Activation and Reconfiguration of Fictive Feeding by the Octopamine-Containing Modulatory OC Interneurons in the Snail Lymnaea

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Received 31 August 2000; accepted in final form 1 May 2001

Vehovszky, Ágnes and Christopher J. H. Elliott. Activation and reconfiguration of fictive feeding by the octopamine-containing modulatory OC interneurons in the snail Lymnaea. J Neurophysiol 86: 792–808, 2001. We describe the role of the octopamine-containing OC interneurons in the buccal feeding system of Lymnaea stagnalis. OC neurons are swallowing phase interneurons receiving inhibitory inputs in the N1 and N2 phases, and excitatory inputs in the N3 phase of fictive feeding. Although the OC neurons do not always fire during feeding, the feeding rate is significantly (P < 0.001) higher when both SO and OC fire in each cycle than when only the SO fires. In 28% of silent preparations, a single stimulation of an OC interneuron evokes the feeding pattern. Repetitive stimulation of the OC interneuron increases the proportion of responsive preparations to 41%. The OC interneuron not only changes both the feeding rate and reconfigures the pattern. Depolarization of the OC interneurons increases the feeding rate and removes the B3 motor neuron from the firing sequence. Hyperpolarization slows it down (increasing the duration of N1 and N3 phases) and recruits the B3 motor neuron. OC interneurons form synaptic connections onto buccal motor neurons and interneurons but not onto the cerebral (cerebral giant cell) modulatory neurons. OC interneurons are electrically coupled to all N3 phase (B4, B4Cl, B8) feeding motor neurons. They form symmetrical connections with the N3p interneurons having dual electrical (excitatory) and chemical (inhibitory) components. OC interneurons evoke biphasic synaptic inputs on the protraction phase interneurons (SO, N1L, N1M), with a short inhibition followed by a longer lasting depolarization. N2d interneurons are hyperpolarized, while N2v interneurons are slowly depolarized and often fire a burst after OC stimulation. Most motor neurons also receive synaptic responses from the OC interneurons. Although OC and N3p interneurons are both swallowing phase interneurons, their synaptic contacts onto follower neurons are usually different (e.g., the B3 motor neurons are inhibited by OC, but excited by N3p interneurons). Repetitive stimulation of OC interneuron facilitates the excitatory component of the biphasic responses evoked on the SO, N1L, and N1M interneurons, but neither the N2 nor the N3 phase interneurons display a similar longer-lasting excitatory effect. OC interneurons are inhibited by all the buccal feeding interneurons, but excited by the serotonergic modulatory CGC neurons. We conclude that OC interneurons are a new kind of swallowing phase interneurons. Their connections with the buccal feeding interneurons can account for their modulatory effects on the feeding rhythm. As they contain octopamine, this is the first example in Lymnaea that monoaminergic modulation and reconfiguration are provided by an intrinsic member of the buccal feeding network.

INTRODUCTION

Rhythmic behavior patterns are produced by a system of central pattern generator (CPG) neurons, while modulatory neurons can contribute by changing the rate of the rhythm, its pattern, or the intensity of action potentials in the network (Katz and Harris-Warrick 1990). The modulatory neurons may be classified as extrinsic or intrinsic to the network (Cropper et al. 1987; Katz 1995; Katz and Frost 1996). Extrinsic modulatory cells receive little feedback from the network and can be situated in a different part of the nervous system and act on multiple targets, e.g., central neurons and peripheral muscles. Intrinsic modulatory neurons receive strong feedback from the CPG neurons and are usually rhythmically active themselves.

Snail feeding systems are productive models for the analysis of the ways in which rhythm patterns are generated and modulated (Benjamin and Elliott 1989; Kupfermann 1997). The pond snail, Lymnaea stagnalis feeds rhythmically using its radula, with three movements of approximately equal duration: protraction of the radula, rasping across the food, and then swallowing the food particles into the esophagus (Fig. 1A). Each feeding cycle takes about 3 s (Kemenes et al. 1986; Vehovszky et al. 1998). A similar rhythmic pattern, called fictive feeding, is produced by the isolated CNS, with rates of up to 20 cycles/min. Many of the neurons that produce this pattern are located in the buccal and cerebral ganglia (Fig. 1, B and C). The buccal motor neurons (B1, B2 ... B10), CPG interneurons (N1, N2, N3), and modulatory interneurons (SO, N1L) have been particularly well characterized (Benjamin and Rose 1979; Elliott and Benjamin 1985a,b; Rose and Benjamin 1979, 1981a,b; Yeoman et al. 1995). The three kinds of N-cells fire in turn, with the N1 interneurons firing in the protraction phase, the N2 during rasping (radula retraction), and the N3 during swallowing (Fig. 1B). Each of these three types of interneurons has been further subdivided into repeatedly recognizable classes, each with consistent anatomy and physiology, and their connections are well described (Fig. 1B) (see Brierley et al. 1997a,b; Elliott and Benjamin 1985a,b; Yeoman et al. 1995). Similar interneurons have been found in other gastropods, e.g., Aplysia (Hurwitz et al. 1994, 1997) and Helisoma (Quinlan and Murphy 1996), and their connections have been used to explain how the rhythm is produced (Brierley et al. 1997a,b; Elliott and Benjamin 1985a,b).

Although many isolated CNS preparations of Lymnaea show...
spontaneous fictive feeding, this can be enhanced in both strength and frequency by depolarizing a modulatory interneuron, SO, of which there is only one between the two buccal ganglia (Elliott and Benjamin 1985b). The SO can also initiate the feeding rhythm in a quiescent preparation, although there are statistically significant differences between the spontaneous and SO-driven patterns (Elliott and Andrew 1991). Similar activation of feeding can be achieved by depolarizing the paired N1L interneurons in the buccal ganglia (Yeoman et al. 1995), or the CV1 interneurons in the cerebral ganglia (McCrohan 1984; McCrohan and Kyriakides 1989). Another cerebral interneuron, the CBWC, has a weaker activating effect (McCrohan and Croll 1997), while the serotonergic cerebral giant cells (CGCs) modulate the rate and strength of the rhythm, but cannot normally initiate a pattern in a quiescent preparation (McCrohan and Audesirk 1987; Yeoman et al. 1994a,b, 1996).
The buccal ganglia of *Lymnaea stagnalis* have a high octopamine content (Hiripi et al. 1998), which was accounted for by just three octopamine immunoreactive neurons called the OC interneurons (Elekes et al. 1993, 1996; Vehovszky et al. 1998). OC interneurons display rhythmic activity pattern during both spontaneous and interneuron-driven fictive feeding. They fire in the third, swallowing (N3) phase of the feeding cycle and form synaptic connections with the feeding neurons of the buccal ganglia, suggesting that the OC interneurons are members of the feeding system (Vehovszky and Elliott 2000; Vehovszky et al. 1998). Their octopaminergic nature is confirmed by pharmacological experiments, as their synaptic connections to the B3 and N3p neurons are blocked by those octopamine antagonists that inhibited feeding in intact animals (Vehovszky et al. 1998, 2000).

Here we show that the OC interneurons modulate all the other interneurons and motor neurons of the buccal feeding network. We also report the diversity of monophasic, biphasic, and long-term effects of OC interneurons on the buccal feeding interneurons. These connections provide the mechanisms by which the OC interneurons reconfigure and modulate the activity of the buccal feeding network. The octopamine content and the octopaminergic output connections of the OC interneurons have already been established (Vehovszky et al. 1998, 2000). Thus this is the first example in *Lymnaea* when aminergic modulation comes from an intrinsic member of the feeding system.

**METHODS**

**Animals**

Adult pond snails (*Lymnaea stagnalis*) were obtained from a dealer (Blades Biological, Kent, UK) kept in standard snail water (Thomas et al. 1976) and fed on lettuce ad libitum.

**Experiments on the isolated CNS**

The CNS, including the buccal ganglia and a short length of esophagus, was isolated and the connective tissue digested for 5 min using a dilute (approximately 0.1%) solution of protease (Sigma type XIV) in standard *Lymnaea* saline (Table 1). Glass capillary electrodes were filled with potassium acetate and used to impale buccal neurons. The large cells were identified visually; the smaller ones by their activity pattern and synaptic relationships with the visually identifiable cells (Benjamin and Elliott 1989; Brierley et al. 1997a,b; Yeoman et al. 1995). The octopamine-containing OC interneurons described in detail here are on the dorsal surface of the buccal ganglia near the buccal commissure (Fig. 1) and are the only cells in this part of the buccal ganglia that have electrical connections with the B4 motor neurons (Vehovszky et al. 1998).

Experiments to identify buccal feeding neurons and to characterize the effects of the OC interneurons on the feeding pattern were performed in normal *Lymnaea* saline (Table 1). To characterize the individual connections the continuous perfusion of standard *Lymnaea* saline was switched to the saline with raised calcium and magnesium (Hi-Di saline, Table 1). The Hi-Di saline raises the action potential threshold (Berry and Pen-tracht 1976; Elliott and Benjamin 1989) and so reduces the spontaneous activity of neurons and the effect of polysynaptic pathways activated by intracellular stimulation of presynaptic neurons. To separate the electrical and chemical connections, a high Mg/Low Ca solution was used (Table 1), which blocks the chemical but not the electrical transmission between neurons (Elliott et al. 1992).

In the experiments with the CGCs, we checked that dissection had not overstretched the cerebro-buccal connectives. These nerves contain the axons of left and right CGCs, which meet in the buccal ganglia with mutually excitatory synapses with each other and form excitatory synaptic connections with the buccal B1 motor neurons (McCrohan and Benjamin 1980a,b). The synchronous firing of CGC neurons, their electrical coupling, and their excitatory effect to the buccal B1 neurons showed that the cerebro-buccal connectives were functional during the experiments.

**RESULTS**

**OC interneurons in the feeding rhythm**

OC NEURONS ARE ACTIVE IN THE SWALLOWING PHASE OF THE FEEDING CYCLE. The OC interneurons display the same pattern of synaptic inputs and firing activity as the other N3 phase neurons of the buccal feeding system (Fig. 2). The OC interneurons are inhibited in the first (N1, protraction) and second (N2, rasp) phases of the feeding pattern and receive excitatory inputs during the N3 (swallowing) phase of the feeding cycle like the B4 motor neurons (Fig. 2, A, C, and E) or N3p interneurons (Fig. 2B). After intracellular activation of the pattern-generating protraction phase SO (Fig. 2, A and C) or N1L (Fig. 2B) interneurons, the feeding pattern gradually builds up until the OC interneurons fire in bursts during the third, swallowing phase of the feeding cycle (Fig. 2, A–C). The intensity of OC firing is from 5 to 22 Hz, depending on the preparation (Fig. 2D) (Elliott and Vehovszky 2000), and varies only slightly within each preparation. The frequency of action potentials in the OC interneuron is approximately the same as that of the B4 swallowing phase motor neurons, (Fig. 2, A, C, and E). One-to-one firing of action potentials is not usually seen, although during the N3 phase, an OC interneuron may show small excitatory postsynaptic potentials (EPSPs) that correspond to the action potentials in the B4 motor neuron (Fig. 2C, small arrows, cf. Fig. 8D) (see also Fig. 1 of Vehovszky and Elliott 2000).

We observed OC firing in 35 of 90 preparations (39%) in which fictive feeding was produced by stimulating the SO interneuron. Detailed analysis of five preparations, in which the OC contributed to some cycles but not in others, showed that the feeding rate is significantly higher (12.8 ± 0.7 cycles/min against 7.2 ± 0.54 cycles/min, mean ± SE, P < 0.001, pairwise measurements on 5 preparations; Fig. 2D), when both the SO and OC neurons are firing in fast bursts (SO in the protraction phase, OC in the swallowing phase). This suggests that the firing activity of OC interneurons contributes to the maintenance of a fast rate of feeding.

**TABLE 1. Composition of the *Lymnaea* salines**

<table>
<thead>
<tr>
<th></th>
<th>Normal Saline, mM</th>
<th>Hi-Di Saline, mM</th>
<th>High Mg/Low Ca Saline, mM</th>
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<tbody>
<tr>
<td>NaCl</td>
<td>24</td>
<td>24</td>
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<tr>
<td>KCl</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>CaCl₂ · 2H₂O</td>
<td>4</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>MgCl₂ · 6H₂O</td>
<td>2</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>NaH₂PO₄ · 2H₂O</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>NaOH</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>HEPES</td>
<td>50</td>
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For all salines, pH 7.9.
OC NEURON STIMULATION EVOKES FICTIVE FEEDING. Intracellular stimulation of OC neurons may also evoke fictive feeding in silent preparations. In 23 of 82 (28%) preparations, a single short depolarizing pulse to the OC interneuron activates fictive feeding (Fig. 2E). As we can only impale one of the three OC interneurons, we applied a relatively strong (25–35 Hz) burst for 2 s to the OC interneuron. This depolarizes the SO interneuron, and as the SO starts to fire faster, both the B4 neuron and the OC interneuron are recruited to the fictive feeding pattern: strong N2 (rasp) inputs occur, as shown by the rapid, synchronized hyperpolarization of the B4 motor neuron and OC interneuron (marked by asterisks on Fig. 2E). On those
preparations when a single pulse to the OC fails to activate feeding pattern, repeated current pulses injected into the OC neurons are more likely to evoke the fictive feeding pattern (12 preparations of 29 tested, 41%, Fig. 2F). A series of 2-s stimuli, separated by 1-s intervals, was applied to the OC interneuron for 10–15 s. Such a pattern of stimulation mimics the rhythm of feeding and has previously been shown to be more effective than tonic stimulation of the OC (Elliott and Vehovszky 2000). This pulsed stimulation gradually leads to the feeding pattern in both motor neurons and interneurons (B1 and N1M in Fig. 2F) and, after four cycles, recruits further OC activity as well. Again, the rhythmic pattern occurs after and lasts longer that the actual duration of the OC stimuli (Fig. 2F).

The activation of feeding neurons, moreover, does not require continuous firing of the OC interneuron, as the depolarization and tonic firing activity on SO and B4 neurons (Fig. 2E) or B1 and N1M neurons (Fig. 2F) is maintained after the end of OC stimulation.

OC ACTIVITY RECONFIGURES THE FEEDING PATTERN. We demonstrated reconfiguration of the network by injecting either depolarizing or hyperpolarizing currents tonically into the OC interneuron, while driving fictive feeding with steady current injection into the SO. When the OC is depolarized (Fig. 3A), the feeding pattern is changed by the removal of the bursting activity in the B3 motor neurons, which project in the dorso- and latero-buccal nerves, and are thought to modulate the buccal glands (Benjamin et al. 1979; Carriker 1946). In the hyperpolarizing experiment, the removal of the OC was correlated with a depolarization of the B3 motor neuron and its recruitment into the feeding rhythm (Fig. 3B1, section b).

The hyperpolarization experiments confirm that the OC interneuron also contributes to the control of the feeding rate (Fig. 3B2, cf. Fig. 2D2). Injecting negative current into one OC during the SO-driven fictive feeding reduces the feeding rate from 20.6 ± 0.75 cycles/min to 15.8 ± 0.62 cycles/min (n = 20, P < 0.001, Fig. 3B2), but the initial feeding rate does not recover within the next few cycles.

The decreased feeding rate during hyperpolarization of OC is caused by prolongation of both the N1 (protraction) and N3 (swallowing) phases of the feeding cycle (N1 = 0.84 ± 0.07 s, and N3 = 1.41 ± 0.13 s when both SO and OC are active, N1 = 1.45 ± 0.17 s, and N3 = 2.0 ± 0.25 s when OC hyperpolarized, P < 0.005 for both N1 and N3 data, n = 11). However, the duration of the N2 phases does not show significant differences whether OC neurons are firing or not (0.82 ± 0.05 s, and 0.91 ± 0.05 s, respectively, n = 11, Fig. 3B3).

Thus the effect of changing the input from the OC is not just to change the feeding rate, but to alter the intensity of the activity of the feeding motor neurons and the time spent in the protraction (N1) and swallowing (N3) phases of the feeding pattern.

Connections from the OC interneurons to the buccal feeding neurons

To account for the firing pattern of the OC neurons, their ability to stimulate feeding rhythm, and the reconfiguration of the firing pattern, we have examined the connections from the OC interneurons to the other buccal feeding neurons in detail. The connections reported below were all monitored in Hi-Di saline, which elevates the firing threshold of the neurons and reduces the spontaneous activity and the polysynaptic effects evoked by presynaptic (OC) stimulation (see METHODS). Therefore our results suggest direct connections between OC and its followers.

SYMmetrical dual connections between OC and N3p interneurons, both containing electrical (excitatory) and chemical (inhibitory) components. The N3p interneurons are, like the OC interneurons themselves, a type of interneuron that receives excitatory inputs or fires in the swallowing phase (Elliott and Benjamin 1985a). The N3p and OC interneurons are electrically coupled to each other with symmetrical connections (n = 36, Fig. 4, A and B). When short (0.5–1 s) negative current is injected into either of these interneurons, the other cell is hyperpolarized, while with depolarizing currents, a symmetrical depolarization, accompanied by electrically transmitted action potentials is seen in the other neuron (Fig. 4, A1 and B1). The connection is, however, very rarely strong enough to evoke 1:1 spikes, unless both cells receive a simultaneous depolarizing input. Furthermore, this is in fact a dual connection with the electrical connection supplemented by a slower chemical component. After suprathreshold OC stimulation, the N3p returns to rest slowly from a more negative potential than it began, indicating an additional hyperpolarizing effect after the electrical response to OC stimulation (n = 35, Fig. 4A2). This is most obvious in the Hi-Di solution in which both the electrical (excitatory) and chemical (inhibitory) components of the responses are clearly visible (Fig. 4, A2 and A3). Similarly, N3p stimulation evokes a complex response on OC interneurons (n = 34, Fig. 4B) when the electrical response is combined with a hyperpolarization of the OC interneuron. In both OC → N3 and N3 → OC directions, 1:1 electrical PSPs can be seen (Fig. 4, A3 and B3), while individual chemical PSPs cannot be resolved. The relative size of the two components (chemical and electrical) of the OC 171 N3p connection depends on the membrane potential level of the target neuron. When the N3p interneuron is deeply hyperpolarized (below −85 mV), the OC neuron produces mainly a depolarizing effect (due to the electrical coupling, Fig. 4C1). As the postsynaptic N3p cell is depolarized, the inhibitory (chemical) component of the OC → N3p connection becomes dominant over the excitatory (electrical) response (Fig. 4, C2 and C3). This change in the relative importance of the electrical and chemical components also happens when the N3p interneuron is stimulated and the OC interneuron membrane potential changes (not shown).

In a high magnesium–low calcium saline, which blocks chemical synapses (Elliott et al. 1992, Table 1) the inhibitory component of the response is abolished, but the excitatory component persists (Fig. 4D2). On return to Hi-Di, this is restored, showing that only the excitatory component is electrical (Fig. 4D3).

OC INTERNEURONS FORM BIPHASIC (INHIBITORY FOLLOWED BY EXCITATORY) CHEMICAL CONNECTIONS WITH PROTRACTION PHASE FEEDING INTERNEURONS. When an OC interneuron is stimulated, a biphasic synaptic input (hyperpolarization followed by slower excitatory response) is seen in the protraction phase SO, N1L, N1M interneurons (SO, n = 19; N1L, n = 13; N1M, n = 12; Fig. 5, A–C). This second, depolarizing component will elicit action potentials if the starting membrane potential is
close to the threshold of action potential of the SO or N1L postsynaptic cells (Fig. 5A2). When the membrane potential of the postsynaptic interneuron lies between \(-70\) and \(-60\) mV, the small initial hyperpolarization is not always visible, while the following slow depolarization is more obvious (Fig. 5, A1, B1, and C1). However, when the protraction phase interneuron is already firing, the inhibitory component dominates, which stops firing the follower interneuron (Fig. 5, B2 and C2). As soon as the OC stimulation (OC burst) is over, the follower cell resumes its firing activity (Fig. 5, B2 and C2).

**OC NEURONS FORM EXCITATORY CONNECTIONS WITH THE VENTRAL N2 INTERNEURONS BUT INHIBIT THE DORSAL N2 INTERNEURONS.** The OC interneuron excites some types of N2 interneurons, namely the N2v interneurons located on the ventral surface of the buccal ganglia (Brierley et al. 1997a,b). OC stimulation is followed by rapid bursts of the N2v neurons, with short latency and rapid decay (Fig. 6A1). After long perfusion in Hi-Di saline, however, when the threshold of action potential generation is higher, and the frequency of the action potentials in the OC bursts is decreased, the synaptically evoked depolarization is clearly separated from the endogenous bursts triggered on N2v interneurons (Fig. 6A2).

The N2d interneurons are located on the dorsal surface. Unlike the ventral N2 interneurons, the N2d interneurons receive hyperpolarizing inhibitory inputs after intracellular stimulation of an OC interneuron (n = 10), occasionally followed by a short depolarization or burst in the N2d neurons (arrows, Fig. 6B1). This excitatory component does not have a constant latency, even in...
normal saline. Furthermore, this second, excitatory component of the N2 response disappears in Hi-Di saline, while the amplitude of the initial hyperpolarization is increased (Fig. 6B2). This suggests that the inhibitory component is likely to be monosynaptic while the occasional short (not longer than 1 s) bursting depolarizations have polysynaptic origin.

**OC INTERNEURONS HAVE DUAL CHEMICAL AND ELECTRICAL EXCITATION WITH THE N3t INTERNEURONS.** The connection of the OC interneurons with the tonically firing N3t interneurons has also been examined in five preparations. Injecting currents of either polarity into the OC interneuron produces a small response on the N3t interneuron with the same sign and duration, suggesting an electrical connection (Fig. 6C1). When suprathreshold currents were applied to the OC, a much larger response is seen in the N3t interneuron, complete with action potentials (Fig. 6C2). The increased response suggests that there is also a chemical excitatory connection from the OC → N3t interneuron.

**SYNAPTIC CONNECTION OF OC INTERNEURONS WITH THE FEEDING MOTOR NEURONS.** The synaptic inputs evoked by OC neurons on most motor neurons correspond to the synaptic responses of the interneurons with which they are simultaneously active during feeding.

OC interneurons have electrical connections with all (B4, B4cluster, B8) motor neurons that fire in the same phase of the fictive feeding (Fig. 7A), although the coupling is the strongest between OC interneurons and B4 motor neurons (Fig. 7A1). Again, the electrical response between an OC and these follower neurons is very rarely large enough to evoke full sized action potentials. Moreover (contrary to the OC → N3p connections), no sign of a chemical connection can be seen on these motor neurons after OC stimulation (Figs. 7A and 8D).

**FIG. 4.** Dual (electrical and chemical) connection between OC interneurons and N3p interneurons. A: after stimulating the OC interneuron in Hi-Di saline with short (up to 1 s) hyperpolarizing or depolarizing current the N3p neuron responds by a corresponding (hyperpolarizing or depolarizing) membrane change due to their electrical coupling (A1), while longer (3 s) OC stimulation evokes a mostly hyperpolarizing response (A2). The 1st section of OC stimulation (recording with higher speed) reveals the 1st component, electrically transmitted postsynaptic potentials in the N3p response (A3). B: stimulation of N3p interneuron in Hi-Di saline also evokes electrical responses on OC neuron (B1). When N3p is stimulated longer, a deep hyperpolarization of OC appears (B2) with electrically transmitted potentials at the beginning of the stimulus (B3). C: the contribution of the excitatory (electrical) and inhibitory (chemical) responses depends on the membrane potential of the follower; at more negative membrane potentials OC stimulation evokes mainly the excitatory (electrical) component (C1), while depolarizing the N3p membrane (C2 and C3) OC mainly evokes hyperpolarization. All 3 parts in Hi-Di saline. D: the inhibitory part of the synaptic responses of N3p interneuron seen in Hi-Di saline (D1) are simplified to a simple electrical response in the low calcium saline applied in D2, while the chemical input is restored in Hi-Di saline (D3).

**FIG. 5.** Biphasic synaptic responses evoked in protraction phase interneurons by OC interneurons. A: OC stimulation evokes a biphasic response, hyperpolarization followed by depolarization (A1), which may evoke action potentials (A2). B: OC stimulation evokes a slow depolarization on N1L interneuron when it is silent (B1), and inhibits the N1L activity when N1L is firing (B2). C: when the N1M interneuron is hyperpolarized it receives a depolarizing input after OC stimulation (C1), but when the N1M interneuron is already firing, OC stimulation strongly inhibits the N1M (C2). Membrane potential values of the followers before OC stimulation are indicated at the beginning of each record. All these experiments in Hi-Di saline.
citatory (depolarizing) inputs after OC stimulation (Fig. 7, B1 and B2), while B5 and B7 motor neurons show a biphasic response, as an initial hyperpolarization is followed by a short depolarization (Fig. 7, C1 and C2).

The B2, B3, and B10 motor neurons principally fire in the N2 (rasp) phase of the feeding cycle, and they are all inhibited by OC stimulation, which is strong enough to stop the motor neurons from firing (Fig. 7D1). The B3 motor neurons display the largest (up to 17 mV) and longer lasting (up to 22 s) inhibitory postsynaptic inputs after OC stimulation (Fig. 7D2). The pharmacological analysis of these connections show that octopamine is the transmitter (Vehovszky et al. 2000).

POSTSYNAPTIC EFFECTS OF OC STIMULATION IS DISSIMILAR TO STIMULATION OF THE ELECTRICALLY COUPLED N3p INTERNEURON.

Both OC and N3p neurons are excited or fire in the swallowing phase of feeding and receive simultaneous inhibitory inputs during N1 and N3 phases (Fig. 2B) (c.f. Elliott and Benjamin 1985a), which indicates that both the OC interneurons and the N3p interneurons are included in the N3 (swallowing phase) interneurons. However, they still may have separate roles in

FIG. 7. Synaptic connection of OC neuron with feeding motor neurons. A: all 3 kinds of feeding motor neurons that fire principally in the N3 phase are electrically coupled to the OC neurons as shown by the responses of B4 (A1) or the or B4 cluster (B4Cl) or B8 motor neurons (A2) after injecting either depolarizing or hyperpolarizing current into OC. B: protraction phase B1 (B1) and B6 (B2) motor neurons are slowly depolarized after OC stimulation. C: burst of OC interneurons evokes biphasic response (hyperpolarization followed by depolarization) on both B5 (C1) and B7 (C2) motor neurons. D: OC stimulation evokes inhibitory responses in retraction (N2) phase motor neurons. OC stimulation inhibits B2 activity (D1), and evokes hyperpolarization on B3 (D2), or B10 motor neuron (D3). All these experiments in Hi-Di saline.
feeding, if their connections to other members of the feeding network are different. Therefore, in the next series of experiments, we compared the effects of either OC or N3p interneurons on their followers recording simultaneously from both OC and N3p interneurons together with other buccal neurons.

The only case where the OC and N3p interneurons have the same effect is their inhibition of the B2 motor neuron (Figs. 7D1 and 8, A1 and A2). All the other feeding motor neurons are affected differently by stimulating the OC or the N3p interneurons (Fig. 8, B–E). Most striking is the fact that OC and N3p interneurons have opposite effects on the B3 motor neurons. These are hyperpolarized by OC stimulation (Figs. 7D2 and 8D1) but, after stimulation of N3p interneurons, the B3 motor neuron is so strongly depolarized that it exhibits a burst of action potentials (Fig. 8B2). B5 motor neurons also receive different synaptic inputs from OC and N3p neurons, as the OC → B5 connection is biphasic (Figs. 7C1 and 8C1), while stimulation of N3p evokes a longer lasting hyperpolarization, without any excitatory component (Fig. 8C2). Similarly, the OC → B4 and N3p → B4 connections are quite different. The OC → B4 interaction is a simple electrical connection (Fig. 8D). In contrast, the N3p → B4 connection is predominantly an inhibitory chemical one with an initial electrical component (Fig. 8E).

The N3p and OC interneurons also make different connections to the feeding interneurons. Retraction phase N2d interneurons receive inhibitory inputs from OC interneurons (Fig. 6B), but no inhibitory responses of N2d neurons were recorded after N3p stimulation.

REPEITIVE STIMULATION OF OC FACILITATES THE EXCITATORY COMPONENT OF THE BIPHASIC SYNAPTIC INPUTS ON PROTRACTION PHASE INTERNEURONS BUT NOT THE DEPOLARIZATION OF N2V NEURONS. As we have already demonstrated (Fig. 2F), repetitive OC stimulation is more effective at evoking fictive feeding than a single stimulus. One reason for this may be that the biphasic postsynaptic effects received on protraction phase feeding interneurons after OC stimulation (Fig. 5) undergo homosynaptic facilitation (Figs. 9 and 10).

After repeated stimulation of OC interneurons, the size of the excitatory (depolarizing) component of the biphasic neuronal response increases on the protraction phase interneurons, N1L, SO, and N1M (Fig. 9). When the OC is stimulated with series of bursts separated by 5- to 7-s intervals, the first inhibitory component gets shorter, but both the amplitude and the time duration of the second, excitatory component increases, even though the follower membrane potential has returned to the same value in between (Fig. 9, A and B). The enhancement of the excitatory component of the response leads to spikes in the protraction phase interneurons (SO in Fig. 9B). When the repeated stimulating pulses injected to OC are more frequent, the facilitation of the excitatory component gradually shifts the membrane potential to a more depolarized level, which lasts even after the OC injecting pulses are switched off (Fig. 9C). This facilitation of the excitatory component is

**FIG. 8.** Differential effects of OC and N3p interneurons on feeding motor neurons. A: stimulating either OC (A1) or N3p (A2) interneuron evokes hyperpolarization on B2 motor neuron. B: a burst in the OC interneuron hyperpolarizes the B3 motor neuron (B1), but N3p stimulation evokes a large depolarization, which triggers action potentials (B2). C: OC neuron evokes biphasic response, hyperpolarization followed by depolarization and spike generation on B5 motor neuron (C1), while stimulating N3p interneuron evokes a single hyperpolarization on B5 motor neuron (C2). D: OC burst is followed by only electrical response on B4 motor neuron (D1), as the higher speed record shows single (electrical) excitatory potentials on B4 motor neuron (D2). E: N3p interneuron stimulation evokes deep hyperpolarization on B4 motor neuron (E1) with short (electrical) excitatory synaptic potentials at the beginning of the response (E2). All these experiments in Hi-Di saline. Note that in A–C the OC and N3p neurons are simultaneously recorded, but only the current injected neuron (OC on A1, B1, and C1, and N3p on A2, B2, and C2) generates burst of action potentials, not its electrically coupled partner.
Specific to the N1 phase interneurons, as the (also excitatory) OC → N2v connection does not show long-lasting increase during or after OC stimulation (Fig. 9C).

Facilitation of the excitatory component in the synaptic connections between the OC neurons and the protractor phase interneurons finally may lead to full fictive feeding, when tonic stimulation for the same period does not evoke feeding pattern (Fig. 10A). An example is shown in Fig. 10, where the OC is recorded simultaneously with the SO and N1L premotor interneurons. During a long-lasting (21 s) single stimulus to the OC, a slight (1.8 mV) depolarization on SO and a weak (4.4 mV) hyperpolarization on the N1L is recorded, but both neurons stay slightly depolarized after the injecting current to OC was switched off (Fig. 10A). With repeated OC stimulation that lasts almost the same duration (20.6 s), however, both SO and N1L interneurons show alternating hyperpolarizing and depolarizing membrane responses (Fig. 10, B and C), due to the biphasic nature of the synaptic inputs they receive from OC interneurons (Fig. 5, A and B). The amplitude of the second (depolarizing) component of the synaptic response gradually increases on SO interneuron, so finally the cell starts firing (Fig. 10, B and C). The N1L interneuron (which is electrically coupled to the SO interneuron) (Yeoman et al. 1995) also fires in the same pattern, and the number of spikes in the depolarizing phases increases with each stimulus. Eventually, the full feeding activity is initiated, with strong N2 inputs seen on the SO and N1L records (Fig. 10B, asterisks), which lasts after the depolarizing current on OC interneuron is switched off. The membrane of the protraction phase interneurons (N1M neuron on Figs. 2F and 9C, SO on Fig. 10C) is also gradually depolarizing during and after the OC stimulating pulses; OC activity therefore increases the chance of protraction phase interneurons to evoke fictive feeding.

It should be noted that the OC neuron itself does not have to be strongly bursting during the whole evoked feeding activity evoked by OC stimulation (Figs. 2F and 10B).

Synaptic inputs received by OC interneurons from buccal feeding interneurons

The OC interneurons receive inhibitory inputs from most of the buccal interneurons. Stimulation of either an SO or N1L interneuron inhibits OC activity (14 of 19 on SO, 10 of 13 on N1L, Fig. 11, A and B), but single inhibitory postsynaptic potentials (IPSPs) are only occasionally seen on the OC records after each SO or N1L presynaptic action potential (not shown). Although OC neurons receive inhibitory inputs when N1M interneurons fire in N1 phase of feeding, intracellular stimulation of N1M neurons usually has much less effect on OC activity; with inhibitory response in only about one-half of the Hi-Di experiments (3 of 7, Fig. 11C) and single IPSPs are not visible.

After stimulation of the N2d interneurons, the inputs on the OC interneurons are rather variable; inhibitory inputs were recorded only on one-half (5 of 10) of the preparations after N2d stimulation. Because of the wide range of latencies between the N2d stimulation and OC response even on the same preparation (Fig. 11, D1 and D2), direct connections from N2d interneurons to OC interneurons are not likely.

In the case of the N2v → OC connections, however, N2v interneurons reliably evoked typical N2 inputs on OC neurons, which are biphasic; a short inhibition is followed by a depolarizing response (8 of 9, Fig. 11E).

The electrical coupling between OC interneurons and N3t interneurons means that either hyperpolarizing or depolarizing N3t interneurons with short pulses should evoke a weak electrical response on the OC interneuron (Fig. 11F). However,

FIG. 9. Repetitive OC stimulation selectively facilitates the excitatory component of the biphasic response on N1 phase interneurons. A: during a series of bursts of OC the excitatory (depolarizing) component of the N1L biphasic response increases, although the parameters of the individual OC bursts (2 s duration, 34-, 36-, and 37-Hz action potentials frequencies) are nearly the same. B: repeating OC bursts (2 s duration, 45, 42, and 40 Hz, respectively) decreases the duration of the inhibitory component of the SO response while increasing the amplitude of the excitatory component, which triggers a burst of small spikes on SO interneuron. C: the facilitation of the synaptic responses is specific to the protraction phase followers. Each of the OC stimulating pulses evokes depolarizing responses on N1M interneuron (top trace) and triggers bursts on N2v interneuron (middle trace). After the OC stimulation, the N1M interneuron and OC itself stay more depolarized than the N2v interneuron. The initial membrane potential level of each neuron before OC stimulation is marked by dotted line; the numbers above the traces indicate the membrane potential shift after OC stimulation. All these experiments in normal saline.
chemically transmitted synaptic response (Fig. 4). Neurons, the former being electrical, while the second is a biphasic (excitatory/inhibitory) synaptic connection with OC asymmetrical, as the N3t connection between the OC and N3t neurons seems to be rather (Fig. 11) than the OC F only a very small effect on the OC (Fig. 11) stimulating the N3t even with longer depolarizing pulses has more depolarized (30 mV) the 1st inhibitory phase of the synaptic responses N1L interneuron the number of action potentials increases. As N1L initially is repetitive OC bursts, the SO interneuron is gradually depolarized, while on the SO and N1L. pattern began, as shown by the strong N2 phase inputs (asterisks) seen in the generation on N1L neuron. After the OC pulses are switched off, the feeding A series of bursts of OC interneuron evokes short hyperpolarizing inputs evokes weak depolarization on SO and hyperpolarization on N1L interneuron. At the end of the OC stimulus, both followers are slightly depolarized (the initial membrane potential level is marked by dotted lines on SO and N1L records). Note that the N1L interneuron has a more positive resting membrane potential level before OC stimulation (numbers above the SO and N1L traces). B: a series of bursts of OC interneuron evokes short hyperpolarizing inputs followed by increasing depolarizing responses on SO and action potential generation on N1L neuron. After the OC pulses are switched off, the feeding pattern began, as shown by the strong N2 phase inputs (asterisks) seen in the SO and N1L: C: part of the record on an expanded scale, showing that, during repetitive OC bursts, the SO interneuron is gradually depolarized, while on the N1L interneuron the number of action potentials increases. As N1L initially is more depolarized (30 mV) the 1st inhibitory phase of the synaptic responses is more visible than on the SO (its membrane potential is −50 mV). All these experiments in normal saline.

stimulating the N3t even with longer depolarizing pulses has only a very small effect on the OC (Fig. 11F2). Thus the connection between the OC and N3t neurons seems to be rather asymmetrical, as the N3t → OC connection is much weaker (Fig. 11F) than the OC → N3t excitatory input (Fig. 6C2).

As we mentioned earlier, N3p interneurons form complex, biphasic (excitatory/inhibitory) synaptic connection with OC neurons, the former being electrical, while the second is a chemically transmitted synaptic response (Fig. 4).

Connection between the OC interneurons and CGCs
We have also looked for connections from the OC interneurons to the serotonergic CGCs, which are feeding interneurons in the cerebral ganglia. The CGCs have no response to either single pulse (Fig. 12A) or repetitive OC stimulation (Fig. 12B) in any of the experiments (neither in normal solution nor in Hi-Di saline, n = 10), even though other buccal neurons (B3 motor neuron, Fig. 12A; B4 motor neuron, Fig. 12B) show their normal responses (OC stimulation, Figs. 7D2 and 8, B1 and D1).

The CGC cells (on the same preparations), however, evoke excitatory responses on OC neurons as well as on feeding motor neurons (Fig. 12, C and D, n = 12), at which the connections are already known (McCrohan and Benjamin 1980b). This confirms that the failure of OC → CGC response is not caused by the lack of connections between the buccal and cerebral ganglia. The spontaneous spikes recorded on CGC are often followed by clearly visible 1:1 excitatory potentials on OC interneurons (Fig. 12E), suggesting that CGC neurons have direct connection with the OC interneurons.

DISCUSSION
The data in this paper show that the octopamine immuno-reactive OC interneurons can stimulate (Fig. 2) and reconfigure (Fig. 3) the Lymnaea feeding system. The OC interneurons also connect to the feeding interneurons and motor neurons (Figs. 4–9, 12, and 13). Here we review the connections and show that they can explain the ability of the OC interneuron to modulate the feeding pattern.

Overview of the synaptic connections formed by OC interneurons
OC interneurons form a wide variety of connections with buccal feeding neurons, which include electrical, chemical, and mixed synapses (Fig. 13). All responses have approximately the same (200–400 ms) latency but different amplitudes and durations (Fig. 13, B and C). Although we did not record single chemical postsynaptic potentials on follower neurons after OC stimulation, all the OC output connections described here persist after 30–60 min of Hi-Di solution, which reduces the effect of polysynaptic connections activated by presynaptic OC stimulation (Berry and Pentreath 1976; Elliott and Benjamin 1989) (see METHODS). Moreover, the OC output effects could not be explained by an interaction passing through any previously known feeding interneuron, as the pattern of inputs recorded in the buccal neurons following OC stimulation is different from that seen when stimulating any one of the N1, N2, or N3 interneurons (Brierley et al. 1997a,b; Elliott and Benjamin 1985a,b; Rose and Benjamin 1981a,b; Vehovszky and Elliott 1995; Yeoman et al. 1995) or OM mechanoreceptor neurons (Elliott and Benjamin 1989). The latency of OC responses, however, is much longer than that of cholinergic chemical postsynaptic potentials in the Lymnaea buccal ganglia (Elliott and Benjamin 1985b; Elliott and Kememes 1992; Vehovszky and Elliott 1995), and this suggests that the OC interneurons may use second-messenger pathways for their effects. Octopaminergic modulation coupled to adenylate cyclase is common in insects (Bounias 1987; Chyb et al. 1999; Nathanson and Greengard 1973; Orchard et al. 1983; Robb et
al. 1994), and some reports suggest that a similar mechanism may be present in molluscan systems (Capasso et al. 1991; Chang et al. 2000; Gerhardt et al. 1997).

The strongest, short-term excitatory connection formed by the OC interneurons is electrical coupling with the N3 phase neurons (B4, B4cl, B8 motor neurons, N3p interneurons, Fig. 13A, traces 1 and 4). The electrical coupling of N3t interneurons and OC neurons is much weaker, but the N3t interneurons receive an additional, rather strong excitatory effect from the OC interneurons (Figs. 6C2 and 13, trace 2). The other excitatory output is the chemically transmitted depolarizing effect on the N2v interneurons, which often triggers a short, quickly terminating burst in the N2v interneuron (Fig. 13A, trace 2), due to its endogenous plateauing property (Brierley et al. 1997a).

On the N3p interneurons, the short (electrical) excitatory inputs are followed by a longer lasting (chemical) inhibitory effect (Fig. 13A, trace 4). These OC 171 N3p connections are symmetrical, as OC interneurons receive similar biphasic (electrical excitatory/chemical inhibitory) input from N3p interneurons (Fig. 4). The synaptic response recorded on protraction phase (SO, N1L, N1M) interneurons is a short inhibitory response followed by a second, longer-lasting excitatory component (Fig. 13A, trace 3). Both responses are chemical ones. The N2d phase interneurons receive a short latency but rather long-lasting hyperpolarization from OC interneurons (Fig. 13A, trace 5).

The synaptic inputs that the motor neurons receive from the OC interneurons are generally similar to those received by the interneurons that fire in the same phase of feeding cycle. For example, N3 phase (B4, B4cl, and B8) motor neurons like N3p interneurons are electrically coupled to OC interneurons, while N2 phase (B2, B3, B10) motor neurons, like N2d interneurons receive hyperpolarizing inputs. The N1 phase B7 motor neurons have similar biphasic (inhibitory than excitatory) synaptic inputs to the SO, N1L, and N1M interneurons. The exceptions are the B1 and B6 protraction phase motor neurons, which receive only excitatory inputs without any inhibitory component.

Although OC interneurons receive excitation from the cerebral CGC neurons, a reverse connection was not found. This confirms the previous suggestion based on morphological results (Vehovszky et al. 1998) that the OC interneurons are local interneurons of the buccal feeding system.

Biphasic outputs of OC interneurons provide activity-dependent effects on the feeding network

A notable characteristic of the OC outputs is that the response is often biphasic (Fig. 13, A and B). This may be achieved by a combination of chemical synapses (inhibition followed by excitation), which is seen on both motor neurons (B5, B7) and interneurons (SO, N1L, N1M) or by the dual (excitatory) electrical then (inhibitory) chemical connection onto the N3p interneurons. This means that the functional consequence of spikes in the OC interneuron depends on the membrane potential of the follower cell. In other words, the OC interneurons will have an activity-dependent effect on the feeding network. First, when the whole feeding system is quiet, the membrane potentials of the follower cells will be near the resting value and any inhibitory inputs produced by stimulating the OC interneurons will be small. Thus the dom-

![Diagram](http://jn.physiology.org/content/86/2/803/F1)

**FIG. 11.** OC interneurons receive inhibitory inputs from the other feeding interneurons. A: strong burst in SO evokes hyperpolarization on OC interneuron, which inhibits its activity. B: N1L interneuron evokes strong hyperpolarization of OC interneuron. C: long (4 s) burst of N1M interneuron is followed by a 2-component inhibitory response on OC interneuron. D: N2d (dorsal) interneuron stimulation evokes biphasic response (inhibition followed by membrane depolarization) on OC neuron, but the latency of the response varies when N2v is stimulated repetitively (1.8 s in D1, and 0.7 s in D2). E: N2v (ventral) interneuron evokes biphasic response of OC with 0.25-s response latency. Note the short burst of N2d after the stimulating current is switched off. Dotted lines under the records in D and E mark the initial membrane potential levels of OC interneuron. F: injection of either hyperpolarizing or depolarizing 0.5-s current pulses into the N3t interneuron evokes a very small electrical response on the OC membrane potential (F1), while a longer depolarizing stimulus to the N3t has a minor depolarizing effect on the OC interneuron (F2). All these experiments in Hi-Di saline.
The wide range of response durations is another especially interesting aspect of the connections formed by OC interneurons (Fig. 13C). The short-term effects (intraphasic modulation) last during the presynaptic OC bursts (marked by dotted lines in Fig. 13, A and C), but other outputs have their main effect after the spiking activity (burst) of the OC interneuron is over.

With its strong, short-term excitatory connections with other N3 phase neurons (electrical coupling with both the N3p interneurons and with the N3t interneurons, supplemented by the chemical excitatory effect on the N3t interneurons), the OC interneurons facilitate the overall firing activity of all the N3 neurons, increasing the outputs to the feeding muscles contracting in swallowing phase. This reflects a general feature of the feeding system as neurons firing in the same phase are electrically coupled (Staras et al. 1998; Yeoman et al. 1995). The short inhibitory effects of OC interneurons on protraction phase neurons, however, prevents them from firing (and the protraction muscles to contract) simultaneously in the swallowing phase.

The longer term outputs are delayed with respect to the burst of the presynaptic OC neuron. After a short inhibitory response recorded in the N1 (protraction) phase interneurons during the OC burst, the following excitatory effect lasts for up to 7–8 s (Fig. 13C), far above the duration of an average feeding cycle (this is 3 s corresponding to the top 20 bites/minute feeding rate of intact animals) (Kemenes et al. 1986; Vehovszky et al. 1998). This fits well with the feeding pattern in which protraction phase neurons are inhibited during swallowing phase, but their membrane can be excited later as the next feeding cycle follows after swallowing. This polycyclic excitatory effect produced by OC neurons seems to be a significant difference between the OC and previously described modulatory interneurons in the Lymnaea feeding system is that these cells (CV1, N1L, and SO) mostly drive feeding as long as the stimulus is maintained (Elliott and Benjamin 1985a,b; McCrohan 1984; McCrohan and Croll 1997; Yeoman et al. 1995) while the effect of the OC interneuron tends to drive fictive feeding once the stimulus is ended (see Figs. 2, D and E, and 10B) therefore promotes the feeding pattern.

The reciprocal biphasic effects between OC and N3p neurons (a short electrical excitatory connection followed by a longer inhibitory chemical one) seem paradoxical first, as both neurons fire in the same N3 phase of feeding. One explanation is that the inhibitory component of the OC connections helps to terminate the N3p bursts (previously facilitated by the short-term electrical coupling). This shortens the feeding cycles and increases the feeding rate (as seen in Fig. 3), therefore the reciprocal OC and N3 inhibitory connections contribute to the pattern generation. Although all previous work in Lymnaea has suggested that the groups of neurons firing in the same phase have mutually (electrical) excitatory effects (Staras et al. 1998; Yeoman et al. 1995), the OC 171 N3p relationship has a similarity to the Aplysia feeding system where the histaminergic B52 neurons fire in the same phase and have reciprocal inhibition, although the B52 neurons are not electrically coupled (Evans et al. 1999). Reciprocal inhibition is suggested to have a central role in the mechanisms of pattern generation (see review by Cropper and Weiss 1996).
The longest duration effects of the OC interneuron are inhibition of the B2 and B3 motor neurons (Figs. 7, D1 and D2, 8, A and B, and 13C). Morphological data (Benjamin et al. 1979; Perry et al. 1998) suggest that these giant neurons innervate of the esophagus. The peristaltic activity of the esophagus may not necessarily be linked tightly to the individual feeding phases produced by the buccal musculature (Perry et al. 1998).

Different outputs of OC and N3p interneurons allow reconfiguration of the feeding network

Although the pattern of inputs and firing activity of OC and N3p interneurons are almost identical to inhibitory N1 and N2 inputs and excitatory inputs during N3 phase (Fig. 2B), they evoke different inputs on (B3, B5, B4) motor neurons (Fig. 8). This indicates that OC and N3p interneurons represent func-
otionally different subgroups in the populations of N3 phase interneurons. The physiological differences between OC and N3p interneurons are reflected by earlier morphological data, as the axonal branching patterns of OC interneurons and N3p neurons are completely different from each other (Elliott and Benjamin 1985a; Vehovszky et al. 1998). Pharmacological differences are also present, as only OC interneurons contain and use octopamine (Vehovszky et al. 1998, 2000); the transmitter for N3p neurons is not established yet. Furthermore, the OC and N3p interneurons will reconfigure the network in different ways, with (for example) recruitment of the OC interneurons reducing the activity of the B3 motor neuron, and recruitment of N3p interneurons increasing the activity of the B3 motor neurons. In addition, the OC interneurons, but not the N3p interneurons, can enhance the overall fictive feeding rate, with the maximal rate (20 cycles/min) occurring only when both the OC and SO fire.

Previous analyses of the rhythmic pattern in *Lymnaea* demonstrated that modulatory interneurons affect the feeding intensity (motor neuron firing rate; CGC) (McCrohan and Audesirk 1987; Yeoman et al. 1996) or rhythmic rate (SO, N1L, CGC, CV1) (Elliott and Benjamin 1985b; McCrohan 1984; McCrohan and Kyriakides 1989; Yeoman et al. 1996). Additionally the SO and CGC interneurons alter the relative proportion of time spent in each feeding phase (Elliott and Andrew 1991; Yeoman et al. 1996). OC interneurons do the same, as the length of N1 and N3 phases decrease during OC activity (see Fig. 3B3). Additionally, OC neurons also change the pattern of motor outputs (due to the differential firing patterns of the motor neurons) during its activity. In *Helisoma* buccal system a similar reconfiguration can be achieved by hyperpolarizing the swallowing phase N3a interneurons, which seems to have many homologous physiological features with the *Lymnaea* OC interneurons (Quinlan and Murphy 1996).

Electrically coupled modulatory neurons that use different transmitters and are able to reconfigure the network in different ways can also be found in crustacean pattern generators (Eisen and Marder 1984; Marder and Eisen 1984). Reconfiguration of the network by a modulatory interneuron may lead to very substantial functional changes; for example, in the crustacean stomatogastric system a new network is constructed from members of the original pattern generating system (Meyrand et al. 1994).

**Role of OC interneurons in the feeding network**

In previous models of the feeding system (Brierley et al. 1997a,b; Elliott and Benjamin 1985a,b; Yeoman et al. 1995), production of the feeding pattern only took place as long as the stimulation of the driving modulatory interneuron (SO, N1L) was maintained. The longer-lasting (polycyclic) excitatory effects of OC interneurons on protraction phase interneurons (SO, N1L, N1M) means that the activity of the OC interneurons contributes to the next cycle of feeding, therefore providing a positive feedback from the swallow (N3) phase to the next feeding cycle, which starts again with radula protraction (N1 phase).

The potential-dependent and long-lasting excitatory effect of OC neurons may facilitate the initial excitatory trigger to the protraction phase interneurons to evoke fictive feeding (Figs. 2 and 10). When the excitatory inputs from OC interneurons to N1 phase interneurons are strong enough, they depolarize the membrane of the follower up to the firing threshold, and finally the pattern-generating protraction phase interneurons evoke a feeding pattern. The electrical coupling between N1L and SO interneurons (Yeoman et al. 1995) further increases the excitatory effect of OC neurons on the coupled partner.

After repetitive OC bursts, the excitatory component of the biphasic response seen in the protraction phase interneurons (SO, N1L, and N1M) facilitates. This means that repetitive stimulation of the OC interneurons is more effective in stimulating these protraction phase interneurons to drive fictive feeding. This is an additional feature of the modulatory effect of the OC interneurons, specific to the protraction phase (pattern-generating) interneurons. As this effect lasts longer than the time for an individual feeding cycle, it may be termed polycyclic modulation. This is a second example of polycyclic excitatory modulation by the OC interneurons, adding to the ability of the OC interneuron to enhance the synaptic output of the SO interneuron (Elliott and Vehovszky 2000). In the *Aplysia* feeding system an example of homosynaptic facilitation that modulates the connections between an interneuron and its followers was described recently (Sanchez and Kirk 2000).

The most crucial question concerning the role of OC interneurons in feeding, however, is how these neurons are activated to produce their modulatory effects on the feeding system?

OC interneurons can be activated by the CPG system during feeding as they depolarize and contribute to the fictive feeding pattern after stimulation of protraction phase interneurons (Fig. 2, A–C). But this activation is unlikely to come from the known pattern-generating interneurons, as we have found inhibitory inputs from SO, N1L, and N1M interneurons to the OC interneurons (Fig. 11). The biphasic inputs (inhibition followed by depolarization) from N2v phase interneurons (Fig. 11E) or the excitatory electrical connection with other N3 phase (B4, N3p, N3t) feeding neurons (Figs. 4, 6, and 7) may also make some small contribution to the activation of the OC interneurons. However, it does not seem to be sufficient to trigger the OC burst.

OC interneurons also receive excitation from the CGCs, as do the B4, N3p (and N3t) (Yeoman et al. 1996) swallowing phase neurons, suggesting an additional extrinsic source of excitatory inputs from the cerebral ganglia. We cannot exclude, however, that the OC interneurons are mainly activated from the periphery (through the sensory system). Establishing this will require further studies in semi-intact preparations, where the CNS is attached to peripheral organs (lip, mouth, buccal mass, salivary glands, etc).

**OC interneurons are intrinsic modulators of the buccal feeding system**

The OC interneurons cannot be considered as CPG interneurons because a rhythm occurs even when they are silent. However, they definitely modulate the network. They receive rhythmic synaptic inputs during fictive feeding and form connections with both CPG and modulatory interneurons of the buccal ganglia but not with the cerebral CGC. This means that the OC interneurons should be considered as intrinsic rather than extrinsic modulators of the feeding network. Here it is worth noting that the axonal branching of the OC, like that of another intrinsic modulator, the SO interneuron, is extensive.
throughout but confined to the buccal neuropil and does not enter other parts of the CNS (Elekes et al. 1996; Elliott and Benjamin 1985b; Vehovszky et al. 1998).

In the buccal ganglia of Lymnaea, known intrinsic modulators as SO and NIL are cholinergic (Vehovszky and Elliott 1995; Yeoman et al. 1993), while in the cerebral ganglia, CBWC neurons contain APGWamide peptide (McCrohan and Croll 1997). The modulatory serotonin, however, arises from an extrinsic modulator (CGC) neuron located in the cerebral ganglia (Pentreath et al. 1982), which can have polycyclic effects on fictive feeding (Benjamin and Elliott 1989). The OC interneurons are octopaminergic as their octopamine content and octopaminergic synaptic contacts are already established (Vehovszky et al. 1998, 2000).

In conclusion, through their synaptic connections with feeding motor neurons and interneurons, OC interneurons can modulate the activity and reconfigure the synaptic outputs of the buccal feeding system in Lymnaea. The effect produced by stimulating the OC interneurons is dependent on the activity (or quiescence) of the feeding network, due to the strongly biphasic outputs of the OC interneurons. As OC interneurons contain octopamine, this is the first example where an intrinsic member of the buccal feeding system in Lymnaea provides aminergic modulation.

This work was supported by Biotechnology and Biological Sciences Research Council Grant S08677.

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