Distinct Synaptic Dynamics of Heterogeneous Pacemaker Neurons in an Oscillatory Network

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Rabbah P, Nadim F. Distinct synaptic dynamics of heterogeneous pacemaker neurons in an oscillatory network. J Neurophysiol 97: 2239–2253, 2007. First published January 3, 2007; doi:10.1152/jn.01161.2006. Many rhythmically active networks involve heterogeneous populations of pacemaker neurons with potentially distinct synaptic outputs that can be differentially targeted by extrinsic inputs or neuromodulators, thereby increasing possible network output patterns. To understand the roles of heterogeneous pacemaker neurons, we characterized differences in synaptic output from the anterior burster (AB) and pyloric dilator (PD) neurons in the lobster pyloric network. These intrinsically distinct neurons are strongly electrically coupled, cooperative, and constitute the pyloric pacemaker ensemble. During pyloric oscillations, the pacemaker neurons produce compound inhibitory synaptic connections to the follower lateral pyloric (LP) and pyloric constrictor (PY) neurons, which fire out of phase with AB/PD and with different delay times. Using pharmacological blockers, we separated the synapses originating from the AB and PD neurons and investigated their temporal dynamics. These synapses exhibited distinct short-term dynamics, depending on the presynaptic neuron type, and had different relative contributions to the total synaptic output depending on waveform shape and cycle frequency. However, paired comparisons revealed that the amplitude or dynamics of synapses from either the AB or PD neuron did not depend on the postsynaptic neuron type, LP or PY. To address the functional implications of these findings, we examined the correlation between synaptic inputs from the pacemakers and the burst onset phase of the LP and PY neurons in the ongoing pyloric rhythm. These comparisons showed that the activity of the LP and PY neurons is influenced by the peak phase and amplitude of the synaptic inputs from the pacemaker neurons.

INTRODUCTION

Many rhythmically active motor networks are driven by pacemaker neurons that act as core oscillators, synchronizing the activity of other network neurons into a coherent rhythmic pattern (Koshiya and Smith 1999; Marder and Bucher 2001; Smith et al. 1991; Tresch and Kiehn 2000; Wallen and Grillner 1987). In such pacemaker-driven pattern-generating networks, it is common that the pacemaker neurons use chemical synaptic inhibition to set the relative activity patterns of the remaining neurons (Eisen and Marder 1984; Tryba and Ritzmann 2000; Wolf 1992) and that the efficacy of these synapses can be altered by neuromodulators to reshape the network output (Harris-Warrick et al. 1998; Marder 1994). Moreover, heterogeneous populations of pacemaker neurons, as in the vertebrate respiratory system, can be differentially targeted by neuromodulators, thus increasing the number of possible network output patterns (Ramirez et al. 2004).

The rhythmically active pyloric central pattern generator of the spiny lobster, Panulirus interruptus, is driven by a pacemaker ensemble that consists of two intrinsically distinct neuron types: the anterior burster (AB) and two pyloric dilator (PD) neurons. The pacemaker neurons exhibit similar, but not identical, synaptic connections to all other follower neurons (Eisen and Marder 1982). During the ongoing pyloric rhythm (frequency 0.5–2 Hz), the AB and PD neurons are active because of their strong electrical coupling and thus produce a compound inhibitory chemical postsynaptic potential onto each of the follower neurons. In a recent study, we characterized the dynamics of the compound synapse from this pacemaker ensemble onto two classes of follower LP and PY neurons (Rabbah and Nadim 2005). The PY neurons always lag the LP neuron in their activity phase; thus we examined the hypothesis that this phase lag is partially explained by distinct synaptic inputs from the pacemaker ensemble. Surprisingly, our results showed that the total synaptic effect of the pacemaker ensemble on these two classes of follower neurons is identical.

Previous studies reported that the AB and PD neurons evoke inhibitory postsynaptic potentials (IPSPs) in the follower neurons that differ in their neurotransmitter type, time course of release, reversal potential, and ion selectivity (Eisen and Marder 1982, 1984; Rabbah et al. 2002). Furthermore, exogenous neuromodulators differentially affect the intrinsic and synaptic properties of these neuron types, subsequently altering their relative synaptic influence on follower neurons and thus on the pyloric oscillation pattern (Ayali and Harris-Warrick 1999; Harris-Warrick et al. 1998; Johnson and Harris-Warrick 1997). It is therefore important to characterize the individual synaptic outputs of the AB and PD neurons. In this study, we examined whether the synapses made by each of the pacemaker AB and PD neurons differ in their short-term plasticity, temporal dynamics, and dependency on presynaptic waveform shapes. We first characterized the dynamics of the synapses from the AB and PD neurons as a combined unit and then individually after blocking each synapse. We activated the synapses using various injection stimuli applied to the voltage-clamped presynaptic neurons and recorded simultaneously from the follower LP and PY neurons. We also examined the correlations between parameters describing the individual pacemaker synapses and the activity phase of the follower neurons. Our results show that the synapses to each of the two classes of follower neurons exhibit distinct dynamics depend-
ing on the presynaptic pacemaker neuron type, AB or PD. These results suggest that neuromodulatory inputs can differentially accentuate one set of dynamics over the other, allowing for selective control of the activity phase of the postsynaptic targets and thus leading to different network outputs.

METH ODS

Preparation and identification of the neurons

Adult male spiny lobsters (P. interruptus) were purchased from Don Tomlinson Fisheries (San Diego, CA) and maintained in artificial seawater tanks at 12–15°C until use. Before each dissection, the animals were anesthetized by cooling in ice for 30 min. The stomatogastric nervous system [STNS; including the stomatogastric ganglion (STG), the esophageal, and the commissural ganglia] was removed using standard methods (Harris-Warrick et al. 1992; Selverston et al. 1976). The STG was desheathed to allow penetration of the cell bodies and superfused using normal saline at 18°C, pH 7.35, containing (in mM): 12.8 KCl, 479 NaCl, 13.7 CaCl2, 10.0 MgSO4, 3.9 NaSO4, 11.2 Trizma base, and 5.1 maleic acid.

For neuron impalement, glass microelectrodes were pulled using a Flaming-Brown micropipette puller (P87, Sutter Instruments, Novato, CA) and filled with 0.6 M K2SO4 + 0.02 M KCl (resistances of 8–13 MΩ). Identification of the neurons was accomplished by matching intracellular action potential recordings to their corresponding extracellular recordings on motor nerves (Harris-Warrick et al. 1992; Selverston et al. 1976). Intracellular recordings were made from the soma of the neurons using Axoclamp 2B amplifiers (Molecular Devices, Foster City, CA) and extracellular recordings were amplified using a Differential AC amplifier model 1700 (A-M Systems, Carlsborg, WA).

Isolation of synapses from the AB and PD neurons to the LP and PY neurons

During the normal ongoing pyloric rhythm, the AB and PD neurons oscillate in synchrony as a result of their strong electrical coupling and so the follower neurons experience a compound inhibitory postsynaptic potential from both the AB and PD neurons (IPSPAB and IPSPPD). Voltage-clamp stimulations of the two PD neurons in control saline are sufficient to elicit synaptic release from both AB and PD neurons (Rabbah and Namid 2005). To characterize the synaptic dynamics of the AB and PD neurons separately, we stimulated the PD neurons while the preparation was superfused with 5 µM picrotoxin (PTX; Marder and Paupardin-Tritsch 1978). PTX blocks the glutamatergic synaptic release from the AB neuron and thus IPSPs recorded in the follower neurons were those elicited solely by the PD neurons (IPSPPD). Because there are no known gap-junctional blockers in the STNS, the IPSPs recorded in the follower neurons were those elicited solely by the PD neurons (IPSPPD).

Comparison of IPSPAB and IPSPPD to the LP and PY neurons

The inhibitory synapses between the pacemaker neurons and the follower neurons use voltage-dependent (graded) release of neurotransmitter as the major form of transmission (Graubard 1978; Graubard et al. 1980; Johnson and Harris-Warrick 1990; Johnson et al. 1995; Maynard and Walton 1975). This form of synaptic release was isolated from the spike-mediated transmission by blocking the latter with Panulirus saline containing 10−7 M tetrodotoxin (TTX; Biotium, Hayward, CA). Spontaneous rhythmic activity and modulatory inputs from anterior ganglia are also blocked by TTX (Raper 1979). The experiments were carried out in voltage clamp to better control the membrane potential of the presynaptic neurons. One PD neuron was voltage clamped with two electrodes to a holding membrane potential (Vhold) of −60 mV. The second PD neuron was impaled with one electrode. The current used to voltage clamp the first PD neuron was scaled up (using the specification of the amplifier) by a Brownlee Precision Amplifier (Santa Clara, CA) and injected into the second PD neuron to effectively voltage clamp the second PD neuron to the same Vhold with only one electrode (see Rabbah and Namid 2005). The LP and PY neurons were impaled with one electrode each and the graded IPSPs, elicited by the stimulation of the PD neurons, were recorded in current clamp (see schematic in Fig. 1B). Only paired recordings of the LP and PY were used in this study.

Properties of the synapses from the pacemaker neurons to the LP and PY neurons were studied using various injection protocols. Single square 2-s depolarizing pulses (amplitudes ranging from 10 to 40 mV) were injected into the voltage-clamped PD neurons to study the synapses in a static context. Trains of five square pulses (fixed 500-ms duration and 40-mV amplitude) with increasing interpulse intervals (IPIs; from 250 to 8,000 ms) were used to study the extent of depression and recovery. Two realistic PD waveforms were built by recording voltage traces of the PD neurons in normal saline during slow (1,358-ms) and fast (623-ms) pyloric cycle periods, averaging them over several cycles and low-pass filtering them at 10 Hz to remove action potentials (and thus isolate the graded transmission). The resulting two realistic waveforms were indexed by their duty cycles (DCs, defined as the percentage of time, in each cycle, that the PD waveform was above its mean membrane potential). The waveform DCs were 17 and 46%, respectively. Hereafter, the two waveforms will be referred to by their DC values. The waveforms were scaled to 40 mV (from trough-to-peak) and played back periodically into the voltage-clamped PD neurons. This allowed us to investigate the effects of varying presynaptic frequencies (from 0.5 to 4 Hz) as well as the shape of presynaptic depolarizations (DC 17% and DC 46%) on the dynamics of the synaptic response in the LP and PY neurons. The same two waveforms were used in all experiments to allow for averaging of the data across preparations.

Whenever trains of pulses were used to activate the presynaptic neurons (square or realistic waveforms), the resulting IPSPs were always measured from resting potential (Vrest) of the postsynaptic neuron as opposed to the respective baseline of each IPSP. This was done because at short interpulse intervals or periods, the IPSPs in each train did not have sufficient time to return to Vrest (see Fig. 3 for example).

Cycle period and the LP and PY neuron burst times were measured using the PD neuron burst onset as reference (see Fig. 1A). The activity phases of the LP and PY neurons were calculated as the time difference (∆t) between the burst onset of that neuron and the onset of the PD neuron burst divided by cycle period.
and each other. PD burst onset was used a reference point (dashed line) to calculate Periodnatural and the LP and PY burst phases. Phase is calculated by dividing neurons oscillate in synchrony and inhibit the follower lateral pyloric (LP) and pyloric constrictor (PY) neurons, which burst out of phase with the AB/PD neurons and each other. PD burst onset was used a reference point (dashed line) to calculate Periodnatural and the LP and PY burst phases. Phase is calculated by dividing

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neurons by chemical inhibitory synapses (Fig. 1 B) and so, during normal ongoing rhythm, the AB and PD neurons produce a compound postsynaptic potential onto the LP and PY neurons.

In a recent publication (Rabbah and Nadim 2005), we compared the dynamics of the synapses between the pacemaker AB and PD neurons as a unit (IPSPAB/PD) to the follower LP and PY neurons. Here, we examine instead the dynamics of the synaptic inputs the LP and PY neurons receive from the individual pacemaker AB (IPSPAB) and PD (IPSPPD) neurons. This analysis was done in three parts: First, we examine whether the intrinsically distinct AB and PD neurons exhibit distinct synaptic dynamics. Thus we start by characterizing various parameters of IPSPAB and IPSPPD onto the LP and PY neurons using paired LP and PY recordings and various injection stimuli into the pacemaker neurons. Second, we examine whether the synapses originating from the same presynaptic neuron (AB or PD) onto two different classes of target neurons (LP and PY) exhibit distinct dynamics. Third, we ask whether these synaptic parameters correlate with and thus help determine the activity phases of the follower LP and PY neurons in the normal ongoing pyloric activity.

Comparison of the dynamics of IPSPAB and IPSPPD to the LP and PY neurons

To measure synaptic strength, we activated the pacemaker synapses using single 2-s presynaptic square pulses of various amplitudes (Fig. 2). Figure 2A shows the synaptic response in the LP neuron when the PD neurons were stepped from −60 to −20 mV (top trace). The red trace represents the IPSPs elicited in the LP neuron in control (TTX) saline. This IPSP consists of the AB and PD components (IPSPAB/PD). In the presence of PTX, the AB glutamatergic release is blocked and thus the LP neuron experiences synaptic release from the PD neurons only (IPSPPD; blue trace). The AB component (IPSPAB; black trace) was then approximated by digitally subtracting the PTX trace from the control trace (see METHODS). All three IPSPs showed a large early peak (Fig. 2A, dotted arrows) that decayed to a sustained value (Fig. 2A, dashed arrows) during the stimulation period. The peak component of IPSPAB decayed faster than IPSPAB/PD or IPSPPD.

Recording, analysis, and statistics

An NI PCI-670-E board (National Instruments, Austin, TX) was used for data acquisition and for current injection with the data-acquisition software Scope developed in the LabWindows/CVI software environment (National Instruments) on a Windows XP operating system. The acquired data were saved as individual binary files and were analyzed with the Readscope software. (Scope and Readscope are software developed in the Nadim laboratory and are available for download at http://stg.rutgers.edu/software/index.htm.) Digital subtraction of traces was done by custom-made programs written in LabWindows/CVI (National Instruments). Statistica (Statsoft, Tulsa, OK), SigmaStat (SPSS, San Rafael, CA), and Origin (OriginLab, Natick, MA) software packages were used for statistical and graphical analysis. Reported statistical significance indicated that the achieved significance level \( P \) was below the critical significance level \( \alpha = 0.05 \). When multiple comparisons were made, the alpha was corrected using the false discovery rate correction method (Curran-Everett 2000). In those cases, the value is given as \( B = \alpha \) corrected value. All error bars shown and error values reported denote SDs.

RESULTS

The electrically coupled AB and two PD neurons compose the pacemaker unit of the pyloric circuit. These neurons oscillate in synchrony (Fig. 1A; top two traces; for simplicity, only one PD neuron is shown) and, together, they drive the pyloric rhythmic activity. Each cycle of the triphasic pyloric rhythm is generated by a simultaneous burst in the pacemaker neurons, followed, with a short delay, by a burst in the LP neuron and then by a burst in the PY neurons (Fig. 1A). The pacemaker AB and PD neurons are each connected to the follower LP and PY neurons by chemical inhibitory synapses (Fig. 1B) and so, during normal ongoing rhythm, the AB and PD neurons produce a compound postsynaptic potential onto the LP and PY neurons.

FIG. 1. Pyloric network and experimental protocol. A: simultaneous intracellular recordings indicate that the anterior burster (AB) and pyloric dilator (PD) neurons oscillate in synchrony and inhibit the follower lateral pyloric (LP) and pyloric constrictor (PY) neurons, which burst out of phase with the AB/PD neurons and each other. PD burst onset was used a reference point (dashed line) to calculate Periodnatural and the LP and PY burst phases. Phase is calculated by dividing the time difference between burst onsets (\( \Delta t \)) by Periodnatural. Baselines for the membrane potential oscillations were −57 mV for AB, −58 mV for LP, and −62 mV for PY. For simplicity, recording of only one of 2 PD neurons is shown. B: synaptic connectivity and the experimental paradigm represented schematically. All chemical synaptic connections (ball and stick symbols) are inhibitory; resistor symbols indicate gap-junctional couplings. All neurons were impaled with one electrode except for one PD neuron that was impaled with 2 for voltage clamp. Current used to voltage clamp that PD neuron was scaled and injected into the other PD neuron to voltage clamp the latter neuron to the same \( V_{\text{clamp}} \) value. Paired recordings of the LP and PY neurons were carried out in current clamp.
At first glance, one may predict that because IPSP_{PD} recorded in PTX was almost as large in amplitude as IPSP_{AB/PD} recorded in control, subtraction of the two traces would yield a much smaller IPSP_{AB}. However, Fig. 2B shows that the delay from presynaptic onset to the onset of the postsynaptic response was different for IPSP_{AB} and IPSP_{PD}. The AB to LP synapse was activated almost instantaneously on presynaptic depolarization, whereas the PD to LP synapse was activated with a nearly 60-ms delay (Fig. 2B, double-headed arrow). Thus IPSP_{PD} reached its maximal peak later in time. Interestingly, once IPSP_{PD} was activated, its rise time was similar to that of IPSP_{AB}, resulting in essentially no inflection on the rise of IPSP_{AB/PD}.

The maximal peak amplitudes of the IPSPs (measure of synaptic strength) elicited in response to all presynaptic depolarization amplitudes ($V_{pre}$ from $-60$ to $-50$, $-40$, $-30$, $-20$ mV) were normalized to the amplitude of IPSP_{AB/PD} recorded in response to a step to $-20$ mV in control conditions. The

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**FIG. 2.** Postsynaptic potentials of the LP and PY neurons in response to stimulations of the PD neurons with a single square pulse. **A**: membrane potentials of the PD neurons were stepped from $-60$ to $-20$ mV (top trace). Red trace: combined inhibitory postsynaptic potentials (IPSPs) from both the AB and PD neurons (IPSP_{AB/PD}; red trace) in control [tetrodotoxin (TTX) saline]. In PTX, the AB synaptic output is blocked and the response in the LP neuron arises from the synapse from the PD neurons only (IPSP_{PD}; blue trace). IPSP_{PD} trace was subtracted from IPSP_{AB/PD} to yield the synapse arising from the AB neuron only (IPSP_{AB}; black trace). All IPSPs exhibited a transient peak (dotted arrows) that decayed to a sustained value (dashed arrows). LP neuron had an initial membrane potential of $-59$ mV. **B**: close-up of the IPSPs in A shows that the time courses of the synaptic response in all conditions were different from each other. Double-headed arrow: delay in onset of the synaptic response of the PD neurons. C: synaptic input–output (I/O) curve for square pulses of 10- to 40-mV amplitudes used to depolarize the presynaptic PD neurons ($n = 6$). Values were normalized to the amplitude IPSP_{AB/PD} in response to a step to $-20$ mV. Amplitude of each IPSP is measured from resting potential ($V_{rest}$) to the maximal value of the IPSP. Results show that for presynaptic depolarization ($V_{pre}$) of $-50$ and $-40$ mV (from $-60$ mV), the compound synapse to the LP neuron was mostly from the AB neuron. IPSP_{PD} required more positive PD potentials to activate compared with both IPSP_{AB/PD} and IPSP_{AB} onto LP. **D**: time to peak of the IPSPs was measured from the beginning of the presynaptic depolarization to the maximal peak of the IPSP (inset). IPSP_{PD} onto the LP neuron reached its maximum peak value more slowly than IPSP_{AB}. Moreover, both IPSPs showed a dependency on the presynaptic potential ($n = 6$). **E**: same as in C, but using the PY neuron as the postsynaptic target. Response is similar to that seen in the LP neuron. **F**: same as in D, but using the PY neuron as the postsynaptic target. Again, the results were similar to those of the LP neuron (* indicates significant difference).
resulting normalized input–output (I/O) relationship is shown in Fig. 2C. Note that $\Delta V_{LP}$ (and $\Delta V_{PY}$) values are plotted as negative in this and subsequent figures to indicate the inhibitory nature of the synapse from the pacemaker neurons onto the LP (and PY) neuron. The IPSP amplitudes elicited in the LP neuron increased with increasing presynaptic potentials as expected from a graded synapse. Interestingly, in response to depolarization steps that shifted the membrane potentials of the presynaptic neurons relatively little ($V_{pre}$ values of $-50$ and $-40 \text{ mV}$), we observed that the amplitudes of IPSP$_{AB/PPD}$ were mostly attributed to the AB neuron (compare red and black traces). In those presynaptic voltage ranges, IPSP$_{PD}$ (blue trace) was significantly smaller in amplitude than IPSP$_{AB/PPD}$ (two-way ANOVA, $P < 0.05$; $B = 1.67 \times 10^{-2}; n = 6$). With larger PD neuron depolarizations ($V_{pre}$ to $-30$ and $-20 \text{ mV}$), the chemical transmission from the PD neurons increased. In those voltage ranges, both IPSP$_{PD}$ and IPSP$_{AB}$ were significantly smaller than IPSP$_{AB/PPD}$ (two-way ANOVA, $P < 0.05$; $B = 1.67 \times 10^{-2}; n = 6$) and not significantly different from each other, indicating that both AB and PD neurons were contributing to the compound synapse.

Blocking the AB to LP direct synapse in PTX removed the AB short time-to-peak component, revealing a slower PD component of synaptic transmission, the time to peak of the IPSPs was an example showing the voltage traces of the LP neuron in Fig. 3A, vertical arrow). Note that the synaptic responses (IPSP$_{AB/PPD}$, IPSP$_{PD}$, and IPSP$_{AB}$) recorded in the PY neuron during single square-pulse stimulation of the PD neurons were not significantly different in strength and time course compared with those elicited in the LP neuron. Moreover, the normalized I/O curve of the IPSPs in the PY neuron (Fig. 2E) showed a trend similar to that observed in the LP neuron (Fig. 2C). Specifically, the compound IPSP$_{AB/PPD}$ consisted mainly of the AB component at $V_{pre}$ values of $-50$ and $-40 \text{ mV}$ and of both the AB and PD components at more depolarized presynaptic voltages. Plotting $\Delta V_{PY}$ versus $V_{pre}$ showed that IPSP$_{PD}$ reached its maximal peak value significantly later at all presynaptic voltage values compared with IPSP$_{AB}$ (Fig. 2F; two-way ANOVA, post hoc Tukey analysis, $P \leq 0.049$ for all $V_{pre}$ values; $n = 6$).

To characterize the parameters of the short-term dynamics of the synapses (extent of depression and time course of recovery) from the pacemaker neurons to the LP and PY neurons, we used a train of square pulses of fixed duration and different interpulse intervals (IPIs: 250–8,000 ms; Fig. 3). Figure 3A is an example showing the voltage traces of the LP neuron in response to a train of pulses with 500-ms IPI into the PD neuron. The synapses from IPSP$_{AB/PPD}$ (red trace) and from the individual AB (black trace) and PD (blue trace) components showed short-term depression: the second and subsequent presynaptic depolarization pulses elicited IPSPs (Fig. 3A, horizontal arrow) that were smaller in amplitude than those elicited by the first pulse (Fig. 3A, vertical arrow). Note that the

FIG. 3. Synaptic dynamics of the IPSPs in response to a train of voltage pulses in the PD neurons. A: PD neurons were stimulated with a series of 5 pulses of 40-mV amplitude from $V_{hold} = -60 \text{ mV}$ with IPI 250 ms in control (red) and PTX (blue) while the response in the LP neuron was monitored. IPSP$_{AB}$ was approximated by subtracting the 2 traces (black trace). All IPSPs in response to the 5th pulse (horizontal arrow) were smaller in amplitude than the 1st pulse IPSPs (vertical arrow), indicating short-term synaptic depression. Note that we measure the amplitude of each IPSP from $V_{rest}$ of the LP neuron. LP neuron had an initial membrane potential of $-59 \text{ mV}$. B: extent of depression of the LP IPSP response was calculated as the ratio of the 5th peak amplitude to the amplitude of the 1st peak for all IPIs used (250–8,000 ms). Data were plotted vs. IPI ($n = 6$). Extent of depression of IPSP$_{PY}$ was similar to that of IPSP$_{AB}$ at all IPIs. C: same as in B using PY as the postsynaptic neuron (* indicates significant difference).
amplitude of each IPSP was measured from the resting potential of the postsynaptic neuron (see METHODS).

The extent of synaptic depression was quantified as the ratio of the final (fifth) IPSP peak amplitude to the first plotted versus IPI (Mamiya et al. 2003; Rabbah and Nadim 2005) (Fig. 3B). At the shortest IPI, IPSP_{PD} and IPSP_{AB} exhibited the greatest depression and as the duration of the IPI increased, the magnitude of depression decreased (ratio of 1 indicates maximum recovery from depression). Even though IPSP_{PD} tended to show more depression compared with IPSP_{AB}, statistically, their degrees of depression (for IPSPs recorded in the LP neuron) were not significantly different from each other (two-way ANOVA, \( P > 0.05; n = 6 \)). This trend was slightly more prominent in the responses recorded in the PY neuron (Fig. 3C; two-way ANOVA, post hoc Tukey analysis, \( P < 0.001 \) for IPI 250 ms; \( n = 6 \)). However, for all IPIs tested, the degrees of depression of the synaptic responses (IPSP_{AB/PD}, IPSP_{PD}, and IPSP_{AB}) recorded in the PY neuron were not significantly different compared with those elicited in the LP neuron (two-way ANOVA, \( P > 0.05; n = 6 \)).

To fully characterize the parameters of short-term synaptic dynamics, we measured the time constant of recovery (\( \tau_{rec} \)) from depression by looking at the relationship between recovery and IPI. In each experiment, we graphed the ratio of the fifth peak/first peak versus IPI and fit it with a first-order exponential decay curve with the equation (1 - D_{max})e^{-\frac{t}{\tau_{rec}}} (for example, see Fig. 4E; also Mamiya et al. 2003). D_{max} is the maximum amount of depression of a synapse as IPI tends to zero and \( \tau_{rec} \) is the time constant of recovery. Average \( \tau_{rec} \) values for the synaptic depression in the LP neuron showed that IPSP_{PD} (\( \tau_{rec} = 311 \pm 28.910 \) ms) tended to recover faster than IPSP_{AB} (\( \tau_{rec} = 478 \pm 103.501 \) ms), although this difference was not statistically significant (paired t-test, \( P = 0.192; n = 6 \); data not shown). Similarly, IPSP_{PD} to the PY neuron tended to recover from depression faster than IPSP_{AB} but not significantly (\( \tau_{rec} \) values of 491 \pm 110.692 and 576 \pm 96.132 ms, respectively; data not shown). The \( \tau_{rec} \) values for all IPSPs (IPSP_{AB/PD}, IPSP_{PD}, and IPSP_{AB}) recorded in the PY neuron were not significantly different compared with those recorded in the LP neuron (two-way ANOVA, \( P > 0.05; n = 6 \)).

In the experiments described to this point, we used the conventional square-pulse method (Johnson et al. 1995; Manor et al. 1997; Rabbah and Nadim 2005) to characterize various properties of the synapses from the pacemaker neurons onto

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**FIG. 4.** IPSPs in the LP neuron in response to a train of 2 realistic PD waveforms. A: PD neurons were stimulated with 2 types of realistic waveforms: duty cycle (DC) 46% (top trace) and DC 17% (bottom trace). Both waveforms were pre-recorded in the PD neuron from 2 different preps, low-pass filtered (at 10 Hz), and amplified to 40-mV amplitudes. They were injected from \( V_h = -60 \) mV in a train of 5 pulses with frequencies ranging from 0.5 to 4 Hz. B: LP response to DC 46% (top) and DC 17% (bottom) with cycle period 500 ms. IPSPs elicited by the individual AB (black trace) and PD neurons (blue trace) and by the compound AB/PD unit (red trace) are superimposed for comparison. C: extent of depression of the IPSPs (5th/1st peak) in response to DC 46% was plotted vs. cycle periods (\( n = 6 \)). D: extent of depression of the IPSPs in response to DC 17% plotted vs. cycle periods showed that IPSP_{AB} exhibited significantly less depression (marked by *) than IPSP_{PD} for cycle periods 250–1,000 ms (\( n = 6 \)). E: time constant of recovery from depression of the IPSPs was calculated by fitting the 5th/1st ratio vs. cycle periods for each experiment with a single exponential fit (dashed line). F: average time constant of recovery (\( \tau_{rec} \)) values. For DC 17%, IPSP_{AB} recovered significantly faster than IPSP_{PD} (\( n = 6 \)) (* indicates significant difference).
the LP and PY neurons. However, it has been shown that square pulses do not fully capture the temporal dynamics of graded synapses (Manor et al. 1997; Olsen and Calabrese 1996). Graded synapses are sensitive to the shape of the presynaptic depolarizations and their frequency (Mamiya and Nadim 2004; Manor et al. 1997; Olsen and Calabrese 1996; Simmons 2002). Therefore, we voltage clamped the presynaptic PD neurons using two different realistic waveforms, prerecorded during fast (623-ms) and slow (1,358-ms) pyloric cycle periods (see METHODS; also Rabbah and Nadim 2005). We indexed the change in waveform shape by the waveform duty cycle (DC) and chose two representative duty cycles—46% (Fig. 4A, top) and 17% (Fig. 4A, bottom)—to stimulate the presynaptic neurons.

The realistic waveforms were applied into the two voltage-clamped PD neurons from a baseline of −60 mV with a fixed 40-mV (trough-to-peak) amplitude and with cycle periods 250 to 2,000 ms (frequency 4 to 0.5 Hz). Figure 4B shows an example of IPSP_{AB/PD} (red traces), IPSP_{PD} (blue traces), and IPSP_{AB} (black traces) elicited in the LP neuron in response to a train of waveform stimulations corresponding to DC 46% (top traces) and DC 17% (bottom traces) and cycle period of 500 ms. As with trains of voltage pulses, all IPSPs showed short-term depression and recovered from depression as the cycle period was increased (Fig. 4, C and D). Also, similar to trains of voltage pulses, the degrees of depression of IPSP_{PD} and IPSP_{AB} in response to DC 46% (Fig. 4C) were statistically similar (two-way ANOVA,  \( P = 0.836; n = 6 \)). Interestingly, however, in response to DC 17%, IPSP_{AB} exhibited significantly less depression compared with IPSP_{PD} at the shortest IPIs tested (Fig. 4D; two-way ANOVA, post hoc Tukey analysis,  \( P = 0.041 \) for periods 250, 500, and 1,000 ms; \( n = 6 \)).

We measured the \( \tau_{rec} \) of the IPSPs in response to the two realistic waveforms as previously described for Fig. 3 (see also Fig. 4E). Figure 4F shows that, in response to DC 46%, the PD component tended to recover from depression faster than the AB component but this tendency was not statistically significant (\( \tau_{rec} \) values of 390 ± 46.303 and 536 ± 98 ms, respectively; Student’s t-test,  \( P = 0.198; n = 6 \)). This trend was similar to that seen with the square-pulse protocol. Interestingly, however, when DC 17% was used, the trend of recovery from depression was reversed: The IPSP that the LP neuron received from the PD neuron recovered significantly more slowly from depression than the IPSP from the AB neuron (\( \tau_{rec} \) values of 510 ± 76.903 and 344 ± 34 ms, respectively; Student’s t-test,  \( P = 0.029; n = 6 \)).

We also activated the synapse between the pacemaker neurons and the PY neuron with the two presynaptic realistic waveforms in control and PTX conditions. Figure 5A shows an example of IPSP_{AB/PD} (red traces; control), IPSP_{PD} (blue traces; PTX), and IPSP_{AB} (black traces) recorded in the PY neuron in response to a train of waveform stimulations corresponding to DC 46% (top traces) and DC 17% (bottom traces) and cycle period of 500 ms. All IPSPs showed short-term depression as the cycle period was increased (Fig. 5, B and C). Unlike what was seen in the LP neuron, in response to both PD realistic waveforms, IPSP_{AB} exhibited significantly less depression compared with IPSP_{PD} (two-way ANOVA, post hoc Tukey analysis,  \( P = 0.042 \) for periods 250, 500, 1,000, 1,500, and 2,000 ms; \( n = 6 \)). However, comparison of the degrees of depression in the LP versus PY neurons attributed to IPSP_{PD} and IPSP_{AB} were not significantly different from each other (two-way ANOVA,  \( P > 0.05; n = 6 \)).

Measurement of the \( \tau_{rec} \) of the IPSPs in response to the two realistic waveforms showed similar trends to the LP neuron.

FIG. 5. IPSPs in the PY neuron in response to a train of 2 realistic PD waveforms. A: PY response to the stimulation of the PD neurons with DC 46% (top) and DC 17% (bottom) corresponding to amplitude 40 mV and cycle period 500 ms. B: ratio 5th/1st peak in response to DC 46% plotted vs. cycle periods showed that IPSP_{AB} depressed significantly less than IPSP_{PD} (\( n = 6 \)). Again, the extent of depression of IPSP_{PD} in response to DC 17% was significantly less than IPSP_{PD}. D: average \( \tau_{rec} \) values of the IPSPs calculated as in Fig. 4E (\( n = 6 \)) (\( * \) indicates significant difference).
(compare Fig. 4F with Fig. 5D). In response to activating the presynaptic neurons with DC 46%, the PD to PY component tended to recover from depression faster than the AB component ($\tau_{rec}$ values of 410 ± 48.304 and 580 ± 136.703 ms, respectively; Student’s t-test, $P = 0.142; n = 6$). Moreover, with DC 17%, IPSP$\text{PD}$ tended to recover more slowly than the synapse from the AB neuron ($\tau_{rec}$ values of 599 ± 110 and 364 ± 80.902 ms, respectively; Student’s t-test, $P = 0.141; n = 6$). Neither of these trends was statistically significant. Also, the $\tau_{rec}$ values for the LP versus PY neurons arising from IPSP$\text{PD}$ and IPSP$\text{AB}$ were not significantly different from each other (two-way ANOVA, $P > 0.05; n = 6$).

The maximal peak amplitude of the IPSP in the LP neuron elicited by either AB or PD components appeared sensitive to the slope of the rising phase of the presynaptic depolarizations (Fig. 6). We focus on the IPSPs in response to the fifth waveform (see Fig. 5A) because it carries information about the dynamics of the “stationary” synapses after transient changes are over. Figure 6A presents a comparison of the amplitudes of the fifth IPSPs elicited with DC 46% (left) with those elicited with DC 17% (right) corresponding to the 500-ms cycle period. We observed that, in response to DC 46%, IPSP$\text{AB}$ was smaller in amplitude than IPSP$\text{PD}$ (Fig. 6A, left; compare black and blue traces). Similar to square pulses, we measure the amplitude of each IPSP from $V_{rest}$. However, in response to DC 17%, IPSP$\text{AB}$ and IPSP$\text{PD}$ appeared equal in amplitude (Fig. 6A, right; compare black and blue traces). To illustrate the relative contribution of the AB and PD components to the total synaptic transmission onto the LP neuron (Fig. 6A, red traces), we normalized the steady-state amplitudes of IPSP$\text{PD}$ and IPSP$\text{AB}$ to the steady-state amplitude of IPSP$\text{AB/PD}$ at frequency 0.67 Hz and plotted the values as a function of presynaptic frequency (Fig. 6, $B$ for DC 46% and $C$ for DC 17%). The results of the data presented in these two panels are twofold. First, IPSP$\text{AB}$ showed no dependency on presynaptic frequency irrespective of the presynaptic waveform used (one-way ANOVA, $P = 0.151$ for DC 46% and $P = 0.438$ for DC 17%; $n = 6$), indicating that the AB synapse is activated to the same degree for all presynaptic frequencies and duty cycles tested. In contrast, the steady-state amplitudes of IPSP$\text{PD}$ significantly decreased as the frequency was increased (one-way ANOVA, $P < 0.001$ for both DC 46% and DC 17%; $n = 6$). Second, the relative contribution of the PD neurons compared with the AB neuron was dependent on the shape of the presynaptic waveform: When the presynaptic waveform with DC 46% was used, IPSP$\text{PD}$ recorded in the LP neuron at all frequencies (except 4 Hz) was significantly larger in amplitude than IPSP$\text{AB}$ (Fig. 6B; two-way ANOVA, post hoc Tukey analysis, $P \leq 0.004$ for frequencies 0.5, 0.67, 1, and 2 Hz; $n = 6$). In contrast, when DC 17% was used, IPSP$\text{PD}$ and IPSP$\text{AB}$ were recruited to similar levels for most frequencies tested (Fig. 6C; two-way ANOVA, post hoc Tukey analysis, $P \leq 0.026$ for frequencies 0.5 and 0.67 Hz only; $n = 6$). These results suggest that, at low frequencies, the synapse from the pacemaker unit onto the LP neuron is dictated mostly by the PD neurons for both presynaptic duty cycles tested. As the frequency of the presynaptic depolarization is increased, the PD neuron contribution decreases and approaches the (unchanging) AB neuron contribution but to a different degree, depending on the duty cycle of the presynaptic waveform.

We repeated all of the above measurements for the PY neuron (Fig. 7). Figure 7A shows a comparison of the amplitudes of the steady-state IPSPs elicited in the PY neuron with DC 46% (left) with those elicited with DC 17% (right) corresponding to a 500-ms cycle period. Similar to the results obtained in the LP neuron at that cycle period, (steady-state) IPSP$\text{AB}$ elicited due to DC 46% was smaller in amplitude than IPSP$\text{PD}$ (Fig. 7A, left), whereas IPSP$\text{AB}$ in response to DC 17% was equal in amplitude to IPSP$\text{PD}$ (Fig. 7A, right). When the steady-state amplitudes of IPSP$\text{PD}$ and IPSP$\text{AB}$ were normalized to the steady-state amplitude of IPSP$\text{AB/PD}$ at a frequency of 0.67 Hz and plotted as a function of presynaptic frequency (Fig. 7, $B$ for DC 46% and...
two-way ANOVA, similar in response to DC 17% at all frequencies (Fig. 7).  

The relative amplitudes of IPSP_{PD} and IPSP_{AB} in the LP versus the PY neurons were not significantly different from each other (two-way ANOVA, $P > 0.05$; $n = 6$).

The time-to-peak of the IPSPs provides information about the time course of synaptic transmission. Thus we compared changes in the peak time of the IPSPs in response to the stationary (i.e., fifth) presynaptic realistic waveform stimulations for all frequencies tested (Fig. 8 for LP and Fig. 9 for PY). Figure 8A (and Fig. 9A for PY) shows a comparison of the time course of synaptic transmission for the stationary IPSP_{PD} and IPSP_{AB} in response to DC 46% (top) and DC 17% (bottom) corresponding to the frequency of 2 Hz. In response to both realistic waveforms in the PD neurons, the onset of the IPSP from the PD neurons was delayed compared with the onset of the IPSP from the AB neuron (Fig. 8A for LP and Fig. 9A for PY; double-headed arrows). This delay in the onset of synaptic response affected the time the response needed to reach its maximal hyperpolarization peak (and decay): For DC 46%, the PD to LP (and PY) transmission reached its maximum peak at the end of the waveform depolarization, whereas the AB to LP (and PY) maximum synaptic peak was perfectly aligned with the presynaptic depolarization peak (Fig. 8A for LP and Fig. 9A for PY, top panels; compare blue and black open circles). For DC 17%, maximum peak IPSP_{PD} occurred after the depolarization was over (Fig. 8A for LP and Fig. 9A for PY, bottom panels; compare black and blue open circles). Similar to the results obtained with the square-pulse stimulation of the presynaptic neurons, these results suggest that the PD synapses exhibit delayed (compared with the AB synapse) inhibitory actions in the LP and PY neurons.

We examined the frequency-dependent effect of this delay in synaptic transmission to the peak phase of the IPSPs in the LP and PY neurons. Phase describes the normalized (by period) time of the IPSP peak in the postsynaptic neuron relative to the presynaptic oscillations. We calculated phase as $\Delta t/\text{Period}_{applied}$, where $\Delta t$ was measured from the most depolarized peak of the presynaptic waveform to the maximum hyperpolarized peak of the IPSP (see example in Fig. 8B, inset). The values were then plotted versus frequency for DC 46% (Fig. 8B for LP and Fig. 9B for PY) and DC 17% (Fig. 8C for LP and Fig. 9C for PY). Note that negative values of peak phase indicate that the IPSP actually reached its maximal hyperpolarized peak before the presynaptic waveform reached its maximal depolarized value. The LP (and PY) IPSP peak phase was significantly delayed because of the synapse from the PD neuron compared with the synapse from the AB neuron, independent of presynaptic waveform type (two-way ANOVA; LP: $P = 0.001$ for DC 46% and $P = 0.015$ for DC 17%; PY: $P = 0.003$ for DC 46% and DC 17%; $n = 6$). Moreover, we found that the peak phase of the IPSPs arising from the individual AB and PD components in response to both presynaptic realistic waveforms showed a dependency on presynaptic frequency in both the LP and PY neurons (one-way ANOVA, $P < 0.001$; $n = 6$). As frequency increased, the postsynaptic IPSP peaked later in phase. There was also a dependency on the

![Fig. 7. Peak amplitude of the IPSPs in the PY neuron appeared sensitive to the slope of the rising phase of the presynaptic depolarizations. A: IPSPs in response to the 5th waveform elicited with DC 46% (left) and DC 17% (right) corresponding to cycle period 500 ms. IPSP_{AB} is smaller in amplitude than IPSP_{PD} for DC 46% and equal in amplitude for DC 17%. B: steady-state amplitudes of IPSP_{PD} and IPSP_{AB} in the PY neurons were normalized and plotted as a function of presynaptic frequency as in Fig. 6. Relative contributions of both the AB and PD components were not significantly different from each other at all frequencies tested ($n = 6$). C: amplitudes of the IPSPs in response to DC 17% were normalized as in Fig. 6 and plotted vs. frequency. Again, no significant difference between the amplitudes at all frequencies tested ($n = 6$) (* indicates significant difference).](https://jn.physiology.org/doi/abs/10.1152/jn.00224.2007)
waveform duty cycle: activating the presynaptic neurons with DC 46% and frequency 0.5 to 1 Hz acted to significantly advance the peak phase of both IPSPPD and IPSPAB in the LP and PY neurons (DC 46% vs. DC 17%: two-way ANOVA, post hoc Tukey analysis, \( P < 0.05 \) for frequencies 0.5, 0.67, and 1 Hz; \( n = 6 \)). The peak phases of IPSPPD and IPSPAB in the LP versus PY neurons for both realistic waveforms were statistically similar to each other (two-way ANOVA, \( P > 0.05 \); \( n = 6 \)).

**Correlation between the dynamics of IPSP\textsubscript{AB} and IPSP\textsubscript{PD} on the phase onset of the LP and PY neurons**

Thus far, we have shown that the synaptic dynamics of the pacemaker AB and PD neurons exhibit distinct dynamics dependent on the frequency and shape of the presynaptic depolarizations. Specifically, the synaptic transmission from the PD neurons is a relatively slow process that serves to delay the peak of the hyperpolarization in the postsynaptic LP and PY neurons (and thus their IPSP peak phase) at all presynaptic frequencies. The opposite is true for the synaptic transmission from the AB neuron. Moreover, the relative contributions of the AB and PD neurons to the total synaptic output onto the LP and PY neurons change as a function of presynaptic depolarization.

![Figure 8](image1.png)

**FIG. 8.** Time course of synaptic transmission of the IPSPs in the LP neuron in response to the realistic waveform stimulations in the PD neurons and its effect on the LP IPSP peak phase. A: IPSPs in response to the 5th waveform elicited with DC 46% (top) and DC 17% (bottom) at cycle frequency 2 Hz showed that IPSP\textsubscript{PD} (blue open circles) reached its maximum peak significantly later in time than IPSP\textsubscript{AB} (black open circles) independent of the duty cycle of the waveform used. Double-headed arrows are meant to represent only the delay between the start of hyperpolarization in the LP neuron arising from the AB vs. the PD synapse. B: LP IPSP peak phase was calculated as \( \Delta t \) (measured from the peak of the presynaptic waveform to the peak IPSP) divided by Period\textsubscript{applied} (inset) and plotted vs. frequency (\( n = 6 \)). In response to DC 46%, the LP IPSP peak phase was significantly delayed because of the synapse from the PD neurons (blue trace). C: similar results were obtained for DC 17% (\( n = 6 \)) (* indicates significant difference).

![Figure 9](image2.png)

**FIG. 9.** Time course of synaptic transmission of the IPSPs in the PY neuron in response to the realistic waveform stimulations and its effect on the IPSP peak phase. A: IPSPs in response to the 5th waveform elicited with DC 46% (top) and DC 17% (bottom) at cycle frequency 2 Hz showed that IPSP\textsubscript{PD} (blue open circles) reached its maximum peak significantly later in time than IPSP\textsubscript{AB} (black open circles) independent of the duty cycle of the waveform used. Double-headed arrows are meant to represent only the delay between the start of hyperpolarization in the PY neuron attributed to the AB vs. the PD synapse. B and C: PY IPSP peak phase was calculated as in Fig. 8B and plotted vs. frequency (\( n = 6 \)). Results were similar to those reported for the LP neuron for both DC 46% (\( B, n = 6 \)) and DC 17% (\( C, n = 6 \)) (* indicates significant difference).
The correlation between the strength of the IPSPs and the LP burst phase does not account for the distinct temporal dynamics of IPSP_{PD} and IPSP_{AB}. Thus we calculated the IPSP time-to-peak with respect to the presynaptic stimuli (see example in Fig. 8B, inset), divided the values by the applied period of 1 s (Period_{applied}), and examined their correlation to the LP burst phase during the natural pyloric rhythm (Mamiya et al. 2003). The right and left panels of Fig. 10C are scatterplots of these results using DC 46% and DC 17%, respectively. Our results show that when the presynaptic stimulus has a duty cycle of 46 or 17%, the burst phase of the LP neuron is not correlated to the IPSP peak phase it receives from either the PD (blue stars) or AB (black stars) neuron. In addition, the peak phase of IPSP_{AB/PD} was not correlated to the LP burst phase with either waveform (Fig. 10D).

In contrast to what was seen in the LP neuron, in response to either presynaptic waveform, the PY burst phase (during the normal ongoing rhythm) showed a weak negative correlation to the amplitudes of both IPSP_{PD} and IPSP_{AB}, i.e., as the IPSP amplitudes increased, the PY phase was delayed (Fig. 11A, left for DC 46%; IPSP_{PD}: \( y = -0.014x + 0.601, r = -0.601, P = 0.251; \) IPSP_{AB}: \( y = -0.019x + 0.651, r = -0.450, P = 0.371; \) Fig. 11A, right for DC 17%; IPSP_{PD}: \( y = -0.015x + 0.592, r = -0.931, P = 0.021; \) IPSP_{AB}: \( y = -0.015x + 0.612, r = -0.611, P = 0.111. \) In response to both waveforms, the amplitude of the compound synapse IPSP_{AB/PD} was also weakly negatively correlated to the burst phase of the PY neuron (Fig. 11B; for DC 46%: \( y = -0.01x + 0.592, r = -0.791, P = 0.112; \) for DC 17%: \( y = -0.015x + 0.572, r = -0.821, P = 0.081. \)

Correlations of the IPSP peak phases with respect to Period_{applied} to the PY burst phase during the natural pyloric rhythm are shown in Fig. 11, C and D. With DC 46% or DC 17%, the burst phase of the PY neuron tended to be very weakly negatively correlated to the IPSP peak phase it receives from the AB neuron only (Fig. 11C; for DC 46%: \( y = -0.51x + 0.589; r = -0.687; P = 0.131; \) for DC 17%: \( y = -1.42x + 0.616; r = -0.602; P = 0.205; n = 6). In addition,
the peak phase of the compound synapse IPSP_{AB/PD} was negatively correlated to the PY burst phase when the presynaptic neurons were activated with either duty cycle (Fig. 11D; for DC 46%: \( y = -0.502x + 0.649; r = -0.731; P = 0.098 \); for DC 17%: \( y = -6.04x + 0.841; r = -0.947; P = 0.004 \); \( n = 6 \)).

**DISCUSSION**

In a pacemaker-driven oscillatory network, the synaptic interactions between the pacemaker neurons and the follower neurons are of crucial importance. These synapses can play a role in setting the activity phase of the follower neurons and the frequency of the rhythmic output (Eisen and Marder 1984; Tryba and Ritzmann 2000; Wolf 1992). However, when neural networks use a heterogeneous population of pacemaker neurons, a greater level of complexity is added to the effect of pacemaker synapses on the relative phase relationships of member neurons. In the lobster pyloric network, the AB and PD pacemaker neurons produce compound chemical synapses to the follower LP and PY neurons. The compound synapses from these pacemaker neurons to the LP and PY neurons are identical in strength and dynamics and thus do not play a role in determining the relative activity phase of these follower neurons (Rabbah and Nadim 2005). However, neuromodulatory inputs to the pyloric network can differentially affect the intrinsic and synaptic properties of the AB and PD neurons as well as the strength of the electrical coupling between them (Harris-Warrick et al. 1998; Johnson and Harris-Warrick 1997; Johnson et al. 1993). In this manner, neuromodulators can emphasize one type of pacemaker neurons (and their synaptic connections) over the other, thus leading to a different functional network output. It is thus important to investigate the dynamics of the synapses originating from the AB and PD neurons separately and not only as a compound synapse.

We report a detailed examination of the temporal dynamics of the AB and PD synapses. Our work builds on previous studies of Eisen and Marder who reported on the neurotransmitter content, reversal potential, and ion selectivities of these two synapses, as well as their time course of release (Eisen and Marder 1982, 1984; Marder and Eisen 1984). We investigated the temporal dynamics of the synapses from the AB and PD synapses separately, in the absence of network neuromodulation, and addressed the functional implications of these synaptic dynamics in predicting the time of activity of the follower neurons.

**Synaptic efficacy depends on presynaptic waveform shape and frequency**

Our results show that the relative contributions of IPSP_{AB} and IPSP_{PD} to the compound AB/PD synapse in the LP and PY neurons are dependent on the shape of presynaptic waveform and frequency. We activated the PD neurons with two distinct realistic waveforms, with short (DC 17%) and long (DC 46%) duty cycles at the same amplitude and frequency, and recorded the postsynaptic responses (Fig. 6 for LP and Fig. 7 for PY). For all frequencies tested except 4 Hz, in response to DC 46%, IPSP_{PD} recorded in the LP and PY neurons was significantly larger than IPSP_{AB}. However, in response to DC 17%, IPSP_{PD} to the LP neuron was significantly larger at low frequencies (0.5 and 0.67 Hz) only. Taken together, these results indicate that the strength of the AB/PD synapse to the LP and PY neurons can be dictated either mostly by the PD neurons (as in the case of DC 46%) or by both the AB and PD neurons (as in the case of DC 17%), in a manner dependent on cycle frequency. Changes in cycle frequency are commonly induced in the pyloric circuit by network neuromodulators (Marder and Thirumalai 2002). In this manner, depending on the state of the network (i.e., which neuromodulator is present), the frequency (and duty cycle) can be altered, allowing either one or both of the pacemaker AB and PD neurons, and the temporal dynamics of their synaptic outputs, to be important determinants of network output.

**Short-term depression of the synapses is dependent on the membrane potential oscillation waveform of the PD neurons**

Short-term depression allows for the dynamic control of synaptic strength between network neurons as a function of cycle period (Manor et al. 2003; Nadim and Manor 2000). Our
results indicate that the short-term depression of IPSP_{AB} and IPSP_{PD} in the LP and PY neurons depends on the membrane potential waveforms of pacemaker neurons. Specifically, in response to waveforms with the sharpest rise time (square pulses) and longest duty cycle (DC 46%) activated with cycle periods comparable to the range of periods of the pyloric rhythm in vivo (Rezer and Moulins 1983; Turrigiano and Heinzel 1992), the synapses from the AB and PD neurons to the LP neuron depressed and recovered from depression to the same extent. However, in response to DC 17%, IPSP_{AB} depressed significantly less and recovered significantly faster. In the case of the PY neuron, the short-term dynamics observed for IPSP_{AB} and IPSP_{PD} was similar for the square pulses but different for both realistic waveforms used. These results indicate that the kinetics of both depression and recovery of the pyloric synapses is tuned so that the synaptic transmission is sensitive to period and duty cycle in the range over which the pyloric network normally operates (Mamiya and Nadim 2004, 2005; Manor et al. 1997). For example, in response to perturbations that increase cycle period and decrease duty cycle of the pacemaker neurons, the amplitudes of the pacemaker synapses to the LP and PY neurons will exhibit less depression and thus grow stronger. Such perturbations, elicited by neuromodulatory or synaptic inputs, can potentially modify the influence of the pacemaker neurons on the activity patterns of the follower neurons merely by changing the pacemaker membrane potential waveforms.

**Measured IPSP peak amplitude as a predictive measure of the burst phase of the follower neurons in the normal ongoing rhythm**

We were interested in investigating whether the relative amplitudes of the AB and PD synapses onto the LP and PY neurons measured in TTX conditions serve as predictive measurements of the burst phase of the LP and PY neurons in the ongoing rhythm. We found that the amplitude of the LP IPSP arising from either the AB or PD neurons was strongly positively correlated to the LP burst phase in the ongoing rhythm; the larger the IPSP, the earlier the LP burst phase. This is consistent with activating a hyperpolarization-activated inward current I_A, a current that is very prominent in the LP neuron and is responsible for the advancement of its burst phase following hyperpolarization (Harris-Warrick et al. 1995a,b; Tierney and Harris-Warrick 1992). In contrast to the findings in the LP neuron, but similar to the findings of Mamiya et al. (2003), a larger IPSP in the PY neuron (evoked by either AB or PD) resulted in a negative correlation to the PY burst phase in the ongoing rhythm. In this case, the larger IPSP most likely serves to activate larger amounts of the fast transient potassium current I_{h}, a subthreshold current involved in the postinhibitory property of the pyloric neurons (Graubard and Hartline 1984; Harris-Warrick 1989; Tierney and Harris-Warrick 1992). Hyperpolarization removes inactivation from this current and, when activated, it can delay the burst onset of the postsynaptic neuron (Harris-Warrick et al. 1995a). A clear understanding of the functional effects of the pacemaker IPSPs on the activity phases of the follower LP and PY neurons, however, requires further experiments that also take into account the synaptic connectivity between the follower neurons (Mamiya and Nadim 2005; Mamiya et al. 2003).

**Measured IPSP peak phase as a predictive measure of the burst phase of the follower neurons in the normal ongoing rhythm**

Synaptic outputs from the PD neurons are relatively slow, producing delayed peaks in the postsynaptic potentials of the LP and PY neurons at all presynaptic frequencies. The opposite is true for synaptic transmission from the AB neuron. It is expected that a delayed IPSP peak phase produce a delayed burst in the postsynaptic neuron. However, when we examined the relationship between the IPSP peak phase at various presynaptic periods and the phase of the LP neuron in the ongoing rhythm, we found no correlation between the two (Fig. 10, C and D). In contrast, when PY was the postsynaptic neuron, the burst phase tended to be (weakly) negatively correlated to the phase of IPSP_{AB}. This latter result is consistent with faster removal of inactivation of an outward current in the PY neuron. The PY neuron, and not the LP neuron, possesses appreciable amounts of I_A (Baro et al. 2000; Graubard and Hartline 1984; Harris-Warrick 1989; Tierney and Harris-Warrick 1992). It is plausible that if the IPSP in the PY neuron peaks earlier, a larger outward current is produced on rebound from inhibition, thus delaying the PY burst onset.

The absence of very strong correlations between the measured synaptic parameters and the burst phase of the follower LP and PY neurons in the ongoing rhythm is not completely unexpected. The pyloric neurons form a multitude of synaptic connections with each other, including mixed electrical and chemical synaptic connections between the LP neuron and PY neurons (Mamiya et al. 2003). Moreover, the pyloric neurons have a variety of nonlinear intrinsic ionic currents (Golowasch and Marder 1992; Johnson et al. 2003; Kloppenburg et al. 1999). Thus in an ongoing rhythm, the activity patterns of the LP and PY neurons are probably determined, not only by inputs from the pacemaker neurons, but by a dynamic combination of their intrinsic properties and various parameters of all their synaptic inputs, including strength, rate of transmission, and extent of depression and recovery (Golowasch et al. 1992; Harris-Warrick et al. 1995a; Hartline and Gassie 1979; Mamiya et al. 2003).

**Contribution of synaptic dynamics from the pacemaker neurons in determining the relative activity phase of the LP and PY neurons**

We previously reported that the dynamics of the synapses that the LP and PY neurons receive from the pacemaker ensemble are not different from each other (Rabbah and Nadim 2005). In this study, we found that the dynamics of the synapses originating from either presynaptic pacemaker neuron (AB or PD) onto the LP neuron versus the PY neuron are not distinct from each other either. The possibility still exists, however, that neuromodulators can alter short-term dynamics of the AB and PD synapses, thus breaking this homogeneity. Modulation of short-term depression was previously reported for the LP to PD synapse in crab *Cancer borealis* by the neuromodulator proctolin, which induced a shift from depression to facilitation of the LP to PD synapse, dependent on the amplitude of presynaptic depolarizations (Atamturktur et al. 2004). In this manner, neuromodulators can affect the total synaptic output of the pacemaker neurons, thus allowing the
AB and PD neurons to play a role in setting the relative activity phase of the LP and PY neurons.

In conclusion, we showed that, in a pacemaker-driven oscillatory network, the neurons that constitute the pacemaker ensemble can exhibit distinct synaptic dynamics onto the same classes of follower neurons. This heterogeneity provides the neural network with a mechanism for differential control of the relative contribution of each class of pacemaker neuron and its effluent targets to the overall network output. The principles reported in this work are not unique to invertebrate systems, but may be important for the generation of oscillatory activity in vertebrates. For example, the rhythmic activity of the inspiratory phase of the mammalian respiratory network is generated by a heterogeneous population of pacemaker neurons (Thoby-Brisson and Ramirez 2001). As in the crustacean pyloric pacemakers, the expression patterns of the mammalian respiratory pacemaker neurons are dependent on the state of the network, allowing for various functional outputs (Peña et al. 2004; Ramirez et al. 2004). It is thus important to emphasize that to fully understand the role of pacemaker neurons in shaping network output patterns we must examine not only their intrinsic and synaptic properties but also how these components can be modified by neuromodulatory inputs.

REFERENCES


