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Oscillating Networks: Control of Burst Duration by Electrically Coupled Neurons

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The pyloric network of the stomatogastric ganglion in crustacea is a central pattern generator that can produce the same basic rhythm over a wide frequency range. Three electrically coupled neurons, the anterior burster (AB) neuron and two pyloric dilator (PD) neurons, act as a pacemaker unit for the pyloric network. The functional characteristics of the pacemaker network are the result of electrical coupling between neurons with quite different intrinsic properties, each contributing a basic feature to the complete circuit. The AB neuron, a conditional oscillator, plays a dominant role in rhythm generation. In the work described here, we manipulate the frequency of the AB neuron both isolated and electrically coupled to the PD neurons. Physiological and modeling studies indicate that the PD neurons play an important role in regulating the duration of the bursts produced by the pacemaker unit.

The functional characteristics of a neural network arise both from the intrinsic properties of individual component neurons and from emergent, collective effects. Central pattern generators are relatively simple networks with well-defined outputs that can be used to study the interplay of intrinsic and emergent phenomena. The pyloric network of the stomatogastric ganglion is a particularly well-studied central pattern generator that produces a three-phase rhythm driving muscles in the

stomach of lobsters and crabs (Selverston and Moulins 1987). This central pattern generator produces the same basic rhythmic pattern over a frequency ranging from about 0.3 to 3 Hz. A pacemaker unit consisting of three electrically coupled cells, the anterior burster (AB) neuron and two pyloric dilator (PD) neurons, plays an important role in regulating network frequency. Experimental studies of isolated AB and PD neurons reveal that they have intrinsic properties quite different from each other (Marder and Eisen 1984; Flamm and Harris-Warrick 1986; Bal *et al.* 1988). Modeling studies reported in this paper suggest how the different intrinsic properties of the AB and PD neurons combine to produce the characteristics of the full pacemaker network. The AB neuron plays a dominant role in rhythm generation whereas the PD neurons regulate the duration of the pacemaker bursts.

A burst from the pacemaker unit forms one element of the three-phase pyloric rhythm. As shown in Figure 1A, the AB and PD neurons depolarize synchronously in periodic bursts. The frequency of the rhythm can be controlled in the laboratory by injecting current into the AB neuron. When the period is modified in this way, the duration of the AB/PD bursts varies in direct proportion to the period. In Figure 1B, AB/PD burst duration is plotted against network period. The duration of the pacemaker burst increases linearly with the period over a wide range of frequency. Defining the duty cycle as the ratio of the burst duration to the period, we see that in the full network the pacemaker unit acts as a constant duty cycle oscillator.

The pacemaker unit consisting of the AB and PD neurons[1] (Fig. 2A) can be isolated from the rest of the pyloric network by blocking glutamatergic synaptic transmission with picrotoxin (Eisen and Marder 1982). The frequency of the AB/PD pacemaker unit can again be modified by injecting current into the AB neuron. In the isolated pacemaker network, as in the full network, the duration of the pacemaker burst increases as the cycle period is increased. This is shown in representative intracellular recordings (Fig. 2A) and in a plot of burst duration versus period (Fig. 2C).

Individual AB or PD neurons can be isolated by killing the cells to which they are coupled (Miller and Selverston 1979). An isolated PD neuron may oscillate but it does so irregularly with a period much longer than that of the normal pyloric rhythm (Bal *et al.* 1988). An isolated AB neuron oscillates at a frequency in the normal pyloric range (Miller and Selverston 1982; Hooper and Marder 1987; Bal *et al.* 1988; Marder and Meyrand 1989). When the frequency of an isolated AB neuron is modified

[1]The AB and PD neurons are also coupled by both electrical and chemical synapses to the ventricular dilator (VD) neuron. The electrical coupling to the VD neuron is weaker than that between the AB and PD neurons and our experiments indicate that the VD neuron does not play an important role in the effects we discuss. For simplicity we have left the VD neuron out of our discussion and diagrams.

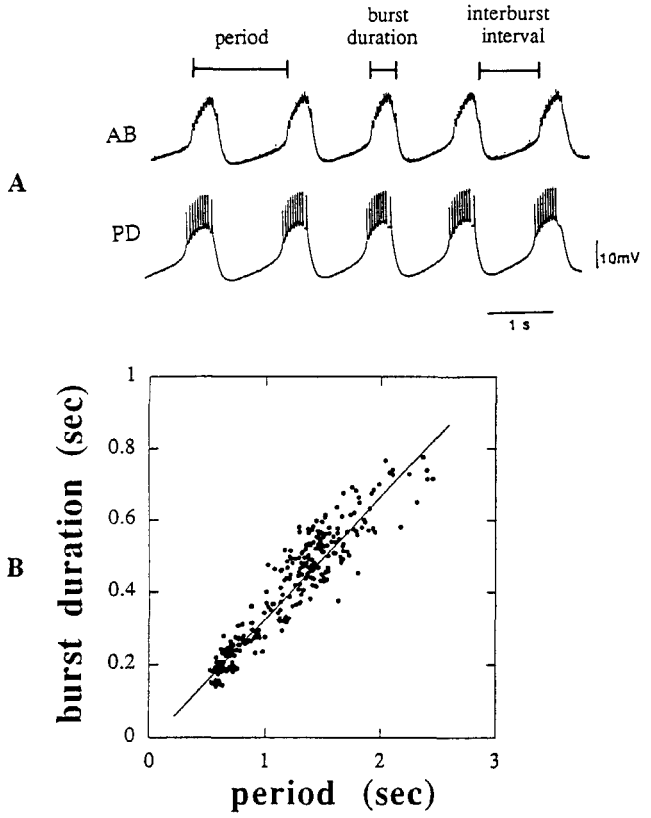

Figure 1: The full pyloric network. (A) Simultaneous intracellular recordings from the somata of the AB and PD neurons. Standard electrophysiological methods (Hooper and Marder 1987) were used. The AB and PD neurons fire during synchronous periodic bursts of depolarization. (B) Burst duration versus period. When the period of AB/PD bursting in the full pyloric network is modified by injecting current into the AB neuron, the AB/PD burst duration increases linearly with the oscillation period.

by current injection, it behaves quite differently than when it is part of the full pyloric or AB/PD pacemaker networks. As shown in Figure 2B and C, the burst duration of an isolated AB neuron remains constant as its period changes. Thus, in isolation the AB neuron acts as a constant burst duration oscillator. When coupled to the PD neurons however, it behaves as a constant duty cycle oscillator.

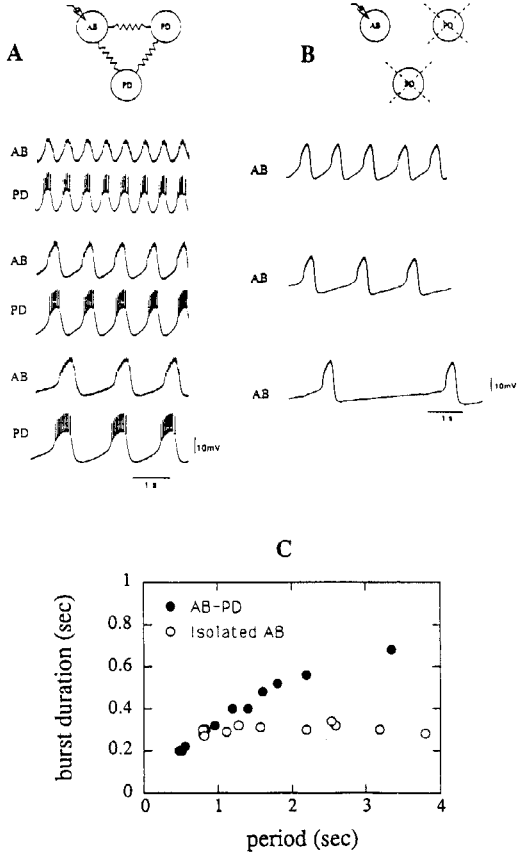

Figure 2: The effect of current injection on the pacemaker network and isolated AB neuron. (A) Intracellular recordings from the AB and PD neurons electrically coupled in the pacemaker network. The first pair of recordings were produced by injection of depolarizing current into the AB neuron. The second pair were at zero injected current and the third pair were with hyperpolarizing current injected into the AB neuron. The increase in period from top to bottom results from increases of both the interburst intervals and the duration of the bursts. (B) Intracellular recording from an isolated AB neuron. The AB neuron was "isolated" by photoinactivating the PD and VD neurons and placing the preparation in picrotoxin (Hooper and Marder 1987). The increase of the period in this case is solely due to an increase in the interburst interval; the burst duration does not change. (C) Burst duration versus period for the pacemaker network and for the isolated AB neuron. Burst duration increases with increased period for the pacemaker but is independent of period for the isolated AB neuron. Isolated AB neuron results include our data and those of Bal *et al.* (1988).

To study how the PD neurons modify the nature of the pacemaker oscillations, we have constructed relatively simple models of the isolated AB and PD neurons and examined the activity the models produce when electrically coupled. We model only a spike-averaged "slow-wave" membrane potential ignoring individual action potentials. [The pyloric network can continue to function when action potentials are blocked by tetrodotoxin (Raper 1979).] To simplify the equations of the model we use units of time in which the cell membrane time constant is one, and choose arbitrary units and an arbitrary zero for the membrane potential v. When results of the model are compared with experimental data we scale and zero the voltage and time variables appropriately.

The models we use to describe the AB and PD neurons are by no means accurate in every detail. For example, the shape of the slow wave for the AB neuron in the model is different from that of the real neuron. However, we have taken care to model accurately those characteristics of the AB and PD neurons which are likely to be important for control and regulation of burst period and duration. Since the AB and PD are resistively coupled it is important to model the amplitude of their respective bursts so that the amount of current flowing through the gap junction is correctly predicted. Likewise, the dependence of the input impedance of each neuron on its membrane potential should be included in the model so that the effect of the current entering through the gap junction can be evaluated.

The AB neuron is represented by a simple oscillator model which mimics the behavior of an isolated AB neuron. The AB membrane potential is governed by the equation of current conservation

$$\frac{dv_{AB}}{dt} = -H_{AB}(v_{AB})[v_{AB}(v_{AB} - 1)(v_{AB} + 1) + u] + I_{ext} + I_R \tag{1.1}$$

The term $v_{AB}(v_{AB} - 1)(v_{AB} + 1)$ represents the rapid I–V characteristics of the neuron as in the familiar FitzHugh–Nagumo equations (FitzHugh 1961; Nagumo *et al.* 1962). In addition, we include the term H_{AB} to account for the large difference in membrane conductance in the hyperpolarized and depolarized regions. $H_{AB}(v_{AB})$ is given by

$$H_{AB}(v_{AB}) = 0.5 + 0.15 \tanh(5v_{AB}) \tag{1.2}$$

The voltage-dependent H_{AB} factor has the important effect of causing the amplitude of the burst oscillations in the model to increase as the model neuron is hyperpolarized and decrease as it is depolarized. With a constant H_{AB} the burst amplitude is independent of injected current. In equation 1.1, I_{ext} represents the external current injected into the cell and I_R is the current entering the AB neuron from the PD neuron,

$$I_R = G(v_{PD} - v_{AB}) \tag{1.3}$$

where G is the coupling conductance of the electrical synapse between the AB and PD neurons. Of course when we discuss the AB neuron in isolation this resistive coupling is set to zero. For simplicity we model the pacemaker network with an AB neuron and a single PD neuron.

Oscillations of the model AB neuron are produced by the variable u, which represents the slow voltage-dependent conductances of the AB neuron responsible for rhythmic bursting. It obeys the equation

$$\begin{aligned} 20\frac{du}{dt} &= [1 - \tanh(5v_{AB})](v_{AB} - u - .1)^3 \\ &\quad + [1 + \tanh(5v_{AB})](1 - u) \end{aligned} \tag{1.4}$$

The form of this equation (which is more complex than the familiar FitzHugh–Nagumo model) was chosen on the basis of reductions of a more complex model of the AB neuron based on realistic ionic conductances (Epstein and Marder 1990). Because of the factors $1 \pm \tanh(5v_{AB})$ the first term on the right side of equation 1.4 governs the behavior of u when the AB neuron is hyperpolarized, while the second term determines the behavior when the neuron is depolarized. The power of three in the first term has been included so that the effect of hyperpolarizing current on the neuron is more correctly modeled. If this power is one as in the FitzHugh–Nagumo model, the frequency of the oscillations is relatively insensitive to hyperpolarizing current until a critical value is reached and the neuron suddenly stops bursting. With the power of three in this term, the oscillation frequency decreases more smoothly as a function of depolarizing current. The constant .1 in equation 1.4 was adjusted to make the ratio of the burst duration to the period match that of the real neuron. The second term on the right side of equation 1.4, which governs the behavior of u when the AB neuron is depolarized, contains the factor $(1 - u)$, which is independent of voltage. Besides being more realistic than the usual FitzHugh–Nagumo form, this makes it easier for the PD neuron (when it is coupled to the AB) to sustain the duration of the combined AB/PD bursts, as it must if it is to act as a burst duration regulator.

The model of equations 1.1, 1.2, and 1.4 duplicates quite well the effect of injected current on both the frequency and amplitude of oscillations for an isolated AB neuron. The oscillating waveform of a model AB neuron and its burst duration at different frequencies are shown in Figure 3B and C. Like the real AB neuron, the model maintains a constant burst duration as its frequency is varied.

The model PD neuron is in some respects similar to the AB model but it lacks the slow current variable u that causes oscillation in the model AB neuron. Instead the PD neuron model includes a slowly varying, voltage-dependent current that allows the PD neuron to oscillate very slowly or to generate plateau potentials. The PD membrane potential

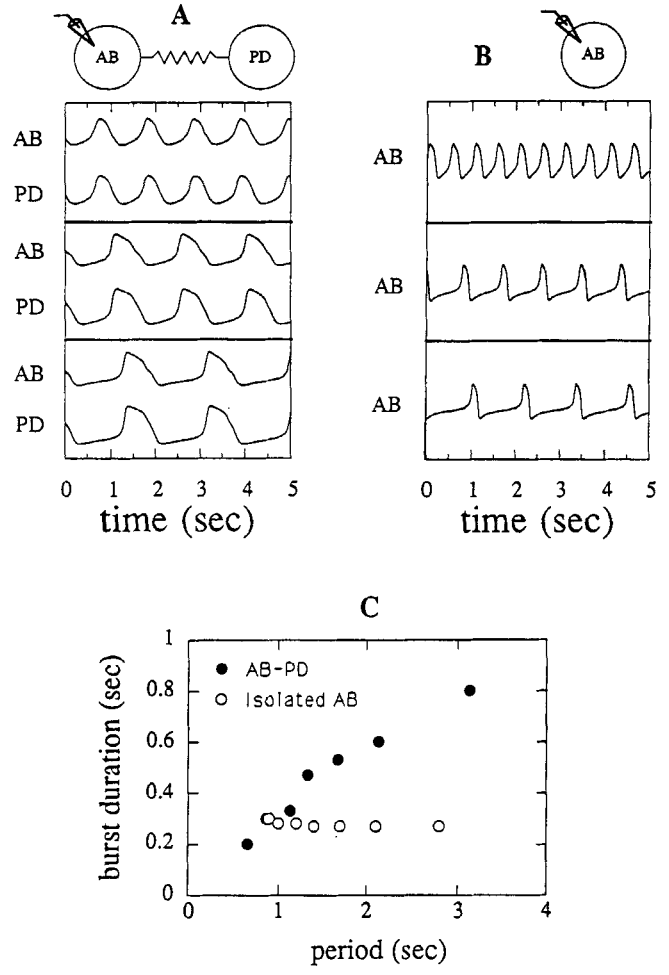

Figure 3: Model pacemaker and AB neuron. (A) Voltage traces for the model pacemaker unit. Only the slow-wave part of the membrane potential is modeled, individual action potentials are not included. (B) Voltage traces for the isolated AB. (C) Burst duration versus period. In the model AB, burst duration is constant as the period is changed by current injection. In the model pacemaker network, burst duration increases linearly with period.

obeys the equation

$$\frac{dv_{PD}}{dt} = -H_{PD}(v_{PD})[v_{PD}(v_{PD} - 1)(v_{PD} + 1) - .5] - I_g - I_R \tag{1.5}$$

where the overall voltage-dependent conductance factor in this case is

$$H_{PD} = 0.2 + 0.06 \tanh(5v_{PD}) \tag{1.6}$$

The factor of .5 in equation 1.5 plays a role similar to the factor .1 in equation 1.4. The particular value chosen allows the AB neuron to drive the PD when the two are coupled with the coupling strength we use. The term I_g is a slowly varying current representing the summed effect of one or more different conductances. This current is predominantly active in the depolarized voltage range. It consists of an outward component that increases slowly in strength when the neuron is depolarized and slowly becomes weaker when the cell is hyperpolarized. This component has characteristics similar to those of a calcium-activated potassium current. In addition I_g may have an inward component that is activated by hyperpolarization and deactivated by depolarization. Specifically, I_g is determined by

$$I_g = \frac{g(v_{PD} + 1)}{1 + \exp(-v_{PD})} \tag{1.7}$$

The voltage dependence we have chosen has the convenient feature of making the model easier to analyze because the current plays a predominant role only when the PD neuron is bursting. The time-dependent conductance strength is given by

$$300\frac{dg}{dt} = \frac{1}{4}[1 + 3\tanh(5v_{PD})] \tag{1.8}$$

The factor of 300 in this equation causes the variation of g to be much slower than the AB neuron oscillation frequency.[2] Note that a term like equation 1.7 with constant g can always be absorbed into the fast part of the PD membrane current. As a result the zero of g can be shifted making it impossible in this model to establish unambiguously the ratio of inward and outward components in the composite current I_g. The factor $1 + 3\tanh(5v_{PD})$ in equation 1.8 is approximately equal to -2 when the PD is hyperpolarized and $+4$ when it is depolarized. This means that g will increase when the neuron is depolarized at twice the rate that it decreases when the neuron is hyperpolarized. As we will see, the ratio of increase to decrease rates will set the ratio of burst duration to period when the PD is coupled to the AB neuron.

[2] In principle, this equation allows g to increase or decrease indefinitely, which is clearly unrealistic. In actual simulations g stays within reasonable bounds but to be safe we sometimes restrict it to a predefined range.

When the model AB neuron is electrically coupled to a model PD, the PD neuron is driven by the AB into rhythmic oscillation (as in the real preparation). Coupling to the PD neuron has the effect of reducing the frequency of the AB oscillations (Kepler *et al.* 1990; Meunier 1990). During each burst the slow current conductance factor g increases and during each interburst it decreases. The integrated effect of the small cycle-by-cycle fluctuations of g depends on the ratio of the burst duration to the length of the interburst interval. If depolarized bursts dominate, g will increase during the bursts more than it decreases during the interbursts and g will become more positive. If the interburst intervals dominate, g will on average decrease. Because I_g is most active when the PD neuron is depolarized, a change in its strength and polarity, determined by g, will affect the length of the AB/PD bursts. A large negative I_g will tend to depolarize the PD neuron causing current to flow through the electrical coupling from the PD to the AB neuron. The resulting injection of current into the AB neuron will prolong the depolarized bursts. A large positive I_g will have the opposite effect shortening the duration of the bursts.

Because the variation of g is slower than the oscillation frequency, the value of g will drift either up or down over several oscillation cycles until an equilibrium condition is reached. The equilibrium is attained when the ratio of the burst duration to the interburst interval matches the ratio of the rate of decrease of the current strength factor g to the rate of its increase. As can been seen from equation 1.8 this ratio has been set equal to 1/2. (For large positive x, $[1 + 3\tanh(x)]/4 \approx 1$ and $[1 + 3\tanh(-x)]/4 \approx -1/2$.) This assures that the correct duty cycle ratio of 1/3 (burst/interburst = 1/2, burst/period = 1/3) will be achieved through a dynamic adjustment of the slowly varying PD current, independent of oscillation frequency. Other duty cycle ratios can be obtained by varying the ratio of decrease to increase rates in equation 1.8. The burst duration and voltage waveforms of the model pacemaker network are shown in Figure 3A and C.

Because the regulating effect of the PD neuron is dynamic, it is quite robust and not overly sensitive to any particular choice of model parameters. For example, we can change the strength of the electrical coupling G by a factor of three without destroying or drastically modifying the constant duty cycle behavior. Likewise the model is not overly sensitive to the values of other parameters such as the time constant (300) in equation 1.8 or the form of the voltage dependence for I_g.

The AB/PD pacemaker unit offers an interesting example of neurons with different characteristics complementing each other to form a network with desirable features not expressed by any single neuron in isolation. The AB neuron by itself can oscillate over a wide frequency range but does so with fixed burst duration. The PD neuron by itself has membrane characteristics much too slow to drive the network at the correct frequency. However, in combination with the more rapid oscillations of the AB neuron, the slow current characteristics of the PD neuron

act to regulate the pacemaker burst duration resulting in a pacemaker network with the desired characteristics needed to produce the pyloric rhythm.

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