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An Identified Histaminergic Neuron Can Modulate the Outputs of Buccal–Cerebral Interneurons in *Aplysia* via Presynaptic Inhibition

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We have identified 2 buccal–cerebral interneurons (BCIs), B17 and B18, that appear to be involved in the coordination of feeding behavior in *Aplysia*. The BCIs have their cell bodies in the buccal ganglion, but send axons to the cerebral ganglion via the cerebral–buccal connectives. The BCIs appear to make monosynaptic connections with neurons in the cerebral ganglion that modulate extrinsic muscles involved in feeding behavior. B17 and B18 are activated antiphasically during a motor program induced by stimulating the esophageal nerve and appear to "read out" different phases of the buccal program to different cells in the cerebral ganglion. B17 and B18 are not necessary, and probably not sufficient, to generate the buccal program. These BCIs, and other cells like them in the buccal ganglion, may be capable of coordinating the activity of the *intrinsic* muscles of the buccal mass with the activity of its *extrinsic* muscles, and perhaps with those of the lips, mouth, and tentacles.

Identified histaminergic neuron, C2, can modulate the outputs of the BCIs onto their synaptic followers in the cerebral ganglion. Firing of C2 inhibits spiking of the BCIs, probably via cerebral–buccal interneurons. C2 also decreases the size of the EPSP that B17 and B18 evoke in cerebral neuron C4. C2 appears to do so monosynaptically, and it decreases the conductance of C4, ruling out one possible postsynaptic mechanism of action. Variance analysis of the EPSPs evoked by B18 supports the hypothesis that C2 acts presynaptically to decrease the release of transmitter. Applications of histamine to the solution bathing the neuron mimic the effect of firing C2 and reduce the size of the EPSPs B18 induces in C4. The bath-applied histamine appears to act directly on B18, since it elicits a voltage-dependent increased conductance hyperpolarization recorded in the soma of B18, and the hyperpolarization persists in a solution in which synaptic transmission has been blocked. Histamine did not produce any marked changes of the duration of a TEA-broadened somatic action potential of B18. To the extent that the soma of B18 reflects the membrane properties of its synaptic terminal region, the data suggest that histamine may produce presynaptic inhibition by hyperpolarizing the synaptic terminal region.

In addition to shaping the output to muscles, the neurons comprising motor pattern generators often provide information to other neurons in the nervous system. This form of information has been termed a corollary discharge (Von Holst and Mittelstaedt, 1950; Gallistel, 1980), and there are several reports suggesting that molluscan feeding motor systems may convey corollary discharges by means of interganglionic interneurons (Davis et al., 1973; Benjamin et al., 1981; Cohan and Mpitsos, 1983a, b; Jahan-Parwar and Fredman, 1983; Elliott and Benjamin, 1985; Chiel et al., 1986). In the first part of this paper we characterize 2 identified interneurons that may serve to coordinate the activity of the buccal and cerebral ganglia during feeding behavior in *Aplysia*. In the second part of the paper we explore the effects of histaminergic neuron C2 on the BCIs. We provide evidence that the outputs of the buccal–cerebral interneurons can be modulated through a mechanism of presynaptic inhibition produced by the activity of C2. Previous work has indicated that C2 is a complex mechanoafferent neuron (Weiss et al., 1986c) that makes a variety of connections to neurons involved in the modulation of feeding behavior (Chiel et al., 1986; Weiss et al., 1986b). C2 and some of its synaptic followers receive powerful excitatory and inhibitory inputs that originate in the buccal ganglion and are active during feeding motor programs (Chiel et al., 1986). The data presented in this report suggest that, in addition to its direct synaptic effects on its follower cells, C2 might also serve to modulate the buccal inputs that impinge on them. A preliminary report of these studies has appeared in an abstract (Chiel et al., 1983).

Materials and Methods

These experiments were done using *Aplysia californica* weighing 100–250 gm. The cerebral and buccal ganglia, with their connectives intact, were pinned out in a Sylgard dish and maintained at room temperature in artificial seawater (ASW) or other solutions (described below). The sheath was removed over the cell groups from which intracellular recordings were made. The recording–stimulating electrodes were double barrelled, and were filled with 2 M K-citrate. Single-barrelled electrodes were sometimes used for impaling small cells. Tip resistances ranged from 5 to 15 MΩ. Spikes in neurons were evoked by constant-current intracellular depolarizing pulses. Recording techniques were standard and are described in detail elsewhere (Rosen et al., 1982). The esophageal nerve was electrically stimulated by means of a suction electrode made of polyethylene tubing. In some experiments, the ganglia were bathed in isotonic solutions containing different concentrations of divalent cations. Unless otherwise indicated, solutions used to suppress polysynap-

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tic activity contained 60 mM calcium (6 × normal concentration) and 153 mM magnesium (3 × normal concentration). Release of transmitter from presynaptic terminals was depressed using a solution containing 0.16 times the normal concentration of calcium (1.6 mM) and 2 times the normal concentration of magnesium (102 mM) or by bathing the preparation in a solution containing 15 mM cobalt. Histamine stock solutions (10^{-1}–10^{-4} M) were made in ASW and kept on ice until they were added to the bath. The effects of histamine on action potential width were studied in preparations bathed in a solution containing 50 mM tetraethylammonium chloride (TEA). In some experiments, the cerebral and buccal ganglia were placed in separate chambers, with the connectives passed through a vaseline seal, so that histamine could be selectively applied to one ganglion. In these experiments, synaptic transmission was blocked by means of a low-Ca^{2+} (5 mM), high-Mg^{2+} (200 mM) solution containing 5 mM Co^{2+}.

Variance analysis of EPSPs was done with the aid of a PDP 11/34 computer. The buccal cell (B18) whose firing evoked EPSPs in cerebral cell C4 was used as a trigger signal for the digitization of the EPSP. For each of these experiments, 5 PSPs each were analyzed during 3 time periods: (1) 1 min before the experimental treatment, (2) during the experimental treatment (firing of cerebral cell C2 at a time when the EPSP reached steady state), and (3) 1 min after the experimental treatment. Every 90 sec, the process was repeated, for a total of 15 runs, i.e., 75 EPSPs. Mean EPSP size (v) and variance of the repeated measures of EPSP size (var v) were calculated by the computer, and estimates of the quantal size, q, and the quantal number, m, were obtained from the following equations: $m = 1/(CV)^2$, $(CV)^2 = \text{var}\ (v)/v^2$, where CV is the coefficient of variation, $q = v/m$ (Hubbard et al., 1969). Martin's correction (Martin, 1955) was not needed since cerebral cell C4 was held far from the equilibrium potential of the EPSP.

To localize buccal–cerebral interneurons (BCIs), the cerebral–buccal connective was backfilled by placing it in a small chamber containing 1 M nickel chloride, while the remainder of the buccal ganglion was bathed in ASW. After an incubation of 48–72 hr at 4°C, the nickel was precipitated by adding a solution of saturated rubeanic acid (Quicke and Brace, 1979). The structure of BCIs was studied by means of Lucifer yellow. Lucifer yellow (from Walter Stewart and Polysciences Corp.) was dissolved in distilled water (5% wt/vol), filtered, and then used to fill single-barrelled electrodes. The dye was injected using hyperpolarizing and depolarizing current pulses of 500 msec duration, with a duty cycle of 50% (Stewart, 1978). The tip resistance was continually monitored, and injection was stopped if it exceeded 100 MΩ (indicating that the tip had clogged). We were generally able to get excellent fills of small cells within 10–20 min. Tissue was fixed in either Carnoys (nickel fills) or formaldehyde (Lucifer fills), dehydrated with ethanol, and cleared with methyl salicylate or Entellan. In several experiments, cells were filled with 5(6)-carboxyfluorescein (Kodak), which generally resulted in superior visualization when unfixed ganglia were viewed. Fluorescence of injected dyes was observed using a Leitz fluorescence microscope.

Results

C2 can suppress or block EPSPs induced in a synaptic follower by cerebral–buccal connective stimulation

Previous results indicated that the buccal ganglion has unidentified neurons that provide synaptic input to follower cells of the histaminergic neuron C2 (Chiel et al., 1986). In order to determine if C2 can modulate these synaptic inputs, we first studied the effects of C2 on inputs to its excitatory follower, C4 (Chiel et al., 1986), during stimulation of the cerebral–buccal connective. Connective stimulation induced an EPSP in C4, and an IPSP in C2 (Fig. 1*A*). We found that firing C2 could reduce the size of the EPSP in C4 or block it completely when the ganglion was bathed in ASW (Fig. 1, *B1* and *2*). In order to reduce the likelihood that C2 was exerting its effect through a polysynaptic connection, we increased the threshold for polysynaptic transmission by bathing the ganglion in a high divalent cation solution (6 × Ca^{2+}, 3 × Mg^{2+}). Firing C2 continued to suppress or block the EPSP in C4 induced by connective stimulation (Fig. 1, *C1* and *2*). These studies suggested that C2 may modulate the synaptic outputs from neurons of buccal origin. In order to direclty explore the functions of these neurons, and the mechanisms by which C2 acted, we undertook to identify the putative BCIs.

Identification of BCIs

Backfills of the cerebral–buccal connective. As a preliminary to searching for BCIs, we used cobalt backfills to visualize buccal neurons that sent axons into the cerebral–buccal connective (CBC). These experiments revealed 30–50 neurons clustered in the rostral and caudal surfaces of the buccal ganglion (Fig. 2). Our studies have concentrated on 2 of these cells, located on the rostral surface of the ganglion (Fig. 2, arrows). In order to identify possible BCIs, we recorded the activity of cerebral neuron C4, while firing candidate neurons in the buccal ganglion.

Identification of BCIs B17 and B18. We have repeatedly encountered 2 BCIs, which we have designated B17 and B18, that we have characterized by (1) their morphology as revealed by dye injections, (2) their position, and (3) their characteristic synaptic actions on cerebral neuron C4. BCI B17 is located on the rostral surface of the buccal ganglion, very close to the fiber bundle that forms the buccal commissure and the radula nerve. It is generally quite close to identified buccal neurons B4 and B11. Dye fills of B17 revealed that it does not send any axons into peripheral nerves. It has a single axon, which it sends into the CBC. When B17 was fired, small facilitating EPSPs appeared in cerebral cell C4. Consistent with the relatively long distance between the cerebral and buccal ganglia, the latency of the EPSP was long, but it was constant and persisted in a high divalent cation solution, suggesting that the connection between B17 and C4 was monosynaptic (Fig. 3).

BCI B18 is also on the rostral surface of the buccal ganglion, but it is located more laterally than B17, near identified motor neuron B15. Dye fills of B18 revealed that it also has only a single axon, which it also sends into the CBC. B18 has various fine processes that extend throughout the ipsilateral buccal hemiganglion. When B18 was fired, cerebral cell C4 exhibited large EPSPs. The initial EPSP of a train was always larger than that evoked by B17, and the B18-evoked EPSPs generally did not facilitate (Fig. 4*A*). The EPSPs followed spikes in B18 with a fixed latency, and persisted in a high divalent cation solution, suggesting that the connection between B18 and C4 was also monosynaptic (Fig. 4*B*). Firing B18 also induced an IPSP in C2 (Fig. 4*B*).

B17 and B18 provide input to cerebral neurons during "feeding motor programs" but are neither necessary nor sufficient for such programs. Since B17 and B18 both excite C4 and C5, it was likely that they provided the cerebral ganglion with information regarding the generation of motor programs concerned with feeding. We next examined whether B17 and B18 are active during rhythmic activity of the buccal ganglion, and if so, what was the nature of the activity. They could have a relatively nonspecific read-out, for example, simply indicating that a buccal motor program was being expressed. Alternatively, they could transmit phase-specific information about buccal motor programs. To explore these issues, we elicited rhythmic motor programs in the isolated buccal–cerebral preparation by continuous electrical stimulation of an esophageal nerve. Our studies of this "motor program" in the isolated feeding head preparation suggested that it may be associated with rejection movements, rather than with biting or swallowing (Chiel et al., 1986; Weiss et al., 1986a). All these behaviors, however, make use of a similar set of muscles and motor neurons, and probably also share

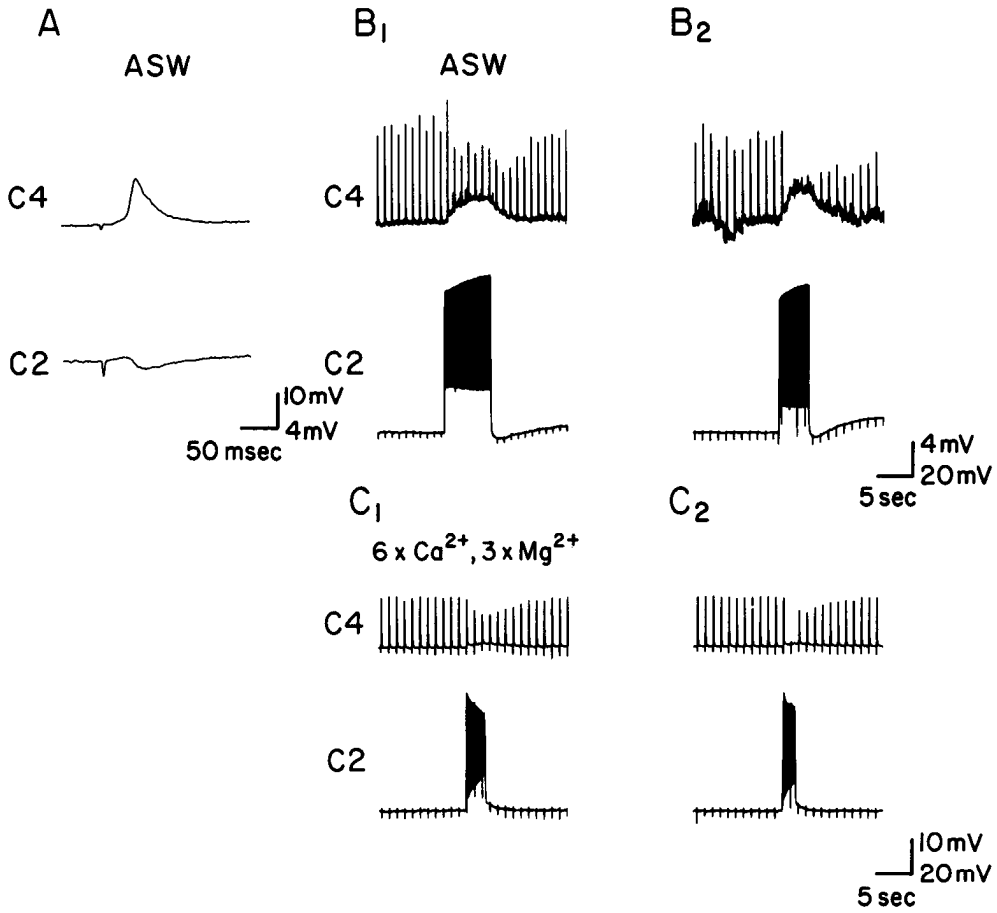

Figure 1. C2 can cause graded reductions or complete block of PSPs induced by stimulation of the CBC. *A,* Electrical stimulation of CBC induces an EPSP in identified neuron C4 and an IPSP in the histaminergic neuron C2. Intracellular recordings done in isolated cerebral ganglion bathed in ASW. Small deflection in record before PSPs is stimulus artifact. *B1,* EPSP induced by stimulating CBC at 0.5 Hz can be decreased in size by firing histaminergic neuron C2 with a steady depolarizing current. Ganglion was bathed in ASW. *B2,* EPSP induced by stimulating CBC at 0.5 Hz can be completely blocked by firing histaminergic neuron C2 with a steady depolarizing current. Ganglion was bathed in ASW. *C1* and *C2,* EPSP induced by stimulating CBC at 0.5 Hz persists in a high divalent cation solution and can be decreased in size (*C1*) or completely blocked (*C2*) by firing histaminergic neuron C2 with steady depolarizing current. Ganglion was bathed in a 6 × Ca^{2+} and 3 × Mg^{2+} solution.

elements of a central pattern generator. During stimulation of the esophageal nerve, we observed that both B17 and B18 received synaptic input during the rhythmic bursts of synaptic input to cerebral cell C4 (Fig. 5*A*), which resembled the synaptic inputs to this cell recorded during feeding behaviors in the isolated feeding head preparation (Chiel et al., 1986; Weiss et al., 1986a). The activation of the BCIs was antiphasic: B17 received excitatory synaptic input at the same time that C4 received its most powerful synaptic inputs (compare to N1 and N2 cells of *Lymnaea*; Elliott and Benjamin, 1985). B17 and C4 were then inhibited at the same time that B18 received excitatory synaptic input. These findings indicate that these 2 cells provide a highly specific "read-out" of different phases of the buccal program to cerebral cells. We further explored this idea by firing B17 or B18, or stimulating the esophageal nerve, while recording the intracellular potentials of a variety of cells in the cerebral ganglion. We found that a number of cells, such as the MCC and B cluster neurons, that did not show prominent synaptic input during the buccal program, did not receive synaptic connections from B17 and B18. On the other hand, B17 and B18 evoked one-for-one EPSPs in a variety of unidentified neurons in the E cluster, and these neurons also received strong input during the buccal program. Some cells received inputs from B17 but not B18, and vice versa. Other cells received inputs from both B17 and B18, but the inputs were of different magnitudes. The synaptic outputs of the BCIs were not solely excitatory: B18 induced an IPSP in histaminergic neuron C2 (Fig. 4*B*; see also Fig. 8, 3 × Ca^{2+}, 3 × Mg^{2+}). The data support the notion that B17 and B18 serve to "read out" different phases of the buccal program to different cerebral neurons in the E cluster.

Are B17 and B18 command neurons for a buccal ganglion program? We determined whether B17 and B18 were necessary for the buccal program to occur, and whether they were the sole source of inputs to cerebral neuron C4, by hyperpolarizing both cells simultaneously, and then stimulating the esophageal nerve. Even when B17 and B18 were hyperpolarized, C4 continued to receive excitatory synaptic inputs, indicating that it receives inputs from other BCIs (Fig. 5*B*). We also observed that the pattern of inputs to B17 and B18 was similar whether they fired action potentials or were prevented from firing by means of hyperpolarizing current. B17 was excited and then inhibited, at which time B18 was excited (Fig. 5*B*). These results indicate that neither B17 nor B18 is necessary for the buccal program to occur. To determine if B17 and B18 were sufficient to induce a buccal program, we fired each one in turn. Firing of B17 or B18 did not induce a rhythmic burst of synaptic inputs to B18 or any other cell, although steady depolarization of B17 sometimes resulted in two to four irregular bursts of synaptic inputs to B17, with concomitant synaptic inputs to C4. Thus B17 and B18 fail both the necessary and sufficient criteria for being command neurons (Kupfermann and Weiss, 1978) for the rhythmic feeding activity, but we cannot preclude the possibility that they are part of a larger command system or a central motor pattern generator.

Histaminergic neuron C2 can modulate outputs of BCIs that impinge on its synaptic follower cells

Having identified BCIs B17 and B18, it was possible to test whether C2 was capable of modulating specific buccal inputs to its synaptic follower cells. We slightly depolarized buccal cell

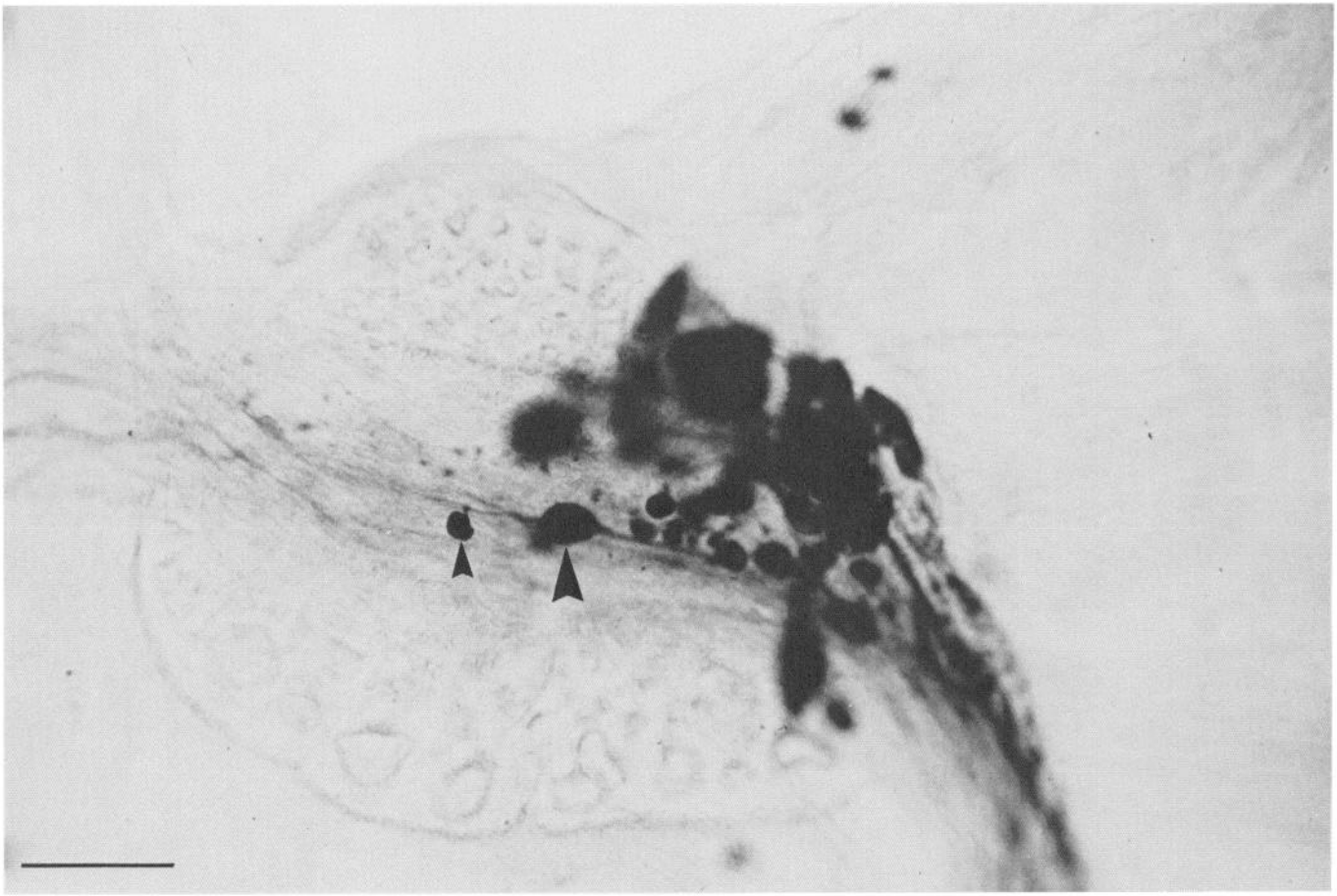

Figure 2. Nickel backfills of the CBC reveal the position of buccal neurons with axons in the connective. The right CBC was backfilled with nickel chloride. This is a view of the right rostral surface. The cell group extends to the caudal surface. *Arrows* indicate the position of B17 (*small arrow*) and B18 (*large arrow*). Scale bar, 250 μm.

B17, so that it fired continuously, and monitored its output by recording from cerebral neuron C4. We then examined the effects of firing the histaminergic neuron C2 on the firing of B17 and on its synaptic output to C4. When C2 was fired, the firing of B17 slowed or stopped, and the EPSPs it evoked in C4 diminished in size (Fig. 6*A*). When B17 was slightly hyperpolarized to prevent it from firing spontaneously, fast small potentials riding on a slow hyperpolarization were observed as C2 was fired (Fig. 6*B*). Since C2 does not send an axon into the CBC, the synaptic inhibition of B17 may be due to one or more interneurons located in the cerebral ganglion that send axons into the connective. The reduction in the size of the EPSPs in C4 evoked by firing B17 is partly due to the reduction in the rate of firing of B17 since the EPSP evoked by B17 strongly facilitates (Fig. 3), and a lengthening of the interspike interval results in greater decay of facilitation. As shown in the following section, the reduction of the EPSP is also likely to be due to a presynaptic action of C2.

The effects of C2 on the EPSPs evoked in C4 when BCI B18 was fired were similar to those seen with B17. When C2 was fired, the firing of B18 slowed and stopped, and the EPSPs it evoked in C4 diminished in size (Fig. 7). In contrast to B17, the diminution in the size of the EPSP that B18 evokes in C4 cannot be explained as a decrease in facilitation, since the EPSP evoked by B18 does not facilitate under these conditions (Fig. 4). Furthermore, C2 reduced the EPSP even when B18 was fired at a constant frequency by means of brief depolarizing pulses (Fig. 9). Firing of C2 also reduced the EPSPs produced by B18 onto C5 (Fig. 8). In addition, we observed that the IPSP evoked in C2 by B18 is reduced for several seconds after C2 fires (Fig. 8, lower trace of second set of traces). Although we have not studied this phenomenon in detail, this reduction does not appear to be due to a conductance increase that is associated with the long-lasting afterpotential seen in C2. In fact, the depolarizing afterpotential in C2 appears to be associated with a conductance decrease (unpublished observations). Furthermore, the IPSP is reduced at a time when the depolarizing afterpotential has returned to baseline. We suspected that the histaminergic neuron C2 might be acting presynaptically to reduce the release of transmitter from the terminals of B18 and B17, and thereby reduce the size of the EPSP they evoked. Because of experimental advantages, we chose to focus on the connection of B18 (which does not facilitate when fired) to C4 (which does not show an increase in conductance when C2 is fired).

Histaminergic neuron C2 may modulate outputs of BCI B18 on cerebral neuron C4 by presynaptic inhibition

Physiological evidence. Since the reduction of the B18 EPSP following activity of C2 cannot be explained by antifacilitation, it is likely to be due to either a postsynaptic shunting of the

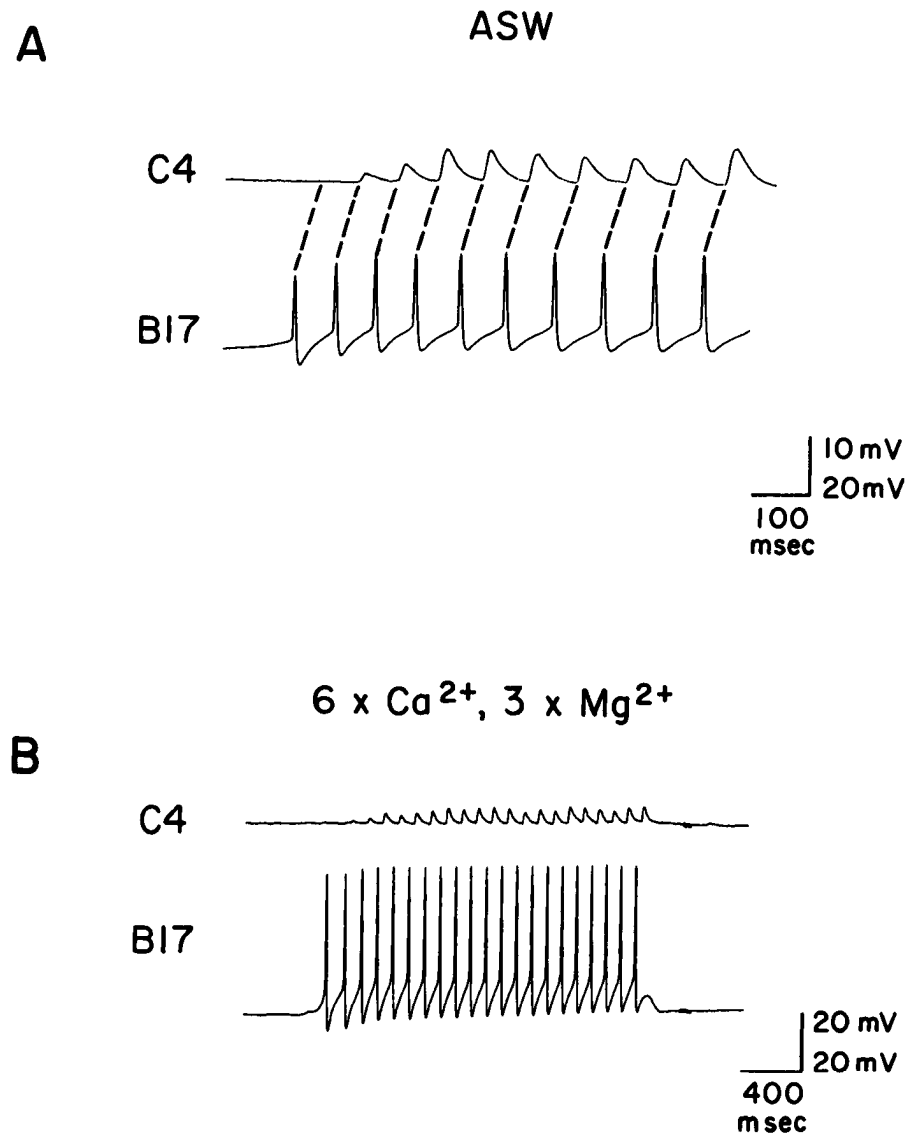

Figure 3. BCI B17 produces one-for-one facilitating EPSPs in cerebral neuron C4. *A*, Buccal and cerebral ganglia were bathed in ASW. B17 was fired by a steady depolarizing current. EPSPs in C4 facilitate and follow spikes in B17 with a fixed latency. *B*, Buccal and cerebral ganglia were bathed in a 6 × Ca^{2+}, 3 × Mg^{2+} solution. Firing B17 still induced one-for-one EPSPs in C4.

EPSP on the follower cell or to a presynaptic action of C2 on the terminals of B18. To test whether C2 was acting through an interneuron, we bathed the ganglia in a high divalent cation solution (6 × Ca^{2+}, 3 × Mg^{2+}), and fired B18 at a steady rate (1 Hz). Since a high divalent cation solution suppresses polysynaptic activity, it should prevent C2 from reducing the EPSP in C4 due to firing B18 if C2 acts via an interneuron. We found, however, that C2 was still capable of reducing the size of the EPSP (Fig. 9).

The possibility of postsynaptic shunting was tested by examining the effects of C2 on the conductance of C4. Using the paradigm described in the previous section, we confirmed that firing of C2 reduces the size of the EPSP in C4 evoked by firing B18 (Fig. 10*A*). Then holding C4 at the same potential, we measured the conductance of C4 by means of constant-current hyperpolarizing pulses and fired C2 again. The hyperpolarizing potentials did not decrease in size, as they should if C2 had increased the conductance of C4. If anything, they increased in size, suggesting that C2 decreased the conductance of C4, which should have increased the size of the EPSPs (Fig. 10*B*). These results are not consistent with a postsynaptic mechanism but are not conclusive, since C2 could still be acting on C4 by a postsynaptic mechanism other than increased conductance. For example, it could decrease the sensitivity of the postsynaptic receptors of C4 to the transmitter released by B18, and thereby diminish the size of the EPSP evoked by B18. In order to further explore this question, we turned to the method of quantal analysis.

Variance analysis. The most accurate techniques of quantal analysis (histogram analysis, failure analysis; see Dudel and Kuffler, 1961) require low release conditions (Hubbard et al., 1969), but in such conditions the release of transmitter by the histamine cell is also lowered, and its effect is diminished. We therefore chose to use a less accurate form of quantal analysis, the variance method. If a treatment or chemical acts primarily on the postsynaptic membrane, it should affect the estimate of the size of each quantum of transmitter released, q; if it acts primarily on the presynaptic membrane, changing the number of quanta released, it should affect the estimate of the number of quanta released, m (Hubbard et al., 1969). The general design of our experiments is illustrated in Figure 9 and described under Materials and Methods. The cerebral and buccal ganglia were bathed

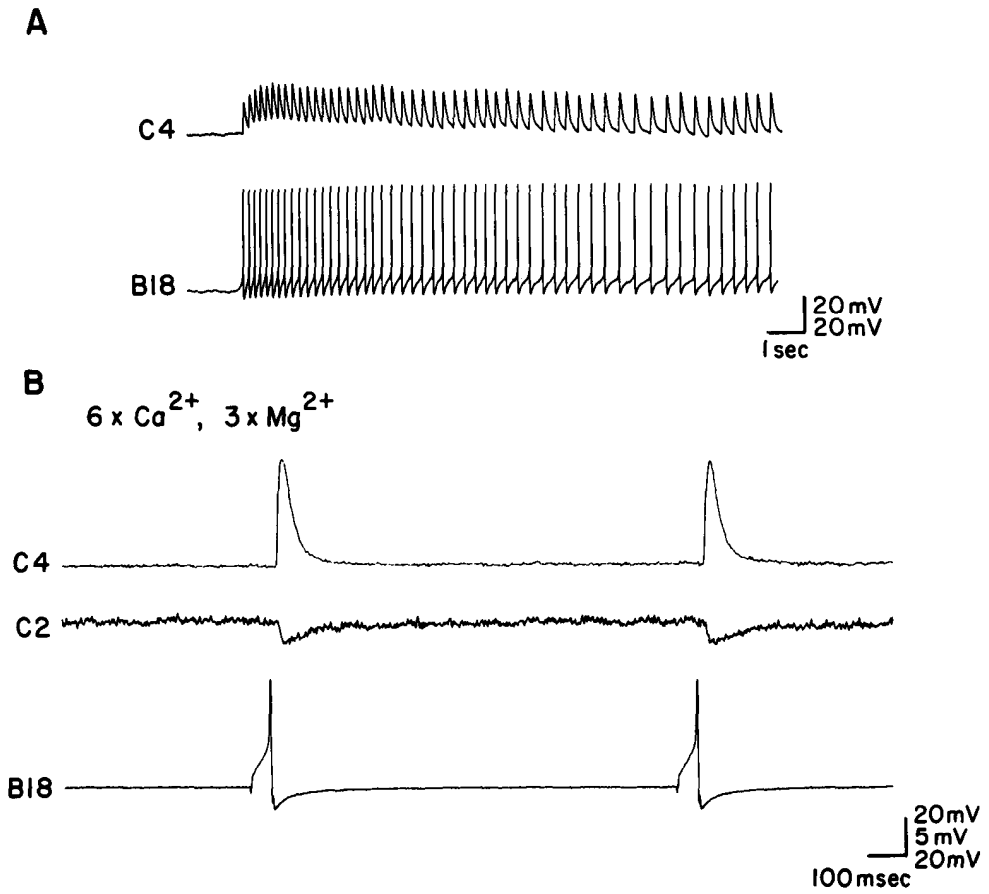

Figure 4. BCI B18 produces one-for-one EPSPs in cerebral neuron C4 which do not facilitate. *A*, Buccal and cerebral ganglia were bathed in ASW. B18 was fired with a steady depolarizing current. The EPSPs induced in C2 follow spikes in B18 with a fixed latency. The EPSPs summated but did not facilitate. *B*, Buccal and cerebral ganglia were bathed in a 6 × Ca^{2+}, 3 × Mg^{2+} solution. B18 was fired once every second by a brief depolarizing current pulse while the potentials of cerebral neurons C2 and C4 were monitored. Note that B18 evoked an IPSP in histaminergic neuron C2.

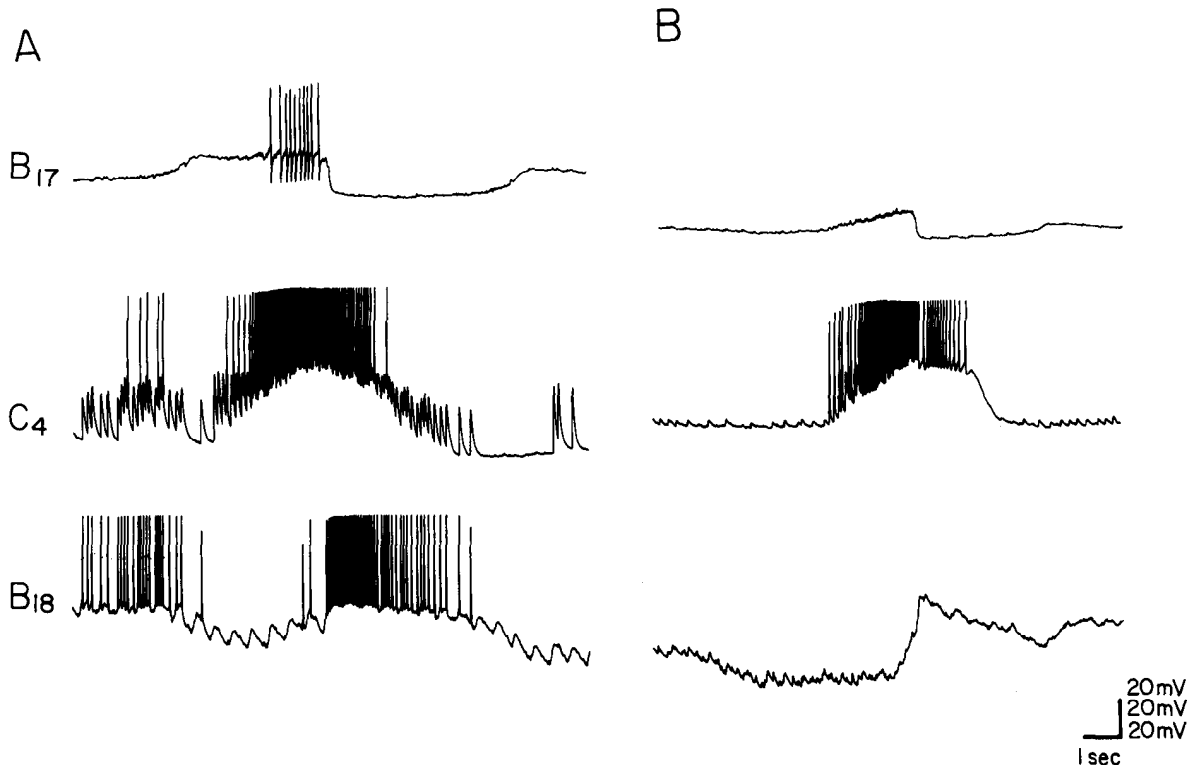

Figure 5. BCIs B17 and B18 are activated during "feeding motor programs." *A*, Stimulation of the esophageal nerve at a rate of 2 Hz induced a rhythmic burst of synaptic activity in buccal cells B17 and B18 and cerebral cell C4. Note that B17 and B18 are activated antiphasically. *B*, Both B17 and B18 were hyperpolarized before the esophageal nerve was stimulated. During nerve stimulation, cerebral cell C4 continued to receive synaptic input, suggesting that other buccal cells also provide input to it. Note the antiphasic excitation of B17 and B18.

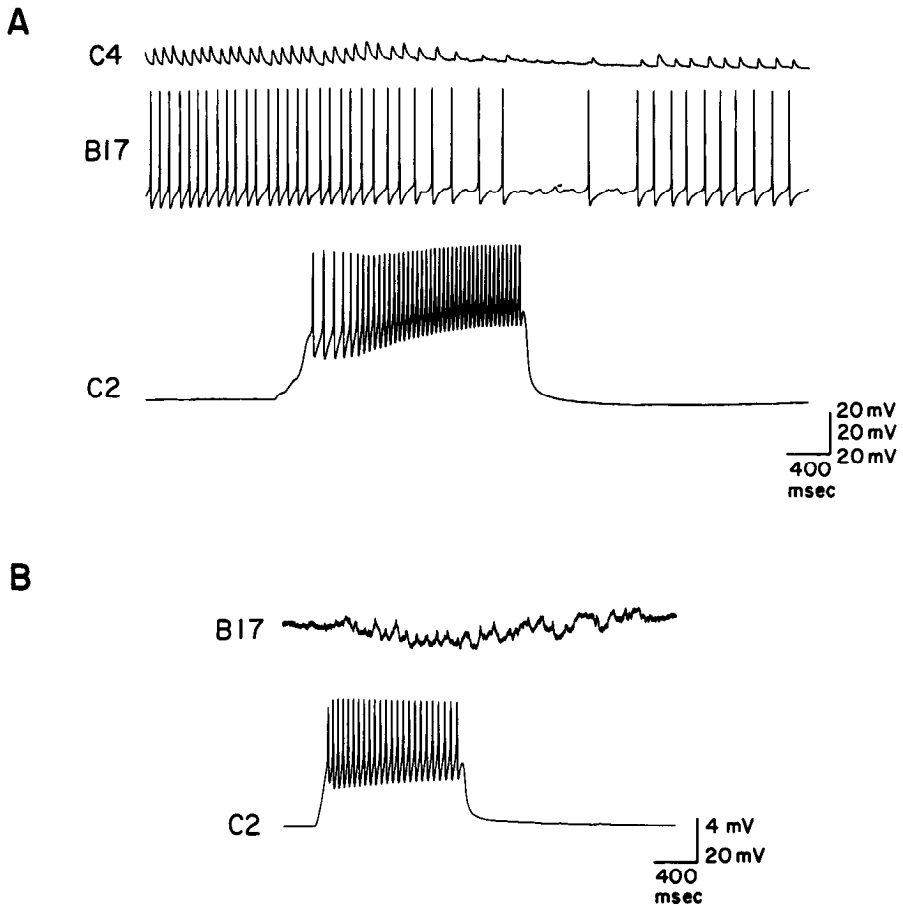

Figure 6. Histaminergic neuron C2 inhibits firing of BCI B17 and blocks its inputs to cerebral neuron C4. *A*, B17 was firing spontaneously at a steady rate. C2 was fired by a steady depolarizing current. B17 stopped firing, and the EPSPs it evoked in C4 diminished in size. *B*, High-gain recording of the potential in B17 during the firing of C2. B17 was slightly hyperpolarized. C2 was fired by a steady depolarizing current. Firing of C2 induced a slow IPSP in B17.

in a high divalent cation solution to reduce polysynaptic activity in C4, and for 15 cycles, periods of rest were alternated with firing of C2. A total of 75 PSPs, elicited by firing B18 and recorded in C4, were analyzed from those that occurred before C2 was fired, 75 were analyzed from those that occurred while C2 was fired, and 75 were analyzed from those that occurred after C2 was fired. We found that q, the estimate of the size of each quantum, was not significantly changed as C2 was fired, but that m, the number of quanta released, was significantly reduced ($p < 0.01$; Fig. 11). The experiment was repeated for a total of 9 independent trials, varying the levels of release by bathing the ganglia in solutions that increased transmitter release (6 × Ca^{2+}, 3 × Mg^{2+}), had no effect (ASW), or decreased transmitter release (0.16 × Ca^{2+}, 2 × Mg^{2+}). In all of the experiments we found that during the firing of C2, m, the quantal number, decreased, while the changes in q were small and inconsistent (Fig. 12). Thus, over a 75-fold variation in transmitter release, firing of C2 appeared to decrease the number of quanta released, supporting our hypothesis that it acts presynaptically.

Pharmacological evidence. Our physiological studies supported the hypothesis that C2 modulated the EPSPs in C4 from buccal cell B18 through presynaptic inhibition. Since consid-

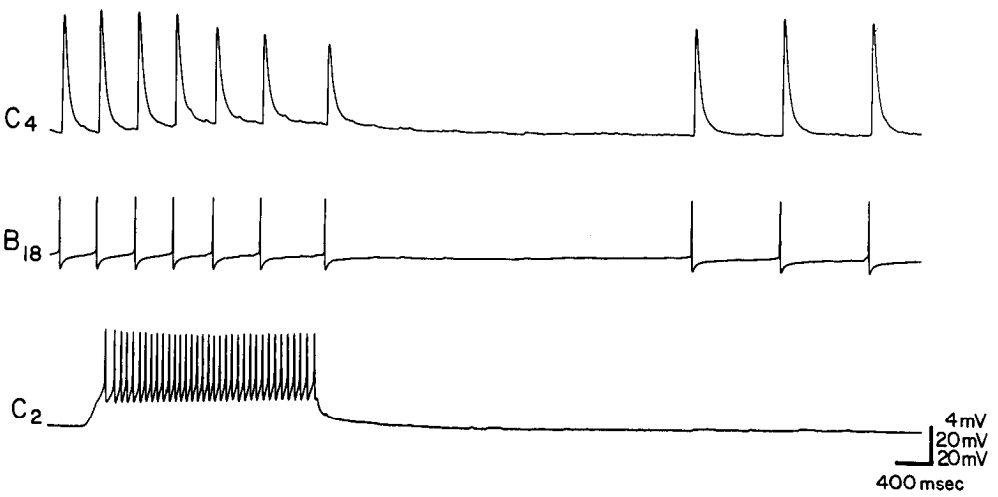

Figure 7. Histaminergic neuron C2 inhibits firing of BCI B18 and blocks its inputs to cerebral neuron C4. Buccal neuron B18 was firing spontaneously at a steady rate. C2 was fired by a steady depolarizing current. Note that the EPSPs evoked in C4 were decreasing in size before B18 stopped firing. Also note that EPSPs in C4 (*top trace*) are at a higher gain than the action potentials in C2 and B18.

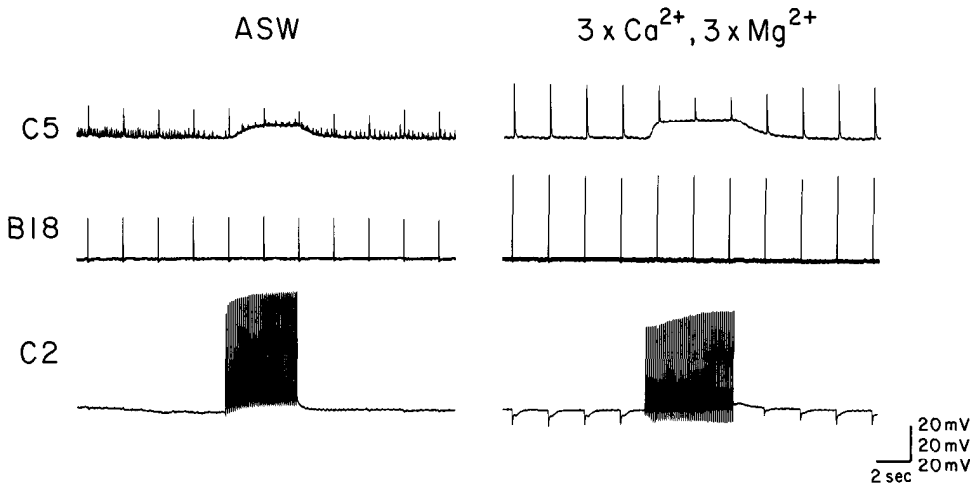

Figure 8. Histaminergic neuron C2 decreases the size of the EPSP in C5 due to firing of B18 in sea water and in a solution of increased divalent cations ($3 \times Ca^{2+}$, $3 \times Mg^{2+}$). B18 was fired with brief depolarizing current pulses. Note that the EPSP in C5 due to firing of B18 becomes smaller as C2 is fired and that the IPSP in C2 due to firing of B18 also becomes smaller.

erable evidence indicates that C2 is histaminergic (Weinreich, 1977; McCaman and Weinreich, 1982, 1985; Schwartz et al., 1986), we examined the actions of bath-applied histamine on the connection between B18 and C4. B18 was fired at a rate of 5 Hz in a high divalent cation solution, used to reduce polysynaptic effects, and the resulting EPSPs in cerebral neuron C4 were recorded. In addition, the conductance of C4 was measured by injecting pulses of hyperpolarizing current (Fig. 13, *A1* and *2*). Histamine (10^{-4} M) perfused over the buccal and cerebral ganglia reversibly depolarized C4 and reduced the size of the EPSPs in C4 from B18. At the same time, it decreased the conductance of C4 (Fig. 13, *B1* and *2*, *C1* and *2*). These effects were similar to those of firing neuron C2 (see Figs. 7 and 8). Thus, the effect of histamine cannot be explained by a change in the conductance of C4. When the histamine was washed out of the bath, the EPSP in C4 and conductance of C4 partially recovered (Fig. 13, *C1* and *2*).

Effect of histamine on the cell body of B18. The cell bodies of molluscan neurons appear to have receptors and channels similar to those of the presynaptic terminal (Geduldig and Gruener, 1970; Stinnakre and Tauc, 1973; Shimahara and Tauc, 1975; Klein and Kandel, 1978). Consequently, we recorded from the cell body of B18 and applied histamine to the bath. B18 was impaled with a double-barrelled electrode and was slightly hyperpolarized to reduce spontaneous activity. Hyperpolarizing pulses were injected into it in order to measure its conductance. The addition of histamine to the bath (final concentration, 10^{-4} M) produced a sustained hyperpolarization and an increase of input conductance of B18 (Fig. 14*A1*). Histamine hyperpolarized B18 even when the bathing solution contained 15 mM cobalt, which would block chemical synaptic transmission of possible interneurons activated by histamine (Fig. 15). When the buccal and cerebral ganglia (with a connective intact) were placed in separate chambers containing low calcium (5 mM), high magnesium (200 mM), and cobalt (5 mM), application of histamine (n = 3) to the buccal chamber also resulted in a

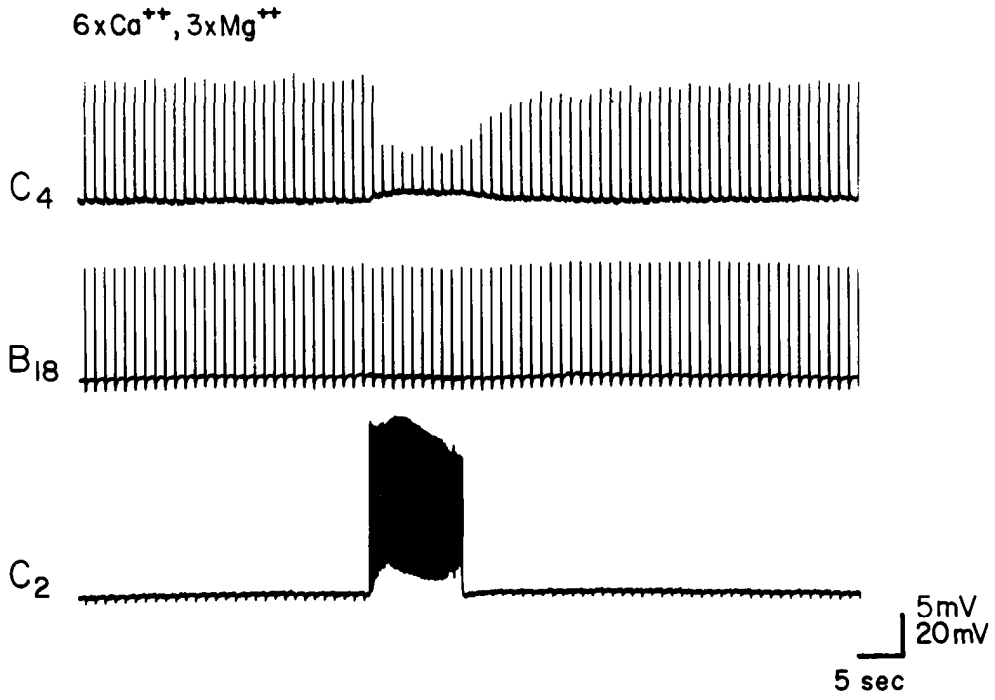

Figure 9. Neuron C2 decreases the size of the EPSP in C4 evoked by firing BCI B18 in the presence of high divalent cation concentration. Buccal and cerebral ganglia were bathed in a $6 \times Ca^{2+}$, $3 \times Mg^{2+}$ solution. B18 was fired once every second by individual depolarizing current pulses. C2 was fired at 15 Hz for 10 sec by individual depolarizing pulses.

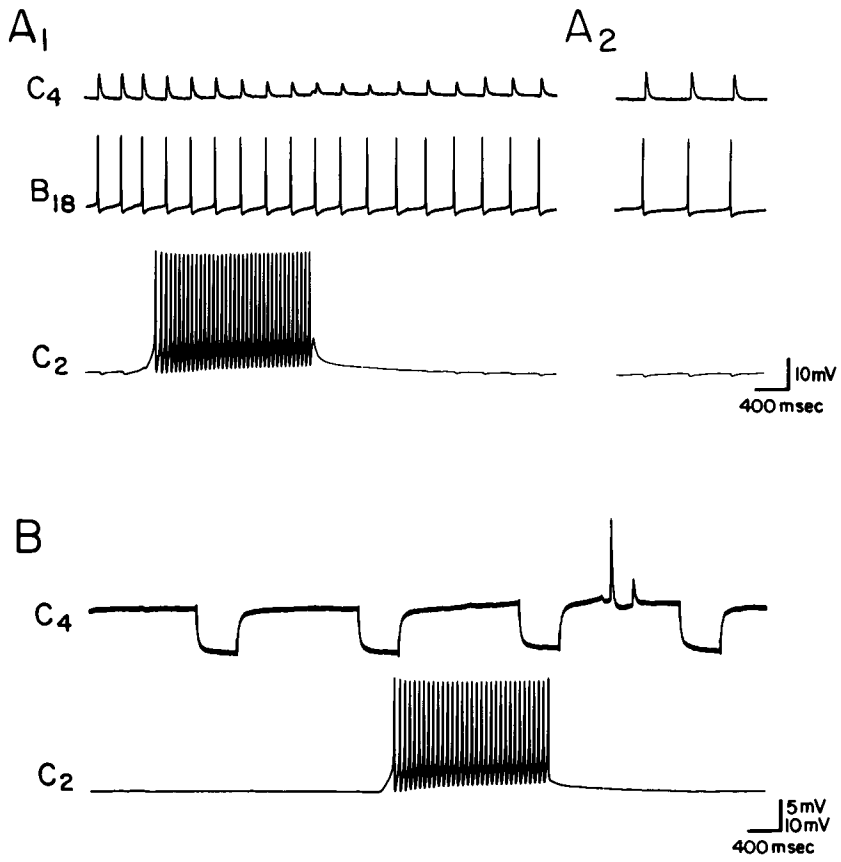

Figure 10. Histaminergic neuron C2 decreases the size of the EPSP in C4 due to firing of BCI B18 but does not do so by increasing the conductance of C4. *A1,* B18 was firing spontaneously at a steady rate. C2 was fired by a steady depolarizing current. C2 decreased the size of the EPSP in C4 evoked by the firing of B18. *A2,* Recovery in size of the EPSP in C4 10 sec after C2 was fired. *B,* Same preparation as in *A.* C4 was held at the same potential, and small hyperpolarizing membrane voltage deflections were produced by constant-current pulses. C2 was fired by a steady depolarizing current. Firing C2 did not decrease the size of these voltage deflections; in fact, it slightly increased their size, suggesting that C2 may have decreased the conductance of C4.

hyperpolarization of B18. Addition of histamine to the cerebral chamber, however, did not result in a hyperpolarization that could be recorded in the soma of B18. This does not imply that the B18 terminals located in the cerebral ganglion were not hyperpolarized by histamine, since the terminals of B18 may be too far from the soma for the membrane potential change to be transmitted by electrotonic spread.

The data indicate that the effect of bath-applied histamine appears to be directly on B18. The measured change of conductance may be partly due to anomalous rectification, since when we hyperpolarized B18 by the amount it had been hyperpolarized by the bath-applied histamine (15 mV), a similar increase in the conductance of B18 was observed (Fig. 14*A2*). When B18 was hyperpolarized by 15 mV, bath-applied histamine had little or no effect on membrane potential (Fig. 14*B*), but it was not possible to reverse the histamine-evoked potential with further hyperpolarization. The decreased responsiveness of B18 to histamine when C2 was hyperpolarized was not due to desensitization, since when the membrane potential was returned to its original value, bath-applied histamine, once again, caused a hyperpolarization (Fig. 14*C*). These results suggest that the histamine-evoked conductance may be voltage dependent.

Is histamine acting by narrowing B18's action potential? Does bath-applied histamine reduce the amount of transmitter released by B18 by narrowing its action potential or by reducing the voltage-dependent influx of calcium? We explored this question by bathing B18 in a solution containing 50 mM TEA, which blocks the delayed rectifying potassium channel, and thus broadens the action potential. The duration of a TEA-broadened spike typically is sensitive to the magnitude of the voltage-dependent calcium current. Neuron B18 was fired with intra-

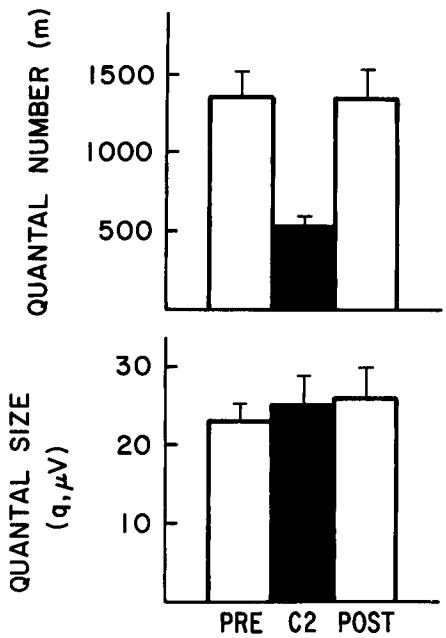

Figure 11. Variance analysis suggests that histaminergic neuron C2 acts presynaptically to decrease the size of EPSPs in C4 evoked by firing BCI B18. An example of the raw data and the description of the experimental paradigm are given in Figure 9. See text for method by which quantal number, *m,* and quantal content, *q,* were estimated. Illustrated here is the analysis of 75 EPSPs (15 repetitions of 5 EPSPs as in the procedure of Fig. 9) before, during, and after firing of C2. The estimate of the number of quanta released by B18 (*m*) is significantly decreased ($p < 0.01$) while C2 is fired; in contrast, the estimate of the size of each quantum released (*q*) is not significantly changed. These results suggest that C2 acts presynaptically to reduce the release of transmitter from the terminals of B18.

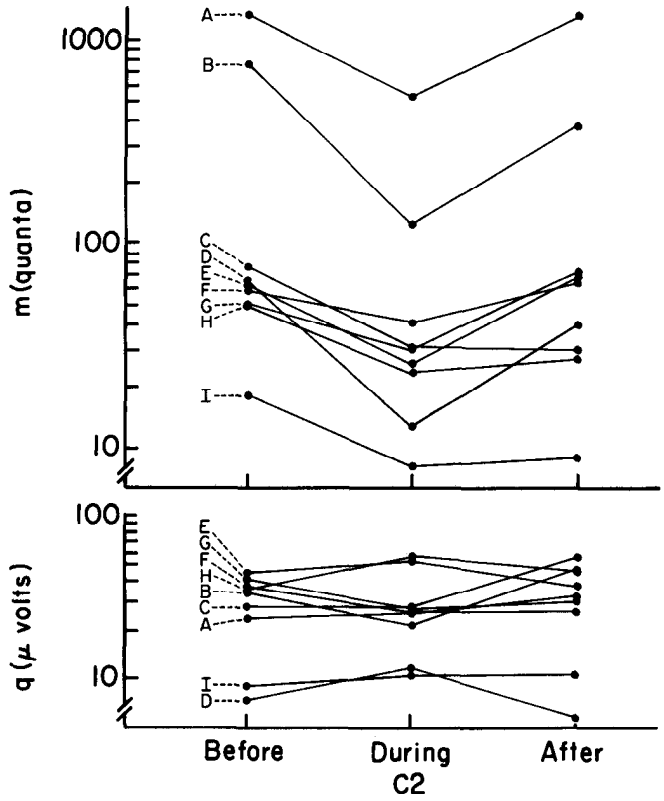

Figure 12. Variance analysis over a wide range of release levels suggests that C2 acts presynaptically to decrease the size of EPSPs in C4 evoked by firing BCI B18. Quantal number, *m*, and quantal content, *q*, were calculated as described in the text and were both plotted on a logarithmic scale. In experiments *A* and *B*, cerebral and buccal ganglia were bathed in a 6 × Ca^{2+}, 3 × Mg^{2+} solution; in experiments *C–H*, ganglia were bathed in ASW; and in experiment *I*, ganglia were bathed in a 0.16 × Ca^{2+}, 2 × Mg^{2+} solution. Note that release, as estimated by *m*, varies 75-fold over the 9 experiments but that firing C2 decreases *m* in each experiment. Also note that firing C2 has no consistent effect on the estimated size of *q*.

cellular depolarizing current at a rate of 0.1 Hz (Fig. 16, *A* and *B1*). It was not possible to monitor the effects of histamine on the EPSP in C4 evoked by firing B18, because the EPSP was blocked by the TEA [TEA is known to block certain cholinergic responses (Kehoe, 1985)]. Adding histamine to the bath (50 μl 10^{-3} M to a 1 ml volume) caused a hyperpolarization of B18 and a depolarization of C4 (Fig. 16*A*). The hyperpolarization was associated with a block of the action potential in B18 (Fig. 16*B2*). Increasing the strength of the depolarizing current injected into B18 did not overcome the block. As B18 repolarized, the action potential returned (Fig. 16, *B3, B4, B5*), and, when the stimulus strength was reduced to control levels, the action potential returned to the size and width it had had before the addition of histamine (Fig. 16*B6*). In this, and 3 other experiments in which histamine was added to a bath containing TEA, no obvious narrowing of the action potential was observed during the hyperpolarization induced by histamine. In these experiments, it appeared that the increase of spike threshold and reduced spike height might have been due, at least in part, to the hyperpolarization induced by histamine. Hyperpolarization of B18 by intracellular current reduced the peak height of the action potential and delayed (Fig. 17*C*) or completely blocked (Fig. 17*B*) its initiation; depolarizing B18 (Fig. 17*D*) had the opposite effects.

Discussion

The following discussion focuses on 3 issues: (1) coordinator neurons in distributed motor systems; (2) evidence that C2 presynaptically inhibits buccal coordinator cell B18; and (3) the possible role of presynaptic inhibition produced by activity of C2.

Role of coordinator neurons

In the present paper, we identified a pair of interganglionic interneurons, B17 and B18. These 2 neurons are part of a cluster of neurons located in the buccal ganglion and send axons to the CBC. In confirmation of Fiore and Geppetti (1985), we have found that most of these neurons do not make synaptic connections with known neurons in the cerebral ganglion. Therefore, B17 and B18 appear to be unique, although it is not unreasonable to expect that other BCIs make synaptic connections that have thus far escaped detection. The map of buccal neurons backfilled from the CBC by Fiore and Geppetti (1985) does not appear to show B17 and B18, but they describe cells only on the caudal surface of the buccal ganglion. We find backfilled cells on both the caudal and rostral surfaces, and B17 and B18 are located on the rostral surface. In terms of the overall configuration of filled neurons, our map confirms theirs.

Neurons B17 and B18 exhibit clear bursts of activity in phase with motor programs in the buccal ganglion. Interneurons that are activated in association with motor programs have been termed corollary discharge or efference copy neurons (Davis, 1973; see also Von Holst and Mittelstaedt, 1950; Gallistel, 1980; Camhi, 1984). Although the neurons we have identified have features of corollary discharge neurons, we prefer to refer to them with the functionally neutral term BCIs (see Cohan and Mpitsos, 1983b). The cell bodies of these neurons are located in the buccal ganglion, but they send their axons to the cerebral ganglion, where they produce synaptic effects on various follower neurons. The synaptic output of the BCIs is suppressed by firing the histaminergic neuron C2, and we provide evidence that this is a result of a presynaptic action of C2 on the BCIs.

Interneurons that appear to coordinate motor activity between ganglia have been described in a number of invertebrates (e.g., Stein, 1971; Stent et al., 1978; Weeks and Kristan, 1978; Pearson et al., 1980; Gillette et al., 1982; Robertson and Moulins, 1984; Elliott and Benjamin, 1985; Nusbaum and Kristan, 1986), and various functions have been ascribed to such neurons, such as commanding specific behaviors (e.g., Weeks and Kristan, 1978; Pearson et al., 1980; Davis et al., 1983; Nusbaum, 1986) or phase coordination of the output from a chain of similar ganglia (Stein, 1971; Stent et al., 1978). It has also been suggested that interganglionic interneurons activated during a particular behavior may inhibit the expression of other, unrelated behaviors (Gillette et al., 1982; Davis et al., 1983). The BCIs we have described in *Aplysia* are likely to be involved in yet another type of function for interganglionic neurons: coordination of dissimilar, but behaviorally related motor acts (see e.g., Cohan and Mpitsos, 1983a; Davis et al., 1984; Robertson and Moulins, 1984). This conclusion derives from a number of observations: (1) the cerebral and buccal ganglia are very different anatomically and functionally, and (2) the BCIs exhibit distinct patterns of activity linked to motor programs that drive buccal muscles, and they make synaptic connections to neurons that appear to be involved in lip and tentacle movements associated with feeding (Chiel et al., 1986).

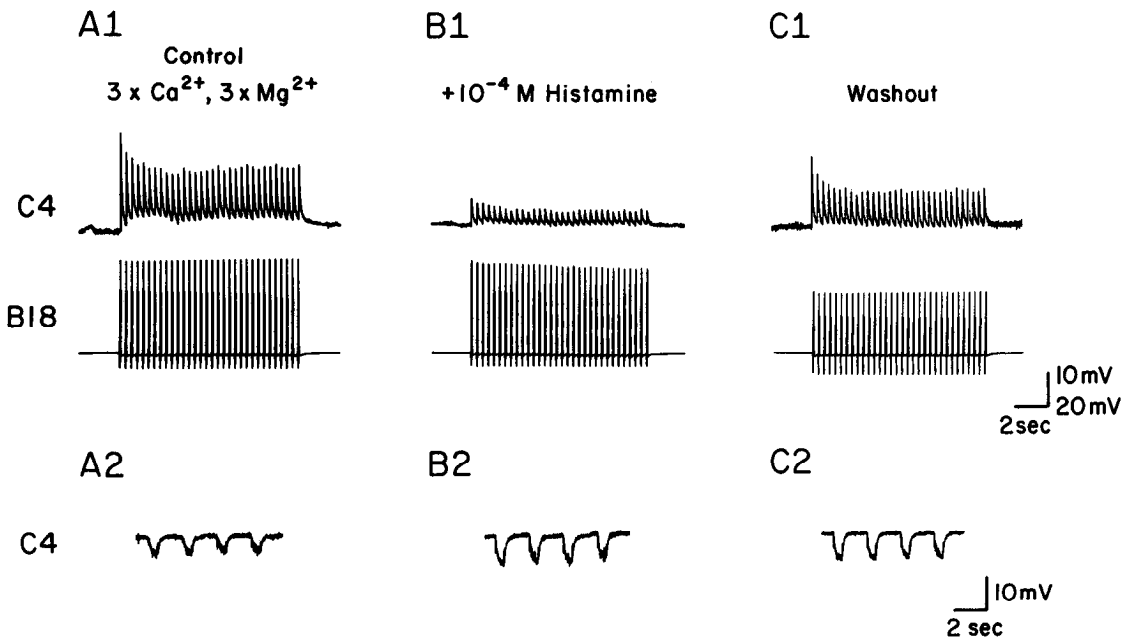

Figure 13. Bath application of histamine reduces size of EPSPs which buccal neuron B18 induces in cerebral neuron C4. *A1,* B18 was fired at 5 Hz with intracellular depolarizing current, inducing EPSPs in C4. The buccal and cerebral ganglia were bathed in a 3 × Ca^{2+}, 3 × Mg^{2+} solution. *A2,* Conductance of C4 was measured by injecting hyperpolarizing current pulses of 0.5 sec duration at a rate of 0.5 Hz. *B1,* Histamine (10^{-4} M) was perfused over the ganglia. Note the decrease in the size of the EPSPs induced in C4 by B18. *B2,* Histamine decreased conductance of C4, as measured by hyperpolarizing current pulses. Thus, the decrease in the size of the EPSP in C4 was unlikely to be due to a shunting effect of histamine. *C1,* Partial recovery of the size of the EPSP was seen after histamine was washed out. *C2,* Partial recovery of the conductance of C4 was seen after histamine was washed out.

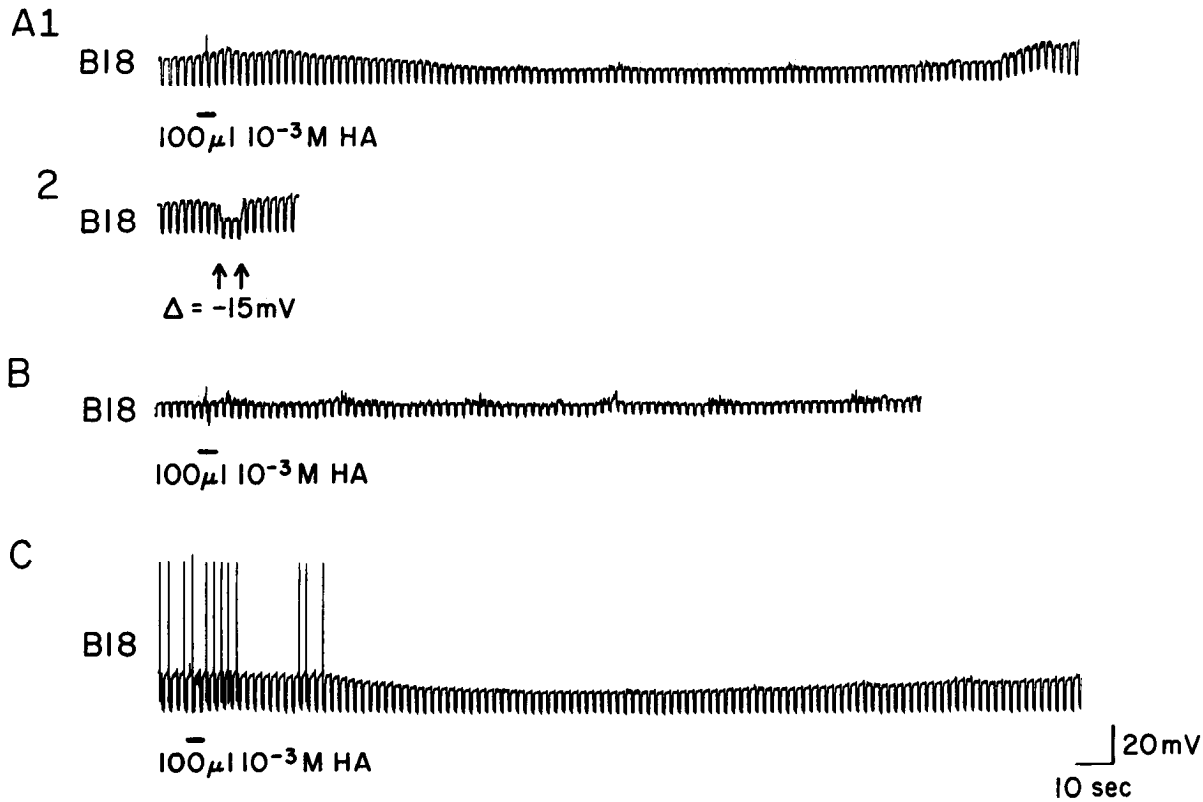

Figure 14. Bath application of histamine induces a voltage-sensitive increased conductance IPSP in buccal neuron B18. The conductance increase may be due, in part, to anomalous rectification. *A1,* Histamine (100 μl 10^{-3} M) was added to a 1 ml bath. The conductance of B18 was measured by intracellular injection of 0.5 sec hyperpolarizing current at a rate of 0.5 Hz. Histamine caused a long-lasting hyperpolarization of B18, during which its conductance appeared to increase. The ganglion was bathed in ASW. *A2,* Hyperpolarizing B18 by 15 mV (the amount it was hyperpolarized by histamine) also caused a conductance increase, suggesting that at least part of histamine's effect was due to anomalous rectification. *B,* Hyperpolarizing B18 eliminates the hyperpolarizing effect of bath-applied histamine. *C,* B18 was then returned to its previous potential, and histamine again induced a long-lasting hyperpolarization of the neuron.

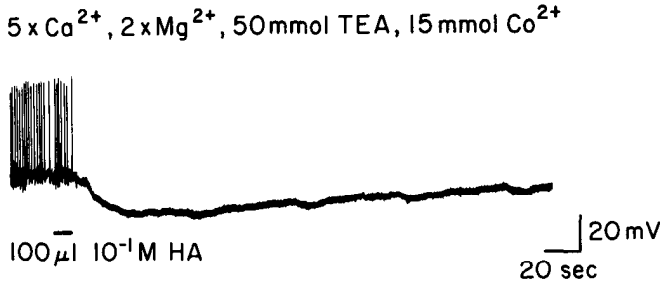

Figure 15. Bath application of histamine induces an IPSP in B18 during complete block of synaptic transmission. The buccal ganglion was bathed in a high divalent cation solution (5 × Ca^{2+}, 2 × Mg^{2+}), 50 mM TEA, and 15 mM Co^{2+} (which blocks synaptic transmission). Histamine (100 µl 10^{-1}) was added to a 1 ml bath and induced a long-lasting hyperpolarization of B18.

Previous evidence suggested that the feeding-related motor programs expressed in the cerebral ganglia of *Aplysia* (Jahan-Parwar and Fredman, 1983; Chiel et al., 1986) and perhaps other gastropods (Cohan and Mpitsos, 1983b, but see Davis et al., 1984) are largely or exclusively driven by a central pattern generator located in the buccal ganglion. Buccal–cerebral neurons B17 and B18 may be important elements that permit the buccal ganglion to drive feeding-related motor programs in the cerebral ganglion. The exact nature of the "feeding" program associated with activity of B17 and B18 remains to be determined, but it has been shown that their follower cells in the cerebral ganglion appear to be active during both ingestion and egestion feeding motor programs (Chiel et al., 1986).

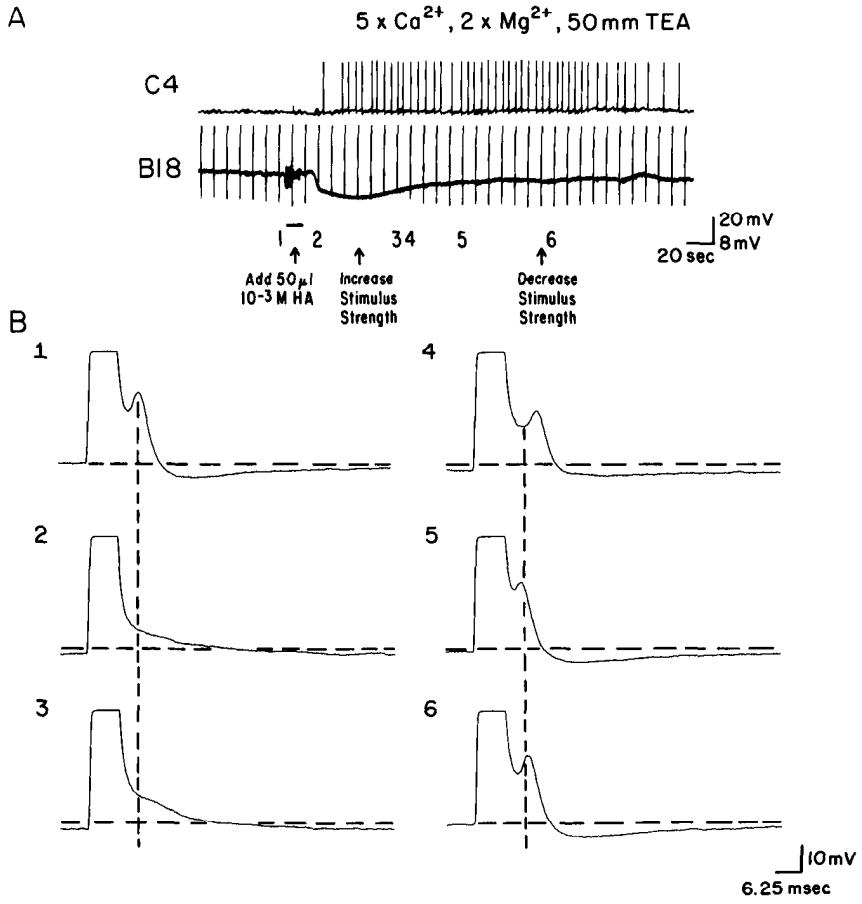

Figure 16. The IPSP in B18 induced by bath application of histamine may block the action potential in B18 but does not appear to be associated with a narrowing of the action potential. *A,* Buccal and cerebral ganglia were bathed in a high divalent cation solution (5 × Ca^{2+}, 2 × Mg^{2+}), containing 50 mmol TEA. Neuron B18 was fired with intracellular depolarizing currents at a rate of 0.1 Hz. TEA blocked the EPSP that B18 oridinarily induced in neuron C4. Histamine (50 µl 10^{-3} M) was added to a 1 ml bath, and induced a long-lasting hyperpolarization of B18. Note that it also caused a long-lasting depolarization of C4, which fired action potentials. *B1,* Expanded record of action potential in B18 (numbered *1* in *A*) before addition of histamine. The *horizontal dotted line* marks the potential of B18 before stimulation; the *vertical dotted line* marks the peak of the action potential. *B2,* Expanded record of action potential in B18 (numbered *2* in *A*) 20 sec after addition of histamine. The neuron hyperpolarized, and the action potential was blocked. The strength of the depolarizing intracellular current in B18 was increased, but the block was not overcome (see *arrow* in part *A*). *B3,* Expanded record of action potential in B18 (numbered *3* in *A*) 70 sec after addition of histamine. B18 was repolarizing, and a very small action potential could be seen after stimulation of the neuron. *B4,* Expanded record of action potential in B18 (numbered *4* in *A*) 80 sec after addition of histamine. B18 continued to repolarize, and an action potential could now be evoked by stimulation. *B5,* Expanded record of action potential in B18 (numbered *5* in *A*) 120 sec after the addition of histamine. The resting potential of B18 was now back to its pre-histamine level, and the threshold for evoking the action potential occurred sooner than it had before the addition of histamine. *B6,* Expanded record of action potential in B18 (numbered *6* in *A*) 180 sec after the addition of histamine. The depolarizing intracellular current in B18 was reduced to the pre-histamine level (second *arrow* in *A*), and the action potential occurred at a time similar to control (compare with *B1*).

Histaminergic modulation of the synaptic output of BCIs

Our evidence indicates that the histaminergic neuron C2 decreases the synaptic potentials produced by the BCIs. Analysis of BCI B18 suggests that this reduction is due to presynaptic inhibition of the synaptic terminals of the BCI, and it is likely that the presynaptic inhibition is due to a direct action of C2. An important observation supporting the idea that the reduction is due to presynaptic inhibition is that the inhibition can occur in the absence of measurable changes in the resting membrane conductance of the postsynaptic neuron. In fact, inhibition can occur when the postsynaptic conductance shows a tonic decrease, which would in itself tend to potentiate synaptic inputs to the cell.

Variance analysis. A second line of evidence supporting the argument for presynaptic inhibition derives from the variance analysis. Although we were unable to make the more direct analyses, the data obtained by variance analysis indicate that following the firing of C2 there is a decreased mean quantal number in the synaptic potentials evoked by the BCI. The accuracy of the variance method in determining quantal number is dependent upon a number of assumptions, which we have not been able to test in this system. Nevertheless, the variance method permits us to draw a qualitative conclusion, and to reject the simple hypothesis that the decrease of synaptic size is the result of a constant decrease in the size of the individual quanta that compose the PSP. This conclusion stems from a consideration that the coefficient of variance $(CV)^2$ is equal to var $(v)/v^2$ (see Materials and Methods for definitions). If each quantal unit were to be multiplied by a constant, k, it can readily be shown that $(CV)^2 = k^2(\text{var } v)/k^2(v^2) = (CV)^2$. That is, it would be unchanged. Since we found that throughout a 75-fold variation in the amount of transmitter release, synaptic depression decreased $(CV)^2$, the most likely interpretation is that quantal number decreased, although the precise quantification of this decrease is uncertain.

Effects of histamine. A third argument supporting a presynaptic action of C2 is that bath application of histamine, the transmitter of C2 (McCaman and Weinreich, 1985), mimics its action on the synaptic potential evoked by the BCIs. Several other examples of histamine decreasing synaptic potentials in *Aplysia* have been described (Hinzen and Riehl, 1985; Kretz et al., 1986a, b; see also Byrne, 1980), but the actual transmitter mediating presynaptic inhibition in these cases has not been conclusively established.

Mechanisms of action of histamine. Not only does histamine mimic the effect of C2, but the response of B18 to histamine indicates that it has histamine receptors. These receptors can be activated in solutions that block chemical transmission from possible interneurons that might be excited by histamine. Histamine produces a hyperpolarization of the somata of B18. Firing of C2 also produces a small hyperpolarization of the BCIs and decreases their firing rates. This occurs even though C2 does not send an axon to the buccal ganglion. The hyperpolarization appears to be mediated, at least in part, by a BCI that is excited by C2.

Our preliminary evidence indicates that the histamine-induced hyperpolarization in B18 is due to a voltage-dependent increase of ionic conductance. These data are consistent with those of Kretz et al. (1986a, b), who have suggested that histamine-induced presynaptic inhibition of neuron L10 of *Aplysia* is partly due to a turning on of a voltage-dependent potassium current. Kretz et al. (1986a, b) also found evidence that histamine produces presynaptic inhibition of L10 by reducing a voltage-dependent calcium conductance (see also Shapiro et al., 1980b, and Dunlap and Fischbach, 1981). Our failure to find that histamine alters the width of the BCI spike (in the presence of TEA) does not support this mechanism for presynaptic inhibition of the BCIs, but our data do not rule out this possibility. For example, because of the relatively small action potential elicited under our experimental conditions, the somatic membrane potential may not have been in the range needed to elicit all relevant conductances. To the extent that the membrane properties of the soma of the BCIs reflect the properties of their terminal regions, our results indicate that at least part of the presynaptic inhibition could be due to hyperpolarization of the synaptic terminal regions. Hyperpolarization or a conductance increase of the terminal region of the BCIs might reduce the size of the presynaptic spike. It could also result in branch block, which could explain the all-or-none disappearance of the PSPs in some instances (see also Swadlow et al., 1980; Tse and At-

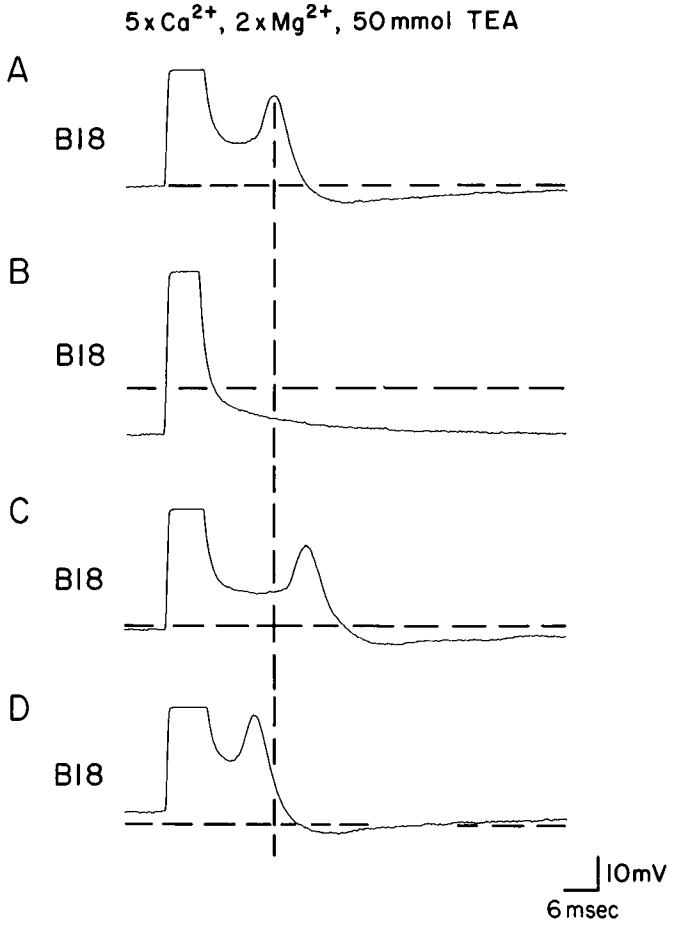

Figure 17. Time of initiation and size of the action potential in B18 appears to depend on its resting membrane potential. *A,* Action potential induced in B18 by intracellular depolarizing current. The ganglion was bathed in 5 × Ca^{2+}, 2 × Mg^{2+}, and 50 mM TEA. *Horizontal dashed lines* mark the resting potential of the neuron; *vertical dashed lines* mark the peak of the action potential. *B,* Hyperpolarizing B18 completely blocked the action potential induced by depolarizing intracellular current. *C,* Slightly hyperpolarizing B18 significantly delayed the time of initiation of the action potential, and reduced the peak height. *D,* Slightly depolarizing B18 significantly reduced the time of initiation of the action potential, and increased the peak height.

wood, 1986). Several investigators have shown that hyperpolarization of presynaptic neurons in *Aplysia* and other animals (Shimahara and Tauc, 1975; Nicholls and Wallace, 1978; Shapiro et al., 1980a) reduces synaptic output, although in some preparations presynaptic depolarization reduces synaptic output (Miledi and Slater, 1966; Blagburn and Sattelle, 1987). Furthermore, in various preparations and under different conditions, presynaptic inhibition correlates either with a recorded presynaptic depolarization (Eccles, 1964; Wall, 1964; Schmidt, 1971; Kennedy et al., 1980; Blagburn and Sattelle, 1987), a presynaptic hyperpolarization (Fuchs and Getting, 1980) or with a conductance increase that is independent of the sign of a change of membrane potential (Baxter and Bittner, 1981; Pearson and Goodman, 1981; Hue and Callec, 1983).

Functional role of presynaptic inhibition of the BCIs

Our results do not permit a definitive conclusion about the functional role of presynaptic inhibition of the BCIs, but several observations need to be taken into account in thinking about this problem. Previous workers have suggested at least 2 functions for presynaptic inhibition: (1) protection of a synapse from dysfunctional depression (Krasne, 1978), (2) shaping of synaptic input to the postsynaptic cell (Schmidt, 1971; Pearson and Goodman, 1981; Blagburn and Sattelle, 1987). It is unlikely that presynaptic inhibition is protecting the BCIs from synaptic depression, since our data do not indicate that the BCI synaptic potentials exhibit any use-dependent depression. Thus, by exclusion, it seems likely that if the presynaptic inhibition has any function, it will contribute to the shaping and sharpening of the outputs of the BCIs. Consistent with this suggestion is the observation that both BCIs we studied exhibit highly phase-specific firing during rhythmic buccal ganglion activity. C2 also fires strongly in phase with buccal ganglion activity not only because it receives phasic inhibitory synaptic input during buccal ganglion programs, but also because it is a proprioceptive afferent that fires in response to movements of the buccal mass (Weiss et al., 1986a, b).

It is interesting to note that while activity of C2 suppresses the excitation that the BCIs produce on C4 and C5, C2 itself produces a slow excitation of C4 and C5. Thus, contrary to what has often been observed (see Hinzen and Riehl, 1985), the presynaptic action of C2 does not augment its postsynaptic action. Instead, C2 appears to produce feed-forward substitution (Kandel and Wachtel, 1968) of its own slow excitation for the rapid excitation produced by the BCIs. The precise, functional effects of C2, however, will depend on the relative time course and magnitude of its postsynaptic and presynaptic actions during normal function, and we currently have not been able to record from all these elements during normal feeding behavior.

In many instances, presynaptic inhibition has been shown to operate at the terminals of sensory afferents (Schmidt, 1971; Krasne, 1978), but instances of presynaptic inhibition at the terminals of interneurons are also now known (Nicholls and Wallace, 1978; Pearson and Goodman, 1981; Krenz and Reichert, 1985). In fact, given that transmitter release is typically depressed by membrane hyperpolarization or increased conductance of synaptic terminal regions, one might expect that in invertebrates, instances of presynaptic modulation of interneurons might be very common. Since the synaptic input and output zones often are anatomically close in invertebrate neurons, conventional postsynaptic inhibition of an interneuron may produce obligatory presynaptic inhibition. In those neurons in which the spike-initiating and synaptic output zones are close, the difference between pre- and postsynaptic inhibition is partly semantic and can depend on what functional parameter is measured (i.e., probability of eliciting a spike or amount of transmitter output per spike).

An added complication in trying to distinguish between pre- and postsynaptic inhibition is that one of the mechanisms of the former appears to be branch block at axonal branches of a neuron (Swadlow et al., 1980; Tse and Atwood, 1986; Weiss et al., 1986b), and branch block can be seen as an outcome of conventional postsynaptic inhibition stabilizing membrane potential at a subthreshold level. For interganglionic interneurons, however, the situation is unique, in that their spike-initiating zone in one ganglion is far removed from their synaptic output zones which are located in another ganglion. Thus, for interganglionic interneurons, the presence of presynaptic modulation is less likely to be an obligatory concomitant of postsynaptic inhibition.

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