Feeding Neural Networks in the Mollusc

**Aplysia**

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**Key Words**

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**Abstract**

*Aplysia* feeding is striking in that it is executed with a great deal of plasticity. At least in part, this flexibility is a result of the organization of the feeding neural network. To illustrate this, we primarily discuss motor programs triggered via stimulation of the command-like cerebral-buccal interneuron 2 (CBI-2). CBI-2 is interesting in that it can generate motor programs that serve opposing functions, i.e., programs can be ingestive or egestive. When programs are egestive, radula-closing motor neurons are activated during the protraction phase of the motor program. When programs are ingestive, radula-closing motor neurons are activated during retraction. When motor programs change in nature, activity in the radula-closing circuitry is altered. Thus, CBI-2 stimulation stereotypically activates the protraction and retraction circuitry, with protraction being generated first, and retraction immediately thereafter. In contrast, radula-closing motor neurons can be activated during either protraction or retraction. Which will occur is determined by whether other cerebral and buccal neurons are recruited, e.g. radula-closing motor neurons tend to be activated during retraction if a second CBI, CBI-3, is recruited. Fundamentally different motor programs are, therefore, generated because CBI-2 activates some interneurons in a stereotypic manner and other interneurons in a variable manner.

**Introduction**

Although relatively simple neural networks mediate feeding behaviors in *Aplysia*, these networks exhibit a great deal of flexibility. For example, feeding is affected by motivational states [1–20], and can be subjected to operant and classical conditioning [21–32]. *Aplysia* feeding has, therefore, proven to be unusually experimentally advantageous for studies of behavioral plasticity.

Initially, *Aplysia* feeding was primarily studied with a top-down approach, i.e., investigators started with behavior then proceeded to circuit analyses [33]. Consequently, the characterization of the *Aplysia* feeding circuitry is still very much ongoing, and it has not been described in detail in a review article. However, see Kupfermann et al. [18] and Kupfermann [34] for reviews of the modulation of feeding and the generation of behavioral states, and...
Feeding Behaviors Most Extensively Studied at the Circuit Level

Although feeding in *Aplysia* consists of both appetitive and consummatory phases, consummatory feeding has been most extensively characterized at the circuit level [for studies of appetitive behavior, see ref. 19, 21–27, 35–43]. More specifically still, a number of studies have focused on movements of the odontophore and radula. The radula is a sheet of semi-hardened tissue covered with rows of chitinous teeth that is used to grasp food and pull it into the buccal cavity (fig. 1a, b) [44].

The radula is bilaterally symmetrical, and consists of two halves (fig. 1b, right) [45]. A number of muscles are directly attached to the radula halves. In general, these muscles are intrinsic to the buccal mass and are therefore referred to with an ‘I’. Extrinsic muscles are given the designation ‘E’ [13, 44, 46]. When some radula muscles contract, the radula halves are pulled apart, i.e., the radula is ‘opened’ (fig. 1b, right; 2b). When other muscles contract, the radula halves are brought together, i.e., the radula is ‘closed’ (fig. 2b). Additionally, the radula can move towards the jaws and towards the esophagus (fig. 2). Often these movements are referred to as protraction and retraction, although they are actually more complex [1, 47, 48].

Three types of consummatory feeding responses have been most extensively characterized; bites, swallows, and rejection movements (fig. 2b) [45]. Bites and swallows are ingestive, i.e., radula opening and protraction occur more or less (but not completely) simultaneously, as do radula closing and retraction (fig. 2b, c). Bites occur when animals make ingestive responses but do not successfully grasp food (fig. 2b) [45]. The radula protracts open, and then retracts closed to return to a neutral state [1]. Bites are converted to bite-swallows when food is ingested [45]. Under these conditions, the radula closing and retraction phase of behavior is enhanced and prolonged so that food will be deposited in the esophagus (fig. 2c) [49]. The enhanced radula retraction that occurs during a swallow is often referred to as hyperretraction. Rejection responses are egestive; radula closing and protraction occur more or less (but again not completely) simultaneously, as do radula opening and retraction (fig. 2d). This combination of radula movements will tend to push an object out of the buccal cavity.

Elliott and Susswein [33] for a review of the comparative neuroethology of feeding control in molluscs. This review differs in that it specifically focuses on *Aplysia* circuitry (i.e., comparative issues are for the most part not discussed), and the emphasis is on basic mechanisms for pattern generation (as opposed to modulation and plasticity).
Fig. 2. Radula movements during consummatory feeding responses. 

**a** Radula in neutral position. Drawing of a partially dissected preparation, which indicates the resting (neutral) position of the radula within the buccal mass. The radula is shown in gray and black.

**b–d** Schematic drawings illustrating radula movements during feeding. In each schematic, gray indicates the initial position of the radula and black represents the final position.

**b** Bite. When *Aplysia* bite, the radula opens and protracts (left) and then closes and retracts (right) to return to the initial (i.e., neutral) position. **c** Bite-swallow. When a bite-swallow is generated, the radula opens and protracts as during a bite (left). Food contact is then detected, and the closing/retraction phase of behavior is enhanced so that food can be pulled into the buccal cavity and deposited in the esophagus (middle). The radula then opens so that food will be released into the esophagus (right). **d** Egestive response. When animals generate egestive responses, phase relationships between radula opening vs. closing and protrac- tion vs. retraction are changed. The radula retracts open (left) and protracts closed (right).

Other consummatory responses that have been described, albeit less extensively, include swallow/tears [47, 50] or cuts [51]. Cuts or tears can be triggered if *Aplysia* are fed strips of food and a sufficient counterweight is attached [51]. Cutting releases food, presumably to prevent it from being pulled out of the buccal cavity [51]. Additionally, *Aplysia* also appear to be capable of making grazing movements in which animals locomote with their mouths against a substrate while rhythmic radula movements occur [52].

To summarize, bites, swallows, and rejection movements have been most extensively characterized in *Aplysia*. Consequently, most studies of feeding motor programs triggered in the isolated nervous system interpret data with respect to these behaviors. As other behaviors are described, however, it may be necessary to refine or reevaluate some of the current classifications of rhythmic activity.

**Circuitry for Ingestive Radula Movements: Biting**

The circuitry that mediates feeding in *Aplysia* is located in two ganglia, the cerebral ganglion and the buccal ganglion (fig. 3a). The buccal ganglion is clearly necessary for all consummatory behaviors [53] since it contains the motor neurons that innervate the feeding musculature [54, 55]. A more controversial question has been whether the cerebral ganglion is essential. In part, the role of the cerebral ganglion appears to be behavior dependent. For biting, the cerebral ganglion does appear to be necessary. Biting responses cannot be triggered if the connection between the cerebral and buccal ganglia (the cerebral-buccal connective) is lesioned or crushed [53, 56–59].

Biting is presumably initiated when cerebral-buccal interneurons (CBIs) are activated (fig. 3a) [60]. These neurons are referred to as CBIs because they have somata in the cerebral ganglion and they project to the buccal ganglion. Currently, approximately 13 CBIs have been identified [61]. A number of CBIs are activated by food-related stimuli (although, as might be expected, this activation is indirect) [60, 62]. In general, the CBIs make synaptic connections with both buccal interneurons and buccal motor neurons (fig. 3a). Thus, buccal motor neurons receive input from the CBIs both directly and indirectly (via buccal interneurons). Buccal motor neurons in turn innervate the feeding muscles, which for the most part are nonspiking (fig. 3a). The total number of motor neuron action potentials in general determines contraction amplitude.
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Fig. 3. a Schematic representation of the circuitry that generates ingestive activity. Motor programs can be triggered via activation of CBIs (notably CBI-2). CBI-2 makes fast excitatory connections with protraction interneurons (some of which are also motor neurons). The protraction circuitry makes inhibitory connections with the retraction circuitry. The retraction circuitry makes inhibitory connections with the protraction circuitry and with CBI-2. Inhibitory connections with CBI-2 are made in both the buccal ganglion and the cerebral ganglion. Some closing/retraction interneurons have peripheral process and dual function, i.e., they also function as afferents.

b Excitatory connections between CBI-2 and the protraction neurons B63, and B31/B32. CBI-2 excites both B63 and B31/B32, but the direct connection with B31/B32 is relatively weak (indicated by the dashed line). B63 makes a chemical excitatory connection with the B31/B32 neurons and is electrically coupled to these cells. During the protraction phase of motor programs, a positive feedback loop is created in that B63 excites B31/B32, which then re-excite B63.

c CBI-2-induced excitation of B63 and one of the B31/B32 neurons. Note that B63 is excited first. Also note the unusually large sustained depolarization in the B31/B32 neuron (according to Hurwitz et al. [70]). pro = Protraction; ret = retraction.

during a burst of activity, i.e., motor neuron firing frequency and duration are both important [63].

One CBI that has been implicated in the generation of biting is the cell CBI-2 [60]. It should be noted, however, that subsequent to the characterization of CBI-2, a second CBI (CBI-12) was identified that has morphological features that are similar to those of CBI-2 [43, 64]. CBI-2 and CBI-12 are not electrically coupled, and activation of one cell does not recruit the other. Nevertheless, motor programs triggered by the two neurons are similar [43, 64]. Because the two CBIs are so alike, it is possible that they have, in some cases, been confused. This is particularly likely to have been true in studies that were conducted prior to the 1999 characterization of CBI-12. Criteria that can be used to distinguish one neuron from the other have now been described [43, 65].

Under physiological conditions, biting motor programs are presumably triggered when CBI-2 is activated by afferent input [60]. Experimentally, however, CBI-2 motor programs are triggered via injection of depolarizing current, i.e., via brief current pulses (fig. 3c, 4) or via injection of direct current (fig. 5). Additionally, CBI-2 can be pharmacologically activated if the nonhydrolyzable cholinergic agonist, carbachol, is applied to the cerebral ganglion [66]. Radula movements induced by carbachol are also primarily biting-like [66]. It has been noted, however, that CBI-2 is not the only CBI activated by carbachol, and that carbachol- and CBI-2 induced motor programs are similar but not identical.

To summarize, biting responses in intact animals are most likely generated when the command-like neuron CBI-2 is activated. Experimentally, fictive biting motor
Internuron B40 is important for expression of CBI-2-elicited ingestive motor programs. a–c Single cycles of feeding motor programs induced by stimulation of CBI-2 with brief current pulses (top traces). Cycles of activity were approximately 2 min apart. The open and filled bars indicate the protraction and retraction phases of activity, respectively (bottom line). In all three cases, CBI-2 stimulation was maintained throughout protraction, but was then terminated when retraction was initiated. a A cycle of ingestive-like activity (the radula closer motor B8 is predominantly active during the retraction phase of the motor program). b The buccal internuron B40 was bilaterally hyperpolarized. This converted ingestive-like activity to an intermediate program (there is increased activity in B8 during protraction). c The B40 hyperpolarization was relieved and the program was again ingestive-like (according to Jing and Weiss [67]). pro = protraction; ret = retraction.

Programs can be generated in the isolated nervous system via stimulation of CBI-2, or by applying carbachol to the cerebral ganglion. Below, we primarily focus on motor programs triggered by CBI-2 because they have been more extensively characterized than carbachol-induced programs.

CBI-2 Elicited Motor Programs

CBI-2 motor programs consist of at least two phases, a radula protraction phase followed by a radula retraction phase (fig. 4, 5). Additionally neurons are activated that produce radula opening and closing movements. To a large extent, the interneurons that generate protraction vs. retraction appear to be different from those that generate opening vs. closing (fig. 6) [67]. Below, we begin by describing the circuitry that generates protraction vs. retraction.

Generation of Radula Protraction and Retraction

When rhythmic activity is triggered via CBI-2, the radula protraction phase of the motor program is always initiated first (fig. 4, 5) [68]. This is a result of the fact that...
Fig. 6. Interneurons activated during CBI-2-evoked motor programs. 

(a) Interneurons that are primarily important for the generation of protraction and retraction are shown in black. Protraction is initiated when CBI-2 excites interneurons such as B63, and interneurons/motor neurons such as B31/B32. During retraction, the protraction circuitry and CBI-2 are inhibited by neurons such as B64. B64 is also electrically coupled to motor neurons that produce radula retraction (not shown). It is currently not clear how protraction/retraction phase transitions are triggered, but the activation of an unidentified neuron (the z cell) may be involved (see text). Gray: neurons that control closing.

(b) Two classes of neurons are shown in black: (1) neurons that are important for the generation of protraction and retraction, and (2) interneurons that are primarily important for the control of the B8 radula closer motor neurons. During CBI-2 motor programs, the B8 neurons can be activated either during protraction, which makes activity egestive-like, or during retraction, which makes activity ingestive-like. B20 is, however, activated during the protraction phase of the motor program. B20 directly excites B8. Additionally, B20 makes a slow excitatory connection with the B4/5 neurons and these cells are activated during the retraction phase of the motor program. The B4/5 neurons make a fast inhibitory connection with B8.

(c) When activity is ingestive-like, CBI-3 is generally recruited by CBI-2. CBI-3 inhibits B20 activity during protraction and B4/5 activity during retraction. Additionally, a cell not active during egestive-like motor programs (B40) is activated. B40 inhibits B8 activity during protraction (via fast inhibition) and promotes B8 activity during retraction (via slow excitation) [65].
CBI-2 makes monosynaptic excitatory connections with a number of protraction interneurons and motor neurons (fig. 3a, b) [64, 69, 70]. Neurons that have been particularly well studied in this context include a group of buccal cells that are thought to be important for generating protraction movements, i.e., the neurons B63, B31, and B32 (fig. 3b) [71–75]. B31 and B32 are electrically coupled, and are virtually indistinguishable [72]. Therefore, they are often referred to together as B31/B32. B63 is electrically coupled to B31/B32, and, in addition to the electrical connection, there is a strong excitatory chemical connection from B63 to the contralateral B31/B32 neurons [71]. B63 and B31/B32 have been described as a functional unit since sufficient depolarization of one of these cells excites all the others [71, 73]. There is a direct relationship between B63/B31/B32 activity and protraction movements since B31/B32 are motor neurons [74, 75]. Specifically, they innervate the I2, which is a major protractor muscle [75]. Thus, protraction is initiated when CBI-2 excites motor/interneurons like B63/B31/B32 (fig. 6a).

If CBI-2 is sufficiently activated, a brief period of stimulation will induce a protraction phase of a motor program that persists after CBI-2 stops spiking (fig. 4). (This is also true with feeding behavior, i.e., once a feeding response is initiated, it is generally completed even if food is removed [45].) In part, the persistence of activity in B63/B31/B32 is likely to be due to the fact that a positive feedback loop is created in this circuitry (fig. 3b) [71]. Thus, during CBI-2-elicited motor programs, B63 is activated first (fig. 3c, 7) [71]. B63 provides excitatory input to the B31/B32 neurons via electrical and facilitating chemical excitation (fig. 3b, step 2) [70, 71]. The B63-induced excitation of B31/B32 is essential for the generation of motor programs, i.e., if B63 is hyperpolarized, programs are not triggered [70]. Presumably, this is because direct connections between CBI-2 and B31/B32 are relatively weak (fig. 3b, step 1b), and in B31/B32, the threshold for excitation is relatively high [70]. As B31/B32 are depolarized, this depolarization is transmitted back to B63 via the electrical coupling, i.e., the loop is closed (fig. 3b, step 3) [71, 73]. Transmission in the B31/B32-to-B63 direction is likely to be facilitated by a number of factors [73]. For example, the B31/B32:B63 coupling ratio is asymmetric (favoring the B31/B32-to-B63 direction). Sustained depolarizations in B31/B32 are also unusually large (i.e., 30–40 mV) (fig. 3c). Presumably, the large depolarizations result from the unusual biophysical properties of the B31/B32 neurons. Namely, plateau or plateau-like potentials are observed in B31/B32, but the somata of these cells do not generate action potentials [72–74].

To summarize, with sufficient activation of the command-like neuron CBI-2, a sustained depolarization is generated in the protraction motor neurons B31/B32. Consequently, if CBI-2 stimulation ceases, B31/B32 do not immediately repolarize. This sustained depolarization may in part result from the fact that a positive feedback loop is created in the protraction circuitry. Additionally B31/B32 generate plateau or plateau-like potentials.

B63 and B31/B32 are not the only neurons active during the protraction phase of CBI-2-induced motor programs. Some of the other cells activated are important for determining whether programs are ingestive-like or egestive-like (as is described below). Still other cells can alter the onset of activity in B31/B32. B34 [71] is a cell in the latter category (fig. 7) [70]. Thus, B34 is directly excited by CBI-2 and is reliably recruited when CBI-2 is activated (recruitment in other motor programs, however, does not always occur) [65, 70]. Although B34 provides excitatory input to B31/B32, hyperpolarization of B34 does not block CBI-2-induced activation of B31/B32 (presumably because the CBI-2-B63/B31/B32 pathway is utilized) [67, 70]. However, the slope of the depolarization in B31/B32 is decreased, i.e., B31/B32 depolarize more slowly. Thus, B34 is not essential for protraction, but it does modify its expression.

Parametric features of protraction can also be affected by the recruitment of another buccal interneuron, B50 [76]. B50 has been of particular interest since it appears to be homologous to a well-characterized interneuron that can trigger rhythmic activity in Lymnaea, the slow oscillator [77, 78]. (To call attention to the homology, the Aplysia neuron was given a designation that resembles the Lymnaea designation, i.e., ‘50’ vs. ‘SO’.) B50 makes excitatory connections with B34 and with the B63/B31/B32 cells [76]. B50 differs from CBI-2 in that direct connections from B50 to B31/B32 are relatively strong [76]. (As discussed above, direct connections between CBI-2 and B31/B32 are relatively weak.) Although CBI-2 directly excites B50, this connection is not strong enough for B50 to be recruited every time a motor program is generated. When B50 is activated, however, parametric features of CBI-2-triggered motor programs are altered, e.g. the protraction phase of the motor program is shortened [76].

While protraction is ongoing, retraction interneurons and motor neurons are inhibited (fig. 3a, 6a). In part, this inhibitory input arises from the interneurons that provide excitatory input to protraction motor neurons; for example, B63 makes a weak inhibitory connection with a retraction interneuron B64 [71, 79] and B34 inhibits B64 [80]. Additionally, however, other cells, i.e. the B52 neu-
rons, are also activated (at least during carbachol-induced motor programs [81, 82]). The B52 neurons make strong inhibitory connections with a number of elements of the retraction circuitry [81–83]. Direct excitatory connections between the B52 neurons and protraction motor neurons have, however, not been described. Moreover, B52 stimulation does not trigger rhythmic activity [83]. At least to some extent, therefore, the generation of protraction and the inhibition of retraction may be mediated by different neurons.

To summarize, a number of buccal interneurons activated during the protraction phase of CBI-2-elicited motor programs have been identified. Some of these neurons appear to be essential for generating protraction movements (i.e., B31/B32 and B63). Others (a) modify parametric features of protraction (i.e., B34), (b) inhibit the retraction circuitry (i.e., B52), and (c) determine activity in the opening vs. closing circuitry (i.e., B20 and B40, which are discussed below).

The protraction phase of a CBI-2-elicited motor program is immediately followed by the retraction phase (fig. 4, 5). The termination of protraction appears to be an active process, e.g. protraction neurons are hyperpolarized below resting membrane potential during retraction [79]. At least in part, this inhibitory input is provided by the retraction interneuron B64 (fig. 6a) [79, 84]. Inhibitory input from B64 is highly effective, i.e., B64 stimulation can phase advance retraction [79, 84]. It is not, however, currently clear how activity in B64 (or the rest of the retraction circuitry) is triggered, i.e., it is not clear what makes protraction-retraction phase transitions occur. In quiescent preparations, B64 does not show postinhibitory rebound excitation at its resting membrane potential.

It has been suggested that B64 activation may be triggered by an as yet uncharacterized circuit element, which has been referred to as the ‘z’ cell (fig. 6a) [85]. The z cell would be activated towards the end of protraction and would provide excitatory input to B64, which would inhibit the protraction circuitry [85]. B64 also makes electrical connections with other retraction neurons. Consequently, it could play a role in driving retraction. Additionally and/or alternatively, some of the characterized circuit elements active during protraction may provide slow excitatory input to retraction neurons [84]. Phase transitions may, therefore, be determined by the balance of excitatory and inhibitory inputs received by the retraction circuitry during the protraction phase of the motor program.

At least two of the interneurons that are depolarized during the retraction phase of CBI-2 motor programs have plateau or plateau-like potentials, i.e. B64 [79] and a second interneuron B51 [83]. B64 and B51 differ in that,
although both cells are depolarized, B51 has a higher threshold for plateau initiation and most commonly does not spike during CBI-2 (or carbachol)-elicited motor programs [86]. The plateau properties of B51 may, however, contribute to the generation of retraction movements since B51 is electrically coupled to B64 and to a number of retraction motor neurons [79, 83, 86]. Thus, a number of the characterized connections in the retraction circuitry are electrical.

While retraction is ongoing, neurons that generate protraction movements are inhibited (fig. 3a, 6a) [79]. Additionally, CBI-2 receives inhibitory input during retraction (fig. 3a, 6). In part, CBI-2 is inhibited by neurons that are referred to as buccal-cerebral interneurons (BCIs) [43, 87]. These cells have somata in the buccal ganglion but project to the cerebral ganglion via the cerebral-buccal connective. One such neuron is B19 [43]. B19 is not, however, always driven to spike during CBI-2-elicited motor programs, and somatic inhibition of CBI-2 is not always observed. CBI-2 is, however, also presynaptically inhibited by B64 in the buccal ganglion (fig. 6a) [84]. B64 is always activated during CBI-2-generated motor programs [70, 80, 84]. Consequently, although somatic spiking can sometimes be observed in CBI-2 during the retraction phase of motor programs, this activity is not transmitted to the protraction circuitry in the buccal ganglion [84].

To summarize, during the retraction phase of motor programs, interneurons with plateau-like potentials are activated (e.g. B64). B64 inhibits protraction interneurons and presynaptically inhibits CBI-2. Consequently, the protraction phase of the motor program is terminated.

Some molluscs (e.g. Lymnaea and Helisoma) have been described as having a three-phase motor program, protraction, retraction, and hyperretraction or swallowing [88, 89]. During CBI-2-elicited motor programs in Aplysia, activity is observed in buccal neurons immediately after the retraction phase of the motor program. It is unlikely, however, that this activity corresponds to the hyperretraction or swallow phase described elsewhere. For example, immediately after retraction, activity is observed in the B52 neurons [81]. The B52 neurons make inhibitory connections with the retraction circuitry, so they certainly do not initiate retraction movements. On the contrary, it has been suggested that B52 activity is important for the termination of retraction [82]. Other cells that can be depolarized immediately after retraction are radula-opening motor neurons, e.g. B48 and B66 [90, 91]. Postretraction activity in B48 and B66 is, however, not always very pronounced when programs are triggered by CBI-2 [90, 91]. Presumably, this is due to the fact that CBI-2-elicited programs are generally biting-like and radula opening at the peak of retraction is not important since food is not ingested. Thus, although there is activity in the buccal circuitry immediately after the retraction phase of CBI-2-elicited motor programs, it is not clear whether this activity will generate functional movements.

CBI-2-induced programs are, therefore, different from the three-phase programs observed in other molluscs, and are generally referred to as two-phase programs, because phases have been traditionally equated with movements, and a movement has not been described for the neural activity that immediately follows retraction.

When motor programs are triggered via stimulation of CBI-2, a single burst of action potentials generally elicits a single cycle of a motor program (fig. 4). For example, if CBI-2 is stimulated throughout protraction and then stimulation ceases, both protraction and retraction phases of activity are generated [62, 65, 67, 70, 80, 84]. Subsequent cycles of motor programs are, however, not necessarily observed. This suggests that the retraction circuitry does not necessarily re-excite the protraction circuitry. In general, this is similar to what has been observed when intact animals bite. Thus, when food contact is maintained, a series of bites can be triggered. If, however, food is immediately removed, it is possible to trigger a single biting response.

The fact that single cycles of CBI-2-evoked activity can be triggered has proven to be experimentally advantageous for studies that have assessed single-cell contributions to motor program generation. Thus, when single cycles of activity are generated approximately once a minute, or once every 2 min, parametric features of evoked motor programs are highly reproducible (fig. 4a vs. 4c) [62, 65, 67, 70, 80, 84]. In contrast, when repetitive activity is triggered via continuous stimulation of CBI-2, evoked activity can stabilize, but often it does not (compare cycles 1 and 3 in fig. 5). When activity is variable, it is obviously more difficult to determine whether the manipulation of a single neuron affects the ongoing motor program. Thus, the single cycle paradigm has proven to be experimentally advantageous, particularly in cases where the manipulation of a single cell alters parametric features of evoked activity but does not completely inhibit or reconfigure it.

To summarize, CBI-2-elicited motor programs are generally referred to as two-phase programs. Phase one corresponds to radula protraction and is triggered by direct excitation of neurons like the interneuron B63 and the
protraction motor neurons B31/B32. Phase two corresponds to radula retraction. It is initiated when cells like the interneuron B64 are activated, and the protraction circuitry and CBI-2 are actively inhibited. It is still not clear how protraction-to-retraction phase transitions occur.

**Radula Opening and Closing during CBI-2-Elicited Motor Programs**

When animals make feeding responses, the radula protracts and retracts. Additionally, the radula opens and closes (fig. 2). Above, we described the circuitry that mediates the protraction vs. retraction phases of CBI-2-elicited motor programs. Below, we will discuss the circuitry that mediates opening vs. closing. This discussion begins with a general consideration of two features of the generation of opening vs. closing that distinguish it from the generation of protraction vs. retraction. Specific connectivity in the opening vs. closing circuitry is then discussed.

**Differences between Protraction vs. Retraction and Opening vs. Closing**

One important difference between protraction/retraction and opening/closing is that protraction/retraction occurs in a stereotypical manner. As described above, protraction and retraction alternate, with protraction occurring first when activity is evoked by CBI-2. CBI-2-induced activation of the opening vs. closing circuitry is, however, variable in that in some cases, radula-closing motor neurons are predominantly activated during the first phase of evoked activity, i.e., during protraction (fig. 4b). In other cases, radula-closing motor neurons are predominantly activated during the second phase of evoked activity, i.e., during retraction (fig. 4a, c). As a result, CBI-2 can generate programs where the protraction and closing circuitry are simultaneously activated, or can generate programs where the protraction and opening circuitry are simultaneously activated. This is of interest since these two types of programs differ functionally. During ingestive behaviors, the radula closes during retraction (fig. 2b, c). In contrast, during egestive behaviors, the radula closes during protraction (fig. 2d).

CBI-2 is, therefore, a command-like neuron that can generate fundamentally different motor programs. At least in part, this occurs because CBI-2 triggers protraction and retraction in a stereotypical manner. Activation of the radula opening and closing circuitry is, however, variable.

Secondly, protraction vs. retraction and opening vs. closing differ in that activity in the protraction and retraction circuitry is almost completely out of phase. During CBI-2-elicited motor programs, the protraction circuitry is initially excited, and the retraction circuitry is inhibited. When retraction is initiated, the protraction circuitry becomes inactive. In contrast, during CBI-2-elicited motor programs, the radula closer motor neurons are generally active, at least to some extent during both the protraction and retraction phases of motor programs (fig. 4) [62]. (It is currently not clear whether this is also true for radula opener motor neurons.) Consequently, CBI-2-elicited motor programs are presumably not either completely ‘ingestive’ (e.g. 100% of the activity in radula closer motor neurons during retraction) or completely ‘egestive’ (e.g. 100% of the activity in radula closer motor neurons during protraction). Often, however, radula closer motor neurons are not equally active during protraction and retraction. Instead, they are predominantly active during either protraction or retraction. For example, in figure 4a and C, the radula closer motor neuron B8 is predominantly active during retraction. In contrast, in figure 4b, B8 is predominantly active during protraction. Motor programs are therefore classified as being either more ingestive-like or more egestive-like [62]. Currently, several systems are used for classifying motor programs [28, 30–32, 62, 67, 92]. Although these methods are likely to produce the same results in some cases, in other cases they may not. The method used for classifying activity can therefore affect data interpretation.

Although CBI-2 activates the protraction/retraction circuitry in a stereotypical manner, the activation of the radula-closing circuitry is highly variable. An important functional consequence of this is that programs triggered by CBI-2 can be egestive-like (radula closing during protraction), ingestive-like (radula closing during retraction), or intermediate (radula closing during both protraction and retraction).

**Connectivity in the Opening/Closing Circuitry**

Because the CBI-2-induced activation of the opening/closing circuitry is so functionally important, much research has gone (and is going) into determining how it occurs. Most studies to date have focused on the control of the B8 neurons, which are radula closers. A number of neurons that can be activated during CBI-2-evoked motor programs make connections with the B8 cells. All of these cells will obviously contribute to the patterning of B8 activity. Not all cells with B8 connections are, however, essential for determining whether motor programs are so altered that their classification as ingestive-like or egestive-like changes. For example, B34 is active during the...
protraction phase of CBI-2 motor programs [67, 70, 71], and it makes a mixed, but predominantly excitatory connection with the B8 neurons [71]. Thus, activation of B34 will tend to make programs more egestive-like. Hyperpolarization of B34 does not, however, completely convert egestive-like motor programs to ingestive-like [67, 70]. In contrast, when other cells are depolarized or hyperpolarized, the ingestive or egestive character of the ongoing motor program is altered. For simplicity, our discussion below focuses on neurons of the latter type.

**Egestive-Like Activity.** When CBI-2-elicited motor programs are egestive-like, a buccal interneuron, B20 [93], is excited (fig. 6b) [65]. B20 receives input directly from CBI-2 [65] and is indirectly excited by CBI-2 via protraction interneurons, e.g. by B34, B63, and B31/B32 (fig. 6b) [65]. B20 makes a fast excitatory connection with the B8 neurons (fig. 6b, 7a) [93], which increases B8 activity during protraction [65]. Additionally, B20 provides slow excitatory input to the retraction phase neurons B4/5 (fig. 6b, 7a) [65]. These cells make monosynaptic inhibitory connections with the B8 neurons (fig. 6b, 7a) [94, 95]. During CBI-2-induced motor programs, therefore, B20 fires during protraction and produces an immediate (i.e., protraction phase) increase in B8 activity (fig. 7a). Additionally, B20 activates B4/5 with a delay, which produces a decrease in retraction phase B8 activity (fig. 7a).

The fact that B20 exerts effects on the B8 neurons via B4/5 is of interest, since the B4/5 neurons are multifunctional and innervate muscles that produce radula opening (i.e. I7–I10) [90]. The B4/5 neurons are not the primary I7–I10 motor neurons, but B4/5 activity can induce I7–I10 contractions, albeit weakly [90]. When B4/5 activity is increased, therefore, radula closing is inhibited and radula opening is simultaneously potentiated.

To summarize, when CBI-2 programs are egestive-like, the protraction interneuron B20 is excited. B20 directly excites the B8 radula closer motor neurons during protraction, and indirectly inhibits the B8 neurons during retraction (via slow effects on B4/5).

**Ingestive-Like Activity.** In most cases, stimulation of CBI-2 induces ingestive-like motor programs (rather than egestive-like programs). More specifically, in semi-intact preparations, evoked movements are most commonly biting-like [96]. At least in part, CBI-2-induced motor programs are likely to be ingestive-like if a second CBI, CBI-3, is recruited (fig. 6c, 7b) [62]. In the isolated nervous system, the variable nature of the recruitment of CBI-3 is in part determined by experimental technique. When CBI-2 is activated via direct current injection, CBI-3 is more apt to be recruited than in experiments where CBI-2 is activated via brief current pulses. Under physiological conditions, however, both cells are likely to be activated by peripheral stimulation. Thus, both neurons are directly activated when food is applied to the lips [60]. Presumably, therefore, CBI-2 and CBI-3 will be coactivated when food is present, which will tend to produce ingestive behavior. Interestingly, however, only CBI-2 is activated when stimuli are applied that are likely to trigger egestive behavior. Namely, stretch of the esophagus, which presumably simulates ingestion of an inappropriate substance, leads to strong inhibition of CBI-3 at a time when CBI-2 is strongly excited [62]. Presumably, therefore, when an ingestive stimulus is presented, only CBI-2 will be activated.

When CBI-3 is recruited, it fires phasically during the protraction phase of CBI-2-elicited motor programs (fig. 7b). In general, CBI-3 has two types of effects. Firstly, it inhibits the egestive-like neurons that are activated by CBI-2, i.e., B20 and B4/5 (fig. 6c). Specifically, CBI-3 generates fast inhibitory postsynaptic potentials in B20, which suppresses B20 activity [65]. Additionally, CBI-3 exerts slow effects on B4/5 and decreases the excitability of these cells (fig. 6c) [65]. At least in part, this slow effect appears to be due to the release of a peptide from CBI-3, APGWaamide [65]. Secondly, CBI-3 provides excitatory input to a protraction phase interneuron, B40 (fig. 6c) [80]. B40 is interesting in that it makes both fast and slow synaptic connections with the B8 radula closer motor neurons (fig. 6c, 7b) [67]. The fast connection is inhibitory. Consequently, when B40 is active during protraction, the B8 neurons are inhibited, which tends to make programs ingestive-like. In contrast, the slow connection between B40 and the B8 neurons is excitatory. Consequently, B40 drives the B8 neurons during retraction, which makes them able to fire above their spontaneous frequency.

Interestingly, B40 and CBI-3 are both GABA immunoreactive and both cells evoke inhibitory postsynaptic potentials in postsynaptic followers that can be blocked by picrotoxin [80]. When CBI-2 motor programs are triggered in the presence of picrotoxin, protraction and retraction phases of motor programs are observed as normal. B8 activity in protraction is, however, increased, which tends to make programs egestive-like [80]. Thus, GABAergic neurons appear to act together to make CBI-2-induced motor programs ingestive-like [80].

To summarize, CBI-2-induced programs are most commonly ingestive-like (presumably biting-like). When programs are ingestive-like, CBI-3 is often recruited during the protraction phase of the motor program. CBI-3 inhibits the ‘egestive’ neurons activated by CBI-2 (B20,
and B4/5), and excites an ‘ingestive’ neuron, B40. B40 tends to make programs ingestive-like because it inhibits the B8 radula closer motor neurons during protraction, and excites the B8 motor neurons during retraction.

**General Characteristics of Neurons That Alter the Ingestive-Like vs. Egestive-Like Nature of CBI-2-Evoked Activity.** In general, neurons that most effectively alter the ingestive-like vs. egestive-like nature of CBI-2-evoked activity exert both fast and slow synaptic actions. Consequently, B8 activity during both protraction and retraction phases of motor programs is adjusted. For example, B40 makes programs ingestive-like in that it decreases B8 activity during protraction via fast inhibitory synaptic input, and increases B8 activity during retraction via slow excitatory effects (fig. 7b) [67]. In a similar vein, B20 makes programs egestive-like in that it increases B8 activity during protraction, and indirectly decreases B8 activity during retraction (via B4/5) (fig. 7a) [65]. Thus, cells that exert both fast and slow synaptic actions most effectively alter the nature of CBI-2-elicited motor programs.

A consequence of this arrangement is that protraction interneurons are utilized to generate both ingestive-like and egestive-like activity. Presumably, this is a result of the fact that it is advantageous for slow synaptic events to be initiated during protraction. In theory, a retraction interneuron could exert slow synaptic actions that are manifested during the subsequent protraction. It is possible that this is not advantageous since biting does not necessarily occur in a repetitive manner. Thus, protraction interneurons appear to play an important role in determining the ingestive-like vs. egestive-like nature of a cycle of CBI-2-elicited activity.

**Additional CBIs Activated by CBI-2**

As is described above, CBI-3 is often recruited by stimulation of CBI-2. Thus, a ‘CBI-2-induced motor program’ is in reality not simply generated by CBI-2 alone. Additional CBIs that are likely to be activated by CBI-2 include CBI-5 and CBI-6 [97]. CBI-5 and CBI-6 are electrically coupled to each other and are so physiologically and morphologically similar that they have been referred to as a single unit (i.e., as CBI-5/6) [97]. CBI-5/6 are interesting in that, although the cerebral somata appear to be capable of generating plateau or plateau-like potentials, the spike initiation site in these neurons is distant (presumably, it is in the buccal ganglion) [97]. Thus, if current is injected somatically, activity above 10 Hz generally cannot be elicited. When motor programs are triggered by CBI-2, however, buccal terminals of CBI-5/6 generate antidromic spikes at frequencies of up to 25 Hz [97]. Because the spike initiation site in CBI-5/6 is so distant, it has been difficult to experimentally manipulate these neurons. Consequently, it has been difficult to study their specific contribution to CBI-2-elicited motor programs. It appears, however, that they are phasically activated during the retraction phase of motor programs and they provide excitatory input to the retraction circuitry, e.g. to the retraction interneurons B64 and B4/5, and to the accessory radula closer motor neuron B15 [97].

Motor programs experimentally evoked by stimulating CBI-2 alone are influenced by the variable recruitment of other CBIs. Coactivation of the CBIs is also likely to occur under physiological conditions. A number of these cells respond to food-related stimuli. The specific contribution of one other CBI (CBI-3) has been examined. Contributions of other cells have not yet been evaluated.

**Sensory Feedback to the Feeding Central Pattern Generator: The Bite to Bite-Swallow Transformation**

Although feeding motor programs can be triggered in the isolated nervous system of *Aplysia*, sensory feedback clearly modifies rhythmic activity under physiological conditions [98]. In particular, sensory feedback is likely to be important when food is ingested, i.e., when bites are converted to bite-swallows. Under these conditions, there is a striking change in radula movements. Specifically, the radula closing/retraction phase of behavior is enhanced so that food will be pulled into the mouth and deposited in the esophagus, i.e., hyperretraction occurs (fig. 2c) [1]. Studies in intact animals have indicated that, at least in part, the enhancement of closing/retraction is mediated via a prolongation of this phase of behavior [49]. Thus, in *Aplysia*, hyperretraction does not appear to occur unless food is ingested. Hyperretractions are therefore not an integral part of all ingestive motor programs. More specifically, CBI-2-induced motor programs most commonly do not appear to have a hyperretraction phase.

In intact animals, at least two classes of sensory neurons are likely to be activated when bites are converted to bite-swallows. Some sensory neurons are radula mechanoaferents. These cells are relatively low-threshold mechanosensors that are activated whenever anything touches the radula [99, 100]. They, therefore, likely to be important for detecting that food has contacted the radula. The largest and best-characterized radula mechanoaferents are B21 and B22 [100]. Other sensory neurons that will be activated when food is ingested are retraction...
At least in part, this appears to be due to the fact that in regulating (or gating) afferent transmission [91, 102]. Subthreshold for spiking, they do play an important role in the initiation phase of CBI-2-elicited motor programs are generally not sufficient to trigger significant activity. In an intact animal, however, B21 and B51 will presumably do not function as interneurons. If peripheral activation of B21 or B51 is ‘mimicked’, however, by inducing spiking during the retraction phase of the motor program, the duration of retraction is significantly increased [101], and retraction movements are enhanced [86]. This is observed when current is injected into a single sensory neuron [86, 101].

To summarize, during biting-like motor programs, B21 and B51 are centrally depolarized, but depolarizations are not sufficient to trigger significant activity. In an intact animal, however, B21 and B51 will presumably be peripherally activated during the retraction phase of behavior (i.e., by food contact and increased resistance to radula retraction). When this occurs, a hyperretraction will be induced, i.e., a bite will be converted to a bite-swell.

Interestingly, experiments with B21 have shown that, although the rhythmic depolarizations during the retraction phase of CBI-2-elicited motor programs are generally subthreshold for spiking, they do play an important role in regulating (or gating) afferent transmission [91, 102]. At least in part, this appears to be due to the fact that centrally induced depolarizations regulate spike propagation in B21 [102]. Specifically, when B21 is at its resting membrane potential, spikes are not actively propagated to all output regions of the cell. Postsynaptic followers are therefore not strongly driven. In contrast, when B21 is centrally depolarized, spikes are actively propagated, and postsynaptic followers are strongly driven. Thus, during CBI-2-elicited motor programs, centrally induced depolarizations during the retraction phase of the motor program are important because they gate in peripherally generated spikes [102].

**Circuitry for Egestive Radula Movements**

As discussed above, *Aplysia* generate egestive as well as ingestive responses. It has been postulated that there is more than one type of egestive behavior in *Aplysia* [59, 103, 104]. In part, this is suggested by the fact that one type of response (i.e., active seaweed rejection) appears to be dependent on the cerebral ganglion [103–105], while a second type (i.e., egestion of a tube) is not [59]. As might be expected, therefore, rejection-like motor programs can be triggered in the isolated nervous system via CBI stimulation. Additionally, they can be triggered in the isolated buccal ganglion without the cerebral ganglion present [for a description of motor programs that would presumably be classified as egestive-like, see ref. 72]. Below, we discuss each type of motor program.

**Without the Cerebral Ganglion (e.g. Stimulation of the Esophageal Nerve)**

The buccal ganglion of *Aplysia* innervates the buccal mass via four bilaterally symmetrical nerves [55], which have been referred to as the esophageal nerve, buccal nerve 1, buccal nerve 2, and buccal nerve 3 [94; for other nomenclature, see ref. 106]. Additionally, a single radula nerve exits in the buccal ganglion from the region of the buccal commissure [94, 106]. In general, these nerves include afferent fibers [55]. Consequently, they can be used to trigger rhythmic activity [13, 28, 72].

Of the motor programs triggered via nerve stimulation, those triggered via the esophageal nerve are perhaps the best understood (at least in terms of their physiological significance). In particular, the esophageal nerve transmits mechanoefferent information from the gut of *Aplysia* to the buccal ganglion. As animals feed, information that is specifically conveyed via the anterior branch appears to be important for positive reinforcement of ingestive behaviors [26–28, 30, 31]. As feeding progresses, however,
the gut is stretched and animals begin to satiate [3, 14, 107]. When animals are satiated, they either fail to respond to food or they egest it, if it is in large pieces.

When the esophageal nerve is stimulated in semi-intact preparations, egestive movements are observed [13]. Similarly when the esophageal nerve is stimulated in the isolated nervous systems, egestive-like motor programs are observed. These motor programs are not as well characterized as programs triggered by CBI-2, but it has been noted that a number of the protraction and retraction neurons activated during CBI-2-elicited motor programs are also activated by esophageal nerve stimulation. For example, B4/5 are activated during retraction [97, 108], and B31/B32 are activated during protraction [72]. Programs are considered egestive-like since radula closer motor neurons predominately fire during the protraction phase of the motor program. As is the case with CBI-2-elicited motor programs, however, quantitative methods are often needed to classify many cycles of activity.

Rejection-Like Activity Triggered by Cerebral Neurons

As described above, rejection-like motor programs can be triggered by CBI-2 if CBI-3 is not recruited. Additionally, a CBI that appears to exclusively trigger rejection activity has been characterized, i.e. the neuron CBI-1. This cell has been identified and characterized in both Aplysia californica [60] and A. kurodai [105]. In A. kurodai, the designation CBM1 is used [105]. In A. californica, CBI-1 responds to touch of the tentacles, lips, and buccal mass [60]. In part, mechanosensory input to CBI-1 is provided by the interganglionic cerebral to buccal mecha-noafferents [60]. Tonic stimulation of CBI-1 generally produces a single cycle of a motor program that is characterized by high frequency activity in B20 during the protraction phase of the motor program and high frequency activity in B4/5 during the retraction phase of the motor program. As described above, this type of activity is generally considered rejection-like.

In A. kurodai, CBM1 is specifically associated with active rejection [105]. Thus, A. kurodai feed well on one type of seaweed, i.e. ulva, but they reject a second type, i.e. gelidium [104]. When gelidium is rejected, distinctive rhythmic patterned movements of the jaws and radula are observed [103]. For example, activity in jaw-closing motor neurons is phase advanced with respect to activity in MA neurons. (The MA neurons are presumably homologous to the B4/5 neurons in A. californica.) This type of change in rhythmic activity is observed when CBM1 is activated [105]. Additionally, imaging studies have indicated that gelidium extracts strongly excite CBM1 [105]. Interestingly, CBM1 (and CBI-1) are dopaminergic [60, 105], as is B65 [109] and B20 [93], i.e. buccal interneurons that have also been associated with egestive-like activity [67, 110].

To summarize, Aplysia generate egestive as well as ingestive responses. These behaviors are interesting in that they do not always require the cerebral ganglion. In general, the egestive circuitry is not as well characterized as the ingestive circuitry but it appears that many of the same essential neurons are utilized. Dopaminergic neurons (e.g. CBI-1) have been particularly implicated as being important for generating egestive-like motor programs.

Concluding Remarks

The Aplysia feeding circuitry is organized in a manner that creates a great deal of potential for flexibility. For example:

(1) The initiation of behavior appears to be a complex process that does not simply involve the recruitment of a single command-like neuron. Instead, multiple cells are likely to be coactivated in a stimulus-dependent manner. The number of possibilities for behavior initiation is, therefore, not simply determined by the total number of command-like neurons. Instead, many different combinations of activity are possible.

(2) Once programs are ongoing, parametric features of motor programs are highly variable. In the context of protraction, this is likely to result from the following. (a) Protraction is in part sustained via a maintained depolarization in B31/B32. In part, this depolarization is induced by plateau-like potentials in B31/B32. Additionally, however, synaptic input is also important. Synaptic input to B31/B32 can be variable since interneurons not essential for protraction movements can be recruited in a behavior-specific manner. (b) Protraction duration is additionally affected by retraction initiation. Retraction initiation is itself under complex control. Thus, the retraction circuitry appears to be both inhibited and excited during protraction. Consequently, protraction duration can be altered either by a change in retraction phase inhibition, or a change in retraction phase excitation.

(3) Parametric features of retraction are also highly variable. In part, this is likely to result from the fact that some retraction interneurons are multifunctional cells, i.e., they have peripheral processes and can function as primary afferents as well as interneurons. These neurons can therefore respond to a change in the environment and
alter the duration of the retraction phase. This can produce a change in the nature of an ongoing motor program (e.g. convert a biting-like program to a bite-swallow-like program).

(4) Finally, the essential nature of a motor program (i.e., whether it is ingestive-like or egestive-like) can be dynamically adjusted. At least in part, this is a result of the fact that a number of interneurons can exert both fast and slow synaptic actions. Consequently, a single cell can modify the two antagonistic phases of a motor program and thereby fundamentally change its nature (e.g. inhibit radula closer motor neurons during protraction and excite radula closer motor neurons during retraction, thereby making a program ingestive-like).

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