The Role of Interneurons in Controlling the Tail-Withdrawal Reflex in Aplysia: A Network Model

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SUMMARY AND CONCLUSIONS
1. The contributions of monosynaptic and polysynaptic circuitry to the tail-withdrawal reflex in the marine mollusk Aplysia californica were assessed by the use of physiologically based neural network models. Effects of monosynaptic circuitry were examined by the use of a two-layer network model with four sensory neurons in the input layer and one motor neuron in the output layer. Results of these simulations indicated that the monosynaptic circuit could not account fully for long-duration responses of tail motor neurons elicited by tail stimulation.

2. A three-layer network model was constructed by interposing a layer of two excitatory interneurons between the input and output layers of the two-layer network model. These interneurons had properties mimicking those of the recently described interneuron LP117, receiving excitatory input from pleural sensory neurons and evoking a biphasic excitatory postsynaptic potential (EPSP) in pedal motor neurons (Cleary and Byrne 1993). The three-layer model could account for long-duration responses in motor neurons.

3. Sensory neurons are a known site of plasticity in Aplysia. Synaptic plasticity was incorporated into the three-layer model by altering the magnitudes of conductance changes evoked in motor neurons and interneurons by presynaptic sensory neurons. In these simulations the excitatory interneurons converted an amplitude-coded input into an amplitude- and duration-coded output, allowing the three-layer network to support a larger range of output amplitudes and durations.

4. Synaptic plasticity at more than one locus modified dramatically the input-output relationship of the three-layer network model. This feature gave the model redundancy in its plastic properties and points to the possibility of distributed memory in the circuitry mediating withdrawal reflexes in Aplysia. Multiple sites of control over the response of the network would likely allow a more diverse repertoire of responses.

INTRODUCTION

The neural circuits that mediate withdrawal reflexes in Aplysia are useful systems for studying the cellular and molecular mechanisms contributing to simple forms of learning. Previous studies of modulation of reflexes in Aplysia have focused mainly on changes in the monosynaptic connection between sensory and motor neurons of the reflex circuit (reviews include Byrne 1987; Byrne et al. 1991; Carew and Sahley 1986; Kandel and Schwartz 1982). For example, enhancement of monosynaptic excitatory postsynaptic potentials (EPSPs) elicited in tail motor neurons by tail sensory neurons accompanies enhancement of both evoked activity in the motor neurons and the tail-withdrawal component of the tail-siphon withdrawal reflex (Walters et al. 1983b). The quantitative contributions of these enhanced monosynaptic EPSPs to behavioral modifications has not been established, however.

Inspection of the physiological data indicates that modulation of the monosynaptic connection between sensory and motor neurons contributes to the early portion of evoked spike trains in motor neurons and hence the early portion of the behavioral response. For example, with regard to the tail-withdrawal response, single action potentials in tail sensory neurons of the pleural ganglion can evoke suprathreshold EPSPs in tail motor neurons of the pedal ganglion. Moreover, tail stimulation evokes bursts of activity in pleural sensory neurons that are coincident with intense bursts of activity in pedal motor neurons (Walters et al. 1983a). On the other hand, given the short (<1 s) durations of responses of pleural sensory neurons to stimulation of the tail, a monosynaptic circuit consisting of simple (i.e., nonbursting) neural elements cannot account for the extended (>10 s) responses of pedal motor neurons to tail stimulation. Because pedal motor neurons do not exhibit bursting behavior in response to depolarizing stimuli (D. V. Buonomano and J. H. Byrne, personal communication) or repeated stimulation by a single sensory neuron (Buonomano and Byrne 1990; Walters et al. 1983a), it seems likely that a polysynaptic circuit drives long-duration responses in motor neurons in response to tail stimulation. A similar line of reasoning can be made with regard to plasticity of the tail-withdrawal response. Modulation of the monosynaptic connection between pleural sensory neurons and pedal motor neurons might be expected to affect the initial response rates of the motor neurons and hence the initial magnitude of the tail-withdrawal response, but a more complex model is necessary to account for changes in sustained firing rates and response durations exhibited by pedal motor neurons in response to relevant stimuli.

Several interneurons participate in the tail-withdrawal response, but the excitatory interneuron LP117 of the pleural ganglion (Cleary and Byrne 1993) seemed particularly likely to make important contributions to long-duration responses in pedal motor neurons. LP117 was shown to receive excitatory input from pleural sensory neurons and to evoke a long-lasting EPSP in pedal motor neurons. This slow EPSP in pedal motor neurons was associated with as much as a 30% decrease in input conductance of the cell and lasted as long as 1 min (Cleary and Byrne 1993). The purpose of the present study was to evaluate the quantitative contributions of the monosynaptic sensorimotor circuit and the polysynaptic circuit including LP117 to the magnitude and duration of the tail-withdrawal response. We showed that the monosynaptic circuit could not account for long-duration responses in motor neurons. In contrast, a model circuit including descriptions of LP117 interneurons could account for spike trains in motor neu-
rons lasting tens of seconds. In addition, the flexibility inherent in the computational approach allowed us to demonstrate the potential for dramatic effects of synaptic plasticity at two novel sites: the sensory neuron-to-LPl17 connection and the LPl17-to-motor neuron connection. Physiologically realistic levels of synaptic modulation at these two sites could support a large (>10-fold) range of amplitudes and durations of spike trains in modeled pedal motor neurons. Portions of this work have appeared in preliminary form (White et al. 1991).

METHODS

Development of the model

Simulations were performed with the use of the program SNNAP (Simulator for Neural Networks and Action Potentials) (Ziv et al. 1991, 1993) (see also APPENDIX). In our SNNAP models, membrane conductances of model neurons were described by Hodgkin-Huxley type equations (Eqs. A1–A4) (e.g., Byrne 1980a; Hodgkin and Huxley 1952; Tam and Perkel 1989). The activation of synaptic conductances was assumed to act as a second-order system (e.g., Jack and Redman 1971; Rall 1967; Wilson and Bower 1989) and was calculated through the numerical solution of a second-order differential equation (Eq. A5) (Ziv et al. 1991, 1993). The forcing function of this differential equation was a pulse of duration equal to that of the presynaptic action potential. The value of the resulting synaptic conductance was directly proportional to synaptic activation for increased-conductance synapses (Eq. A6) and inversely proportional to synaptic activation for decreased-conductance synapses (Eq. A7). The set of differential equations describing the neural network was solved numerically by the use of Euler’s method.

ELEMENTS OF THE MODEL: SENSORY, MOTOR, AND INTERNEURONS. Responsiveness of sensory neurons were simulated by the use of Hodgkin-Huxley type equations, the parameters of which were based on data from voltage- and current-clamp studies (Baxter and Byrne 1990; Byrne et al. 1990; Canavier et al. 1991). The model sensory neuron included a description of a fast Na⁺ conductance, three K⁺ conductances (a delayed K⁺ conductance, a fast K⁺ conductance, and an S-type K⁺ conductance), and a Ca²⁺ conductance (Tables 1 and 2). Action potentials in the sensory neurons were activated by a series of short, suprathreshold pulses. The duration of the sensory neuron spikes, in turn, regulated transmitter release (see Eq. A5).

Figure 1A shows an experimentally measured monosynaptic EPSP elicited in a hyperpolarized tail motor neuron by a single spike in a tail sensory neuron and its simulated counterpart. The motor neuron was identified on the basis of its size, location in the pedal ganglion, and its antidromic activation by shocking nerve P9 (Walters et al. 1983a). The conductance underlying these EPSPs was modeled as a variable conductance of maximal value 0.16 μS, associated with a reversal potential of +30 mV (Table 2). These values were chosen to match the amplitude and waveform of a recorded EPSP. The model of the tail motor neuron was derived from the Byrne (1980a) model of ink motor neurons. It included a fast Na⁺ conductance, a delayed K⁺ conductance, and a Ca²⁺ conductance. Parameters were adjusted to fit experimental excitability data from tail motor neurons (Fig. 1B; Tables 1 and 2). In neither experimental nor simulated results was sustained firing seen after the 1-s depolarizing pulse.

Elements representing the excitatory interneuron LPl17 were included in some simulations as well. Figure 2 shows experimental (Cleary and Byrne 1993) and simulated data from hyperpolarized tail motor neurons in response to a 1-s, high-frequency (~20 Hz) burst of activity in an excitatory interneuron. The biophysical properties of the interneuron were modeled with the use of a Hodgkin-Huxley model with parameters of the fast Na⁺ conductance and delayed K⁺ conductance adjusted to match experimental excitability data (Tables 1 and 2). The EPSP produced in a motor neuron by LPl17 is biphasic, with a fast, increased-conductance component and a slow, decreased-conductance component (Fig. 2A). The fast component was modeled as a maximal conductance of 0.05 μS with an associated reversal potential of +30 mV. The slow component was modeled as a decrease in membrane conductance (maximal value: 0.035 μS) with an associated reversal potential of −70 mV (Tables 1 and 2). The actual reversal potential may be different from the values used in these simulations, and evidence indicates that the slow EPSP may be rectified rather than reverse at hyperpolarized potentials (e.g., Fig. 3 of Cleary and Byrne 1993). A reversal potential of −70 mV allowed us to use a simple, voltage-independent model to match the slow component of the biphasic EPSP (Fig. 2). The development of a voltage-dependent model must await a more detailed voltage-clamp analysis of the slow EPSP. Its omission from the present model is not critical, because the divergence between experimental and simulated results would be at hyperpolarized values of membrane potential that are never achieved in the present simulations.

RESULTS

Two-layer network model does not produce long-duration responses in motor neurons

To assess the contributions of the monosynaptic circuit to the tail-withdrawal response, simulations were performed with the use of the two-layer network model (Fig. 3A). Individual sensory and motor neurons and the synaptic connection between them were modeled with the use of
TABLE 1. Parameters describing membrane currents

<table>
<thead>
<tr>
<th>Neuron</th>
<th>( E_r ), mV</th>
<th>( g_{\text{max}} ), ( \mu S )</th>
<th>( h_x ), mV</th>
<th>( s_x ), mV</th>
<th>( \tau_{A(\text{max})} ), ms</th>
<th>( \tau_{A(\text{min})} ), ms</th>
<th>( \tau_{B(\text{max})} ), ms</th>
<th>( \tau_{B(\text{min})} ), ms</th>
<th>( \beta_{\text{min}} ), ms</th>
<th>( \beta_{\text{max}} ), mV</th>
<th>( S_{\text{R}} ), mV</th>
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</thead>
<tbody>
<tr>
<td>SN</td>
<td>70.0</td>
<td>10.0</td>
<td>18.2</td>
<td>8.8</td>
<td>2.0</td>
<td>0.56</td>
<td>9.0</td>
<td>7.0</td>
<td>-40</td>
<td>3.2</td>
<td>0.0</td>
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<td>MN</td>
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<td>-20.7</td>
<td>-26.0</td>
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<td>5.0</td>
<td>33.8</td>
<td>2.9</td>
<td>-49.3</td>
<td>23.3</td>
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<td>-9.5</td>
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<td>10.0</td>
<td>-46.0†</td>
<td>-6.5†</td>
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<td>MN</td>
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<td>0.39</td>
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<td>1.0</td>
<td>-42.8</td>
<td>21.8</td>
<td>-16.3</td>
<td>7.9</td>
<td>0.24</td>
</tr>
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<td>2.8</td>
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<tr>
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<td>-15.0</td>
<td>10.0</td>
<td>37.0</td>
<td>3.2</td>
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<td>2.8</td>
<td>-9.0</td>
<td>7.0</td>
</tr>
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<td>-19.0</td>
<td>0.033</td>
<td>0.003</td>
<td>0.001</td>
<td>-23.0†</td>
<td>-13.3†</td>
<td>372.6</td>
<td>67.1</td>
<td>-40.1</td>
<td>33.3</td>
</tr>
</tbody>
</table>

| MN     | -80.0        | -50.0           | -18.0       | -8.8   | 3.0             | 0.56            | -9.0            | 7.0             | -37.0           | 3.2             | 0.0             |
| MN     | -90.0        | 0.2             | -3.7        | -9.5   | 3.0             | 28.0            | 2.8             | 22.0            | 17.5            | -22.9           | 12.4            |
| MN     | 87.0         | 0.2             | -1.3        | -10.8  | 1.0             | 1.0             | -42.8           | 21.8            | -16.3           | 7.9             | 0.24            |
| MN     | -70.0        | 4.2             | -3.7        | -9.5   | 28.0            | 2.8             | 22.0            | 17.5            | -22.9           | 12.4            | 0.0             |
| MN     | 75.0         | 250.0           | -15.0       | 10.0   | 37.0            | 3.2             | 0.0             | 10.0            | 2.8             | -9.0            | 7.0             |
| MN     | -90.0        | -19.0           | 0.033       | 0.003  | 0.001           | -23.0†          | -13.3†          | 372.6           | 67.1            | -40.1           | 33.3            |

**TABLE 2. Parameters describing individual neurons and synaptic connections**

<table>
<thead>
<tr>
<th>Neuron</th>
<th>( V_r ), mV</th>
<th>( E_{\text{syn}} ), mV</th>
<th>( g_{\text{max}} ), ( \mu S )</th>
<th>( E_{\text{syn}} ), mV</th>
<th>( \tau_{\text{syn}} ), ms</th>
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</thead>
<tbody>
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<td>0.033</td>
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<tr>
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<td>0.103</td>
<td>0.01</td>
<td>-53.0</td>
</tr>
<tr>
<td>MN†</td>
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<td>-19.0</td>
<td>0.035</td>
<td>0.01</td>
<td>-19.0</td>
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<tr>
<td>LPI17</td>
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<td>-51.0</td>
<td>0.02</td>
<td>0.001</td>
<td>-51.0</td>
</tr>
<tr>
<td>SN</td>
<td>0.16</td>
<td>+30</td>
<td>2.7</td>
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<td></td>
</tr>
<tr>
<td>MN*</td>
<td>0.007</td>
<td>+30</td>
<td>4.0</td>
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</tr>
<tr>
<td>MN†</td>
<td>0.05</td>
<td>+30</td>
<td>7.0</td>
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<tr>
<td>LPI17</td>
<td>0.035</td>
<td>-70</td>
<td>6,000</td>
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</tr>
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</table>

**F**, reversal potential; \( g_{\text{max}} \), maximum conductance; \( h_x \), half-activation voltage; \( s_x \), slope parameter of activation function; \( \tau_{A(\text{max})} \), maximum value of activation time constant; \( \tau_{A(\text{min})} \), minimum activation time constant; \( \tau_{B(\text{max})} \), maximum value of inactivation time constant; \( \tau_{B(\text{min})} \), minimum value of inactivation time constant; \( h_y \), voltage at which inactivation time constant is half-maximal; \( s_y \), slope parameter of inactivation time constant function; \( \beta_{\text{min}} \), half-inactivation voltage; \( \beta_{\text{max}} \), slope parameter of inactivation function; \( \beta_{\text{min}} \), minimum value of inactivation function; \( \beta_{\text{max}} \), maximum value of inactivation function; \( \beta_{\text{min}} \), minimum value of inactivation time constant; \( \beta_{\text{max}} \), maximum value of inactivation time constant; \( h_x \), voltage at which activation time constant is half-maximal; \( s_x \), slope parameter of activation time constant function; \( h_y \), half-inactivation voltage; \( s_y \), slope parameter of inactivation function; \( g_{\text{max}} \), maximum value of the synaptic conductance (Eq. A6). The amplitude of the motor neuron response was defined as the number of spikes fired by the modeled motor neuron. The duration of the motor neuron response was defined as the difference in time between the first and last spikes fired by the motor neuron. The duration of the motor neuron response increased approximately linearly over a 5-fold range in response to a 12-fold change in \( \Delta g \). In contrast, the duration of the motor neuron response seemed to saturate at a value <600 ms, which was far shorter than the duration of the normal response to tail stimulation (Walters et al. 1983a). A series of simulations were performed to test the ability of the two-layer network to support plasticity in the motor neuron response. In these simulations the magnitude of the conductance change (\( \Delta g \)) underlying the monosynaptic EPSP in the motor neuron was altered systematically, at levels both above and below the control level, and the resulting effect on the magnitude and duration of the response of the motor neuron noted. Such manipulations are compatible with a model of neural plasticity in which the shape of the presynaptic action potential or the mobilization of synaptic vesicles is altered (see Byrne et al. 1991; Pieroni and Byrne 1992). \( \Delta g \) was altered by scaling \( g_{\text{max}} \), the maximum value of the synaptic conductance (Eq. A6). The amplitude of the motor neuron response was defined as the number of spikes fired by the modeled motor neuron. The duration of the motor neuron response was defined as the difference in time between the first and last spikes fired by the motor neuron. Figure 3C shows these measures of response amplitude and duration as functions of \( \Delta g \), the magnitude of the conductance change evoked in the motor neuron by sensory neuron input. The amplitude of the motor neuron response increased approximately linearly over a 5-fold range in response to a 12-fold change in \( \Delta g \). In contrast, the duration of the motor neuron response seemed to saturate at a value <600 ms, which was far shorter than the duration of the normal response to tail stimulation (Walters et al. 1983a). Inclusion of network elements representing excitatory interneurons allows the model to account for long-duration responses. We next tested the ability of a polysynaptic circuit model (Fig. 4A) including a physiologically based description of LPI17 neurons (Cleary and Byrne 1993) to support long-
A Evoked EPSPs in MNs

Experimental

Simulated

3 mV

40 msec

B Excitability in MNs (1-sec pulse)

FIG. 1. Models of sensory to motor neuron synaptic potential and of motor neurons (MNs) firing properties. A: experimental and simulated excitatory postsynaptic potentials (EPSPs) of pedal motor neurons (MNs) in response to a single action potential (not shown) in a presynaptic sensory neuron. In both cases the membrane potential of the motor neuron has been hyperpolarized to -90 mV. B: experimental and simulated data reflecting excitability in pedal motor neurons. The number of spikes in the motor neuron is plotted vs. the magnitude of an injected current pulse of 1-s duration. Parameters describing the simulated neurons are given in Tables 1 and 2.

duration (> 10 s) responses to sensory neuron activation in tail motor neurons. Results from one such simulation are shown in Fig. 4B. In this simulation, short-duration responses in the modeled sensory neurons and interneurons led to long-duration responses in the motor neuron. This result was not simply due to the additional excitation of the motor neuron provided by adding two more presynaptic cells; a two-layer network with six presynaptic sensory neurons failed to produce long-duration responses, even with greatly enhanced synaptic strengths (data not shown).

Thus results from the three-layer network model indicate that LP117 interneurons could make a quantitatively important contribution to tail-withdrawal responses.

Having demonstrated that the three-layer network model can account for long-duration responses in tail motor neurons, we next tested the ability of this network to simulate changes in responses of motor neurons as a result of changes in the magnitudes of the postsynaptic conductance changes in the motor neuron and interneurons resulting from sensory neuron input. The parameter $\Delta g$ was adjusted by scaling $g_{\text{max}}$, for the particular synapse. Figure 4C shows plots of response amplitude and duration as functions of $\Delta g$ for all synapses for which a sensory neuron is the presynaptic cell (A, Fig. 4A). The three-layer network supported a large range (~50-fold) of both response amplitudes and response durations. Control levels of $\Delta g$ gave intermediate levels of both response amplitude and response duration. As a result, the three-layer network supported simulations of both enhanced and diminished tail-withdrawal responses. For levels of $\Delta g \geq 100\%$ of control values, the number of spikes varied from 27 to 65, and the response duration varied from 16.3 to 23.2 s. For levels of $\Delta g \leq 100\%$ of control values, the number of spikes varied from 2 to 27, and the response duration varied from 0.2 to 16.3 s. The extended ranges of response magnitude and duration supported by the three-layer network were due to the effects of interneuron-driven slow EPSPs in the motor neuron.

Potential contributions of multiple sites of neuronal plasticity

Plasticity at synapses from sensory neurons to both interneurons and motor neurons exerts a strong influence on the output of the three-layer network. We were interested in examining the possibility that the response of the network is sensitive to synaptic modifications at the connections from LP117 interneurons to the motor neuron (A, Fig. 5A). Although plasticity at this synapse has not yet been investigated experimentally, the mathematical simulation is a useful tool for examining the potential contribution of plasticity at this synapse to the output of the circuit. Figure 5B shows the response of the three-layer network with only this connection changed from control levels. For this simulation, $g_{\text{max}}$ of the fast, increased-conductance component of this connection was set to 200% of its control value. The magnitude of the decrease in conductance associated with the slow component of the connection was approximately doubled as well, by doubling $\alpha_{\text{sc}}$ in Eq. A7. These changes (Fig. 5B) led to an increase in response amplitude (from 27 spikes to 31 spikes) and response duration (from 16.3 to 21.6 s; cf. Fig. 4B; note the change in time scales).
FIG. 3. Two-layer network model, representing the monosynaptic component of the tail-withdrawal reflex, does not support long-duration responses in simulations of responses of motor neurons. A: 2-layer network model. An input layer consisting of 4 modeled sensory neurons (SN1–SN4) sends convergent excitatory connections to a single motor neuron (MN) constituting the output layer of the model. Parameters describing individual neural elements and the connections between them are given in Tables 1 and 2. B: simulated responses of 1 of the sensory neurons (SN1) and the motor neuron (MN) to tail stimulation with levels of all model parameters at control levels (see Tables 1 and 2). C: number of spikes fired by the motor neuron (•) and duration of the response of the motor neuron (○) as functions of the size of the evoked conductance change (Δg) triggered in the motor neuron by each presynaptic action potential. Δg was scaled by altering the value of gmax in Eq. A6.

5C shows summary results from several such simulations. In this figure, response amplitude and duration are plotted as functions of the magnitude of the postsynaptic conductance changes induced in the motor neuron by the excitatory interneurons. The results are similar to those of Fig. 4C. For levels of conductance changes ≤100% of control values, the number of evoked spikes varied from 6 to 27, and the response duration varied from 0.5 to 16.3 s. For levels of conductance changes ≥100% of control values, the number of spikes varied from 27 to 66, and the response duration varied from 16.3 to 24.2 s.

We performed additional simulations to examine the interactive effects of the magnitudes of sensory neuron- and LPI17-induced conductance changes in determining the amplitude and duration of the motor neuron response. Examples of the results of such simulations are shown in Fig. 6. Figure 6A shows the modeled motor neuron spike train under control conditions (i.e., with both sensory neuron- and LPI17-induced conductance changes at control levels; reproduced from Fig. 4B). Figure 6B shows the motor neuron response with sensory neuron-induced conductance changes augmented by 100% and LPI17-induced conductance changes at control levels. The magnitude of the response (as measured by the number of evoked action potentials) was increased by 85%, and its duration was increased by 25%. Figure 6C shows the motor neuron response with LPI17-induced conductance changes increased by 100% and sensory neuron–induced conductance changes at control levels (reproduced from Fig. 5B). This response was very similar to that of Fig. 6B. Its amplitude was increased 89% and its duration 38% relative to control.

Figure 6D shows the response of the motor neuron with both sensory neuron– and LPI17-induced conductance changes augmented by 100% relative to control levels. The magnitude of the response in Fig. 6D was increased 181% relative to its control level, indicating that manipulations of the magnitudes of sensory neuron– and LPI17-induced conductance changes summed roughly linearly within this range of response characteristics (85 + 89%). Similarly, the increase in response duration due to facilitation at both sites was the sum of that at each site individually (25 + 38%).

DISCUSSION

We used a physiologically based neural network model to examine the functional consequences of a parallel channel of information to tail motor neurons provided by the pleural interneuron LPI17. Inclusion in the model of elements representing LPI17 neurons allowed the model to
mimic responses of realistic amplitudes and durations in tail motor neurons. In the model the excitatory interneurons play an important role in transforming an amplitude-coded input into an amplitude- and duration-coded output that they deliver to the simulated tail motor neuron (see Byrne 1983; Frost et al. 1991; Trudeau and Castellucci 1992b for similar conclusions regarding the role of interneurons in the siphon-gill withdrawal reflex). In this way the model predicts that physiologically realistic modulation of synaptic connections between sensory neurons and LP117 neurons can exert a causal effect on both the magnitude and duration of the motor neuron response. In addition, plasticity at the synapse from LP117 units to motor neurons is effective in modulating the amplitude and duration of the modeled response. This modeled plasticity alters the properties of the amplitude-to-duration conversion and suggests the possibility that behavioral training can induce synaptic plasticity at two sites: synapses of the pleural sensory neurons and synapses of the LP117 neurons. Thus these simulations imply that memory in the circuit mediating the tail-withdrawal reflex may be spatially distributed among sensory and interneurons. A similar suggestion has been proposed for the circuit underlying the gill-siphon withdrawal reflex (Frost et al. 1988).

The results of this study, taken with those of the previous paper (Cleary and Byrne 1993), suggest that LP117 interneurons in *Aplysia* may serve a rather complex function, delivering differentially encoded sensory information to multiple sites within the CNS. Given that LP117 makes mutually excitatory connections with the abdominal ganglion interneuron 1.29 (Cleary and Byrne 1993), which may be involved in mediating long-duration responses of the siphon-withdrawal reflex (Frost et al. 1988, 1991), LP117 may also be part of a more general system of neurons controlling coordinated, long-duration withdrawal responses.

A principal advantage of the computational approach applied in this study is that it allows the examination of issues that are difficult or impossible to study experimentally. For example, these models allowed us to examine activity patterns in multiple neurons simultaneously. In addition, we were able to alter specific synaptic connections and examine the resulting effects. Thus, to the degree that our model captured with sufficient accuracy the properties of the sensory neuron-LP117-motor neuron circuit, we were able to explore the causal relationships between the properties of this neural circuit and its input-output characteristics.

Although simulation of a realistic range of motor neuron responses (and, consequently, behavioral responses) with the three-layer network model is encouraging, the results
FIG. 5. Synaptic plasticity at the interneuron-to-motor neuron synapse is effective in modulating the magnitude and duration of the motor neuron response. A: 3-layer network model. In these simulations, sensory neuron-to-LPI17 and sensory neuron-to-motor neuron connections (△) were held at control values; fast and slow LPI17-to-motor neuron connections (●) were modified. B: responses of 1 of the sensory neurons (SN1), 1 of the interneurons (LPI17), and the motor neuron with LPI17 induced conductance changes at 200% of control levels. C: number of spikes and response duration in the motor neuron as functions of Δg, which in this case represents the magnitudes of both fast and slow conductance changes evoked in the MN by LPI17. The fast, increased-conductance component of biphasic EPSP was scaled by changing the value $g_{max}$ in Eq. A6. The slow, decreased-conductance component was altered by changing the value of $\alpha_{DC}$ in Eq. A7.

Presented here must be interpreted with caution. For example, although the three-layer network can mimic long-duration responses to tail stimulation, an examination of the details of simulated and experimental responses to tail stimulation reveals subtle differences. Experimentally measured responses of tail motor neurons are characterized by an initial burst of action potentials, the firing rate of which gradually decreases to a steady-state level (Cleary and Byrne 1993). In contrast, the simulated responses of motor neurons presented here exhibit a rapid burst of action potentials followed by an extended response at a lower rate of firing, with a silent period of 0.5-2 s between the two bursts. This difference emphasizes the fact that other excitatory and inhibitory interneurons provide input to tail motor neurons and participate in the tail-withdrawal reflex (Buonomano et al. 1992; Cleary and Byrne 1993; Xu et al. 1991; J. L. Raymond, and J. H. Byrne, personal communication). A future goal will be to characterize these other cells and include them in the network model.

Assumptions of the network model

Issues that must be addressed in any modeling study are those of the validity and effects of the assumptions made in development of the model. There are two main assumptions of the model presented here that need to be verified experimentally. First, because the necessary experiment of simultaneous current clamp of an LPI17 interneuron and voltage clamp of a postsynaptic pedal motor neuron has not yet been performed, we were forced to design our model of this synaptic connection with the main goal of reproducing the current-clamp results shown in Fig. 2. Although the fit is good, a more thorough examination of the postsynaptic effects of LPI17 interneurons is merited, particularly at lower firing rates in presynaptic LPI17 neurons. This model will almost certainly be refined after voltage clamp analysis of the slow EPSP has been performed, but the precise values of details like the reversal potential of the slow EPSP have only small effects on the results of the simulations (data not shown).

A second assumption of the three-layer network is that all sensory neurons connect to the motor neuron and to both LPI17 interneurons and that both interneurons connect to the motor neuron. The detailed connectivity of this circuit has not yet been studied systematically. Nevertheless, there is some evidence to support this assumption. Convergent input from sensory neurons to motor neurons has been demonstrated (Buonomano and Byrne 1990, Walters and Byrne 1983, 1985; Walters et al. 1983a). In addition, multiple LPI17s have been identified in a single ganglion (Cleary
and Byrne 1993), and there is some evidence for convergent input to motor neurons (data not shown). Thus, although it is unlikely that sensory neurons, interneurons, and motor neurons are fully connected in vivo, there is experimental evidence for some degree of convergent synaptic connectivity.

Amplitude-to-duration conversion and distributed memory

Modification of reflex responses in Aplysia typically involves large changes in the duration of those responses (see, e.g., Byrne et al. 1991; Carew et al. 1981; Pinsker et al. 1973). Previous cellular models of plasticity, which emphasized the magnitude of the monosynaptic connection between sensory and motor neurons (Kandel and Schwartz 1982), were unlikely to account for these changes in response duration, given the short durations of sensory neuron responses to tail stimulation both before and after training (Walters et al. 1983a,b). Indeed, the network of L25 interneurons in the abdominal ganglion was proposed to transform changes in EPSP amplitude into changes in the duration of the siphon-gill withdrawal response (Byrne 1983). In that case the proposed mechanism of this amplitude-to-duration conversion involved the interactions of mutually excitatory interneurons. Similarly, a complex mechanism involving disinhibition of an excitatory interneuron (L29) and posttetanic potentiation at the slow synapse between L29 and the small siphon motor neurons (LFs) has been proposed to regulate the duration of siphon withdrawal (Frost et al. 1988). The circuit described here has an interneuron that evokes a slow EPSP as an essential element. Like interneurons in other circuits (Byrne 1980b, 1981; Frost et al. 1991; Lieb and Frost 1992), LP117 contributes to the amplitude-to-duration conversion directly without the necessity for long-lasting responses in the interneuron itself.

The proposed role of LP117 as a neuron that transforms an amplitude-coded input into an amplitude- and duration-coded output has interesting implications regarding the consequences of distributed plasticity in this neural circuit. Simple modulation of either the sensory neuron-to-LP117 connection or both components of the LP117-to-motor neuron connection gave similar effects. In both cases the magnitude and duration of the motor neuron response were increased by similar amounts (cf. Figs. 4C and 5C). Thus plasticity at these two synaptic sites can act to give the tail-withdrawal system redundancy and to extend its dynamic range (Fig. 6). Other types of plasticity could alter the properties of the evoked motor neuron response in different ways. For example, a selective alteration of the time constant of the slow EPSP would be expected to change the duration of the motor neuron response. Changes in the excitability of LP117, which could be accomplished by modulating a K+ conductance, might be expected to alter the characteristics of the amplitude-to-duration conversion in any of a number of ways. Moreover, selective changes in either the sensory neuron-to-LP117 connection or the sensory neuron-to-motor neuron connection could allow independent control of initial and sustained rates of firing in motor neurons. Such selective control of different aspects of reflex responses has been postulated in other reflex systems in Aplysia (Frost et al. 1988).

Studies of network circuitry in Aplysia

In recent studies of learning and memory in Aplysia, an increasing amount of emphasis has been placed on network properties rather than those of individual neurons. As men-
tioned above, several of these studies have highlighted the role of interneurons in the control of the duration of reflexive responses (Byrne 1983; Frost et al. 1988; Lieb and Frost 1992; Trudeau and Castellucci 1992b). In addition, studies have focused on sites of plasticity other than the monosynaptic connection between sensory and motor neurons (Fischer and Carew 1993; Frost et al. 1988; Lukowiak and Colebrook 1988–1989; Trudeau and Castellucci 1992a,b; Xu et al. 1992), the large number of neurons that may be involved in responses to stimulation of the skin (Zecvcevic et al. 1989), and the roles of interneurons in inhibiting withdrawal responses (Blazis et al. 1993; Buonomano et al. 1992; Fischer and Carew 1993; Hawkins et al. 1981; Mackey et al. 1987; Small et al. 1992; Xu et al. 1991). More generally, the abilities of small, physiologically based neural network models to simulate higher-order forms of learning have been assessed (Buonomano et al. 1992; Ray-
man et al. 1992). The physiologically based model presented here provides the opportunity to study the role of other excitatory interneurons as well as inhibitory inter-
eurons (Fischer and Carew 1993; Frost et al. 1988; Lukowiak

**APPENDIX**

In SNNAP (Simulator for Neural Networks and Action Poten-
tials) a network of \( n \) neurons is represented mathematically as a system of \( n \) coupled first-order, nonlinear ordinary differential equations. For neuron \( i \), the following differential equation was solved

\[
\frac{dV_i}{dt} = \frac{I_{\text{leak}}(V_i, t) + \sum_{k=1}^{m} I_{\text{ion}}(V_i, t) + \sum_{j=1}^{n} I_{\text{syn}}(V_i, t)}{C_{m(i)}} \quad (A1)
\]

In Eq. A1, \( V_i \) is the membrane potential of neuron \( i \). The numerator of the right side of Eq. A1 represents the total membrane current in cell \( i \) at time \( t \). Three classes of membrane current contribut to total membrane current: leakage current, current due to voltage-dependent ionic conductances, and current due to synaptic conductances. \( I_{\text{leak}}(i) \) is the leakage current. The currents due to \( m \) ionic conductances were summed at each time step. \( I_{\text{ion}}(ik) \) represents the current in neuron \( i \) due to ionic conductance \( k \). The currents driven by as many as \( n \) synaptic conductances were summed as well. \( I_{\text{syn}}(ik) \) represents the synaptic current in cell \( i \) due to the influence of presynaptic cell \( j \). Total membrane current was divided by the membrane capacitance \( C_{m(i)} \) of neuron \( i \). Each current \( I_x \) was obtained by solving the equation \( I_x = g_x(V_i - E_x) \), where \( g_x \) is the underlying conductance and \( E_x \) is the reversal potential associated with the conductance.

Voltage-dependent ionic conductances were represented with the use of a Hodgkin-Huxley type formulation. Each ionic conductance \( g_{\text{ion}}(ik) \) was evaluated by solving the equation

\[
\frac{dA_{\text{ion}}}{dt} = -\frac{A_{\text{ion}}(ik)}{\tau_{\text{ion}}(ik)} \quad (A2)
\]

where \( g_{\text{max}}(ik) \) is the maximal value of \( g_{\text{ion}}(ik) \), \( A_{\text{ion}} \) and \( B_{\text{ion}} \) are the respective activation and inactivation functions associated with \( g_{\text{ion}}(ik) \), and \( p \) is the power to which \( A_{\text{ion}} \) was raised. \( A_{\text{ion}} \) and \( B_{\text{ion}} \) were given by the solutions to the differential equations

\[
\frac{dA_{\text{ion}}}{dt} = \frac{A_{\text{ion}}(ik) - A_{\text{ion}}(ik)}{\tau_{\text{ion}}(ik)} \quad \frac{dB_{\text{ion}}}{dt} = \frac{B_{\text{ion}}(ik) - B_{\text{ion}}(ik)}{\tau_{\text{ion}}(ik)} \quad (A3)
\]

where \( \tau_{\text{ion}}(ik) \) and \( \tau_{\text{ion}}(ik) \) are the voltage-dependent, steady-state values of the activation and inactivation functions, respectively. \( \tau_{\text{ion}}(ik) \) and \( \tau_{\text{ion}}(ik) \) are the time constants of the activation and inactivation functions, respectively. The values of \( A_{\text{ion}}(ik) \), \( B_{\text{ion}}(ik) \), and \( g_{\text{syn}}(ik) \), and \( \tau_{\text{ion}}(ik) \), \( \tau_{\text{ion}}(ik) \), and \( \tau_{\text{ion}}(ik) \) were determined from the following equations

\[
A_{\text{ion}}(ik) = \frac{1}{1 + \exp \left( \frac{V_i - h_{A(ik)}}{s_{A(ik)}} \right)} \quad (A4a)
\]

\[
B_{\text{ion}}(ik) = \frac{1}{1 - B_{\text{min}}(ik)} \quad (A4b)
\]

\[
\tau_{\text{ion}}(ik) = \frac{\tau_{\text{ion}}(max)(ik) - \tau_{\text{ion}}(min)(ik)}{\left( 1 + \exp \left( \frac{V_i - h_{\text{ion}}(ik)}{s_{\text{ion}}(ik)} \right) \right)} \quad (A4c)
\]

\[
\tau_{\text{ion}}(ik) = \frac{\tau_{\text{ion}}(max)(ik) - \tau_{\text{ion}}(min)(ik)}{\left( 1 + \exp \left( \frac{V_i - h_{\text{ion}}(ik)}{s_{\text{ion}}(ik)} \right) \right)} \quad (A4d)
\]

Expressions of the symbols in Eqs. A4a–A4d are given with Table 1. A two-exponential model of activation \( t \) with \( n \exp = 2 \) in Eq. A4c was used to solve for \( \tau_{\text{ion}}(ik) \) in sensory neurons and \( \tau_{\text{ion}}(ik) \) in the motor neuron. In all other cases a single-exponential model, with \( n \exp = 1 \) in Eq. A4c, was used (see Table 1).

Synaptic activation functions were obtained by solving the second-order ordinary differential equation

\[
\frac{d^2A_{\text{syn}}(ij)}{dt^2} = \frac{2 \frac{dA_{\text{syn}}(ij)}{dt} - \tau_{\text{syn}(ij)} A_{\text{syn}}(ij) + X(t)}{\tau_{\text{syn}(ij)}^2} \quad (A5)
\]

where \( A_{\text{syn}}(ij) \) is the synaptic activation function evoked in postsynaptic neuron \( i \) by presynaptic neuron \( j \), \( \tau_{\text{syn}(ij)} \) is the time constant of the synaptic driving function, and the \( X(t) \) is the driving function of the differential equation. \( X(t) \) was set equal to one for the width of the presynaptic spike; \( X(t) \) equaled zero for all other times. For increased-conductance synapses, the synaptic conductance was given by the function

\[
r_{\text{syn}(ij)} = \frac{g_{\text{max}(ij)} - g_{\text{syn}(ij)}}{1 + \alpha_{\text{DC}} A_{\text{syn}}(ij)} \quad (A6)
\]

where \( \alpha_{\text{DC}} \) is a scaling factor. For decreased-conductance synapses, the synaptic conductance was given by

\[
r_{\text{syn}(ij)} = \frac{g_{\text{max}(ij)}}{1 + \alpha_{\text{DC}} A_{\text{syn}}(ij)} \quad (A7)
\]

In Eq. A7 the scaling factor \( \alpha_{\text{DC}} \) was set to a value of 100 to match physiological data [with \( g_{\text{max}(ij)} = 0.035 \mu S \)]. The value of \( \alpha_{\text{DC}} \) was altered to examine the effects of the magnitude of the slow synaptic-induced decrease in membrane conductance on the modeled response (Fig. 5C).

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