C-PR Neuron of *Aplysia* Has Differential Effects on “Feeding” Cerebral Interneurons, Including Myomodulin-Positive CBI-12

ITAY HURWITZ,† RAY PERRINS,† YUANPEI XIN,‡ KLAUDIUSZ R. WEISS,† AND IRVING KUPFERMANN‡

*Department of Physiology and Biophysics, Mount Sinai School of Medicine, New York 10029; ‡Center for Neurobiology and Behavior, College of Physicians and Surgeons, Columbia University, New York City, New York 10032

Hurwitz, Itay, Ray Perrins, Yuanpei Xin, Klaudiusz R. Weiss, and Irving Kupfermann. C-PR neuron of *Aplysia* has differential effects on “feeding” cerebral interneurons, including myomodulin-positive CBI-12. *J. Neurophysiol.* 81: 521–534, 1999. Head lifting and other aspects of the appetitive central motive state that precedes consummatory feeding movements in *Aplysia* is promoted by excitation of the C-PR neuron. Food stimuli activate C-PR as well as a small population of cerebral-buccal interneurons (CBIs). We wished to determine if firing of C-PR produced differential effects on the various CBIs or perhaps affected all the CBIs uniformly as might be expected for a neuron involved in producing a broad undifferentiated arousal state.

We found that when C-PR was fired, it produced a wide variety of effects on various CBIs. Firing of C-PR evoked excitatory input to a newly identified CBI (CBI-12) the soma of which is located in the M cluster near the previously identified CBI-2. CBI-12 shares certain properties with CBI-2, including a similar morphology and a capacity to drive rhythmic activity of the buccal-ganglion. Unlike CBI-2, CBI-12 exhibits myomodulin immunoreactivity. Furthermore when C-PR is fired, CBI-12 receives a polysynaptic voltage-dependent slow excitation, whereas, CBI-2 receives relatively little input. C-PR also polysynaptically excites other CBIs including CBI-1 and CBI-8/9 but produces inhibition in CBI-3. In addition, firing of C-PR inhibits plateau potentials in CBI-5/6. The data suggest that activity of C-PR may promote the activity of one subset of cerebral-buccal interneurons, perhaps those involved in ingestive behaviors that occur during the head-up posture. C-PR also inhibits some cerebral-buccal interneurons that may be involved in behaviors in which C-PR activity is not required or may even interfere with other feeding behaviors such as rejection or grazing, that occur with the head down.

**Introduction**

Complex behavioral acts such as feeding are typically composed of a number of different but interrelated behavioral acts. The behavioral sequences that provide the components of each behavioral act are generated by systems of interconnected neurons termed pattern generators (PGs) or central pattern generators (CPGs). Studies in several invertebrates suggest that the activity of pattern generating circuits often is initiated or modulated by the firing of a small number of higher-order interneurons (Arshavsky et al. 1988, 1989; Bartos and Nusbaum 1997; Delaney and Gelperin 1990; Frost and Katz 1996; Kupfermann and Weiss 1978; Rosen et al. 1991; Wiersma and Ikeda 1964). Those higher-order neurons that exert relatively large effects have been termed command neurons (Kupfermann and Weiss 1978; Wiersma and Ikeda 1964), command-like neurons (Deodhar et al. 1994), or influential neurons (Arshavsky et al. 1988). Analogous higher-order neurons, rather than directly participating in the generation of behaviors, contribute to the generation of motivational states, which function to coordinate and optimize the functioning of the various somatic and visceral behaviors that comprise complex behavioral acts (Teyke et al. 1990). An understanding of the interactions between the various higher-order neurons that control behavior may provide insights into how the nervous system generates decisions about what behavior to execute and how to modify the chosen behavior based on the specific conditions of the environment and the internal state of the organism.

In the cerebral-ganglion of *Aplysia*, an identified neuron, C-PR, is important in generating a feeding posture (head up) and other manifestations of an appetitive arousal motivational state associated with feeding behavior (Nagahama et al. 1994; Teyke et al. 1990, 1991). Consummatory feeding behaviors, such as biting, are generated by neurons primarily located in the buccal ganglion, and this circuitry is regulated by a population of approximately 12 bilateral cerebral-buccal interneurons (CBIs) that are located in the cerebral ganglion and that project to the buccal ganglion (Church and Lloyd 1994; Perrins and Weiss 1998; Rosen et al. 1991; Xin et al. 1999). The CBIs are excited by stimuli contacting the tentacles, lips, and perioral zone, and firing of certain individual CBIs drives one or another rhythmic buccal motor program (BMP) or components of the various interrelated programs that underlie ingestive and egestive consummatory feeding-behaviors. Previous studies (Teyke et al. 1990) suggested that C-PR may affect the firing of a CBI, indicating that there is cross-communication between the neurons involved in appetitive behaviors and those involved in consummatory behaviors. In the present research, we examined the nature of the synaptic input that C-PR evokes in various CBIs. The research explored the question of whether C-PR excites all CBIs involved in feeding, as might be expected for a neuron involved in producing a broad undifferentiated arousal state. Alternatively, if C-PR has differential effects on various CBIs, what are the various effects? Could the firing of C-PR, which is involved in appetitive arousal, contribute to consummatory behaviors and promote a subset of the various buccal motor programs that can be generated? In answering these questions, we also hoped to obtain evidence that could prove useful in understanding the various roles of the very diverse population of cerebral-buccal interneurons and more generally gain insights into the question of how a small population of neurons controls a complex behavior. In the course of these experiments, we identified a new myomodulin-positive CBI-12.
containing CBI that is excited by C-PR, and the present paper describes some of the characteristics of this cell.

METHODS

The experimental subjects in this research were wild-type *Aplysia californica* weighing 150–400 g (Marinus, Long Beach, CA) that were maintained and prepared for experiments as previously described (Xin et al. 1999). More than 60 preparations were used.

Recording apparatus and bathing solutions

Intracellular recordings were obtained from isolated ganglia or from reduced preparations maintained at room temperature (17–23°C), in a clear Lucite recording chamber that was divided into two compartments, each containing artificial sea water (ASW). The first compartment contained the cerebral and buccal ganglia, whereas the second compartment contained the pedal and the pleural ganglia. The chamber contained grooves in which the C-PL and C-P connectives were placed, and the grooves were filled with petroleum jelly (Vaseline) to maintain a watertight seal between compartments. Suction electrodes were attached to selected nerves or connectives as needed for extracellular recording of unit activity. In some experiments, a solution containing increased concentrations of divalent cations (Hi-Di) was used and contained three times the normal concentration of magnesium and calcium.

Electrophysiology

For intracellular recording and stimulation, neurons were impaled with double-barreled microelectrodes that were made of thin-walled glass and contained 1.9 M potassium acetate and 0.1 M potassium chloride. The electrodes were beveled so that their impedances ranged from 10 to 15 MΩ. For the purposes of identifying cells and examining their morphology, the potassium acetate in the stimulating electrode was replaced by a solution of 3% 5(6)-carboxyfluorescein dye (Kodak) in 0.05 M potassium citrate, titrated to pH 8.0 with KOH (Rao et al. 1986). These electrodes were beveled so that the impedance of the electrode containing the dye was ~10 MΩ and the impedance of the potassium acetate electrode was ~6 MΩ. Up to three simultaneous intracellular recordings were obtained using conventional electrometers. Nerve recordings were made with polyethylene suction electrodes and AC amplifiers. The nomenclature for the nerves follows that of Gardner (1971).

Morphology

The locations, sizes, and shapes of cerebral neurons with axons in the C-B connectives first were determined by back-filling the connectives with cobalt or nickel chloride followed by treatment with rubeanic acid (Quicke and Brace 1979). At the termination of many electrophysiological experiments, selected neurons were injected with carboxyfluorescein dye. To reduce the active transport of the dye from the cells, probenecid (1 mM final concentration) was added to the ASW bathing medium (Rosen et al. 1991; Steinberg et al. 1987), and the preparation was kept for 24–48 h at 4°C. The living ganglia were cleaved in 50% glycerol in ASW and viewed with a fluorescence microscope. Confirmation of cell morphology was made with nickel-chloride injections followed by treatment with rubeanic acid, fixation in paraformaldehyde, and clearing in 50% glycerol in phosphate buffer.

Immunohistochemistry was performed on whole-mount preparations treated as previously described (Miller et al. 1991; Xin et al. 1999). In some preparations, cells first were identified and filled with biocytin. After fixation and permeabilization with Triton, the cell was labeled fluorescently by incubating the tissue with streptavidin Bodipy FL conjugate (50 μg/ml) (Molecular Probes, Eugene, OR)/PBS Triton at 4°C for 12–24 h. This then was followed by immunostaining with a myomodulin antibody and a Cy-3 second antibody.

Identification of CBIs

A number of criteria were used to identify individual CBIs. Not all criteria were employed for every cell, but the properties of the CBIs we studied included soma location, branching patterns of the cell processes, spontaneous excitatory or inhibitory postsynaptic poten-
and that firing of C-PR contributes to the evocation of a complex of responses that represent the appetitive central motive state that precedes consummatory movements (Teyke et al. 1990). Consummatory feeding movements are inhibited by C-PR in the buccal ganglion. Previous data indicated that stimulation of the rhinophores or tentacles with food produces excitation of C-PR and that firing of C-PR contributes to the evocation of a complex of responses that represent the appetitive central motive state that precedes consummatory movements (Teyke et al. 1990). Consummatory feeding movements are inhibited by C-PR in the buccal ganglion. 

### RESULTS

#### Morphology of C-PR

Previous data indicated that stimulation of the rhinophores or tentacles with food produces excitation of C-PR. Previous data indicated that stimulation of the rhinophores or tentacles with food produces excitation of C-PR. Previous data indicated that stimulation of the rhinophores or tentacles with food produces excitation of C-PR. Previous data indicated that stimulation of the rhinophores or tentacles with food produces excitation of C-PR.

#### Morphology of C-PR

Previous data indicated that stimulation of the rhinophores or tentacles with food produces excitation of C-PR. Previous data indicated that stimulation of the rhinophores or tentacles with food produces excitation of C-PR. Previous data indicated that stimulation of the rhinophores or tentacles with food produces excitation of C-PR.

#### Morphology of C-PR

Previous data indicated that stimulation of the rhinophores or tentacles with food produces excitation of C-PR. Previous data indicated that stimulation of the rhinophores or tentacles with food produces excitation of C-PR. Previous data indicated that stimulation of the rhinophores or tentacles with food produces excitation of C-PR.

#### Morphology of C-PR

Previous data indicated that stimulation of the rhinophores or tentacles with food produces excitation of C-PR. Previous data indicated that stimulation of the rhinophores or tentacles with food produces excitation of C-PR. Previous data indicated that stimulation of the rhinophores or tentacles with food produces excitation of C-PR.

### FIG. 3

**A1**: Firing of C2 led to a hyperpolarizing potential in CBI-2 and a depolarization in CBI-12, when the cells were at resting potential. **A2**: Firing of C2 while both CBI-2 and CBI-12 were depolarized just below threshold evoked a pure, brief inhibition in CBI-2 and a mixed fast EPSP/slow IPSP in CBI-12. **B1**: Brief burst of spikes in B19 produced small, fast one-for-one IPSPs and a late slow depolarization in both CBI-2 and CBI-12. **B2**: During more prolonged and intense firing in B19, the slow excitation was more evident in both cells but was much larger in CBI-12. **C1**: Firing of B18 produced a slow excitation in both CBI-12, regardless of their membrane potential (C2).

### FIG. 4

**A**: Firing of CBI-2 lead to a rhythmic-BMP as indicated by the bursts of activity in buccal nerve 2 and the radula nerve. Each major burst of firing in the radula nerve overlapped in part with firing in nerve 2. **B**: Firing of CBI-12 also evoked rhythmic activity, but it was not as regular as that evoked by CBI-2, and the bursting in the two nerves was not well coordinated. Section of the trace marked C is shown in expanded time scale in C of the figure. **C**: Expanded time record of portion of CBI-12 trace shown in B to illustrate the weak inhibition seen during the retraction phase of the buccal program.
evoked when food contacts the perioral region and excites cerebral-buccal interneurons (CBIs), which drive buccal motor programs or components of the programs. A previous study using silver intensification of cobalt-filled cells described the basic morphology of C-PR (Teyke et al. 1997). Using fluorescent dye-fills, we confirmed the basic morphology of the cell, examined in detail the location of its processes in the cerebral ganglion, and determined the location of its cell body relative to that of the CBIs located in the same region of the ganglion. C-PR has three main processes that arise directly from the soma (Fig. 1A). One of the three main trunks reaches the ventral side of the M cluster and is folded toward the dorsal side. The second trunk of C-PR travels medially toward the C cluster and gives off fine branches. The third trunk projects through the cerebral-pleural connective, and previous data indicate that it branches extensively in the ipsilateral pedal ganglion and continues to the contralateral pedal ganglion (Teyke et al. 1997). The M cluster contains the somata of a number of CBIs including CBI-1, CBI-2, CBI-3, CBI-4, and the newly identified CBI-12 (Fig. 1B). The C-PR soma is located posterior to the CBIs in the M cluster and is just posterior to CBI-3 (Fig. 1B).

Effects of firing of C-PR on CBI-2 and on newly identified CBI-12

Previous results suggest that the CBIs constitute a diverse population of interneurons that may be involved differentially in the various ingestive and egestive motor programs that the buccal ganglion controls. We first examined the effect of C-PR on CBI-2, a CBI that drives a robust buccal program and generates responses of the buccal mass that resemble biting. We identified CBI-2 according to its location in the M cluster and its ability to drive a robust BMP. Other features that aided in the identification of the cell was the presence of frequent multiple IPSPs and a characteristic appearance of its processes as revealed by dye fills (Rosen et al. 1991). In previous work, CBI-2 was shown to drive a rhythmic buccal program (Rosen et al. 1991). At that time, the evidence suggested that CBI-2 was the only CBI in the M cluster region that was capable of driving a rhythmic buccal program. In the current series of experiments, however, it appeared that in addition to CBI-2, there was a second cell that also could drive rhythmic buccal activity. CBI-2 and the second cell were located close to one another at a position adjacent to the large motor neurons (Fig. 1B) C-11 and C-12 (Rosen et al. 1991; Teyke et al. 1993). The presence of a previously unidentified CBI is consistent with
nickel-chloride back-fills of the cerebral-buccal connectives (CBCs), which indicated that the ventral M cluster may contain a CBI in addition to the three that had been characterized previously in that region (Rosen et al. 1991; Xin et al. 1999).

A preliminary screen of the two cells in the M cluster that could drive buccal programs revealed that the more laterally positioned cell was the previously reported CBI-2. This cell showed inhibition sometimes followed by some excitation when C-PR was fired (Fig. 2). For the other, more medially and superficially positioned cell, firing of C-PR produced a slow excitation (Fig. 2) that could effectively fire the cell if it was depolarized tonically or had a low resting potential (see later section). We term the more medially and superficially positioned cell CBI-12. As described in the following text, the two cells have some similarities but are different in a number of features.

**CBI-2 and CBI-12 share similar morphology**

To better define the newly identified CBI-12 neuron and distinguish it from CBI-2, we determined the effects of firing of C-PR on the cells and then filled them with dye to determine their morphology. Nine of nine lateral cells that were presumed CBI-2 cells were not excited by C-PR and were found to have the morphological pattern previously described for CBI-2 (Rosen et al. 1991). A dense group of processes extended directly from the soma, primarily in the medial direction (see Fig. 3 of Rosen et al. 1991). This pattern is clearly different from that exhibited by CBI-1 and CBI-3, which are located anterior and posterior, respectively, to CBI-2. Of 11 more medially situated neurons that received excitation from C-PR and are presumably CBI-12 cells, 6 showed a morphology similar to that of CBI-2. In 5 of the 11 presumptive CBI-12 neurons, however, the processes in the neuropile spread more widely than those typically observed for CBI-2. This pattern is similar to that exhibited by CBI-3, but the cells clearly were not CBI-3 cells, which can be distinguished easily by their larger sizes and more posterior positions (Fig. 1B). In three preparations, we filled both the lateral (CBI-2) and medial (CBI-12) cells, and in all these cases the cells could not be distinguished morphologically and showed the CBI-2 pattern. Because the morphology of CBI-2 and CBI-12 did not clearly differentiate the cells, we studied a number of other parameters.

**CBI-2 and CBI-12 have different inputs and outputs**

We examined the inputs to CBI-2 and CBI-12 from the cerebral mechanoefferent neuron C2 and the buccal-to-cerebral interneuron B19. These neurons previously were shown to make extensive connections to cells in the cerebral ganglion (McCaman and Weinreich 1985; Rosen et al. 1991; Weiss et al. 1986). Firing of C2 produced a hyperpolarizing potential in CBI-2 but a depolarization in CBI-12 when the two CBIs were at their resting potential (Fig. 3A1). When both CBIs were depolarized by intracellular current to just below threshold, C2 evoked an initial depolarization followed by a relatively slow hyperpolarization in CBI-12. The hyperpolarization decayed back to baseline with a time course of several seconds. By contrast CBI-2 exhibited a pure hyperpolarization that had a rapid onset and rapid decay (Fig. 3A2).

The two CBIs also could be distinguished from one another on the basis of the their responses to firing of neuron B19. While at resting potential, a brief burst of spikes in B19 evoked what appeared to be very similar discrete IPSPs in both CBIs (Fig. 3B1). The two CBIs behaved very differently during more prolonged firing in B19. In response to a relatively prolonged and high frequency of B19 firing, CBI-12 exhibited a barely detectable hyperpolarization followed by a substantial lasting depolarization that fired the cell (Fig. 3B2). By contrast, during the firing of B19, CBI-2 exhibited a weak, slow, hyperpolarization followed by a slow depolarization. The differences in input from C2 and B19 were found to be very consistent between preparations.

Not all interneurons had differential effects on CBI-2 and CBI-12. For example, buccal cerebral neuron B18 produced a slow excitation in both CBIs (Fig. 3C1) that fired the cells similarly when they were slightly depolarized (Fig. 3C2).

The rhythmic synaptic inputs the cells receive during buccal motor programs also is different in the two CBIs. On the basis of observations from semi-intact ganglion-buccal mass preparations (Rosen et al. 1988), during the phase of radula retraction, a barrage of IPSPs always occurs in CBI-2. A similar
A barrage of IPSPs occurs during programs elicited from isolated ganglia. Typically after a warm-up period of one to five cycles, the IPSPs completely block CBI-2 from firing (Fig. 4A) (see also Rosen et al. 1991), but the IPSPs rarely effectively block CBI-12 from firing (Fig. 4, B and C), although they can reduce the firing frequency of the cell.

In addition to examining the synaptic input to the cells, we compared their ability to drive BMPs. In 41 of 43 preparations CBI-2 drove rhythmic buccal-ganglion bursts throughout a 2- to 3-min period when the cell was fired, generating 6–10 cycles of activity. By contrast, in 17 of 30 preparations, firing of CBI-12 failed to sustain rhythmic bursting and only generated two or three cycles during the 2–3 min period of stimulation. In nine preparations, both CBI-2 and CBI-12 were impaled, and in five of these preparations, both cells evoked multiple cycles of activity throughout the period that the cells were fired (Fig. 4). The bursting, however, produced by CBI-12 (Fig. 4B) was not as robust or as regular as that produced by CBI-2 (Fig. 4A), and unlike what is seen during behavioral programs, the activity in the radula nerve and nerve 2 was not well coordinated. These findings are consistent with the idea that an individual CBI-2 can initiate a functional buccal motor program, whereas CBI-12 may operate in a more modulatory role, together with other CBIs.

CBI-12 but not CBI-2 exhibits myomodulin immunoreactivity

An unidentified small cell medial to CBI-2 has been reported to be myomodulin positive (Miller et al. 1991) and immunocytochemistry combined with back-fills of cerebral-buccal connectives have shown that a number of CBIs, including one in the M cluster, are myomodulin immunopositive (Xin et al. 1999). We therefore examined the possible myomodulin immunoreactivity of CBI-2 and CBI-12. Myomodulin immunoreactivity was observed in one large cell in the M cluster, identified as C12 (not shown), and in only one other small cell the position of which suggested that it is CBI-12. On the basis of identification by means of electrophysiological characteris-
Effect of C-PR on CBIs is polysynaptic

Previous studies (Teyke et al. 1997) provided evidence that the effects of C-PR on cerebral B motor neurons and on the metacerebral cell are mediated by interneurons located in the pedal or pleural ganglion. To determine the location of possible interneurons mediating the effects of C-PR on CBI-12, we placed the cerebral ganglion and the pedal-pleural ganglia in different chambers, leaving the connectives intact. Thus we could change selectively the solution bathing the cerebral or pedal-pleural ganglia. We studied the effect of raising the firing threshold of pleural and pedal-ganglion neurons by first establishing that firing of C-PR evoked a depolarization in CBI-12 and then determining if the effect remained after bathing the pedal-pleural ganglia in a solution containing increased divalent cations (Hi-Di, 3 times normal Ca; 3 times normal Mg) (n = 4). For conditions shown in Fig. 7, C-PR was fired similarly as indicated by the bottom recording. In these experiments, we also recorded extracellularly from a CBC to monitor the activity of other CBIs the firing of which was affected by C-PR. In normal ASW, firing of CBI-12 depolarized CBI-12 and also excited other CBIs as indicated by the recording from the CBC (Fig. 7A). Exposure of the pedal-pleural ganglia to Hi-Di completely blocked the effect of firing of C-PR on CBI-12 and on other CBIs recorded in the CBC (Fig. 7B). Partial recovery of the responses occurred when the pedal-pleural ganglia were re-exposed to ASW (Fig. 7C).

Effects C-PR firing are mediated by ipsilateral and contralateral cerebral-pleural connectives

It was shown previously that the effect of firing C-PR on cerebral neurons such as the MCC and B cells was mediated by pedal-pleural interneurons that send their axons through cerebral-pleural or cerebral-pleural connectives (Teyke et al. 1997). To explore the pathway by which C-PR affects CBIs, ipsilateral or contralateral pedal or pleural connectives were sequentially sectioned (n = 4). The effect of firing of C-PR on the excitability of CBI-12 or the activity of other CBIs recorded from a CBC was not substantially altered by cutting both cerebral-pleural connectives (Fig. 8, A and B). Cutting a single cerebral-pleural connective contralateral to C-PR reduced the effects of C-PR on CBI-12.

FIG. 7. Selective cutting of individual connectives reveals pathways by which C-PR affects CBIs. Effects of firing C-PR on CBI-12 and on units recorded from the CBC after successive cuts of the pedal and pleural connectives are shown. A low level of depolarizing current was injected into the left CBI-12, and additional repeated depolarizing current pulses were used to evoke spikes. After each cut of a connective, C-PR was fired (shown only in D and indicated by horizontal lines in the other parts) A: in the intact ganglion, as indicated by the slow depolarization and enhanced evoked spikes, activation of C-PR excited CBI-12. Other presumptive CBIs also were excited, as indicated by the recording from the CBC. B: after cutting both cerebral-pleural connectives, the effects of C-PR were still present. C: cutting the cerebral pedal-connective contralateral to C-PR (which does not contain the axon of the stimulated C-PR), reduced the input to CBI-12 and to cells projecting to the CBC. D: additional lesion of the contralateral cerebral-pleural connective and consequently of the axon of C-PR blocked all the effects of firing of C-PR.

Firing of C-PR evokes voltage-dependent excitation of CBI-12

The excitatory effect of C-PR on CBI-12 varied significantly between preparations: in some preparations a strong excitation of >15 mV was recorded, whereas in other preparations the depolarization was much smaller. Variations of the magnitude appeared to be related to the membrane potential of the cell. Indeed, we found that when CBI-12 was undamaged, and at its resting potential, firing of C-PR produced relatively little and sometimes no obvious depolarization of the cell. A clear excitation was observed, however, when CBI-12 was depolarized 5 mV by means of constant current injection (Fig. 6A). When CBI-12 was depolarized by >10 mV, C-PR firing brought CBI-12 above firing threshold (Fig. 6B). The EPSP evoked in a tonically depolarized cell, greatly outlasted the duration of the firing of C-PR, but the expression of the slow EPSP could be terminated immediately by stepping the cell membrane potential back to rest (Fig. 6C, 1st set of traces). Conversely if C-PR was fired while CBI-12 was at resting potential (Fig. 6C, 2nd set of traces), no depolarization was evident in CBI-12, but a slow depolarization could be uncovered, by passing a constant depolarizing current into the cell at the termination of firing of C-PR (n = 4). The overall data suggest that firing of C-PR produces a long-duration voltage-dependent EPSP in CBI-12.

Effect of C-PR on CBIs is polysynaptic

Previous studies (Teyke et al. 1997) provided evidence that the effects of C-PR on cerebral B motor neurons and on the metacerebral cell are mediated by interneurons located in the pedal or pleural ganglion. To determine the location of possible interneurons mediating the effects of C-PR on CBI-12, we placed the cerebral ganglion and the pedal-pleural ganglia in different chambers, leaving the connectives intact. Thus we could change selectively the solution bathing the cerebral or pedal-pleural ganglia. We studied the effect of raising the firing threshold of pleural and pedal-ganglion neurons by first establishing that firing of C-PR evoked a depolarization in CBI-12 and then determining if the effect remained after bathing the pedal-pleural ganglia in a solution containing increased divalent cations (Hi-Di, 3 times normal Ca; 3 times normal Mg) (n = 4). For conditions shown in Fig. 7, C-PR was fired similarly as indicated by the bottom recording. In these experiments, we also recorded extracellularly from a CBC to monitor the activity of other CBIs the firing of which was affected by C-PR. In normal ASW, firing of CBI-12 depolarized CBI-12 and also excited other CBIs as indicated by the recording from the CBC (Fig. 7A). Exposure of the pedal-pleural ganglia to Hi-Di completely blocked the effect of firing of C-PR on CBI-12 and on other CBIs recorded in the CBC (Fig. 7B). Partial recovery of the responses occurred when the pedal-pleural ganglia were re-exposed to ASW (Fig. 7C).

Effects C-PR firing are mediated by ipsilateral and contralateral cerebral-pleural connectives

It was shown previously that the effect of firing C-PR on cerebral neurons such as the MCC and B cells was mediated by pedal-pleural interneurons that send their axons through cerebral-pleural or cerebral-pleural connectives (Teyke et al. 1997). To explore the pathway by which C-PR affects CBIs, ipsilateral or contralateral pedal or pleural connectives were sequentially sectioned (n = 4). The effect of firing of C-PR on the excitability of CBI-12 or the activity of other CBIs recorded from a CBC was not substantially altered by cutting both cerebral-pleural connectives (Fig. 8, A and B). Cutting a single cerebral-pleural connective contralateral to C-PR reduced the effects of C-PR.
C-PR on CBIs (Fig. 8C), whereas cutting both pedal-cerebral connectives completely blocked the effects of C-PR (Fig. 8D). Similar results were seen in those preparations in which C-PR had some actions on CBI-3 \((n = 3)\). The order in which the connectives were cut did not appear to be significant as long as the last connective cut was the ipsilateral pedal-cerebral connective (which contains the C-PR axon that projects to the pedal-pleural ganglion).

**Effects of pleural and pedal ganglion neurons on CBIs also may involve cerebral interneurons**

Although the effects of firing of C-PR on CBI-12 and other CBIs appear to be mediated by interneurons located in the pedal-pleural ganglia, it is possible that these interneurons do not directly innervate the CBIs but rather synapse on intermediate interneurons located in the cerebral ganglion. To test this possibility, the cerebral ganglia but not the pedal-pleural ganglia were bathed in a Hi-Di solution while C-PR was fired. Under these conditions, the effect of firing of the C-PR also was blocked \((n = 4)\) (normal ASW, Fig. 9, A1 vs. high-divalent sea water, A2). In two experiments on CBI-3, in normal ASW firing of C-PR evoked what appeared to be a mixture of excitatory and inhibitory synaptic potentials (Fig. 9B1), and these effects were blocked (Fig. 9B2) when the concentration of calcium in the solution was increased to five times normal. The high-calcium solution raises cell thresholds while presumably not decreasing synaptic output of neurons. In these experiments, the effects of the divalent cations were not clearly reversed when the preparation was returned to ASW.

**Different CBIs exhibit various inputs when C-PR is fired**

Twelve CBIs have been identified, and most of them have different but characteristic effects on buccal programs (Perrins and Weiss 1998; Rosen et al. 1991; Xin et al. 1999), suggesting that different CBIs or different combinations of CBIs may evoke or modulate the various behaviors that are known to be mediated by the buccal ganglion. We therefore examined the effect of firing of C-PR on various CBIs, concentrating on those that can be approached from the ventral surface of the cerebral ganglion (the surface containing the cell body of C-PR).

Firing of C-PR evoked a burst of what appeared to be discrete polysynaptic EPSPs in CBI-1 (Fig. 10A). Firing of C-PR also evoked a burst of PSPs in CBI-3, but the PSPs were
relatively small and appeared to be comprised of both EPSPs and IPSPs (Fig. 10B). As shown in a later section, based on changes in the excitability of CBI-3 the cell apparently receives waves of inhibitory inputs when C-PR is fired (see Fig. 14). CBI-4 received little or no input when C-PR was fired.

**Effect of C-PR on plateau potentials of CBI-5/6**

Firing of C-PR did not appear to evoke any obvious synaptic input into the newly identified pair of CBIs, CBI-5/6 (Perrins and Weiss 1998), either at resting potential or when CBI-5/6 was depolarized. Furthermore, firing of C-PR did not produce any change in the input resistance of CBI-5/6, as measured with constant-current hyperpolarizing pulses (Fig. 11A). Because CBI-5/6 can generate plateau potentials (Perrins and Weiss 1998), we determined the effect of firing of C-PR on the capacity of CBI-5/6 to initiate these responses. The amount of current needed to produce a reliable plateau response first was determined for a given CBI-5/6 (Fig. 11B), and that level then was presented repeatedly (note that under conditions in which repeated pulses are given, the plateau response of CBI-5/6 appears as an active response that does not outlast the depolarizing pulse). When a 6-s burst of spikes was evoked in C-PR, the capacity of CBI-5/6 to generate a plateau potential was suppressed even though no obvious IPSP could be observed (Fig. 11C; n = 4). The onset of the suppression of the plateau was typically very slow and often not clearly present until the 6-s burst of C-PR spikes was terminated.

**Effects of C-PR on CBI 8/9**

Simultaneous recordings from dorsal CBIs that have been identified recently (Xin et al. 1999) and the ventrally located C-PR were obtained by pinning the ganglion, dorsal side up, and twisting the anterior portion of the cerebral ganglion so that C-PR was exposed. CBI-8 and -9 consist of two similar cells located at the base of the AT nerve. These cells received a slow excitation when C-PR was fired (Fig. 12).

**Effects of C-PR on the excitability of CBIs**

Although C-PR excited a number of CBIs, activity of C-PR never evoked buccal motor programs even when both C-PRs were fired at high frequency. Furthermore we have found that firing of C-PR leads to an inhibition in many buccal CPG elements such as B31/B32, B63, and B34 (Hurwitz, Weiss, and Kupfermann, unpublished observations). Therefore the actions of C-PR may be primarily modulatory and might be most apparent when it is active together with other inputs to the CBIs. To reveal possible modulatory effects of C-PR, CBIs were injected suprathreshold constant current depolarizing pulses that were adjusted so that they evoked three to five action potentials. We then determined whether the number of evoked spikes was affected by C-PR, which was fired by constant current pulses adjusted to evoke a 6-s train of spikes at 20 Hz, which is within a physiological range (Teyke et al. 1991).

The effect of firing of C-PR was determined for four to eight cells of a given type in three to six runs. C-PR primarily enhanced the excitability of CBI-1, CBI-8/9, and CBI-12 (Fig. 13, A–C) and had no effect on the excitability of CBI-4 (Fig. 13D). The effect of C-PR on CBI-2 consisted of a very small decrease of excitability (Fig. 13E). CBI-3 exhibited multiple effects (Fig. 13F): an early decrease of excitability followed by an increase of excitability and finally a second decrease of excitability.

We obtained more quantitative data on CBI-1, -2, -3, -4, -8/9, and -12 by recording from six identical cells for at least three runs for each cell, and averaging the number of action potentials per cell per run (3 times before C-PR was fired, 3 times while C-PR was fired and 11 times after termination of C-PR firing). The data were plotted as number of evoked spikes as a percent of initial control. Figure 13 shows the...
average and standard error of six normalized individual runs for each of the six CBIs that was tested, before, during, and after C-PR was fired (period of firing indicated by thick horizontal bars). The data confirm our previous observations. CBI-12 exhibited a large and relatively prolonged increase in excitability. CBI-1 exhibited a smaller increase of excitability, CBI-8/9 exhibited a yet smaller increase of excitability, and CBI-4 exhibited only a very small increase in excitability. CBI-2 appeared to exhibit a small decrease of excitability, and finally CBI-3 showed an initial decrease followed by a return to baseline or small increase of excitability and finally a late inhibition of long duration. Note that in Fig. 13 the vertical scales for the effects on CBI-3, -2, and -4 have been magnified compared with the scales for CBI-12, -1, and -8/9.

**DISCUSSION**

These studies illuminate two questions. Does a neuron such as C-PR, which plays a role in generating a motivational state, interact directly or indirectly with command-like neurons for specific behaviors? Can a population of command-like neurons be differentiated on the basis of synaptic input from C-PR?

The cerebral ganglion contains a small number of neurons that project axons out the cerebral-buccal connective. Some of these are the somata of primary mechanoaferents, and one is the extrinsic modulatory neuron, the metacerebral cell. The remaining neurons are termed CBIs and have been postulated to be involved in the generation of one or more of the stereotyped motor programs generated by the buccal ganglion. The CBIs consist of ~12 cells. The buccal ganglion generates what appear to be at least six distinct but related motor programs (grazing, rejection, withdrawal, biting, swallowing, and cutting) (Howells 1942; Hurwitz and Susswein 1992; Kupfermann 1974a; Kupfermann and
Carew 1974; Morton and Chiel 1993), and thus it is likely that at least some of these behaviors involve the combined activity of several CBIs. Consistent with this suggestion are the observations that buccal interneurons and afferent stimulation, provide synaptic inputs to more than one CBI at a time (Rosen et al. 1991). One approach to understanding how the various CBIs act together to generate behavior is to examine their differential inputs. In the current study, we determined the effects of firing of C-PR on CBIs. C-PR was of particular interest because there is considerable evidence that it is an important element of the neural circuitry that generates various manifestations of the appetitive arousal that precedes some consummatory behaviors. For example, the cell fires when animals assume the head-up feeding posture (Teyke et al. 1991), and animals exhibit deficits in the intensity and rate of consummatory feeding responses when the connectives that contain the C-PR axon are severed (Kupfermann 1974b).

Cerebral M cluster contains a novel myomodulin-containing CBI

We examined the input to a variety of identified CBIs located in the M and E clusters of the cerebral ganglion. In the course of these studies, we encountered a previously undescribed CBI, which we have named CBI-12. Previous studies reported that only a single cell in the M cluster drives a maintained buccal motor rhythm. In the current research, we found a second M cluster neuron (termed CBI-12) that can drive rhythmic activity, although compared with CBI-2, the rhythm driven by CBI-12 is not as robust or coordinated between various buccal nerves. Thus the effects of CBI-12 may be primarily in modulating an ongoing program rather than in initiating a complete program. The morphology of CBI-12 was similar to that of CBI-2, and it is possible that previous studies of CBI-2 on occasion were performed on CBI-12. We now find, however, that the two cells can be distinguished based on a combination of features, including their exact position in the M cluster, the nature of the programs they evoke, and inputs from sensory cells (such as C2) and interneurons (such as B19 and C-PR). The single feature that most definitively distinguishes the cells is that CBI-12 exhibits immunoreactivity to the peptide, myomodulin (MM), whereas CBI-2 is myomodulin negative. The MM immunoreactivity of CBI-12 is consistent with the previous evidence that the cerebral ganglion transports large amounts of MM to the buccal ganglion (Lloyd 1988).

C-PR, a neuron involved in appetitive arousal, has differential effects on cerebral-buccal interneurons

Firing of C-PR was found to evoke strong synaptic input to some CBIs but little or no input to other CBIs. Furthermore for the CBIs that received input, some were excited, some inhibited, and one pair (CBI-5/6) appeared to exhibit a change in an intrinsic membrane property that affected their capacity to exhibit plateau potentials. The synaptic effects of C-PR on CBIs are not consistent with the hypothesis that C-PR produces an indiscriminate general arousal but rather are most compatible with the idea that the firing of C-PR promotes the activity of only a subclass of feeding-related behaviors, such as grazing during locomotion, rather than biting, during stationary feeding (see next section).

Although both CBI-12 and CBI-2 can drive rhythmic buccal ganglion activity, the two cells receive very different synaptic inputs when C-PR is fired. C-PR primarily inhibits CBI-2. By contrast, C-PR evokes a slow EPSP in CBI-12. The effect of the EPSP is modulatory in that the amplitude of the EPSP is

![Diagram](http://jn.physiology.org/Downloaded_from.jpg)
enhanced greatly when the cell is depolarized. In other words, C-PR on its own will not have much effect on CBI-12 but will modulate the effects of inputs that depolarize CBI-12. Other cells that are excited by firing of C-PR are CBI-8/9, a pair of cells that are similar to CBI-12 in that they contain myomodulin. Similar to CBI-12, CBI-8/9 can drive rhythmic buccal activity, but the rhythm is not robust and appears to include primarily neurons in buccal nerve 3 rather than in other buccal nerves (Xin et al. 1999). CBI-3, a cell that appears to inhibit ongoing buccal motor programs (Rosen et al. 1991), receives a slow inhibition when C-PR is fired, whereas CBI-1, which drives a single cycle of a buccal motor program, receives relatively weak excitation. It may be significant that CBI-2 and CBI-4, the two cells in which C-PR produces the least modulation of their firing, drive the most robust and reliable rhythmic buccal motor programs. Thus it is possible that the cells that receive more substantial inputs from C-PR and from the interneurons that C-PR drives function more in a modulatory role in adjusting ongoing programs rather than directly eliciting programs.

**C-PR may promote a subset of feeding-related motor programs**

Our data suggest that the synaptic inputs evoked by firing C-PR are mediated by interneurons that are located in the pedal or pleural ganglia and that send their axons to the cerebral ganglion via the cerebral-pedal connectives and possibly excite secondary cerebral interneurons. Most of the effects of C-PR on the CBIs appear to involve slow onsets and decay, because they either are mediated by slow synaptic potentials or are mediated by small fast potentials produced by interneurons that have slow onset and offset times. In either case, the effects of C-PR do not appear to be appropriate for producing phasic effects but rather appear to involve relatively slow changes in the excitability of the CBIs.

Because one of the main effects of C-PR on the pedal
ganglion is to excite neurons that are involved in generating a head-up posture, it is possible that the interneurons that mediate the effect of C-PR on the CBIs are part of the neural circuitry involved in the head-up posture or other aspects of appetitive arousal. Thus it can be postulated that those CBIs that are excited by these interneurons may be active during the ingestive behaviors that are associated with appetitive arousal. Neurons that are inhibited or receive no input may be related to egestive behaviors. Alternatively these CBIs may be related to ingestive behaviors, such as grazing (Kupfermann and Carew 1974), that occur in the absence of a head-up posture. Previous data suggested that firing of C-PR excited pedal or pleural interneurons that mediate synaptic excitation of the serotonergic metacerebral cells or inhibition of Bn cells, which appear to be involved in defensive withdrawal responses (Teyke et al. 1989). The inputs to the Bn cells appeared to travel via the cerebral-pleural connectives, whereas the inputs to the MCC travel via the cerebral-pedal connectives. Thus the inhibitory input to the Bn cells must be mediated by a set of interneurons that are different from those that excite the MCC and the CBIs (Teyke et al. 1997).

It was suggested previously that one reason the connections of C-PR to cerebral neurons such as Bn cells and the MCC take an indirect route, via the pedal ganglion, is that this serves to ensure appropriate sequencing of feeding behavior (Teyke et al. 1997). Feeding involves initial appetitive responses that are mediated by neurons located in the pedal and pleural ganglia. These responses precede consummatory responses, which are mediated by neurons contained primarily in the cerebral and buccal ganglia. The current data suggest that food stimuli initially may activate C-PR, which then activates pedal ganglion circuitry, which in turn activates a specific subset of CBIs. Thereby the appetitive aspects of feeding (head lifting and orientation) will be promoted to precede specific classes of the consummatory aspects of feeding. It is of interest that based on the use of high-divalent cation solutions, our evidence suggests that at least part of the effect of the presumptive pedal-pleural interneurons that mediate the effects of C-PR on CBIs may involve intermediary interneurons located in the cerebral ganglion.

The differential input to CBIs cells from C-PR suggests that one form of feeding behavior may be potentiated by circuitry associated with generating a head-up posture, whereas another form of feeding may be depressed by the head-up posture. Behavioral observations indeed suggest that feeding may occur either during the head-up posture or during grazing, during which the head of the animal is on the substrate and the animal slowly locomotes while feeding (Kupfermann and Carew 1974). A testable prediction of this hypothesis is that circuitry associated with locomotion may have actions opposite to those produced by firing of C-PR, namely excitation of CBI-2 and inhibition of CBI-12. Definite conclusions about the specific role of C-PR in the generation or modulation of buccal programs must await further information about the firing patterns and rate of C-PR and of the CBIs during the various behaviors that are mediated by the buccal ganglion.

We thank A. Klein and S. Rosen for comments on the study.
This work was supported by National Institutes of Health Grants MH-50235, MH-36730, GM-32909, and K05-MH-01427 and by Human Frontier Science Program LT-0464/1997.

Present address of R. Perrins: School of Biological Sciences, Woodland Road, University of Bristol, Bristol BS8 1UG, UK.
Address for reprint requests: I. Kupfermann, Center for Neurobiology and Behavior, Columbia University, 722 W. 168 St., Box 25, New York City, NY 10032.
Received 6 March 1998; accepted in final form 22 October 1998.

REFERENCES


