac activity is an OSF increase followed by a decrease. *A*: extracellular VD neuron recording from the ventricular dilator nerve (*vdn*). *B*: across experiment average of VD neuron OSF vs. time relative to cardiac sac beginning.

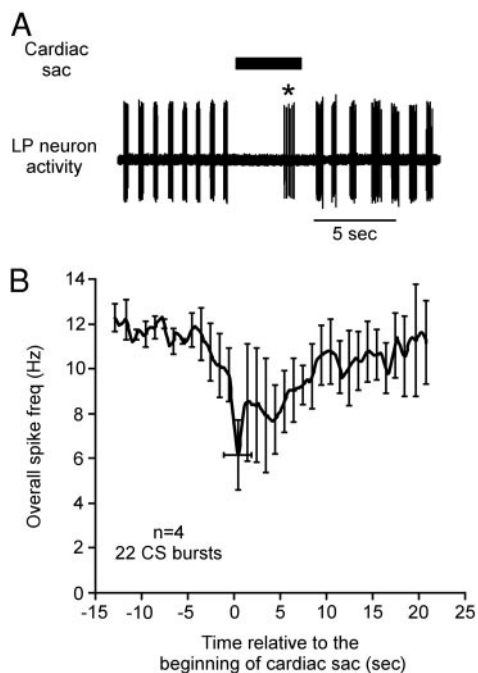


FIG. 4. LP neuron response to cardiac sac activity is an OSF decrease. *A*: extracellular LP neuron recording from the lvn. Bar represents cardiac sac burst. For this neuron and the PY, PD, and IC neurons, *cycle 0* (asterisk) was defined as the first cycle after the beginning of cardiac sac activity. *B*: across experiment average of LP neuron OSF vs. time relative to cardiac sac beginning.

creased from 9 to 11 Hz at the beginning of the cardiac sac burst, decreased to 7 Hz during the burst, and increased to 19 Hz after it. The late OSF increase was significant ($P < 0.002$), but the other two changes were not ($P < 0.42$, trough; $P < 0.16$, first peak). Unlike the VD neuron, this was not due to cardiac sac burst duration changes resulting in peaks, troughs, and control data being averaged together, but because in two experiments neither the first peak nor the trough were present. As noted in DISCUSSION, these changes

in PD neuron response presumably reflect the PD neuron's complex response to cardiac sac activity (Russell and Hartline 1981; Sigvardt and Mulloney 1982) and differences in ivnTF firing in the different experiments.

The IC neuron had a biphasic response, with its OSF decreasing at the beginning of cardiac sac activity and strongly increasing when cardiac sac activity ceased. Figure 8*A* shows an extracellular recording from the inferior cardiac nerve (icn), a branch of the mvn containing only the IC neuron's axon; Fig. 8*B* shows the IC neuron across experiment OSF average ($n = 5$, 27 cardiac sac bursts). IC neuron OSF decreased from 6 to 4 Hz at the beginning of the cardiac sac activity and increased to 11 Hz afterward, taking some 10–20 s to return to baseline. Both changes were significant ($P < 0.01$, $P < 0.0006$, respectively).

DISCUSSION

We performed a quantitative analysis of the variation of pyloric neuron OSF as a function of cardiac sac activity. Figure 9 summarizes the effects of cardiac sac activity on pyloric network activity; the PD and IC neuron traces have been time-shifted to compensate for using cardiac sac ending instead of beginning as their reference point. The numbers to the left of each trace are each neuron's average baseline OSF; the bar at the bottom is the average duration of the cardiac sac bursts from the 6 experiments used. The VD neuron has a biphasic response consisting of an initial OSF increase followed by a decrease. The LP and one type of PY neuron respond with a simple OSF decrease, and the other type of PY neuron with a simple OSF increase. The PD neuron has a triphasic increase-decrease-increase response and the IC neuron has biphasic response consisting of an OSF decrease followed by a prolonged increase. The return to baseline OSF levels for all neurons except the PY neurons is slow (10–20 s).

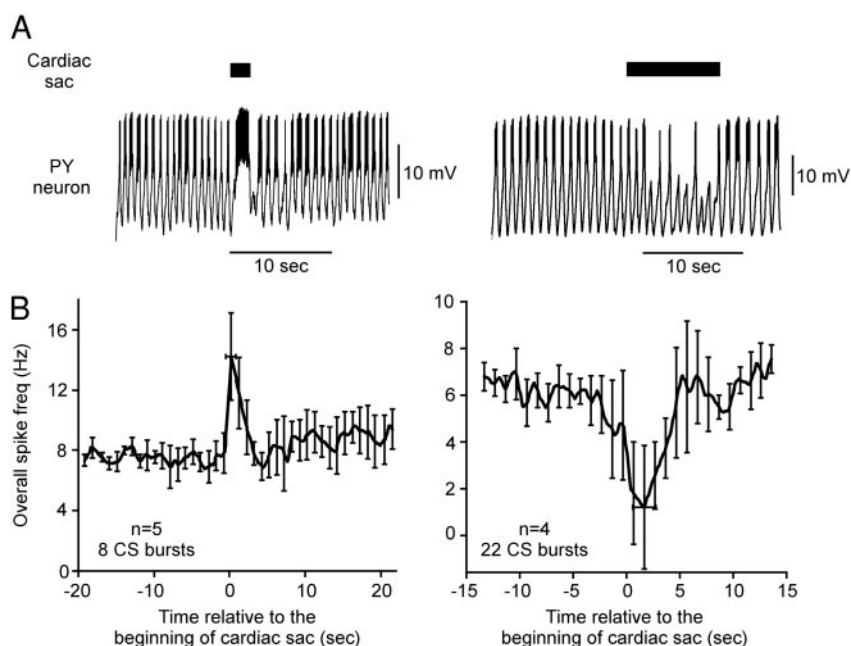


FIG. 5. PY neurons had two responses to cardiac sac activity, an OSF increase (*left*) and a decrease (*right*). *A*: intracellular PY neuron recordings. Bars represent cardiac sac bursts. *B*: across experiment averages of PY neuron OSF vs. time relative to cardiac sac beginning.

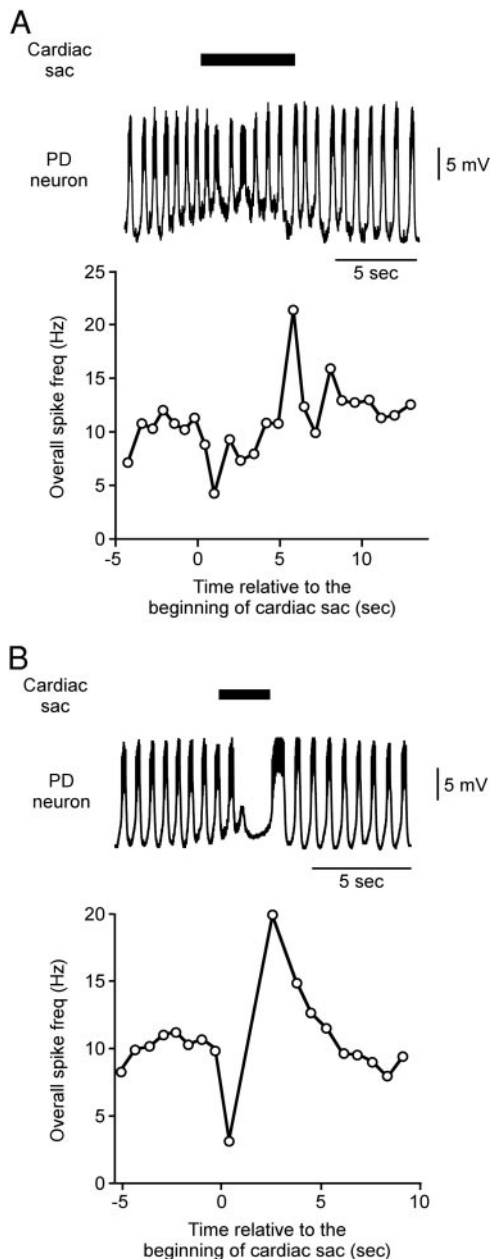


FIG. 6. PD neurons had 2 visually different, but OSF similar, responses to cardiac sac activity. *A*: PD neuron continues to burst throughout cardiac sac burst (bar). OSF decreases early in the cardiac sac burst and then increases. *B*: PD neuron stops firing during the cardiac sac burst (bar). OSF again decreases early in the cardiac sac burst and then increases. In both *A* and *B* the top panel is an intracellular PD neuron recording and the bottom panel is the corresponding OSF vs. time relative to cardiac sac beginning plot.

Choice of VD neuron as a monitor of cardiac sac activity

The VD neuron is a follower of cardiac sac activity, but does not help generate the cardiac sac rhythm. We nonetheless believe that, particularly since we are concerned here with the effects of cardiac sac activity on pyloric network output, using the VD neuron as a monitor of cardiac sac activity is highly appropriate. ivnTF bursts often begin with a variable period of low-frequency firing, and it is unclear when in this firing ivnTF spike frequency is sufficient to begin to alter pyloric activity. This concern is well demon-

strated in the ivn trace in Fig. 2A, in which the first ivnTF spike occurs almost 4 s before the VD neuron begins to fire tonically, and there is a 2 s period of low-frequency ivnTF firing in which, although VD neuron OSF increases somewhat (Fig. 2B, *cycle -1*), the VD neuron does not fire tonically. Comparison of different cardiac sac bursts shows considerable variation in the details of ivnTF firing at the beginning of ivnTF bursts, and in when the VD neuron begins to fire tonically relative to the first ivnTF spike. Using the first spike of ivnTF bursts as a monitor would thus induce considerable variation in which pyloric cycle was chosen as the 0 cycle, and the alternative of choosing a particular ivnTF spike frequency to define ivnTF burst beginning seemed arbitrary. Using a pyloric network neuron as a monitor of cardiac sac activity obviated these difficulties by ensuring that, regardless of the details of ivnTF firing, we were always measuring cardiac sac activity from the time at which cardiac sac activity was great enough to change pyloric activity. The VD neuron was an obvious choice due to its particularly well-defined response (tonic firing) to cardiac sac activity. It is clear, however, that this reference point is actually slightly subsequent to the true time at which ivnTF firing becomes sufficient to alter pyloric activity, as small changes in pyloric neuron activity occur before tonic VD neuron firing begins (Fig. 9), which correspond to the low spike-frequency portion of the ivnTF burst.

Cellular basis of cardiac sac effects on pyloric activity

In the pyloric network, the changes an input induces in a given pyloric neuron's activity often do not occur because the input has direct effects on the neuron, but rather because the input directly alters the activity of other pyloric neurons, and these changes in turn change the activity of nondirectly affected neurons (Hooper and Marder 1987; Hooper and Moulins 1990). Consideration of the known cardiac sac synaptic connections to the pyloric network, and of the pyloric network's connectivity pattern (Fig. 1), suggests that this may be the case for some cardiac sac effects as well. The cardiac sac effects described here on the VD and PD neurons are consistent with the strong excitatory synapses the ivnTF make on the VD neuron, and the complex excitatory-inhibitory-delayed slow excitatory synapses they make on the PD neurons (Claiborne and Selverston 1984; Russell and Hartline 1981; Sigvardt and Mulloney 1982). No direct connections from cardiac sac neurons to any other pyloric neuron, however, are known. Some of the cardiac sac effects on these other neurons can be explained as indirect consequences of changes in VD and PD neuron activity. For instance, the LP and PY neurons are inhibited by the VD, PD, and AB neurons (the AB neuron fires with the PD neurons). Thus LP and PY neuron inhibition during cardiac sac bursts may be due to increased inhibition from the excitatory portions of the VD and PD neuron responses to cardiac sac activity. The IC neuron inhibition during the cardiac sac burst may similarly be due to inhibition from the VD neuron, and its initial increase in firing after the cardiac sac burst from postinhibitory rebound. The source of its subsequent long-lasting increase in activity, however, is unclear. The excitatory PY neuron response is also difficult to explain as arising from an indirect, intra-pyloric network mechanism. It is thus possible

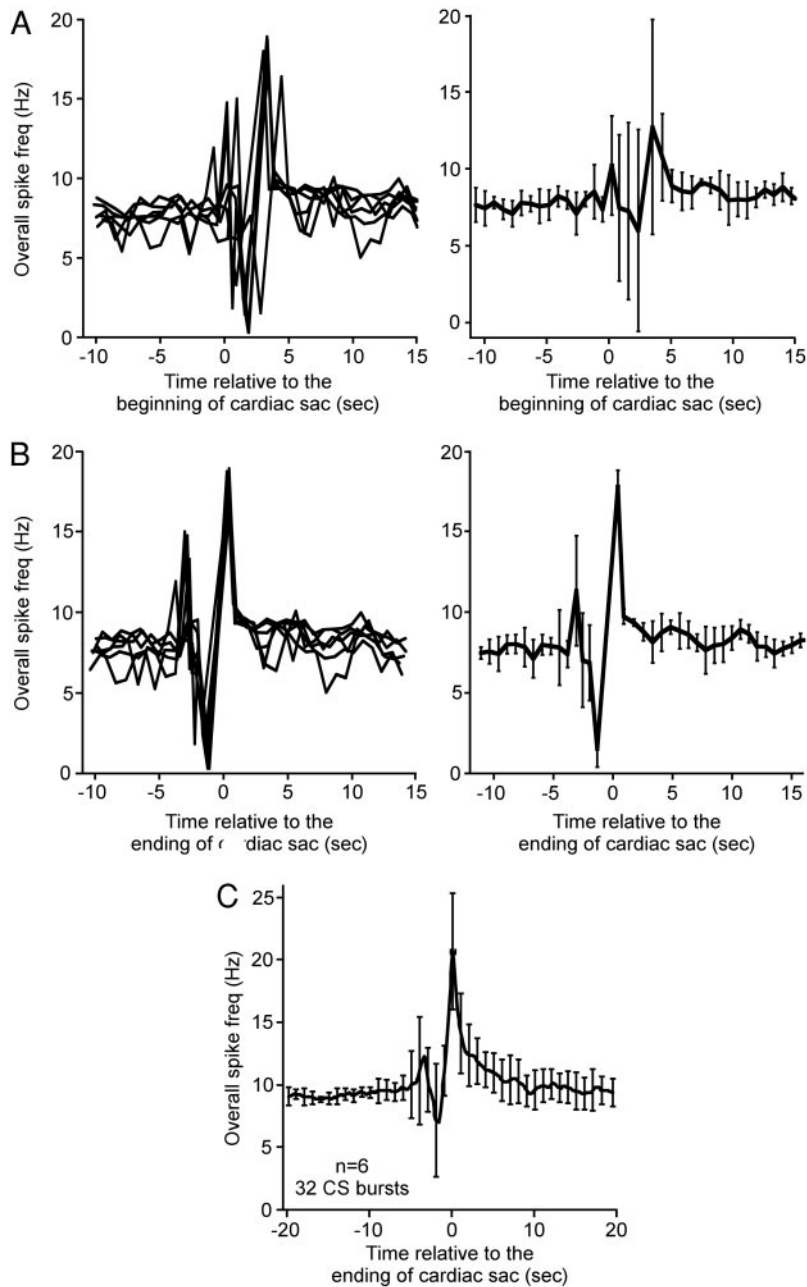


FIG. 7. PD neuron response is better averaged relative to cardiac sac burst ending. *A*: 6 PD neuron responses plotted vs. time relative to cardiac sac beginning (*left*) and their average (*right*). *B*: same 6 PD neuron responses plotted vs. time relative to cardiac sac ending (*left*) and their average (*right*). Note decreased SD, and more accurate capture of the shape of the individual responses, in *B* vs. *A*. IC neuron response was also more accurately captured when averaged relative to cardiac sac ending (data not shown). *C*: PD neuron response to cardiac sac activity is a triphasic increase-decrease-increase in OSF.

that as yet undescribed cardiac sac inputs exist to these neurons.

Multiple PY neuron responses

Although it is clear there are two PY neuron responses, we never observed a PY neuron switch its response type. However, we also never observed PY neurons of both response types in a single preparation. It is thus an open question whether the two responses observed arise from two classes of PY neurons or from preparation-specific changes in PY neuron responsiveness or cardiac sac input properties. Hartline et al. (1987) identified two PY neuron types, PY early and PY late neurons. We routinely identify, when possible, PY neurons using the Hartline et al. classification; the different PY neuron responses to cardiac sac input are

not a function of whether the neuron is a PY late or early neuron.

Multiple PD neuron responses

Unlike the PY neurons, there is no evidence that the two PD neurons differ in terms of cellular properties and synaptic output and input patterns. The two PD neuron responses therefore almost certainly arise either from the PD neurons being in different states in different preparations or from changes in ivnTF firing in different cardiac sac bursts. Sigvardt and Mulloney (1982) noted that PD neuron responses would vary in the manner shown in Fig. 6 depending on whether the ivnTF fired at low (Fig. 6*A*) or high (Fig. 6*B*) frequency. In our data set each PD neuron expressed only one type of response. However, in each of the exper-

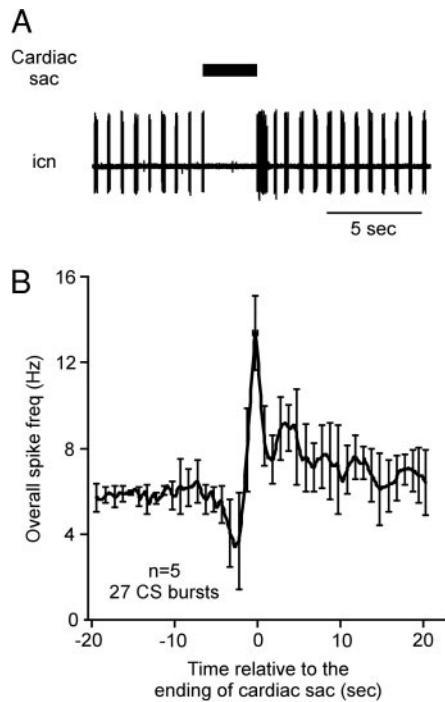


FIG. 8. IC neuron response to cardiac sac activity is a biphasic OSF decrease followed by an increase. *A*: extracellular IC neuron recording from the inferior cardiac nerve (icn). Bar represents cardiac sac burst. *B*: across experiment average of IC neuron OSF vs. time relative to cardiac sac ending.

iments cardiac sac burst spike frequency and duration (as reflected in VD neuron activity) were quite regular from burst to burst, and thus differences in PD neuron response inside an experiment would not be expected. Comparison across experiments of the VD neuron bursts associated with Fig. 6, *A* and *B*, responses showed no obvious correlation of VD neuron spike frequency or burst duration with response type. However, although VD neuron bursts are a good monitor of the beginning and ending of ivnTF activity, they may not be for ivnTF spike frequency. It is thus very possible that the two PD neuron responses arise from changes in ivnTF activity that VD neuron firing does not reflect.

On a more general level, it is important to stress again that, even though in the one case the PD neurons continue to burst during cardiac sac activity, and in the other the neurons stop bursting, OSF variation (and thus likely muscle activity, Morris and Hooper 1998a) is similar in both. This observation highlights the fact that responses that differ in some characteristics (for instance, burst patterning) may be similar in others (OSF), and that to assess properly whether different presynaptic activities will generate different postsynaptic responses it is essential to know which characteristics determine postsynaptic partner response. For example, in the case at hand, the PD neurons make synapses both onto pyloric muscles and on neurons inside the pyloric network. It is thus possible that the responses in Fig. 6, *A* and *B*, could be identical on the muscle level (because changes in OSF, not burst patterning, primarily determine muscle response to PD neuron activity), but nonetheless elicit different responses centrally (because the patterning of PD neuron activity, not just its OSF, helps determine response for postsynaptic neuronal targets).

Reference frame

We have noted elsewhere the importance of correct reference frame selection for accurate representation, and even simple recognition, of neuronal activity changes (Hooper 1997; Thuma and Hooper 2002). The need in the present data to use the ending of cardiac sac activity to accurately capture the changes in PD (Fig. 7) and IC neuron activity is another example of this phenomenon. Unfortunately, we know of no *a priori* method of correctly choosing a reference frame and have always relied on comparing individual and averaged data in different reference frames to select the best frame. This method is time-consuming and tedious. However, the improvement in data averaging produced by choosing the correct reference frame can be dramatic (*right panels* of Fig. 7, *A* and *B*) and suggests that reference frame selection should be a general concern in analyzing data where multiple reference frames are possible. On this issue, it is also important to stress that, of course, reference frame selection must be in some way related to the source that is inducing the neuronal change of interest. For instance, in the case at hand reference frame must be selected relative to cardiac sac burst, and it is essential if a repeating pattern of neuron response (e.g., the triphasic PD

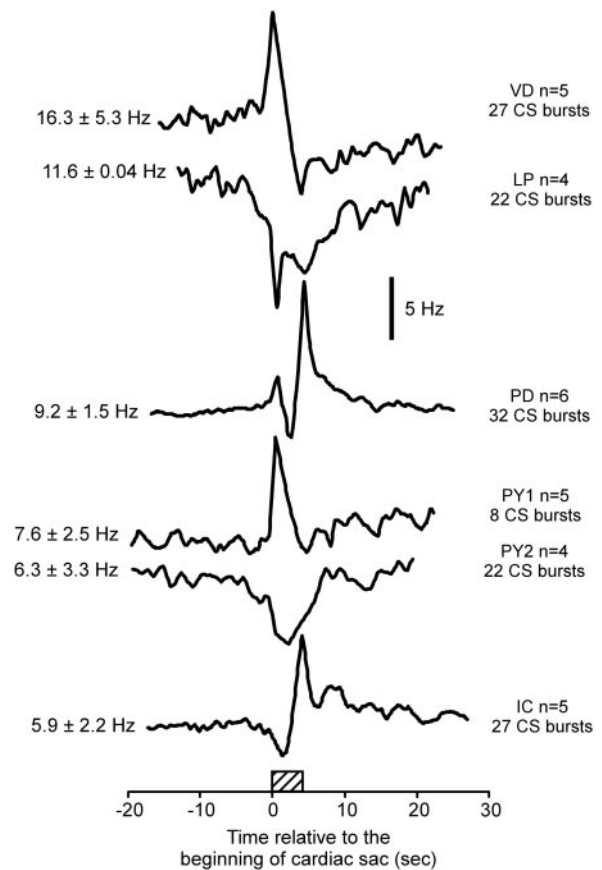


FIG. 9. Summary of all pyloric neuron responses to cardiac sac activity. PD and IC neuron responses have been time shifted to compensate for being averaged relative to cardiac sac ending. The VD neuron response is a biphasic OSF increase followed by a decrease. The LP and 1 type of PY neuron respond with an OSF decrease, and the other type of PY neuron responds with an OSF increase. The PD neuron has a triphasic increase-decrease-increase response and the IC neuron response is a biphasic OSF decrease followed by a prolonged increase. The return to baseline OSF levels for all neurons except the PY neurons takes 10–20 s.

neuron response) is used to identify the reference frame, that this response never occur without the source being active (which is true for all data reported here). That said, however, whether to use the beginning, middle, end, or other time relative to the burst to define the reference frame can only be chosen by determining in which reference frame the timing of the response is most repeatable.

On the neurobiological level, the fact that the PD and IC neurons are best analyzed with respect to the end of cardiac sac activity, and the other neurons with respect to the beginning of cardiac sac activity, presumably reflects some difference in the mechanisms of cardiac sac action. With respect to the changes in the various neurons that occur during the cardiac sac bursts, it is unclear to us why one frame would be better than the other. However, both the PD and the IC neurons, and only the PD and IC neurons, show large activity increases after the cardiac sac bursts. The mechanisms underlying these increases are likely different [for the PD neurons the increase is due to both postinhibitory rebound and an increase in PD neuron intrinsic "burstiness," Russell and Hartline (1981), whereas for the IC neuron it may be due to only postinhibitory rebound]. However, in each case neither increase can be fully expressed until the cardiac sac burst is over (because of the ivnTF inhibition for the PD neurons and the VD neuron inhibition for the IC neurons). With respect to the late increase in PD and IC neuron OSF, it is thus not surprising that cardiac sac ending is a better reference frame.

Implications for relating pyloric neural activity to pyloric behavior

The pyloric neural network is among the best understood of all neural networks, and yet, due to the internal location of the lobster stomach and small size of the pylorus, the behavior it generates is almost completely unknown. Without technical advances in noninvasive imaging, this inability to directly measure pyloric behavior is likely to continue. As such, at present the only means by which pyloric neural activity and behavior are likely to be linked is by computer modeling of the pyloric anatomy and by using data on pyloric muscle response to neural input to simulate pyloric motions (Geier et al. 2002; Hoover et al. 2001; Morris and Hooper 1998a,b, 2001; Morris et al. 1999, 2000). We have shown that the changes in pyloric neuron OSF reported here are sufficient to change pyloric muscle contractions (Morris et al. 1999, 2000) and are developing models of pyloric muscles that predict contraction in response to physiological neuronal activity (Geier et al. 2002). The present quantitative description of cardiac sac effects, and earlier work quantifying gastric mill effects, on pyloric neuronal activity (Thuma and Hooper 2002) provide the data necessary to drive these models and thus are necessary steps toward linking neuronal activity and behavior in this system.

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