Mechanisms Underlying Fictive Feeding in *Aplysia*: Coupling Between a Large Neuron With Plateau Potentials Activity and a Spiking Neuron

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INTRODUCTION

Central pattern generators (CPGs) are circuits of neurons that organize repetitive movements even in the absence of phased sensory input (Getting 1989a; Pearson 1993; Selverston and Moulins 1985). The movements controlled by CPGs can differ in cycle length, duty cycles, complexity, or sensitivity to regulation by external stimuli. CPGs can be built from circuit elements with a wide variety of different features. An important question in neurobiology is to determine how the features used in the design of a particular CPG are appropriate for the control of the specific behavioral patterns that are expressed.

Computer simulations are an important tool in investigating such questions. The use of simulations allows one to build a circuit that has elements that are thought to be important for a particular function and then systematically put in, take out, or change a particular feature of a neuron or a network and thereby determine how the feature affects the activity of the simulated circuit. Computer models are generally of two types: theoretical versus realistic. (The relative benefits of each approach are discussed in Abbott and Marder 1998; Calabrese et al. 2001; Marder and Abbott 1995; Marder et al. 1998; Muloney and Perkel 1988; Reeke and Sporns 1993; Segev 1992; Selverston 1993.) Realistic models try to include as many features as are thought relevant to the function of a particular neural circuit and then to examine whether the included elements are adequate to describe the properties of the circuit (for examples, see Baxter et al. 1999; Canavier et al. 1991; Getting 1989b; Golowasch et al. 1992; Hill et al. 2001; Hodgkin and Huxley 1952; Nadim et al. 1995; Olsen et al. 1995; Warshaw and Hartline 1976). By contrast, abstract models are used to explore the functional features of a theoretical circuit that are not necessarily tied to a specific existing circuit (e.g., Abbott and LeMasson 1985; Calabrese et al. 1997; Chow and Kopell 2000; Deodhar et al. 1993; Guckenheimer et al. 1997; Jung et al. 1996; Kupfermann et al. 1992; Rowat and Selverston 1997; Skinner et al. 1994; Van Vreeswijk et al. 1994).

The present study presents a model that has features of both realistic and theoretical models. It is inspired by some features...
of neurons that are part of a CPG that controls consummatory feeding movements in *Aplysia*, although it is not designed to mimic these neurons explicitly. Although detailed voltage-clamp data are not available for these neurons, a great deal of current-clamp data are available, and these data provided biological underpinnings for the development of the model. The circuit that we have modeled consists of neurons that are active synchronously and that are coupled to one another via both chemical and electrical synapses. Such a configuration is a prominent feature of the *Aplysia* feeding CPG (Hurwitz et al. 1997), and similar patterns of interconnections are also found in other CPGs (Arshavsky et al. 1997; Calabrese 1995; Cropper and Weiss 1996; Marder and Calabrese 1996; Stein et al. 1997) as well as in other neural circuits (Shepherd 1998). Our aim was to design a circuit that has some of the properties of the *Aplysia* system and then to examine the possible function of the combined electrical and chemical coupling in this circuit.

The present study focuses on some of the features that characterize protractor-phase neurons B31/B32 and B63 in the buccal ganglia of *Aplysia*. The consummatory phase of *Aplysia* feeding consists of sequential protractions and retractions of the toothed radula (Kupfermann 1974). The CPG controlling this movement consists of groups of mutually inhibitory protraction- and retraction-phase interneurons (Hurwitz and Susswein 1996; Hurwitz et al. 1997; Susswein and Byrne 1988). B31 and B32 are a pair of strongly electrically coupled, seemingly identical neurons found in each buccal hemiganglion (Susswein and Byrne 1988). These neurons are also strongly coupled to some additional buccal ganglia neurons (Susswein and Byrne 1988). B31/B32 have some unusual features. They are among the few *Aplysia* neurons with somata that fail to sustain conventional fast action potentials (Susswein and Byrne 1988). In addition, a brief depolarization initiates a slow sustained depolarization that resembles a plateau potential in other systems (Russell and Hartline 1982; Tajaki and Cooke 1979) in that the depolarization outlasts the initial stimulus (Susswein and Byrne 1988). Many small (<10 mV) fast depolarizations are superimposed on the slow depolarization. Some of these fast depolarizations represent recurrent chemical and electrical excitatory postsynaptic potentials (EPSPs) from neurons that are activated by the sustained depolarization, and some arise from spikes in the B31 and B32 axons that fail to invade the somata (Hurwitz et al. 1994, 1997; Susswein and Byrne 1988). B31/B32 have spiking axons that leave the buccal ganglia and innervate the I2 protractor muscle (Hurwitz et al. 1994, 1996). However, the functions of the axon and soma compartments are somewhat separate. The slow plateau-like potentials in the soma function as part of the CPG, whereas axonal spikes are driven by the slow depolarization in the soma, and function to drive motor activity (Hurwitz et al. 1994).

B63 is a bilaterally symmetrical neuron that monosynaptically excites the contralateral B31/B32 neurons via a mixed chemical/electrical synapse, with the chemical component of the EPSP undergoing moderate (~50%) facilitation (Hurwitz et al. 1997). Activation of the B63 and B31/B32 neurons is an essential component of the protraction phase of a buccal motor program (Hurwitz et al. 1997). The B63 and B31/B32 neurons can be activated via a wide variety of stimuli. Of particular interest is direct excitation from cerebral-buccal interneurons (CBIs), command-like neurons in the cerebral ganglion (Rosen et al. 1991). In most instances, activity in the B63 and B31/B32 neurons is terminated by an abrupt hyperpolarization, which represents a large inhibitory postsynaptic potential (IPSP) caused by the firing of retraction interneuron B64 (Hurwitz and Susswein 1996). However, in some cases, B64 fails to fire, and the sustained depolarization stops spontaneously (Susswein and Byrne 1988), presumably by events that are endogenous to the protraction circuit.

In this study, we recorded the patterns of activity of the B63 and B31/B32 in the buccal ganglia. We then used SNAP (Ziv et al. 1994) to simulate some of the features of a single B63 and a single B31/B32 neuron and the connections between them. The B31/B32 neurons are morphologically complex with different compartments of a neuron having distinct physiological properties. The cells and their connections were modeled as being much simpler than in reality (Fig. 1).

The properties of the currents that were used to build the simulated B63 and B31 neurons (Table 1) were designed to give rise to cells that behave in a manner that is very reminiscent of those in the buccal ganglia. However, there have been no systematic studies of buccal ganglia neurons B63 and B31/B32 in conditions of voltage clamping, and therefore little data are available on the currents that underlie the electrical activity of these neurons. For example, the mechanism that is responsible for the sustained depolarization in B31/B32 is not known. Thus several different mechanisms (e.g., incorporating a plateau-like potential directly into B31/B32 vs. producing the slow depolarization via a slow EPSP from B63 to B31/B32)
were implemented and examined. By determining which model more closely simulated properties of the buccal ganglia and by selectively changing aspects of the model and examining how such changes affected the activity of the network, these simulations provided insights into the function of some of the systems of interconnected neurons with similar properties.

In the model system, we systematically altered some of the features of the neurons, or of their interconnections, to determine how these features contribute to the observed properties of the simulated circuit. The simulations indicated that the combined electrical and chemical coupling with moderate facilitation, in addition to some unusual properties of one of the simulated neurons, gave rise to a circuit that was extremely sensitive to inputs preceding the initiation of a preprogrammed burst, and these inputs could control the timing of the burst onset. However, once a burst of activity was initiated, inputs to the circuit produced little effect. This pattern of activity was consistent with the behavioral function of the feeding circuit and suggested that combined electrical and chemical coupling could have a similar function in other circuits.

METH O DS

Simulations

All but one series of the simulations were performed using version 5.0 of SNAP (simulator for neural networks and action potentials), which runs on JAVA. These simulations were performed using the JAVA development kit 1.2 and were run under Windows 98 on a Pentium III-450. An additional series of simulations (that examined the effects of stochastic fluctuations) was run using version 7.0 of SNAP, running on JAVA development kit 1.3.1. The latest versions of SNAP can be found at http://snapp.uth.tmc.edu.

The features simulated by the model corresponded to those generally seen when recording from a single B63 neuron in one hemiganglion and a single B31 neuron in the opposite hemiganglion.

B63. In all but one simulation, B63 was built from two compartments, which represented the soma and axon. The soma and axon compartments differed primarily in that the input resistance of the axon was larger than that of the soma. This was modeled by building the axon with a smaller leak conductance than that of the soma. In both, conventional fast time- and voltage-dependent Na⁺ and K⁺ currents were present, with no additional slow currents (Table 1). A simulated electrical synapse was used to represent the connection between these compartments. Designing B63 with separate soma and axon compartments was meant to simulate the fact that the synapse from B63 to B31 is in the contralateral buccal ganglion and is therefore electrically distant from the B63 soma.

B31. B31 was simulated with a single compartment model, which was built to mimic the properties of the B31 soma, without including the conventionally spiking B31 axon (Table 1). The B31 soma was modeled with a low input resistance, achieved by a large leak conductance, to simulate strong coupling to a second B31/B32 neuron and other neurons (Susswein and Byrne 1988). Although variants of the model were examined (see RESULTS), the majority of the simulations involved a model of B31/B32 that included slowly activating and inactivating Na⁺ and K⁺ currents. These currents generated a plateau-like potential in B31/B32. In contrast to B63, B31 did not have conventional fast Na⁺ and K⁺ currents and thus did not generate action potentials. The B31 neuron was also modeled as having a larger capacitance than that did B63, reflecting the larger surface area of the neuron and its coupled compartments.

SYNAP TIC CONNECTIONS BETWEEN B63 AND B31. The B63 axon and the B31 soma were built as communicating via electrical coupling (Table 2). The coupling between the B63 soma and axon, and the B31 soma, was adjusted to be similar to that reported previously between

### Table 1. Parameters of the neurons

<table>
<thead>
<tr>
<th>Neuron</th>
<th>$C_m$</th>
<th>Current</th>
<th>$E_C$</th>
<th>$g_{max}$</th>
<th>$h_{max}$</th>
<th>$\tau_{a(max)}$</th>
<th>$\tau_{a(min)}$</th>
<th>$g_{leak}$</th>
<th>$h_{leak}$</th>
<th>$\tau_{r}$</th>
<th>$h_{r}$</th>
<th>$\tau_{b(min)}$</th>
<th>$s_{r}$</th>
<th>$s_{b}$</th>
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<tbody>
<tr>
<td>B31</td>
<td>0.011</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$I_{Na}$</td>
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<td>$-45.0$</td>
<td>3.0</td>
<td>0.4*</td>
<td></td>
<td>$-40.0$</td>
<td>0</td>
<td>1</td>
<td>1.2</td>
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<td>$-30.0$</td>
<td>7.0</td>
<td>4.0</td>
<td></td>
<td>$-57.1$</td>
<td>0</td>
<td>23.3</td>
<td>0.14</td>
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<td>$-37.0$</td>
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<td></td>
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<td>soma</td>
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<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
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<td>0.0015</td>
<td></td>
<td>$-32.7$</td>
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<td>51.5</td>
<td>0</td>
<td>9.2</td>
<td>0.01</td>
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<td></td>
<td></td>
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<td>25.0</td>
<td>$-26.4$</td>
<td>4.0</td>
<td>0.027</td>
<td></td>
<td>$-24.0$</td>
<td>11.7</td>
<td>16.4</td>
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<td>$-51.0$</td>
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<td>$I_{Na}$</td>
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<td>-70.0</td>
<td>30.0</td>
<td>$-26.4$</td>
<td>8.4</td>
<td>0.027</td>
<td></td>
<td>$-24.0$</td>
<td>11.7</td>
<td>16.4</td>
<td>0.15</td>
<td>2</td>
<td>1.5</td>
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<td></td>
<td>$I_{leak}$</td>
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<td>0.0012</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>$-51.0$</td>
<td>11.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The time constants for activation and/or inactivation were constants (i.e., not described by voltage-dependent functions). ‡ A double-exponential function was used to describe the voltage dependence of the activation time constant (see APPENDIX).

### Table 2. Parameters of the connections between the neurons

<table>
<thead>
<tr>
<th>Chemical synapse from B63 axon to B31</th>
<th>Synaptic Current ($I_s$)</th>
<th>Synaptic Plasticity (SP)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$E_C$, mV</td>
<td>$g_{r(s)}$, $\mu S$</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Electrical coupling</th>
<th>Presynaptic Cell/Compartment</th>
<th>Postsynaptic Cell/Compartment</th>
<th>$g_{rec}$, $\mu S$</th>
</tr>
</thead>
<tbody>
<tr>
<td>B63 soma</td>
<td>B63 axon</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>B63 axon</td>
<td>B63 soma</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>B63 axon</td>
<td>B31</td>
<td>0.045</td>
<td></td>
</tr>
<tr>
<td>B31</td>
<td>B63 axon</td>
<td>0.12</td>
<td></td>
</tr>
</tbody>
</table>

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the contralateral B63 and B31 somata: 5.5:1 for the coupling from B31 to B63, and 12:1 for the coupling from B63 to B31 (Fig. 6 of Hurwitz et al. 1997). The asymmetry in coupling arises, in part, from the differences in input resistance among the three components of the model. In addition, firing in B63 elicited a weakly facilitating EPSP in B31/B32 (see Fig. 1). The neurons were modeled as being connected via a chemical synapse with B63 presynaptic to B31 (Table 2). The chemical synapse showed a moderate degree of facilitation, which was achieved by using the arbitrary SP function (Eq. A8) that describes an activity-dependent change in transmitter release (Ziv et al. 1994). The parameters chosen for the neurons are shown in Table 1. The equations are detailed in the Appendix (see also Ziv et al. 1994).

In some simulations, the parameters of the connections between B63 and B31 or the properties of B31 were changed to test a particular hypothesis. These changes are described in RESULTS.

**ROBUSTNESS ANALYSIS.** The robustness of the simulated circuit was tested by running a series of simulations in which the values for some parameters were either randomly altered at the beginning of a simulation or subjected to stochastic fluctuations throughout a simulation. To randomly alter parameters, the initial value of a given parameter (shown in Table 1) was used as the seed to a random-number generator that returned an evenly distributed random number between ±15% of the control value. This procedure was accomplished with the random number function in Excel 2000 (Microsoft, Redmond, WA), and the new values were entered by the user at the beginning of each simulation. To subject parameters to stochastic fluctuations throughout a simulation, the control value of a given parameter was used as the seed to a random-number generator that returned a normally distributed random number (i.e., a Gaussian distribution). The mean of the Gaussian distribution was the control value of the given parameter. The magnitude of the SD of the distribution was defined by the user. The user also defined the frequency at which new random numbers were selected throughout the simulation. This procedure was accomplished with the Gaussian function in the JAVA programming language (version 1.3.0.02, Sun Microsystems, Palo Alto, CA).

**Recordings**

Experiments were performed on *Aplysia californica* (100–200 g) purchased from Marine Specimens Unlimited (Pacific Palisades, CA). Prior to dissection, animals were anesthetized with 25–50% of the body volume of isotonic MgCl₂. The buccal ganglia were then removed from the animals and placed in a chamber filled with a solution containing 70% filtered artificial seawater (ASW) and 30% isotonic MgCl₂. The connective tissue sheath overlying the neurons was then surgically removed. Following the desheathing, the bathing solution was replaced with ASW. Intracellular recording and stimulation were performed at room temperature (22–24°C) using 3- to 10-MΩ electrodes filled with 3 M K acetate. B31 and B63 were identified on the basis of previously described properties (Hurwitz et al. 1997; Susswein and Byrne 1988).

**RESULTS**

The aim of this study was to explore the functional role of some of design features of the B63 and B31 neurons by creating computational models resembling these cells and their interconnection and then systematically changing the models so that specific features were altered or eliminated. Before systematically altering features of the simulation and then determining the effects of such changes, it was first important to demonstrate some of the properties of B63 and B31/B32 and then show that the computational model simulated many of the features of the B63 and B31/B32 neurons.

**Properties of the B63-B31/B32 system in the buccal ganglia**

Simultaneous recordings from B63 and a B31/B32 neuron showed that a brief depolarization in either B63 or B31/B32 was sufficient to initiate a patterned burst of activity that was recorded in both neurons. The threshold for eliciting a burst was lower for current injected into B63 than into B31/B32. The bursting in B63 and B31/B32 corresponds with the protraction phase of a buccal motor program (BMP). The burst was maintained after the initiating stimulus was terminated. In B63, the burst was characterized by a series of action potentials riding on a 10- to 15-mV sustained depolarization, whereas in B31 the burst was characterized by a slow, sustained 20- to 25-mV plateau-like depolarization. Superimposed on the sustained depolarization were EPSPs elicited by the firing in B63. This activity was sustained for ~5 s (Fig. 2A). The retraction phase in B63 and in B31/B32 corresponds to the hyperpolarization seen in both neurons that follows the sustained burst.

Sustained depolarization of either B63 or B31/B32 was able to elicit continuous cycles of bursting, with the threshold for initiating bursting lower in B63 than in B31/B32 (Fig. 2B).

An interesting property of the system is that a series of properly spaced subthreshold depolarizations to either neuron could initiate a sustained burst (Fig. 2C). In addition, once a burst was initiated, even large hyperpolarizing pulses to either neuron were relatively ineffective in terminating the burst (see Fig. 8) (Hurwitz et al. 1997).

The delay to onset of bursting and the continuous rhythmic activity in the presence of sustained stimuli were reminiscent of features of the feeding behaviors that are controlled by the B63 and B31 neurons. When *Aplysia* are first stimulated with food, there may be a substantial delay before the animal begins to bite (Kupfermann 1974; Susswein et al. 1978). If the food stimulus is maintained, the animal will make repetitive, rhythmic feeding movements (Kupfermann 1974; Susswein et al. 1978). In addition, once a feeding movement is initiated, it is completed even if the food is removed (Kupfermann 1974). Thus the features of the B63-B31/B32 system are similar to features observed in the behaving animal.

**Properties of the simulated circuit**

The neurons and their interconnections were designed to produce a pattern of activity similar to those observed in B63 and in B31/B32 during BMPs (see Fig. 2). In particular, the pattern resembled that seen when retractor interneuron B64 fails to fire (see Fig. 12B in Susswein and Byrne 1988). B64 usually acts to terminate the protraction phase of a buccal motor program and initiates the retraction phase. However, in some cases B64 fails to fire, and the protraction phase is ended as a result of a seemingly endogenous processes.

**ACTIVITY OF SIMULATED B63 AND B31 NEURONS**

As in the buccal ganglia, a brief depolarization in either a simulated B63 or B31 neuron was sufficient to initiate a patterned burst of activity that was recorded in both neurons (Fig. 3). Threshold for eliciting a burst was lower for current injected into B63 than into B31. The burst was initiated after a delay of ~3 s and was maintained well after the initiating stimulus was terminated. In B63, the burst was characterized by a series of action potentials riding on a 9-mV sustained depolarization, whereas in B31 the burst was characterized by a slow, sustained 45-mV
FIG. 2. Recordings from B63 and B31/B32 in the buccal ganglia. A: just threshold (400-ms duration) current pulses to both B31/B32 (1) and to B63 (2) induced similar bursts of activity in both neurons, following a delay. However, the threshold for eliciting a burst was higher for B31/B32 than for B63. B: in the buccal ganglia, continuous depolarization of either a B31/B32 neuron (1) or a B63 neuron (2) induced continuous bursting for as long as the stimulus was maintained. For this figure, the amplitude of the stimulus was adjusted so bursting was maintained at a rate of ~4 per 100 s. Note the difference in the current amplitudes to the two neurons that were needed to elicit this rate. C: a series of subthreshold depolarizations to B63 also elicited a burst in the buccal ganglia.

FIG. 3. Just threshold (400-ms duration) current pulses to both B31 (A) and to B63 (B) induced similar bursts of activity in both neurons, following a delay. However, the threshold for eliciting a burst was higher for B31 than for B63. In this and in all subsequent figures showing data from the computational model, the B63 trace shows a recording from the soma.
plateau-like depolarization. Superimposed on the sustained depolarization were EPSPs elicited by the firing in B63. This activity was sustained for \(~5\) s.

The initiation of a burst was affected by a positive feedback loop (see Fig. 4). Even small depolarizations of B31 began to activate the sustained \(\text{Na}^+\) current in B31. The depolarizing effects of this current were transferred to B63 via the electrical coupling. The additional depolarization of B63 elicited additional spiking in B63, more EPSPs, and more depolarization of B31, and thus a cycle of positive feedback. The burst was terminated when the \(\text{K}^+\) conductance in B31 was sufficiently activated to repolarize B31. This positive feedback loop was examined in greater detail in the following text.

We examined the stimulus parameters required to elicit a burst. As might be expected, for currents injected into either neuron, the threshold was dependent on the pulse width, with shorter pulses requiring larger current amplitudes to initiate a burst. For all pulse widths, the threshold for inducing a burst was substantially lower for current pulses delivered to the simulated B63 than to the simulated B31 (not shown). We also examined the minimal current required to elicit a burst, when a neuron is tonically depolarized. The absolute threshold was also considerably lower for the simulated B63 than for the simulated B31.

In the buccal ganglia, (e.g., Fig. 2A), the patterned burst often is seen only after a relatively long delay that follows the end of the current pulse. In the simulated system, the delay was inversely proportional to the amplitude of the current pulse, relative to the threshold needed to elicit a burst. The longest delays were seen at just threshold amplitude for eliciting a burst. As the amplitude increased, the delay decreased systematically (not shown). Although a delay was observed following stimuli to either the simulated B63 or to the simulated B31, it was systematically longer following just-threshold stimuli to B31 than to B63 (not shown). The delay was a result of the slow activation kinetics and voltage dependency of the sustained \(\text{Na}^+\) current in the simulated B31. Near the resting potential, very little \(I_{\text{Na}}\) was activated, and it develops slowly, enhancing the prolonged delay. With more intense stimuli, greater amounts of \(I_{\text{Na}}\) were activated, which in turn more rapidly activated the positive feedback loop that underlies bursting (see Fig. 4).

Sustained depolarization of either simulated neuron was able to elicit continuous cycles of bursting (Fig. 5). The burst frequency was dependent on the amplitude of the injected current (not shown).

A SERIES OF BRIEF STIMULI TO THE SIMULATED B31 OR B63 CELLS ELICITS A BURST. The simulated circuit also displayed the feature that a series of properly spaced sub-threshold depolarization to either neuron could initiate a burst (Fig. 6). The ability of a series of pulses to initiate activity emerged from the slow activation and inactivation of the inward currents in B31. These were sufficiently slow so that depolarizations spaced a few hundred milliseconds apart may still affect one another.

An additional property of the B63-B31/B32 system in the buccal ganglia is that the ability of a depolarizing pulse to elicit a burst is exquisitely sensitive to the timing of the pulse. In the simulated B63-B31 circuit, when two different pulses were delivered with a 5-s interval separating them (Fig. 7A), the second pulse elicited a burst. However, delaying the pulse by an additional 1 s led to a failure to elicit a burst (Fig. 7B). This resulted from the slow inactivation built into the B31 \(\text{Na}^+\) current (as well as to the slow kinetics of the \(\text{K}^+\) current—see

![FIG. 4. Mechanism of burst generation in the model circuit.](image-url)
IN THEIR EFFECTS ON A BURST. The preceding data showed that
HYPERPOLARIZING PULSES IN THE SIMULATED B31 CELL DIFFER
from sustained currents. The inability to induce a burst is
Fig. 4B), which prevented the pulse from inducing a voltage
change that crosses threshold. The inability to induce a burst is
was initiated blocked or delayed the onset of the burst. The
effect of a hyperpolarizing pulse to B31 before a burst was
exquisitely dependent on the amplitude and the pulse width as
well as on the timing of the pulse (Fig. 8, A–C). Even small
changes in any of these factors could have different effects
with regard to a block or delay of the burst.

In contrast to the effects of a brief hyperpolarization before
a burst, the same hyperpolarization of B31 after the onset of a
burst was found to have little effect on the burst (Fig. 8D).
Increases in the pulse amplitude to as much as −90 nA did not
stop the bursting (although such pulses could affect the burst
length). The relative lack of effect of an injected hyperpolar-
izing current pulse arises from the large currents induced by the
slow plateau in B31 during a burst that were difficult to turn off
via an injected current pulse. Thus the effects of a hyperpolar-
izing pulse were very different before and after the burst was
initiated.

The effects of hyperpolarizing pulses to B63 were also
investigated (not shown). Previous data showed that small
amplitude depolarizing pulses to the simulated B63 were effec-
tive in eliciting bursts, whereas larger-amplitude pulses to the
simulated B31 were required (Fig. 3). For hyperpolarizing
currents, however, slightly larger amplitude pulses to the
simulated B63 were required to produce the delay, block, or
termination of a burst than for pulses to the simulated B31.
This finding suggests that the effects of hyperpolarization were
produced via the coupling to the simulated B31.

Robustness analysis

Although each element of the model was designed to resemble
the empirically measured properties of the cells and syn-
aptic connections, there are nevertheless no detailed voltage-
clamp data with which to constrain the parameters in the
model. Thus it is not clear to what extent the pattern generating
abilities of the neural network might be linked to a specific
value or set of values for a parameter(s). To assess the quality
of the model in terms of its consistency and robustness, a
parameter sensitivity analysis was undertaken. This analysis
consisted of three groups of simulations. One group of simu-
lations assessed the values selected for synaptic conductances

**FIG. 6.** A series of subthreshold depolarizations elicited a burst. In the
example shown, 4 400-ms pulses were delivered to the simulated B63 neuron,
with 400-ms rests between pulses. Note that a single 400-ms pulse would have
elicited a burst in response to −1 nA (see Fig. 3), whereas the 4 pulses in this
example elicited a burst with less than half of this current.

**FIG. 7.** Inactivation of the active response in the simulated B31. In all four
traces, a subthreshold, 9.80-nA depolarizing pulse was injected into B31 and
somewhat later a 9.82 nA was then injected. A: 5 s after the 1st pulse, the 2nd
pulse elicited a burst. B: 1 s later, the 2nd pulse no longer elicited a burst. C:
the 2nd pulse follows the first by 18 s. There was still inactivation, and no burst
was elicited. D: 4 s later the inactivation had mostly worn off, and a burst was
elicited, although only with a sustained depolarization of B31 prior to the burst.

**HYPERPOLARIZING PULSES IN THE SIMULATED B31 CELL DIFFER
IN THEIR EFFECTS ON A BURST.** The preceding data showed that
the effects of a series of depolarizing pulses were cumulative
and thereby affected the ability of the system to sustain a burst.
This suggested that hyperpolarizing pulses delivered in the
period before a burst was initiated could also affect the burst.
Accordingly, we examined the effects of brief hyperpolarizing
pulses injected into either the simulated B63 or B31 in the
period between the termination of a suprathreshold depolariz-
ing pulse and the start of a burst as well as after the burst had
begun.

Hyperpolarizing the simulated B31 for 0.5 s before a burst

**FIG. 5.** In the simulated circuit, continuous depolarization of either neuron
induced continuous bursting for as long as the stimulus was maintained. A:
depolarization of B31. B: depolarization of B63. For this figure, the amplitude
of the stimulus was adjusted so bursting was maintained at a rate of ~4/min.
Note the difference in the current amplitudes to the 2 neurons that were needed
to elicit this rate.
(both chemical and electrical). In this first group of simulations, all five synaptic conductances were randomly assigned new values that were within ±15% of their control values (\(g_{ec}\) and \(g_{sc}\) in Table 2). After these randomly assigned values were incorporated into the network, stimuli were applied to the simulated B63 (and B31) cells, and the ability of the modified network to generate both a single pattern of activity and continuous rhythmic activity was determined. This procedure of randomly altering all synaptic conductances and attempting to generate patterned activity was repeated 10 times. All 10 variants of the neural network produced both single patterns of activity and continuous rhythmic activity that were similar to that generated by the control circuit (i.e., Figs. 3 and 5).

A second group of simulations assessed the impact of values selected for the membrane conductances (\(g_{\text{max}}\) in Table 1). In this second group of simulations, these nine membrane conductances were randomly assigned new values that were within ±15% of their control values, and these new values were incorporated into the neural network. As described in the preceding text, this procedure was repeated 10 times, and each variant of the network was tested for its ability to generate both a single pattern of activity and continuous rhythmic activity in response to stimulation of the simulated B63 (and B31) cells. Five of the 10 variants of the model produced both a single pattern of activity and continuous rhythmic activity similar to that generated by the control circuit (i.e., Figs. 3 and 5). An additional four variants produced a single pattern of activity, similar to that of the control simulation, but did not exhibit cyclical rhythmic activity in response to continuous depolarization. Only a single variant model produced neither a single pattern of activity nor continuous rhythmic activity in response to stimulation.

A further analysis revealed that all simulations that did not display continuous bursting had a critical factor in common, a relatively low total \(K^+\) conductance. Because all elements are electrically coupled, the conductance of one cell affects another, and thus it is meaningful to examine whether the total sodium and potassium conductances can influence the system. Figure 9 indicates that the model can tolerate variations in total \(Na^+\) conductances, but if the total \(K^+\) conductance fell below \(\approx 57 \mu S\), the model begins to fail.

Finally, in a third group of simulations, all 14 conductances in the model (i.e., \(g_{\text{max}}, g_{ec}\), and \(g_{sc}\) in Tables 1 and 2) were subjected to continuous stochastic fluctuations throughout the simulations, and the ability of the model to produce patterned activity was evaluated. As in the preceding text, the model continued to produce both single patterns of activity and continuous rhythmic activity when the SD of the stochastic fluctuations was ±15%. Indeed, the model continued to function appropriately until the SD of the stochastic fluctuations ≥25%.
These simulations indicate that the ability of the network to simulate aspects of fictive feeding did not result from the arbitrary selection of any single value or set of values for parameters in the model. Rather, these results indicate that the ability to generate pattern activity was a moderately robust property that emerged from the neural network as a whole.

**Contribution of specific features of the model to the properties of the circuit**

The simulated B63 and B31 circuit was designed with many of the features of the B63 and B31 neurons in the living animal. These features gave rise to patterns of activity that were similar in the simulation and in the behaving animal. An advantage of a simulation is that features can be selectively included or excluded, thereby revealing the contribution of the feature of interest to the simulated pattern of activity. We systematically examined the contribution of some features of the simulated B63-B31 system to the pattern of activity in an attempt to understand their functional role in contributing to the properties of the circuit. These simulations revealed that slow time- and voltage-dependent active currents, and positive feedback from B31 to B63, play pivotal roles in the circuit.

**Time- and Voltage-Dependent Depolarization in B31.** In our model, the plateau-like depolarization of B31 and the simultaneous firing in B63 were achieved by designing an endogenous time- and voltage-dependent slow Na\(^+\) current in B31. However, to date there are no empirical data on the currents underlying the sustained depolarization of B31. To determine whether a completely different mechanism for achieving a sustained depolarization could give rise to a similar pattern of activity, the model was altered. The slow voltage-dependent Na\(^+\) current in B31 was removed, and in its place a slow EPSP from B63 to B31 was inserted. Thus transmission from B63 to B31 was characterized by both fast and slow chemical EPSPs in addition to the electrical coupling. The slow EPSP was adjusted to have the same reversal potential (40 mV) as the slow Na\(^+\) current that had been removed. The time constant was set to 750 ms and the maximum synaptic conductance was 4 \(\mu\)S. The slow EPSP was also designed with facilitation properties identical to those in the fast EPSP (see Table 2). All other features of the simulation, including the slow voltage-dependent K\(^+\) current, were retained, because there is empirical evidence supporting their existence even if their precise values are not known.

Replacing the voltage-dependent slow Na\(^+\) current with a slow EPSP led to retention of some of the response properties of the simulated B63-B31 system, but many of them were markedly changed. As in the simulation with a voltage-dependent depolarizing current, brief depolarization of either B63 or B31 in the simulation with a slow EPSP caused sustained activity that long outlasted the stimulus. In addition, the threshold for initiating such long-lasting activity was lower for depolarization of B63 than of B31. However, the amplitude and the duration of the sustained activity were now strongly dependent on the amplitude of the stimulus to either B63 or B3 with no clear threshold for an all-or-none burst seen. In addition, for low-amplitude stimuli to either B63 or to B31, there was no delay between the end of the stimulus and the start of the slow depolarization (Fig. 10). Finally, no repetitive bursting was observed in response to a maintained depolarization.

Tonic suprathreshold depolarization of either the simulated B63 or the simulated B31 neuron led to a period of increased depolarization of B31 and an increased firing rate in B63 that was followed by a steady-state depolarization of B31 and a steady-state firing in B63 (not shown). These findings are inconsistent with empirical observations on the B63 and B31/ B32 neurons in the buccal ganglia, which display a sustained plateau after a delay and only after a specific suprathreshold stimulus. In addition, in recordings from the B63 and B31 neurons in the buccal ganglia, the amplitude and duration of the plateau are not obviously dependent on the amplitude of the stimulus. These findings suggest that a slow EPSP with no active time and voltage dependence cannot alone account for the integrative properties seen in the B63-B31/B32 system of the buccal ganglia. Rather, time- and voltage-dependent depolarizing currents are likely to play a role.

**Electrical Coupling.** The contribution of the electrical coupling between B63 and B31 to the ability of a pulse to drive the neurons was systematically examined by removing the coupling and then determining the threshold for bursting using different pulse widths.

Removing the coupling had major effects on the threshold for eliciting activity via a direct depolarization of either the simulated B63 or B31 cell. In all cases, the threshold was increased when the coupling was removed (Fig. 11). For pulses delivered to B63, the effects of the coupling were more prominent with longer pulses than with shorter pulses. For longer pulses, removing the coupling increased the threshold by \(\sim 100\%\) of that seen in the presence of the coupling. As the pulses were shortened this value declined to \(\sim 40\%\). For pulses delivered to B31, removing the coupling increased the threshold by \(\sim 60\%\) with little difference seen between longer and shorter pulses.

With electrical coupling restored to the network, the contribution of the electrical coupling to the ability of the simulated B63 to initiate a burst was evident when pulses of different lengths were examined (Fig. 12). For all pulses, there was a period from the start of the pulse until the first action potential in B63 in which both B31 and B63 gradually depolarize. In addition, the depolarization in both cells became progressively larger even after the start of spiking in B63. The depolarization of B31 caused activation of slow currents in this neuron. The coupling to B63 in turn depolarized B63 still further, causing an increase in firing frequency, which in turn increased the depolarization of B31. Thus the electrical coupling between B63 and B31 served to amplify the exogenous depolarization applied to B63, by causing positive feedback to B63 as a result of the subthreshold activation of B31 (see Fig. 4A). This effect was particularly evident for longer pulses because the slow Na\(^+\) current in B31 has a very long time constant of activation.

We also examined the contribution of the electrical synapse between the simulated B63 to B31 in driving bursts by removing the chemical synapse between these neurons and thereby leaving the cells connected exclusively via the coupling pathway. When the chemical synapse was removed, even a 200-nA depolarization of the B63 soma for 1.0 s was insufficient to drive a burst (not shown). Thus coupling alone was extremely ineffective in driving activity via B63, although it had a major effect in amplifying the ability of B63 to drive a burst via the chemical synaptic connection.

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FACILITATION. The function of the facilitation in the chemical synapse from B63 to B31 was examined by removing this feature from the synapse. The threshold for eliciting bursts with different pulse widths was then determined (Fig. 11). Removing the facilitation raised the threshold for driving bursts via B63. Because direct stimulation of B31/B32 elicited a burst primarily via activating the plateau potential, removing facilitation had little effect on bursts that were initiated by depolarization of B31. In contrast to the effects of coupling on B63-induced bursts, facilitation reduced the threshold primarily in response to short pulses and had relatively little effect on longer pulses. The effects of facilitation were evident in just-threshold recordings with pulses to B63 of different lengths (Fig. 12). For short pulses, the facilitation caused a marked increase in the amplitude of EPSPs in B31, but the facilitation was too weak to affect the EPSPs in response to longer pulses. The combined effect of the synaptic facilitation and the electrical coupling to the ability of B63 to elicit a burst can be seen in greater detail in Fig. 13, which displays response to a just-threshold stimulus in the presence of both coupling and facilitation and then displays the response to the same stimulus in the absence of facilitation and of coupling. Removal of the facilitation reduced the overall depolarization of B31 caused by the spikes in B63. Consequently, the depolarization of B31 was not sufficient to elicit a regenerative burst of activity. Removal of coupling reduced the number of spikes elicited by the depolarization of B63 because the depolarization was not amplified by the feedback from the active subthreshold depolarization of B31.

COUPLING VIA A CONTRALATERAL PROCESS. In the buccal ganglia, the B63 axon crosses the buccal commisure and synapses via a mixed electrical-chemical synapse with the contralateral B31/B32 neurons. Thus the synapse is located at a relatively large electrical distance from the B63 soma. This was simulated by building B63 with both soma and axon compartments and placing the synapse to B31 in the axon compartment of B63. We explored the functional consequence of the relatively distant coupling from the B63 soma by removing the B63 axon from the simulation. The coupling strengths between the B63 and B31 somata were then adjusted so as to fit the amplitude of that observed in the ganglion.

Placing the interconnections between the somatic compartments of B63 to B31 had two major effects: the firing rate of B63 was strongly reduced (Fig. 14A) and removal of the electrical coupling now had no consistent effect on the threshold to initiate a burst via depolarization of B63 (Fig. 14B). For some pulse widths, the threshold was increased in the absence of coupling, whereas for others, it was decreased. Even when the removal of coupling increased the threshold, the increase was ≤10%. Both effects are explained by the relatively large input resistance of the B63 axon. When the B63 axon was present, its large input resistance caused a larger voltage change in response to the same current change in B31, leading...
to a greater firing frequency in B63 and a greater amplification in the positive feedback loop between B63 and B31. These findings suggest that the coupling via a contralateral process that is distant from the B63 soma contributes to the positive feedback loop that amplifies the ability of B63 to drive a burst.

LARGE SIZE OF B31. The simulated B31 neuron was modeled with a large leak current, to simulate a low input resistance. In the buccal ganglia, B31/B32 also have a low input resistance, probably as a result of their extensive electrical coupling. We

FIG. 13. Effects of facilitation and coupling on simulated B63 firing frequency via feedback from B31. A: a just-threshold stimulus to B63 elicited 8 spikes in B63 during the 680 ms in which the current was delivered, and sufficient depolarization of B31 to initiate further spikes in B63 after cessation of the current pulse. No facilitation, when the facilitation in the chemical synapse was removed, the same stimulus still elicited 8 spikes, but these elicited a smaller depolarization of B31 and a burst was not initiated. No coupling, when the electrical coupling between B63 and B31 was removed, while the facilitation was retained, only 6 spikes were elicited in B63.

FIG. 14. The effect of removing the B63 axon, and creating a direct electrical connection between the somata of B63 and B31. A: a just-threshold stimulus to B63 elicited a burst, but the firing frequency in B63 was much reduced (compare to Fig. 3). B: a comparison of removal of the coupling when the axon is present (removal of coupling via axon; note these data were re-plotted from Fig. 11A), and when the coupling is directly to the B31 soma (removal of coupling via soma). Unlike removal of the coupling via the axon, removal of coupling via a soma to soma connection had mixed, but relatively small effects on the threshold for eliciting a burst. In no case did removal of the coupling reduce the threshold by much more than 10%, and in some cases removal of the coupling reduced the threshold. Thus the role of the electrical coupling as part of a positive feedback loop was enhanced because the coupling was relatively distant from the B63 soma.
examined how the large size of the simulated B31 neuron affected the properties of the circuit. To produce a cell with properties similar to a smaller B31 neuron, the leak conductance and the capacitance of the simulated B31 were reduced by 50%. In general, the properties of circuits with smaller and larger B31 neurons were similar. Bursts of similar waveforms were seen after a delay in response to just threshold depolarizations. In addition, a series of subthreshold depolarizations was also able to induce a burst (not shown). However, reduction in the size of B31 caused a decrease in the threshold in response to stimuli delivered to either B63 or B31, particularly in response to brief pulses.

PLACING SLOW AND FAST CONDUCTANCES INTO THE SAME NEURON. A prominent feature of the B63-B31 system is that fast and slow currents are in separate neurons (fast currents in B63 and slow currents in B31). We examined the consequences of combining both fast and slow currents in a single neuron. As an initial step in examining this question, the fast Na\(^+\) and K\(^+\) currents from the simulated B63 soma were added to a simulated B31 in addition to the slow currents that were already included in the simulated B31 neuron. Addition of the fast currents had little discernable effect on the activity of the circuit: thresholds and waveforms were identical to those seen before the fast currents had been added. We also examined how the fast Na\(^+\) and K\(^+\) currents affected the simulated B31 neuron in isolation. The electrical and chemical connections between B63 and B31 were removed, and the threshold and waveform of the B31 slow potential were examined in the presence and absence of the fast currents. These were very similar. The relative lack of effect of the fast currents presumably arose from the large size of the simulated B31 neuron. Fast conductances that were appropriate for generating spikes in B63 were unable to generate spikes in B31, which had a much larger leak conductance, as well as a much larger capacitance.

It was of interest to determine the effects on B31 of fast currents that were scaled up to be effective in causing fast spikes in B31. Such currents caused a hyper-excitable system, unless the thresholds of the spikes were reduced, so that the currents were activated only as a result of a depolarization of \(\pm 20\) mV. After these changes, the properties of the circuit remained essentially as they had been before the addition of the fast conductances except that B31 now displayed conventional spikes superimposed on its slow depolarization. Note that firing in the simulated B31 neuron begins well after firing in the simulated B63 and after the plateau-like depolarization of B31 was already established.

**DISCUSSION**

When its lips are stimulated with food, an *Aplysia* decides whether or not to perform a consummatory feeding sequence, consisting of a protraction and then a retraction of the radula (Kupfermann 1974). Many factors potentially influence this decision. These factors include the taste or the texture of the food (Kupfermann 1974), the time from the last encounter with food (Susswein et al. 1978), the time from the last feeding response (Kupfermann 1974), the degree to which the anterior gut is distended (Susswein and Kupfermann 1975), as well as associative and nonassociative learning resulting from previous experiences with the food (Botzer et al. 1998; Brembs et al. 2001; Kupfermann and Pinsker 1968; Lechner et al. 2000; Schwarz et al. 1988; Susswein et al. 1986). However, once a consummatory sequence begins, it is completed (e.g., it is a fixed act) (Kupfermann 1974), although the strength and speed of the movement are subject to modulation (Kupfermann et al. 1991).

Chronic recordings from intact behaving animals have shown that sustained bursts of activity in the B63-B31/B32 neurons are correlated one for one with the protraction phase of a consummatory movement (Hurwitz et al. 1996). Thus the neural events that underlie the decision on whether to initiate a consummatory movement are the subthreshold electrical events that precede and trigger a sustained burst of activity in the B63-B31/B32 neurons. The properties of the B63-B31/B32 neurons are appropriate for their decision-making function. Subthreshold depolarizations and hyperpolarizations can summate over a fairly long period and affect the initiation of the first stage of a patterned burst, protraction. However, once protraction is initiated, it is relatively difficult to stop (Hurwitz et al. 1997).

The aim of the present study was to explore some of the cellular and network properties that may contribute to the decision-making function in the B63-B31/B32 neurons. To this end, a computational model was created that displays some of key features of these neurons and of their interconnections.
Simulations showed that the slow kinetics of the active currents that underlie the sustained depolarization of the simulated B31 and the positive feedback loop between the simulated B31 and B63 cells are key features contributing to the ability of these cells to summate information over an extended period before a decision is made to initiate a feeding sequence. The large amplitudes and slow kinetics of the slow currents in the simulated B31 give rise to the property that protraction can be stopped after it is initiated only by either very large or prolonged inhibition. These results suggest that slow active currents in B31/B32, as well as a positive feedback loop with B63, may also be key features in the decision-making function of the real B63-B31/B32 neurons in the buccal ganglia.

COMMON FEATURES OF THE MODEL SYSTEM AND THE BUCCAL GANGLIA. In both the model system and in the buccal ganglia, the protraction phase is dependent on two different cell types. B63 is able to sustain conventional action potentials, whereas B31 is a large neuron that displays only slow potentials. These neurons are electrically coupled and are also connected via a facilitating EPSP from B63 to B31. In the buccal ganglia, the B31/B32 neurons respond to a brief depolarization with a slow sustained depolarization that resembles a plateau potential. This is reflected by a parallel sustained depolarization in B63 that underlies spiking in this neuron. In the model, B31 was constructed with slow voltage-dependent conductances that give rise to a plateau potential. B63 was constructed without active slow conductances, but the coupling with B31/B32 was sufficient to cause a sustained depolarization in B63 as well as conventional spiking.

POSSIBLE DIFFERENCES BETWEEN THE MODEL SYSTEM AND THE BUCCAL GANGLIA. It is important to note that there is no direct evidence that the slow depolarization in the B31/B32 neurons arises as a result of endogenous voltage-dependent conductances as in the model. In principle, a slow depolarization could arise via other mechanisms, such as slow synaptic transmission (perhaps via peptide transmitters) from B63 to B31/B32. Indeed, preliminary evidence indicates that slow synaptic transmission may contribute to the plateau-like activity in the B63-B31/B32 complex (Hurwitz et al. 1999). However, it is unlikely that a purely ligand-gated slow process could alone account for the sustained depolarization of B31/B32 because subthreshold depolarizations of B31/B32 can cause inactivation of the plateau-like potentials similar to that shown in the simulation (Fig. 7). Slow transmission would have to be partially voltage dependent to account for the voltage-dependent nonlinearities observed in the activity of B31/B32 (see Fig. 2). In addition, designing a simulation in which a ligand-gated slow EPSP was responsible for the depolarization of the simulated B31 led to a system with many differences in its behavior from that observed in the buccal ganglia (Fig. 10). These findings do not rule out the possibility that a slow synaptic potential underlies some of the depolarization of B31/B32, but a slow EPSP is likely to initiate a voltage-dependent process.

The ability of the simulated B63-B31 circuit to oscillate (e.g., Fig. 5) derives from the depolarization of B31, whose origin is in the positive feedback provided by the connections between B63 and B31, and the voltage-dependent slow depolarization in B31, followed by the repolarization of B31, which derives from a slow, voltage-dependent K⁺ current. Our analyses have shown that the ability to oscillate is highly dependent on the magnitude of the total K⁺ current in the system (Fig. 9). We have no empirical data on whether the endogenous currents driving repolarization in the B63-B31/B32 system in the buccal ganglia are sufficient for oscillations. In the buccal ganglia, a major contributor to repolarization is neuron B64, which produces powerful IPSPs in B63 and B31/B32 (Hurwitz and Susswein 1996). This cell is not included in the current model. It is important to note that in vivo, B31/B32 also repolarize even in the absence of B64 activity (Susswein et al. 1988), indicating that an endogenous mechanism for repolarization is likely to be present in these neurons.

In spite of the likely differences in the features of the plateau-like potential between the simulated B31 neuron and the B31/B32 in the buccal ganglia, the most interesting features of the B63-B31 model developed in the preceding text are likely to be present in the ganglia because they are not dependent on the precise mechanism of the plateau potential. In particular, features that arise from the combined electrical and chemical coupling between the neurons and from their different passive electrical properties will probably be found in recordings from the real B63 and B31/B32 neurons in the buccal ganglia.

FUNCTION OF B63. In the buccal ganglia, synaptic inputs from cerebral ganglion command-like neurons affecting the initiation of a buccal motor program generally act on both B63 and B31/B32 (Hurwitz et al. 1997; Rosen et al. 1991). However, our model suggests that excitatory inputs affect burst initiation primarily via effects on B63 because the threshold for initiating a burst is lower for depolarizations of the simulated B63 than for the simulated B31 (Figs. 3 and 5). Thus B63 may represent a key element in receiving excitatory inputs and translating them into a buccal motor program. In the simulation (and presumably in the buccal ganglia), the threshold difference between B63 and B31 arises from the difference in input resistance between the two neurons. The larger input resistance of B63 leads to a larger depolarization in response to a similar current. The depolarization of B63 causes a depolarization of B31 via both electrical and chemical synapses. The combined electrical and chemical synapses, as well as the active response of B31, produces a positive feedback loop that amplifies the initial depolarization in B63 (see Figs. 4 and 11–13).

FUNCTION OF B31. In the simulation, B31 is the source of the sustained depolarization that drives the protraction phase. The origin in B31 of the currents driving protraction accounts for the longer delay to initiate a burst in response to a just-threshold depolarization to the simulated B31 than to B63 (Fig. 3).

The low input resistance and large capacitance of B31 apparently are not essential factors contributing to the ability of the neuron in initiating a burst. Thus models in which B31 was made smaller still retained most of the properties seen with a larger neuron. However, the thresholds for eliciting a burst via either B63 or B31 were consistently lower in models with a smaller B31. This finding suggests that the large size of B31 contributes to matching the synaptic inputs with the desired threshold for initiating a burst. In addition, the difference in input resistance between B31 and B63 causes an asymmetry in the coupling ratio, so that coupling is more effective for currents from B31 to B63 than vice versa (Fig. 1). This functions as part of the positive feedback that amplifies the activity of...
B63. Slow active depolarizations in B31 are effectively fed back to B63 and thereby affect its activity.

In the buccal ganglia, the low input resistance in B31 arises from the strong (2:1) coupling between B31 and B32 as well as from weaker coupling with other neurons (Susswein and Byrne 1988). An unusual feature of many identified Aplysia neurons is that they come in pairs of seemingly identical neurons (e.g., B4/B5, B8a/b, B52a/b, B61/B62, CB1-5/6, CB1-8/9) (see Evans et al. 1999; Gardiner 1977; Morton and Chiel 1993; Perrins and Weiss 1998; Xin et al. 1999). At least for B31/B32, it is possible that the duplication in the cell, and the coupling between the duplicate cells, is related to the need to decrease its input resistance to levels appropriate for initiating a burst in response to the synaptic inputs produced in this system. However, the duplication of the B31/B32 neurons may also be related to additional functions, such as having neurites that extend into somewhat different areas of the buccal ganglia (Hurwitz et al. 1994) or that innervate somewhat different areas of the I2 muscle.

INEXITABILITY OF B31. We have also addressed ourselves to the functional implications of the lack of conventional excitability in B31.

Although most Aplysia neurons have fully excitable somata, in some neurons (e.g., B4/B5), spikes recorded in the soma are relatively fast and do not display an afterhyperpolarization, suggesting that these are axon spikes that fail to invade the soma, but whose failure point is quite close to the soma. Our data indicate that the large impedance mismatch between the soma and axon of B31/B32 is likely to account for the small size (5–15 mV) of the axon spikes recorded in the soma. When fast currents appropriate for spiking in B63 were added to modeled B31, there was virtually no change in the properties of the neuron presumably because of the impedance mismatch. A previous preliminary report (Chiel and You 1991) reported that when a single B31 neuron is removed from the buccal ganglia and is grown in tissue culture, the neuron displays spikes. This could be explained by a decrease in the impedance mismatch between the axon and the soma because the leak resistance of the soma is increased as a result of its isolation from neurons to which it is electrically coupling in the ganglion.

In many neurons, the ionic bases of the soma and axon spikes may differ considerably (e.g., Alving 1968; Clarac and Cattaert 1999; Evans and Cropper 1998; Kramer 1986; Perrins and Weiss 1998; Weiss et al. 1986). Therefore the lack of conventional spikes in the B31 soma cannot be explained merely as a result of an impedance mismatch but rather represents a specific absence of channels that underlie fast excitability. Our data indicate that the absence of fast spikes in B31 may be a design feature in this neuron. When B31 was built with fast conductances that cause spikes, the system became hyper-excitable unless the threshold of the spikes was increased (Fig. 15). One means of increasing the threshold for spiking is to place the spike initiating zone at an electrically distant site from the source of the slow currents that drive the spikes. This seems to be the mechanism used by the B31/B32 in the ganglion, in which the spiking axon is electrically separate from the nonspiking soma (Hurwitz et al. 1994).

POSITIVE FEEDBACK BETWEEN B63 AND B31. A striking feature of the simulated B63-B31 system is a positive feedback loop that amplifies the inputs to B63 and thereby contributes to the ability of this neuron to induce a burst. We have identified three components of this positive feedback loop: the electrical coupling between B63 and B31, the facilitating chemical synapse from B63 to B31, and the contralateral connection between B63 and B31, which leads to coupling between a fine axon and a large soma. These three features were identified by selectively removing them from the simulated circuit and characterizing the circuit with and without the features.

Positive feedback loops between CPG neurons that are active in the same phase of a program are also found in other systems (e.g., Byrne 1983; Koester 1989; Kupfermann and Kandel 1970; Norekian 1999). In these systems, mutual chemical and electrical synapses underlie the positive feedback. In addition, in Clione, positive feedback in part is mediated by a self-excitatory synapse (Norekian 1999). Although B63 and B31 are separate neurons with different physiological properties, they are always active together. These neurons may be regarded as a single functional unit with different physiological properties localized to different portions of the unit. The synaptic excitation of B31 by B63 may be functionally equivalent to a self-excitatory synapse.

SIMILARITY TO EFFECTS OF A CURRENT. A striking feature of the simulated B63-B31 circuit is that bursts often occur after a delay (Figs. 3, 6, and 8). The extent of the delay inversely depends on the amplitude of the depolarization. In many systems, similar delays in activity are explained by the presence in a neuron of an A current (e.g., Byrne 1980a,b; Hurwitz et al. 1997). In these systems, depolarization activates a transient K+ current, which counteracts the effects of the depolarization and thereby introduces a delay in activity. By contrast, in our system, the delay arises from the slow kinetics of the B31 active currents and the time needed to activate the positive feedback loop between B63 and B31. In principle, it would be easy to design a single cell with both an A current and fast Na+ and K+ currents that would have many of the functional properties of the B63-B31 system, and it is possible that the B63-B31 circuit is an evolutionary alternative to one built on an A current.

MODEL SYSTEM AND THE BUCCAL GANGLIA IN THE LIVING ANIMAL. It is important to note that an all-or-none system with a delay could have been built from neurons with properties radically different from those of the B63-B31/B32 system. Our aim is to suggest functional explanations of some of the unusual properties of the neurons in the Aplysia protraction circuit, recognizing that a functionally similar circuit could have been built from different design features.

It is also important to note that the B63-B31/B32 circuit in the buccal ganglia is considerably more complex than is the simulated circuit. Future studies will be needed to determine whether features that were not included in the model (e.g., the presence of >1 B63 and B31/B32 neuron, complex geometries of B63 and B31, additional protraction-phase interneurons), or features included in the model that may operate somewhat differently in life (e.g., the biophysical mechanisms underlying the slow currents in B31) affect the functional properties of the circuit. However, in addition to explaining some of the functions of the neurons B63 and B31, our model indicates that circuits built along the lines that we have modeled may be generally useful as parts of the architecture of
many neural systems. We may expect to encounter circuits similar to those modeled in many additional systems.

**APPENDIX**

Cell B31/B32 was modeled as a single, isopotential compartment, whereas cell B63 was modeled with two compartments: one representing the axon and the other representing the soma (see Fig. 1). The equivalent electrical circuit for each compartment consisted of a membrane capacitance ($C_m$) in parallel with a leakage conductance ($g_l$) with its associated equilibrium potential ($E_l$) and two voltage- and time-dependent conductances with their associated equilibrium potentials: one for Na$^+$ (i.e., $g_{Na}$ and $E_{Na}$) and another for K$^+$ (i.e., $g_K$ and $E_K$). The membrane potential ($V_m$) of each compartment was given by the differential equation

$$-C_m \frac{dV_m}{dt} = \left( \sum I_{m} + \sum I_{es} + \sum I_{in} \right) - I_{lim} \quad (A1)$$

where $C_m$ is the membrane capacitance of a given compartment, $\sum I_{m}$ is the sum of the ionic currents in that compartment, $\sum I_{es}$ is the sum of the chemical synaptic currents in that compartment, $\sum I_{in}$ is the sum of the electrical synaptic currents in that compartment, and $I_{lim}$ is an extrinsic stimuli, which can be applied to any compartment. The parameters that described each compartment were adjusted to match empirically observed biophysical properties (see Table 1 and following text). For example, the somatic compartment of B31/B32 did not support conventional, fast action potentials, whereas both compartments of B63 did.

Each ionic current was obtained by solving the equation $I_{m} = g_{ion}(v, t) (V_m - E_{ion})$, where $g_{ion}(v, t)$ is the voltage- and time-dependent conductance and $E_{ion}$ is the reversal potential associated with each current. $g_{ion}(v, t)$ was represented by a Hodgkin-Huxley-type formulation and each ionic conductance was evaluated by solving the general equation

$$g_{ion} = g_{max(ion)} A_{ion}(v, t) B_{ion}(v, t) \quad (A2)$$

where $g_{max(ion)}$ is the maximal value of $g_{ion}$, $A_{ion}(v, t)$ and $B_{ion}(v, t)$ are functions describing the voltage- and time-dependent activation and inactivation, respectively, associated with $g_{ion}$ and $p$ is the power to which $A_{ion}$ was raised. $A_{ion}$ and $B_{ion}$ were given by the solution to the general differential equations

$$\frac{dA_{ion}}{dt} = A_{ion} - A_{ion} \tau_{A(ion)} \quad (A3a)$$

$$\frac{dB_{ion}}{dt} = B_{ion} - B_{ion} \tau_{B(ion)} \quad (A3b)$$

where $A_{ion}$ and $B_{ion}$ are the voltage-dependent steady-state values of the activation and inactivation functions, respectively. $\tau_{A(ion)}$ and $\tau_{B(ion)}$ are the voltage-dependent time constants of the activation and inactivation functions, respectively. The values of $A_{ion}$, $B_{ion}$, $\tau_{A(ion)}$, and $\tau_{B(ion)}$ were determined from the general equations

$$A_{ion} = \frac{1}{1 + \exp((V_m - h_{ion})/b_{ion})} \quad (A4a)$$

$$B_{ion} = \frac{1 - A_{ion}}{1 + \exp((V_m - h_{ion})/b_{ion})} + B_{max(ion)} \quad (A4b)$$

$$\tau_{A(ion)} = \frac{\tau_{A(max(ion))} - \tau_{A(min(ion))}}{1 + \exp((V_m - h_{ion})/b_{ion})} + \tau_{A(ion)} \quad (A4c)$$

$$\tau_{B(ion)} = \frac{\tau_{B(max(ion))} - \tau_{B(min(ion))}}{1 + \exp((V_m - h_{ion})/b_{ion})} + \tau_{B(ion)} \quad (A4d)$$

where $h$ and $z$ are the position and slope parameters, respectively, for these generalized Boltzman-type equations. In some cases (see Table 1), the time constant for activation and/or inactivation ($\tau_A$ and $\tau_P$, respectively) were taken to be voltage independent and in some cases the denominator of $\tau_A$ was the product of two exponentials (Table 1; see Ziv et al. 1999).

Currents due to chemical synapses ($I_{es}$) were calculated by solving the general equation

$$I_{es} = g_{max(ion)} A_{ion}(v, t) (V_m - E_{ion}) \quad (A5)$$

where $g_{max(ion)}$ is the maximal conductance, $A_{ion}(v, t)$ describes the time-dependent activation, and $E_{ion}$ is the reversal potential associated with each synapse. The time-dependent synaptic activation was assumed to act as a second-order system of the following form

$$\frac{d^2A_{ion}}{dt^2} = \frac{X - 2\tau_{A(ion)} \left( \frac{dA_{ion}}{dt} - A_{ion} \right)}{\tau_{A(ion)}^2} \quad (A6)$$

where $A_{ion}$ is the variable that describes the second-order system, $X$ is the forcing function, and $\tau_{A(ion)}$ is the time. The forcing function $X$ in Eq. A6 is the synaptic driver, which may represent the pool of available transmitter (TP) during a presynaptic action potential, and thus

$$X = \begin{cases} TP & \text{during a presynaptic spike} \\ 0 & \text{in the absence of a presynaptic spike} \end{cases} \quad (A7)$$

One method of implementing synaptic plasticity (e.g., homosynaptic depression or facilitation) is to regulate TP. If TP is a constant, then the synapse does not manifest plasticity. In the present study, the magnitude of TP was equal to 1 for a duration of 3 ms at nonplastic synapses. At synapses that manifest plasticity, TP was equal to a synaptic-plasticity (SP) variable. The initial magnitude of SP was equal to 1 and changes in the SP variable were described by

$$dSP/dt = \begin{cases} -SP\tau_{SP-1} & \text{during presynaptic spike} \\ (1 - SP)\tau_{SP-2} & \text{in absence of presynaptic spike} \end{cases} \quad (A8)$$

where $\tau_{SP-1}$ and $\tau_{SP-2}$ are time constants for the development of and recovery from plasticity, respectively. Note that assigning positive values to $\tau_{SP-1}$ is one method for producing homosynaptic depression, whereas assigning negative values is a method for producing homosynaptic facilitation.

Currents ($I_{es}$) due to electrical coupling between any two cells (i.e., cells 1 and 2) were calculated by solving the general equations

$$I_{es(cell1)} = g_{es(1-2)} (V_{m(cell1)} - V_{m(cell2)}) \quad (A9a)$$

$$I_{es(cell2)} = g_{es(2-1)} (V_{m(cell2)} - V_{m(cell1)}) \quad (A9b)$$

where $g_{es(1-2)}$ is the coupling conductances from cell 1 to cell 2; $g_{es(2-1)}$ is the coupling conductances from cell 2 to cell 1, and $V_{m(cell1)}$ and $V_{m(cell2)}$ are the membrane potentials of cells 1 and 2, respectively.

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