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Dynamic Control of Irregular Bursting in an Identified Neuron of an Oscillatory Circuit

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Elson, Robert C., Ramon Huerta, Henry D. I. Abarbanel, Mikhail I. Rabinovich, and Allen I. Selverston. Dynamic control of irregular bursting in an identified neuron of an oscillatory circuit. *J. Neurophysiol.* 82: 115–122, 1999. In the oscillatory circuits known as central pattern generators (CPGs), most synaptic connections are inhibitory. We have assessed the effects of inhibitory synaptic input on the dynamic behavior of a component neuron of the pyloric CPG in the lobster stomatogastric ganglion. Experimental perturbations were applied to the single, lateral pyloric neuron (LP), and the resulting voltage time series were analyzed using an entropy measure obtained from power spectra. When isolated from phasic inhibitory input, LP generates irregular spiking-bursting activity. Each burst begins in a relatively stereotyped manner but then evolves with exponentially increasing variability. Periodic, depolarizing current pulses are poor regulators of this activity, whereas hyperpolarizing pulses exert a strong, frequency-dependent regularizing action. Rhythmic inhibitory inputs from presynaptic pacemaker neurons also regularize the bursting. These inputs 1) reset LP to a similar state at each cycle, 2) extend and further stabilize the initial, quasi-stable phase of its bursts, and 3) at sufficiently high frequencies terminate ongoing bursts before they become unstable. The dynamic time frame for stabilization overlaps the normal frequency range of oscillations of the pyloric CPG. Thus, in this oscillatory circuit, the interaction of rhythmic inhibitory input with intrinsic burst properties affects not only the phasing, but also the dynamic stability of neural activity.

INTRODUCTION

Coordinated, oscillatory activity occurs in neural assemblies in many parts of the nervous system, including sensory circuits (Laurent 1996; Singer 1993), thalamic and cortical networks (Steriade et al. 1993), and motor centers (Grillner et al. 1995; Marder and Calabrese 1996). How can such networks produce reliable, rhythmic bursting when the intrinsic activity of individual neurons may be irregular (Mainen and Sejnowski 1995; van Steveninck et al. 1997) or chaotic (Abarbanel et al. 1996; Hayashi and Ishizuka 1992)? We suggest that the intrinsic instabilities of circuit neurons may be regulated by their synaptic interactions. To begin testing this hypothesis, we have studied an identified, irregularly bursting neuron of an oscillatory network, characterizing its dynamic response to periodic current pulses and to phasic inhibitory input from presynaptic, pacemaker neurons.

Central pattern generators (CPGs; the circuits that produce and control rhythmic movements) are favorable subjects for

this type of analysis. CPGs generate stereotyped patterns of rhythmic activity, and their component neurons and synaptic connections can be identified. Much detailed study (mainly in invertebrates) has yielded a qualitative understanding of rhythm generation in terms of interacting cellular and synaptic properties (Arshavsky et al. 1993; Getting 1989; Grillner et al. 1995; Harris-Warrick et al. 1992; Marder and Calabrese 1996; Selverston and Moulins 1985). However, more fundamental questions remain unresolved. For example, although most CPG circuits are dominated by inhibitory synaptic connections (Getting 1989; Marder and Calabrese 1996; Selverston and Moulins 1985), the functional advantage of connecting bursty neurons by inhibition rather than by alternative patterns of excitation is not clear. Further analysis requires the use of quantitative methods. Applying these techniques in appropriate experimental systems, we can start to dissect the effects of synaptic connectivity on the dynamics of neurons within circuits. In this paper we report an analysis of the dynamic behavior of an identified neuron within a well-characterized CPG.

The pyloric network of the lobster stomatogastric ganglion (STG) comprises 14 neurons whose identity and connectivity are completely established (Harris-Warrick et al. 1992; Miller 1987). Under normal modulatory influences, the circuit oscillates at 0.5–2 Hz. A set of three, electrically coupled, “pacemaker” neurons [anterior burster cell (AB) and 2 pyloric dilator cells (PDs)] burst rhythmically and inhibit all the other circuit neurons (Fig. 1A1). These rebound to fire regular bursts of spikes at phases set by their own active membrane properties and inhibitory synaptic interactions (Miller 1987).

The lateral pyloric neuron (LP) sits at an important node of the circuit. It interacts with several other pyloric neurons and provides strong synaptic feedback to the pacemaker group. LP itself receives phasic inhibition from the pacemakers and from several other circuit neurons (Fig. 1A1). In this setting, LP produces regular bursts of spikes within the pyloric rhythm. However, when isolated from the phasic inhibition provided by other pyloric neurons (Fig. 1A3), it bursts in an irregular pattern (Bal et al. 1988).

We term this irregular bursting of LP its *free-running* activity. The bursting persists in the absence of obvious phasic synaptic inputs, although its expression requires continued modulatory input from the anterior ganglia of the stomatogastric nervous system (Bal et al. 1988). The detailed origin of the irregularity is not examined here. Instead, we study the regulatory effects of different polarities and frequencies of phasic

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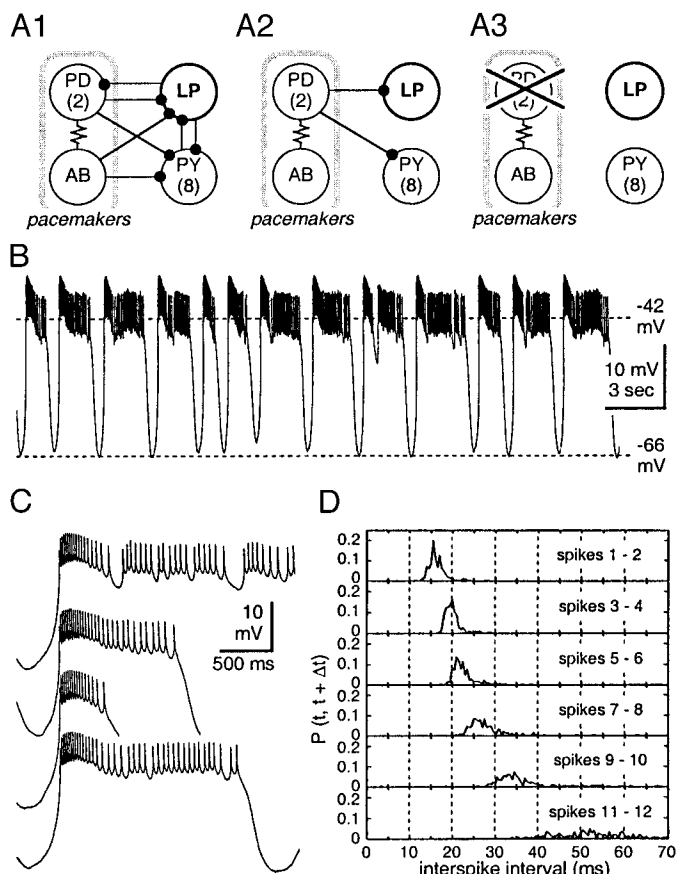


FIG. 1. Lateral pyloric (LP) neuron: circuitry and burst dynamics. *A*: simplified circuit diagrams of the pyloric central pattern generator [CPG; for simplicity, we omit the ventricular dilator neuron (VD) and inferior cardiac neurons]. In all cases the modulatory input provided by anterior ganglia of the stomatogastric nervous system is retained. *A1*: schematic of intact circuit. The LP neuron is emphasized by the thicker outline. *A2*: subcircuit for pacemaker inhibition of LP [obtained by killing VD and blocking some synapses with picrotoxin (PTX)]. *A3*: LP neuron, deprived of chemical synaptic inputs from pyloric circuit neurons. Minimally, the VD neuron is killed, PTX is applied, and the pyloric dilator (PD) neurons are killed or deeply hyperpolarized. Resistor, electrical synapse; black dot, inhibitory chemical synapse; number of members of the cell type (where >1) are given in parentheses; see text for abbreviations. *B*: intracellular voltage recording showing irregular, free-running bursting in an LP cell that is deprived of chemical synaptic inputs from other pyloric neurons [conditions: PTX, VD and anterior burster (AB) killed, both PD neurons deeply hyperpolarized; no offset current in LP; no 4-aminopyridine (4-AP)]. *C*: 4 successive bursts from the free-running activity of *B*, aligned by their 1st spike. *D*: probability distribution of interspike intervals for the 1st 12 spikes in each of 200 free-running bursts of another synaptically isolated LP. The ordinate, $P(t, t + \Delta t)$, plots the probability that the interval between a given pair of spikes occurs between a time t (specified on the abscissa) and $t + \Delta t$, where the bin size $\Delta t = 0.5$ ms.

input, as a first step in understanding how irregular bursting behavior is controlled synaptically within an oscillatory circuit.

We study the dynamic behavior of LP as follows: 1) when free-running in isolation from phasic inhibition; 2) when receiving periodic current pulses; and 3) when rhythmically inhibited by the pyloric pacemaker group (PD/AB) (Fig. 1A2). Rhythmic inhibitory inputs regularize LP's bursting activity, provided that they act within a dynamic time frame (set by the interaction of the synaptic input with intrinsic burst properties). This time scale overlaps the normal range of rhythm frequencies in the intact circuit. In contrast, the regularizing action of periodic excitatory input is much less. Our quantitative analy-

sis shows that rhythmic inhibition does not simply interrupt or "entrain" the irregular bursting, but produces dynamic stabilization. Thus inhibitory coupling can promote ordered bursting in irregular neurons of oscillatory circuits.

A preliminary account of this work appeared as an abstract (Elson et al. 1997).

METHODS

Experimental methods

Adult spiny lobsters, *Panulirus interruptus*, were caught locally and kept in running seawater until use. The stomatogastric nervous system, consisting of the STG and anterior (commissural and oesophageal) ganglia and their connecting and motor nerves, was removed from the foregut (Mulloney and Selverston 1974) and pinned out in a silicone elastomer (Sylgard)-lined dish, filled with normal saline (in mM: 479 NaCl, 13 KCl, 14 CaCl₂, 6 MgSO₄, 4 Na₂SO₄, 5 HEPES, and 5 TES; pH 7.4). The STG was separately superfused by a continuous flow of chilled saline (14–17°C; temperature variation was kept within 1°C during each recording session), to which drugs were added as needed. The ganglion was desheathed and the somata of pyloric neurons impaled by microelectrodes (filled with 3 M-KCl; resistance ~20 MΩ). Neurons were identified by their characteristic phase of bursting and by correlation of spikes with impulses in motor nerves. Voltage signals were amplified by conventional electrometers and stored on video tape. Quantitative analysis (see *Analytic methods*) was applied to results from experiments in seven preparations.

We focused on the single, LP within the pyloric circuit (Fig. 1A1). Synaptic inputs to LP were reduced in two stages (always retaining the normal modulatory influence of the anterior ganglia).

INHIBITORY SUBCIRCUIT (FIG. 1A2). Fast, glutamatergic inhibition was blocked with 7.5 μM picrotoxin (PTX) (Eisen and Marder 1982) and the cholinergic, ventricular dilator neuron (VD) was photoinactivated (Miller and Selverston 1979). In this configuration, the only remaining circuit input to LP came from the two PD cells, which, together with the AB cell, form a group of electrically coupled pacemaker neurons (PD/AB). The normal synaptic feedback to the pacemakers was blocked by the PTX (Fig. 1A2). The PD/AB group bursts as a single unit, in a nearly periodic pattern.

Single microelectrodes were placed in LP and both PDs. Pacemaker bursting was slowed by injecting hyperpolarizing offset currents into one PD while monitoring voltage activity in the other. Additional, periodic current pulses (~50 ms, +5 nA) were used to entrain pacemaker bursting at a stable frequency (slightly greater than that obtained with the offset current alone). Pacemaker output could be terminated by deep hyperpolarization of both PD neurons. This was a temporary method of removing all phasic inhibition from LP.

ISOLATION OF LP FROM INHIBITORY SYNAPTIC INPUT (FIG. 1A3). Lasting isolation of LP from the phasic, chemical synaptic input provided by other pyloric neurons was obtained by applying PTX and photoinactivating VD and both PDs (Bal et al. 1988; Bidaut 1980; Miller 1987). [A weak, rectifying electrical connection remains between LP and some pyloric (PY) neurons (Graubard and Hartline 1987; Johnson et al. 1993). The extent to which this interaction influences LP's bursting is presently unknown.] As long as modulatory influences from the commissural ganglia are maintained, LP will continue to burst, but in an irregular manner (Bal et al. 1988). The voltage activity of LP under these conditions is termed "free-running."

Two microelectrodes were placed in LP for separate current passage and voltage recording. To enhance voltage-dependent bursting, in three experiments we injected a small, constant hyperpolarizing current (in the range of -0.4 to -1.4 nA) (thought to compensate for the removal of background inhibition normally provided by the PD neurons) (Bal et al. 1988); in two of these, we also added 1 mM 4-aminopyridine to the STG superfusate (this reduces the A-current

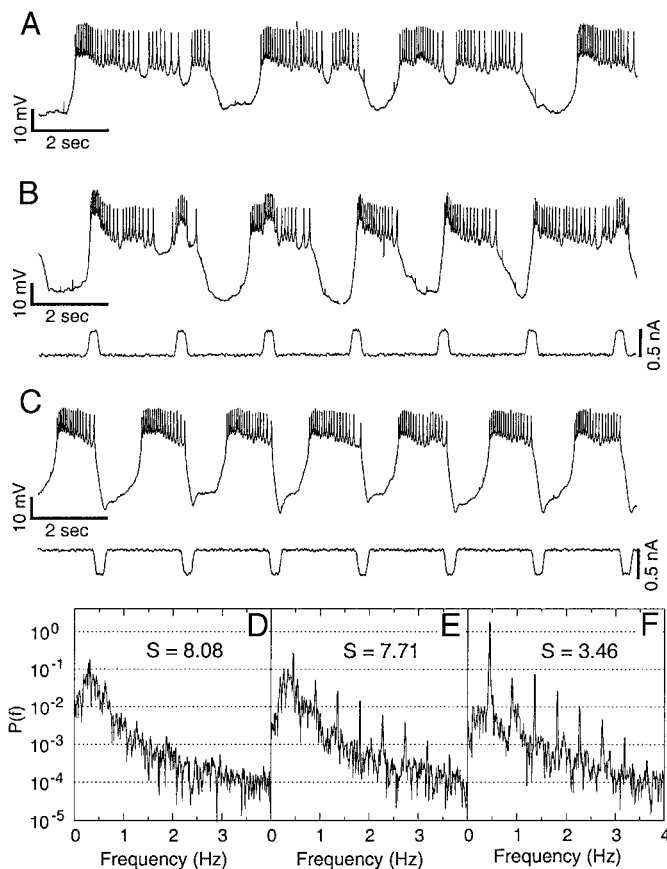


FIG. 2. Regulation of irregular bursting activity by injection of periodic current pulses. Recordings (A–C) and analysis (D–F) of voltage activity in an LP neuron after removal of chemical synaptic input from other pyloric neurons. A: free-running activity (no current pulses). B: forcing by periodic, depolarizing current pulses (frequency, 0.45 Hz). C: forcing by periodic hyperpolarizing pulses at the same frequency. D: Fourier power spectrum for free-running voltage activity of LP. E: spectrum for activity with periodic, depolarizing pulses. F: spectrum for activity with periodic, hyperpolarizing pulses. Note the logarithmic scale for power, $P(f)$. Entropy values (S) are given in bits. Conditions: VD and both PDs killed; PTX; 1 mM 4-AP. Current pulses (amplitude [0.4] nA, monitored in B and C, bottom trace) are superimposed on a constant d.c. offset of -1.6 nA.

by $\sim 25\%$) (Tierney and Harris-Warrick 1992). When used, these conditions are noted in the figure legends; however, irregular, free-running bursting activity could be observed in their absence (at least 4 experiments: cf. Figs. 1 and 4). To approximate synaptic input from bursting pacemaker neurons, we injected additional, smoothed pulses of current, ~ 200 ms in duration, 0.3–0.8 Hz in frequency, of either polarity and of constant amplitude (range between experiments, [0.3] to [0.5] nA).

Analytic methods

Long time series of voltage activity (5–6 min) were digitized at 2 kHz for off-line analysis. To evaluate the degree of irregularity of the observed oscillations, we utilized the power spectrum for each measurement and a measure of the entropy for the harmonics of the power spectra for frequencies up to 40 Hz. We first calculated the fast Fourier transform for $2^{19} = 524,288$ points (sampled every 0.5 ms) of voltage time series (cf. Figs. 2 and 4). Digitized voltage signals received no further filtering before Fourier analysis. The power in harmonics up to 40 Hz, $P(n)$ for $n = 0 \dots 20,976$, were converted to probability values by defining $p(n) = P(n)/\sum P(n)$. An entropy function (in bits), $S = -\sum_n p(n) \log_2 [p(n)]$, was then evaluated. S gives a

quantitative measure of the complexity of the power spectrum associated with the observed time series. In this measure, a power spectrum with a single peak (a linear periodic system) yields $S = 0$ bits. A spectrum with two isolated peaks (namely, a linear quasiperiodic system with two possible states) gives $S = 1$ bit. A flat or white spectrum extending to 40 Hz (or $n = 20,976$) yields $S = \log_2 20,976 = 14.35$ bits. Low values of S , relative to 14.35 bits, are then interpreted as associated with lower complexity in the oscillations of the system.

Within a given time series, we also calculated the variance between the voltage trajectories of successive bursts. Bursts were aligned at the site of minimum variance, and an average trajectory calculated. We computed the variance of individual trajectories from the average and plotted this value as a function of time (Fig. 5).

RESULTS

Irregular, free-running bursting of LP

When effectively isolated from the chemical synaptic inputs normally provided by the rest of the network (see Fig. 1A3 and METHODS; slow modulatory influences of anterior ganglia were retained), LP continued to burst, but in an irregular pattern (Fig. 1B) (Bal et al. 1988). In this free-running activity, bursts of spikes (which appear attenuated in somatic recordings) were driven by the slow oscillations of membrane potential. Each depolarized phase is underlain by a plateau potential (Bal et al. 1988; Russell and Hartline 1978, 1982). The plateaus were of variable duration and could develop smaller-amplitude oscillations; these features accounted for much of the irregular appearance of the time series (Fig. 1B). Nevertheless, the initiation of each burst was relatively stereotyped, with a similar initial waveform and pattern of spikes (Fig. 1C). This initial phase lasted ~ 500 ms. Thereafter, the slow waveform and spike pattern began to vary. The time of burst termination was highly variable (Fig. 1, B and C). An analysis of interspike intervals showed that spike timings were sharply defined at burst onset, but became increasingly variable as the burst continued (Fig. 1D).

Regularizing effects of periodic current pulses

This irregular, free-running activity contrasts markedly with the regular burst pattern that is produced when LP is connected to the rest of the circuit (Bal et al. 1988). In the intact network, the neuron receives rhythmic synaptic inputs that regulate the timing of its bursts (Miller 1987).

In our experiments reported here, we have begun a study of the dynamic response of LP to rhythmic inputs. Initially, we examined the reaction of the synaptically isolated neuron to injection of periodic current pulses (Figs. 2 and 3; $n = 3$ preparations). The amplitude and duration of pulses were adjusted to mimic those of rhythmic barrages of synaptic input from other pyloric cells.

We found strikingly different results, depending on pulse polarity (Fig. 2). With no current pulses, LP generated a typical, irregular burst pattern (Fig. 2A). The corresponding power spectrum showed a wide distribution with a broad peak centered at ~ 0.3 Hz (Fig. 2D). From this, we derived an $S = 8.08$ bits. With LP in this isolated state we applied periodic current pulses at a slightly higher frequency, 0.45 Hz. Depolarizing pulses approximating excitatory input affected both the timing and duration of bursts but produced little regularization

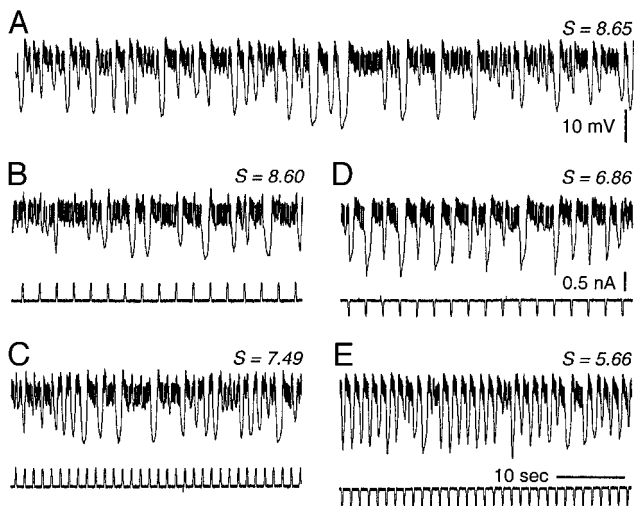


FIG. 3. Frequency-dependent regularization of bursting by periodic current pulses, illustrated by extended time series. Recordings are made from an LP neuron, after the removal of chemical synaptic input from other circuit neurons. *A*: free-running activity. *B*: depolarizing pulses at 0.4 Hz. *C*: depolarizing pulses at 0.8 Hz. *D*: hyperpolarizing pulses at 0.4 Hz. *E*: hyperpolarizing pulses at 0.8 Hz. Entropy values for each data set are given (S , in bits). Conditions: VD and both PDs killed; PTX; 1 mM 4-AP. Current pulses (monitored in *B–E*, bottom traces) are superimposed on a constant d.c. offset of -0.4 nA. (Different preparation from that in Fig. 2).

of the pattern (Fig. 2*B*). Falling during the interburst, a depolarizing pulse could trigger the onset of a new burst; falling during an ongoing burst, it had variable actions, sometimes triggering burst offset. Cycle-by-cycle, the bursting remained irregular (Fig. 2*B*). The corresponding Fourier spectrum showed little structural change, apart from the addition of local peaks at the stimulus frequency and its multiples. The entropy value was $S = 7.71$ (Fig. 2*E*).

In distinct contrast, hyperpolarizing pulses, mimicking inhibitory input, strongly affected burst timing and regularized the pattern (Fig. 2*C*). Falling during the plateau, the hyperpolarizing pulse could terminate the burst. Subsequently, the neuron underwent a hyperpolarized phase (outlasting the pulse) before it recovered to generate a new burst. Repetitions of this sequence produced a nearly, but not completely, periodic pattern of bursts (Fig. 2*C*). The Fourier spectrum underwent a structural change: power was now concentrated in sharp peaks located at the forcing frequency and its harmonics, and the entropy dropped to $S = 3.46$ (Fig. 2*F*).

Extended time series, taken from a similar experiment, show the regularization produced by pulses at different frequencies (Fig. 3). The free-running neuron displayed typical, irregular bursting and high entropy (Fig. 3*A*). At low frequency (0.4 Hz), depolarizing pulses produced little regularization and almost no reduction in entropy (Fig. 3*B*). At higher-frequency stimulation (0.8 Hz), the bursting showed episodic coordination, with a small decrease in entropy; however, the behavior was unstable (Fig. 3*C*). Hyperpolarizing pulses produced longer episodes of 1:1 and 1:2 coordination. Accordingly, burst patterns were more regular and showed larger reductions in entropy (Fig. 3, *D* and *E*). Greater regularization occurred at higher stimulus frequency. An intermediate stimulus frequency, 0.6 Hz, produced intermediate reductions in entropy for both pulse polarities (data not shown).

Regularization by synaptic inhibition from pacemaker neurons

Next, we examined the dynamic regularization of LP by a biological input (Figs. 4 and 5). We used the simple subcircuit shown in Fig. 1*A2*, in which the only phasic input to LP was the slow inhibitory postsynaptic potential evoked by the two PD neurons of the electrically coupled (PD/AB) pacemaker group (Eisen and Marder 1982). We then used current injection to alter the frequency of bursting in the pacemakers ($n = 3$ preparations).

Figure 4, *A–D*, left column, illustrates simultaneous intracellular recordings from LP and one of the PDs during subcircuit activity. With no current injection, the pacemakers generated rhythmic bursts (here at ~ 1.3 Hz; Fig. 4*A*, spontaneous). In each cycle, the PD neurons fired and inhibited LP, which then rebounded into its own burst before being inhibited again by the next burst of the pacemakers (Fig. 4*A*). Using current injection, we then forced the PDs to burst at progressively lower frequencies: 1 Hz (Fig. 4*B*), 0.65 Hz (Fig. 4*C*), and 0.4 Hz (Fig. 4*D*). Finally, we applied strong hyperpolarization to shut off the pacemakers completely (Fig. 4*E*: only the LP trace is shown). As the frequency of pacemaker input was reduced, LP developed increasingly irregular behavior (Fig. 4, *B–D*). On removal of inputs, the typical, free-running activity emerged (Fig. 4*E*).

The corresponding power spectra of LP activity are shown the Fig. 4, right column. During spontaneous subcircuit activity, the LP displayed a power spectrum with strong peaks at the pacemaker frequency and its harmonics (Fig. 4*A*). The entropy of LP activity was $S = 5.1$ bits. Forcing the pacemakers to burst more slowly caused the LP spectra to broaden and lose structure (while retaining local peaks at the forcing frequency and its harmonics; Fig. 4, *B–D*). When the pacemakers were shut off, LP activity displayed a broadband spectrum (Fig. 4*E*): the entropy increased to $S = 7.9$ bits.

To study effects on burst structure in LP, we analyzed the burst-by-burst variability of voltage oscillations (Fig. 5). The variance between voltage trajectories of individual bursts was computed for time series recorded in each condition (spontaneous input, forced input, etc.). The point of minimum variance occurred at the upswing of membrane potential preceding burst onset. Using this reference point (designated as *time 0 ms*), we aligned and superimposed the voltage traces of individual bursts (Fig. 5, left), and plotted the variance of trajectories as a function of time elapsed during the average burst cycle (Fig. 5, right).

With no circuit inputs (free-running activity, Fig. 5*E*) voltage traces converged before burst onset, remained correlated during the first ~ 500 ms of the burst, and then diverged again (Fig. 5*E*, left). The variance declined exponentially as the trajectories converged, jumped to a stable level for the initial stage of the burst, and then increased exponentially during the divergent tail (Fig. 5*E*, right: small oscillations on the plateau reflect correlated spike activity). This behavior resembles that of a typical nonlinear dynamic system showing chaotic oscillations with some additive noise.

Periodic inhibition introduced several changes (Fig. 5, *A–D*). At all frequencies, the inhibitory input terminated the preceding burst, forcing a strong, early convergence of voltage trajectories (with a corresponding drop in variance) before the

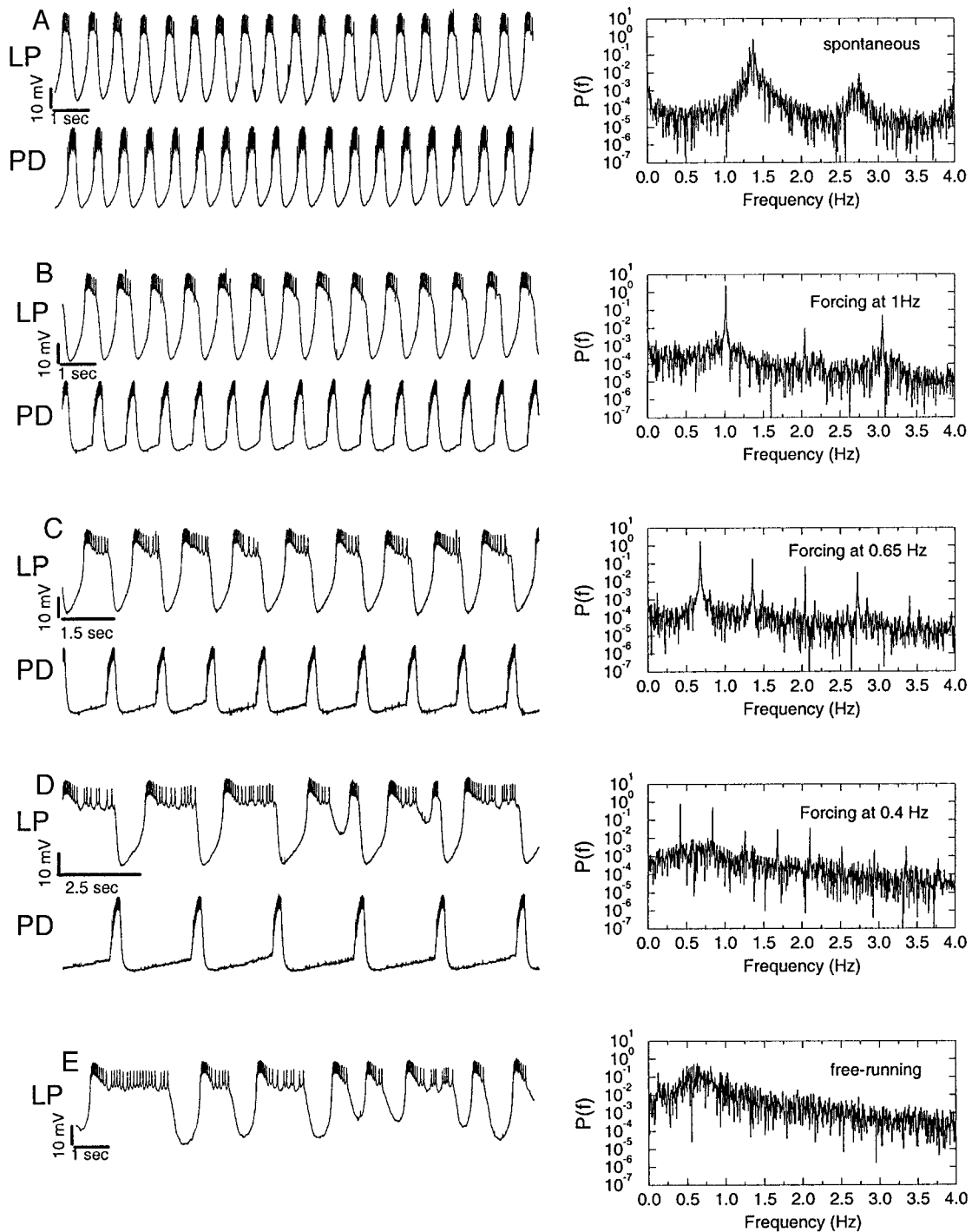


FIG. 4. Control of irregular bursting by rhythmic synaptic inhibition: intracellular recordings and Fourier power spectra. In this configuration, the only strong, phasic synaptic input to LP comes from the PD neurons, which are part of the pacemaker group (subcircuit in Fig. 1A2). The rhythmic bursting of the pacemakers was altered by current injection (see METHODS). *Left column*: simultaneous intracellular recordings from LP (*top trace* of each pair) and 1 PD neuron (*bottom trace*, except E, where PD is omitted). *Right column*: corresponding Fourier power [$P(f)$] spectra for LP membrane potential, evaluated from long time series. The displayed frequency range encompasses the slower voltage oscillations. A: spontaneous activity of subcircuit (no forcing of PD). B: PD forced to burst at 1 Hz. C: PD forcing at 0.65 Hz. D: PD forcing at 0.4 Hz. E: free-running activity of LP when PD bursting was shut off by deep hyperpolarization. Conditions: VD killed; PTX; no 4-AP; no offset current on LP.

initiation of a new burst on rebound. At the lowest input frequency (0.4 Hz), enough time elapsed between inputs to allow the tail of the burst to become unstable, although the rise in variance was delayed and slowed (Fig. 5D). At 0.65 Hz, the

onset of divergence was slowed further, so that the burst remained relatively stable until terminated by the next input (Fig. 5C). As the input frequency increased, bursts were terminated sooner, before the strong divergence of trajectories

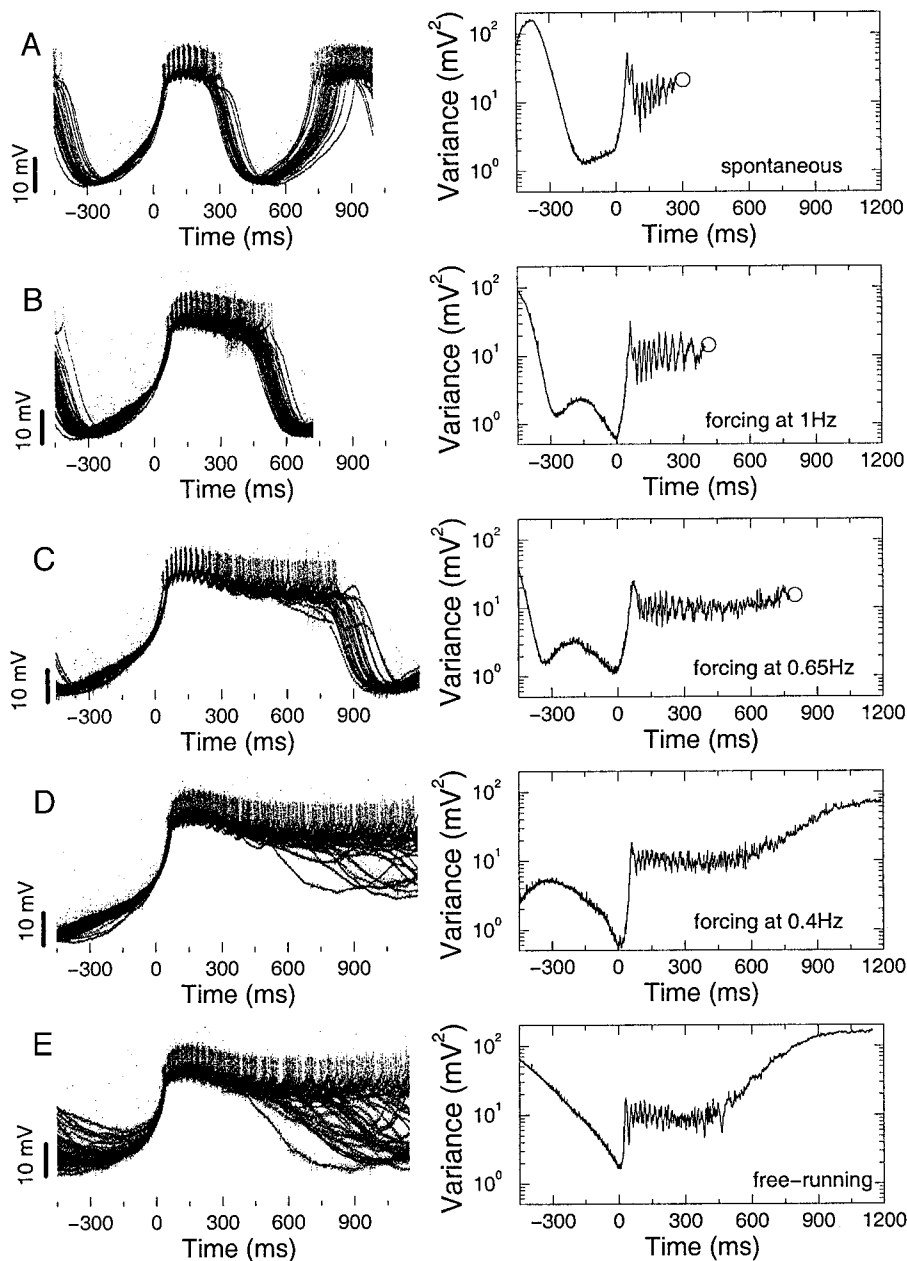


FIG. 5. Frequency-dependent effects of periodic synaptic inhibition on waveform and variability of bursts in LP (same experiment and data sets as in Fig. 4). *Left column*: overlays of individual burst trajectories. *Right column*: variance between voltage traces of individual bursts. Columns show corresponding time frames, measured relative to the averaged burst trajectory. *Time 0 ms* marks the point of alignment. This was the point of minimum variance (except in A, where aligned traces were shifted into register with the other rows). Negative times show the hyperpolarized phase preceding burst onset. In A–D this phase is determined by a wave of inhibition from the presynaptic PD neurons. The PD burst (not shown) lasts ~ 300 ms and ends at about -400 ms. Positive times show the evolution of the LP burst: open circles mark the mean time of burst termination. Each variance plot is calculated from a set of >165 bursts, of which the corresponding overlay shows a representative sample ($\sim 30\%$ of the total).

and exponential growth of variance could develop. The correlation, between bursts, of the initial sequence of spikes was also increased (Fig. 5, A and B). This indicates a regularizing effect on spike discharge.

DISCUSSION

Our results show that irregular bursting in LP, a component neuron of the pyloric CPG, is subject to dynamic regulation by rhythmic synaptic inhibition from the pyloric pacemaker cells (PD/AB). The regularizing effect of periodic inhibition is frequency dependent within a range determined by the time scale of the developing instability. This range overlaps the normal burst frequencies of the presynaptic pacemakers. Interestingly, periodic excitation was much less effective.

Dynamics of bursting in component neurons of the pyloric CPG

When the pyloric circuit of the STG is intact and activated by modulatory input from the anterior ganglia, it generates a regular, rhythmic pattern of activity in which the bursts of its component neurons are tightly coordinated. Each component neuron expresses a particular set of burst-generating membrane properties. Its bursting activity in the intact circuit is a product of the interaction of these intrinsic burst properties and phasic synaptic inputs from other pyloric neurons (Harris-Warrick et al. 1992; Miller 1987). When circuit synaptic interactions are blocked or inactivated, each component neuron can continue to burst, but with different temporal properties. The AB neuron generates regular, rhythmic bursts; in all other pyloric neurons, however, free-running bursts show irregularities (Bal et al. 1988).

The free-running bursts of LP are produced by repetitive plateau potentials of variable duration (Bal et al. 1988). We show that each burst actually begins with a similar slow wave of membrane potential and similar initial spike discharge. Subsequently, the plateau phase developed exponentially increasing variability. This instability could arise from intrinsic mechanisms, extrinsic influences, or both. We eliminated strong, phasic inputs, but could not exclude the possibility of residual electrotonic interactions with other pyloric neurons, or undetected, phasic synaptic interactions with interneurons from the anterior ganglia. However, known extrinsic factors (electrical coupling to some PY neurons, inputs from commissural "P" interneurons) are weak or absent under our recording conditions (Bidaut 1980; Johnson et al. 1993); irregular, free-running burst patterns could occur without any obvious, strong phasic inputs (e.g., Fig. 4E). Moreover, the variability in plateau duration and spike pattern were strongly voltage dependent (Abarbanel et al. 1996; Bal et al. 1988). This suggests a major (although not exclusive) contribution from intrinsic mechanisms. How could cellular mechanisms produce irregular bursting? One possibility, indicated by modeling studies (e.g., Chay 1996; H. Abarbanel, M. Falcke, R. Huerta, and M. Rabinovich, unpublished data), is the generation of chaotic voltage activity by the interaction of membrane conductances with slow intracellular calcium dynamics.

Regardless of its underlying mechanisms, the complex, irregular voltage behavior of LP allows us to study the regulatory action exerted by phasic synaptic inputs. From a dynamic perspective, the exponential changes in variance and the convergent-divergent trajectories of membrane voltage suggest the presence of deterministic behavior (in addition to inevitable noise). These dynamics contribute to the regularizing action of rhythmic inputs.

Dynamic control of irregular bursting by rhythmic inhibition

Rhythmic synaptic inhibition, such as that provided by the pyloric pacemaker neurons, produces frequency-dependent stabilization of irregular bursting in the LP. Regularization occurs through a sequence of events. 1) At each cycle, inhibitory input triggers active termination of the ongoing burst and sets the neuron to a similar, hyperpolarized state. 2) Subsequently, the neuron recovers to generate a new burst. The initial, stable phase of this burst is extended, and the development of instability is slowed. 3) At high enough input frequencies, the stable phase extends to the arrival of the next inhibitory input. At low input frequencies, the LP neuron can develop instability during the interval between inputs, and irregular oscillations occur.

Regularization therefore occurs within a dynamic time frame. In our experiments, rhythmic input reduced and stabilized the variance between voltage trajectories for a period lasting up to 1.3 s (Fig. 5, C and D). This suggests that periodic inhibition can stabilize bursting at frequencies ≥ 0.7 Hz, but not much lower. In fact, stabilization began to fail at ~ 0.3 – 0.4 Hz. The normal burst frequency of the pacemakers (and of the pyloric CPG as whole) ranges from ~ 0.5 to 2 Hz (Miller and Selverston 1979), encompassing the time frame for effective stabilization.

We worked with a simplified subcircuit of the pyloric CPG (Fig. 1A2). In the intact circuit, LP neuron receives phasic

inhibition from two main sources: the pyloric pacemaker group and the PY neurons (Fig. 1A1). Like LP, the PYs are inhibited by the pacemakers; however, they rebound more slowly. When they begin their delayed burst, they inhibit LP (Miller 1987). Their action further truncates the LP burst, but does not evoke the same, reliable hyperpolarization as subsequent, pacemaker input. The PY pathway augments, but does not qualitatively change, the stabilization produced by direct pacemaker inhibition.

Reliable, phasic hyperpolarization may extend the stable phase of a subsequent LP burst by enhancing and regularizing the activation of burst-generating currents. In dynamic terms, each periodic input forces the neuron into a similar, convergent region of its state space, thereby decreasing the subsequent divergence of trajectories during burst evolution. The weaker regularizing action of periodic excitation (depolarizing input) is now readily understood. Depolarizing pulses can trigger the onset of a new burst but do not stabilize its subsequent development; they can terminate an ongoing burst, but this action is unreliable (Fig. 2 and data not shown). Neither effect allows for a stable cooperation with burst dynamics.

Regularization and dynamic stabilization must be distinguished from simple "interruption" or "entrainment." Interruption of ongoing irregular activity should produce no change in the underlying slow dynamics. Entrainment refers to phase locking of one oscillator to another. This concept is difficult to apply when the driven oscillator is irregular. Beyond phase locking, however, our analysis points to regularization of both slow oscillations and spike patterns. Regular output is by no means the inevitable result of periodic forcing of a nonlinear oscillator (Dymirtiev and Kislov 1982; Ueda and Akamatsu 1981).

Stabilization of bursting in oscillatory circuits

We suggest that rhythmic inhibition stabilizes the irregular burst activity of LP by resetting the neuron to a reproducible region of its parameter space where essential, burst-generating currents undergo voltage-dependent activation or deactivation with appropriate kinetics. Conceivably, other bursting neurons may possess other combinations of ionic currents with different properties, such that optimal resetting could occur in depolarized voltage ranges. These cells would operate with different dynamic rules.

Nevertheless, the effects described here may have some general relevance. Neurons of many oscillatory circuits possess low-threshold, inward currents that allow them to fire relatively stereotyped bursts of spikes following phasic inhibition (Alonso and Llinas 1992; Getting 1989; Huguenard 1996; Llinas 1988; Marder and Calabrese 1996). Plateau potentials extend the duration of burst firing in a variety of motor and central circuits (see Pearson and Ramirez 1992, for review). Rhythmic synaptic inhibition, arising from pacemaker-like (Gray and McCormick 1996) or network (Traub et al. 1996) activity, is an important element in the oscillatory activity of vertebrate brains (Buzsaki and Chrobak 1995). Inhibitory interconnections also predominate in CPGs, where they are implicated in rhythmogenesis and phasing (Arshavsky et al. 1993; Getting 1989; Harris-Warrick et al. 1992; Marder and Calabrese 1996; Selverston and Moulins 1985). Our results suggest that a neuron with irregular plateau potentials will experience

greater stabilization from phasic inhibition than phasic excitation. We conclude that rhythmic inhibition can exert dynamic control over bursting activity, affecting not only its phase, but also its stability and regularity.

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