Intrinsic and Extrinsic Modulation of a Single Central Pattern Generating Circuit

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Morgan, Peter T., Ray Perrins, Philip E. Lloyd, and Klaudiusz R. Weiss. Intrinsic and extrinsic modulation of a single central pattern generating circuit. J Neurophysiol 84: 1186–1193, 2000. Intrinsic and extrinsic neuromodulation are both thought to be responsible for the flexibility of the neural circuits (central pattern generators) that control rhythmic behaviors. Because the two forms of modulation have been studied in different circuits, it has been difficult to compare them directly. We find that the central pattern generator for biting in Aplysia is modulated both extrinsically and intrinsically. Both forms of modulation increase the frequency of motor programs and shorten the duration of the protraction phase. Extrinsic modulation is mediated by the serotonergic metacerebral cell (MCC) neurons and is mimicked by application of serotonin. Intrinsic modulation is mediated by the cerebral peptide-2 (CP-2) containing CBI-2 interneurons and is mimicked by application of CP-2. Since the effects of CBI-2 and CP-2 occlude each other, the modulatory actions of CBI-2 may be mediated by CP-2 release. Although the effects of intrinsic and extrinsic modulation are similar, the neurons that mediate them are active predominantly at different times, suggesting a specialized role for each system. Metacerebral cell (MCC) activity predominates in the preparatory (appetitive) phase and thus precedes the activation of CBI-2 and biting motor programs. Once the CBI-2s are activated and the biting motor program is initiated, MCC activity declines precipitously. Hence intrinsic modulation prefacilitates biting, whereas intrinsic modulation occurs during biting. Since biting inhibits appetitive behavior, intrinsic modulation cannot be used to prefacilitate biting in the appetitive phase. Thus the sequential use of extrinsic and intrinsic modulation may provide a means for premodulation of biting without the concomitant disruption of appetitive behaviors.

INTRODUCTION

Rhythmic movements such as locomotion, respiration, and mastication are generated by ensembles of interconnected neurons [central pattern generators (CPGs)] that control the timing and intensity of the discharges of motor neurons (Delcomyn 1980; Marder and Calabrese 1996). The ability to modify behavior, without disrupting the proper phase and amplitude relations of its components, is thought to derive from a coordinated modulation of multiple elements of a CPG (Kiehn and Katz 1999). Over the years a number of neurons, biogenic amines, and neuropeptides that act from outside CPGs have been shown to act as extrinsic modulators (Beltz et al. 1984; Christie et al. 1997; Dickinson et al. 1990, 1993; Flamm and Harris-Warrick 1986; Heinzl 1988; Hooper and Marder 1987; Jankowska et al. 1997; Jovanovic et al. 1996; Kemnitz 1997; Nagy et al. 1981; Parker and Grillner 1999; Satterlie and Norekian 1996; Skiebe and Schneider 1994; Tierney et al. 1997; Turrigiano and Selverston 1990; Woolston et al. 1994; Yeoman et al. 1996). Although there is evidence that some extrinsically modulated CPGs contain neuromodulators, there is no direct demonstration of a single circuit that is modulated both extrinsically and intrinsically. Indeed, intrinsic modulation by identified CPG neurons has only recently been described (Katz and Frost 1995; Katz et al. 1994).

We sought to obtain a better understanding of the functions of extrinsic and intrinsic modulation by examining these two forms of plasticity acting on a single CPG, the feeding CPG of Aplysia. This preparation is attractive for the study of multiple forms of modulation because the feeding behavior of this animal is well characterized and there is indirect evidence that suggests that it may be subject to multiple forms of modulation. The serotonergic metacerebral cells (MCCs) are purely modulatory neurons that are considered to be extrinsic to the feeding CPG because their activity does not affect the CPG on a cycle-to-cycle basis, because they are only weakly coupled to CPG activity, and indeed because the majority of their activity in the living animal occurs prior to CPG activation (Kupfermann and Weiss 1982). The MCCs have been shown to contribute to but not account in total for the increase in the frequency of biting that occurs when an animal is aroused by food (Rosen et al. 1983, 1989; Weiss et al. 1986). Specifically, stimulation of the MCCs in a semi-intact preparation accelerated the feeding behavior, whereas lesions of the MCC reduced the initial frequency of biting and increased the duration of the protraction phase of biting (Rosen et al. 1983). However, in lesioned animals an acceleration of the feeding behavior and concomitant decrease in the duration of protraction was observed, but only after a considerable delay (Rosen et al. 1983, 1989). These findings suggested that the feeding CPG might be subject to an additional source of modulation that produces the delayed acceleration in feeding behavior.

In this study we present evidence that cerebral neuron CBI-2 (Rosen et al. 1991) may be this additional source of modulation...
tion. CBI-2 is activated by food stimuli, and firing CBI-2 initiates the biting program, so it was originally identified as a command-like neuron (Rosen et al. 1991). However, Rosen et al. (1991) also showed that bursts of stimulation of CBI-2 produced motor programs that followed the frequency of bursts at two different burst frequencies. This result demonstrated that CBI-2 transmits phasic information to the feeding rhythm on a cycle-per-cycle basis. As CBI-2 activity affects the motor rhythm on a cycle-per-cycle basis and is itself phasically active during motor programs (Church and Lloyd 1994; Rosen et al. 1991), CBI-2 is also an element of the CPG (Marder and Calabrese 1996). Hence, CBI-2 fulfills more than one role in the circuit. As CBI-2 is an element of the CPG, the modulatory actions of CBI-2 represent intrinsic modulation. Thus the feeding CPG in Aplysia is subject to dual modulation, and with the extensive information that is available on the control of feeding behavior in Aplysia, we are able to discuss the relative contributions and limitations of each form of modulation.

![Image](http://jn.physiology.org/doi/fig/10.1152/jn.1996.275.4.1187)

**FIG. 1.** Stimulation of the metacerebral cell (MCC) prior to and during CBI-2 stimulation decreases the cycle period and protraction to retraction ratio of the CBI-2–induced motor rhythm (B8 is a motor neuron in the buccal ganglion that acts on buccal musculature; B4 is a multifunction buccal neuron; BN2 is buccal nerve number 2, which contains axons from motor neurons in the buccal ganglion). A: 5-Hz stimulation of CBI-2 induces a slow motor program. B: 5-Hz stimulation of one MCC for 30 s prior to and during CBI-2 stimulation decreases the cycle period and protraction to retraction ratio of the CBI-2 motor program. Horizontal scale bar is 15 s, vertical scale bar is 40 mV; open and closed bars within the traces show representative protraction and retraction phases, respectively.

**FIG. 2.** The duration of cycles and the protraction to retraction ratio decrease with successive cycles within the initial stimulation of CBI-2. Horizontal scale bar is 15 s, vertical scale bar is 40 mV; open and closed bars within the traces show representative protraction and retraction phases, respectively. CBI-2 was stimulated with short current pulses at 15 Hz and fired consistently with those pulses except during the retraction phase of the biting rhythm, when feedback inhibition from buccal neurons acts on CBI-2, and momentarily at the beginning of the protraction phases that followed retraction phase inhibition.

**METHODS**

**Animals**

Experiments were performed on *Aplysia californica* weighing 100–250 g (Marinus, CA). The animals were maintained at 14–16°C in holding tanks for 3–7 days then transferred to room temperature tanks (22–24°C) and kept there for 2 days prior to use. This treatment was shown to increase the probability of feeding behavior being elicited from semi-intact preparations. The animals were anesthetized by the injection of isotonic MgCl₂ solution (50% body wt) into the body cavity. The cerebral and buccal ganglia were removed with the cerebral to buccal connective nerves (CBCs) intact. The ganglia were pinned to a silicone elastomer (Sylgard; Dow Corning, Midland, MI)–bottomed dish filled with 50% artificial sea water (ASW) and 50% isotonic MgCl₂ solution at room temperature. The connective tissue sheath that covers the neurons was surgically removed from the ganglia, and the bathing solution was replaced with 100% ASW. The bathing solution on the cerebral and buccal ganglia was separated into two compartments by the placement of a polyethylene ring around the buccal ganglion. Silicone vacuum grease (Dow Corning, Midland, MI) was used to make a water-tight seal between the separator ring and the Sylgard without damaging the CBCs (ASW, in mM: 460 NaCl, 10 KCl, 11 CaCl₂, 55 MgCl₂, and 10 HEPES 10, at pH 7.6; all salts from Sigma, St. Louis, MO).

**Electrophysiology**

Intracellular recordings were made using single-barreled microelectrodes filled with 2 M potassium acetate and beveled to a resistance of 6–8 MΩ. An Axoclamp 2A (Axon Instruments, Burlingame, CA) and two homemade amplifiers were used for the recordings. Neurons were identified based on size, morphology, membrane properties, and synaptic connections. Extracellular recordings were made using a polyethylene suction electrode placed on the nerve of interest (Morton and Chiel 1993) and connected to a model P15 AC amplifier (Grass Medical Instruments, Quincy, MA). Neurons were stimulated either by the injection of a constant, DC current, or by the injection of short pulses that each elicited a single spike with frequencies ranging from 5 to 15 Hz produced by a model S88 stimulator (Grass Medical Instruments). The bathing solution was kept at 16–18°C.

The protraction phase of the fictive feeding rhythm is characterized by a smooth depolarization of B8 with action potentials, a smooth depolarization of B4 without action potentials, and the appearance of a repeating unit in buccal nerve 2 (Church and Lloyd 1994; Rosen et al. 1991) and is indicated by the open bar underneath the traces (Figs. 1–4). The retraction phase is characterized by the sharp depolariza-
tions and action potentials in B8 and B4 and the appearance of multiple units in buccal nerve 2 and is indicated by the closed bar underneath the traces. The concurrence of the strong activity in B8 and B4 suggests that radula closure (monitored by B8 activity) occurs during the retraction phase (monitored by B4 activity). Radula closing during retraction characterizes ingestion (food is held by the radula as the radula moves toward the esophagus).

Measurements of average cycle period, protraction phase duration, and retraction phase duration of the CBI-2–induced motor program were made. Intracellular recordings from buccal neurons B4 and B8 and extracellular recordings from buccal nerve 2 were typically used for the measurements. The beginning of the protraction phase was defined for the purposes of measurement as the simultaneous appearance of the signs of protraction in two or more records. The beginning of the retraction phase (which is also the end of protraction) and the end of retraction were defined similarly. The average cycle period was defined as the time from the beginning of the first retraction phase to the beginning of the last retraction phase divided by the number of cycles in the stimulation minus one. In some cases, B16 replaced B8, and the I2 nerve replaced buccal nerve 2.

Immunohistochemistry

After electrophysiological identification, neurons were filled with 3% 5(6)-carboxyfluorescein in 0.1 M potassium citrate, titrated to pH 8.0 with KOH (Rao et al. 1986), by iontophoresis (10–15 min of 500 ms, 2-nA pulses at 1 Hz). Immunohistochemical methods used have been described (Miller et al. 1991). Ganglia were fixed with 4% paraformaldehyde in phosphate-sucrose buffer for 2–4 h at room temperature. The ganglia were washed repeatedly in phosphate buffer and were placed in triton-azide-phosphate buffer and kept at 4°C. The ganglia were exposed to 0.5% normal goat serum (Jackson Immuno Research) for 2 h, and subsequently a 1:200 dilution of primary antiserum for CP-2 (Phares and Lloyd 1996) was added. Following a 24-h incubation, the ganglia were washed repeatedly in phosphate buffer and left in phosphate buffer for 24 h. The ganglia were then placed in triton-azide-phosphate buffer with a 1:100 dilution of rhodamine-conjugated secondary antibody (Jackson Immuno Research) for 24 h. Following this incubation the ganglia were again washed repeatedly in phosphate buffer and left for 48 h in phosphate buffer. During this 48 h the phosphate buffer solution was exchanged every 12 h to facilitate removal of the secondary antibody. Ganglia so prepared were mounted on depression slides and examined on a microscope equipped with filter packs for viewing rhodamine and carboxyfluorescein epifluorescence [buffers (in mM): phosphate buffer: 20 K2 HPO4, 40 K H2 PO4, and 140 Na2 HPO4; phosphate-sucrose buffer: phosphate buffer diluted 1:2 in H2O with 30% sucrose final concentration; phosphate-triton-azide buffer: phosphate buffer diluted 1:2 in H2O with 2% Triton X-100 and 0.1% NaN3, both final concentrations; all salts and sucrose from Sigma].

RESULTS

The study of the organization and function of CPGs has been greatly facilitated by the use of isolated nervous systems in

FIG. 3. CBI-2 is CP-2 immunopositive. A: CBI-1, CBI-2, and CBI-12 were identified electrophysiologically and injected with carboxyfluorescein. The soma of CBI-1 is indicated by an asterisk, the somata of CBI-2 and CBI-12 are indicated by the numbers 2 and 12, respectively. B: CBI-2 and CBI-12 were shown to contain CP-2 immunoreactivity by using a primary antiserum for CP-2 and a rhodamine conjugated goat, anti-rabbit secondary antiserum (Jackson Immuno Research Laboratories). The MCC is not CP-2 immunoreactive (not visible in figure).

FIG. 4. Modulatory actions of CBI-2 and CP-2. A–D: increasing the stimulation frequency of CBI-2 or adding CP-2 exogenously modulates the CBI-2 motor rhythm. A: 5-Hz control stimulation of CBI-2 shows a shorter cycle period than the 5-Hz stimulation. B: 10-Hz control stimulation of CBI-2 shows a shorter cycle period than in control. C: 5-Hz stimulation of CBI-2 in 10 μM CP-2 shows a shorter cycle period than in control. D: 10-Hz stimulation of CBI-2 in 10 μM CP-2 shows a cycle period comparable to that in C. Horizontal scale bar is 15 s; vertical scale bar is 40 mV; open and closed bars within the traces show representative protraction and retraction phases, respectively.
which it has been possible to induce and monitor fictive motor rhythms. Here we used the isolated cerebral and buccal ganglia preparation of *Aplysia* in which CBI-2 firing was shown to induce fictive biting rhythms (Church and Lloyd 1994; Rosen et al. 1991) that correspond to actual biting movements (Rosen et al. 1998).

Previous work demonstrated that activity of the MCCs in *Aplysia* modulates the frequency of rhythmic feeding behavior (Rosen et al. 1983, 1989; Weiss et al. 1986). Because the MCCs innervate peripheral tissues and previous experiments were not performed on the isolated nervous system, the possibility that the MCCs modulate the frequency of feeding indirectly by affecting muscle contractions and/or sensory feedback remained. Here we demonstrate that in the absence of peripheral tissues, i.e., in the isolated nervous system, the MCCs modulate the frequency of the CPG. When one MCC was stimulated prior to and during a 5-Hz stimulation of CBI-2, both the average cycle period and the protraction to retraction ratio were reduced (Fig. 1, A and B; average cycle period went from 44.7 ± 5.1 s, mean ± SE, in control to 30.8 ± 4.7 s after MCC stimulation; the protraction to retraction ratio went from 1.6 ± 0.1 in control to 1.1 ± 0.1 after MCC stimulation; *n* = 7; *P* < 0.01 and *P* < 0.05, respectively).

Stimulation of CBI-2 was shown previously to initiate the *Aplysia* biting motor program (Church and Lloyd 1994; Rosen et al. 1991). We observed a subtle effect of CBI-2 stimulation that was present consistently in previously unstimulated preparations. We found that the initial stimulation of CBI-2 to fire at 15 Hz produced a rhythm that was at first slow and accelerated with continued stimulation of CBI-2 (Fig. 2). Thus from the first cycle to the third cycle the duration of the cycle decreased from 39.6 ± 5.8 s to 21.0 ± 1.8 s (Fig. 2); the protraction to retraction ratio decreased from 2.3 ± 0.2 to 1.3 ± 0.2 (Fig. 2). Equally, the cycle period and the ratio of protraction to retraction remained relatively stable (Fig. 2).

The neuropeptide CP-2 previously was shown to be present in cerebral neurons in *Aplysia* and to affect motor neurons in the buccal ganglia when applied exogenously (Phares and Lloyd 1996). Using immunostaining we found that CBI-2 is immunoreactive for CP-2 (Fig. 3). With the possibility that CBI-2–induced rhythms may be self modulating and that transmitter released by CBI-2 (e.g., its classical transmitter) could have contributed to the observed modulation. Even if CP-2 is the mediator of the effects seen with high-frequency CBI-2 stimulation, this does not mean that CP-2 alone is necessarily capable of such modulation. Rather, the action a classical transmitter released by CBI-2 (Rosen et al. 1991) even at low-frequency stimulation may produce effects that enable the putative CP-2 modulation we see (Blitz and Nusbaum 1999).

We also found that the cycle period and the proportion to retraction ratio were correlated, i.e., the preparations that had relatively long cycle periods also had high proportion to retraction ratios (Fig. 6A). This relationship was maintained following bath application of the neuropeptide CP-2, but the average values of the period and the proportion to retraction ratio were decreased markedly (Fig. 6B; the average value of *P* = 1.9 ± 0.3 in control and 1.0 ± 0.1 in CP-2; *P* < 0.05; the average value of the period is 40 ± 7 s in control and 20 ± 2 s in CP-2; *P* < 0.05). Furthermore, the degree of effect of bath application of CP-2 depended on the initial cycle period; that is, the effect of CP-2 on the initial cycle period was more pronounced when the initial cycle period was high (Fig. 6C). Along with the findings illustrated in Figs. 4 and 5, these findings suggest that CBI-2 may release CP-2 in a frequency or number of spikes dependent manner (Vilim et al. 1996) to modulate the cycle with both the period of the biting rhythm...
The CBI-2–induced feeding CPG rhythm in *Aplysia*. In addition, the exogenous application of the neuropeptide C-2 or an increase in firing of CBI-2 produces similar modulatory effects on the same rhythm. Therefore we provide evidence that the feeding CPG in *Aplysia* is extrinsically modulated by the MCC and its transmitter serotonin, and intrinsically modulated by neuron CBI-2 and its neuropeptide CP-2. The two forms of modulation share a number of similarities; however, with the knowledge gained by previous work we suggest that the two forms of modulation are not redundant and that each of them makes a unique contribution to the generation of feeding rhythms.

Previous work has shown that both in *Aplysia* and in related mollusks the MCCs modulate several aspects of feeding behavior (Arshavsky et al. 1985; Rosen et al. 1983, 1989; Weiss et al. 1975, 1978, 1981, 1986; Yeoman et al. 1996). In *Aplysia*, the MCCs modulate contractions of feeding musculature (Fox and Lloyd 1998; Lloyd et al. 1984; Weiss et al. 1978), they increase the frequency of feeding behavior (Weiss et al. 1981), and as suggested by the MCC lesion experiments, they shorten and the relative duration of the protraction and retraction phases affected.

The modulatory actions exerted by bath application of CP-2 or by higher frequency (10–15 Hz) stimulation of CBI-2 are almost identical to those produced by stimulation of the MCC (Fig. 1). The similarity of the effects of CBI-2/CP-2 and MCC/serotonin suggest that the effects of two modulatory systems may ultimately converge on the same elements of the CPG. We found that the actions of the MCC and CBI-2 occluded each other, i.e., stimulation of the MCC prior to and during 10-Hz CBI-2 stimulation had no additional effects on the already shortened cycle period and reduced the protraction to retraction ratio (average cycle period and protraction to retraction ratio with 10-Hz CBI-2 stimulation were 25.3 ± 2.3 and 1.1 ± 0.1, respectively; after MCC stimulation average cycle period and protraction to retraction ratio were 24.1 ± 2.8 and 1.0 ± 0.1). Similarly, following bath application of CP-2 or serotonin (which mimicked MCC stimulation), MCC stimulation produced no additional modulatory effect (Fig. 7). Barring a ceiling effect as discussed before, these occlusion experiments suggest that the actions of CP-2 and serotonin may converge on the same elements in the feeding CPG.

**DISCUSSION**

The exogenous application of serotonin or stimulation of the MCC decreases the period and protraction to retraction ratio of the CBI-2–induced feeding CPG rhythm in *Aplysia*. In addition, the exogenous application of the neuropeptide CP-2 or an increase in firing of CBI-2 produces similar modulatory effects on the same rhythm. Therefore we provide evidence that the feeding CPG in *Aplysia* is extrinsically modulated by the MCC and its transmitter serotonin, and intrinsically modulated by neuron CBI-2 and its neuropeptide CP-2. The two forms of modulation share a number of similarities; however, with the knowledge gained by previous work we suggest that the two forms of modulation are not redundant and that each of them makes a unique contribution to the generation of feeding rhythms.

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the duration of protraction (Rosen et al. 1983, 1989). The present work indicates that both the increase in the frequency of the rhythm and alterations of the protraction-retraction phase ratio in fictive feeding rhythms are extrinsically modulated by the MCCs at the CPG level.

Our finding that the feeding CPG is modulated not only by the MCC but also by CBI-2 helps explain how, despite the drastic reduction in the firing rate of the MCC at the onset of biting, the animal maintains its speed of biting. As an intrinsic element of the feeding CPG, CBI-2 maintains its activity and hence continues to exert its modulatory effect throughout a bout of biting. Consequently, the elevated frequency of biting is maintained even in the face of reduced activity in the MCCs. Furthermore, the existence of the intrinsic modulatory system helps to explain why animals in which the MCCs have been lesioned are gradually able to increase their biting frequency. Since, in the MCC lesioned animals, the up-modulation of biting that is normally exerted by the firing of the MCC during the appetitive phase is absent, the speed of biting is at first slow. However, our data indicate that as the CBI-2s initiate biting and begin to exert their modulatory actions the speed of biting progressively increases (Fig. 2). That the rate increases in lesioned animals but reaches a level that is lower than that observed in unlesioned animals suggests that extrinsic modulation by the MCC continues to make a contribution while the animal is biting. Indeed, MCC activity, although decreased, continues for some time after the onset of biting (Kupfermann and Weiss 1982). Furthermore, since we found that when stimulated sufficiently either the MCC or CBI-2 modulatory action occludes that of the other, it appears that, during feeding in MCC-lesioned animals, CBI-2 modulation does not reach its in vitro maximum.

MCC/5-HT and CBI-2/CP-2 exert similar modulatory actions on the fictive biting rhythm. Findings from previous work, however, make it unlikely that these modulatory actions serve identical roles in feeding. Specialized functions of the two modulatory systems may derive from a differential pattern of activation in various stages of feeding behavior. Similar to many behaviors and to the feeding behavior of other animals, feeding behavior in *Aplysia* is organized into phases. The appetitive phase, which occurs when food is detected in the environment, serves to localize food and bring the mouth of the animal in contact with food. This is followed by the ingestive phase, which consists of biting and is triggered when food contacts the mouth (Kupfermann 1974a–c). The MCCs are strongly activated during the appetitive phase of behavior, a phase during which the activity of an identified neuron (the cerebral-pedal regulatory neuron) increases the excitability of or activates many neurons that are involved in feeding, including the MCC, and decreases the excitability of neurons that are involved in competing behaviors, e.g., defensive behaviors (Hurwitz et al. 1999b; Teyke et al. 1990a, 1997). Although predominant in the appetitive phase, MCC activity itself has no effect on the various aspects of appetitive behavior; rather, the effects of MCC activity are manifested in the beginning of the ingestive phase (Rosen et al. 1989).

Although the MCC and CBI-2 are both involved in feeding behavior, the different phases of feeding behavior in which these neurons are predominantly active are themselves competing behaviors. During the appetitive phase of feeding behavior, head turning is important for the localization of food; however, once food is centered on the mouth and a biting response is elicited, head turning is inhibited by over 90% (Teyke et al. 1990b). Thus biting inhibits head turning and thereby prevents the animal from turning away from food that has already touched the mouth and is immediately available for

![Fig. 7. CP-2, MCC stimulation, and serotonin have similar effects on the cycle period (A) and protraction to retraction ratio (B) of the CBI-2 motor program (a total of 9 experiments were performed: control, n = 9; CP-2 alone, n = 7; MCC alone, n = 7; CP-2 and MCC, n = 7; serotonin alone, n = 4; serotonin and MCC, n = 4). Analysis of variance of the control and experimental groups showed that there was a significant difference between the groups (P < 0.05 for both period and protraction to retraction ratio data). Analysis of variance of the experimental groups with the control group excluded showed no statistical difference between the experimental groups (P > 0.5 for both period and protraction to retraction ratio data). Dunnett’s tests for multiple comparisons to control indicated statistically significant differences from control for each of the experimental groups (P < 0.05 for each average cycle period and protraction to retraction ratio except P < 0.01 for the MCC stimulation alone for average cycle period).

![Fig. 8. Diagrams showing proposed roles of MCC and CBI-2 in the progression and modulation of biting behavior. A: the MCC may act as a bridge between appetitive and ingestive behaviors. A food stimulus outside of the mouth initiates appetitive behavior and the stimulation of the MCC. MCC activity modulates early ingestive activity initiated by a food stimulus to the mouth area. Ingestive circuit activity inhibits appetitive circuit activity, and MCC activity declines. B: 2 proposed stages of modulation of the *Aplysia* feeding CPG. Initially (left), MCC modulation is active and CBI-2 modulation is inactive during the appetitive phase of feeding and the beginning of biting. Later, during established biting (right), MCC modulation is reduced and CBI-2 modulation is active.](http://jn.physiology.org/doi/10.220.32.247/FIG.7.png)
ingestion. Importantly, since intrinsic modulation involves activation of the ingestive CPG whose activity inhibits head turning behavior, it is clear that the intrinsic circuitry cannot be used during the appetitive phase of behavior without disrupting the process of food localization and acquisition. Therefore we suggest that, whereas modulation by CBI-2 acts predominantly in the ingestive phase, activation of the MCCs during the appetitive phase of behavior constitutes a bridge between the appetitive phase of feeding and the ingestive phase in which the presence of food on the mouth triggers the biting response, and appetitive behavior (including MCC activity) is inhibited (Fig. 8A).

The difference between the function of MCC and CBI-2 modulation is not limited to the timing of when each takes place, however. It has been noted previously (Cropper et al. 1987; Katz 1995) that extrinsic modulatory systems tend to act on a large number of elements that are involved in the generation of a specific behavior, whereas intrinsic modulation tends to act on a more limited number of circuits or elements within a circuit. Such is the case for the extrinsic modulators of biting, the MCCs. Unlike the CBI-2s, the MCCs act not only on the CPG for biting but also modulate the contraction of feeding muscle directly. The actions of the MCCs on the musculature are not selective; i.e., when the MCCs are active all of the muscles innervated by the MCCs are modulated. This generalized modulation limits the effect of selective modulation of individual muscles, a type of modulation that may be necessary to adjust the contraction of individual muscles to specific demands imposed by the characteristics of food. When CBI-2 is brought into play, the contribution of the MCCs to the overall modulation decreases; this allows for selective modulation of individual muscles through specific sensory input (Fiore and Geppetti 1981; Miller et al. 1994) (Fig. 8B). Interestingly, extensive literature documents that this modulation may be exerted by modulators that are intrinsic to the motor systems as they are released from individual motorneurons (Church et al. 1993; Cropper et al. 1987; Fox and Lloyd 1997).

Complex behaviors generally occur as sequences of stages that are characterized by patterns of muscle activity and outcomes. The successful transition from one stage to the next requires that the neural circuitry responsible for generating the coming stage of behavior be appropriately prepared. However, in some behaviors, a successful transition from one stage of behavior to the next mandates the inhibition of the first stage. Under such conditions, modulation that controls the ongoing behavior of the second stage cannot be used. To overcome this problem and allow for a smooth transition, a bridging form of modulation that prepares the neural circuitry for the next stage of behavior could be active while the current stage of behavior progresses. This bridging modulation might have many similarities to the modulation that is active during the next stage of behavior, but unlike that coming modulation, would most likely be stereotyped, and not tunable to small differences in the environment. Here we suggest that an extrinsic modulator (MCC-serotonin) primes the Aplysia feeding CPG for ingestion during the appetitive phase of behavior and an intrinsic modulator (CBI-2/CP-2) acts during the ongoing expression of ingestive behavior. More generally, extrinsic modulation may act both centrally and peripherally to facilitate or “prime” behavior (Jacobs and Fornal 1995), whereas intrinsic modulation affects individual components, be they central or peripher-eral, that are involved in the generation of the behavior. Since a number of findings suggest that many vertebrate and invertebrate CPGs may be subject to both intrinsic and extrinsic modulation (Katz 1995), it is possible that in those CPGs the two forms of modulation play roles similar to those that we have suggested here.

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