

Phase Maintenance in the Pyloric Pattern of the Lobster (*Panulirus interruptus*) Stomatogastric Ganglion

SCOTT L. HOOPER

Neurobiology Program, Department of Biological Sciences, Ohio University, Athens, OH 45701

Hooper@ohiou.edu

Received August 14, 1996; Revised October 9, 1996; Accepted October 15, 1996

Action Editor: Eve Marder

Abstract. The extent to which individual neural networks can produce phase-constant motor patterns as cycle frequency is altered has not been studied extensively. I investigated this issue in the well-defined, rhythmic pyloric neural network. When pyloric cycle frequency is altered three- to fivefold, pyloric inter-neuronal delays shift by hundreds to thousands of msec, and all pyloric pattern elements show strong phase maintenance. The experimental paradigm used is unlikely to activate exogenous inputs to the network, and these delay changes are thus likely to arise from phase-compensatory mechanisms intrinsic to the network. Pyloric inter-neuronal delays depend on the time constants of the network's synapses and of the membrane properties of its neurons. The observed delay shifts thus suggest that, in response to changes in overall cycle frequency, these constants vary so as to maintain pattern phasing.

Keywords: stomatogastric system, pyloric neural network, crustacea (lobster, *Panulirus interruptus*), motor pattern, phase maintenance

Introduction

Organisms produce functional versions of most rhythmic motor patterns over a wide frequency range. For certain motor patterns (particularly those with distinct power and return strokes) the duration of only one phase of the pattern is varied as cycle frequency changes (Grillner, 1981). In other motor patterns (e.g., lamprey swimming (Cohen et al., 1992)), pattern phase is highly maintained. However, these motor patterns are produced by chains of coupled oscillators, and whether (and how) individual neural networks can maintain phase constancy as overall cycle frequency changes is less understood.

This question is interesting because phase maintenance requires that the time between presynaptic activity and postsynaptic response must proportionally change as overall cycle frequency changes for all synaptically connected neurons in the network. This

time depends on synaptic delay, passive (RC) postsynaptic neuron membrane properties, and active (voltage and/or second messenger activated) postsynaptic neuron membrane conductances. Phase maintenance thus requires that one or more of these processes precisely change such that pre- to postsynaptic time varies proportionally with presynaptic neuron cycle frequency.

Do any of these processes have characteristics such that one would necessarily expect them to change appropriately so as to maintain phase? Synaptic delay is largely determined by Ca^{++} entry into the presynaptic terminal, vesicle fusion, and transmitter diffusion across the synaptic cleft and thus presumably varies relatively little as presynaptic activity changes. With respect to passive membrane properties, changes in presynaptic activity that alter the amplitude of the postsynaptic potential will alter (in the absence of active conductances) the time it takes the postsynaptic neuron to subsequently reach any given membrane

potential. However, there is no *a priori* reason these time variations should vary proportionally with presynaptic neuron cycle frequency. Due to the dependence of active conductances on voltage or second messenger concentrations, the timing of postsynaptic responses that depend on these conductances can shift by hundreds of milliseconds as presynaptic activity changes (McCormick and Huguenard, 1992). To maintain phase these conductances would need to change precisely so as to vary inter-neuronal delay proportionally as cycle frequency changes. Again, there is no *a priori* reason this should occur.

However, in the absence of appropriate changes in inter-neuronal delay, neural networks cannot produce the “same” pattern at different frequencies. Consider the neural network shown in Fig. 1A. This network

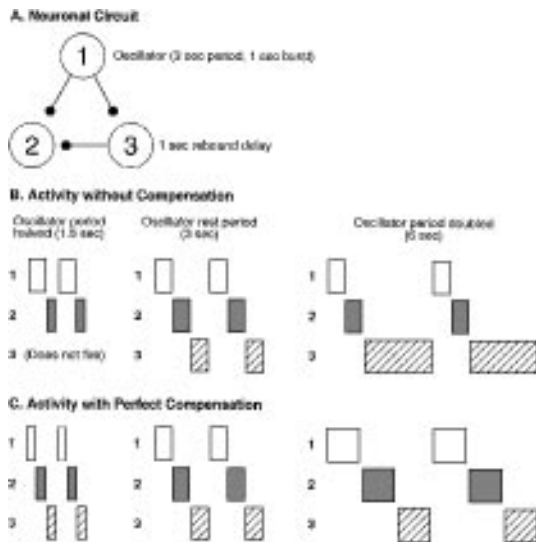


Figure 1. Phase constancy is not an automatic property of networks with complex active conductances. **A:** Neuron 1 is an oscillator neuron with a 1 sec burst duration and 3 sec period. Neuron 1 inhibits neurons 2 and 3; neuron 3 inhibits neuron 2. Neurons 2 and 3 depolarize and fire after inhibition due to postinhibitory rebound. Neuron 3 has additional properties that delay its firing for 1 sec after inhibition. **B:** Without compensatory mechanisms, the pattern is not maintained as period changes. Middle panel: activity at rest. Neuron 2 fires immediately after neuron 1's burst, and neuron 3 fires 1 sec later. Neuron 3 inhibits neuron 2 and is itself inhibited by the next neuron 1 burst. Each neuron thus fires for 1 sec. Right panel: activity when neuron 1's period is doubled. Neurons 1 and 2 still fire for 1 sec, but neuron 3 fires for 4 sec since neuron 1's next burst does not occur until then. Left panel: network activity with neuron 1's period halved. The next neuron 1 burst inhibits neuron 2 after only 0.5 sec, and hence neuron 3 cannot fire at all. **C:** Network activity with perfect compensation. If neuron 1's burst and interburst intervals, and neuron 3's rebound delay, proportionally vary as period changes, the network's pattern is preserved.

produces a rhythmic output as a result of the active conductances of its neurons and the network's synaptic connectivity. Neuron 1 is an endogenous burster with a 3 sec period and a 1 sec burst duration. Neurons 2 and 3 are plateau potential neurons (Russell and Hartline, 1982) with postinhibitory rebound. This means they respond to a release from inhibition by depolarizing above rest to a quasistable, suprathreshold membrane potential, at which they remain until they are next inhibited. Neuron 3 recovers from neuron 1's inhibition one second more slowly than neuron 2. Thus, neuron 2 fires immediately at the end of neuron 1's burst, and neuron 3 begins to fire one second later. Neuron 3 shuts off neuron 2, which thus has a 1 sec burst duration. The next neuron 1 burst turns off neuron 3, and so neuron 3 also fires for 1 sec.

The middle panel of Fig. 1B shows a block diagram of this network's output. What happens if current is injected into neuron 1? Depending on the mix of active conductances in their membranes, endogenous oscillator neurons can alter their cycle period in a variety of ways (e.g., burst duration remains constant while interburst interval alters, burst duration alters while interburst interval remains constant, both burst duration and interburst interval proportionally alter). For the purposes of this example, assume that neuron 1 alters cycle period by changing only its interburst interval (when isolated from the network, the pyloric network pacemaker neuron responds in this manner (Abbott et al., 1990)).

If the activation level of the conductances that underlie the active responses of neurons 2 and 3 achieves steady state within the 1 sec inhibition, then the firing delays of neurons 2 and 3 will not change when the cycle period is doubled to 6 sec (right panel, Fig. 1B), and hence neuron 2 will still burst for only 1 sec. However, neuron 3 will fire until it is inhibited by the next neuron 1 burst and thus fire for 4 secs. Alternatively, if the period is halved to 1.5 sec (left panel, Fig. 1B), the next neuron 1 burst will occur only 0.5 sec after the end of the first. This burst will shut off neuron 2, which will therefore fire only 0.5 sec, and neuron 3 will not fire at all. Clearly, without compensatory mechanisms the pattern is not maintained as period changes, and thus if this was a motor pattern, the movements generated at different cycle frequencies would be very different.

What compensatory mechanisms are needed to maintain phase as cycle period changes (Fig. 1C)? First, the oscillator neuron must compensate: its burst

duration must vary proportionally with its period. Second, since neuron 3 shuts off neuron 2 and thus determines neuron 2's burst duration, neuron 3's delay following neuron 1 must also compensate. For instance, during high-frequency oscillation neuron 1's burst duration would be 0.5 sec. To maintain the pattern, neuron 3's delay must halve so that neuron 3 inhibits neuron 2 after 0.5 sec.

The response of the endogenous oscillator neuron to injected current, and the delay shifts of the follower neurons, depend on the mix of active conductances present in these neurons. It follows that studying individual neural networks that produce phase-constant outputs could give insight into what sets of conductances can give rise to such specific neuronal temporal properties. The literature suggests that at least three individual neural networks—crab gill bailer (DiCaprio, 1997), leech heartbeat (Arbas and Calabrese, 1984), pyloric (Eisen and Marder, 1984; Abbott et al., 1990)—may maintain phase as cycle frequency is altered *in vitro*. However, to my knowledge a thorough description of phase regulation as frequency is altered has not been carried out in any neural network that is well understood on the cellular level. I therefore characterized pyloric network activity as pyloric frequency was changed and report that this network shows significant phase maintenance over a three- to fivefold frequency range.

Some of this work has been previously published in abstract form (Hooper, 1993).

Methods

Preparation

Male and female *Panulirus interruptus* (500 to 1000 g) were obtained from Don Tomlinson (San Diego, CA) and maintained in aquaria with circulating artificial sea water at 12°C. Stomatogastric nervous systems were dissected and prepared for recording using standard techniques (Selverston et al., 1976). All preparations were continuously superfused (3 to 10 ml/min) with chilled (12° to 15°C) *Panulirus interruptus* saline (Selverston et al., 1976).

Electrophysiology

All electronics and techniques were standard. Extracellular nerve recordings were made with stainless steel pin electrodes and an A-M Systems differential

amplifier. Intracellular recordings from, and current injection into, neuronal somata were made with glass microelectrodes filled with 0.55M K₂SO₄, 0.02M KCl (resistance 10 to 20 MΩ) and an Axoclamp 2A. Neurons were identified by matching intracellular spikes with extracellular activity in the appropriate nerves. Signals were displayed on an Astro-Med MT9500 electrostatic chart recorder and recorded on a Microdata DT-800 digital tape recorder.

Cycle Period Control

Period was altered by current injection into the anterior burster neuron soma; current levels were maintained for at least 10 cycles. Period was altered three- to tenfold (depending on the preparation) until the pattern was disrupted (until one of the neurons failed to fire with the pattern). The stomatogastric nerve was intact and unblocked so that all pyloric neurons would be active (Russell, 1979). Data were not collected during or immediately after cardiac sac (another stomatogastric network that alters pyloric activity) bursts.

Data Analysis

The pyloric dilator (PD) neuron burst beginning (PDB) was used as the reference point for cycle period and the delays of the other pyloric pattern elements. Delays were measured for all 9 pattern elements (PD neuron burst ending (PDE) and beginning and ending of the inferior cardiac (IC; ICB, ICE), lateral pyloric (LP; LPB, LPE), pyloric (PY; PYB, PYE), and ventricular dilator (VD; VDB, VDE) neuron bursts either by hand or using Spike II scripts after transfer (Cambridge Electronic Design 1401 laboratory interface) to a Gateway 2000 P5. Phase was calculated on a Macintosh Quadra 950 using Kaleidagraph (Synergy Software) scripts. Statistical analyses (two-tailed Student's *t*-test) were performed using JMP Statistical Visualization (SAS Institute) software. The data shown here are from 13 experiments and comprise some 16,000 points. Two steps were taken to ensure data validity. First, all computer measurements were checked by two people. Second, for each preparation the data of each type (e.g., ICB delay) were plotted over the entire frequency range and visually checked for outliers. Data giving rise to outliers were then verified.

In four preparations either or both the PY or IC neurons were silent or only weakly active, and in one preparation PD neuron burst duration did not change

as the cycle period was altered. These changes from “normal” pyloric activity were associated with two alterations in the activity of the other pyloric neurons. First, in preparations with weak or no PY or IC neuron activity, VDB delay and phase did not vary linearly with period (see Figs. 4C, D). VDB data from these preparations are shown as fine dotted lines in Figs. 5 and 6, and were excluded from grouped analyses (Fig. 7). Second, although their delays varied linearly, the activity of certain elements in these preparations was nonetheless altered from that normally observed (e.g., when PD neuron burst duration was constant, LPB delay was abnormal). Data for pyloric elements from these preparations were excluded from grouped analyses if the slope or intercept of the fitted lines in the delay versus period, or phase versus frequency, representations was an extreme and was more than twofold different from the group average. Excluded data are shown as fine dotted lines in Figs. 5 and 6.

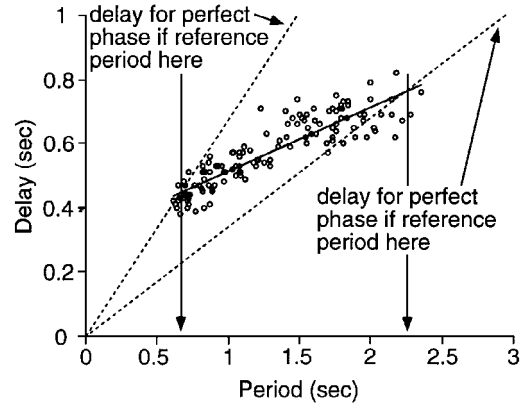
Perfect and Constant Delay Lines; Comparison of Data Fits to These Lines

As is shown in the Results, most pyloric pattern elements do not perfectly maintain phase. It was therefore desirable to find methods to quantify the extent to which pyloric phase regulation differed from perfect compensation. In order to provide a measure of the degree of phase maintenance that did occur, it was also desirable to compare pyloric phase compensation to the phase changes expected if delays remained constant as frequency was altered. These comparisons are not straightforward because the slopes of some of these theoretical lines (in delay versus period representations, the “delay for perfect phase” line; in phase versus frequency representations, the “constant delay” line) depend on which cycle period is chosen as reference period.

Consider the LPE data from one preparation shown in Fig. 2A (delay versus period representation). The data are fit well with a linear regression; how different is this line’s slope from the slope of the line expected if phase were perfectly maintained? To maintain phase perfectly, delay would proportionally decrease to zero as cycle period decreases to zero. Thus, if the fitted line is $y = a + mx$, the slope of the “delay for perfect phase” line referenced to a cycle period of x_0 would be

$$\Delta y / \Delta x = [y(x_0) - y(0)] / (x_0 - 0) = (a + mx_0) / x_0.$$

A. Slope of “Delay for Perfect Phase” Line Depends on Reference Period



B. Slope of “Phase with Constant Delay” Line Depends on Reference Frequency

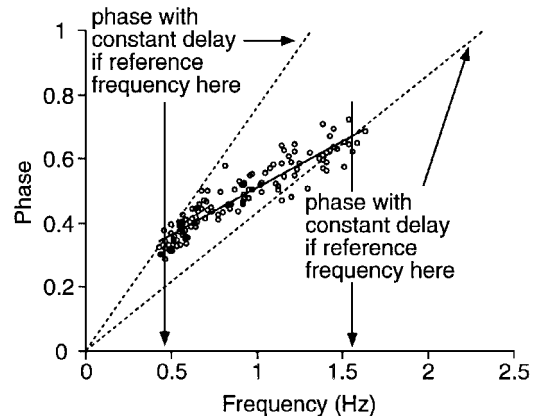


Figure 2. “Delay for perfect phase” (in the delay versus period representation, A) and “Phase with constant delay” (in the phase versus frequency representation, B) lines (dashed) depend on the chosen reference period (frequency). In the delay representation, if a short reference period is chosen, the perfect phase line will have a greater slope than the equivalent line if a long reference period is chosen. Exactly analogous reasoning applies to the constant delay lines in the phase representation. It is therefore necessary to choose the same reference period for all preparations. In this article the reference period (frequency) was 1 sec (1 Hz). See Methods for discussion of other concerns in comparing the slopes of the average, delay for perfect phase, and constant delay lines in the two representations.

This dependence of perfect phase slope on reference period in delay plots is shown graphically in Fig. 2A, in which the dashed lines are the perfect lines using short and long reference periods. The slope of the dashed line calculated at the short reference period is much greater than the slope of the same line calculated at

the long reference period, and these data would thus be considered to keep much worse phase if they were compared to the short reference line than if they were compared to the long reference line. An exactly analogous line of reasoning applies in phase versus frequency plots to “constant delay” lines (dashed lines, Fig. 2B). Thus, when data from different preparations are compared, the same reference cycle period must be chosen in each case. The pyloric pattern typically has a period of approximately 1 sec *in vitro*, and using this period is particularly convenient for various calculations. A reference period of 1 sec (cycle frequency 1 Hz) was therefore used in all comparative and grouped analyses.

These considerations lead to another concern. Average, perfect phase, and constant delay slopes are calculated to compare how well different pattern elements maintain phase. For instance, if in the delay representation, the average and perfect slopes of PDE were 0.32 and 0.4 and the average and perfect slopes of LPB were 0.24 and 0.6, which element keeps phase better? One might compare the ratios of each element’s average and perfect slopes, in which case PDE would be considered to keep phase twofold better than LPB (80% of perfect phase for PDE versus 40% for LPB).

However, ratios are not a good comparison because they also depend on reference period. Continuing to consider the delay versus period representation, if the linear fit to a pattern element—say, PDE—has a slope m_{PDE} , the associated perfect slope is $(a_{\text{PDE}} + m_{\text{PDE}}x_0)/x_0$. The ratio of the average and perfect slopes is therefore

$$\begin{aligned} r_{\text{PDE}} &= m_{\text{PDE}} / [(a_{\text{PDE}} + m_{\text{PDE}}x_0) / x_0] \\ &= m_{\text{PDE}}x_0 / (a_{\text{PDE}} + m_{\text{PDE}}x_0). \end{aligned}$$

The ratio of the ratios of two pattern elements (say, PDE and LPB) is therefore

$$\begin{aligned} r_{\text{PDE}} / r_{\text{LPB}} &= \frac{m_{\text{PDE}}x_0 / (a_{\text{PDE}} + m_{\text{PDE}}x_0)}{m_{\text{LPB}}x_0 / (a_{\text{LPB}} + m_{\text{LPB}}x_0)} \\ &= \frac{m_{\text{PDE}} / (a_{\text{LPB}} + m_{\text{LPB}}x_0)}{m_{\text{LPB}} / (a_{\text{PDE}} + m_{\text{PDE}}x_0)}, \end{aligned}$$

which depends on reference period (x_0). As such, if by this measure PDE kept phase twofold better than LPB at one reference period, it would not keep phase twice as well at another. Ratios are therefore not a good comparison of how well different pattern elements maintain phase because they depend on reference period.

A comparison between pattern elements that is independent of reference period is the *difference* between

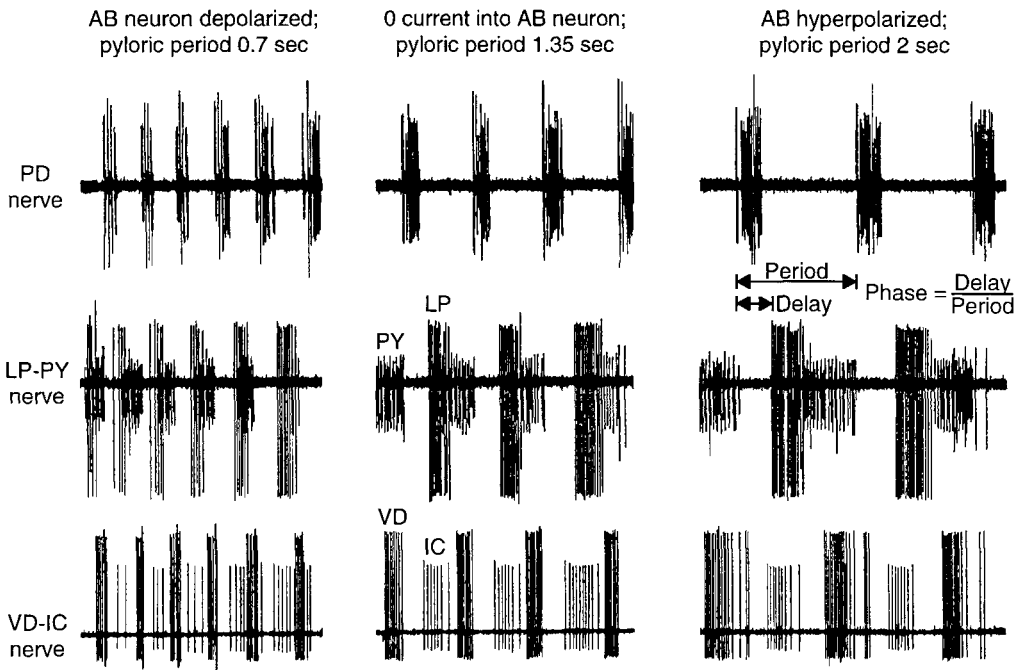
the observed and perfect slopes, $\Delta m = m - (a + mx_0)/x_0 = -a/x_0$. For two different pattern elements, $\Delta m_1 / \Delta m_2 = (-a_1/x_0) / (-a_2/x_0) = a_1/a_2$, and hence (since a_1/a_2 is independent of x_0) if the difference between observed and perfect slopes of one pattern element is twice the difference of another element at one reference period, it will be twice at all reference periods. An exactly analogous argument applies to the difference between observed and constant delay slopes in phase versus frequency representations. Grouped data (Fig. 7) are therefore expressed as simple average slopes; the ratio of the *differences* between the average and perfect-phase (or constant-delay) slopes of any two elements would be the same at any reference period.

Results

The pyloric network generates the motor pattern of the pylorus of the lobster stomach (Selverston et al., 1976). This network is an advantageous preparation in which to study phase regulation because the pyloric pattern is produced over a wide (two- to fivefold) frequency range *in vivo* (Rezer and Moulins, 1983), and the pyloric network is in many ways similar to the network shown in Fig. 1 (see below). Figure 3A (middle panel) shows the pattern the network typically produces *in vitro*. The pattern consists of an ordered sequence of neuron bursts that repeats each cycle. If the pyloric dilator (PD) neurons (top trace) are taken as the pattern reference point, the firing sequence within a cycle is as follows: the PD neurons fire first, then the inferior cardiac (IC; small unit, bottom trace) and lateral pyloric (LP; large unit, middle trace) neurons fire, then the pyloric (PY; small units, middle trace) neurons fire, and finally the ventricular dilator (VD; large unit, bottom trace) neuron fires. The remaining pyloric neuron, the anterior burster (AB) neuron (which generally serves as the network’s pacemaker and can alter pyloric frequency when current is injected into it) is not shown but is electrically coupled to the PD neurons and fires with them (Selverston et al., 1976).

The pyloric network has been studied intensively (Harris-Warrick et al., 1992), and its complete synaptic connectivity (Selverston et al., 1976; Eisen and Marder, 1982) and many of the characteristics that underlie its rhythmicity and firing pattern are known (Raper, 1979; Miller and Selverston, 1982a, 1982b; Russell and Hartline, 1982; Bal et al., 1988; Golowasch and Marder, 1992). Of particular interest here is that

A. The Pyloric Pattern is Maintained as Pyloric Cycle Period is Altered



B. Phase Normalized Plots of Above Data

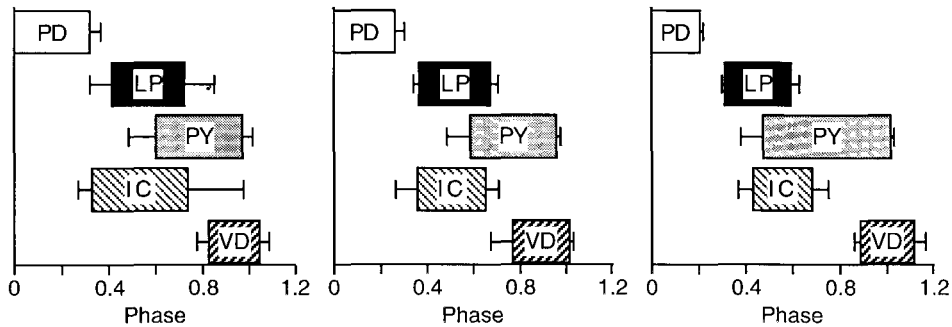


Figure 3. The pyloric pattern qualitatively maintains phase as period is altered. **A:** Extracellular recordings of pyloric activity when period is altered by current injection into the AB neuron. Top traces, PD neuron activity; middle traces, LP (large unit) and PY (small unit) neuron activity; bottom traces, VD (large unit) and IC (small unit) neuron activity. Left: AB neuron depolarized; middle: 0 current injected into AB neuron; right: AB neuron hyperpolarized. Note that burst durations and firing delays change to preserve the pattern at different frequencies and that the period range is sufficient that, without compensatory mechanisms, the PY and VD neurons would not have time to fire at the period shown in the left panel. **B:** Phase normalization (see inset in right panel, A) of data shown in A. This procedure normalizes with respect to period, and shows that the pattern is qualitatively maintained over this frequency range. Error bars are standard deviations of the mean.

the network's activity critically depends on active postsynaptic responses similar to those discussed earlier. All the network's chemical synapses are inhibitory, and neuronal firing (except for the endogenously oscillating AB neuron) depends on postinhibitory rebound and plateau potentials. Furthermore, PY neuron postinhibitory rebound is considerably delayed as a result of properties intrinsic to the neurons (Hartline, 1979;

Tierney and Harris-Warrick, 1992). The pyloric network thus has neurons analogous to those shown in Fig. 1 (the AB neuron corresponds to neuron 1, the LP and IC neurons to neuron 2, the PY neurons to neuron 3), and, for reasons similar to those shown in Fig. 1, in the absence of compensatory mechanisms the pattern would not be expected to be maintained as period changes.

However, as is shown in the left and right panels of Fig. 3A, when period is changed by current injection into the AB neuron, neuronal firing delays and burst durations change sufficiently to maintain the pattern. Note that, as in Fig. 1, these changes in cycle period are large enough that without compensation the pattern would be destroyed. In particular, at the period shown in the left panel, neither the PY nor the VD neurons would have time to fire if compensatory changes in their firing delays did not occur.

Although these records show that sufficient phase maintenance exists that the pattern is not destroyed as period changes, it is difficult to discern from them precisely how well the pattern is maintained (e.g., does PD neuron burst duration change in strict proportion as period changes?). It is therefore useful to analyze these data in terms of phase. As is shown in the inset in Fig. 3A (right panel), phase is equal to delay divided by period. This analysis normalizes delay with respect to period and thus allows direct comparison of patterns with different periods. Figure 3B shows Fig. 3A's data after this analysis; it is apparent that the pattern is qualitatively maintained as period is altered.

However, this presentation of the data does not show how delay changes as period changes, does not show phase maintenance as an explicit function of frequency, does not allow comparison of actual and perfect phase maintenance, and is insufficiently compact to present data from multiple preparations in a single plot. If delay varies linearly with period, these goals can be achieved by plotting delay versus period on a cycle-by-cycle basis and calculating a linear fit to the data. Such plots explicitly show delay as a function of period, and comparing the fit's slope to the slope of the theoretical perfect phase line (delay doubles when period doubles) allows the degree of phase maintenance to be quantified. Although it is unlikely that pyloric neuron conductances reach steady-state activation levels (and hence the pattern elements would show constant delays as in Fig. 1) within the durations of inhibition typically present in the pattern, it is also useful to plot the constant delay (slope = 0) line because it marks the boundary within which at least some phase compensation occurs. Although the two representations of the data are equivalent (frequency = 1/period, phase = delay/period), it is also useful to plot the data in the phase/frequency representation because the nonlinearity of the transformation makes it difficult to deduce phase relationships from delay plots and *vice versa*. Both representations are therefore shown throughout this article.

Figures 4A and B show such plots for LP neuron burst beginning (LPB) in three preparations. The data clearly vary linearly as period changes, and hence linear fits are appropriate. In preparations in which all pyloric neurons were active, delay varied linearly with period for all pattern elements (PD neuron burst ending (PDE), LP neuron burst ending (LPE), etc.). However, in preparations in which the IC and/or PY neurons were silent or only weakly active, VDB delay did not vary linearly. Figures 4C and D show VDB in a preparation in which the PY neurons were silent. VDB delay is constant at periods less than 1.5 sec, shows a discontinuous jump at this period, and then increases linearly. Linear fits to these data are therefore inappropriate (although fits to the data on either side of the discontinuity were made and are shown as fine dotted lines in the VDB panels in Figs. 5 and 6), and VDB data from these preparations were excluded from further analysis. Figures 4E and F show VDB data from two preparations in which all the pyloric neurons were active; it is apparent that in these cases linear fits to the data are appropriate.

Note also that the data from the individual preparations shown in Figs. 4A, B, E, and F cluster tightly around their fitted lines and thus that data from preparations with different linear fits segregate into separate populations with relatively little overlap (compare diamonds and squares in Figs. 4A and B). This tight clustering of the data, and segregation of the data into separate populations, was observed for all pattern elements (data not shown).

It is apparent from Figs. 4A and B that LPB keeps neither constant phase (in the phase representation (Fig. 4B), constant phase would be a horizontal line) nor constant delay (in the delay representation (Fig. 4A), constant delay would be a horizontal line). One therefore needs to quantify the extent to which pyloric activity differs from perfect compensation and constant delay in both representations. Comparison of actual versus perfect (in the phase representation), and actual versus constant (in the delay representation) is straightforward since the theoretical lines have 0 slope in these representations. Full characterization of phase maintenance, however, also requires comparing (1) the delays in the delay representation to the delays that would occur with perfect phase compensation (delay doubles as period doubles), and (2) the phases in the phase representation to the phase variation that would occur with constant delay (phase doubles as frequency doubles). As noted in the Methods, these comparisons are not straightforward because the slopes of these lines

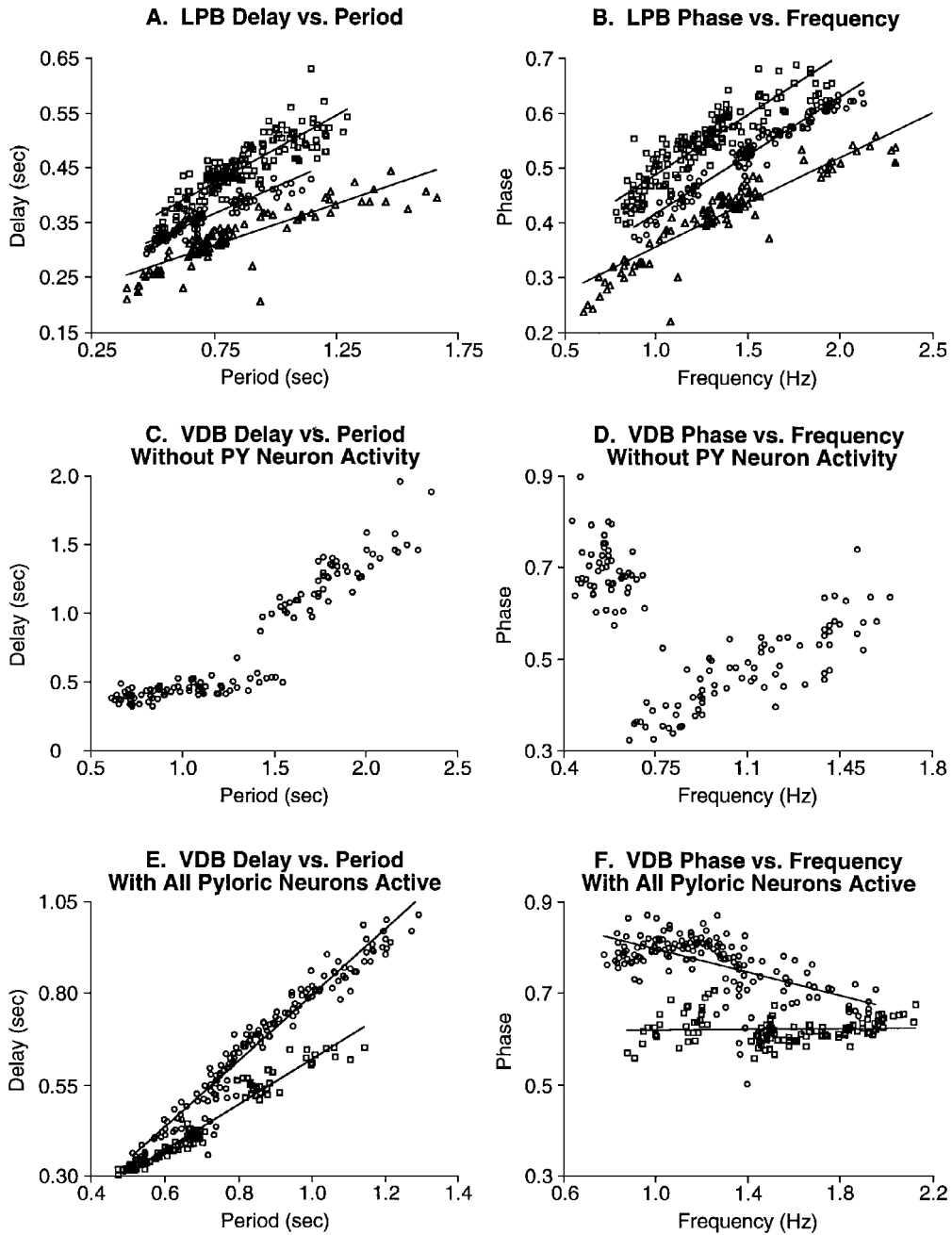


Figure 4. In pyloric patterns in which all neurons are active, delays (phases) vary linearly as period (frequency) changes. A: LPB delay (left) and phase (right) as period is altered for three preparations (triangle, circle, square); lines are linear fits to the data. Note that the scatter around the fit is less than the separation between fits—that is, data from different preparations segregate into distinct populations. B: VDB delay and phase from a preparation in which the PY neurons were silent. In preparations in which the PY and/or IC neurons were silent or only weakly active, VDB delay (phase) was always nonlinear. C: VDB delay and phase from two preparations in which all neurons were active. Note that VDB varies linearly and that the data from the two preparations again segregate.

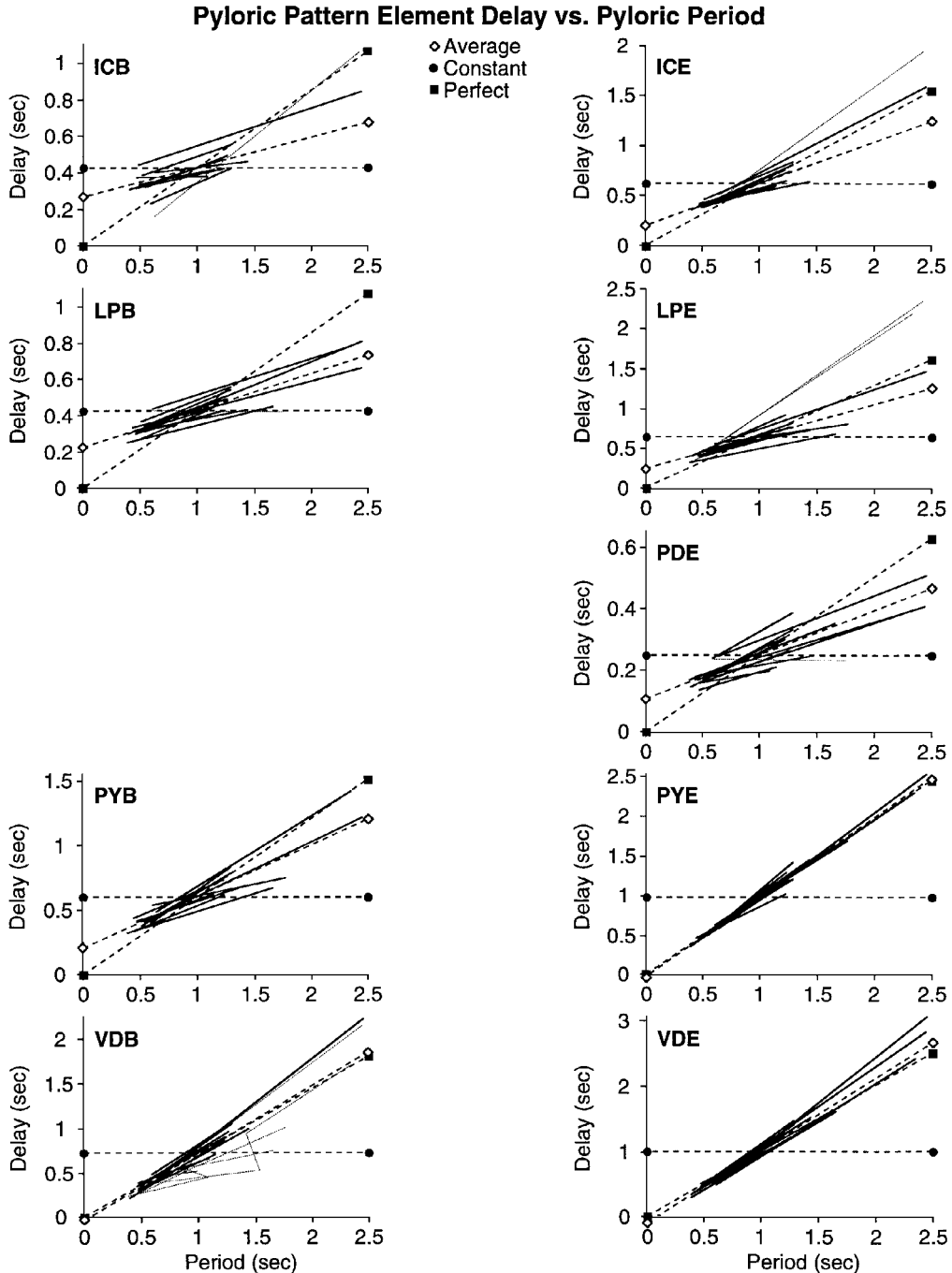


Figure 5. Pyloric pattern element delay versus period. Key: solid lines are linear fits to the data that met inclusion criteria; fine dotted lines are fits to excluded data (see Methods). Dashed lines with open diamonds are the averages of the included fits. Dashed lines with circles are constant delays; dashed lines with squares are the delays expected for perfect phase; both lines were calculated from the average fit line. Note that for most pattern elements (1) a wide range of delays is observed at any given period, (2) how well a given pattern element maintains phase is generally consistent across preparations, (3) all pattern elements show considerable phase maintenance, and (4) the corresponding delay changes can be very large (up to 2 sec). Abscissa scales vary because of the wide range of delays present among the various pattern elements (e.g., PDE varies between only 0.1 and 0.6 sec, whereas VDE varies between 0.5 and 3 sec).

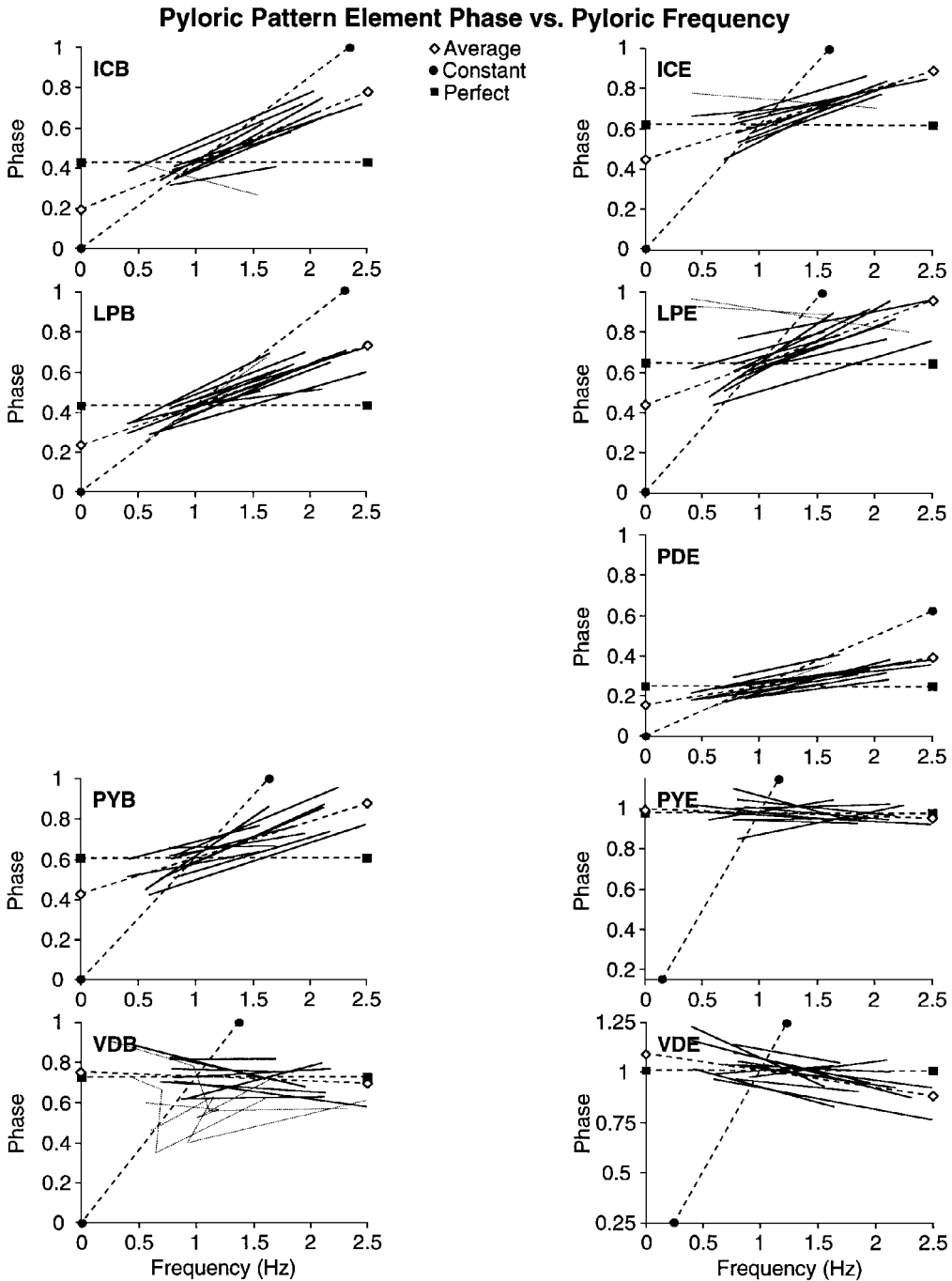


Figure 6. Pyloric pattern element phase versus frequency. Key the same as Fig. 5. Note that (1) all elements show considerable phase maintenance (all average fit lines have slopes much less than the constant delay lines) and (2) a wide range of phases is observed at any given frequency. Abscissa scales are the same on all plots to facilitate comparison among the pattern elements.

depend on which period (frequency) is chosen as reference. In all plots (Figs. 5 and 6) with these lines the reference period (frequency) was 1 sec (1 Hz).

Figures 5 and 6 show linear fits to each preparation's data for all pyloric pattern elements in, respectively, the delay and phase representations. Also shown are (1) the averages of the data fits (open diamonds) and (2) the perfect phase lines (squares; positive slope in delay, 0 slope (horizontal) in phase) and the constant delay lines (circles; 0 slope (horizontal) in delay, positive slope in phase) calculated from the averaged data fit line.

There are three points to make about these data. First (Fig. 5), all pattern elements show considerable phase maintenance (the fits are far from horizontal), and the corresponding delay changes over the shown frequency range can be as large as 2 sec (PYE, VDB, VDE). Second (Fig. 6), at any given frequency all pattern elements (except PYE) show considerable preparation specific phase variability (e.g., at 1 Hz ICB phase can be anywhere from 0.3 to 0.5 depending on the preparation). As noted earlier (Figs. 4A, B, E, and F), the data giving rise to these fits show little scatter in comparison to the distance between the fits. These data thus suggest that considerable differences in pyloric phasing exist between preparations. Third (Fig. 6), how well a given pattern element maintains phase (the slopes of the individual fits) is generally consistent from preparation to preparation.

Although these presentations are instructive, they have a drawback. The theoretical lines with nonzero slope (the perfect line in the delay representation and the constant line in the phase representation) in these plots were calculated using the value of the average data line (open diamonds) at the reference period (frequency) of 1 sec (1 Hz). However, these theoretical lines depend on the delay/phase at the reference period/frequency (see Methods), and comparison of the fits for individual preparations to these lines thus can be misleading. For instance, in the ICB panel in Fig. 5 the perfect slope for the short delay preparation ("lowermost" solid line) would be 0.3, while for the long delay preparation ("uppermost" solid line) it would be 0.5. It would therefore be better to determine the theoretical lines individually for each preparation, average these preparation specific slopes, and then compare these averages with the average data slopes.

Figure 7 shows the result of this analysis. In the delay representation (Fig. 7A) the average observed slopes are the dark bars, the average perfect slopes are the light bars, and the constant delay slope would be 0 (not plotted). In agreement with the visual impression in Fig. 5, all pyloric elements show large phase com-

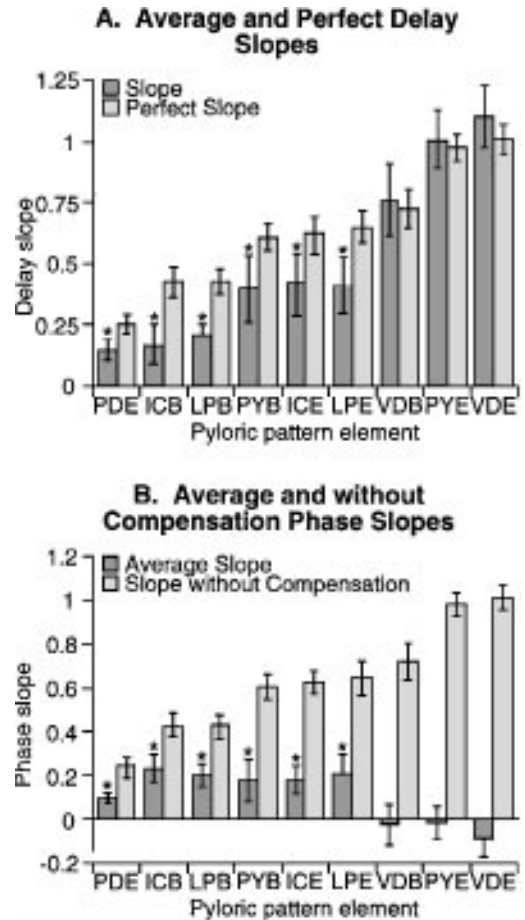


Figure 7. Summary of the slopes of the average and perfect phase delay lines (A) and of the slopes of the average and constant delay phase lines (B). Slopes were calculated individually for each pattern element in each preparation. The averages of these slopes were then used to create these plots. Error bars are standard deviations of the mean. In both plots the pattern elements are ordered according to their occurrence in the pattern. A: The average data slopes are all different ($p < 0.0001$) from the constant delay slope of 0 (not shown on plot). PDE, ICB, LPB, PYB, ICE, and LPE maintain phase less well (average (dark bars) and perfect (light bars) slopes are different ($p < 0.0001$, asterisks)) than VDB, PYE, and VDE. The delay changes as period is altered are quite large. The smallest (PDE) is a shift of 200 msec for each 1 sec period change, and the largest (VDE) is 1.1 sec for each 1 sec period change. B: All pyloric elements show large phase compensation (all dark and light bar pairs are different ($p < 0.0001$)) but PDE, ICB, LPB, PYB, ICE, and LPE maintain phase less well (different ($p < 0.0001$, asterisks)) from perfect phase compensation (0 slope, not shown on plot) than VDB, PYE, and VDE.

ensation as frequency is altered (the average slope of all pattern elements differ from a constant (0) slope ($p < 0.0001$)). Note that these slopes are quite large. The smallest slope (PDE) is a shift of 200 msec for a 1 sec period change, and the largest (VDE) is 1.1 sec for a 1 sec period change. However, the PDE, ICB,

LPB, PYB, ICE, and LPE average slopes (dark bars) are far from being large enough to perfectly maintain phase (average (dark) and perfect (light) slopes differ at $p < 0.0001$, asterisks). VDB, PYE, and VDE average slopes are much closer to their perfect slopes, and these elements thus maintain phase better than do the other pyloric elements.

In the phase representation (Fig. 7B) the average observed slopes are the dark bars, the average constant delay slopes are the light bars, and the perfect phase slope would be 0 (not plotted). As they must, since the two plots in Fig. 7 are different representations of the same phenomenon, these data agree with those in Fig. 7A. In particular, again (1) all pattern elements show large-phase compensation (all dark and light bar pairs differ at $p < 0.0001$) but (2) PDE, ICB, LPB, PYB, ICE, and LPE maintain phase less well (different from perfect (0 slope) at $p < 0.0001$, asterisks) than VDB, PYE, and VDE.

Discussion

Implications for Pyloric Network Mechanisms

The data presented here show that when pyloric cycle frequency is altered over a three- to fivefold range, pyloric pattern element delays change by hundreds to thousands of msec so as to maintain phase, depending on the pattern element, from 50 to 100% (referenced to a 1 sec period) of that required for perfect maintenance. It is not possible from these data to unambiguously ascribe these delay changes to cellular (as opposed to "network" based) mechanisms, but many pyloric pattern delays are due to the temporal characteristics of postsynaptic neuron responses (e.g., the long PY neuron delay; Hartline, 1979; Tierney and Harris-Warrick, 1992). Furthermore, I have shown elsewhere (Hooper, 1993) that certain pyloric neurons, when isolated from the network, alter their postinhibitory rebound times in response to varying temporal patterns of simulated inhibitory input.

It is thus likely that some of the delay shifts seen here result from changes in the intrinsic membrane properties of the neurons themselves. What mechanisms could underlie these changes? As an example, consider what changes in input a neuron experiences when pyloric period is increased. First, the neuron receives less frequent inhibitions since period is longer. However, these inhibitions are of increased duration since the burst durations of the neurons presynaptic to the

neuron are longer. Finally, depending on how presynaptic firing changes as burst duration changes, the amplitude of inhibition could change. All these changes could alter the average activation level of a large number of the neuron's active conductances. Remarkably, the sum of these conductance changes result in linear phase compensating shifts in all the pyloric neurons that can be as large as 2 sec as period changes from 0.5 to 2.5 sec. Which conductances are involved in this process, and how they accomplish this feat, is as yet unknown, but the experimental advantages of this system should permit this process to be fully understood on the conductance level.

It is important to note that the method used here to alter frequency (current injection into the AB neuron) is equivalent to opening a voltage independent current in the AB neuron soma and is very unlikely to mimic the mechanisms that actually alter AB neuron cycle frequency. However, this method is specific and well-controlled and hence is a good tool for investigating the mechanisms underlying network phase regulation.

A second possible difficulty in interpreting this work is that multiple modulatory processes extend to the stomatogastric ganglion from higher centers in lobster and related crustacean nervous systems (Russell, 1979; Nagy and Dickinson, 1983; Beltz et al., 1984; Marder et al., 1986; Katz and Harris-Warrick, 1989; Nusbaum and Marder, 1989; Turrigiano and Selverston, 1989; Cazalets et al., 1990; Hooper et al., 1990; Coleman et al., 1992; Nusbaum et al., 1992; Weimann et al., 1993; Coleman and Nusbaum, 1994; Nagy and Cardi, 1994; Skiebe and Schneider, 1994; Blitz et al., 1995; Christie et al., 1995; Harris-Warrick et al., 1995; Johnson et al., 1995; Norris et al., 1996), and the activity of at least some of these inputs are altered by pyloric activity (Selverston et al., 1976; Moulins and Nagy, 1983; Nusbaum et al., 1992; Coleman and Nusbaum, 1994; Norris et al., 1996). It is thus possible that changing pyloric activity by current injection into the AB neuron may activate inputs to the pyloric network and that these inputs are responsible for the observed phase maintenance. Although this remains an intriguing possibility, it is unclear whether the inputs to the pyloric network known to be affected by pyloric network activity have phase-maintaining effects. Furthermore, the disrupted phase maintenance observed in preparations in which various neurons are silent (fine dotted lines; Figs. 5, 6) suggests at least some aspects of pyloric phase maintenance are intrinsic properties of the network.

Functional Implications for Pyloric Motor Pattern Production

The pyloric network generates the neural input to the pyloric muscles and hence helps generate the pyloric motor pattern. If the muscles were faithful followers of this input, the data presented here would suggest the motor pattern maintains phase as period changes. However, research in *Panulirus* and related crustacea shows at least some pyloric muscles dramatically transform the neural input they receive (e.g., respond to rhythmic neural input with sustained contraction; Meyrand and Moulins, 1986; Meyrand and Marder, 1991; Morris and Hooper, 1994). Thus, without a more complete understanding of the input-output relationship of the pyloric musculature, it is premature to assert that phase maintenance of the pyloric neural pattern implies phase maintenance of the motor pattern.

How Consistent is the Pyloric Pattern across Animals?

Prior studies of pyloric phase (Eisen and Marder, 1984; Flamm and Harris-Warrick, 1986; Hooper and Marder, 1987) did not independently vary frequency and thus could not distinguish whether phase differences in different preparations were preparation specific or due to the different rest frequencies present in different preparations. The data presented here show that, although phase is maintained within broad ranges, any given element's phase shows considerable preparation specific variability independent of cycle frequency. This variability could arise from the lobsters being in different behavioral states prior to dissection. However, all lobsters were maintained under identical conditions, all protocols were identical, and data were never gathered until at least six hours after the nervous system was removed (and hence any behavioral state influences would have to be very long lasting to produce the observed differences). The most parsimonious conclusion is therefore that pattern delays (phases) show considerable variation between animals.

Comparison to Earlier Work

In earlier work in *Panulirus*, Abbott et al. (1990) found that PDE maintained phase perfectly when current was injected into the AB neuron, whereas here PDE maintained phase considerably less well. This difference

may stem from Abbott et al. working with stomatogastric ganglia isolated from anterior influences, whereas here the stomatogastric nerve was kept intact. This apparent state-dependence of pyloric phase maintenance (at least for the pacemaker ensemble) suggests that neuromodulatory inputs may alter the degree to which different pyloric elements maintain phase. However, neuromodulator application to preparations in which the stomatogastric nerve is intact has relatively little effect (data not shown), and I have not further investigated this possibility.

Implications for Other Systems

Considerable work has been performed on how chains of coupled oscillator networks produce phase constant outputs (Stein, 1976; Grillner, 1981; Kopell and Ermentrout, 1986; McClellan and Sigvardt, 1988; Rand et al., 1988; Cohen et al., 1992; Williams, 1992; Friesen and Pearce, 1993). In much of this work the individual unit oscillator is abstracted into a single rhythmic equation, and hence it is difficult to directly compare the present data with this work. However, it is clear that constant duration delays could pose difficulties for phase maintenance in coupled oscillator chains (see Cohen et al., 1992). Furthermore, many of the unit networks that comprise these chains contain neurons with complex cellular properties (e.g., neurons with postinhibitory rebound and plateau properties are present in lamprey oscillator networks (Grillner and Wallén, 1985)), and thus the problems noted in Fig. 1 could also apply to these systems. The data presented here showing that individual neural networks can produce relatively constant phase outputs suggests that the unit oscillators in coupled oscillator chains could also, and thus these problems may not pose a difficulty. However, it is important to note that there is no *a priori* reason to believe that this capability is necessarily present in all neural networks.

Acknowledgments

This research was supported by grants from the National Science Foundation, the Human Frontier Science Program, OU COM, OURC, and OU A&S. I thank R.A. DiCaprio and T. Kepler for discussion and advice, H.L. Atwood for the extremely kind donation of micromanipulators, J.B. Thuma for excellent technical assistance, and anonymous reviewers for useful comments on presentation and style.

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