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ORIGINAL PAPER

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Inhibition of mating in *Aplysia fasciata* by food stimuli in the environment, but not by post-ingestion stimuli causing satiation

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Abstract *Aplysia* mating is inhibited when animals have steady-state access to food. We examined which stimuli provided by food inhibit mating. Pre-feeding animals to satiation caused a decrease in mating immediately after the meal. This effect could be mimicked by placing food into the water for the length of time of a meal just before the animals were allowed to mate, but not by filling the gut with non-nutritive bulk, which inhibits feeding. The presence of food in the water while animals were allowed to mate caused a stronger inhibition of mating. When food was maintained in the water for 24 h, animals adapted to this stimulus, indicating that the maintained presence of food could not alone account for the inhibition of mating in steady-state conditions of access to food. However, food in the water for 24 h, coupled with an occasional touch of food to the lips while animals had access to mates, caused strong inhibition of mating. We tested the hypothesis that food initiates a state of food arousal, which competes with sexual arousal, and thereby inhibits mating. If this hypothesis is correct, gut fill should facilitate mating, by inhibiting food arousal. However, this effect was not observed. The data support the hypothesis that food stimuli directly inhibit sexual arousal, rather than acting via an excitation of food arousal.

Key words *Aplysia* · Mating · Food · Feeding · Competition

Introduction

Mating and feeding in *Aplysia* are regulated in tandem. Some stimulus conditions excite both behaviors, whereas others inhibit both. The previous paper examined the effects of pheromones released into the environment by animals with access to mates, which facilitate both mating (Audesirk 1977) and feeding (Botzer et al. 1991). Tissue homogenates containing the atrial gland, or homologues of the atrial gland have similar effects, as do bag cell peptides that are homologous to peptides expressed in the atrial gland (Blumberg and Susswein 1997; Painter et al. 1989; Susswein and Benny 1985; S. Blumberg et al., unpublished observations). These data suggest that pheromones released by animals with access to mates are likely to be synthesized in tissues homologous with the atrial gland, and are likely to be peptides that are homologous with those expressed in the neuroendocrine bag cells.

The present study examines a stimulus condition that causes inhibition of feeding and mating, namely steady-state access to food. Food initially excites feeding by initiating an arousal state (Kupfermann 1974; Susswein et al. 1978), as well as by acting as a stimulus that initiates feeding (Kupfermann 1974; Rosen et al. 1982). However, subsequent consumption of food leads to a gradual inhibition of feeding as a result of satiation (Kupfermann 1974). Food also inhibits mating, as shown by the observation that *Aplysia* having steady-state access to both food and mates spend less time mating than do food-deprived animals with steady-state access to mates (Susswein 1984).

Many of the inhibitory effects of food on feeding behavior can be mimicked by filling the gut with non-nutritive bulk similar in quantity to that of the food present in the gut after a meal, indicating that satiation is likely to be caused in part by activating gut mechanoreceptors (Susswein and Kupfermann 1975a,b). It was previously suggested that inhibition of mating by food could be caused by the same post-ingestion mechanical stimuli that inhibit feeding (Susswein 1984); however, no experiments were performed to examine this hypothesis. In the present study, we have tried to determine the stimuli provided by access to food that inhibit mating. The inhibition of mating could be caused

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by the animals' sensing the presence of food in the water, by the occasional touch of food to the animals, or by post-ingestional stimuli such as the presence of food in the gut, or of nutrients in the hemolymph. We have found that exposure to food per se, rather than post-ingestion stimuli inhibits mating. Thus, the inhibition of feeding and of mating are caused by different stimuli provided by the food.

Materials and methods

Animals

Experiments were performed during the summers of 1994 and 1995 on *Aplysia fasciata* weighing 150—200 g. The conditions in which animals were maintained are described by Blumberg and Susswein (1997). Animals were food deprived for 1 week prior to use in an experiment, but had access to conspecifics and were able to mate until being transferred to the experimental chambers.

Experimental procedures

Animals were transferred from the storage tanks to the experimental chambers 12-17 h before the start of an experiment. These were 10-1 plastic containers of aerated seawater maintained at 21-22 °C. Unless otherwise noted, three animals were transferred together to an experimental chamber. Initially, the animals were prevented from mating by separating them with plastic mesh partitions that allowed water and substances in solution to flow freely, but which prevented the animals from touching one another. The experiment began by feeding animals, or by applying a procedure that mimicked an aspect of feeding After these procedures, the partitions separating the animals were removed, and animals were permitted to mate. Over the next 4 h. animals were sampled every 5 min to determine whether or not they were mating. As in previous experiments (Susswein 1984, Susswein et al. 1983, 1984: Ziv et al. 1991a,b,c), animals were classified as mating when the head of the animal acting as a male was buried between the parapodia of the female, or when the penis of the male was seen entering between the parapodia of the female. Mating as a male, as a female, or as both simultaneously were each noted as a single instance of mating.

Animals were fed meals to satiation by stimulating the lips with strips of food that were approximately 1 cm wide and 10-15 cm long, and then allowing the animals to consume the strip. All three animals in the chamber were fed simultaneously. Animals were presented with a new strip of food within 1 min of completing a strip. Satiation and cessation of a meal was defined by a period of 5 min without a response to food. The partitions separating the animals were removed after the last of the three animals in the chamber completed its meal.

In some animals, food was placed in the experimental chamber but did not touch the animals. The length of this procedure was matched to the length of time needed to satiate animals. The food (~20 g) was placed into a small mesh cage, which was immersed in the experimental chamber.

In some animals some of the post-ingestive consequences a meal were mimicked by injecting into the gut 15-20 ml of non-nutritive bulk, in accordance with procedures described previously (Susswein and Kupfermann 1975b; Susswein and Markovich 1983). The material used in these experiments was beads of poly-acrylamide (Bio-Gel P-60, medium, 130 ± 40 μm (wet), Bio-Rad Laboratories, Richmond, Calif., USA) that were hydrated by placing them in an excess of seawater for 3-12 h prior to use. The bulk was injected via a 10-ml syringe that was attached to a tube (approximately 2 mm in diameter) that the animals were induced to swallow by first touching the lips with food, and then placing the tube into the mouth when it opened in response to the food. The tube was gently pushed into the gut, while palpating the animal to determine the location of the tip of the tube. When the tip was felt to be close to the back of the crop the contents of the syringe were injected. Animals were induced to swallow the tube twice, so that a sufficient quantity of gel could be injected. In all cases, animals were unwilling to swallow the tube a third time, probably because they were either satiated, or close to satiation.

In some animals, the lips were stimulated with food while animals were permitted access to one another. Food was touched to the lips every 2—3 min and was maintained in contact with the lips until the animal responded with a bite. The food was withdrawn at the bite, preventing the animals from ingesting the food.

Results

Pre-feeding *Aplysia* inhibits mating

It was previously shown that animals with steady-state access to food and mates spend less time mating than do food-deprived animals with constant access to mates (Susswein 1984). The present experiments were designed to determine which stimuli provided by access to food inhibit mating. The previous experiments measured the total time spent mating over a full day of observation. Experiments that examine behavior over a full day are laborious and difficult to perform. In addition, measures of behavior in steady-state conditions do not provide information regarding the dynamics of an effect. For these reasons in the current experiments we examined the effect on mating of introducing a food-related stimulus into the environment.

A preliminary experiment was performed to determine whether pre-feeding animals to satiation inhibits mating. In this experiment nine animals ate a mean of 18 g of seaweed during a meal (excess water was removed before the food was weighed, but the food was still somewhat moist). Immediately after being fed they were allowed access to conspecifics for 4 h, and mating during this period was noted. Mating over 4 h was also measured in control animals that were not pre-fed. For analysis, the 4-h experiment was divided into four periods of 1 h each (Fig. 1). A two-way analysis of variance was used to determine whether mating was affected by the pre-feeding, by the hourly intervals, and by the interaction between these variables.

Animals that were pre-fed to satiation spent significantly less time mating than did hungry controls ($P = 0.02$, $F = 5.42$, $df = 1,88$). There was also a significant increase in mating over the 4 h of observation ($P = 0.003$, $F = 4.94$, $df = 3,88$), with no significant interaction between these two variables ($P = 0.72$, $F = 0.44$, $df = 3,88$; two-way analysis of variance with repeated measures). These data extend the previous finding that steady-state access to food inhibits mating, by showing that pre-feeding animals to satiation inhibits mating after the meal.

Pre-exposure to food inhibits mating

Inhibition of mating as a result of pre-feeding animals to satiation could arise from post-ingestive stimuli similar

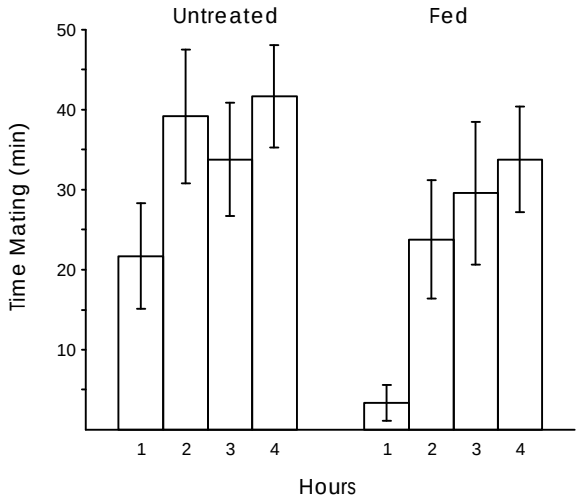

Fig. 1 Pre-feeding animals to satiation inhibits mating. One group of animals *(Untreated)* was not treated in any way, whereas a second group *(Fed)* was fed to satiation. Immediately after the meal both groups were allowed to mate, and the time spent mating was measured over the next 4 h. There was a significant decrease in mating in fed animals

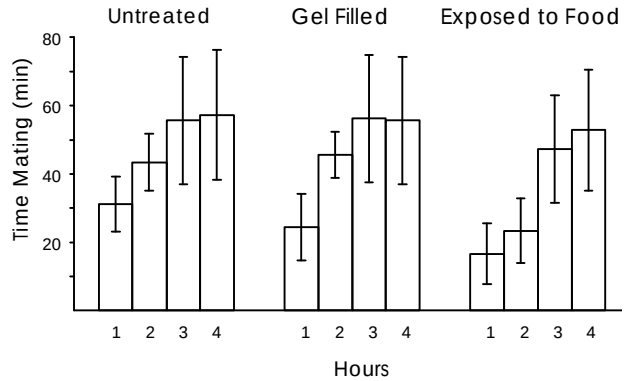

Fig. 2 Pre-exposure to food inhibits mating. One group of animals (*Untreated*) was not treated prior to being allowed to mate. In a second group (*Gel Filled*) satiation of feeding was mimicked by filling the gut with non-nutritive bulk (polyacrylamide gel), and after this procedure the animals were exposed to conspecifics and allowed to mate In the third group (*Exposed to Food*), food was present in the environment for a period of time equal to a meal, and the food was then withdrawn immediately prior to exposure to conspecifics. Of more interest was that the gel fill did not significantly affect mating, whereas there was a significant decrease in mating seen in animals that had been exposed to food

to those inhibiting feeding (Susswein and Kupfermann 1975a). Previous studies have shown that satiation can be mimicked by filling the gut with non-nutritive bulk similar in quantity to the food that is present in the gut after a meal (Susswein and Kupfermann 1975b). If similar stimuli inhibit feeding and mating, injecting non-nutritive bulk into the gut before exposing animals to conspecifics should significantly reduce the time spent mating. An alternative possibility is that the exposure to food per se inhibits mating. If this hypothesis is correct, simply placing food into the water for a period comparable to that needed to satiate animals should inhibit subsequent mating. We determined which of these two possibilities explains the inhibition of mating (Fig. 2). In one group of *Aplysia* (gel-filled) the gut was filled with non-nutritive bulk (beads of polyacrylamide gel) in a procedure similar to that which was shown previously to inhibit feeding (Susswein and Kupfermann 1975b; Susswein and Markovich 1983). In a second group of animals (exposed to food) food was placed in the cage along with the animals. Food was in the cage for 3 h. The food was placed behind a mesh partition, allowing the animals to smell the food but not to touch it. Animals were then allowed access to conspecifics for 4 h, and the time spent mating every hour was measured. A third group of animals was exposed to conspecifics without being pre-exposed to either of these procedures (untreated). *A* two-way analysis of variance tested for possible differences between these groups over the 4 h.

There was a significant difference between the three groups in the time spent mating *(P* = 0.024; *F = 3.85; df* = 2,96). A post hoc test (Duncan's Multiple Range Test) showed that animals pre-exposed to food mated significantly less than did the other two groups *(P* < 0.01), whereas there was no significant difference between untreated and gel-filled animals *(P*> 0.10). As in the previous experiment, there was also a significant increase in mating over the 4 h of observation *(P* < 0.001, *F* = 14.69, *df* = 3,96), and there was no significant interaction between the different pre-treatments and the hour that mating was measured *(P* = 0.80, *F = 0.50, df* = 6,96; two-way analysis of variance with repeated measures). These data show that the inhibition of mating caused by pre-feeding animals to satiation can be attributed to the effects caused by exposure to the food, with no additional inhibition of mating caused by the post-ingestional mechanical stimuli that inhibit feeding.

The lack of inhibition of mating seen in animals that were gel filled was somewhat surprising, since it was previously suggested (Susswein 1984) that inhibition of feeding and mating are caused by common stimuli, and filling the gut with gel was shown previously to inhibit feeding (Susswein and Kupfermann 1975b). To be certain that gel fills identical to those used to test mating indeed inhibit feeding, we re-examined this point (Fig. 3). Six animals were filled with gel in a procedure identical to that used above to examine mating. An additional six control animals were induced to swallow a cannula, and seawater was injected into the gut. Previous data (Susswein and Kupfermann 1975b) have shown that injecting seawater does not induce satiation, since this procedure preferentially fills areas of the gut not involved in satiation. After these procedures, the latency to respond to food was measured. In addition, the number of bites elicited by stimulating the lips with food for *5* min was also measured. In gel-filled animals, the latency to respond to food was significantly in-

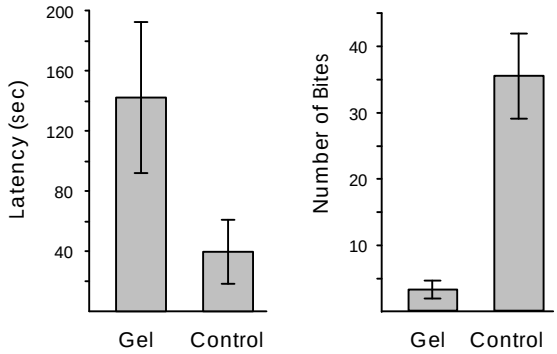

Fig. 3 Gel fill inhibits feeding. In six animals gel was injected into the gut (*Gel*), whereas an additional six control animals were induced to swallow a cannula. but gel was not injected. The latency to respond to food was significantly increased, and the number of bites induced in 5 min of food stimulation was significantly decreased, in the gel-filled animals

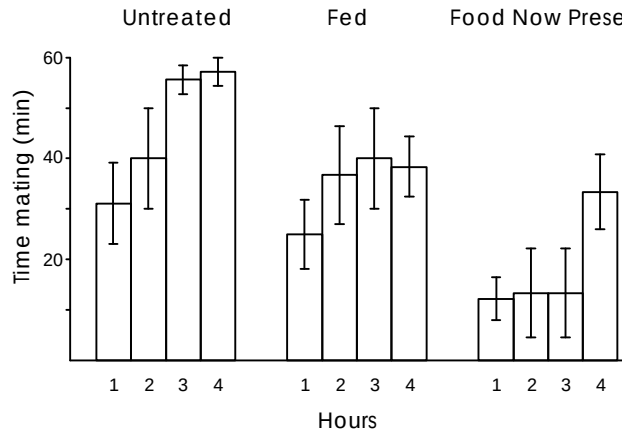

Fig. 4 Simultaneous exposure to food and mates inhibits mating. One group of animals (*Untreated*) was not treated prior to being allowed to mate. A second group (*Fed*) was fed to satiation before being exposed to conspecifics and allowed to mate. In the third group (*Food Now Present*) food was placed in the environment simultaneous with the exposure to conspecifics. Both pre-feeding animals, and th(presence of food inhibited mating. However, the presence of food had a significantly larger effect

creased (P = 0.044, t = 1.9, df = 10, one-tailed t-test), and the number of bites elicited by food was significantly decreased (P < 0.001, t = 5.0, df = 10, one-tailed 1-test). These data confirm that different stimuli inhibit feeding and mating.

Food in the environment inhibits mating

The previous experiment indicated that pre-exposure to food inhibits subsequent mating. A possible explanation of this inhibition is that feeding and mating are competing behaviors, which are rarely performed simultaneously. The presence of food biases animals to try to find food. Although food was no longer present in the water when animals were subsequently exposed to conspecifics and allowed to mate, nutrients that are still in the water may elicit food-seeking behavior which competes with mating. If this interpretation is correct, the presence of food in the water releasing nutrients while the animals are allowed to mate should inhibit mating more strongly than does pre-feeding animals. We ex-examined this possibility.

Two groups of animals were tested (Fig. 4). One group was pre-fed to satiation before being allowed to mate (fed), whereas the second group was exposed to Food placed in the water behind a mesh partition while simultaneously being allowed to mate (food now present). In this group, the food and the potential mates were introduced to the experimental animals simultaneously. Data were also compared to those from animals hat had not been treated prior to being exposed to conspecifics (untreated). Note that the untreated group is identical to that in the previous experiment, as the experiments were performed at the same time. A comparison of the time spent mating in untreated and in pre-fed animals showed a significant decrease in animals that had been pre-fed (P = 0.04, F = 4.20, df = 1.64, two-way analysis of variance with repeated measures), confirming the earlier finding (see Fig. 1) that pre-feeding inhibits mating.

Significantly less time was spent mating in animals that were exposed to food while also allowed to mate, with respect to mating in animals that were pre-fed to satiation (P < 0.004; F = 9.08; df = 1,64, two-way analysis of variance with repeated measures). In this experiment there were no significant differences in mating over the 4 h of observation (P = 0.20, F = 1.60, df = 3,64), and there was no significant interaction between the treatments and the time (P = 0.51, F = 0.77, df = 3,64; two-way analysis of variance with repeated measures). These data showed that the simultaneous exposure to food and to mates inhibits mating more strongly than does pre-feeding. The experiment supports the hypothesis that the inhibition of mating arises from competition between mating and feeding, with food inducing food-seeking behavior at the expense of mating.

Adaptation to food stimuli blocks inhibition of mating

The previous experiment showed that exposure to food stimuli inhibits mating. This effect is sufficient to explain the inhibition of mating caused when animals are pre-fed. However, it is possible that exposure to food is insufficient to explain the inhibition of mating seen in earlier studies (Susswein 1984), in which animals had steady-state access to food. The maintained presence of food stimuli in the water in steady-state conditions is likely to cause adaptation to the stimulus, and adaptation should lead to a decrease in the inhibitory effect of food on mating. To determine the possible food stimuli that inhibit mating when animals have continuous access to food, an additional experiment was performed

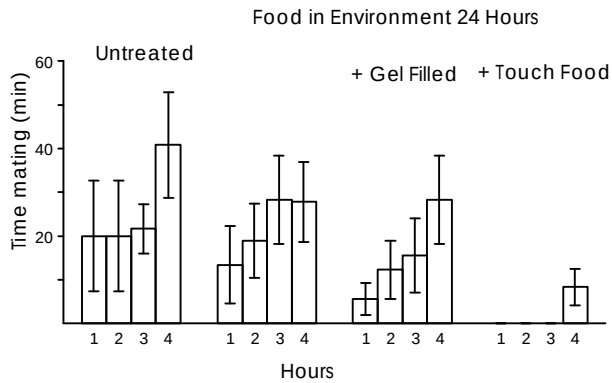

Fig. 5 Adaptation of the effect of food on mating. One group of animals (*Untreated*) was not treated prior to being allowed to mate. In three additional groups (*Food in Environment 24 Hours*). food was placed in the environment 24 h prior to the experiment, and remained in the environment throughout the experiment. In one of these three groups, no additional procedures were performed. In a second group (+ *Gel Filled*) polyacrilamide gel was injected into the gut just before the experiment. In a third group (+ Touch Food) food was periodically touched to the rhinophores and lips during the 4-h experiment. The animals that were touched with food mated significantly less than did untreated animals, whereas the other three groups were not significantly different from one another

(Fig. 5). In this experiment, three groups of animals were maintained in the presence of food for 24 h prior to being exposed to conspecifics, as well as throughout the 4-h period that they were allowed to mate (food in environment 24 h). This treatment would presumably allow animals to adapt to the maintained presence of the food. One group received no additional procedure. In a second of these three groups, the gut was filled with non-nutritive gel (+ gel-filled) just prior to allowing the animals to mate. This procedure mimicked the effects of post-ingestive stimuli that are also present in animals that have steady-state access to food. In a third group the rhinophores and lips of the animals were touched with food approximately every 2-3 min during the period in which they were allowed to mate (+ touch food). Since animals with steady-state access to food are also able to touch the food, this procedure examined the possible effect on mating of steady-state chemostimuli, plus occasional touch of the food. Mating in these three groups was compared to that in a group of hungry animals that had not been pre-exposed to food, and that were not treated with gel or with touch of food (untreated).

There was a significant difference between the four groups in the time spent mating ($P = 0.0002$; $F = 7.10$; $df = 3,116$). A post hoc test (Duncan's multiple range test. $P < 0.05$) showed that animals which were touched with food mated significantly less than did the untreated animals, whereas none of the other groups differed significantly from one another. As in the previous experiments there were significant increases in mating over the 4 h of observation ($P = 0.02$, $F = 3.28$, $df = 3, 116$), and there was no significant interaction between the treatments and the time ($P = 0.97$, $F = 0.31$, $df = 9, 116$; two-way analysis of variance with repeated measures). These data indicate that the inhibition of mating in steady-state access to food cannot be explained by the presence of food in the medium alone, or in combination with post-ingestive mechanical stimuli. The inhibition apparently arises from the repeated contacts with food, which presumably overcome the adaptation and re-initiate food-seeking behavior that competes with mating.

Satiation does not drive mating

Food in the environment excites feeding, whereas gut fill inhibits it. Previous studies have shown that food initiates an arousal state (Kupfermann 1974), and as a result of this state animals locomote and head wave, presumably in an attempt to find food (Kupfermann 1974; Susswein et al. 1978). Gut fill leads to an inhibition of the food arousal (Susswein et al. 1978). These findings suggest that food in the environment could inhibit mating indirectly, via its effect on food arousal, which in turn inhibits sexual arousal. If this hypothesis is correct, one would predict that gut fill could excite mating indirectly, via its inhibitory effect on food arousal (Fig. 6A). However, the data above (Figs. 2, 5) did not show increases in mating as a result of gut fill. It is possible that excitation of mating via gut fill was missed because of a ceiling effect, since sexual arousal was already close to its maximal state. The conditions of the experiment were designed to detect an *inhibition* of

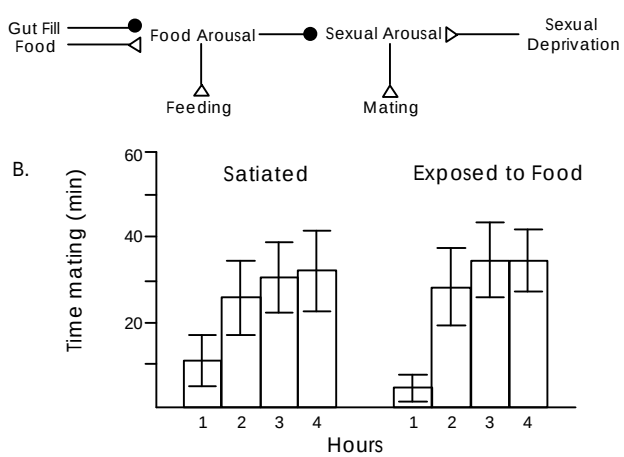

Fig. 6A,B Gut fill does not increase mating. **A** A diagram showing the hypothesis tested (open circles excitation, closed circles inhibition). Feeding and mating are controlled by arousal mechanisms. Food excites, and gut fill inhibits food arousal, which inhibits sexual arousal. The inhibition of mating by food is explained by the effect of food on food arousal This flow diagram predicts that gut fill will facilitate mating, via disinhibition. **B** Satiation did not significantly increase the time spent mating, with respect to that seen in animals that were exposed to food. Thus, we found no evidence that gut fill could facilitate mating via disinhibition

mating, rather than an excitation. For this reason, in the previous experiments animals were deprived of contact with conspecifics for 12—17 h before being tested. Depriving *Aplysia* of mates causes an increase in sexual arousal (Ziv et al. 1989).

An additional experiment was performed to test the possibility that gut fill increases mating under less drastic conditions of sexual deprivation. In this experiment, 20 pairs of animals had access to conspecifics until just before the start of the experiment. In addition, animals were allowed limited access to food (one piece of food measuring ~0.5 cm^2 approximately every 0.5 h) prior to the experiment. At the start of the experiment, each pair was prevented from mating. Half of the pairs were then fed to satiation, whereas in the other half food was placed in the water. When the fed animals stopped eating, the pairs were again allowed to mate. Thus, all of the animals were equally exposed to food, but only half of them were satiated. All incidence of mating were then observed over the next 4 h (Fig. 6B). As in the previous experiments, an analysis of variance was used to determine whether there were differences in mating between satiated and unsatiated animals, and between the 4 h of observation.

There was no significant difference between the time spent mating in satiated and unsatiated animals (P = 0.91, F = 0.01, df = 1,72). As in previous experiments, there were significant differences between the 4 h of observation (P = 0.005, F = 4.61, df = 3,72). There was no significant interaction between these two variables (P = 0.904, F = 0.19, df = 3,72). These data indicate that satiation does not cause an increase in mating, even in conditions in which sexual arousal is relatively depressed (note that controls were stimulated with food, and were not sexually deprived, which would cause a decrease in sexual arousal).

Discussion

We have confirmed and extended a previous finding that feeding inhibits mating in *A. fasciata.* Earlier data (Susswein 1984) showed that animals with steady-state access to food spend less time mating than do food-deprived animals. The previous study did not determine the stimuli provided by food that cause a decrease in mating. However, it was suggested that post-ingestion stimuli which inhibit feeding could also inhibit mating. We have now directly examined which food-related stimuli cause a decrease in mating. Pre-feeding an animal with a single meal to satiation was found to cause a decrease in subsequent mating (Fig. 1). This effect could not be mimicked by filling the gut with non-nutritional bulk (Fig. 2), which mimics the effects of satiation on feeding (Fig. 3; and Susswein and Kupfermann 1975b; Susswein et al. 1983). Thus, a common stimulus does not inhibit both feeding and mating. The decrease in mating could be mimicked by exposing animals to food that was placed in the water along with them (Fig. 2), even if the food did not contact the animals, indicating that distance chemoreceptors sensing the presence of food signal the decrease in mating. This effect was enhanced when the food was present while animals were permitted to mate (Fig. 4). The maintained presence of food in the water led to an adaptation of the effect of food on mating (Fig. 5). This adaptation could be overcome by touching the animal occasionally with food (Fig. 5), suggesting that the decrease in mating that is seen when animals have steady-state food access arises because animals touch the food from time to time.

Arousal and the control of *Aplvsia* behavior

A number of arousal systems affect feeding and sexual behaviors in *Aplysia.*

Food arousal

Food initiates an increase in responsiveness to food which has been termed food-induced arousal (Kupfermann et al. 1991). Arousal is characterized by appetitive behaviors that function in locating the food, and by the facilitation of consummatory feeding responses (Kupfermann 1974; Susswein et al. 1978; Teyke et al. 1990, 1992). Food-induced arousal is modified by the animal's previous feeding history. An increase in hunger amplifies the effect of food arousal, whereas satiation inhibits it (Susswein et al. 1978). Pheromones modify both appetitive and consummatory components of feeding (Blumberg and Susswein 1997; S. Blumberg et al., unpublished observations), as well as exciting the C-PR (T. Teyke and A.J. Susswein, unpublished observations), a neuron thought to command aspects of food-induced arousal, indicating that pheromones act in part by amplifying food-induced arousal.

Sexual arousal

Sexual behavior consists of appetitive behaviors which are followed by consummatory responses, male and female mating. In addition to courtship, which is an appetitive behavior that is specifically related to subsequent mating (Ziv et al. 1989), head-waving and crawling similar in form to those preceding feeding also precede mating (Susswein et al. 1984). Pheromones released during mating and egg-laying facilitate sexual behavior (Audesirk 1977), and presumably increase the level of sexual arousal. An increase in sexual deprivation causes an increase in sexual arousal (Ziv et al. 1989).

Common arousal

Since common stimuli affect feeding and mating, it was suggested that an additional, common arousal system

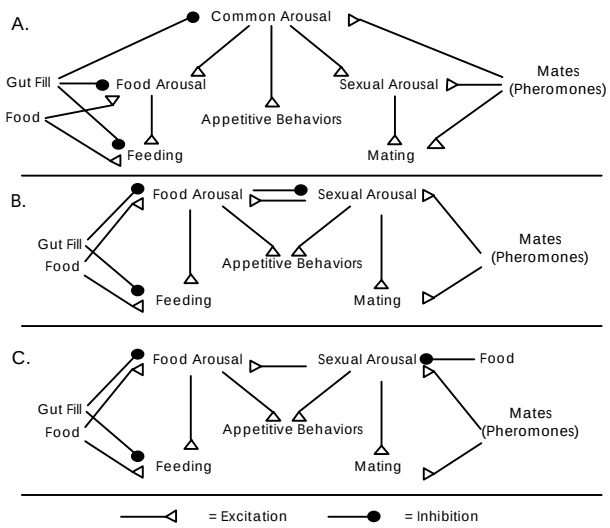

Fig. 7A—C Three models of the interactions between feeding and mating. **A** Interactions between feeding and mating are via a common arousal system affecting both behaviors, whereas lower-level arousal systems have more circumscribed effects on each behavior. **B** The separate arousal systems controlling feeding and mating interact, but have opposite effects on one another. **C** Food directly inhibits sexual arousal. See text for details

affects both behaviors (Botzer et al. 1991; Ziv et al. l991b,c). This hypothesis suggests that an increase in common arousal biases *A. fasciata* to increase the overall time spent feeding and mating (Fig. 7A). The hypothesis is supported by a number of findings: (1) similar appetitive behaviors precede feeding (Kupfermann 1974) and male mating (Susswein et al. 1984; Ziv et al. 1989); (2) animals that are aroused and are about to mate as males immediately feed when stimulated with food without the need to arouse them (Susswein et al. 1984); (3) at times of the year when animals spend large amounts of time on reproductive behaviors there is also an increase in the time spent on feeding (Susswein et al. 1991; Ziv et al. 1991b); (4) a formula that estimated the level of common arousal based the time spent feeding and mating successfully predicted the relative time spent moving in place (Susswein et al. 1991), a second monitor of arousal (Ziv et al. 199 le).

At what level of organization do feeding and mating interact?

Our previous studies showed that access to mates amplifies feeding, whereas access to food decreases mating. We suggested previously that these effects can be explained by the opposite effects of pheromones and of post-ingestive stimuli on the common arousal system (Fig. 7A). Common arousal is increased by the presence of pheromones in the water, in an effect parallel to the effect of pheromones on sexual arousal and on sexual behavior, whereas post-ingestive stimuli signaling satiation inhibit both the common arousal as well as the lower-level feeding arousal, and feeding behavior (Botzer et al. 1991).

We have now found that the inhibition of feeding and of mating arise from different stimuli provided by access to food. The inhibition of feeding is caused by post-ingestive stimuli arising from filling the gut with bulk, whereas the inhibition of mating is in response to chemostimuli released from food that is present in the water, as well as from touch of food to the animals. Thus, a postulated parallel inhibition of food arousal and of common arousal by post-ingestion stimuli cannot account for the inhibition of feeding and mating caused by access to food.

An alternate model of the joint control of feeding and mating (Fig. 7B) does away with the notion of common arousal. This model postulates that both food and sexual arousal produce common appetitive behaviors. In addition, food arousal is excited by food and is inhibited by gut fill, whereas sexual arousal is excited by pheromones. The asymmetry in the effects of food and of pheromones arises from an asymmetric relationship between food arousal and sexual arousal: food arousal inhibits sexual arousal, whereas sexual arousal excites food arousal. The inhibitory effect of food on mating is accounted for by an increase in food arousal, which inhibits mating. The excitatory effect of pheromones on feeding is accounted for by an increase in sexual arousal, which excites food arousal. Excitation of feeding by sexual arousal can account for many of the phenomena previously explained by common arousal, such as the immediate response to food in sexually aroused animals. and the increased feeding seen in seasons characterized by increased mating. However, this model is inconsistent with some of our data. The model predicts that gut fill should cause an increase in mating, via disinhibition, since gut fill inhibits food arousal, which in turn inhibits sexual arousal. We were unable to find such an effect (see Figs. 2, 5, 6), even in animals that were not sexually deprived, thereby decreasing the sexual arousal (Fig. 6).

Our data can be explained by an additional model (Fig. 7C), which is a minor variation of the previous model. In this model, food is postulated to inhibit sexual arousal directly, rather than via its effects on food arousal. In addition, food arousal has no effect on sexual arousal, whereas sexual arousal excites food arousal. The inhibition of mating by the presence of food is accounted for by the direct effect of food on sexual arousal, whereas the excitation of feeding by pheromones and by variables that enhance sexual arousal occurs via the excitatory effect of sexual arousal on food arousal. Support for the direct inhibitory effect of food on sexual arousal, rather than via food arousal, comes from the finding that pre-feeding animals to satiation inhibits mating (Figs. 1, 3), even though food arousal is depressed in these animals.

The finding that food, rather than gut fill, inhibits mating is reminiscent of a previous finding in *Pleuro-*

branchaea, in which food inhibits the righting reflex, independent of the satiation state of the animal (Davis et al. 1977)

Why do pheromones amplify feeding?

It is likely that the primary function of pheromones is to signal the willingness of an animal to mate, and thereby to excite or amplify sexual arousal in adjacent animals, so that mating can then occur. In addition to facilitating sexual behavior, we have shown that pheromones also strongly facilitate feeding behavior (Botzer et al. 1991). To date, we have not addressed ourselves to the adaptive function served by the pheromone effect on feeding, which at first glance seems to be counter-intuitive. The data in this paper suggest an explanation for this effect. Our data have shown that feeding behavior interferes with mating, perhaps because the two behaviors compete for a common block of time. An increase in food arousal at the wrong time of day could seriously impede the efficacy of mating. *Aplysia* often mate in large groups in which animals frequently change sexual roles, and mate with many individuals (Susswein et al. 1984). In addition, animals may spend up to 50 % of their time mating (Susswein et al. 1983). If animals become hungry and begin to eat during the periods of time that should be devoted to mating, the patterns of mating would be severely disrupted. One solution may be for pheromones to amplify feeding, so that in conditions of high sexual arousal feeding becomes more efficient. When feeding is initiated animals will eat vigorously and effectively, and consequently will become thoroughly satiated by the end of a meal. The urge to feed will then be relatively low during much of the rest of the day, which can then be devoted to effective mating. Support for this hypothesis comes from the observation that in natural conditions feeding is highly synchronized among animals, with virtually all of the animals feeding during a 3- to 4-h period during which little mating is seen, with little feeding seen at other times of day (Susswein et al. 1983). By contrast, in the absence of mates feeding occurs in many bouts distributed throughout the day (Ziv et al. 1991 a).

This hypothesis could be tested by preventing animals from feeding to satiation, and causing them to take a number of smaller meals throughout the day. One would predict that this procedure would lead to a decrease in mating, with respect to that seen in animals fed comparable quantities of food during larger meals.

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