Identification and Characterization of Catecholaminergic Neuron B65, Which Initiates and Modifies Patterned Activity in the Buccal Ganglia of *Aplysia*

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Kabotyanski, E. A., D. A. Baxter, and J. H. Byrne. Identification and characterization of catecholaminergic neuron B65, which initiates and modifies patterned activity in the buccal ganglia of *Aplysia*. J. Neurophysiol. 79: 605±621, 1998. Catecholamines are believed to play an important role in regulating the properties and functional organization of the neural circuitry mediating consummatory feeding behaviors in *Aplysia*. In the present study, we morphologically and electrophysiologically identified a pair of catecholaminergic interneurons, referred to as B65, in the buccal ganglia. Their processes innervate both the ipsi- and contralateral neuropil, and separate branches of B65 appeared to innervate the somata of both ipsi- and contralateral B4/5 neurons. B65 exhibited patterned burst(s) of activity during spontaneous cycles of fictive feeding. Patterned activity in B65 also was elicited by stimulation of the radula nerve, by depolarization of the pattern initiating neurons B31/32 or B63, and by bath application of l-3,4-dihydroxyphenylalanine (DOPA). B65 appeared to be a member of the projection group of neurons. Action potentials in B65 elicited fast one-for-one excitatory postsynaptic potentials (EPSPs) in neurons B4/5, B8A/B, B31/32, B63, and B64. In turn, B31/32 and B63 excited B65 and B64 inhibited B65. Some of the synaptic connections of B65 were plastic. For example, the fast EPSPs elicited in B4/5 and B64 decremented, whereas those in B31/32 and B8A/B facilitated. In addition to fast EPSPs, B65 elicited slow postsynaptic potentials in some of its follower cells. Depolarization of B65 elicited cycles of patterned activity indicative of fictive feeding in buccal neurons, including B65 itself. During series of B65-induced patterns, the properties of the buccal motor programs appeared to change. In particular, the activity of radula closure motor neurons B8A/B, which initially coincided mainly with the retraction phase of a cycle, gradually extended to overlap mostly with the retraction phase. This observation suggests that prolonged activity in B65 may play a role in transitioning from rejection-like to ingestion-like fictive feeding. The phase shift of the activity of B8A/B appears due, at least in part, to a decrease in activity of B4/5, and thus a reduction in inhibition from B4/5 onto B8A/B, during the retraction phase. The functional properties and synaptic connections of B65 suggest that it may play an important role in determining features of patterned neural activity in the buccal ganglia.

**INTRODUCTION**

Rhythmic behaviors in invertebrates provide excellent opportunities to understand, at the level of individual neurons and synapses, how such behaviors are generated, controlled, and modified. One useful preparation for such studies is feeding behavior in *Aplysia*.* Aplysia* usually start feeding with appetitive responses that lead to contact with food (Bablanian et al. 1987; Kupfermann 1974a,b; Teyke et al. 1990, 1992). The following consummatory phase of feeding consists of a series of well-coordinated rhythmic movements of the lips, jaws, odontophore, and esophagus, which result in either ingestion (biting or swallowing) or rejection of food (Howells 1942; Kandel 1979; Kupfermann 1974a; Morton and Chiel 1993a; Susswein et al. 1976). In general, a cycle of ingestion or rejection, starts with protraction of the odontophore, a muscular tongue-like organ covered with toothed radula, and concludes with its retraction (Hurwitz et al. 1995, 1996; Kupfermann 1974a; Morton and Chiel 1993a). If the two halves of the radula are open during the protraction phase and closed during the retraction phase, then food (seaweed) is grasped and transported into the buccal cavity (i.e., ingestion). Alternatively, if the radula is closed during protraction and open during retraction, then objects are expelled from the mouth (i.e., rejection) (Church and Lloyd 1994; Kupfermann 1974a; Morton and Chiel 1993b).

The rhythmic movements of the jaws and radula are believed to be under the control of a neural circuit located in the buccal ganglia that functions as a central pattern generator (CPG) (for recent reviews of CPGs, see Arashavsky et al. 1993; Cropper and Weiss 1996; Getting 1989; Harris-Warrick 1993; Kupfermann 1994; Selverston 1992). The understanding of the buccal CPG has progressed significantly in recent years. Many premotor and motor neurons expressing patterned activity that underlies aspects of feeding or are critical for generating different patterns have been identified (Church and Lloyd 1994; Church et al. 1991, 1993; Cohen et al. 1978; Cropper et al. 1990; Gardner 1971, 1977; Hurwitz et al. 1994; Kirk 1989; Plummer and Kirk 1990). The relative phase of spike activity in some of these neurons has been characterized and, accordingly, they have been classified into the protraction- or the retraction-phase groups or the radula closure group (Church and Lloyd 1994). For example, neurons B31/32, B35, and B63 appear to be critical for generating the projection phase of the buccal motor programs, whereas B64 appears to be critical for generating the retraction phase (Hurwitz and Susswein 1996; Hurwitz et al. 1993, 1996; Susswein and Byrne 1988; Susswein et al. 1996; see also Kabotyanski et al. 1994b; Ziv et al. 1994), and motor neurons B8A/B, B16 control the closure of the radula (Church and Lloyd 1994; Cropper et al. 1990; Morton and Chiel 1993b). Finally, a distinction between ingestion and rejection-like motor programs was established based on the timing of activity in the radula.
closure group neurons relative to the activity of protraction and retraction-groups. An ingestion-like buccal motor program (fictive feeding) is characterized by the firing of radula closure neurons mainly in phase with retraction-group neurons, whereas a rejection-like fictive feeding is characterized by the firing of radula closure neurons mainly in phase with protraction-group neurons (Church and Lloyd 1994; Morton and Chiel 1993b; Nargeot et al. 1997). The neuronal mechanisms of this phase shift underlying switching between different types of motor programs are not well understood, however.

In other CPGs, for example in the crustacean stomatogastric CPG, functional reconfiguration accompanied by phase shifts in the patterned activity have been shown to be induced by modulatory transmitters (Harris-Warrick et al. 1995). A possible candidate for the transmitter that could change functional properties in the buccal CPG of Aplysia is dopamine. The buccal ganglia receive an abundant catecholaminergic input from the foregut via the esophageal nerves (Susswein et al. 1993) and from the cerebral ganglia via the cerebro-buccal connective (Rathouz and Kirk 1988; Rosen et al. 1991). In addition, several catecholamine-containing neurons have been observed in the buccal ganglia (Goldstein and Schwartz 1989; Hawkins 1989; Rathouz and Kirk 1988; Salimova et al. 1987; Teyke et al. 1993). The principal catecholamine in the CNS of Aplysia and other mollusks is dopamine (Fanchini et al. 1985; Guthrie et al. 1975; Juorio et al. 1989; Teyke et al. 1993). Previously, we found that dopamine or its precursor L-3,4-dihydroxyphenylalanine (DOPA) initiated or substantially accelerated rhythmic activity in the buccal CPG (Baxter et al. 1995; Kabotyanski et al. 1993, 1994b). Moreover, both dopamine or DOPA biased the phase relationships among the elements of the feeding circuit toward that characteristic of an ingestion-like motor program (Baxter et al. 1995; Kabotyanski et al. 1994b).

To examine further the cellular mechanisms of dopamine-dependent transitions from rejection to ingestion, the present study focused on finding catecholaminergic neurons that may contribute to these processes in the buccal ganglia. We identified and characterized a lateral pair of midsize catecholaminergic neurons that we refer to as B65. We also examined the possible role that B65 may play in generating different types of buccal motor programs. Repeated firing of B65 led to a gradual transition from rejection-like to intermediate and ingestion-like patterns. In addition, the activity of B4/5 neurons appeared to decrease gradually during B65-induced buccal motor programs, and we examined how this effect may contribute to the phase shift of activity in radula closure neurons B8A/B. Preliminary reports of aspects of this work have appeared previously (Kabotyanski et al. 1994a,b).

METHODS

Aplysia californica were obtained from the Aplysia Resource Facility, University of Miami (Miami, FL), and Marine Specimens Unlimited (Pacific Palisades, CA). Animals (150–350 g) were maintained in aquaria with aerated artificial seawater (Instant Ocean, Aquarium Systems, Mentor, OH) at 15°C on 12 h dark/light cycle and fed regularly with dried seaweed (Hang Loong Marine Products, Hong Kong).

Before dissection, animals were anesthetized by injection of a volume of isotonic MgCl₂ solution equal to 30% of their body volume, and then 10–20 ml of high [Mg²⁺] hemolymph was drawn. The buccal ganglia were excised, usually treated for 2.5 min with 0.4% solution of Pronase E (Sigma) at room temperature, then washed with cold hemolymph (15 min), pinned to the silicone elastomer (Sylgard, Dow Corning, Midland, MI)-covered bottom of a recording chamber in 1:1 mixture of artificial seawater and high [Mg²⁺] hemolymph, and surgically desheathed.

Glyoxylate-induced histofluorescence of catecholamines was obtained in whole-mount preparations as described in Kabotyanski and Sakharov (1990). Briefly, desheathed ganglia were incubated in freshly made glyoxylic-acid-containing solution at 4°C for 2 h, placed on a glass slide, dried with air blower at room temperature for 1.5–2 h, heated in an oven at 66°C for 9 min, covered with paraffin oil, coverslipped, and viewed under an Axioskop fluorescence microscope (Carl Zeiss, Oberkochen, Germany) with filter set 05 (green-blue fluorescence for catecholamines and yellow fluorescence for indolamines, i.e., serotonin). The incubating solution contained (in mM) 500 sodium glyoxylate (Fluka Chemical, Ronkonkoma, NY), 40 N-2-hydroxyethylpiperazine-N' 2-ethanesulfonic acid (HEPES) buffer, and 100 sucrose, dissolved in deionized water, pH 7.0 (Sigma Chemical, St. Louis, MO). The final pH of the solution was adjusted to 7.0 with either glyoxylic acid (Fluka) or sodium bicarbonate crystals. Control experiments used either an incubating solution in which sodium glyoxylate was substituted in equimolar amounts for sodium chloride or specific fluorescence was extinguished with water (Sakharov and Salimova 1980).

For electrophysiological experiments, ganglia were pinned either caudal side up or twisted along the commissure, allowing simultaneous access to the caudal surface of one ganglion and rostral surface of the other. The preparation was superfused continuously with artificial seawater at a flow rate of ~2 ml (the volume of the recording chamber) per minute. The composition of artificial seawater (in mM) was 450 NaCl, 10 KCl, 30 MgCl₂, 20 MgSO₄, 10 CaCl₂, 2.5 NaHCO₃, and 10 HEPES, pH adjusted to 7.5. In some experiments, artificial seawater contained DOPA (Calbiochem, La Jolla, CA) in concentration 40 μg/ml. In some experiments, the bathing medium was used that contained three times higher concentrations of divalent ions (3×[Ca²⁺], 3×[Mg²⁺] artificial seawater) with the following composition (in mM): 300 NaCl, 10 KCl, 130 MgCl₂, 20 MgSO₄, 30 CaCl₂, 2.5 NaHCO₃, and 10 HEPES, pH adjusted to 7.5. Intracellular recordings were performed using glass micropipettes filled with a solution containing 3 M potassium acetate and 0.1 M potassium chloride (10–15 MΩ) or with a fluorescent dye, which was used for double-labeling experiments (15–25 MΩ). Intracellular recordings were performed with conventional current-clamp techniques. Neurons were identified based on their position and physiology. In addition, the identification of B8 was verified by its ability to produce axonal spikes in the radula nerve, and the identification of B63 was verified by its morphology. Extracellular recordings from one of two radula nerve branches were performed using plastic suction electrodes and a differential AC-coupled amplifier. When needed, the radula nerve was stimulated via the same electrodes with train of five pulses (3–6 V, 75 ms, 1 Hz) applied via stimulus isolator. The preparations were maintained at 15–16°C.
of Fast Green, as well as with filter set 05 for catecholamines. Preparations were photographed on Kodak Technical Pan film (Eastman Kodak, Rochester, NY) and drawn using the Neurulucida program (MicroBrightField, Baltimore, MD).

For statistical analysis, data were averaged within each experiment first and then averaged across experiments. When phase ratio was analyzed with Student’s t-test (Figs. 13 and 14), data in each experiment were first transformed using arcsine square root transformation to meet assumption of normality because proportions are binomial (Zar 1984).

RESULTS

Localization of catecholamines

Before electrophysiological identification, we obtained preliminary information about the number, relative positions, sizes, and branching patterns of catecholaminergic neurons. The mapping technique in the present study had to be compatible with the double-labeling procedure in subsequent experiments. In pilot studies, a commercially available antidi-pamine immunostaining procedure was found incompatible with the use of fluorescent labels because preparations had intense nonspecific background fluorescence. Thus we used a modification of glyoxylic-induced fluorescence technique (Kabotvanski and Sakharov 1990), which revealed the anatomic features of monoaminergic cells in greater detail than other histo-fluorescent procedures such as the FaGlu method (Goldstein and Schwartz 1989).

In 46 of 47 preparations, the paired buccal ganglia contained five midsized (40–60 μm diam), morphologically identifiable cells, and two lateral clusters of small (5–10 μm diam) cells, that exhibited bright green-blue fluorescence associated with catecholamines (Figs. 1A and 2A). Of the five midsized catecholamine-containing neurons, one was unpaired and usually located in the right hemiganglion. The other four neurons formed two bilaterally symmetric pairs. These results are consistent with the previous findings of Goldstein and Schwartz (1989). In one of the 47 prepara-tions, we observed only three midsized catecholamine-con-taining neurons. In 27 preparations, we also observed an additional relatively large (~100 μm diam), paired cell, exhibiting pale green-blue fluorescence and located in the dorso-medial quarter on the caudal side of the ganglia (not shown). This observation suggests that additional catechol-amine-containing neurons probably could be found in the buccal ganglia using more sensitive techniques (e.g., immuno-cytochemistry) or using animals in different behavioral states.

The cells of the medial pair recently were characterized and designated B20 (Teyke et al. 1993). The unpaired neuron, like B20, is bipolar; it sends one of each processes into one of each cerebro-buccal connectives. The lateral pair of cells appeared to send processes only into the con-tralateral ganglion via the buccal commissure (see further). This lateral pair of neurons was identified anatomically and electrophysiologically in the present study and designated B65.

The somata of B65 usually were covered by at least two layers of neurons on the caudal side. Some of the outer cells were B31–B37 (Susswein and Byrne 1988), which were covered in turn by neurons B1 and B2. The somata of B65 were found consistently within an arc formed by two axonal bundles that enter the buccal neuropil from the esophageal nerve (e.n.) and buccal nerve 1 (b.n.1; Fig. 2A) (nerve nomenclature from Gardner 1971).

The histofluorescence technique also revealed catechol-amine-containing processes in many nerves of the buccal ganglia and in the neuropil. The radula nerves were the only nerves that failed to consistently contain catecholaminergic processes. In 12 preparations, however, we observed one or two fine catecholamine-containing neurites with varicosities on the surface of the radula nerves. The most abundant cate-cholaminergic processes appeared in the esophageal nerves, which previously have been shown to contain numerous neu-rites of catecholamine-containing neurons located in esopha-gus (Susswein et al. 1993). In addition, at least five bright catecholaminergic-containing processes were observed consist-ently in each cerebro-buccal connective (Fig. 2A). Three of these processes were apparently from the two B20 cells and the unpaired catecholamine-containing neuron. The fourth axon was probably from the cerebral catecholaminergic neuron CBI-1 (Rosen et al. 1991). The origin of the remaining catecholamine-containing process(es) in the cerebro-buccal connective is unknown.

We regularly observed a catecholaminergic braiding cov-ering some neurons in the buccal ganglia, similar to that reported by Salimova et al. (1987). Somata of visually identifi-cable cells B1, B2, B3, B4, and B5 and some other unidenti-fied cells were surrounded by a dense web of fine catechol-amine-containing varicose neurites. Most prominent was the braiding around cells B4 and B5 (Figs. 1, B and C, and 3, A and C). We show below that, in part, these neurites are from B65.

In the control experiments (5 with chloride for glyoxylic in the incubating solution and 5 with specific fluorescence extinguished with water), no fluorescing neurons were found.

Double labeling and morphology of B65

Because the somata of B65 were covered by other cells, they were inaccessible for identification based on simple visual criteria. The double-labeling procedure was employed routinely to verify that data collected in the electrophysiologi-cal experiments were obtained from B65. For this approach, microelectrodes filled with Fast Green-containing solution were used to impale a candidate cell. After experiment, the neuron was filled ionophoretically with Fast Green (~2 to ~5 nA, 15–40 min) until the soma acquired a light-blue color. The preparation then was processed for histofluores-cence of monoamines (see METHODS) to determine whether green-blue fluorescence of catecholamines colocalized with the red fluorescence of Fast Green in the candidate cell. All electrophysiological data presented in this paper were obtained in experiments with positive double labeling (n = 24). Figure 2 illustrates one such experiment. Figure 2A shows histofluorescence of catecholamines in the buccal ganglia with arrowhead pointing to the right B65. Switching to the red fluorescence filter set (Fig. 2B) demonstrated that the neuron was filled with Fast Green during the electrophys-iological experiment.

Intracellular injections of Fast Green revealed details of the morphology of B65. B65 usually had a major process
that extended into the contralateral ganglion (Figs. 2B and 3A). In some preparations (3 of 15 fluorescent preparations, 16 of 24 double-labeling preparations), at least one B65 of the pair had a second relatively large process branching in the proximity of the soma (e.g., see Fig. 1A, inset). In all cases, B65 branched extensively in the ipsilateral neuropil (Fig. 3, A and B), forming varicosities and terminals mainly along the neural tracts that enter the ganglion from the esophageal nerve and buccal nerve 1. In 5 of 24 double-labeling preparations, we observed fine branches that terminated at the entrance point of the esophageal nerve. In the medial zone of ipsilateral ganglion, the major axon sent a distinct branch toward the cell bodies of ipsilateral B4 and B5 (Fig. 3A, arrow). This branch formed a fine covering around B4/5 (Fig. 3C). In the contralateral ganglion, another branch, or several branches, were sent toward and formed braiding around contralateral B4/5 somata (Fig. 3A, arrowhead). The major process terminated in the contralateral ganglion, forming many large varicosities within the neuropil, although it did not branch as extensively as the ipsilateral portion (Figs. 2B and 3A). No branches of B65 were found in the buccal nerves or connectives. Thus B65 appeared to be an interneuron. Its functional properties were studied in electrophysiological experiments (see further).

**Electrophysiological properties of B65**

B65 exhibited patterned activity. The resting potential of B65 ranged from −44 to −60 mV, with an average of $-52.2 \pm 1.1$ mV (mean ± SE; $n = 24$). Average spike amplitude was $70.3 \pm 1.9$ mV. B65 was typically silent, sometimes producing a burst of spikes on impalement, but in preparations that exhibited patterned activity, B65 fired bursts of spikes that were phase-locked with cycles of the buccal motor program (Fig. 4A).

In the buccal ganglia, a cycle of a buccal motor program usually appears as a stereotypic, recurring sequence, or pattern, of phasic bursts of activity in a number of neurons. The cycle starts with a burst of spikes in protraction-group neurons and is followed by synaptic inhibition in these neurons coincident with a burst in retraction-group neurons. Activity in closure-group neurons shifts between these two phases depending on whether an ingestion-like or a rejection-like buccal motor program is produced. A useful monitor of the buccal motor programs is the radula nerve, in which a
cycle of a buccal motor program occurs as a distinct pattern of extracellular activity (e.g., Figs. 4 and 11). The largest units recorded in the radula nerve during the pattern (e.g., Figs. 12C and 13, A and B) are produced by two bilateral radula closure neurons B8A/B (Morton and Chiel 1993b). Therefore simultaneously recording from a protraction- or retraction-group neuron and from a closure-group neuron or the radula nerve allows for discriminating between ingestion- and rejection-like patterns.

In addition to firing during spontaneous buccal motor programs, B65 was also active phasically during patterned activity that was elicited by stimulation of the radula nerve (Fig. 4B; 30 observations in all 8 experiments in which this was tested) or by depolarization of the pattern initiating neurons B31/32 (5 observations in 5 experiments) or B63 (13 observations in 3 experiments). When continuous rhythmic activity was elicited by bath application of DOPA, B65 also exhibited patterned activity (Fig. 4C; 3 experiments). Note that the bursts in B65 were associated with, and preceded by, a strong excitatory synaptic input to B65 from an unidentified source. Patterns in Fig. 4, A and B, were rejection-like, and pattern in Fig. 4C was ingestion-like.

When B65 fired during spontaneous or induced patterned activity, it was active primarily in phase with protraction-group neurons (e.g., B31/32, B63) and in antiphase with retraction-group neurons (e.g., B4/5 and B64). Moreover, B65 received a strong hyperpolarizing input during the retraction phase of patterned activity (Figs. 4, 10, 11, 12, and 13). This strong inhibitory input during the retraction phase is a distinguishing characteristic of the protraction-group neurons (Church and Lloyd 1994). Thus B65 was classified as a protraction-group neuron.

SYNAPTIC CONNECTIONS OF B65. We explored the synaptic connections among B65 and some of the neurons known to exhibit patterned activity in the buccal ganglia. The multifunctional neurons B4/5 commonly are used to monitor intracellularly patterned activity in the buccal ganglia (e.g., Gardner 1977; Hurwitz et al. 1996; Plummer and Kirk 1990;
FIG. 3. Morphology of B65. A: Neuro-lucida drawing of the buccal ganglia in which the right B65 was injected with Fast Green (same orientation as in Fig. 1). Arrow points at smaller branch that extended toward the cell bodies of neurons B4/5 located in ventromedial quadrant of the ipsilateral ganglion. This branch forms a part of catecholamine-containing wrapping around the B4/5 somata. A similar branch(es) is sent to the somata of contralateral B4/5 (arrowhead). B: high-power microphotograph taken from the preparation drawn in A. Cell body and proximal axon with a host of fine neurites terminating in the neuropil are seen. C: high-power micrograph taken from the ipsilateral ventromedial quadrant of the same preparation that was marked with arrow in A. A fine varicose neurite surrounds the cell body of B4/5. Calibration bar: 200 µm for A, 40 µm for B, 50 µm for C. Abbreviations are as in Fig. 1.

FIG. 4. B65 fires phasically along with other neurons during patterned activity. A: spontaneous cycle of rejection-like patterned activity. Cycle begins with activation of protraction-group neurons B65 by a synaptic input from unknown source (see text for explanation of phase relationships of B65) and is followed by activation of retraction-group neuron B4 concomitantly with inhibition in B65. Activity in radula closure neuron B8 is in phase with B65 and antiphase with B4, which is indicative of a rejection-like buccal motor program. B: cycle of rejection-like patterned activity elicited in the same preparation by electrical stimulation (A) of the radula nerve. The radula nerve trace was blanked during stimulation of the nerve. C: an ingestion-like patterned activity that was induced in the same preparation by bath-applied L-3,4-dihydroxyphenylalanine (DOPA). Abbreviations: r.n., radula nerve; R, right; L, left.
Firing B65 elicited fast EPSPs in contralateral B31/32 that followed one-for-one spikes in B65. In one experiment, B65 elicited biphasic, excitatory followed by inhibitory PSPs in B8. EPSPs in the contralateral B8A/B also were observed when the concentration of divalent ions was elevated (7 observations in 2 experiments). B65 produced no fast one-for-one PSPs in the ipsilateral B8 (Fig. 5D; 9 observations in 3 experiments). B8 produced no synaptic input to B65 (not shown; 50 observations in 7 experiments). No electrical coupling was found.

We also examined the connections between B65 and the multifunctional neurons B31/32, which previously were shown to be critical for initiating patterned activity (Hurwitz et al. 1994; Susswein and Byrne 1988). These cells belong to the protraction-group neurons (Hurwitz et al. 1995, 1996). Firing B65 elicited fast EPSPs in contralateral B31/32 that followed one-for-one spikes in B65 with constant delay and no failures under different rates of firing (Fig. 6C; 71 observations in 10 experiments). These PSPs summed and their amplitude increased with the increased firing rates in B65 (Fig. 6, A and C). In addition to fast time-locked EPSPs, B65 usually evoked polysynaptic excitatory input to B31/32 (Fig. 6, A and C). In turn, activation of B31/32 depolarized B65 (not shown, 7 observation in 5 experiments), but it was difficult to further assess the B31/32 to B65 connection because B31/32 generate plateau potentials and do not produce conventional spikes in their somata. We recorded from both cells ipsilaterally in only one experiment. In this case, B65 or B31/32 induced slow depolarization in each other, but we did not observe fast PSPs. B65 also was coupled electrically with B31/32 (Fig. 6B; 18 observations in 5 experiments). The coupling ratio between contralateral B31/32 and B65 cells was ~1:20 and was much lower between ipsilateral cells. Because EPSPs in B31/32 followed spikes in B65 with ~30-ms delay and electrotonic depolarization in B31/32 was negligible (Fig. 6C), the B65 to B31/32 synapse appears to be primarily chemical.

Recently, a new element of the buccal CPG has been identified as bilateral neuron B63, which is sufficient and necessary for the generation of buccal motor programs (Hurwitz et al. 1993; Susswein et al. 1996). We examined the connections between B63 and B65. Recordings were made in three experiments from a total of four B63 cells, which were identified based on location of their somata, morphology (all 4 were stained), and characteristic electrophysiolog-

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**Fig. 5.** Synaptic connections between B65 and B4/5 and B65 and B8A/B. A: spikes in B65 elicit one-for-one excitatory postsynaptic potentials (EPSPs) in contralateral neurons B4 and B8. Note that EPSPs in B4 gradually decremented, whereas those in B8 were facilitated. B: shaded segment of experiment shown in A was played back from tape recorder and displayed on a storage oscilloscope. Superimposed traces were obtained by triggering the oscilloscope with the rising phase of spikes in B65. EPSPs in B4 (top trace) and B8 (middle trace) followed spikes in B65 (bottom trace) with constant delay and no failures. C: B65 has asymmetric effects on ipsilateral and contralateral B4/5. EPSPs in ipsilateral B4 (middle trace) were of lower amplitude and did not exhibit gradual depression typical of EPSPs in the contralateral B4 (top trace). D: B65 also has asymmetric effects on ipsilateral and contralateral B8. Firing B65 elicited fast EPSPs only in contralateral B8. Recordings in A and B were from the same experiment, and recordings in C and D were from a separate experiment.
FIG. 6. Synaptic connections between B65 and pattern-initiating neurons B31/32. A: firing B65 elicited in B31/32 fast EPSPs that followed one-for-one spikes in B65 under different rates of firing. B: B65 and B31/32 were electrically coupled. C: data from shaded segments 1–3 of experiment in A were redisplayed in spike-triggering mode (C1, C2, and C3, correspondingly). EPSPs in B31/32 followed spikes in B65 with constant delay and no failures. Note that with higher rates of firing of B65, EPSPs in B31/32 increased in amplitude. Increase in amplitude of the EPSPs was associated with spike broadening in B65.

FIG. 7. Synaptic connections between B65 and the protraction-group neuron B63. A: B65 elicited EPSPs in contralateral interneuron B63. B: data from experiment in A redisplayed in spike-triggering mode. EPSPs in B63 followed spikes in B65 with constant delay and no failures. C: B63 produced a slow depolarization but no fast PSPs in B65. D: B65 and B63 were coupled electrically. All data are from the same experiment.

LONG-LASTING SYNAPTIC ACTIONS OF B65. In addition to fast synaptic potentials, B65 elicited slow and relatively persistent synaptic potentials in B8A / B (Figs. 5A and 9). In addition, the depression of B65-elicited activity in B4 / 5 appeared to be slow to recover (Fig. 9).

B65 ELICITED PATTERNED ACTIVITY. The observation that B65 exhibited synaptically driven patterned discharges during spontaneous or induced episodes of patterned activity and had synaptic connections with neurons implicated in the CPG for feeding suggested that B65 was an element of the CPG. An important test for direct involvement of this neuron in pattern generation is whether B65 is sufficient to induce a pattern. Indeed, in initially quiescent preparations, firing B65 elicited patterned activity in B65, as well as in neurons B4 / 5, B8A / B, B31 / 32, B63, and B64 (e.g., Fig. 10; multiple observations in all 18 experiments in which this was tested). Usually, threshold currents for eliciting patterned activity were +1.5 to +2 nA. We used long (1–3 min) or sustained depolarizing currents of twice the threshold to study B65-elicited patterns.
Although B65 invariably induced patterned activity, the number of cycles that were elicited varied. Most often, only two cycles of activity were elicited with an average period of $35.8 \pm 4.0\text{ s}$ (11 experiments). Sometimes, multiple cycles of rhythmic activity were initiated (e.g., Fig. 10) with an average cycle period of $13.7 \pm 2.8\text{ s}$ (5 experiments). In only two experiments, the B65-induced rhythmic activity was sustained as long as B65 was depolarized. Firing B65 also modulated the frequency of ongoing rhythmic activity (Fig. 11). In this experiment, we used a relatively low concentration of DOPA ($40\mu g/ml$) to induce sustained cyclic activity of the CPG. Depolarization of B65 resulted in an increase ($\sim 4$ times) in the rate of cyclic activity.

Brief activation of B65 was also sufficient to induce a pattern. Firing B65 with a brief depolarizing current pulse could elicit activity in other neurons that outlasted the depolarizing pulse and resulted in a cycle of patterned activity (Fig. 12C; note that the depolarizing pulse was much shorter than the initial protraction phase of the cycle). This finding (multiple observations in all 12 experiments) indicates that B65 recruits elements of the CPG with regenerative properties, such as B31/32 (Susswein and Byrne 1988). A possible mechanism of pattern initiation may involve B65-elicited facilitating EPSPs in B31/32 and B63 as well as triggering polysynaptic input to B31/32 (Figs. 6 and 7).

Figure 12 also illustrates that B65 appears to be the source of characteristic synaptic input to the contralateral neurons B4/5 (Fig. 12C, arrows) that usually is observed during a cycle of fictive motor activity. This input often evoked spiking in B4/5 during the protraction phase of patterned activity. Moreover, blocking spikes in B65 by hyperpolarizing current blocked this characteristic input to the contralateral B4/5 (not shown), suggesting that one B65 is sufficient and necessary to produce this synaptic input in the contralateral B4/5. This input to B4/5 therefore can serve as a monitor of activity in contralateral B65 (see further).

Blocking activity in one of the paired B65 neurons with hyperpolarizing current failed to prevent the spontaneous or radula nerve-elicited patterned activity in other buccal neurons (9 observations in 4 experiments) nor did it change the phase relationship of patterns. This result suggested that B65 may not be necessary for the expression of this type of patterned activity, which usually was rejection-like. Rather B65 may play a role in modifying the pattern (see further).

B65 ELICITS VARIOUS TYPES OF MOTOR PROGRAMS. What is the nature of buccal motor programs (ingestion-like or rejection-like) that are initiated by B65? The answer to this question depends, in part, on the time at which activity in the radula closure group neurons (e.g., B8A/B) occurs relative to the protraction and retraction phases. In the present study, activity of closure-group neurons was monitored in two ways: using either intracellular recordings from radula closure motor neuron B8A/B or extracellular recordings from the radula nerve, where spikes of B8A/B are recognized as the larger units (Morton and Chiel 1993b).

To determine whether ingestion-like, rejection-like or intermediate patterns were elicited by B65, we reanalyzed the 18 experiments in which depolarizing B65 produced patterned activity. In 13 of 18 experiments, it was possible to monitor activity of closure-group neurons. We used a crite-
rion similar to that developed by Morton and Chiel (1993a) to establish the type of B65-elicited patterns. We estimated the portion of large unit activity in the radula nerve (Morton and Chiel 1993a), or the portion of B8A/B activity, that overlapped with the retraction phase of a cycle of B65-elicited buccal motor programs. Cycles with an overlap of 0.1–0.7 were considered intermediate, smaller overlap indicated rejection-like, and larger overlap indicated ingestion-like activity.

B65 was activated with long (1–3 min) depolarizing currents. Each depolarization elicited one or more cycles of patterned activity. An average percentage of rejection, ingestion-like and intermediate cycles was determined for each preparation and then for all 13 preparations. Most patterns (50 ± 11%) were classified as being rejection-like (e.g., 1st, 2nd, 3rd, and 6th cycles in Fig. 10); 38 ± 11% were intermediate (e.g., 4th and 5th cycles in Fig. 10); and 12 ± 7% were ingestion-like (e.g., last 5 cycles in Fig. 11). This result is in contrast with the effects of prolonged applications of dopamine and DOPA on the phase relationships within CPG, as these drugs bias the patterned activity toward ingestion-like motor programs (Baxter et al. 1995; Kabotyanski et al. 1994b, 1995).

**REPEATED ACTIVATION OF B65 AFFECTS PHASE RELATIONSHIPS BETWEEN BUCCAL CPG NEURONS.** In three experiments in which firing B65 with constant depolarizing currents elicited five or more cycles, there appeared to be a trend for the phase distribution of B8 activity to shift from rejection-like to intermediate (e.g., Fig. 10) or to ingestion-like. Similar shifts occurred when B65 was depolarized during application of DOPA (e.g., Fig. 11). Figure 11 illustrates, for example, that the phasing of B8A/B neurons changed with each cycle during B65 stimulation until it became ingestion-like.

Because sustained depolarizations of B65 usually did not provide sustained patterned activity (see preceding text), in a separate set of five preparations, B65 was stimulated with series of trains of depolarizing current pulses to investigate the development of the apparent phase changes. The brief depolarizing pulses (80-ms, +6 nA/pulse, 40-ms interpulse interval) produced one spike per pulse and thereby provided a constant firing rate of B65 in all experiments. The frequency of the stimulus train was chosen to be similar to the firing rate of B65 (~8 Hz) during bursts of patterned activity induced by the stimulation of radula nerve or buccal nerve 2. In these experiments, the stimulation of B65 was terminated at the beginning of retraction phase of each induced cycle—a point in time when activity of B65 naturally is inhibited by synaptic input. Finally, we found that patterned activity could not be evoked repeatedly if the stimulation was delivered too often. A 4-min rest period was needed between stimulations in most preparations. In each preparation, stimulus trains were applied to B65 ~10 times.

Figure 13 illustrates two of these experiments: one in which B65 and B4 were recorded contralaterally (Fig. 13A and B2), and another in which B65 and B4 were recorded ipsilaterally (Fig. 13B1 and B2). Typically, the first one to three trains in B65 induced a rejection-like pattern because B8 activity, monitored either intracellularly or by extracellular recording from the radula nerve, occurred almost exclusively during protraction phase (Fig. 13, A1 and B1). During subsequent cycles, a shift in phase distribution of spike activity in B8 occurred in all experiments. By the 10th cycle, B8 was active either during both protraction and retraction phases (Fig. 13A2; 3 experiments) or primarily during the retraction phase (Fig. 13B2; 2 experiments). Although the search was not exhaustive, we did not observe reproducible changes in the phase distribution of activity of cells other than B8. There was noticeable variability of neuronal activity within and between experiments, however, and this variability underlined some differences in activities of some cells in Fig. 13.

Summary data for the phase shift in B8A/B are illustrated in Fig. 13C. Data were quantified by counting spikes in a single B8A/B that coincided with retraction phase and dividing this number by the total number of spikes generated in the cell during that cycle. These values represented a measure of phase distribution of B8A/B activity, referred here to as phase ratio. The phase ratio did not significantly change during first four trains, but it gradually increased about sixfold from the 5th to the 10th trains. Statistical analysis indicated that this increase was significant in trains 9–10 as compared with trains 1–2 (paired-sample t-test, 2-tail, P < 0.03, df = 3, t = 3.18). This phase shift in the activity of the radula closure neurons indicated that repeated activation of a single B65 can lead to a transition from rejection-like toward ingestion-like fictive feeding.

In control experiments, we examined whether repeated induction of patterned activity via neural elements other than B65 could cause the phase shift in B8A/B. In four experiments, the same mode of stimulation described above was delivered to the pattern initiating neurons B31/32, while spike activity in one of the paired B65 was blocked by hyperpolarizing current. In these cases, we did not observe changes in the phase ratio of B8 activity (not shown). In another set of three experiments, we repeatedly evoked patterned activity by stimulating the radula nerve. In these experiments, all induced patterns were rejection-like. In addition, thephase relationships in the CPG during spontaneous patterned activity in the isolated buccal ganglia do not significantly change over time (Baxter et al. 1995). These results suggest that different means of pattern initiation can preferentially elicit different types of buccal motor programs, and repeated activation of B65 appears to be associated with ingestion-like patterns.

In addition to the phase shift in B8A/B, there was an increase of the total number of spikes in B8. Moreover, the level of activity in B4/5 during retraction phase appeared to gradually decrease during successive cycles of B65-elicited patterned activity (e.g., Fig. 13, A and B). This effect was manifested most prominently in those B4/5 that were ipsilateral to the B65 that was being repeatedly fired (compare Fig. 13B, 1 and 2, top traces). This decrease of the activity in neurons B4/5 was unlikely to be due to spike adaptation because we observed full bursts of spikes in B4/5 when patterned activity was elicited by stimulating the radula nerve between and after B65-elicited patterns (not shown). Thus the changes in the level of activity of B4/5 during retraction phase appeared to be specific to B65-induced activity. At the same time, this effect was transient, and after
a rest period of ~10 min, B4/5 regained a high frequency of action potentials in response to reactivation of B65.

**B4/5 AFFECTS PHASE DISTRIBUTION OF B8A/B ACTIVITY.** Previously, B4/5 were reported to produce IPSPs in motor neurons B8A/B (Gardner 1971, 1977; Gardner and Kandel 1977), thus a decrease in activity of B4/5 during the retraction phase may contribute to concurrent increase of B8A/B activity. To explore this possibility, we first examined the B4/5 to B8A/B connection during periods of spontaneous bursts of activity in B8. In these experiments, B4 was hyperpolarized continuously to prevent it from spiking during spontaneous bursts; the current was released briefly to allow B4 to fire a burst of rebound spikes. During quiescent periods, B4/5 elicited small IPSPs in the ipsilateral B8 (Fig. 14A1). On arrival of a spontaneous burst in B8, the postinhibitory rebound burst of spikes in B4 reduced or interrupted firing in the ipsilateral B8 (Fig. 14A2; 5 of 6 experiments where this was tested). This effect was noticeable even though the other three B4/5 neurons in the ganglia were not hyperpolarized. These experiments illustrated that activation of B4/5 can inhibit spiking in the ipsilateral B8A/B during bursts of activity in B8. We next examined whether decreasing the activity in the B4/5 cells could affect the phase ratio of activity in B8A/B during fictive-feeding cycles.

Normally, spontaneous patterned activity in isolated buccal ganglia is predominantly rejection-like, i.e., B8A/B firing primarily during the protraction phase (Fig. 14B1). When activity in one of the four B4/5 neurons was depressed by applying a strong hyperpolarizing current at the onset of the retraction phase of a spontaneous cycle, the ipsilateral B8 produced more spikes during the retraction phase, indicative of an intermediate pattern (Fig. 14B2). Rejection-like patterned activity was restored in subsequent cycles if B4 was not hyperpolarized (Fig. 14B3; 6 experiments). Similar effects were observed when patterned activity was elicited by depolarization of neurons B31/32 (not shown). One B4/5 controlled the phase distribution of activity in both ipsilateral B8A/B cells in a similar manner (not shown). A summary of these experiments is plotted in Fig. 14C. These experiments indicated that decreasing the level of activity in the B4/5 cells during the retraction phase led to a significant concomitant increase in the activity of B8A/B (paired-sample t-test, 2-tail, $P < 0.0002$, df = 5, $t = 2.57$) and suggested a mechanism underlying the B65-induced phase shift.

**DISCUSSION**

Previous results showed that bath applied dopamine or DOPA elicited or increased the frequency of rhythmic activ-

**FIG. 10.** B65 is sufficient to induce patterned activity in the buccal ganglia. The pattern was characterized by a gradual decrease in the firing rate of B4 neurons during the retraction phase in successive cycles and by a gradual increase in the activity of B8. Note that B65 fired in phase with B63, which belongs to the protraction-group neurons, and in antiphase with the most intense bursts of activity in B4, which is a retraction-group neuron. The patterned activity was not sustained despite the continued depolarization of B65.

**FIG. 11.** Depolarization of B65 accelerates ongoing rhythmic activity. Periodic patterned activity was elicited by bath application of DOPA (40 min). Driving B65 with depolarizing current led to a fourfold increase in the cycling rate.
ity in isolated buccal ganglia and biased the phase relationships of this activity toward an ingestion-like one (Baxter et al. 1995; Kabotyanski et al. 1993, 1994b). In addition, in semi-intact preparations dopamine or DOPA induced biting-like movements (Kabotyanski et al. 1994b, 1995). These observations suggested that the ingestive behaviors of *Aplysia* may be controlled by catecholaminergic neural elements in the buccal ganglia.

**CATECHOLAMINE-CONTAINING CELLS IN THE BUCCAL GANGLIA.** Estimates of the number of catecholamine-containing neurons within the buccal ganglia from earlier histochemical studies varied. For example, Salimova et al. (1987) reported a total of two catecholamine-containing neurons in both ganglia, Rathouz and Kirk (1988) observed three to five mid-sized cells, and Hawkins (1989) found eight cells. In the present study, we addressed this issue using a glyoxylate technique modified for marine invertebrates (Kabotyanski and Sakharov 1990). In agreement with Goldstein and Schwartz (1989) and Teyke et al. (1993), we found that the buccal ganglia contain two symmetrical, lateral clusters of small-sized catecholamine-containing neurons, and five mid-sized cells, two paired and one unpaired. One pair of these cells is lateral and the other pair is medial. In addition, in >50% of preparations we observed an additional pair of relatively large cells in the dorso-medial quarter. The variations of the number of catecholamine-containing neurons in these studies could be explained by variations of sensitivities of different histofluorescent techniques that were used. The variations observed in our study could be caused by seasonal or behavioral variations of the level of catecholamines in individual neurons. The lateral pair of catecholamine-containing neurons was identified in this study as B65. On the basis of intracellular staining, B65 appeared to be an interneuron that was restricted to the buccal ganglia and that apparently formed synapses in both the ipsi- and contralateral ganglia. Because of its histochemistry, morphology, and position, B65 appears to be homologous to a catecholaminergic interneuron N1a that was recently identified in the buccal ganglia of *Helisoma* (Quinlan et al. 1997).

The specific catecholamine associated with the buccal neurons remains to be determined because the histochemical reaction used to reveal them visualizes all catecholamines and DOPA (Falk et al. 1962; Lindval and Bjorklund 1974; Lindval et al. 1974), and no effort was made to distinguish among them. Nevertheless, various biochemical studies have indicated that dopamine is a major (Carpenter et al. 1971; Juorio and Killick 1972), if not the only (McCaman et al. 1973, 1979), catecholamine in *Aplysia*. Therefore, there is a high probability that these neurons are dopaminergic.

**CENTRAL CATECHOLAMINE-CONTAINING CELLS SHARE SOME SIMILAR PROPERTIES.** Another pair of catecholamine-containing neurons located within the buccal ganglia is B20 (Teyke et al. 1993). In addition, the buccal ganglia are innervated by the catecholamine-containing cerebral-to-buccal interneuron CBI-1 (Rosen et al. 1991). B65, B20, and CBI-1 have similar effects in that they can all drive patterned activity. However, the types of activity elicited by their activation differ. For example, CBI-1, unlike B20 and B65, fires tonically and does not exhibit rhythmic activity on depolarization (Rosen et al. 1991). In addition, depolarization of CBI-1 always elicited only one cycle (Rosen et al. 1991), whereas B20 drives sustained cyclic activity (Teyke et al. 1993) and B65 induces mostly double or multiple cycles. Patterns driven by B65 appeared to exhibit a progressive, cycle-by-cycle decay in the level of activity of B4/5 (see also Kabotyanski et al. 1994b), which, judging from published data, apparently is not the case for B20-driven activity (Rosen et al. 1991; Teyke et al. 1993). In other respects, the features of B65 and B20 are similar. They both have similar connections with the neurons of the buccal CPG, they elicit patterned activity in those neurons, and they exhibit phase-locked rhythmic activity. In addition, both B20 (based on data from Teyke et al. 1993) and B65 belong to the protraction-group neurons. It would be interesting to find whether B20 also can bias buccal motor programs toward ingestion.

**B65 MAY BE AN ELEMENT OF THE BUCCAL CPG.** Several lines of evidence suggest that B65 may be an element of the buccal CPG for feeding. First, direct activation of B65 elicited patterned activity in B65 itself as well as in neurons that have been identified previously as elements of the CPG. Second, B65 exhibited patterned activity during both spontaneous and induced buccal motor programs, and the activity of B65 was in phase with other protraction-group neurons. Third, B65 affected the rate of ongoing rhythmic activity...
Repeated firing of B65 changes phase distribution of the activity in radula-closure neurons B8A/B, resulting in a shift toward intermediate and ingestion-like fictive feeding. Shaded areas below the B65 traces indicate the time of application of the stimulus train. Dashed vertical lines mark point of transition from protraction phase (to the left) to retraction phase (to the right). Data in A1 and A2, are from an experiment in which B65 and B4 were located contralaterally. A1: rejection-like patterned activity induced by the 2nd train of pulses. A2: intermediate pattern induced by the 10th train of pulses. Majority of spikes in B8 occurred during the retraction phase (33 spikes vs. 26). B1: rejection-like patterned activity induced by the 2nd train of pulses in another preparation in which B65 and B4 were located ipsilaterally. In this preparation, the B8 neuron that was monitored did not exhibit spikes during the first 3 trains. Nevertheless, large unit activity in the radula nerve indicated that other B8 neurons were active exclusively during protraction phase. Sequence of gradually decrementing EPSPs (arrows) in B4 is a likely indicator of the beginning and the rate of firing in contralateral B65. It appears that firing in the contralateral B65 is delayed and less intense than that elicited by train of pulses in the impaled (ipsilateral) B65. B2: ingestion-like patterned activity during the 10th train of pulses. Spikes in B8 occurred almost exclusively during the retraction phase. C: summary data for the shift in the phase distribution of activity in neurons B8A/B as represented by the phase ratio (see text). In one experiment, the impaled B8A/B neuron was not spiking during first 3 cycles (e.g., B1), so the ratio was calculated based on large units activity in radula nerve. Data were averaged for a corresponding train number across all experiments and plotted. Numbers in bars show how many data points were included in each average (if a train in B65 elicited an incomplete cycle, i.e., lacking distinct protraction or retraction, it resulted in a missing point).
FIG. 14. Control of radula closure group neurons B8A/B by ipsilateral neurons B4/5. All data taken from the same experiment. A1: during a quiescent episode, B4 elicited weak IPSPs in ipsilateral B8. A2: during a spontaneous burst in B8, B4 elicited inhibition of B8 that was sufficiently strong to interrupt B8 activity. B1: inhibiting the firing of neuron B4 increased the activity of B8 during retraction phase. B2: inhibiting B4 with hyperpolarizing current resulted in an increase in B8 activity during the retraction phase. B3: spontaneous activity returned to rejection-like after removal of inhibition in B4. C: summary data from 6 experiments identical to that illustrated in B. Phase ratio values were determined for each cycle in each experiment, and 2 groups of values were obtained: one for cycles in which B4/5 was not hyperpolarized (control) and another for cycles in which B4/5 was hyperpolarized (B4 hyperpolarized). Data were averaged across all experiments and plotted. Phase ratios in 2 groups are significantly different (paired-sample t-test; 2-tailed, \( P < 0.0002 \)).

(e.g., Fig. 11). Fourth, B65 had strong, probably monosynaptic, connections with other CPG neurons, particularly with pattern-initiating neurons B31/32 and B63. On the other hand, blocking spikes in one B65 with hyperpolarizing current did not prevent rejection-like patterned activity from occurring spontaneously or in response to stimulation of the radula nerve. This observation may indicate that B65 is not necessary for generation of rejection-like patterns, but rather that it is required for a specific (e.g., ingestion-like) activity.

**SOME EFFECTS OF B65 ARE LONG LASTING AND SITE SPECIFIC.** In addition to fast PSPs, B65 produced longer-lasting effects in some of its follower neurons. Some of these effects, such as synaptic depression in B4/5 and facilitation in B8A/B of B65-elicted EPSPs, developed rather rapidly. Others, such as decay of activity in B4/5 during the retraction phase (in this case, B4/5 activity is driven by retraction phase interneurons, e.g., B64), developed more gradually, after repeated activation of B65. Although we cannot currently establish whether these effects are pre- or postsynaptic, direct or indirect, these observations raise the intriguing possibility that B65 may be an example of an intrinsic modulatory CPG element, similar to the previously reported DSI neurons in Tritonia (Katz et al. 1994; Katz and Frost 1995).

The B65 activity-dependent changes in synaptic efficacy were site specific. Although connections from both ipsi- and contralateral B65 converge on ipsi- and contralateral B4/5, decrementing EPSPs are exhibited only in the contralateral B4/5. In contrast, the depression of the firing in B4/5 is most prominently expressed in the ipsilateral B4/5. The synaptic depression of B65-elicted EPSPs in the contralateral B4/5 neurons represents an example of site-specific plasticity of synaptic strength, which has been reported previously at other synapses in the buccal (Gardner 1993) and abdominal ganglia (Clark and Kandel 1993) of Aplysia and in crustacean neuromuscular junctions (Wojtowicz et al. 1994).
FUNCTIONAL ROLE OF B65. An important issue is the functional role of B65 in the feeding network. On the basis of the effects of dopamine and DOPA on this system (Baxter et al. 1995; Kabotyanski et al. 1993, 1994b), we expected that B65 could initiate ingestion-like buccal motor programs. However, a single activation of B65 usually elicited patterns that were indistinguishable from spontaneously occurring rejection-like or intermediate patterns. In contrast, repeated bursts of B65 activity produced a significant phase shift in the firing of closure-group neurons B8A/B that indicated a transition from rejection-like toward ingestion-like buccal motor programs. We suggest that repeated bursts of activity in B65 may be necessary to release sufficient levels of dopamine and thereby reconfigure phase relationships between elements of the CPG. This intensive stimulation of B65 may have been required simply to compensate for the lack of stimulation of the second B65. Alternatively, it may represent a natural mode of activation of B65 by food stimuli. The finding that repeated stimulation of B65 results in the production of intermediate and ingestion-like patterns supports our hypothesis that dopamine is involved in the control of ingestive feeding behavior in Aplysia (Kabotyanski et al. 1994b, 1995). In the stomatogastric CPG, dopamine also produces phase shifts in the pyloric motor pattern (e.g., Harris-Warrick et al. 1995), which raises a question of the homology of the roles that this neurotransmitter may play in these evolutionary distant feeding systems.

B65 also fired during repetitive radula nerve-elicited or spontaneous buccal motor programs in control experiments when no phase shift was observed. Under these conditions, however, bursts in B65 were associated with, and preceded by, the strong excitatory synaptic input from an unidentified source(s) (Fig. 4, A and B). Therefore the rejection-like phasing of these patterns could not be linked causally to B65 because they were initiated by a different source. On the other hand, when patterned activity was elicited by repeatedly firing B65, the phase shift in B8 was linked causally to B65. Apparently, the buccal CPG is a multifunctional circuit that underlies various types of motor programs, and specific elements may play different roles in eliciting different patterns under specific conditions.

B65-ELICITED CHANGES IN B4/5 ACTIVITY MAY UNDERLIE TRANSITIONS TOWARD INGESTION-LIKE PATTERNED ACTIVITY. During activity induced by repeated stimulation of B65, we observed a gradual decay of the rate of firing in B4/5. This apparent effect was particularly interesting because it was similar to the apparent decay of B4/5 firing that accompanied the transition to ingestion-like patterned activity in presence of dopamine or DOPA (Kabotyanski et al. 1994b, 1997). In addition, the firing frequency of B4/5 appeared to be more than two times lower during ingestion-like buccal motor programs than during rejection-like buccal motor programs (Church and Lloyd 1994). This effect may contribute to the phase shift in B8A/B that occurs in series of B65-induced patterns. Indeed, inhibiting activity in B4/5 during retraction phase led to a concomitant increase of B8A/B activity (Fig. 14B), which is believed to be critical for biasing the buccal CPG toward generating ingestion-like patterns. The results further suggest that control of B4/5 activity may be one of several mechanisms controlling phase distribution of closure-group neurons during switching between rejection and ingestion caused by food or inedible objects.

CIRCUIT DIAGRAM. Figure 15 illustrates a summary of connections between B65 and neurons that were examined in this study. The connections on which data were collected in this study, including the B4 to B8 connection (Gardner 1971, 1977), are depicted with thick lines, and connections re-
ported elsewhere with thin lines. To reflect functional properties of these neurons, we aligned vertically those cells that fire simultaneously during two types of patterned activity—rejection-like (Fig. 15A) and ingestion-like (Fig. 15B) patterns. There are no anatomic differences between the two. Dashed lines in Fig. 15 separate neurons active during retraction phase (left) and those active during the retraction phase (right). The excitatory connections between B65 and B31/32 and B63 underlie the ability of B65 to initiate patterned activity because B31/32 and B63 are critical for the initiation of buccal motor programs (Hurwitz et al. 1993, 1996; Susswein and Byrne 1988; Susswein et al. 1996). After the onset of the pattern, protraction-group neurons discharge followed by a burst in B64, which starts the retraction phase by inhibiting B31/32, B63 and B65, and activating B4/5. During a rejection-like pattern (Fig. 15A), the inhibition from B4/5 prevents firing of B8A/B during retraction phase. With repeated activation of B65, however, B4/5 fires fewer spikes, which reduces its inhibitory effects on B8A/B. Thus B8A/B now fires during retraction, and the circuit becomes physiologically reconfigured to produce ingestion-like buccal motor program (Fig. 15B).

Further studies of the modulatory abilities of B65 to alter synaptic interconnections and intrinsic properties of the elements of the buccal CPG may provide better insights into functional significance of this cell in the buccal CPG as well as into mechanisms by which this CPG may be reconfigured to produce ingestive-like feeding motor programs.

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