Control of Feeding in Aplysia With Ad Libitum Access to Food: Presence of Food Increases the Intervals Between Feeding Bouts

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Hurwitz, Itay, Anat Harel, Silvia Markowitz, Ohad Noy, and Abraham J. Susswein. Control of feeding in Aplysia with ad libitum access to food: presence of food increases the intervals between feeding bouts. J Neurophysiol 95: 106–118, 2006. First published September 7, 2005; doi:10.1152/jn.00705.2005. The patterning of feeding and the quantity eaten in Aplysia californica with ad libitum food access cannot be explained by the effects of three variables previously shown to control the patterning of consummatory feeding responses and the quantity eaten in animals hand-fed individual meals. Feeding in ad libitum conditions is regulated primarily by varying the time between feeding bouts rather than by modulating bout lengths or the efficacy of consummatory movements within a bout. Aplysia with steady-state food access are in a newly characterized feeding state in which they are relatively unresponsive to food. They eat very little (1–4% of the time), and the quantity eaten is unrelated to the quantity of food in the anterior gut. The steady state can be maintained by the presence of food, even if animals do not contact food. The chemosensory rhinophores signal the presence of food that maintains the steady state. Up to 24 h without food is needed for animals to recover from the inhibition of feeding by steady-state presence of food. Recovery from the steady state is partially governed by postigestion stimuli as shown by a faster recovery in animals that have not been in contact with food. Inhibition of feeding during the steady-state is mediated in part via humoral factors because bathing the cerebral and buccal ganglia in hemolymph from animals in the steady state inhibits the ability to elicit buccal motor programs via a cholinomimetic thought to simulate stimulation of the lips with food. After food deprivation that is sufficiently long so that the steady-state decays, animals eat a large meal the size and dynamics of which are consistent with regulation via the three variables previously identified. This large meal is modulated by pheromones secreted by conspecifics even in sexually immature Aplysia.

INTRODUCTION

Experiments designed to understand the neural basis of behavior are usually performed in carefully controlled laboratory conditions, using protocols in which the effects can be determined of a single variable on behavior or on the nervous system. It is assumed that the variables identified as controlling behavior and the nervous system in controlled experiments also operate when the animal behaves naturally and when the experimenter lacks control over the animal’s behavior and nervous system, and the animal decides which neural circuits and behaviors to perform. It is often very difficult to test whether variables known to operate in the laboratory in fact account for ad libitum patterns of behavior. The present study was designed to test whether the variables known to control a well-characterized behavior and the neural circuit giving rise to the behavior can account for patterns of the behavior in freely behaving animals.

The investigation focused on Aplysia feeding, a model system for examining how a relatively simple neural circuit controls complex behavior. Of particular interest is modulation of consummatory feeding responses (biting, swallowing, rejection) because the neural circuitry controlling these responses has been explored (for review, see Elliott and Susswein 2002). Previous studies examining feeding patterns in controlled laboratory conditions identified stimuli that affect consummatory responses and thereby would be thought to control ad libitum patterns of feeding. The effects of many of these stimuli on the nervous system have been characterized (Elliott and Susswein 2002). This study examined feeding in freely behaving A. californica to determine whether feeding patterns in this condition are explained by data from more controlled conditions and, if not, to identify additional variables affecting feeding and the neural circuitry underlying feeding.

Four major variables regulating feeding have been identified. 1) Food arousal is initiated by touching food to the lips and decays when food is removed. It causes animals to bite more quickly (Kupfermann 1974; Susswein et al. 1978) and with greater intensity (A. J. Susswein, K. R. Weiss, and V. I. Kupfermann, unpublished data) in response to food. Food arousal is partially mediated by identified neurons, particularly neurons C-PR, C2, and MCC, which respond to touch of food to the lips as well as by release of peptide modulators from motor neurons innervating the musculature producing consummatory responses (for review, see Kupfermann et al. 1991). 2) Satiation causing inhibition of consummatory responses is signaled by activating mechanoreceptors in the anterior gut (Susswein and Kupfermann 1975a,b; Susswein et al. 1976). Satiation interacts with food arousal: it becomes progressively more difficult to arouse animals to eat, and arousal decay more rapidly, as the gut is filled (Susswein et al. 1978). Thus a partially satiated animal that briefly loses contact with food may not begin eating again when food is re-encountered because arousal will decay more quickly, and will be initiated more slowly, as a result of the effects of the partial satiation on arousal (Susswein et al. 1978). 3) Sensory adaptation or habituation of chemoreceptors (Schwarz et al. 1988) can terminate a meal before the anterior gut is filled (Horn et al. 2001). 4) In A. fasciata, pheromones regulating sexual behavior also modulate feeding, in part via modulating the amplitude of swal-
lowing (Blumberg and Susswein 1998; Blumberg et al. 1998; Botzer et al. 1991; Teyke and Susswein 1998; Ziv et al. 1991a).

Previous data provided contradictory expectations for the current experiments. A hungry *A. californica* fed a single meal eats ~15% of its body weight in a 2- to 3-h meal. The quantity eaten, and the patterning of feeding, are explained by the volume required to fill the anterior gut (Susswein and Kupfermann 1975b; Susswein et al. 1976). Twenty-four hours later animals are unresponsive when the lips are stimulated with food for 2 min. Responses to food are restored over the next few days (Kupfermann 1974). However, if the lips are stimulated for 15 min 24 h after a satiating meal, the animals respond (Susswein et al. 1978), eating a smaller meal than that eaten the previous day. The amount eaten, and the patterning of feeding, are explained quantitatively by the volume of food present in the anterior gut from the previous meal (Susswein and Kupfermann 1975b; Susswein et al. 1976). These data suggest that in ad libitum conditions, *Aplysia* may eat every few days, when the gut becomes empty, perhaps appended by brief meals initiated after long contact with food. By contrast, experiments on another *Aplysia* species, *A. fasciata*, showed that animals ate a number of small meals daily (Ziv et al. 1994). Field studies on both *A. californica* and *A. fasciata* found that some animals eat large meals in natural conditions (Kupfermann and Carew 1974; Susswein et al. 1984).

Our data indicated that patterns of feeding in *A. californica* with constant access to food were different from those expected and could not be explained on the basis of the effects of the previously identified variables on consummatory movements. First, feeding was regulated by controlling the initiation of a feeding bout rather than via regulating the consummatory behaviors within a bout. Thus the portions of the neural circuit governing feeding that have been described in greatest detail are unlikely to have a major role in the modulation of ad libitum feeding. Second, the constant presence of food was found to initiate a previously undescribed state in which *Aplysia* eat very little. This newly defined state operates at least in part via circulating factors that inhibit the ability of an otherwise adequate stimulus to initiate feeding. The major stimulus condition causing *A. californica* to eat was found to be the introduction of food after a period of time without food.

**Experimental conditions**

Animals were transferred from the storage tanks to 5- or 10-l aerated experimental aquaria 24 h prior to an experiment. The aquaria were kept at 23°C. Animals were generally kept one to a 5-l aquarium. In one experiment, animals were kept two to a 10-l aquarium with the two animals separated by a partition that prevented contact between the animals but that allowed free water flow. In some experiments, a single animal was kept in a 10-l aquarium with a partition with food behind the partition that animals were able to smell but could not contact.

**STEOY-STATE FOOD ACCESS.** In animals examined in this condition, food (*U. lactuca*) was available ad libitum for ≥1 wk prior to the experiment both in the storage tanks and in the experimental aquaria.

**FOOD DEPRIVED.** Animals examined in this condition were food-deprived for 5 days prior to the experiment.

**PARTIALLY DEPRIVED.** These experiments were designed to test the effects of specific periods of food deprivation on feeding. Prior to the experiment animals were kept with steady-state access to food. The food was then removed for periods of a few hours to a few days, and the food was then restored to the animals, and the quantity of food eaten was examined. When the deprivation period was >12 h, to prevent a large temperature change just prior to the experiment, animals were transferred 24 h before the experiment to experimental aquaria at 23°C with food and were then transferred to aquaria without food for the period appropriate to the experiment.

**FOOD IN THE ENVIRONMENT.** After a period with steady-state access to food in the storage tanks, animals were transferred to a 10-l aquarium in which a partition separated the animal from food on the other side of the partition. To test feeding, food was then added to both sides of the partition.

In a second experiment, after 24 h in a 10-l aquarium with food behind the partition, all of the water and food were removed, and fresh water without food placed in the aquarium. Food was added to the aquarium 3 h later.

**Experimental measures**

**PERCENT TIME SPENT FEEDING.** Two procedures were used to measure the percentage of time spent feeding. Both procedures have been used in previous experiments describing time budgeting and bout patterning of *A. fasciata* behavior (Ziv et al. 1991b, 1994) and have been described in detail previously. In one, animals were continuously observed and the time of onset and offset of all feeding bouts was noted. In the other, animals were sampled every 5 min, and feeding was noted. The number of times that feeding was observed per unit time was used to estimate the percent time spent feeding during that time period (Susswein et al. 1983; Ziv et al. 1991b). Use of each procedure is noted in the text. In some experiments, feeding was sampled during the dark phase of the day or spanned a number of hours that included the transitions from light to dark or the reverse. For these experiments, the timers controlling the laboratory lights were adjusted a number of days previously, so that the day and night were reversed or were offset by 6 h. As in previous experiments (Ziv et al. 1991b), animals were observed during the dark phase by using dim incandescent lighting that pointed down and away from the animals. Such lighting provided illumination of <0.5 lux to the animals but was nonetheless sufficient to observe feeding.

**WEIGHT OF FOOD EATEN.** In some experiments, a preweighed quantity of food was placed in the experimental aquarium at the start of the observation period, and the food remaining was weighed again at the end of the experiment. The difference in weight was attributed to consumption during the observation period. For animals in steady-state conditions, the food already in the aquarium was removed before

**METHODS**

**Animals**

Experiments were performed on *A. californica* weighing 50–120 g that were purchased from Marinus (Long Branch, CA) and from Marinus Scientific (Garden Grove, CA). The animals were stored in 600-l tanks of aerated, filtered Mediterranean seawater maintained at 17°C. Lighting was light:dark 12:12. Animals were generally kept one to a 5-l aquarium. For these experiments, the timers controlling the laboratory lights were adjusted a number of days previously, so that the day and night were reversed or were offset by 6 h. As in previous experiments (Ziv et al. 1991b), animals were observed during the dark phase by using dim incandescent lighting that pointed down and away from the animals. Such lighting provided illumination of <0.5 lux to the animals but was nonetheless sufficient to observe feeding.

**Food**

The food used in all experiments was the seaweed *U. lactuca*, which was gathered at various sites along the Mediterranean coast of Israel and then stored frozen. Food was thawed before use in an experiment.
adding the preweighed food. Previous experiments (Botzer et al. 1991) have shown that the food used in these experiments retains its integrity over the experimental period and the weight change is a reliable estimate of the quantity of food eaten.

Surgical procedures

Animals were placed in a 5-1 chamber partially filled with seawater and with ice made from seawater. When animals stopped moving and lost contact with the substrate because of lack of muscle tone, the tips of the chemosensory rhinophores were cut off. Animals were then restored to the holding tanks until used in an experiment. Control animals were treated in the same way as were the animals without rhinophores, except that the tip of the rhinophores was not cut.

Extracellular recording

The cerebral and buccal ganglia connected via the cerebrobuccal connectives were dissected from animals that previously had steady-state access to food. Before dissection, animals were injected with 50% of their body weight of isotonic MgCl2. The cerebral and buccal ganglia were removed and placed in a solution of 1:1 ASW, isotonic MgCl2. To limit possible damage, the ganglia were not desheathed. The two ganglia were pinned and then separated from one another by building a wall around the cerebral ganglion with petroleum jelly (Vaseline). The cerebral-buccal connectives crossed the wall isolating the cerebral and buccal ganglia. Possible leak of the Vaseline seals was determined by filling the cerebral ganglion chamber almost to overflow and observing if the level changed over 2–5 min. In addition, the fluid in the cerebral ganglion chamber was stained with Fast Green to detect possible leakage of the dye to the other chamber. Suction electrodes were used to record extracellular nerve activity and were placed on the radula nerve and buccal nerve 2 (Gardner 1971) [these are nerves 1 and 5 in the terminology of Scott et al. (1991)]. The volume of the cerebral ganglion chamber was 1 ml, whereas the buccal ganglia chamber contained 10 ml. After completing the dissection and pinning of the ganglia, the bathing solution was initially changed to artificial seawater (ASW) with the following composition (in mM): 460 NaCl, 10 KCl, 11 CaCl2, 55 MgCl2, and 5 NaHCO3 at pH = 7.64.

To induce activity related to feeding the nonhydrolyzable cholinergic agonist carbachol (CCh, \(2.5 \times 10^{-3} \) M) was applied to the cerebral ganglion for 10 min. Previous data (Susswein et al. 1996) has already shown that CCh applied to the cerebral ganglion induces organized buccal motor patterns. These were recorded via the suction electrodes. A total of seven trials of CCh application were run. These were separated from one another by 10-min intervals in which the cerebral ganglion was bathed in ASW. To determine how hemolymph from either hungry animals or from animals in the steady state affects the activity induced by CCh, hemolymph was applied along with CCh during the fourth and the fifth runs. After the fourth run, the CCh was washed and was replaced with hemolymph alone. Thus the ganglion was exposed to hemolymph from the start of the fourth run in CCh, throughout the 10-min period between the fourth and the fifth runs, and was removed only after the fifth run. Data were tabulated and are shown from the exposure to carbachol immediately preceding the exposure to hemolymph and from the second run in carbachol in the hemolymph. The effects of hemolymph from hungry versus steady-state animals on buccal motor program were tested using a blind procedure: the experimenter was unaware of whether the hemolymph applied had been extracted from previously hungry animals or from animals with steady-state access to food.

Hemolymph was extracted by pricking animals with an empty syringe that penetrated through the body wall into the hemocoele. Animals were ~100 ml in volume, and 5–7 ml of hemolymph were extracted. Hemolymph from two to four animals was extracted just prior to an experiment. The hemolymph from the different animals was combined. Both the buccal and cerebral ganglia were bathed with the hemolymph.

**RESULTS**

The aim of these experiments was to determine the patterns of ad libitum feeding in *A. californica* and then examine whether such patterns can be explained by previous data on the control of individual meals when animals are hand-fed.

**Regulation of consummatory behaviors does not contribute to regulation of food intake**

An initial experiment was designed to determine how animals regulate the total quantity of food eaten, and the patterning of feeding, when they have ad libitum access to food. Feeding was examined in animals that had been food deprived for 1 wk as well as in animals that had had steady-state access to food for a number of days prior to the experiment. Feeding behavior was observed for 6 h from ~3–9 h after the onset of light. Animals were observed continuously, thereby allowing us to determine the total time devoted to feeding, the length of all feeding bouts, and the length of inter-bout intervals. The weight of the food consumed during the 6-h observation was also measured. The 6-h observation was divided into 12 half-hour intervals, and the percent time spent feeding was calculated for each interval.

**REGULATION OF INTER-BOUT INTERVALS CAUSES A DECREASE IN FEEDING DURING MEALS.** Previously food-deprived animals ate well when allowed steady-state access to food. There was a significant decrease in the percent time spent feeding over the 6 h of observation (Fig. 1A). The decrease presumably reflects satiation as the hungry animals consumed progressively larger quantities of food. It is important to note that the percent feeding decreased gradually over the 6-h observation.

The decrease in time devoted to feeding as animals satiate could be a result of a decrease in the lengths of feeding bouts, as a result of longer intervals between bouts, or both. To determine whether feeding bouts became shorter, we examined the length of successive feeding bouts. There was a rapid decrease in bout length over the first five bouts from a mean of 145.7 ± (SD) 7 s for the first bout to a mean of 29.3 ± 4.4 s

![Figure 1](http://jn.physiology.org/DownloadedFrom/html_jn/2006/095/Jan/2006/jn.00006.2005.3.11005_Fig1.jpg)

**FIG. 1.** The percent time spent feeding during a 6-h period of continuous observation during the daylight hours in previously hungry *Aplysia californica* (A) and in animals that had been kept in steady-state conditions of access to food (B). The data were divided into half-hour periods, and the mean ± SE of percentage of time feeding was calculated for each half hour. The data show a significant decrease in time devoted to feeding in previously hungry animals but not in animals with steady-state access to food that display relatively little feeding. In previously hungry animals, there was a significant decrease in the percent time spent feeding over the 6 h observation [\(P = 0.006, F(1,155) = 2.75\)]. By contrast, in steady-state animals there was no significant change in the time spent feeding during the 6-h observation period [\(P = 0.79, F(12,65) = 0.65\); 1-way ANOVA with repeated measures].
for the fifth bout. The bout length was then maintained at a relatively constant value of 49.9 ± 44.0 s for the rest of the 6-h interval. There was a significant difference between the mean bout length during the first bout and the means of bout 5 and onward (Fig. 2A).

Could the decrease in bout lengths explain the decrease in percent time spent feeding? Animals performed a mean of 5.67 ± 2.5 (SD) feeding bouts in the first 30 min after the start of the feeding. Because the decrease in bout length is seen at most only over the first 5 feeding bouts, the decrease in bout length could have contributed to the decrease in time spent feeding only during the first 30 min of the meal. The slow decrease in the time spent feeding after the first 30 min must arise as a result of longer intervals between feeding bouts. Either a decrease in the likelihood to encounter food or a decrease in the likelihood to initiate feeding after food is encountered will explain the increase in interbout intervals as animals become satiated.

The possible effect of feeding on interbout intervals was directly assessed (Fig. 2C). For this analysis, the 6-h observation was divided into 1-h bins because some interbout intervals were >30 min. There was a significant increase in interbout intervals over the 6-h observation, particularly in the latter part of the observation.

REGULATION OF INTER-BOUT INTERVALS CAUSES A DECREASE IN FEEDING IN STEADY-STATE CONDITIONS. *Aplysia* that had been maintained with steady-state access to food prior to the observation ate remarkably little. Only four of six animals ate at all during the 6-h observation. The six animals devoted to feeding a mean of 9.87 ± 4.15 min (or 2.75 ± 1.15% of the 6-h time period; Fig. 1B). As would be expected for animals in steady-state conditions, in the four animals that fed, there was no significant change in the time spent feeding during the 6-h observation period.

The difference in time spent feeding in steady-state conditions and in previously hungry animals could arise from shorter bout lengths, from longer inter-bout intervals, or from both. Feeding bouts were significantly longer in steady-state conditions than in previously hungry animals (Fig. 2B). These data indicate that the time spent feeding in steady-state conditions is decreased because of longer intervals between feeding bouts, in spite of an increase in mean bout length. To estimate the mean interbout interval in steady-state conditions, the total time not spent feeding in the six animals was divided by the total number of feeding bouts observed. This gave a value of 67.7 min. A survey of the interbout intervals showed that shortest interval was 14 s and the longest was longer than the 6-h observation period. Thus changes in either food-finding behavior, or in the likelihood of initiating feeding after food is encountered, account for the difference in time spent feeding between previously deprived animals and animal with steady-state access to food.

REGULATION OF THE QUANTITY OF FOOD EATEN. Animals that were in steady-state conditions consumed significantly less food than did previously hungry animals (Fig. 3A). This is not surprising because previously hungry animals spent significantly more time spent feeding than did steady-state animals (Fig. 3B). However, the difference in the quantity eaten could also arise in part via a difference in the efficacy of feeding behavior. When animals are hand-fed individual meals, the amplitude of biting responses decreases (Susswein et al. 1976). To determine whether the efficacy of consummatory responses is regulated, we divided the time spent feeding by the quantity eaten to obtain a measure of the active feeding time needed to consume 1 g of food. Calculating the time needed to consume 1 g of food in previously hungry and in steady-state animals showed that there was no significant difference in efficacy of feeding (Fig. 3C). Both previously hungry and steady-state animals required a mean of ~20 min of active feeding to consume a gram of food. These data indicate that changes in the amplitude of biting and swallowing movements produced by changes in the feeding state are unlikely to contribute
we found that none of these factors operated and that the observation that animals with steady-state access to food eat little was confirmed.

**TIME SPENT FEEDING AT OTHER TIMES OF DAY.** Because *A. californica* feeding is primarily a diurnal activity (Kupfermann 1974; Lyons et al. 2005), and the observations reported in the preceding text were during the daylight hours, feeding should have been observed. Nonetheless, it is possible that relatively little feeding was seen because of significant differences in diurnal versus nocturnal feeding between animals with steady-state access to food (as in the present experiments) and animals that are intermittently exposed to food (as in previous experiments showing diurnal feeding). In steady-state conditions, feeding may be more prominent at other times of day. To examine this possibility, feeding was sampled every 5 min in conditions of steady-state access to food during 6 h of night (from 3 to 9 h after the lights went out), during the 6 h of transition from day to night (3 h before and 3 h after the lights extinguished), and during the 6 h of transition from night to day (3 h before and 3 h after the lights turned on). Together with the observations in the preceding text during the daylight hours, these observations would reveal feeding at all hours of the day, if it was present.

The rates of feeding were extremely low during all hours of the day (Fig. 4). There was no significant difference between the rates of feeding seen during the day or night or during the transition periods. Thus animals with steady-state access to food eat very little throughout the day-night cycle.

**DO APlysia EAT LARGE MEALS EVERY FEW DAYS?** The data in the preceding text suggest that *Aplysia* with steady-state access to food eat little and generally act as though they are close to satiated. When do they eat to become satiated? It is possible that in steady-state conditions, animals occasionally eat a large meal, which causes them to become satiated, and they then eat again only after digesting the meal completely. The interval between meals may be a number of days, and one might need a large sample of animals to observe the occasional large meal eaten by an animal.

To test this possibility, feeding was sampled every 5 min in seven *Aplysia* with steady-state access to food that were observed for 6 h during the daytime, for 3 days in succession, providing a larger data set to catch an animal eating a large meal.

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**FIG. 3.** Control of the quantity consumed. *A:* animals that were previously hungry consumed significantly more food than were animals with steady-state access to food [P < 0.001, t(10) = 5.15]. *B:* animals that were previously hungry spent significantly more time feeding than did animals with steady-state access to food [P < 0.001, t(10) = 6.43]. *C:* there was no significant difference in the time required to eat 1 g of food between animals that were previously hungry and those that were in the steady state. Because in the steady-state animals eat relatively little, 2 separate groups of animals were examined in steady-state conditions to increase the sample size and attain a better estimate of the efficacy of feeding. One group (continuous) is the same as that whose data are shown in Figs. 1 and 2, *A* and *B.* In the other group (n = 12; sampling), animals were sampled every 5 min over 6 h. There was no significant difference among the 3 groups [P = 0.98, F(2,18) = 0.15; 1-way ANOVA].

Fig. 4. The percentage of time devoted to feeding in 6-h samples of behavior from different parts of the day. The data from the daytime are those in Fig. 1B. SE is shown. There was no significant difference between the rates of feeding seen during the day or night or during the transition periods [P = 0.15, F(3,77) = 10.2]. The percent time spent feeding is low at all hours of the day.
meal. No large meals were observed. Animals ate a mean of 0.50 ± 1.1% of the time. The longest meal seen was 3.3% of the total observation period.

IS THE ANTERIOR GUT FULL? Previous data on animals fed once daily showed a strong correlation between the volume that fills the gut and the satiation state of an animal (Susswein and Kupfermann 1975b). At satiation, the anterior gut is close to full. The best predictor of satiation is the ratio of the weight of the gut contents divided by the weight of the empty anterior gut (Susswein and Kupfermann 1975b). In satiated animals, this ratio is 8.8 ± 0.9 (SE). To determine whether animals with steady-state access to food acted as though satiated or close to satiated because the gut was full, or close to full, animals (n = 7) were dissected, and the gut contents and the empty gut were weighed. The mean ratio of gut contents to the empty gut was found to be 3.47 ± 2.66 (SD), with a range of 7.55 (close to full) to 0.52 (empty). Thus the animals were very heterogeneous, with some animals having gut contents indicating that they were close to satiated and others with a virtually empty gut. Nonetheless, all of the animals were not eating before being dissected and would probably have eaten very little over the subsequent few hours.

DOES THE STEADY STATE DEPEND ON TEMPERATURE? Previous behavioral experiments on A. californica feeding were performed on animals that were cooled. In addition, A. californica are found in relatively cool waters. Previous studies have shown that temperature can have major effects on the modulation of the feeding musculature (Vilim et al. 1996; Zhurov and Brezina 2005). In our experiments, animals were stored at 17°, but experiments were performed at room temperature. To test whether the steady-state conditions arose as a result of the relatively warm temperature at which experiments were performed, ad libitum feeding was observed in 16 animals (8 hungry, 8 steady-state) at 17° for 4.5 h. Hungry animals ate a mean of 2.55 ± 0.56 g, whereas animals in the steady-state ate only 0.3 ± 0.44 g. Hungry animals spent a mean of 22.05 ± 5.18% of the time eating, whereas the steady-state animals spent a mean of 1.14 ± 1.35% time eating. These data indicate that the steady-state inhibition of feeding is intact at cooler temperatures. Nonetheless, some modulation by the temperature was seen in hungry animals. Patterning in the hungry animals was different from that seen in the previous experiments at room temperature. Animals ate more vigorously at the start of the meal and became satiated more rapidly. Thus during the first half hour, animals ate a mean of 77.08 ± 18.52% of the time, but by the start of the second hour, animals were eating only 4.17 ± 7.22% of the time.

Is a period without food a signal to eat?

Our data have shown that animals having steady-state access to food eat little, but animals that have been food-deprived eat a large meal. This suggests that the signal to begin eating may be a period of food-deprivation. How long a deprivation is needed to induce an increase in feeding similar to that seen in hungry animals? To examine this question, animals were allowed steady-state access to food for a number of days. The animals were then food-deprived for 1, 2, 3, 4, 5, 6, or 8 days, and feeding was then sampled every 5 min for 4.5 h (Fig. 5), enough time to sample a large meal after the deprivation. In all of the groups, the rate of feeding gradually declined during the first 2 h. For all periods of food deprivation, there were significant differences between the percent time spent feeding during the first half hour of exposure to food and the last half hour of the test (P < 0.05, paired t-test for all 7 groups). These data confirm that a period of food deprivation is a signal to begin feeding when food is restored. The data also indicate that a 24-h period of food deprivation is sufficient to induce feeding.

Although a 24-h deprivation is sufficient to cause animals to eat, it is possible that 24 h is not a sufficiently long deprivation to induce a maximal meal. To examine whether the time devoted to feeding during a meal is affected by the length of the previous deprivation, it is necessary to establish a criterion for deciding that a meal is over and that animals have reached the steady state. After defining the length of a meal, the parameters of the meal can then be determined.

Previous data had shown that in steady-state conditions in different experiments animals ate for a mean of 0.5–4% of the time (see preceding text). In the present experiment, the mean percent time spent feeding in the last hour of the observation in all groups was 3.37 ± 0.98%, suggesting that animals had reached the steady state by the end of the observation. How long does it take the animals to reach the steady state? For
successive hourly intervals, we tested whether the 95% confidence interval of the percent time spent feeding over an hour reached steady-state conditions in comparable periods of time, it is likely that the hourly feeding rate is partially obscured by the gray bar, until the end of the experiment. Note that after 1–6 days of feeding deprivation, the animals reach the steady state from 1.5 to 2 h after the start of feeding.

Although animals that are food-deprived for 1–6 days reach steady-state conditions in comparable periods of time, it is possible that there are differences in the percent time devoted to feeding before they reach steady-state conditions. There were no significant differences between the groups in the percent time spent feeding during the first 2 h (Fig. 7). These data indicate that a 24-h period of food deprivation is no less efficient in inducing feeding than are periods of 2–6 days. Because the quantity eaten is directly related to the time spent feeding (see preceding text), these data indicate that the quantities eaten immediately after 24 h without food are unlikely to be different from the quantities eaten immediately after longer periods of food deprivation.

**DO SHORTER PERIODS OF FOOD-DEPRIVATION INDUCE FEEDING?** As a result of the findings in the preceding text, we examined the efficacy of periods of <24 h without food to initiate feeding. In this experiment, animals were kept without food for 3, 6, 12, or 24 h and were then transferred to an aquarium with food. Controls that were transferred to a new observation chamber without a time period with no food were also examined, and these animals provided a baseline value for steady-state feeding in these experiments (Fig. 8).

Data on 24 h of deprivation were similar to those in the previous experiment. There was a significant difference in the percent time feeding between the first and last 30-min observation period. In addition, the 95% confidence interval of the hourly percent time spent feeding was significantly different from that in steady-state conditions. For the first 2 h after food was introduced (note the down arrow in the figure that marks when the 95% confidence interval overlaps the steady state). With only 12 h of deprivation, there was also a decrease in the percent time spent feeding between the first and last 30-min observation period, and the percent time spent feeding was significantly higher than in steady-state conditions for the first 1.75 h after the introduction of food. By contrast, with 6 h of deprivation, there was no significant difference between the first and last 30 min of the observation, and the percentage of time spent feeding was not significantly higher than that in steady-state conditions at any time after the food was introduced (note the
FOOD IN THE ENVIRONMENT SIGNALS THE STEADY-STATE. After animals are in a condition of steady-state access to food, a period of 24 h without food will initiate a large meal. The 24-h period without food removes a number of separable stimuli that are provided to the animals by the steady-state access to food. In the presence of steady-state food, animals sense the food in the environment, which stimulates chemoreceptors. In addition, animals contact the food, and touch of food will stimulate mechanoreceptors as well as contact chemoreceptors. Finally, the occasional consumption of food will activate a variety of postingestion stimuli. The continued or intermittent presence of any or all of these stimuli could contribute to the animal’s maintained steady-state behavior, and the removal of one or all of these stimuli could contribute to the initiation of feeding after the 24 h without food. To test the possible contribution of food in the environment that is not contacted to the maintenance of the steady-state, animals were first given access to steady-state food for 3 days. They were then transferred for 24 h to an environment in which food was present behind a partition, allowing the animals to smell the food, but the partition prevented them from touching or eating the food. At the end of the 24 h in the presence of inaccessible food, animals were given 4.5 h of access to food, and feeding was examined. During the test of feeding after 24 h in the presence of inaccessible food, animals ate a mean of 1.6 ± 4.2% of the time (mean ± SD; n = 15), similar to that seen in animals with steady-state access to food (Fig. 9A). These data indicate that food in the environment is sufficient to maintain the steady-state condition, even if the animals never contact the food or consume it.

THE RHINOPHORES SENSE SIGNALS INDUCING THE STEADY STATE. A number of chemosensory organs (e.g., rhinophores, anterior tentacles, osphradium) could potentially sense the presence of food in the environment that maintains the steady state. The most likely distance chemosensory organ for signaling the presence of food is the rhinophores. One would predict that lesioning the rhinophores would prevent animals from sensing food in the water and that animals sharing seawater with seaweed for 24 h, but with the rhinophores ablated, should behave like animals that were kept for 24 h without food. We examined this possibility. In half the animals, the rhinophores were ablated, whereas the other half had undergone an identical procedure of anesthesia and manipulation of tissue but without ablation of the rhinophores. Three days later the animals were placed in an environment with steady-state food. After 3 days in the steady state, animals were transferred for 24 h to an aquarium with food present but inaccessible. Animals were then given 4.5 h of access to food, and feeding was examined. There was a significant difference in time spent feeding between the two groups (Fig. 9B). In sham-operated controls, food in the water inhibited feeding as it had in the previous experiment. However, the behavior of animals without the rhinophores was similar to that of animals that had been deprived of contact with food for 24 h (compare Fig. 9, A and B). In addition, the pattern of feeding was similar to that seen in animals that had experienced an absence of food for 24 h (data not shown), with a decline over the first 2.5 h from ~40% to ~2% of the time spent feeding. These data indicate that the rhinophores sense food in the water that is a sufficient stimulus for maintaining the steady state. The data also show that stimuli not sensed by the rhinophores are sufficient to initiate the steady state when previously hungry animals encounter food.

ARE THE RHINOPHORES NECESSARY? The preceding experiment showed that chemostimuli sensed by the rhinophores are suf-
ficient to maintain the steady state. Are they also necessary or can other receptors responding to food substitute for the rhinophores in maintaining the steady state? To test whether other receptors may also contribute to maintaining the steady state, animals without rhinophores and sham-operated controls were allowed steady-state access to food for 3 days, and feeding was then measured for 4.5 h. There was no significant difference between the two groups. Both groups showed little feeding (Fig. 9C), similar to that seen previously in animals in steady-state conditions. These data, coupled with the previous experiment, indicate that the rhinophores are not necessary for either initiating or maintaining the steady state, even though they are a sufficient input pathway for maintaining the steady state. Other input pathways are also able to signal the presence of food and thereby initiate and maintain the steady state.

**Fig. 9.** Comparison of the percent time spent feeding during 4.5 h immediately after a number of procedures. All of the procedures followed a number of days in which the animals had steady-state access to food. The experiments tested whether a procedure maintained the steady state or caused an initiation of feeding. A: effect of 24 h in the presence of inaccessible food in the environment is compared with the effect of an immediate transfer to the test aquarium after steady-state access to food and after 24 h in which food was not present. A 1-way ANOVA showed a significant difference among the 3 groups [P < 0.001, F(2,32) = 17.46]. A post hoc test showed that animals tested after 24 h of inaccessible food in the environment and animals tested immediately after transfer from accessible food were not significantly different, whereas both differed significantly from animals tested 24 h after removal of food (α = 0.05, Student-Neuman-Keuls test). The data show that food in the environment maintains the steady-state and inhibits feeding. B: effect of 24 h in the presence of inaccessible food in the environment in animals in which the rhinophores were ablated and in sham-operated controls. There was a significant difference between animals with and without rhinophores [P = 0.001, t(18) = 3.89]. The data show that ablation of the rhinophores blocks the effect of food in the environment in maintaining the steady state because the rhinophores sense the food in the environment. C: effect of steady-state access to food in animals in which the rhinophores were ablated and in sham-operated controls. There was no significant difference between the 2 groups [P = 0.52, t(18) = 0.65]. The data show that even after ablation of the rhinophores, feeding is blocked in the presence of accessible food, indicating that stimuli other than food sensed by the rhinophores can also cause inhibition of feeding and maintenance of the steady state. D: 1 group had access to food prior to the 3 h without food (data are the same as that in Fig. 7, 3 h), whereas the other groups was in the presence of inaccessible food for 24 h prior to the 3 h without food. The effect of a 3-h period without food on subsequent feeding was then measured. There was a significant difference between the 2 [P = 0.04, t(16) = 2.23]. The data show that feeding is largely restored even after 3 h without food, if animals had not contacted the food in the previous 24 h.

**Neural correlates of the steady state**

To determine whether correlates of the steady state can be measured by recording from the *Aplysia* nervous system, we took advantage of a finding that buccal motor programs that are correlates of ingestive movements can be elicited by bathing the cerebral ganglion in the cholinomimetic carbachol (Susswein et al. 1996). Carbachol is thought to be effective in inducing buccal motor programs because sensory neurons sensing food on the lips are thought to be cholinergic (Susswein et al. 1996). The buccal motor programs are measured by extracellular recordings from buccal nerves, which innervate the buccal muscles producing consummatory feeding movements. Preliminary studies (A. J. Susswein and I. Kupfermann, unpublished results) had suggested that hemolymph from animals in the steady state has a role in modulating the ability of
FIG. 10. Effect of hemolymph on buccal motor programs. The cerebral ganglia were exposed to carbachol for 10 min. After a delay, this treatment induces buccal motor programs that are recorded in the buccal ganglia. The buccal motor programs are monitored by extracellular recordings from the radula nerve and buccal nerve 2. The graph shows the latency to the initiation of buccal motor programs in the presence of hemolymph from steady-state animals and from hungry animals. A lack of response during the 10-min test was scored as a latency of 10 min. The bars show the mean latency to the 1st buccal motor program, and the dashed lines show the latency of each preparation in the sample (n = 7 preparations tested with hemolymph from the steady state, n = 8 animals tested with hemolymph from hungry animals). Note that 6 steady-state preparations and 2 hungry preparations showed no responses. Because of the large number of nonresponsive preparations, a nonparametric test was used to determine whether the 2 populations are significantly different. A Mann-Whitney U test showed that the latency was significantly longer in animals with hemolymph from the steady state than in animals with hemolymph from hungry animals (P = 0.001, U = 3).

carbachol to induce buccal motor programs. We examined the buccal motor programs induced by a 10-min exposure to carbachol when the ganglia were bathed in hemolymph taken from animals that had steady-state access to food, as well as from animals that had been food deprived (Fig. 10). The presence of hemolymph reduced the ability of carbachol to induce buccal motor programs with respect to programs induced in the presence of artificial seawater. In the absence of hemolymph, carbachol induced a mean of 9.50 ± 11.18 (SD) buccal motor programs with a mean latency of 50.33 ± 81.76 s (compare this value to those in Fig. 10). However, hemolymph taken from animals that had been in the steady state produced a much larger inhibition of feeding than did hemolymph from previously hungry animals. Indeed, in six of seven preparations in hemolymph from animals that had been in the steady state, the carbachol was unable to induce any buccal motor programs. These data indicate that inhibition of feeding in steady-state conditions occurs in part via release of chemical factors into the hemolymph. These factors in turn reduce the ability of stimuli that normally initiate consummatory feeding patterns to do so.

Do conspecifics affect steady-state feeding in A. californica?

The preceding data indicate that A. californica with steady-state access to food eat between 2 and 4% of the time. However, previous data on another Aplysia species, A. fasciata, showed that animals with steady-state access to food eat close to 20% of the time (Susswein et al. 1983). These animals also had access to conspecifics, and to egg cordons, which release pheromones into the water that strongly modulate A. fasciata feeding (Blumberg and Susswein 1998; Blumberg et al. 1998; Botzer et al. 1991; Teyke and Susswein 1998; Ziv et al. 1991a). It is possible that the difference between the large time investment in feeding observed in A. fasciata and the small investment observed in the current experiments stems from the fact that the experiments in A. californica were on isolated animals. Had other animals been present, the time devoted to feeding might be increased to values observed previously in A. fasciata. The possible modulation of feeding by pheromones has not been previously examined in A. californica.

To examine the possibility that pheromones secreted by conspecifics might increase feeding in A. californica, animals were maintained either one to an experimental aquarium or two to an aquarium with a partition separating the two animals from one another. Feeding was observed for 6 h, both in steady-state conditions of access to food, as well as in previously hungry animals (Fig. 11). There was no significant difference in the percent time spent feeding between isolated animals and those maintained together in steady-state conditions. However, in previously hungry animals the presence of a conspecific significantly increased the percent time spent feeding over that in an isolated animal. These data indicate that pheromones secreted by conspecifics affect feeding in A. californica but cannot explain the difference in percent feeding in steady-state conditions observed in these experiments and in those on A. fasciata.

We also examined the pattern of feeding in previously hungry animals in the presence and absence of conspecifics. In the absence of conspecifics, the percentage of time feeding was similar to that described previously. During the first half hour after the introduction of food, animals ate ~40% of the time. The time devoted to feeding declined rapidly over the first 2–3 h of access to food. By contrast, in animals maintained with conspecifics the decline in the percent time spent feeding was much slower, and animals had not reached steady-state values by the end of the 6 h observation (Fig. 12). Even during the last 2 h of the observation, animals that were in the presence of conspecifics spent significantly more time feeding than did the isolated animals [P = 0.04, t(19) = 2.11, 2-tailed t-test].

FIG. 11. Effects of conspecifics in the seawater. In animals with steady-state access to food, there was no significant difference in the percent time spent feeding between isolated animals and animals sharing an aquarium with a conspecific [P = 0.11, t(22) = 1.69]. By contrast, in previously hungry animals there was a significant increase in the percent time spent feeding in animals sharing the seawater with a conspecific [P = 0.03, t(18) = 2.42; 2-tailed t-test] when compared with isolated animals.
DISCUSSION

Previous experiments provided expectations on factors controlling feeding in animals with ad libitum food access. Hungry A. californica hand-fed small pieces of food eat ~15% of their body weight. Feeding causes decreases in consummatory response amplitude and increases in latency (Susswein et al. 1976). The size and dynamics of a meal are set by passive fill of the anterior gut (Susswein and Kupfermann 1975a,b). Sensory adaptation also contributes to the initiation and termination of consummatory responses (Horn et al. 2001). In addition, food arousal is initiated by touching food to the lips; arousal decays when animals lose contact with food. Food arousal interacts with satiation: as animals satiate, it becomes more difficult to arouse them, and arousal declines more rapidly (Susswein et al. 1978). It was suggested (Susswein et al. 1978) that the interaction between satiation and arousal could contribute to spacing of feeding bouts. When animals are hand-fed a single daily meal they consume 60% less than when they are hungry, and the anterior gut contents are 60% full at the start of the meal, from food remaining in the gut from previous meals. Patterning of feeding is similar to that in the last 40% of the large meal eaten by hungry animals (Susswein et al. 1976). These experiments suggested that in ad libitum conditions, animals would eat large meals that are terminated when the anterior gut is close to full or as a result of sensory adaptation. During the meals, consummatory feeding responses would become less effective, thereby causing a progressive decrease in food consumption per unit time spent feeding. Meals would be initiated as the gut becomes empty or as sensory adaptation decays.

We found that in ad libitum conditions, feeding is not easily explained by the previously identified factors regulating consummatory responses. First, consummatory responses were minimally regulated. Second, in steady-state conditions, the quantity of food eaten is unrelated to the quantity of material in the anterior gut. Third, a newly identified factor, the presence of food in the environment, is a major variable inhibiting feeding.

Lack of regulation of consummatory responses

Feeding is regulated primarily by regulating interbout intervals rather than by regulating feeding bout lengths. Thus in steady-state conditions, animals eat less than after food deprivation, but bouts are longer. In addition, in previously deprived animals, the time spent feeding decreases over a number of hours, but bout length decreases only during the first half hour.

These findings contrast with data showing that regulation of bout length contributes to learning affecting feeding behavior (Chiel and Susswein 1993; Susswein et al. 1986). Thus Aplysia can regulate bout lengths in other behavioral contexts. Regulation of consummatory behavior amplitude also did not contribute to regulation of the quantity eaten. Consummatory response amplitude is modulated by learning (Chiel and Susswein 1993) and by pheromones (Blumberg and Susswein 1998). In addition, a previous study showed that the amplitude of consummatory movements is decreased as Aplysia satiate (Susswein et al. 1976). It is likely that in our experiments consummatory response amplitude was regulated, but this regulation was difficult to pick up in experiments not explicitly designed to find it.

Regulation of interbout intervals indicates that regulating bout initiation is more important than is the regulating consummatory responses within a bout. However, much more is known about the neural circuitry giving rise to consummatory behaviors, i.e., repetitive biting, swallowing, and rejection (for review, see Elliott and Susswein 2002) than about the neural circuitry influencing the decision to initiate consummatory behaviors. It will be important to gather more information on the neural mechanisms underlying the decision to initiate a bout.

Steady-state conditions

Aplysia with constant access to food are in a novel state in which they eat little. The little eaten is distributed into bouts ~150 s with ~70 min separating bouts.

ENTRY, MAINTENANCE, AND EXIT FROM THE STEADY STATE. In previously hungry animals a 2- to 3-h meal causes animals to enter the steady state. We did not explore which aspects of the meal initiate the steady state. Possible contributors include the presence of food, touch of food to the animals, the performance of feeding behaviors, or postingestion stimuli. However, initiating the steady state is not dependent on the rhinophores because animals without rhinophores enter the steady state as well as do intact animals.

The steady state is maintained by the presence of food, which is sensed by the rhinophores, even if the animals do not contact the food. However, the effect of only 24 h of food in the water was examined. Animals might eventually leave the steady state, even if food is present, if the period without food contact is longer. In addition, the steady state can be maintained in the absence of the rhinophores, indicating that other stimuli can also maintain the steady state.

Animals leave the steady state after a period without food. Three hours without food was sufficient to terminate the steady state when animals had not contacted food for 24 h. However, termination of the steady state is influenced by postingestion stimuli because 3 h without food was not effective in terminating the steady state in animals that had been in contact with food over the previous 24 h. The nature of the postingestion stimuli affecting steady-state termination remains to be determined.

HEMOLYMPH AND NEURAL CORRELATES OF THE STEADY STATE. The steady state is maintained in part via humoral factors. Thus application of the cholinomimetic carbachol onto the cerebral ganglion either failed to elicit buccal motor program, or elic-
cluded fewer programs, with a longer delay, in preparations bathed in hemolymph from animals that had had steady-state access to food with respect to buccal motor program elicited in hemolymph from hungry animals. The identity of the hemolymph factors inhibiting feeding has not been explored. In addition, the mechanism by which stimuli signaling the steady state cause an eventual change in the hemolymph has not been explored. The finding that a hemolymph factor following a meal affects Aplysia feeding behavior is consistent with a previous study suggesting that hemolymph factors cause an increase in heart rate that is correlated with decreased feeding when Aplysia are fed once every 3 h over 3 days (Dieringer et al. 1978).

Other behaviors affected by food. The finding that food stimuli maintain the steady state is reminiscent of a previous finding that steady-state food access inhibits sexual behavior in A. fasciata (Nedvetski et al. 1998; Susswein 1984). However, inhibition of sexual behavior required animals to touch the food occasionally (Nedvetski et al. 1998), presumably because without touch, the animals become adapted to the food. Food may cause continued inhibition of feeding, but not sexual behavior, by activating two sensory pathways with only one displaying adaptation without food contact. Food stimuli could also habituate in motor systems controlling feeding but not in those controlling sexual behaviors. Food could also differentially affect feeding and mating because a hemolymph factor is an intermediary in the inhibition of feeding but not sexual behavior. The hemolymph factor may maintain the block of feeding after adaptation of the receptors responding to food.

Nutritional factors and control of feeding. In the steady state, feeding is not controlled by variables that monitor nutritional state directly or indirectly. Previous studies have shown that Aplysia regulate nutritional variables such as hemolymph glucose concentrations, but these do not affect feeding behavior (Horn et al. 1998). Our data now show that gut fill also does not affect feeding behavior in the steady-state. The dissociation of Aplysia feeding from monitors of nutritional state is superficially surprising. However, this dissociation is seen when Aplysia are in an environment of abundance with food constantly available. In this condition, the small amount of food eaten is presumably adequate to support the metabolic needs. It is unlikely that a 24-h deprivation, which induces a large meal, increases the metabolic costs of the animal so greatly that it must now compensate for the deprivation. The large meal probably reflects a change in strategy in response to a patchy food supply. When food is only sometimes present, a useful strategy may be to feed maximally because the source and time of the next meal is not predictable. Previous studies have shown that in nature, A. californica eat large meals (Kupfermann and Carew 1974). If our interpretation is correct, these were seen in an environment with a patchy food distribution. Natural environments harboring Aplysia are variable; sometimes food is constantly available, at other times it is variably present (Susswein et al. 1984). Previous studies (Susswein et al. 1976) showed that animals hand-fed daily also eat sizeable meals. Our data suggest that these meals provided energy well beyond that needed to support the metabolism. The large daily meals may have arisen from an experimental protocol that maximized the total quantity of food eaten.

It is important to note that the small time investment in feeding was observed in sexually immature animals. Previous studies on ad libitum feeding in sexually mature A. fasciata showed that they budgeted 15–20% of their day to feeding (Susswein et al. 1983). However, sexually mature isolated A. fasciata devote no more than 4% to feeding, similar to our present findings. In A. fasciata, pheromones released by conspecifics and by egg cordons significantly increase feeding both in hungry animals and in animals with steady food access (Ziv et al. 1991a). Our data showed that the presence of conspecifics amplified feeding in previously hungry animals but not in animals in steady-state conditions (Figs. 10 and 11), indicating that the presence of pheromones could not account for the low level of feeding in our experiments. However, it is possible that the pheromones would modulate feeding in steady-state conditions in sexually mature A. californica. Pheromones may increase feeding to compensate for the high metabolic cost of reproductive behaviors, such as mating and egg-laying.

Although the primary function of pheromonal modulation of feeding may be to compensate for the metabolic cost of reproduction, some pheromonal modulation of feeding is also found in immature Aplysia. Thus in the present study pheromones did affect feeding in hungry animals. A previous study on A. fasciata (Schwarz et al. 1998) also found some modulation of feeding by pheromones in sexually immature animals.

Meals in hungry Aplysia

The large meal observed after food deprivation is qualitatively similar to meals observed previously when hungry animals were hand-fed (Susswein et al. 1976). The gradual decrease of feeding during the meal probably arises as a result of stimuli that were previously described as causing satiation, e.g., the gradual fill of the anterior gut, modulated by interruptions caused by sensory adaptation and the build-up and decay of food arousal. However, the effect of these variables on the initiation of a feeding bout is more important than are their previously examined effects on consummatory behaviors within a bout. A number of points are consistent with the hypothesis that the quantity of food eaten, and the pattern of feeding, are determined at least in part by bulk stimuli provided by food in the anterior gut. First, as the period of food deprivation following steady-state access to food increases from 3 to 24 h, animals eat progressively more. In addition, progressively more time is devoted to feeding at the start of the meal as the deprivation period increases. Finally, when feeding is initiated after only 3 h without food in the environment, but 24 h after the last access to edible food, animals eat a meal comparable to that after 24 h without food.

Gut emptying and control of feeding

A. californica hand-fed a single daily meal eat 60% less than animals that are food-deprived for a week (Susswein et al. 1976). The decreased feeding was explained by the anterior gut contents, which were 60% full 24 h after the meal. However, the current experiments found no significant increases in feeding when animals were food deprived for 24 h or for longer periods, suggesting that the anterior gut is empty after 24 h. The difference between the previous and the current data can be explained by the difference in feeding protocol and in the food used. Hand-feeding with an unnaturally attractive food (the

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previous experiments used dried artificial seaweed, whereas the present experiments used a natural food) leads to an unphysiologically large meal, whereas ad libitum feeding on a natural seaweed leads to a large but normal meal. Only the former fills the gut to such a degree that it does not empty within 24 h.

Implications

Previous experiments showed that the circuit underlying consummatory feeding movements can be regulated. Our experiments indicate that little of this regulation is expressed in ad libitum feeding conditions. Thus the neural sites most accessible for studying modulation of feeding give rise to modulation that is minimally expressed in freely behaving animals.

Our experiments also provide evidence for a new state controlling *Aplysia* feeding in which animals are relatively uninterested in food. This state is maintained by food in the environment sensed by the rhinophores. The neural mechanisms underlying the state are in principle accessible to study: by mapping the effects of tonic stimulation of the rhinophores on the neural circuit controlling feeding and by determining the effects of humoral factors initiated by the steady state on the neural circuit controlling feeding and by determining the effects of tonic stimulation of the rhinophores. The neural mechanisms underlying the state are in principle accessible to study: by mapping the effects of tonic stimulation of the rhinophores on the neural circuit controlling feeding and by determining the effects of humoral factors initiated by the steady state on the neural circuitry initiating feeding.

Our experiments showed that it is difficult to extrapolate the behavior of an animal when the animal does what it wants from experiments in the laboratory. This is maintained by food in the environment sensed by the rhinophores. The neural mechanisms underlying the state are in principle accessible to study: by mapping the effects of tonic stimulation of the rhinophores on the neural circuit controlling feeding and by determining the effects of humoral factors initiated by the steady state on the neural circuitry initiating feeding.

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