

# Social Isolation Blocks the Expression of Memory After Training That a Food Is Inedible in *Aplysia fasciata*

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Isolating a sexually mature *Aplysia fasciata* for either 1 or 24 hr immediately after training that a food is inedible blocks the subsequent expression of memory measured 24 hr later. Isolation that is delayed for 1 hr after training, but not for 12 hr after training, is also effective in blocking memory. Isolation affects memory because of a specific effect caused by the absence of pheromones secreted by conspecifics rather than by a nonspecific change in the chemical environment, because transferring animals to a novel environment (120% seawater) that contains a conspecific does not affect memory. Isolation also does not affect memory in sexually immature *Aplysia*, even though immature animals are able to sense one another's presence. Isolation may affect memory because social (and sexual) isolation is a form of stress in mature *A. fasciata*, and stress after training affects retention in many animals.

Learning and memory can be modified by events that accompany or follow a training experience (Krasne, 1978; Squire, 1987). In humans and animals, emotionally charged or stressful events can either enhance or depress learning and memory (McGaugh, 1989; Schacter, 1996). Learning and memory can also be affected by physiological analogues of stress obtained by the application of hormones or of modulatory transmitters (McGaugh, 1989). A number of different types of stress can modulate learning and memory. In a variety of animals (Arnold & Spear, 1995; De Vaus, Gibbs, & Ng, 1980; Laudien, Freyer, Erb, & Denzer, 1986; Diamond, Fleshner, Ingersoll, & Rose, 1996), including gastropod mollusks (Kemenes & Benjamin, 1994; Schwarz & Susswein, 1992a, 1992b), isolation, or a change to a novel environment, are stimuli causing stress that can modulate learning and memory. This study examines the effects on memory in the gastropod mollusk *Aplysia fasciata* of changing the animal's environment after training from one in which a conspecific is present to one in which the animal is isolated.

Previous data have shown that *A. fasciata* is a highly social animal (Susswein, Gev, Achatuv, & Markovich, 1984). In the field animals are often found together in large groups (Susswein et al., 1984). Adults spend 25 to 50% of their time mating (Susswein, 1984) as males or females, or as both simultaneously (the animals are simultaneous hermaphrodites), with frequent exchange of partners. They are

also often in physical contact with one another, even when not mating (Susswein et al., 1984). An isolated *Aplysia* actively locomotes toward conspecifics (Lederhendler, Herriges, & Tobach, 1977).

The presence of conspecifics is a major modulator of many aspects of behavior in *A. fasciata*. In the absence of conspecifics, animals spend less time exploring the local environment and more time either completely immobile or actively swimming (Ziv, Markovich, Lustig, & Susswein, 1991). Isolated animals also eat less food (Ziv, Botzer, Markovich, & Susswein, 1991) because of a decrease in the likelihood to encounter food and because of an inhibition of feeding behavior after the food is encountered (Blumberg, Haran, Botzer, Susswein, & Teyke, 1998; Blumberg & Susswein, 1998). Isolation also suppresses the ability of *A. fasciata* to learn and remember an associative learning task affecting feeding behavior (Schwarz & Susswein, 1992a, 1992b). In this task, *Aplysia* are fed a palatable food that is physically too tough to swallow. In the presence of conspecifics, animals learn to stop responding to the food (Susswein, Schwarz, & Feldman, 1986) and maintain a decreased responsiveness to the tough food for at least 3 weeks after the training (Schwarz, Feldman, & Susswein, 1991). When animals are maintained in the absence of conspecifics, learning and memory are blocked (Schwarz & Susswein, 1992a, 1992b).

The neural circuitry controlling *Aplysia* feeding has been intensively studied. Many neurons that control or modulate various aspects of feeding have been identified (Hurwitz, Kupfermann, & Susswein, 1997; Hurwitz & Susswein, 1996; Perrins & Weiss, 1996; Plummer & Kirk, 1990; Rosen, Teyke, Miller, Weiss, & Kupfermann, 1991; Susswein & Byrne, 1988; Teyke, Rosen, Weiss, & Kupfermann, 1993; Teyke, Weiss, & Kupfermann, 1990; Weiss, Cohen, & Kupfermann, 1978). One neuron that facilitates feeding, the C-PR, is excited by stimuli that are related to the presence of conspecifics (Teyke & Susswein, 1998). Access to key neurons in this circuit opens the possibility of

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We thank Irving Kupfermann and Aron Weller for comments on the manuscript. This work was supported by a grant from the German–Israeli Foundation for Scientific Research and Development.

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examining the mechanisms underlying how stimuli such as isolation modulate behavior and learning.

The present report examines the effect on memory of transferring *A. fasciata* subsequent to training that a food is inedible from an environment in which conspecifics are present to one in which the animals are isolated. Training animals in the presence of a conspecific and then isolating them for 1 hr was found to block the expression of memory tested after 24 hr. This effect is attributed to the specific absence of pheromones in the environment when animals are isolated rather than to a nonspecific change in the environment. The effect of isolation on memory may be an analogue of the modulation of memory by changes in emotional state that occurs in higher animals.

A preliminary report describing some of these data has appeared (Schwarz & Susswein, 1992a).

## Method

### Animals

*Aplysia fasciata* weighing 10 to 200 g were collected along the Mediterranean coast of Israel. Animals were stored in groups of 5 or 6 in plastic mesh cages immersed in 900-L tanks of aerated, filtered Mediterranean seawater at 17 ° to 18 °C with lighting at a 12:12-hr light—dark cycle. They were fed one to two times weekly with *Ulva lactuca* that was gathered with *A. fasciata* and was stored frozen.

Some experiments were performed on sexually immature animals. In mature *A. fasciata*, the yellow gonad is usually seen through the semitransparent dorsal skin, just underneath the mantle. This tissue was not seen in immature specimens. In addition, the immature animals were kept together for 2 weeks before and 1 week after the experiments. The animals were never observed mating and never laid eggs. By contrast, mature animals spend 25 to 50% of their time mating and laid eggs every few days (Susswein et al., 1984; Ziv, Markovich, et al., 1991).

### Training Procedure

One week before an experiment, animals were transferred to 10-L experimental aquaria that were constantly aerated and maintained at 17 °C. Water was changed daily. The aquaria were divided into two compartments by a partition made of black plastic net. The partition allowed the free diffusion of chemical stimuli dissolved in the water, but prevented animals kept on one side of the partition from physical access to food and mates on the other side. Throughout the week before the experiment, a single experimental animal was kept on one side of the partition, whereas on the other side food (approximately 5 g) and a second *Aplysia* were present. The experimental animal was food deprived during this period.

Because *A. fasciata* are nocturnally active animals (Susswein et al., 1984), experiments were done during the dark portion of the light cycle, as described previously (Susswein, Schwarz, & Feldman, 1986). As in previous studies (Susswein et al., 1986; Schwarz & Susswein, 1986; Schwarz et al., 1991; Schwarz, Markovich, & Susswein, 1988), training began by touching a small piece of *Ulva* wrapped in plastic net to the rhinophores. The animal responded by lifting the head and centering food on the lips. It then bit the food, leading to entry of the food into the buccal cavity. Food in the buccal cavity initiates swallowing responses. However, because netted food physically cannot be swallowed, it becomes

lodged in the buccal cavity, where it produces repetitive failed attempts to swallow. Food eventually is pushed out of the buccal cavity. The netted food continues to stimulate the lips, producing further bites, which again lead to failed swallows. As training proceeds, many responses fail to lead to entry of food into the buccal cavity. When food enters the buccal cavity, it stays within the cavity for progressively shorter periods, eliciting fewer attempted swallowing responses. Finally, the animal stops responding to the netted food. The criterion for cessation of responsiveness was 3 min with only a single feeding response. Biting responses and entry and exit of food into and from the buccal cavity were observed visually, and their occurrences were noted by pressing the appropriate button of a three-button mouse connected to a computer. A program noted the time at which a mouse button was touched. In addition, swallowing responses that were felt by the experimenter as an inward pull on the netted food were also noted. Two parameters of learning and memory were used: (a) the time to stop responding to food; (b) the time spent by food in the buccal cavity at the start of a training or test session. For this purpose, the first 5 min of a session were used. A third parameter measured in previous experiments, the number of attempted swallowing responses during the first 5 min of a session, was measured and examined in some experiments but was not analyzed in detail. Swallows are initiated by food in the mouth (Kupfermann, 1974), and previous studies (as well as the present experiments) showed that this parameter is strongly correlated with the time spent in the mouth (Susswein et al., 1986).

All experiments that tested the effects of isolation on memory used a blind procedure. Control animals were handled the same as the isolated animals, except that they were returned to their original cages, where there were conspecifics. They were then tested along with the previously isolated animals.

### Modulation of Feeding

The possible influence of conspecifics or mating on the total amount of food eaten over a full day was examined in three groups of 4 immature animals, using the same experimental procedures as in previous experiments that examined the effects of pheromones on feeding (Botzer, Blumberg, Ziv, & Susswein, 1991; Ziv, Botzer, et al., 1991). In one group, each animal was housed alone in a 10-L aquarium. In a second group, 4 animals were placed in a 40-L aquarium. Animals were separated from one another by plastic net partitions that prevented contact between animals, but allowed the free diffusion of chemicals in the water. In the third group, a pair of immature animals was separated from one another and from 3 sexually mature animals by plastic net partitions. The mature animals had continuous access to one another and were not fed and, therefore, spent much of their time mating. The immature animals were placed in these environments with an excess of food for 1 day before the start of the experiment. The experiment consisted of weighing the quantity of food eaten every day over 5 days, providing 20 daily measures of feeding in each of the three groups. During these 5 days, the food was replaced every 24 hr. Before being placed in the aquaria, water was squeezed out of the food by hand. The *Ulva* was then blotted twice between two sheets of filter paper for 2 min. Dried seaweed was then weighed on a scale accurate to 0.1 g. The food was weighed again when it was removed after 24 hr, and the difference in weight was used as a measure of the quantity consumed. This value was normalized to animal weight, which was the mean of the animals' weights measured before and after the day's food consumption (the animals were weighed daily).

Results

*Isolation Immediately After Training  
Blocks Expression of Memory*

Animals were trained that a food is inedible in the presence of a conspecific. Immediately after the training, they were transferred to a medium lacking a conspecific, where they remained for 24 hr. At the end of this period, they were transferred back to a medium with a conspecific. Memory was then tested 24 hr after the animals were returned to the medium with a conspecific. Memory was indicated by decreases during the test in the time to stop responding and in the time that food was in the mouth at the start of the test. Controls were trained and rested along with the animals that were isolated. In the isolated animals, no memory was shown, as indicated by a lack of a significant difference between the time to stop responding during the initial training and during the test 24 hr after the end of the isolation ( $p = .35$ ),  $t(6) = 1.01$ . There was also no significant difference in the time that food was in the mouth at the start of the training and testing sessions ( $p = .84$ ),  $t(6) = 0.22$ . By contrast, the control animals showed clear memory, as measured by significant decreases during the test in both the time to stop responding ( $p = .001$ ),  $t(7) = 5.36$ , and in the time that food was in the mouth ( $p = .016$ ),  $t(7) = 3.14$ , all tests are two-tailed paired  $t$  tests, with respect to the

values seen during the initial training. These data indicate that 24 hr of isolation after training blocks the expression of memory measured 24 hr later.

We examined whether testing animals with inedible food 24 hr after the isolation could itself lead to subsequent memory, when animals are tested a second time 24 hr later (48 hr after the isolation). If so, this finding would indicate that the isolation did not cause permanent damage to the animals or block their subsequent ability to learn and remember. Rather, the isolation only blocks memory that would have been caused by the immediately preceding training session. There were significant decreases during the second (48-hr) test in both the time to stop responding to food ( $p = .006$ ),  $t(6) = 4.78$ , and in the time spent in the mouth during the first 5 min ( $p = .008$ ),  $t(6) = 4.53$ . Both tests are two-tailed paired  $t$  tests with Bonferroni correction, with respect to the values seen during the initial training. Values for the second test in control animals were similar to those during the first test. Thus, isolation does not block the subsequent ability of animals to learn or remember after they are restored to an environment with conspecifics.

A second experiment examined whether a briefer isolation can also block memory. In this experiment, animals were isolated from conspecifics for only 1 hr immediately after training rather than for 24 hr (Figure 1B). They were then tested 24 and 48 hr after training, using a blind procedure.

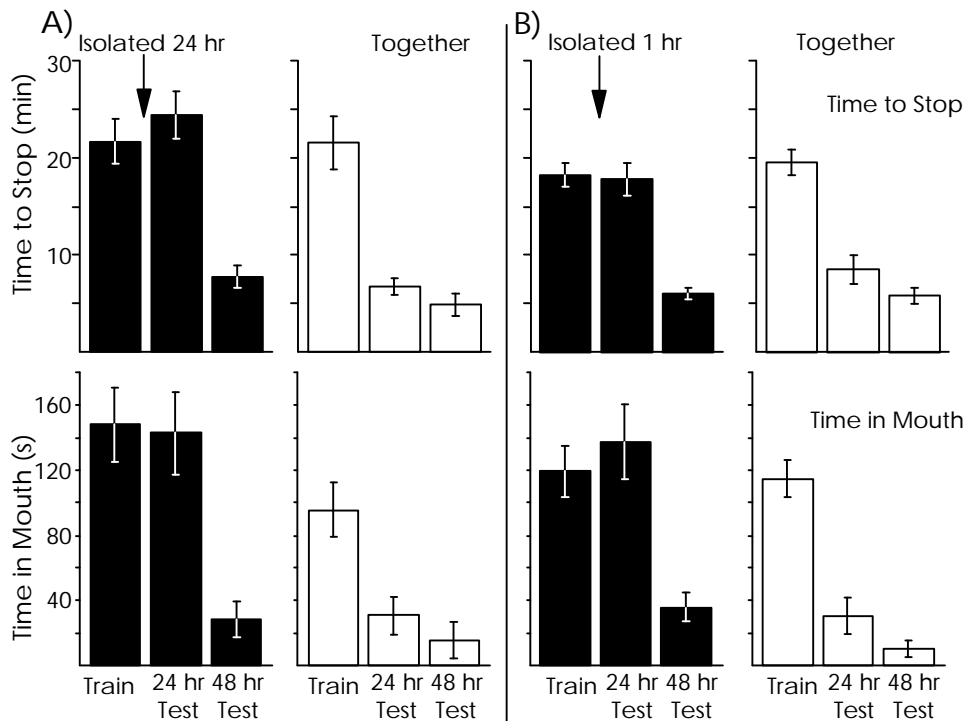


Figure 1. Isolation blocks the expression of memory 24 hr after training. (A) Animals were trained that a food is inedible. Immediately after the training, they were isolated for 24 hr (shaded bars) ( $n = 7$ ). Memory was then tested 24 and 48 hr after animals were restored to the presence of conspecifics. Two parameters were used to determine memory: (a) the time to stop responding to food (upper bars); (b) the time that food was in the mouth during the first 5 min of a training or testing session (lower bars). Means and standard errors are shown. Together (open bars;  $n = 8$ ) are control animals that were handled as were the isolated animals but were in the presence of a conspecific. (B) Procedures and data are as in Part A, except that the isolation was for only 1 hr immediately after training;  $n = 6$  isolated and  $n = 6$  control animals.

The effects caused by only 1 hr of isolation were similar to those caused by 24-hr isolation. There were no significant differences in the isolated animals between the initial training session and the first test for either the time to stop responding ( $p = .85$ ),  $t(5) = 0.20$ , and for the time spent in the mouth during the start of the initial training session ( $p = .38$ ),  $t(5) = 0.94$ . By contrast, there were significant differences in the control animals between the initial training session and the first test for both the time to stop responding ( $p < .001$ ),  $t(5) = 7.96$  and the time spent in the mouth during the start of the session ( $p < .001$ ),  $t(5) = 6.56$ , two-tailed paired  $t$  tests. As in animals that were isolated for 24 hr, during the second test there were significant decreases in both the time to stop responding to food ( $p = .001$ ),  $t(5) = 6.53$  and the time spent in the mouth during the first 5 min ( $p = .014$ ),  $t(5) = 4.02$ , two-tailed paired  $t$  tests with Bonferroni correction, with respect to values during the initial test. As expected, values for the second test in control animals were also reduced from the values in the initial experiment. These data indicate that 1 hr of isolation immediately after training is sufficient to block memory tested 1 hr later.

#### Isolation 1 hr After Training Blocks Expression of Memory

Posttraining treatments that affect memory are generally strongly time dependent. Their effect on memory is usually seen only if the treatment occurs within a narrow time

window after training (Squire, 1987; McGaugh, 1989). We examined whether 1 hr of isolation after training *A fasciata* that a food is inedible would also block memory if the isolation occurred some period of time after the training rather than immediately after the training. In this experiment, the animals were trained as previously mentioned. Either 1 or 12 hr after the training, they were transferred to a chamber lacking a conspecific for 1 hr and were then returned to an environment with a conspecific. They were then tested 24 hr after the initial training using a blind procedure (Figure 2). There were no significant differences between the training and testing sessions for animals isolated 1 hr after training in both the time to stop responding to the food ( $p = .83$ ),  $t(7) = 0.22$ , as well as in the time that food was in the mouth during the first 5 min of a session ( $p = .31$ ),  $t(7) = 1.10$ , two-tailed paired  $t$  tests. Thus, a 1-hr delay in the isolation was still effective in blocking the expression of memory. By contrast, for animals isolated 12 hr after training, there were significant decreases in the test session in the time to stop ( $p = .01$ ),  $t(4) = 4.36$ , as well as in the time that food was in the mouth ( $p = .03$ ),  $t(4) = 3.16$ , two-tailed paired  $t$  tests. Thus, a delay in the isolation of 12 hr no longer causes an effect on memory. Savings were also seen in the control animals that were not isolated (combined data from both control groups), as measured by the time to stop responding to food ( $p = .01$ ),  $t(8) = 3.19$ , as well as the time that food was in the mouth in the first 5 min ( $p = .02$ ),  $t(8) = 2.81$ , two-tailed paired  $t$  tests.

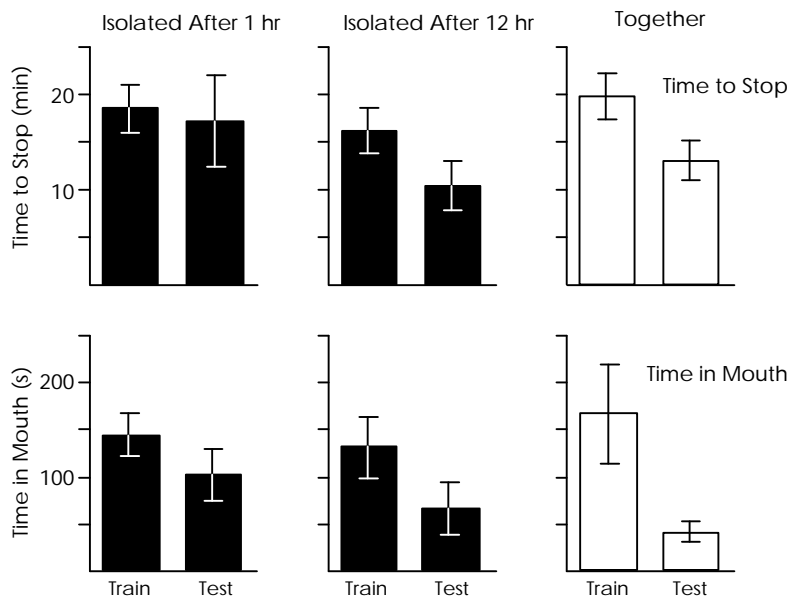


Figure 2. Isolation 1 hr after training blocks memory. In this experiment, animals were isolated for 1 hr either 1 ( $n = 8$ ) or 12 ( $n = 5$ ) hr after the training, and they were then tested 24 hr after the training (shaded bars). Controls (together [open bars]) were handled as were the isolated animals but were always in the presence of a conspecific. Note that the data from the two control groups are combined ( $n = 5$  one hour after training;  $n = 4$  twelve hours after training). Means and standard errors are shown.

*Altered Seawaters Do Not Block Expression of Memory*

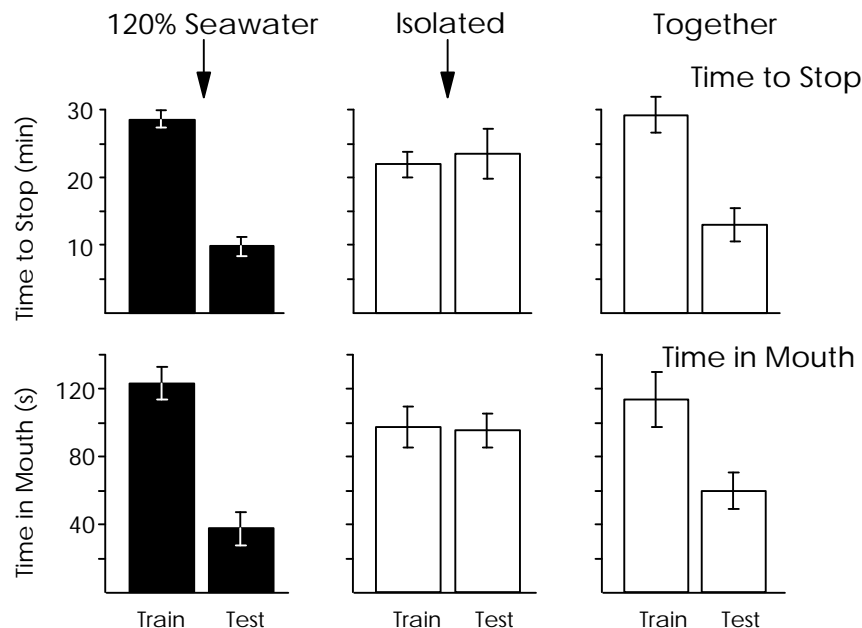
*Aplysia* communicate via pheromones that are sensed by chemoreceptors (Levy, Blumberg, & Susswein, 1997). Isolation changes the chemical environment surrounding animals by placing them in an environment lacking in pheromones. The effect of isolation on memory that was shown previously could be attributed to one of two effects: (a) a specific effect caused by the absence of pheromones that signal the presence of conspecifics; (b) a nonspecific effect caused by any change in the chemical environment. To test these possibilities, we determined whether placing animals immediately after training for 1 hr in an altered chemical environment that nonetheless contains a conspecific (which presumably secretes pheromones) also blocks memory after training. The altered chemical solution used was seawater that had been concentrated to 120% of its initial salinity. Previous studies that examined the effects of altered concentrations of seawater on behavior (Levy, Susswein, & Susswein, 1993) have shown that this stimulus does not elicit overt changes in behavior in naive *A. fasciata*. However, the animals are able to sense the presence of this stimulus, as shown by learned changes in behavior in response to the stimulus after it is paired with a noxious stimulus (Levy, Weller, & Susswein, 1994). Two groups of animals were trained along with those exposed to 120% seawater: (a) isolated: animals were placed in 100% seawater without conspecifics for 1 hr immediately after the training; (b) together: animals were placed in a container of 100% seawater with a conspecific for 1 hr immediately after the

training. Twenty-four hr after the training, animals were tested by determining the time to stop responding to the inedible food and the time that food was in the mouth during the first 5 min. The test was performed using a blind procedure (Figure 3).

In animals that had been exposed to 120% seawater for 1 hr, there were significant decreases during the test in both the time to stop ( $p < .001$ ,  $t(4) = 8.81$ ), and in the time that food was in the mouth at the start of the session ( $p < .001$ ),  $t(4) = 12.97$ , two-tailed paired  $t$  tests, with respect to the values seen during the initial training. By contrast, as was seen previously, in animals that were isolated for 1 hr after the training, there were no significant differences between the training and the test in either the time to stop ( $p = .53$ ),  $t(5) = 0.67$ , or in the time that food spent in the mouth ( $p = .90$ ),  $t(5) = 0.14$ , two-tailed paired  $t$  tests. In animals that were together, significant decreases were seen in both the time to stop ( $p = .004$ ),  $t(4) = 6.00$ , and the time that food was in the mouth ( $p = .004$ ),  $t(4) = 5.96$ , two-tailed paired  $t$  tests. These data show that memory is retained after *Aplysia* are transferred to chemically altered seawater that contains a conspecific.

*Isolation Does Not Block Expression of Memory in Immature Animals*

These data indicate that isolation after training is likely to block the expression of memory as a result of a specific lack of pheromones rather than as a result of a nonspecific change in the chemical environment. If this is so, one might predict that isolation would not block the expression of memory in

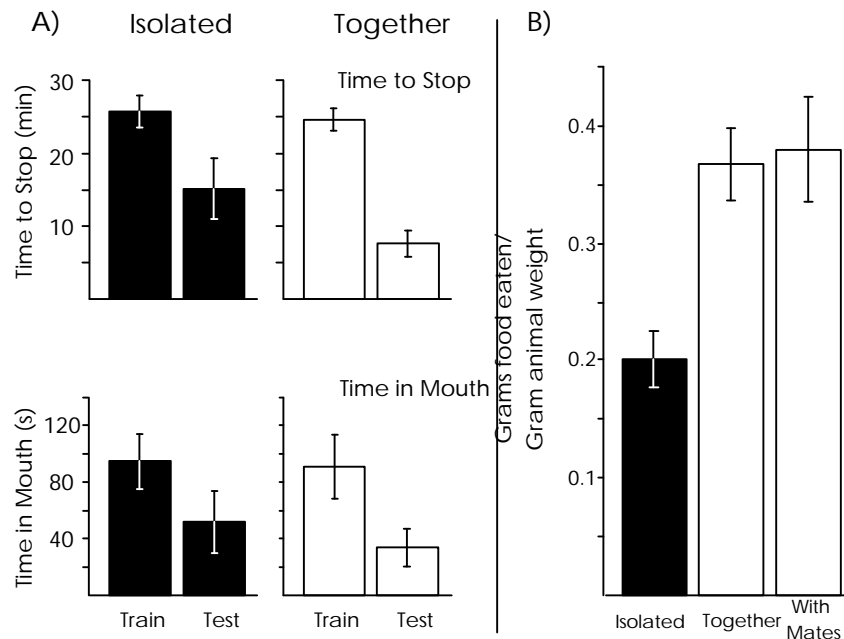


*Figure 3.* Changes in chemical stimuli not signaling isolation do not block memory. Animals were trained that a food is inedible and were then immersed in 120% seawater containing a conspecific for 1 hr ( $n = 5$ ). Memory was rested 24 hr later. Two control groups were run. In one (isolated;  $n = 6$ ), animals were transferred to a medium without a conspecific in 100% seawater for 1 hr after training. In the other (together;  $n = 5$ ), animals were handled as in the other two groups, but a conspecific was always present in 100% seawater. Means and standard errors are shown.

sexually immature animals, in which pheromones are less likely to be important in regulating the animal's behavior. We tested this prediction by repeating these experiments on isolation after training in sexually immature animals (Figure 4A). As in the previous experiments, animals were trained and tested in the presence of a conspecific, which was also sexually immature. One group of immature animals was isolated for 1 hr immediately after training, whereas the control group was handled as were the isolated animals but were always in a medium with another sexually immature animal. There were significant reductions in both the time to stop ( $p = .01$ ),  $t(3) = 5.32$ , and the time spent in the mouth ( $p = .07$ ),  $t(3) = 2.69$ , two-tailed paired  $t$  tests, in the isolated animals. In the controls, the time to stop was significantly reduced during the test ( $p < .001$ ),  $t(4) = 8.27$ , but there was no significant reduction for the time that food was in the mouth ( $p = .12$ ),  $t(4) = 2.00$ , two-tailed paired  $t$  tests. These data indicate that memory is preserved after isolation in immature *Aplysia*.

A possible explanation of the finding that isolating immature animals does not affect the ability to learn and

remember is that these animals do not secrete and are unable to sense pheromones. Thus, immature animals do not sense a change in the chemical environment when they are isolated. We tested this possibility by measuring the quantity of food eaten per day in three conditions: (a) sexually immature animals were kept in isolation; (b) sexually immature animals were kept together; (c) sexually immature animals were kept with mature, mating animals (Figure 4B). There was a significant difference between the quantity of food eaten daily between these three groups,  $F(2, 45) = 9.58$ ,  $p < .001$ ; two-way analysis of variance in which the main effects were the presence vs. absence of other animals and the 5 days over which the animals were measured). There was no significant difference between the 5 days,  $F(4, 45) = 1.61$ ,  $p = .19$ , and no significant interaction,  $F(8, 45) = 1.72$ ,  $p = .12$ . A post hoc test (Student-Newman-Keuls,  $\alpha = .05$ ) showed that the isolated animals ate significantly less than the other two groups, whereas there was no significant difference between the animals that were merely in one another's presence versus those in the presence of mating conspecifics. These data indicate that immature animals



**Figure 4.** (A) Isolation does not block memory in sexually immature *Aplysia*. One group of sexually immature animals was trained in the presence of a conspecific, and then the animals were isolated for 1 hr immediately after training (shaded bars;  $n = 5$ ). Control immature animals (together [open bars]) were handled in the same manner but were always in the presence of conspecifics ( $n = 4$ ). Means and standard errors are shown. Normal memory was shown after isolation in these animals. (B) Isolated immature animals eat less than immature animals that are together. In this experiment, 4 animals were kept with steady-state access to food while isolated (shaded bar). Four additional animals were kept together, and an additional 4 animals were kept in the same medium as mature mating animals (open bars). For each animal, the quantity of food eaten daily was measured over 5 days. The food eaten was normalized to the animal's weight. Isolated animals ate significantly less than animals that are together and animals in the presence of mature mating animals, indicating that immature animals sense one another's presence. However, the increase in feeding caused by mating over that seen when animals are together was not evident, confirming that immature animals do not respond normally to all pheromone-related signals.

release and sense pheromones that affect feeding behavior. However, these pheromones do not have the same effects as in sexually mature animals. The presence of mating does not increase feeding over the level seen in nonmating conspecifics, as it does in mature animals (Botzer et al., 1991).

### Discussion

Our data indicate that isolating a mature *A. fasciata* immediately after training blocks the expression of memory measured 24 hr later (see Figure 1). This is consistent with previous findings that *A. fasciata* are social animals that spend much of their time mating (Susswein et al., 1984), and that many aspects of the animals' behavior are modified when they are isolated (Ziv, Markovich, et al., 1991). Sexual or social isolation may represent a form of stress for *A. fasciata*. In many animals, stress before or after training can be a strong modulator of learning and memory (McGaugh, 1989). Isolation from a permanent mate (Castro & Matt, 1997; Crawley, 1984) or from conspecifics in social animals (Boissy & Le Neindre, 1997) are well-recognized stimuli used to examine the effects of depression or stress on various aspects of behavior and physiology. Isolation during training and testing can also affect learning and memory in day-old chicks (De Vaus et al., 1980) and in goldfish (Laudien et al., 1986). A 3-hr isolation from either parents or siblings after training also disrupts retention in 18-day-old rat pups (Arnold & Spear, 1995).

The effects of isolation on *A. fasciata* behavior are consistent with the hypothesis that isolation causes a change in animal state. When isolated, the animals' behavior has many of the properties characteristic of depression or stress in higher animals. In the presence of conspecifics, *A. fasciata* spend much of their time performing appetitive movements (crawling and head waving) that allow them to explore the local environment and find stimuli such as food and potential mates. Additional blocks of time are spent feeding and mating. By contrast, isolated animals spend much of the day immobile and much of the night swimming (Ziv, Lustig, Ben-Zion, & Susswein, 1991). The latter is a form of locomotion that allows the animal to cover large distances fairly rapidly and is likely to represent an attempt to escape from a hostile environment. Isolation also causes a large decrease in the percentage of time spent feeding (Ziv, Botzer, et al., 1991). Finally, maintaining the animal in isolation during and after training and testing that a food is inedible prevents the animal from learning (Schwarz & Susswein, 1992b).

In mammals, transfer to a new environment can act as a stressful stimulus that affects the expression of memory (Diamond et al., 1996). Transferring the animal to a novel chemical environment also modifies the ability to learn and remember a feeding-related task in the gastropod mollusk *Lymnaea* (Kemenes & Benjamin, 1994). We examined the possibility that the effect of isolation could be attributed to a change in the environment rather than being caused by a specific effect resulting from the lack of a conspecific. Two

experiments argued against this possibility. In one, the chemical environment was changed by immersing animals in 120% seawater containing a conspecific. The animals are able to sense 120% seawater, as shown by their ability to use this stimulus as a conditioned stimulus (CS) for learned changes in respiratory pumping (Levy et al., 1994). Before conditioning, 120% seawater elicits no changes in respiratory pumping or other behaviors (Levy et al., 1993). Immersing animals in this solution in the presence of a conspecific did not affect memory after training (see Figure 3). In a second experiment, the effect of isolation was examined in sexually immature animals. Isolation did not affect memory consolidation in these animals (see Figure 4A). A possible explanation of this finding is that immature animals do not secrete and are unable to sense pheromones. However, additional evidence indicates that pheromones can affect other aspects of behavior in sexually immature animals, indicating that they can sense the change in the environment caused by isolation. First, such animals are often found contiguous with larger mating animals (Susswein et al., 1984), suggesting that pheromones secreted by adults attract immature animals. Second, sexually immature animals that are kept together in the same aquarium eat more over a full day than do immature animals that are isolated (see Figure 4B), suggesting that immature animals are able to sense one another's presence. The lack of effect of isolation on memory in immature animals indicates that isolation may be less stressful than in sexually mature individuals. In mature animals, the absence of pheromones may be stressful because of the strong drive to mate in the near future. This drive is unlikely to be present in immature animals. Further evidence supporting the contention that immature animals are less sensitive than adults to sexual signals from other animals comes from the finding that the presence of mature, mating animals did not increase feeding over that seen when immature animals were together (see Figure 4B). In mature *A. fasciata*, the presence of mating conspecifics amplifies feeding over that seen when animals are together (Blumberg et al., in press; Botzer et al., 1991).

Isolation in immature mammals can affect memory (Arnold & Spear, 1995), whereas isolation in immature *Aplysia* did not affect memory. This difference probably arises because even mammals that lead relatively solitary lives as adults have strong social contacts with their mother and siblings while young, whereas *A. fasciata* are strongly social only when they are sexually mature.

Differences in feeding in the presence and absence of a conspecific are unlikely to be caused by factors such as the ability of *Aplysia* to see one another or by stimuli such as vibrations caused by the movement of the other animal. First, the eyes of *Aplysia* are unlikely to be useful in resolving clear images of features in the environment (Kandel, 1979). In addition, the partitions dividing an experimental animal from a conspecific were made from a black plastic mesh that largely prevented animals from seeing across the partition. Finally, the experimental cages were constantly aerated, thereby creating vibrations and

water movements that were at least as large as those created by movements of the conspecific.

Previous studies have shown that *Aplysia* are able to discern one another by secreting and sensing pheromones (Audesirk, 1977). Pheromones allow the animals to detect one another, even when they are not in contact (Ziv, Botzer, et al., 1991). Pheromones secreted during mating as well as those deposited on egg cordons have additional effects (Botzer et al., 1991; Painter, Gustavson, Kalman, Nagle, & Blankenship, 1989). The pheromones secreted as a result of mating and egg laying are sensed by the rhinophores (Blumberg & Susswein, 1998; Levy et al., 1997). The rhinophores could also sense the pheromones that affect learning and memory. However, additional chemoreceptors on the lips, tentacles, and osphradium are also present (Xin, Weiss, & Kupfermann, 1996). These could also have a role in sensing the presence of conspecifics and regulating learning and memory. Future studies are required to determine this point.

The effects of pheromones secreted during mating on feeding behavior can be partially mimicked by placing into the water egg-laying hormone (ELH) and other peptides that are naturally synthesized in the neuroendocrine bag cells (Blumberg et al., 1998; Blumberg & Susswein, 1998). These peptides are homologues of those synthesized in and released from atrial gland tissues that are embedded in the walls of the common hermaphroditic duct (Nagle, Painter, & Blankenship, 1989). Considerable indirect evidence suggests that atrial gland peptides serve as pheromones regulating sexual behavior and interanimal communication (Blumberg et al., 1998; Blumberg & Susswein, 1998; Painter et al., 1989; Susswein & Benny, 1985). Studies have also identified a novel peptide, termed attractin, that is synthesized in the albumen gland and is secreted onto egg cordons and which attracts conspecifics (Fan, Wu, Nagle, & Painter, 1997; Painter, Clough, Fan, & Nagle, 1996). It will be of interest to determine whether placing atrial or albumen gland tissue extracts or peptides into the water overcomes the effects of isolation, so that memory is maintained in the presence of extracts or peptides even in the absence of a conspecific.

A single training session such as that used in the present experiments elicits short-term memory that is evident for less than 1 hr after training and long-term memory that is expressed only 12 to 24 hr after training. From 1 to 12 hr after training, neither short-term nor long-term memory is expressed (Botzer, Markovich, & Susswein, in press). During this period, short-term memory has decayed, whereas long-term memory has not yet been fully consolidated. The expression of long-term, but not short-term, memory can be blocked by cooling animals to ~20° C for 15 min immediately after the training (Botzer et al., in press). Our experiments have shown that isolation blocks the expression of long-term memory, but we have not yet examined the possible effect of isolation or of a change in the environment on short-term memory. We have also not examined whether posttraining experiences such as isolation or cooling directly interfere with the formation of long-term memory or modu-

late memory retrieval processes. Nonetheless, our data are consistent with the notion that the formation of long-term memory is dependent on a gradual consolidation process, which can be interrupted even a considerable time after training. However, in many animals, posttraining experiences affect memory only when presented within one or a few minutes after training (e.g., Hammer & Menzel, 1995; Yamada, Sekiguchi, Suzuki, & Mizukami, 1992), suggesting that memory consolidation may be relatively slow in our learning task.

In mammals, the expression of memory can be modified by the central or peripheral effects of a variety of modulatory neurotransmitters (McGaugh, 1989). Many of the same substances (e.g., dopamine, serotonin, acetylcholine; see Susswein, Rosen, Gapon, & Kupfermann, 1996; Teyke et al., 1993; Weiss et al., 1978) also modulate the neural circuitry controlling *Aplysia* feeding, and it is possible that these transmitters have a role in modulating the ability to learn and remember in this circuit.

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Received September 4, 1997

Revision received December 4, 1997

Accepted January 20, 1998 .

