Modulation of Fictive Feeding by Dopamine and Serotonin in *Aplysia*

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INTRODUCTION

The feeding behavior of *Aplysia* consists of a sequence of appetitive and consummatory behaviors (Kupfermann 1974a,b; Kupfermann et al. 1991). First, food (seaweed) in the immediate environment of the animal activates appetitive behaviors, such as locomotion and head waving, which bring the animal in contact with the food (Teyke et al. 1990, 1992). Second, contact with the food activates consummatory behaviors, such as ingestion (biting and swallowing) or rejection (Kupfermann 1974a; Susswein et al. 1976). Consummatory behaviors involve rhythmic movements of feeding organs, including the lips, jaws, buccal mass, odontophore, and esophagus (Drushel et al. 1997; Kupfermann 1974a; Morton and Chiel 1993a; Rosen et al. 1997; Weiss et al. 1986). During a bite, the odontophore is rotated forward (i.e., protraction) as the jaws open. Initially, the two halves of the radula (toothed grasping surfaces of the odontophore) are separated during protraction. Before the peak of protraction, however, the radula begins to close and grasp the food. The radula remains closed as the odontophore retracts (backward rotation), which brings the food into the buccal cavity, and the jaws close. Swallowing consists of rhythmic movements of the odontophore and radula, which are similar to those during biting, and is associated with peristaltic contractions of the esophagus. Unlike biting, swallowing is not associated with opening of the jaws. In addition to biting and swallowing, the feeding apparatus can produce rejection movements in response to inedible material in the buccal cavity. During rejection, the radula is closed as the odontophore retracts and open as it retracts, causing the unwanted material to be ejected from the buccal cavity. Thus the motor programs that mediate consummatory feeding behaviors can be described, in general, as having two phases: a protraction phase followed by a retraction phase. During ingestion, the radula is open during the initial phase of protraction and closed during retraction, whereas during rejection, the radula is closed during protraction and open during retraction. Thus the two-phase feeding motor program of *Aplysia*, which is a browser, differs from other herbivorous gastropods (e.g., *Helisoma*, *Helix*, *Limax*, *Lymnaea*, *Planorbarius*, etc.), which are rapers and have three phases of feeding cycle (for review, see Kupfermann 1974a,b; Willows 1985).

Using a variety of intact, semi-intact, and reduced preparations, recent studies have begun to relate specific patterns of neural activity recorded in vivo and in situ to aspects of consummatory feeding behaviors (Chiel et al. 1986; Church and Lloyd 1994; Cropper et al. 1990; Evans and Cropper 1997; Evans et al. 1996; Fiore et al. 1992; Hurwitz et al. 1996; Jahan-Parwar and Fredman 1983; Kabotyanski et al. 1997, 1998a; Kupfermann and Weiss 1982; Morton and Chiel 1993a,b; Nagahama and Takata 1987, 1988, 1990; Nargeot et al. 1997, 1999a–c; Perrins and Weiss 1996, 1998; Rosen et al. 1991, 1997; Scott et al. 1995; Susswein et al. 1996; Weiss et al. 1978, 1986). Two types of buccal motor programs (BMPs) have been characterized during recordings in vivo. One type of BMP was associated primarily with ingestion, whereas the Metabolism of the odontophore protracts and opens during the initial phase of protraction, and is associated with peristaltic contractions of the esophagus. Unlike biting, swallowing is not associated with opening of the jaws. In addition to biting and swallowing, the feeding apparatus can produce rejection movements in response to inedible material in the buccal cavity. During rejection, the radula is closed as the odontophore retracts and open as it retracts, causing the unwanted material to be ejected from the buccal cavity. Thus the motor programs that mediate consummatory feeding behaviors can be described, in general, as having two phases: a protraction phase followed by a retraction phase. During ingestion, the radula is open during the initial phase of protraction and closed during retraction, whereas during rejection, the radula is closed during protraction and open during retraction. Thus the two-phase feeding motor program of *Aplysia*, which is a browser, differs from other herbivorous gastropods (e.g., *Helisoma*, *Helix*, *Limax*, *Lymnaea*, *Planorbarius*, etc.), which are rapers and have three phases of feeding cycle (for review, see Kupfermann 1974a,b; Willows 1985).

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Effects of DA and 5-HT in Progressively More Reduced Preparations. Perfusing a semi-intact head preparation with DA (50 μM) induced feeding-like movements that were similar to bites in the intact animal. Similarly, perfusing isolated ganglia preparations with DA (50 μM) elicited ingestion-like BMPs. Serotonin (5 μM) also induced feeding-like movements when perfused into the semi-intact preparation. The 5-HT-induced movements were similar to swallows, however. Perfusing isolated buccal ganglia with 5-HT (5 μM) did not significantly change the number of BMPs nor did it significantly bias the output of the CPG toward ingestion-like BMPs. Finally, the effects of DA and 5-HT on several cells (e.g., B4/5, B8, B31/32, B34, B35, B51, B63, and B64) and synaptic connections within the CPG were examined. The results of the present study suggest that DA and 5-HT regulate the functional configuration of the CPG and thereby play distinctive roles in organizing different aspects of feeding. Preliminary reports of some of these results have appeared in abstract form (Baxter and Byrne 1993; Baxter et al. 1995; Cushman et al. 1995; Kabotyanski et al. 1993, 1995, 1998b).

METHODS

Aplysia californica (150–300 g) were obtained from Alacrity Marine Biological Services (Redondo Beach, CA), Marine Specimens Unlimited (Pacific Palisades, CA), Marinus (Long Beach, CA), and Pacific Biomarine (Venice, CA). Animals were kept in aerated aquaria containing artificial seawater (ASW; Instant Ocean, Aquarium Systems, Mentor, OH), which was maintained at 15°C. Animals were fed dried seaweed (Hang Loong Marine Products, Japan) three times a week. Before dissections, animals were anesthetized by an injection of isotonic MgCl₂.

Semi-intact head preparation

The semi-intact head preparation (Fig. 1) consisted of the anterior portion of the animal (i.e., an isolated head) that retained, in situ, the external structures of the head (e.g., lips, tentacles, and rhinophores), the feeding organs (e.g., jaws, buccal mass, and esophagus), and the buccal, cerebral, pleural, and pedal ganglia (see also Chiel et al. 1986; Drushel et al. 1997; Nagahama and Takata 1987, 1988; Rosen et al. 1991; Weiss et al. 1986b). Before each experiment, animals were food-deprived for 2 days. The head was cut from the rest of the animal slightly rostral to the parapodia (dorsally) and slightly caudal to the beginning of the foot (ventrally). The esophagus was severed rostrally to the crop and was cannulated with a polyvinyl tube, 80 mm long and 3 mm in diameter. The anterior aorta was severed slightly caudal to the pedal artery. Another polyvinyl tube, 80 mm long and 2 mm in diameter, was inserted in the aorta and protruded rostrally into the buccal cavity. One ligature secured the buccal artery to the tube, and another constricted the anterior aorta so that the perfusion of the buccal artery bypassed the pedal and cephalic arteries. The peripheral nerves from the pleural-pedal ganglia that projected caudally (e.g., PL2, pleuroabdominal connectives, P7, P8, P9, and P10) were severed. Once the gross dissection was completed, the head was transferred to an experimental perflex chamber, which contained ASW. Within the chamber, the preparation was mounted on a polyurethane tube, which had a diameter similar to that of the “neck” of the isolated head. The head was mounted on the tube by pinning the cut edges of the neck to the tube as well as tying a ligature around the overlapping portions of the neck and tube. The polyurethane tube was attached on a perflex tube that was attached to one wall of the experimental chamber. This wall had sliding gate/door (not shown) through which the two canula were drawn outside the chamber. The gate then was closed and sealed off with petroleum jelly (Vaseline).
Thus the preparation had two isolated compartments: one outside the head and another inside (Fig. 1). The volume of the internal compartment (30–40 ml) depended on the size of the head.

The canulated buccal artery was used to pump ASW into the arterial system and thereby perfuse the tissue and simulate the normal hydroskeletal inflation of the animal. The rate of perfusion was 1–2 ml/min. To maintain hydroskeletal inflation, positive hydrostatic pressure was provided inside the head by elevating the open end of the outflow tube 30–40 mm above the surface of ASW in the chamber. A thread was used to measure feeding movements. It was attached to a lever of isotonic transducer and was drawn into the mouth, between the two halves of the radula and through the esophagus and its canula—outside the chamber. A counterbalance weight was attached to the opposite arm of the transducer’s lever so that a small constant outward force of 0.2 g was applied to the thread, ensuring that the thread always was stretched and transduced movements in both directions. At the beginning of each experiment, the transducer’s lever was positioned so that it was possible to measure both inward and outward movements.

In vitro preparation

An isolated buccal ganglia preparation was used to make extracellular recordings of BMPs and intracellular recordings from identified cells. Typically the peripheral nerves of the buccal ganglia were severed at the point where they entered the buccal mass, esophagus, and salivary glands. The cerebral-buccal connectives (C-B conn.) were severed close to the cerebral ganglion. The buccal ganglia were removed from the animal and pinned to the floor of a recording chamber, which was coated with a silicone elastomer (Sylgard 184, Dow Corning, Midland, MI). In those preparations where only extracellular recordings were made, the connective tissue sheath that covers the buccal ganglia was left intact and the cut ends of the R n. and buccal nerves 1, 2, and 3 (n.1, n.2, and n.3) were drawn into extracellular electrodes. (The nerve designations are from Gardner 1971;
see also Church and Lloyd 1994; Morton and Chiel 1993b; Scott et al. 1991.) In those preparations in which both intracellular and extracellular recordings were made, the buccal ganglia were pinned to the floor of the recording chamber so that the rostral surface of the right buccal ganglion and caudal surface of the left buccal ganglion were accessible. The ganglia were desheathed, and only the R n. was drawn into an extracellular electrode. For intracellular recordings, neurons were identified by their position, size, projection of axons in peripheral nerves, nature of synaptic connectivity, characteristic patterns of firing during BMPs, and intrinsic biophysical properties (see RESULTS for details).

Conventional techniques were used for extracellular and intracellular recordings of neural activity. Briefly, extracellular recordings of neural activity were made with polyethylene suction electrodes and AC coupled amplifiers (Model 1700, AM Systems, Everett, WA). The suction electrodes also were used to stimulate buccal nerves. Intracellular electrodes were filled with a solution of 3 M potassium acetate and 100 mM potassium chloride and had an impedance of 6–15 MΩ. The signals from the intracellular electrodes were amplified using DC-coupled amplifiers (models Axoclamp 2A and Axoprobe 1A, Axon Instruments, Burlingame, CA). The extracellular and intracellular signals were amplified further and displayed on a chart recorder, and an oscilloscope and were recorded on magnetic tape.

**Solutions**

Generally, control saline consisted of ASW, to which 10 mM of either TRIZMA or HEPES buffer (Sigma Chemical, St. Louis, MO) was added and the pH was adjusted to 7.4. Solutions containing L-3,4-dihydroxyphenylalanine, a metabolic precursor of catecholamines (DOPA; Calbiochem, La Jolla, CA), 3-hydroxytryptamine HCl (dopamine, DA; Calbiochem), and 5-hydroxytryptamine creatinine sulfate complex (serotonin, 5-HT; Sigma Chemical) were made immediately before use. Solutions of DA and DOPA also contained an equimolar concentration of an antioxidant (ascorbic acid; Sigma Chemical). In those experiments in which either DA or DOPA was used, the control saline also contained ascorbic acid.

Unless otherwise noted, 50 μM DA, 250 μM DOPA, or 5 μM 5-HT was used. These concentrations were selected on the basis of preliminary studies that examined the effects of 1–500 μM DA, 0.5–50 μM 5-HT, and 100–1,000 μM of DOPA. Concentrations were selected that elicited reliable, stable, but not maximal responses. Moreover these concentrations were similar to concentrations used in previous studies (e.g., Ascher 1972; Gospe and Wilson 1980; Kabotiantski et al. 1994; Kramer and Levitan 1990; Ocorr and Byrne 1985; Shozushima 1984; Sossin et al. 1987; Teyke et al. 1993; Weiss and Drummond 1981; see also Gospe 1983; Trimble and Barker 1984; Yeoman et al. 1994). In previous studies, levels of DA and 5-HT were measured in the CNS, specific ganglia and their neurips, monoamine-containing neurons, and even vesicles (e.g., Chien et al. 1990, 1995; McCaman et al. 1973, 1979). These measurements, however, do not provide information about effective concentrations acting at synaptic contacts. It is possible to estimate such concentrations by comparing cellular responses to exogenous transmitters with responses elicited by stimulating DA- or 5-HT-containing neurons or by studying binding properties of DA and 5-HT receptors. These concentrations are on the scale of 10^{-6} M for 5-HT, and 10^{-3} M for DA (e.g., Ascher 1972; Fox and Lloyd 1998; Gospe 1983; Magoski et al. 1993; Shozushima 1984). Thus we believe that the concentrations used in the present study are in the physiological range.

Summary data were represented as means ± SE or as medians with interquartile range (1st quartile to 3rd quartile). The statistical methods of analyses were indicated in the RESULTS and P < 0.05 was taken as indicating significant differences.

**RESULTS**

**DA and 5-HT coordinate different feeding-like movements a semi-intact head preparation**

As a first step toward investigating the role of biogenic amines in feeding, it was important to determine the behavioral significance of the DA- and 5-HT-induced activity. To address this issue, we developed semi-intact head preparations in which either DA or 5-HT could be applied and behavior could be measured in quantitative and controllable manner.

**Results of pilot experiments**

The final design of the semi-intact preparation was an outcome of extensive preliminary study. First, we assessed the behavioral effects of DA and 5-HT by injecting them into the intact animals (n = 5). These results were unclear. For example, DA and DOPA induced changes in posture, local skin contractions, and foot disattachment as well as brief movements of the buccal mass. Because the main focus of this study was the modulation of consummatory feeding, we then used a more reduced preparation of head with only buccal ganglia attached to segregate consummatory feeding component from the rest of behavioral effects of the transmitters. This approach was based previous findings suggesting the buccal ganglia contain the circuit sufficient to generate consummatory feeding (e.g., Kirk 1989; Kupfermann 1974b; Morton et al. 1991). In this preparation (n = 4), DA elicited periodic movements of the buccal mass, but they were weak, did not appear as bites, or swallows or rejections, and produced only weak inward displacements of thread placed on radula. Successful feeding involves coordination of many muscle groups besides the buccal mass (foot, body wall, and extrinsic buccal muscles), re-distribution of hydroskeleton, etc. In the next set of preparations (n = 4), we retained the cerebral, pedal, and pleural ganglia. We also cannulated the anterior aorta and perfused it under small hydrostatic pressure to inflate tissues and provide some hydroskelatal support. When DA was perfused first through the cephalic and pedal arteries, however, we observed a mixture of effects: local contractions and withdrawals, penis protrusion as well as abortive feeding movements. We then adopted the preparation described in METHODS. In this preparation, the pedal, pleural, and cerebral ganglia were preserved, but bypassed during perfusion of ASW or monoamines. The advantage of this preparation was that the isolated head was able to assume a feeding-like “posture” and exhibit recognizable and strong feeding movements. Bypassing the cephalic and pedal arteries and perfusing DA or 5-HT through the buccal artery allowed the drugs to have their predominant effects on the buccal ganglia. After this preparation was tested (n = 9), quantitative experiments were performed in the final setup (Fig. 1).

The role of periphery also was addressed in the pilot experiments (n = 3) in which we removed all the CNS and perfused the head and the buccal mass with DA. No feeding movements were observed; this indicated that the DA-induced activity in the head preparation was not of peripheral origin.

**DA induced bite-like movements**

Figure 2 compares a bite that was elicited by presenting food (seaweed) to a freely behaving animal (Fig. 2A) to feeding-like
movements that were elicited by perfusing a semi-intact preparation with DA (Fig. 2B). In the freely behaving animal, the bite began with the jaws opening to accommodate the protrusion of the odontophore (Fig. 2A, 1 and 2). During this initial phase of protraction, the two halves of the radula were open. At the peak of the protraction phase (Fig. 2A3), the two halves of the radula were closed, and they remain closed as the odontophore retracted and the jaws closed (Fig. 2A, 4 and 5).

DA induced a similar sequence of movements in the semi-intact preparation (Fig. 2B). These movements began with the jaws opening as the odontophore protracted (Fig. 2B, 1 and 2). During this initial phase of protraction, the two halves of the radula were open. At the peak of the protraction phase (Fig. 2B3), the two halves of the radula began to close, and they remained closed as the odontophore retracted and the jaws closed (Fig. 2B, 4 and 5). The recording from the isotonic force transducer for the sequence of DA-induced movements (Fig. 2, B, 1–5, insets) indicated that the thread was drawn into the mouth ~4 mm. Figure 2B, bottom, illustrates the complete record of that experiment from the isotonic force transducer. Before perfusion with DA, feeding-like movements occurred infrequently and there was little net displacement of the thread.
Soon after the preparation was perfused with DA, the frequency of feed-like movements increased, and ~13 min into the perfusion, these movements began to produce a net inward displacement of the thread. By the end of the experiment, ~100 mm of thread had been drawn into the foregut and examination of the video record indicated that the jaws opened and closed rhythmically during these feeding-like movements. These results suggest the DA induced biting-like movements.

It should be noted that the effects of perfusing preparations with a transmitter, such as DA, may not reflect the normal role of the transmitter. For example, perfusion will activate all receptors for the transmitter that under normal conditions might not be active together. To partially address this issue, semi-intact head preparations were perfused with the metabolic precursor of catecholamines, DOPA. Precursors, such as DOPA, are presumed to be accumulated selectively and metabolized by neurons that use the transmitter and to act via increased release of the transmitter from synaptic terminals of those cells (e.g., Kabotyanski and Sakharov 1988, 1991; Kabotyanski et al 1994; McCaman et al 1984; Mitchell et al. 1992). As a result, precursors affect neural circuitry via endogenous mechanisms, reach their targets in physiologically relevant order and timing, and produce gradual and long-lasting effects.

Perfusing with DOPA increased the frequency of feeding-like movements, produced a net inward displacement (i.e., ingestion) of the thread (Fig. 3B), and the DOPA-induced feeding-like movements were similar to those induced by DA (not shown). Both the DA- and DOPA-induced inward displacements of the thread were accompanied by coordinated opening and closing of the jaws as the odontophore rhythmically protracted and retracted (i.e., movements similar to biting in freely behaving animals). The only notable difference between perfusing with DA and DOPA was the greater time required for the feeding-like movements to develop in presence of DOPA. The onsets of sustained feeding rhythm (Fig. 3C1) and net inward displacement of thread (i.e., ingestion; Fig. 3C2) were delayed for ~15 min longer in DOPA than in DA (Fig. 3C). This presumably reflected the time required for dopaminergic cells to accumulate DOPA and metabolize it into DA. Nevertheless a weak agonist also can have delayed effects. If this was the case, the effects would be weaker, however. Yet the effects of DOPA, although delayed, were notably stronger than effects of DA (Fig. 3, A and B). Thus delayed but stronger effects support the hypothesis that DOPA acts as the metabolic precursor of DA and not as a weak agonist.

### 5-HT induced swallow-like movements

Perfusing semi-intact preparations with 5-HT also induced a net inward displacement of the thread (i.e., ingestion; Fig. 2C). However, 5-HT-induced ingestion was different from DA- or DOPA-induced movements. As illustrated by the video record in Fig. 2C, 1–4, the jaws remained closed while the thread was drawn into the mouth (net inward displacement for this sequence was ~10 mm). Figure 2C, bottom, illustrates the complete record from the isotonic force transducer. Before perfusing with 5-HT, there was no net change in the position of the thread. Within ~15 min of perfusing with 5-HT, large inward displacements of the thread were recorded (i.e., ingestion-like movements). By the end of the experiment, ~80 mm of thread had been drawn into the foregut. On average, one 5-HT-induced movement displaced more thread than one DA-induced bite-like movement (2.730 ± 0.546 vs. 0.614 ± 0.076 mm; 2-sample t-test, t = -3.26, 2-tailed P < 0.025). There were fewer movements in 5-HT than in DA (Fig. 3), however, so the resulting average total net displacement was smaller in serotonin (Fig. 3). Examination of the video record indicated that the jaws remained closed during these feeding-like movements. In addition, we could clearly see through transparent experimental chamber that each inward displacement of the thread was associated with strong movement of the buccal mass inside the “head.” On the basis of definitions of swallowing during behavioral observations (Kupfermann 1974a),
these results suggest that 5-HT induced swallow-like movements.

Figure 3 summarizes data from semi-intact preparations that were perfused with DA, DOPA (Fig. 3, A–C) or 5-HT (Fig. 3D). DA and DOPA had similar effects. First, DA and DOPA increased the frequency of feeding-like movements from 0.0056 ± 0.0029 to 0.0343 ± 0.0071 Hz and from 0.0086 ± 0.0021 to 0.0452 ± 0.0010 Hz, respectively. Paired t-tests indicated that these increases were significant (DA: \( t_2 = -4.54, 1\text{-tailed} \ P < 0.025 \); DOPA: \( t_2 = -15.32, 1\text{-tailed} \ P < 0.0025 \)). [As mentioned in the preceding text, before these experiments using a simplified design. Thus we had specific hypotheses for the direction of the effects, which warranted 1-tailed tests used in this section]. Second, the feeding-like movements that were induced by DA or DOPA produced a net inward displacement of the thread. Before perfusion with DA, the average displacement of the thread was 0.3 ± 0.2 mm/h. During perfusion with DA, the average displacement of the thread was 78.8 ± 25.7 mm/h. Similarly, before perfusion with DOPA, the average displacement of the thread was −3.2 ± 2.2 mm/h, whereas during perfusion with DOPA, the average displacement was 117.5 ± 31.6 mm/h. Paired t-tests indicated that both of these changes were significant (DA: \( t_2 = -3.31, \text{one-tailed} \ P < 0.05 \); DOPA: \( t_2 = -3.59, \text{one-tailed} \ P < 0.04 \)). Although effects of DA and DOPA were similar in amplitude, the time courses of their development were different. DA elicited sustained feeding-like movements with a latency of 59 ± 53 s after the start of perfusion, whereas the effects of DOPA had a latency of 890 ± 18 s (Fig. 3C). The difference is statistically significant (2-sample t-test, \( t_4 = -22.09, \text{one-tailed} \ P < 0.00002 \)). Moreover the number of feeding-like movements elicited during first 10 min after drug application was 25 ± 2.6 for DA and 6.0 ± 1.5 for DOPA; this was significantly different (\( t_1 = 6.30, \text{1-tailed} \ P < 0.002 \)).

Net inward thread displacement started after 532 ± 130 s of DA perfusion versus 1,410 ± 206 s after DOPA perfusion (Fig. 3C; \( t_2 = -3.60, \text{1-tailed} \ P < 0.02 \)). Because the latter effect of DOPA began ~20 min after start of perfusion, the observation times were ~1 h for DA and 1.33 h for DOPA. At 1 h, though, the average total net displacements were similar for the both compounds (90 ± 6.3 mm for DOPA and 86.5 ± 43.3 mm for DA). This results suggests that DOPA-induced movements, although delayed, appeared to be more vigorous than DA-induced movements.

Perfusing semi-intact preparations with 5-HT did not significantly increase the frequency of feeding-like movements (Fig. 3D1). In control saline, the average frequency of feeding-like movements was 0.00375 ± 0.00083 Hz, and in the presence of 5-HT, the average frequency was 0.00667 ± 0.00170 Hz. A paired t-test indicated that this difference was not significant (\( t_2 = -1.21 \)). In contrast, perfusing semi-intact preparations with 5-HT significantly increased the inward displacement of the thread (Fig. 3D2). In control saline, the average displacement of the thread was −3.7 ± 1.5 mm/h, and in the presence of 5-HT, the average displacement was 60.1 ± 11.2 mm/h. A paired t-test indicated that this difference was significant (\( t_3 = -5.58, \text{1-tailed} \ P < 0.006 \)).

The results from the semi-intact preparations indicated that both DA and 5-HT induced and organized ingestion-like movements. However, the results indicated that the two transmitters...
organized different aspects of ingestion. Bites appeared to be organized by DA, whereas 5-HT appeared to organize swallows. The actions of these two transmitters can result from modulation of either elements in the CNS (e.g., cellular and/or synaptic properties within the CPG) and/or elements in the periphery (e.g., the properties of muscle fibers, release at neuromuscular junctions, and/or sensory feedback). To investigate the neural mechanisms that underlie the actions of these two transmitters, we began by characterizing the motor programs that were expressed in preparations of isolated buccal ganglia.

Characterization of spontaneously occurring BMPs in vitro

Besides the buccal ganglia, other central ganglia play a role in integrating a correct feeding response in the head preparation, and their removal may change the parameters of feeding. However, previous studies showed that rhythmic movements of the odontophore and radula are mainly supported by the buccal ganglia (e.g., Kupfermann 1974b), and the monoamines in our experiments with the semi-intact preparation were applied primarily to the buccal ganglia via the buccal artery. Hence the analysis that follows focuses on the effects of DA and 5-HT on the CPG for rhythmic movements of the odontophore and radula in the isolated buccal ganglia.

During feeding, the rhythmic movements of the radula and odontophore are produced by coordinated contractions of muscles in the buccal mass, which in turn, are innervated by motor neurons located in the buccal ganglia. These motor neurons project to the buccal mass via four peripheral nerves; the R n. and n.1, n.2, and n.3, respectively (e.g., Nargeot et al. 1997; Scott et al. 1991). Thus extracellular recordings from these four nerves provide a comprehensive monitor of centrally generated buccal motor output. Before investigating the effects of DA and 5-HT on BMPs, we examined the patterns of neural activity that were generated in preparations of isolated buccal ganglia, which were perfused with control saline.

Figure 4 illustrates an extracellular recording of spontaneous rhythmic neural activity from a preparation of isolated buccal ganglia. A BMP was defined as a sequence of bursts of large-unit activity in all four buccal nerves. Spontaneous BMPs occurred most often as discrete, individual patterns (e.g., the neural activity indicated by the box in Fig. 4A), but occasionally BMPs occurred as a “chain” of multiple cycles of bursting activity (e.g., the neural activity indicated by the bar in Fig. 4A). Although all BMPs had some features in common (e.g., each began and ended with a burst of large-unit activity in n.1), several types of BMPs could be distinguished, in part, by the phase relationship between activity in R n. and n.2. For example, in Fig. 4B, the bursts of large-unit activity in R n. terminated before the burst of large-unit activity in n.2 (see also Nargeot et al. 1997). This temporal relationship was similar to the neural correlate of rejection described by Morton and Chiel (1993a,b).

In addition to the rejection-like BMP (Fig. 4B), the isolated ganglia spontaneously expressed several other types of BMPs. A second type of BMP was characterized by a substantial overlap of large-unit activities in R n. and n.2 (Fig. 4C) (see also Nargeot et al. 1997). The temporal relationship was similar to the neural correlate of ingestion described by Morton and Chiel (1993a,b). A third type of BMP was characterized by two bursts of large-unit activity in R n. (Fig. 4D). The first burst in R n. occurred before large-unit activity in n.2 and the second burst overlapped with large-unit activity in n.2. This temporal relationship was similar to a pattern that was observed in vivo mainly during transitions to or from other types of responses (Morton and Chiel 1993a,b). A fourth type of BMP was the chain pattern (Fig. 4E). The chain BMP had some features similar to those of the individual BMPs. For example, the chain BMP began and ended with bursts of large-unit activity in n.1, and the large-unit activity in R n. preceded that in n.2. Some differences were observed constantly, however. First, the chain BMP contained a burst of medium-unit activity in n.1 that overlapped with the burst of large-unit activity in n.2. Whenever this medium-unit activity was present, the rhythmic activity continued, and whenever this medium-unit activity was absent, the chain pattern terminated. Second, the bursts of large-unit activity in n.2 and n.3 did not overlap substantially. At present, the behavioral relevance of the chain BMP is unknown.

Extracellular recordings indicated that individual preparations could spontaneously express several different types of BMPs. For example, the rejection- and transitional-like BMPs illustrated in Fig. 4, B1 and D1, were recorded from the same preparation. Preparations did not express the different types of BMPs in equal numbers, however. Figure 5 illustrates the distribution of spontaneously expressed BMPs that was observed in 55 preparations. A median of 43% [interquartile range (IR): 9.5–69.5%] of the BMPs were rejection-like, 20% (IR: 0–48.5%) were transitional-like, 0% (IR: 0–21%) were ingestion-like, and 0% (IR: 0–17.5%) were categorized as other (e.g., chain patterns). For analyses of the distribution of different types of BMPs, we used Friedman’s test—a nonparametric test analogous to the two-factor ANOVA, repeated-measures design (Zar 1996). The types of BMPs were the fixed-level factor, and subjects were the random-effect factor with within subjects’ repeated measures. The test does not depend on normal distribution assumptions and on scale of measurements. Medians were used to characterize the populations. The data are ranked and then χ² test statistic is calculated. Friedman’s test indicated a statistically significant difference in the median values among the four categories (χ² = 29.896, P < 0.001). Post hoc Dunnett’s all pairwise multiple comparison test indicated that preparations of isolated buccal ganglia spontaneously expressed significantly more rejection-like BMPs than any other type (2-tailed P < 0.05). Thus although the CPG in the isolated buccal ganglia preparation could generate several different BMPs, its spontaneous activity appeared to be biased toward generating the rejection-like BMP.

Effects of DA and 5-HT on spontaneous BMPs in vitro

MODULATING THE FREQUENCY OF SPONTANEOUSLY OCCURRING BMPs. To determine whether the frequency of spontaneously occurring BMPs changed over time in vitro, 16 preparations of isolated buccal ganglia were perfused with control saline for ≥2 h. The average frequency of BMPs was 0.0019 ± 0.0003 Hz during the first hour and 0.0021 ± 0.0003 Hz during the second hour (Fig. 6A). A paired t-test indicated that this change was not significant (t₁₅ = −0.84). Thus the frequency of spontaneously occurring BMPs was relatively constant for several hours in vitro. The effects of DA (50 μM) on the frequency of spontaneously occurring BMPs were examined in
Before application of DA, the average frequency of spontaneous BMPs was 0.00276 ± 0.00049 Hz. During perfusion with DA, the average frequency increased to 0.00701 ± 0.00109 Hz. A paired t-test indicated that this increase was significant (t_{18} = 4.09, 2-tailed P < 0.001).

Similar results were obtained in preparations in which simultaneous extracellular and intracellular recordings were used to monitor BMPs (Fig. 7). During intracellular recordings, the protraction phase of a BMP was monitored via recordings from cell B34, which functions as an element of the CPG and the activity of which is correlated primarily with rejection-like BMPs (Hurwitz et al. 1997). The retraction phase was monitored via recordings from cells B64 and B4/5 (Church and Lloyd 1994; Hurwitz and Susswein 1996; Morton and Chiel 1993b), and the closure phase was monitored with both intracellular recordings from radula-closer motor neuron B8 and extracellular recordings from R n.

Before application of DA, the average frequency of spontaneous BMPs was 0.00149 ± 0.00045 Hz, and during perfusion with DA, the average frequency increased to 0.01246 ± 0.00286 Hz (Fig. 7B). A paired t-test indicated that this increase was significant (t_{8} = 4.18, 2-tailed P < 0.005). Similar results also were observed during simultaneous extracellular and intracellular recordings in nine preparations that were perfused with DOPA. Before

FIG. 4. Characterization of buccal motor programs (BMPs) in preparations of isolated ganglia. A: buccal ganglia were perfused with control saline and efferent activity was recorded extracellularly from the radula nerve (R n.) and buccal nerves 1, 2, and 3 (n.1, n.2 and n.3, respectively). BMPs were defined as sequential bursts of large-unit activity in all 4 buccal nerves. (Occasionally, bursts of activity were observed in individual nerves or in subsets of nerves. These “incomplete” patterns were not included in the present study.) BMPs occurred most often as individual patterns (e.g., activity outlined by the dashed box in A), but occasionally a pattern occurred as a “chain” of multiple bursts (e.g., the activity indicated by the bar in A). Using the criteria developed in previous studies (Kabotyanski et al. 1998a; Morton and Chiel 1993a; Nargeot et al. 1997), individual BMPs were categorized into 3 groups. **B1**: rejection-like BMPs were characterized by large-unit activity in R n. ending before the onset of large-unit activity in n.2. **C1**: ingestion-like BMPs were characterized by a substantial overlap of the large-unit activity in R n. and n.2. **D1**: transitional-like BMPs were characterized by 2 bursts of large-unit activity in R n., 1 before the onset of large-unit activity in n.2 and a 2nd burst that overlapped with large-unit activity in n.2. **B2, C2, and D2**: schematic diagram summarizing the sequences of large-unit activity in the 4 buccal nerves during spontaneously occurring BMPs. Boxes represent the averaged (± SE) start and stop times of large-unit activity that was measured in a pseudorandom sample of 100 individual BMPs in 20 preparations. (Selection process was not truly random because chain BMPs were excluded and only 5 BMPs were selected from each preparation.) Filled boxes correspond to large-unit activity in n.1, n.2, and n.3, and the open boxes correspond to large-unit activity in R n. All times were relative to the start of large-unit activity in n.1. **B2**: averaged data from 47 rejection-like BMPs. **C2**: averaged data from 20 ingestion-like BMPs. **D2**: averaged data from 33 transitional-like BMPs. Note the phase shifts of the large-unit activity in R n. (open boxes) that distinguished the different types of BMPs. **E**: a chain pattern, which was indicated by the horizontal bar in A, plotted with an expanded time scale. Asterisk indicate the medium-sized units that were recorded in n.1 and that appeared to be unique to the chain BMP.
 application of DOPA, the average frequency of spontaneous BMPs was 0.00077 ± 0.00044 Hz. During perfusion with DOPA, the average frequency increased to 0.05547 ± 0.01769 Hz. A paired t-test indicated that this increase was significant (t = 3.16, 2-tailed P < 0.02).

The effects of 5-HT (5 μM) on the frequency of spontaneously occurring BMPs were examined in 12 preparations (Fig. 6C). In control saline, the average frequency of BMPs was 0.00426 ± 0.00131 Hz, and in presence of 5-HT the average frequency somewhat decreased to 0.00188 ± 0.00037 Hz. A paired t-test indicated that this decrease was not significant (t = 1.89).

The results described in the preceding text indicated that, at least regarding the level of activity, the semi-intact and isolated buccal ganglia preparations responded similarly to DA. In both preparations, DA increased the frequency of ongoing feeding-related activity (Figs. 3A1 and 6B2). Also, in both semi-intact and isolated buccal ganglia preparation, 5-HT did not alter significantly the frequency of feeding-like movements or BMPs (Figs. 3D1 and 6C2). Because both DA and 5-HT induced ingestion-like movements in the semi-intact preparation, we also examined whether DA or 5-HT had an effect on which types of BMPs were expressed.

DA but not 5-HT biased the spontaneous output of the CPG toward ingestion-like BMPs. In addition to increasing the frequency of spontaneously occurring BMPs, DA modulated the type of BMP that was expressed. Two examples of this second action of DA are illustrated in Fig. 8. During the 30 min that the preparation illustrated in Fig. 8A was perfused with control saline, five spontaneous BMPs were recorded and all five of these were rejection-like BMPs (e.g., Fig. 8A1). During the 30 min that this preparation was perfused with DA (50 μM), 11 BMPs were observed and all of these BMPs were ingestion-like (e.g., Fig. 8A2). Similar results were obtained in preparations in which simultaneous extracellular and intracellular recordings were used to monitor BMPs (Fig. 8B). Figure 8B1 illustrates a rejection-like BMP that was recorded in control saline, and Fig. 8B2 illustrates an ingestion-like BMP that was recorded from the same preparation after application of DA (50 μM). Recent studies have indicated that high levels of spiking activity in cells B4/5 and B34 were correlated with rejection-like BMPs (Hurwitz et al. 1997; Kabotyanski et al. 1997, 1998a). The level of spiking activity of B4/5 and B34 appeared to be reduced during the DA-induced ingestion-like BMP (Fig. 8B2), which suggested that DA may bias the functional configuration of the CPG toward generating ingestion-like BMPs by decreasing the excitability of these cells (see following text).

Figure 9 summarizes the effects of DA and 5-HT on the expression of different types of BMPs. The effects of DA or 5-HT were calculated for each preparation by determining the distribution of BMPs in control saline (e.g., Fig. 5), determining the distribution in the presence of either DA or 5-HT, and subtracting the values for the control distribution from the values for the distribution in either DA or 5-HT. Thus positive values indicated an increase in the proportion of a particular BMP and negative values indicated a decrease. To determine whether the distribution of BMPs changed over time, 16 preparations were perfused with control saline for ≥2 h and net change in the distribution of BMPs between the first and second hours was calculated (Fig. 9A). In control experiments, the median net change in the proportion of rejection-like BMPs was 0% (IR: −33–15%), transitional-like BMPs was 0% (IR: −0.25–13.5%), ingestion-like BMPs was 0% (IR: −19.75–20%), and others patterns was 0% (IR: 0–10.5%). Friedman’s test indicated that there was no significant difference among these values (χ^2_3 = 0.84). Thus the types of BMPs that were expressed spontaneously in vitro were stable over time.

Figure 9B summarizes the effects of DA in 19 preparations. In the presence of DA, the median net change in the proportion of rejection-like BMPs was −28% (IR: −51–0%), transitional-like BMPs was 0% (IR: −34–14%), ingestion-like BMPs was 50% (IR: 4.5–62%), and other patterns was 0% (IR: −13–1.5). Friedman’s test indicated that there was a significant difference among these values (χ^2_3 = 17.14; P < 0.001). Post hoc Dunn’s multiple comparison analysis indicated significant differences between the change in ingestion-like BMPs as compared with the changes in all other types of BMP (for each pair-wise comparison, P < 0.05). Similar results were observed in three preparations that were perfused with DOPA. Thus DA appeared to bias the output of the buccal CPG toward the ingestion-like BMP.

In contrast, 5-HT did not appear to modify the expression of BMPs by the CPG in 12 preparations (Fig. 9C). In the presence of 5-HT (5 μM), the median net change in the proportion of rejection-like BMPs was −7.5% (IR: −48.5–2%), transitional-like BMPs was −4.5% (IR: −42.5–20), ingestion-like BMPs was 0% (IR: 0–59.5%), and other patterns was 0% (IR: −7.25–0%). Friedman’s test indicated that there were no significant differences among these values (χ^2_3 = 4.92). These results need to be interpreted with caution. The low power of the test (0.28) and the large variability of the data render the results of these experiments rather inconclusive.

Modulation of cellular and synaptic properties by 5-HT and DA

To understand the mechanisms and the role of modulation of the CPG for feeding by DA or 5-HT, we have begun to examine sites in the CPG at which the transmitters might exert their actions. As a first step, it was necessary to characterize the
cellular events associated with the action of modulators that occur in the CPG and output neurons under the same pharmacological conditions that led to fictive feeding (i.e., while existing synaptic connections were maintained). Figure 10 illustrates some of the loci within the feeding neural circuitry that were modulated by application of 5-HT. At least three biophysical properties of B31/32 were consistently modulated in the presence of 5-HT (n = 8 preparations). First, the excitability of B31/32 was reduced (Fig. 10A). Second, an oscillation in the resting membrane potential of B31/32 was induced (Fig. 10B). Third, the plateau-like potential in B31/32 appeared to be reduced (Fig. 10C). Similar 5-HT-induced changes were observed in B33 (not shown). In the presence of 5-HT, the membrane potential of B33 oscillated and its excitability was decreased (n = 3 preparations). In contrast to its actions on B31/32 and B33, 5-HT did not induce oscillations or any other observable change in the membrane potential of cell B35. It did, however, appear to increase the excitability of B35 (Fig. 10D) and increased the strength of the B35 synaptic connection to B4/5 (n = 4 preparations). Finally, although the presence of 5-HT did not induce a change in the resting membrane potential of cells B4/5, it did decrease the excitability of these cells.
and decreased the strength of the B4/5 synaptic connection to B31/32 (Fig. 10E; n = 7 preparations).

Some of the same sites that were modulated by bath application of 5-HT also were modulated by presence of DA, albeit in different ways. For example, bath application of DA depolarized the membrane potential of cells B31/32 (n = 7 preparations). This depolarization immediately preceded and appeared to induce the sustained rhythmic activity in the CPG. The depolarizations of membrane potentials in the presence of DA also were observed in cells B8 (n = 8 preparations), B34 (n = 5 preparations), and B65 (n = 2 preparations). In contrast, in the presence of DA, the membrane potentials were hyperpolarized in cells B4/5 (n = 14 preparations), B51 (n = 8 preparations), B63 (n = 2 preparations), and B64 (n = 5 preparations).

Figure 11 illustrates some additional effects of DA. In the presence of DA, the excitability of cell B34 appear to be reduced (Fig. 11A; n = 6 preparations) and the strength of the excitatory synaptic connection from B34 to B31/32 was decreased. Similarly the excitability of cells B4/5 (Fig. 11B; n = 14 preparations) (see also Kabotyanski et al. 1994, 1997, 1998a) was reduced. At the same time, B4/5 exhibited a lower rate of activity in presence of DA, and in ~20% of preparations, it produced only one to two spikes per cycle. In addition, bath application of DA decreased the strength of the synaptic connections from B4/5 to B8 (Fig. 11C; n = 5 preparations). The excitability of B51 appeared to be reduced in the sustained presence of DA, and this cell was usually not active during DA-induced rhythm (not shown; n = 5 preparations). Although the excitability of B64 was somewhat reduced in the presence of DA (Fig. 11, D and E; n = 6 preparations), the strength of its inhibitory synaptic connection to B31/32 (Fig. 11D; n = 3 preparations) and B34 (not shown; n = 3 preparations) appeared to be increased. In contrast, the excitatory connection from B64 to B4/5 was reduced in the presence of DA (Fig. 11E; n = 5 preparations). Finally, bath application of DA led to an increase in the excitability of B8 and appeared to enhance posthyperpolarization rebound excitation in this cell (Fig. 11F; n = 10 preparations). Although the actions of DOPA have not been studied extensively, similar results have been observed. In the presence of DOPA the excitability of cells B4/5, B34, and B64 appeared to be reduced. The strength of the inhibitory synaptic connection from B64 to B31/32 appeared to be enhanced, whereas the strength of the excitatory synaptic connection from B64 to B4/5 appeared to be reduced.

These results indicate that bath application of DA and 5-HT to isolated buccal ganglia elicit diverse and cell-specific changes in identifiable neurons while they are still incorporated in the network. More detailed and quantitative experiments will be necessary to investigate these modulatory actions under conditions that minimize polysynaptic influences.

**DISCUSSION**

The present study examined the actions of DA and 5-HT in the feeding system of *Aplysia* at the levels of behavior, neural network activity, and cellular properties. At the behavioral level, both DA and 5-HT induced movements that transported...
a thread into the foregut of a semi-intact preparation. This result indicated that the DA- and 5-HT-induced movements were ingestion-like. There were several important differences between the features of DA- and 5-HT-induced feeding-like movements, however. First, DA induced feeding-like movements at a high frequency, whereas 5-HT did not alter the frequency of feeding-like movements. Second, the amplitude of individual DA-induced displacements was relatively small as compared with the amplitude of individual 5-HT-induced displacements. Third, DA-induced movements involved opening and closing of the jaws as the radula/odontophore protruded and retracted. In contrast, the jaws remained closed during 5-HT-induced movements. These observations suggested that DA induced bite-like movements, whereas 5-HT induced swallow-like movements.

At the level of the isolated buccal ganglia, 5-HT did not significantly change frequency of spontaneously occurring BMPs nor did it significantly change the proportion of ingestion-like BMPs. This latter result seems to differ from the results in semi-intact preparation in which we observed strong ingestion induced by 5-HT. The reasons for this difference are unclear. One possibility is that additional central and/or peripheral elements may be required for 5-HT to induce neural correlates of swallowing. For example, to organize swallowing, 5-HT may require feedback from peripheral structures (e.g., Cropper et al. 1996; Evans and Cropper 1997; Jahan-Parwar et al. 1983; Scott et al. 1995), and/or 5-HT-mediated modulation of the “physical plant” may be a key factor in organizing the appropriate movements (e.g., Chiel and Beer 1993; Kupfermann et al. 1997; Weiss et al. 1992). The absence of the cerebral ganglion in the in vitro experiments did not seem to be a key factor in that 5-HT produced similar effects in preparations consisting of isolated cerebral-buccal ganglia (unpublished observations).

The effects of DA at the network level were consistent with its actions at the behavioral level. Perfusing preparations of isolated buccal ganglia with DA increased the frequency of BMPs. The activity of the CPG was biased toward ingestion-like BMPs by DA. Moreover the activity of the CPG was biased toward ingestion-like BMPs by DA. Furthermore, DA biased the output of the central pattern generator (CPG) toward ingestion-like BMPs. DA biased the output of the central pattern generator (CPG) toward ingestion-like BMPs. - - -, presumed transition from the protraction phase of the BMP (to the left) to the retraction phase (to the right). A: extracellular recordings were used to monitor BMPs in control saline (A1) and after bath application of 50 μM DA (A2). A1: rejection-like BMP (i.e., large-unit activity in R n. preceded large-unit activity in n.2) recorded in the presence of DA. B: ingestion-like BMP (i.e., large-unit activity in the R n. coincided with large-unit activity in n.2) recorded in the presence of DA. B1: simultaneous intracellular and extracellular recordings during spontaneous BMPs in control saline (B1) and in the presence of 50 μM DA (B2). B1: rejection-like BMP (from Fig. 7A1). As previously reported, B8 has an axon in the R n. and contributed to the large-unit activity recorded extracellularly from R n. Closure activity occurred primarily during the protraction phase (i.e., coinciding with activity in cell B34). B2: ingestion-like BMP (the 3rd cycle in Fig. 7A2). In the presence of DA, the majority of closure activity was shifted to the retraction phase (i.e., coinciding with activity in cell B64).
system. First, DA has an overall activating effect on patterned motor output of the buccal ganglia, and second, DA modifies the functional configuration of the CPG such that it generates more ingestion-like BMPs and fewer rejection-like BMPs.

At the cellular level, bath application of DA and 5-HT had diverse effects on cells and synaptic connections within the CPG. To evaluate the functional implications of these changes, however, it is necessary to understand how the CPG functions and which elements of CPG mediate different aspects of its function. Recent studies have identified several cells that mediate specific functions of the CPG, such as cells that initiate rhythmic activity (e.g., B31/32 and B63) and cells that shape the patterns (e.g., B4/5, B34, B51, and B65) (Hurwitz and Susswein 1996; Hurwitz et al. 1994, 1997; Kabotyanski et al. 1997, 1998a; Nargeot et al. 1999, 2000; Susswein and Byrne 1988). Moreover recent computational models have begun to reconstruct the circuitry of the CPG and to explore its function (Baxter et al. 1997, 1999, 2000; Thorne et al. 1997; Ziv et al. 1994). These studies provide a conceptual framework within which the functional consequences of DA- and 5-HT-induced changes in cellular and synaptic properties can be considered.

Cells that are important for initiating rhythmic activity are targets for modulation by both DA and 5-HT. Previous studies have indicated that a key step for initiating a BMP is to sufficiently depolarize B31/32 so as to elicit a plateau-like potential (Baxter et al. 1997; Hurwitz et al. 1994, 1997; Susswein and Byrne 1988; Thorne et al. 1997). 5-HT decreased the excitability and the plateau-like potential of B31/32, which might explain, in part, why the frequency of BMPs did not increase in 5-HT. Conversely, DA depolarized B31/32, which might explain, in part, why BMPs were more likely to occur in DA. Additional factors that may contribute to DA-induced rhythmicity are the decreased excitability of B64 and the enhancement of its inhibitory input to B31/32. Simulations studies have indicated that posthyperpolarization excitation of B31/32 can result from briefer, more intense B64-mediated inhibition and that this posthyperpolarization excitation can contribute to genesis of rhythmic activity (Baxter et al. 1997, 2000).

Cells that are important for shaping the patterns of activity are also targets for modulation by DA and/or 5-HT. Previous studies have indicated that the levels of activity in cells B4/5 and B34 play important roles in organizing rejection-versus ingestion-like BMPs (Baxter et al. 1997; Hurwitz et al. 1997; Kabotyanski et al. 1997, 1998a). High levels of activity in B4/5 and B34 appear to contribute to rejection-like BMPs, whereas low levels of activity favor ingestion-like BMPs. In the present study, bath application of DA led to a reduction in the excitability and synaptic strengths of both B4/5 and B34, and B34 was usually not spiking in the presence of DA, which might explain, in part, why more ingestion-like patterns and fewer rejection-like BMPs were generated in DA. The excitability of B4/5 also was reduced by bath application 5-HT, which might contribute to the ingestion-like feeding movements that 5-HT induced in the semi-intact preparation. In addition, recent studies have indicated that B51 is another cell that helps to shape BMPs (Nargeot et al. 1999a,b). Activity in B51 was correlated with ingestion-like patterns in isolated buccal ganglia. Moreover direct depolarization of B51 during rhythmic activity increased the number of ingestion-like BMPs, whereas hyperpolarizing B51 decreased the number of ingestion-like BMPs. In the present study, bath application of DA led to a hyperpolarization of the membrane potential of B51 and appeared to reduce its excitability. In addition, intracellular recordings indicated that B51 generally was not active during DA-induced rhythmic activity. These results suggest that the ingestion-like BMPs in which B51 is active may represent neural correlates of swallowing rather than biting.

Other actions of DA and 5-HT have yet to be explained, in part, because the functions of the cells that they modulate are not well understood. For example, cells B33 and B35 were modulated in the presence of 5-HT, but the roles of these cells in the CPG are unknown. Similarly the presence of DA ap-

FIG. 9. Modulation of the types of spontaneously occurring BMPs in isolated buccal ganglia. A: in 16 control preparations, no significant changes in the distribution of BMPs were observed between the 1st and 2nd hours of recordings. B: in 19 preparations, bath application of DA (50 μM) biased the output of the CPG away from rejection-like BMPs and toward ingestion-like BMPs. C: in 12 preparations, perfusing isolated buccal ganglia with 5-HT (5 μM) did not significantly alter the distribution of the types of BMPs.
induced ingestion-like BMPs in the isolated buccal ganglia and features. The results of the present study indicated that DA activation of B65, the BMPs began to express ingestion-like dopaminergic neurons (B65) in the buccal ganglia. Depolarization of B65 that were active during BMPs and that could drive BMPs retraction, which is required for the production of ingestion-like BMPs. Smaller of the spike-like potentials are believed to represent spikes in the electrically coupled cells such as B63, whereas the larger spike-like potentials are believed to represent axonal spikes in B31/32 (see Hurwitz et al. 1997.) In the presence of 5-HT, identical stimuli elicted far less of the plateau-like potential (D2). D: excitability of B35 and the strength of its excitatory synaptic connection to B4/5 were enhanced in the presence of 5-HT. Two electrodes, one for passing current and another for monitoring membrane potential, were placed in both the presynaptic and postsynaptic cells (B35 and B4/5, respectively). In control saline (D1), a 2-nA, 1-s depolarizing current pulse elicited 5 spikes in B35 and a train of excitatory postsynaptic potentials (EPSPs) in B4/5, the first of which was -2 mV in amplitude. In the presence of 5-HT (D2), an identical stimulus elicted 11 spikes in B35 and the amplitude of the first EPSP in B4/5 was increased to ~3 mV. Membrane potentials of both cells were adjusted to control levels. E: bath application of 5-HT led to a decrease in the excitability of B4/5 and a decrease in the strength of synaptic connections from B4/5 to B31/32. In control saline, a 2.5-nA, 3-s current pulse elicited a train of 31 spikes in B4/5 and PSPs in B31/32. In the presence of 5-HT, an identical stimulus elicted only 6 spikes in B4/5 and no PSPs were observed in B31/32. In both cells, only a single electrode was used to monitor the membrane potential and/or inject current pulses.

**DA is a possible candidate transmitter for the control of biting**

The data of the present study and others suggest that DA plays important roles in initiating and organizing ingestive behaviors such as biting. Teyke et al. (1993) characterized a pair of putative dopaminergic neurons in the buccal ganglia (B20) that were active during BMPs and that could drive BMPs if depolarized. Moreover, the B20-induced BMPs had several features in common with the BMPs that are driven by the putative bite-command neuron CBI-2. In addition, Kabotyan ski et al. (1998a) characterized a second pair of putative dopaminergic neurons (B65) in the buccal ganglia. Depolarization of B65 initiated rhythmic activity and during repetitive activation of B65, the BMPs began to express ingestion-like features. The results of the present study indicated that DA induced ingestion-like BMPs in the isolated buccal ganglia and bite-like movements in the semi-intact preparation. In addition, although bath application of DA led to a variety of different effects on various cells, some of the actions of DA appear to operate in synergy consistent with reconfiguration of the CPG and biasing its activity toward ingestion. For example, depolarization of B31/32 and B65, associated with an enhanced inhibition from B64 to B31/32, could help to increase the frequency of BMPs. In addition, depolarization of B65 in presence of DA indicates that these neurons may be an element of a positive-feedback mechanism by which DA maintains sustained rhythmic activity. On the other hand, bath application of DA reduced the excitability and synaptic strength of cell B34. This cell is believed to play an important role in organizing rejection-like BMPs (Hurwitz et al. 1997), and its inhibition should promote the expression of ingestion-like patterns. At the same time, decrease of excitation from B64 to B4/5 and decrease of excitability of B4/5 could account for reduced firing in B4/5 during retraction. The reduced firing, together with decreased inhibition from B4/5 to B8A/B, could lead to disinhibition and firing of B8 during retraction (see also Kabotyanski et al. 1997, 1998a). As a result of these reconfigurations, the balance of B8 activity shifts toward firing mostly during retraction, which is required for the production of ingestion-like BMPs.
Although the results described in the preceding text indicate that DA is sufficient to induce ingestion-like activity, they do not indicate whether DA is necessary for pattern generation. Recent studies of the effects of the DA antagonist ergonovine, however, address this issue. Teyke et al. (1993) reported that ergonovine blocked B20-induced BMPs at concentrations of $10^{-7}$ and $10^{-8}$ M. Similarly, we found that ergonovine (EC$_{50}$ $\sim$8 $\times$ $10^{-8}$ M) blocked rhythmic activity that was elicited via tonic stimulation of an afferent nerve to the buccal ganglia (n. 2,3) (Baxter et al. 1998; Nargeot et al. 1999c). These results suggest that DA is necessary for pattern generation in the buccal ganglia.

Roles of DA and 5-HT in the feeding systems of gastropod mollusks

The effects of exogenous DA and 5-HT on rhythmic activity have been investigated in a number of gastropods in addition to gastropods. In Aplysia. In Limax (Wieland and Gelperin 1983), exogenous DA ($3 \times 10^{-5}$ M) induced rhythmic feeding motor programs in reduced preparations. Moreover, the frequency and phase relations of the DA-induced motor programs were similar to lip-stimulated fictive feeding. In contrast, exogenous 5-HT ($10^{-5}$ M) induced broad excitation of multiple buccal motor units, but this activity exhibited little or none of the synchronization found in feeding motor programs. Finally, application of ergonovine ($0.5 \times 10^{-6}$ M) blocked both DA- and lip-stimulus-induced expression of fictive feeding. These results suggested that DA was both sufficient and necessary for the genesis of feeding motor programs in Limax. In Helisoma (Arnett 1996; Granzow and Kater 1977; Quinlan et al. 1997; Trimble and Barker 1984), exogenous DA ($10^{-6}$ to $10^{-4}$ M) induced fictive feeding in reduced and semi-intact preparations. Moreover, DA antagonists (i.e., sulpiride or haloperidol) blocked fictive feeding. Bath-applied 5-HT ($10^{-6}$ to $10^{-3}$ M) also was reported to induce feeding motor programs, and these motor patterns are believed to mediate repetitive swallowing. In Helix (Galanina et al. 1986), bath-applied DA also has been reported to induce fictive feeding, whereas 5-HT modulated the effects of exogenous DA and 5-HT on rhythmic activity. Although the results described in the preceding text indicate that DA is necessary for pattern generation in the tonic stimulation of an afferent nerve to the buccal ganglia (n. 8, 7 and 10), these studies were performed in the presence of DA, spikes in B4/5 failed to elicit PSPs in B8 (C1). In control saline, spikes in B4/5 elicited PSPs in B8 (C1) phase of the presynaptic spike and 3 spikes in B8 (C2) elicited a summating IPSP was increased to $\sim$17 mV. In the presence of DA, an identical stimulus failed to elicit spiking (not shown). At least 4 nA was necessary to elicit spiking, and this larger stimulus elicited only 2 spikes (B2). C: in the presence of DA, the strength of the inhibitory synaptic connections from B4/5 to B8 was decreased. Membrane potential of B8 was hyperpolarized beyond the reversal potential of the inhibitory postsynaptic potential (IPSP) by a sustained injection of $\sim$4 nA. Oscilloscope display was triggered by the raising phase of the presynaptic spike and 3 spikes in C1 or 4 spikes in C2 successive sweeps were superimposed. In control saline, spikes in B4/5 elicited PSPs in B8 (C1). In the presence of DA, spikes in B4/5 failed to elicit PSPs in B8 (C2). D: excitability of B64 was decreased in the presence of DA, but the strength of its excitatory synaptic connection to B31/32 was increased. In control saline, a 3-nA current pulse injected into B64 (E1) elicited a burst of spikes and a summating IPSPs in B31/32 with a peak amplitude of $\sim$10 mV (D1). In the presence of DA, an identical stimulus failed to elicit spiking in B64 (not shown). Current of $\sim$5 nA was necessary to elicit a burst of spikes in B64 (D2). Peak amplitude of the IPSP was increased to $\sim$17 mV. E: bath application of DA led to a decrease in the strength of the excitatory synaptic connection from B64 to B8. In the presence of DA, an identical stimulus elicited 5 spikes in B8 (E2). (Note that hyperpolarization in B8 saturated the downward display of the pen recorder.)
nanomolar range did not induce fictive feeding but did facilitate the production of feeding motor programs (Yeoman et al. 1994), which supports the hypothesis the 5-HT has a modulatory rather than command-like role. Finally, injections of the neurotoxins 5,6-DHT and 6-hydroxydopamine, which ablate serotonergic and dopaminergic systems, respectively, indicated that DA was necessary for a basic feeding response to food to occur, whereas 5-HT has a predominantly modulatory role in feeding behavior.

These data indicate that DA plays a major role in initiating and organizing consummatory feeding responses in gastropods. Perfusing reduced preparations with DA is sufficient to initiate fictive feeding, and blocking the actions of DA or depleting the nervous system of DA impairs rhythmic activity and feeding. Generally, 5-HT is believed to play a modulatory role in feeding behavior. This circuitry is multifunctional and can switch between generating rejection- and ingestion-like BMPs. Initiating rhythmic activity in the CPG and switching among different functional reconfigurations may be mediated, in part, by the transmitter DA. Bath application of DA led to modulation of several loci within the CPG and thereby biased its output toward ingestion-like BMPs. Although the BMPs in isolated buccal ganglia may not account for all aspects of consummatory feeding (e.g., 5-HT-induced swallowing), this reduced preparation does appear to retain many key elements of the feeding circuitry and thus can provide a useful model system for cellular and biophysical analyses of the functional reconfiguration of pattern generating circuitry and their modulation by learning (e.g., Colwill et al. 1997; Lechner et al. 1997; Nargeot et al. 1997).

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