Social Learning of Food Preferences in Rodents: Rapid Appetitive Learning

BASIC PROTOCOL

For the neuroscientist studying the consequences of manipulation of specific regions of the brain on learning in rodents, the social transmission of food preference provides a reliable, efficient method for rapidly inducing a learned appetitive behavior in rats, mice, or gerbils. The procedure consists of three stages (Fig. 8.5D.1). First, "demonstrator" rats, mice, or gerbils are given one of two distinctively scented foods to eat. Each demonstrator is then permitted to interact briefly with a conspecific "observer." During this period of interaction between demonstrator and observer, observers have the opportunity to smell the scented food on the breath of their respective demonstrators. After a delay which can last minutes to weeks, each observer is given a choice between the two distinctively scented foods, one of which was eaten by its demonstrator. Observers show an enhanced preference for whichever of the two scented foods their respective demonstrators ate.

Retention of such socially induced changes in the food preferences of Norway rats is sensitive to effects of hippocampal damage (Winocur, 1990; Bunsey and Eichenbaum, 1995) and hormonal state (Fleming et al., 1994). CREB mutant mice show a deficit in long-term retention of such socially-learned food preferences (Kogan et al., 1996), and galanin transgenic mice are significantly impaired on the task (Steiner et al., 2001).

The major advantages of the procedure are that: (1) learning is rapid, (2) little effort or skill on the part of the experimenter is needed to train subjects, (3) large numbers of trained subjects can be produced at relatively little cost, (4) learning is appetitively (rather than aversively) motivated, so subjects need never be deprived or subjected to stress of any kind, and (5) the learning has ecological validity. The procedure has been in use for many years, and there is an extensive literature describing both causes and consequences of social effects on food preference in rodents (reviewed in Galef, 1988, 1996).

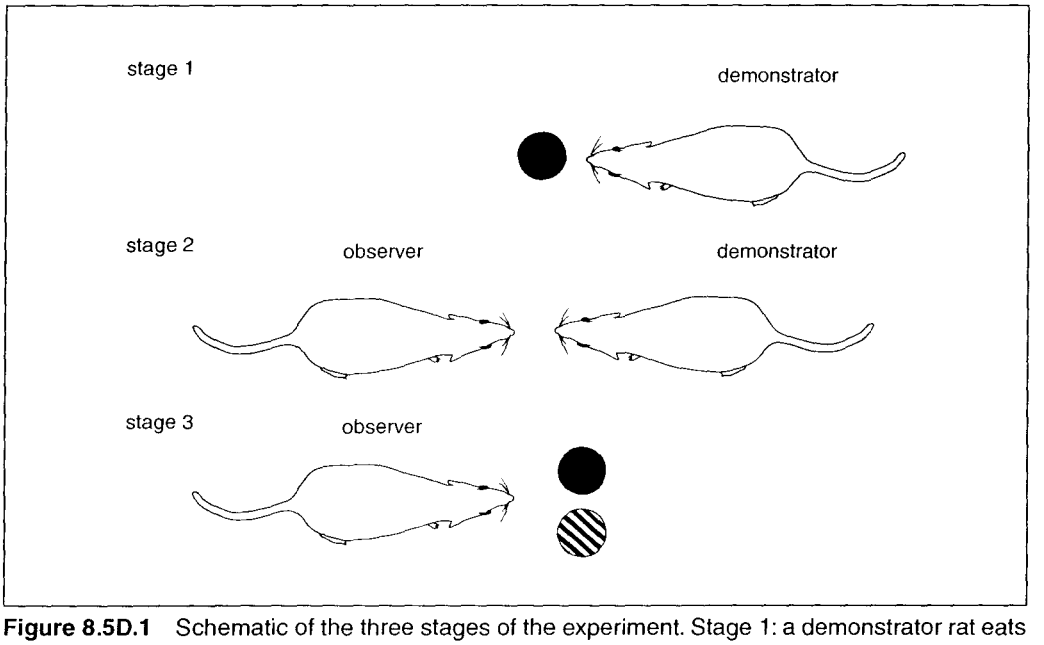

Figure 8.5D.1 Schematic of the three stages of the experiment. Stage 1: a demonstrator rat eats one of two distinctively flavored diets (represented by the circles with the two different shadings). Stage 2: The demonstrator interacts with an experimentally naive observer rat. Stage 3: The observer chooses between the diet its demonstrator ate and a second distinctively flavored diet. Best results are obtained if half the demonstrators are fed each distinctively flavored diet during stage 1.

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Current Protocols in Neuroscience (2002) 8.5D.1-8.5D.8
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Social induction of food preference has been most extensively studied in young (6- to 8-week-old) female Norway rats, so a procedure appropriate for use with such subjects is described here. However, with relatively minor modifications (see Commentary), the procedure is also suitable for use with adult rats, house mice (*Mus musculus*; Valsecchi and Galef, 1989), and Mongolian gerbils (*Meriones unguiculatus*; Galef et al., 1998; Valsecchi et al., 1996). The author has not had success with golden hamsters (*Mesocricetus auratus*).

A wide variety of diets (liquid, solid, or mash) can be used in the procedure (see Commentary). However, the procedure gives strongest results when the two distinctively scented diets used in an experiment are roughly equipalatable (Galef and Whiskin, 1998). Because different samples of natural ingredients (such as cocoa and cinnamon) can vary considerably in palatability to rats, before undertaking a series of experiments, use extra animals from the colony to assess the relative palatability of diets. Social induction of food preferences can be demonstrated even when there is a marked difference in the palatability of diets offered to observers by allowing each observer to interact repeatedly with demonstrators fed the less palatable diet (Galef, 1989).

Materials

Ground cinnamon
Unsweetened powdered cocoa
Powdered rat chow (PMI Rodent Diet 5001, available from W.F. Fisher & Son) and rat chow in pellet form
Rats (preferably young female Norway rats 6 to 8 weeks of age)
Granulated sugar

Air-tight food containers
Top-loading scale accurate to 0.1 g
Individual cages (preferably hanging cages with screen floors) suitable for rats
Water bottles
Food cups (200-ml, 2-in. deep stainless steel demi-moon feed and water cups; Lenderking Metal Products) and hardware for attaching them to cage walls
Felt-tipped pens

Prepare diets and determine whether a pair of diets are roughly equipalatable

1. In separate air-tight containers, place sufficient ground cinnamon and unsweetened powdered cocoa to last several months, assuming that each demonstrator and observer rat will eat ~30 g of each food mixture (cinnamon- and cocoa-flavored; see step 2) on each day that it is in the experiment. Homogenize the contents of each container thoroughly and keep refrigerated at 4°C.

   When kept refrigerated in sealed containers, diet ingredients can be used for at least 3 months.

2. Prepare 500 g each of cinnamon- and cocoa-flavored diet.

   a. To make cinnamon-flavored diet mix 5 g ground cinnamon with 495 g powdered rat chow (1% w/w).

   b. To make cocoa-flavored diet mix 10 g unsweetened powdered cocoa with 490 g powdered rat chow (2% w/w).

3. Place six rats in individual cages each equipped with a water bottle.

   Hanging cages with screen floors are preferable to cages with solid floors covered with bedding because (1) hanging cages do not permit animals to hoard food, and (2) spillage is easily detected on paper towels placed under hanging cages beneath food cups.

4. Half fill six food cups with cinnamon-flavored diet and six food cups with cocoa-flavored diet. Place at least 30 g of food in each cup, so that even if a subject eats from only one cup, it will have sufficient food.

   Each cup should be only half-filled, to reduce the frequency of spillage.

5. Label the food cups in a location that rats will not be able to reach when the cups are in the cage.

6. Weigh the food cups and record the weights.

7. Place a pair of food cups, one containing cinnamon-flavored and the other containing cocoa-flavored diet, in the cage of each rat.

8. Place paper towels under each hanging cage beneath both food cups.

   Paper towels make it easy to detect excessive spillage, should it occur.

9. Leave the six subjects undisturbed for 24 hr.

10. At the end of the 24-hr choice test, reweigh food cups.

11. Calculate the amount of each diet eaten by each rat and the percentage of each subject's total 24-hr intake that was either cinnamon- or cocoa-flavored diet.

12a. *If the mean percent intake by subjects of each of the two diets was roughly 50% (in the range of 43% to 57%):* Proceed to the main experiment (step 13).

12b. *If the mean percent intake of either of the two diets is outside the range of 43% to 57%:* Add some granulated sugar (start with 1% w/w) to the less preferred diet and test again using the same or different subjects until approximate equipalatibility of diets is achieved.

   Fresh equipalatable cinnamon- and cocoa-flavored diets should be prepared daily for the main experiment according to the formula determined in this step.

Establish subjects in cages for the main experiment

13. Place 12 "demonstrator" and 12 "observer" rats in individual hanging cages with screen floors. Provide ad libitum access to water

14. Mark the tail of each demonstrator rat with a permanent marker.

   Marking is necessary to distinguish demonstrators from observers after they have interacted.

Train demonstrators

15. Place the demonstrators on a 23 hr/day schedule of food deprivation. Give each demonstrator rat a weighed cup half-filled with plain powdered rat chow for 1 hr/day for two successive days, introducing the food cup into each demonstrator's cage at the same hour on each day.

16. At the end of each feeding period, give each demonstrator one pellet of rat chow for overnight.

17. While training demonstrators, give each observer rat ad libitum access to pellets of rat chow.

Induce food preferences in observers

18. Deprive each demonstrator rat of food for a third 23-hr period. At the end of this period, place a labeled, weighed food cup half-filled with freshly prepared cinnamon-flavored diet in the cage of each of six demonstrators and a labeled, weighed food

cup half-filled with freshly prepared cocoa-flavored diet into the cage of each of the remaining six demonstrators.

Label each food cup using a felt-tip pen with a demonstrator number (1 to 12) in a location that will be inaccessible to the demonstrator when the food cup is in the cage.

19. Leave demonstrators undisturbed to eat for 1 hr.
20. Remove and weigh each food cup to determine how much each demonstrator ate.

    Demonstrators that have eaten less than 3 g of flavored diet should not be used, as they may provide insufficient signals to observers.

21. Immediately after removing food cups from the cages of demonstrators, place a demonstrator rat into the cage of each observer rat.
22. Leave demonstrator and observer rats undisturbed for 30 min.
23. Remove each demonstrator rat from the cage of its observer, and return each demonstrator to its home cage.

    Delays from minutes to a month can be interposed between the interaction of demonstrators and observers and testing of observers. If the delay is more than 23 hr, observers should be fed rat chow in their home cages.

    To reduce expense, demonstrators may be kept on their food-deprivation schedule and used again with a new cohort of observers.

Test observers

24. Half fill 12 food cups with fresh cinnamon-flavored diet and 12 food cups with fresh cocoa-flavored diet, using at least 30 g of each diet.
25. Using a felt-tip marker, label each food cup with both a subject number (1 to 12) and the flavor of diet placed in the cup. Weigh each of the food cups and record the weight.
26. For 24 hr, offer each observer a choice between two weighed food cups, one containing a sample of cinnamon-flavored and the other cocoa-flavored diet. To control for position effects, counterbalance across observers the positions in each cage of the food cups containing cinnamon-and cocoa-flavored diet.

    For example, food cups containing cinnamon-flavored diet should be closer to the front (or right side) of half the cages used to test subjects and closer to the back (or left side) of the remaining cages.

27. At the end of the 24-hr choice period, weigh the two food cups from each observer's cage, and record the weights.
28. Calculate either the percentage of each observer's total intake that was either cinnamon- or cocoa-flavored diet or the percentage of each observer's total intake that was the diet eaten by each observer's demonstrator.

    The latter measure is particularly useful for comparisons between conditions.

COMMENTARY

Background Information

Procedures such as that described in this unit have been in use for 20 years (Galef and Wigmore, 1983; Strupp and Levitsky, 1984), and social induction of food preferences has been reported dozens of times from many laboratories. Many variables have been tested to determine whether they affect the outcome of the procedure, but almost invariably, if demonstrators and observers are given an opportunity to interact within an hour of demonstrators having ingested a flavored substance, observers will

show a significant enhancement of their preferences for foods that their respective demonstrators have eaten. Below, a number of variations on the basic experiment are discussed that have little effect on its outcome.

Flavorants

Over the years, several different flavorant pairs (in addition to cinnamon and cocoa) have been successfully used in the basic procedure. These include the following (Galef et al., 1984, 1990a):

Solids:

2% (w/w) ground marjoram and 1% (w/w) ground anise

0.4% (w/w) ground cloves and 0.5% (w/w) ground cumin

0.5% (w/w) ground rosemary and 0.5% (w/w) ground cardamom

Liquids:

2.1% (w/v) instant, decaffeinated coffee and 3.2% (v/v) cider vinegar.

Critical Parameters

Socially induced food preferences have been observed in wild and domesticated Norway rats (*Rattus norvegicus*), as well as in young and old rats, male and female rats, food-deprived and replete rats, and protein-deprived and replete rats. The phenomenon has also been observed in mice and gerbils. The authors have used anesthetized demonstrators (Fig. 8.5D.2; Galef and Wigmore, 1983), demonstrators experiencing gastrointestinal distress (Galef et al., 1990b), demonstrators that were familiar and unfamiliar to their observers, and demonstrators genetically related and unrelated to their observers (Galef et al., 1998). Other experimental scenarios have included allowing observers and demonstrators to interact in one anothers' home cages, as well as in neutral arenas. Demonstrators have also been separated from observers by hardware-cloth screens while they interacted. None of these variations in procedure have made much difference. In every case, so long as observers interacted with their respective demonstrators within several hours of the demonstrators having eaten a distinctively flavored, unfamiliar food (Galef and Kennett, 1985), observer rats exhibited enhanced preferences for the food eaten by their respective demonstrators. The robustness of the phenomenon permits investigators considerable latitude in design of experiments.

Different animals, however, require slightly different procedures. Because the social induction of food preference depends on an observer acquiring olfactory information by sniffing near the mouth of its demonstrator (Galef et al., 1985; Galef and Stein, 1985), interactions between demonstrators and observers that reduce the probability of observers coming close to the mouths or noses of their demonstrators also reduce the probability of successful social induction of food preference. For example, adult male Norway rats that are unfamiliar with one another are likely to interact aggressively when first placed together in a small cage. If such animals are used as demonstrators and observers, they have to be separated by a 1.25-cm screen partition while they interact (Galef and Wigmore, 1983; Galef et al., 1998). Because mice eat only small amounts of food in 24 hr and tend to spill powdered food, special feeding dishes are needed to accurately measure their food intake (Valsecchi and Galef, 1989).

More complex procedures

Observer rats are able to socially learn preferences for two or more diets simultaneously. For example, observer rats offered a choice between cinnamon- and cocoa-flavored diets or between marjoram- and anise-flavored diets will eat more of the former member of each diet pair after interacting with demonstrators fed both cinnamon- and marjoram-flavored diets than after interacting with demonstrators fed both cocoa- and anise-flavored diet (Galef et al., 1990a).

It is also possible for demonstrators to socially induce preferences for two different diets in a single observer in successive weeks, and evidence of both induced preferences can be observed a month after the induction of each (B.G. Galef, unpub. observ.). Consequently, studies in which rats socially learn two flavor preferences, one before and one after an intervention, should be possible.

Troubleshooting

Social induction of food preference has not been detected in observers when there is a marked discrepancy in the palatability of the diets offered for choice during testing of observers (Galef and Whiskin, 1998). Such a problem is obvious because, during testing of observers, all observers eat far more of one diet than the other, regardless of which diet their respective demonstrators ate. The anticipated result has also not been observed when ob-

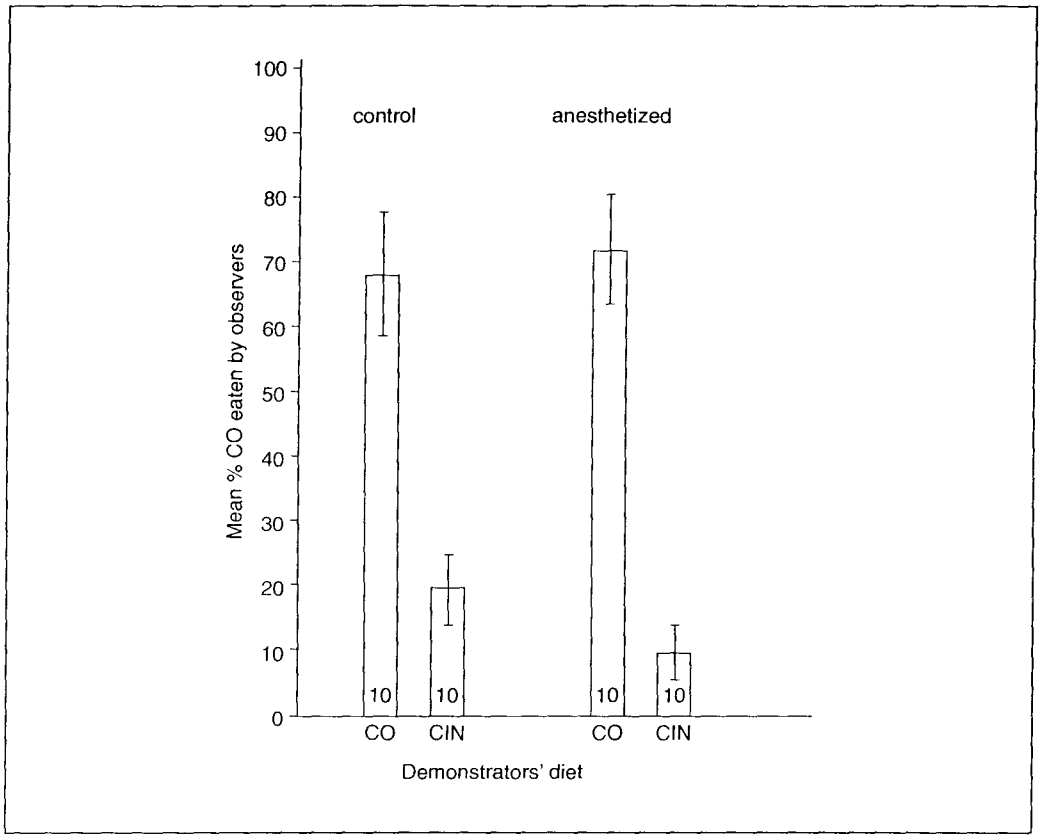

Figure 8.5D.2 Amount of cocoa-flavored diet (CO), as a percentage of total intake, eaten by observer rats that interacted during stage 2 (See Fig. 8.5D.1) with a demonstrator rat fed either CO or cinnamon-flavored diet (CIN) in stage 1. The figure illustrates the impact on observers' food choices during stage 3 of interaction with demonstrators either anesthetized by intraperitoneal injection or injected with saline just before stage 2. Numbers within the bars represent *n* per group. Reprinted from Galef and Wigmore (1983) with permission.

servers have been suffering from upper respiratory infections that interfered with their ability to detect the distinctive scent of the food that their respective demonstrators had eaten.

If there is (or has been) sneezing among the animals in the colony room in which subjects are kept, and there is failure of social induction of food preference, flavor-aversion conditioning (*UNIT 8.6E*) can be used to determine whether observers are suffering an olfactory deficit that makes it impossible for them to distinguish the odors of a pair of foods. This is done as follows:

1. Place six subjects on a 1-hr/day feeding schedule;
2. Feed three subjects cocoa-flavored diet and three subjects cinnamon-flavored diet for 1 hr;
3. Inject all six subjects intraperitoneally with 1% of body weight 13 mM lithium chloride solution (see *UNIT 8.6E*);
4. The next day offer all six subjects a choice between cinnamon- and cocoa-flavored diets. If subjects fail to avoid the food they ate before being poisoned with lithium, then they are unable to discriminate between the foods offered to them, and olfactory deficits are probably responsible for the failure to see an effect of demonstrators on observers' food preferences.

Anticipated Results

After a single interaction with a demonstrator, healthy rats will show a significantly enhanced preference for the diets that their respective demonstrators have eaten (Fig. 8.5D.2). Consequently, if some observers have interacted with a demonstrator fed cinnamon-flavored diet and other observers have interacted with a demonstrator fed cocoa-flavored diet, when offered a choice between cinnamon and cocoa-flavored diets, observers that interacted with demonstrators fed cinnamon-flavored diet will eat a greater percentage of cinnamon-flavored diet (and a smaller percentage of cocoa-flavored diet) than observers that interacted with a demonstrator fed cocoa-flavored diet (Galef and Wigmore, 1983). Significant effects of demonstrators on observers' food choices (even replete observers) can be seen as soon as

2 to 4 hr after the observers are given a choice of foods to eat. If testing of observers is delayed for hours, days, or even weeks after interaction with demonstrators, there is little diminution of the effect (Galef and Whiskin, in press).

Once testing starts, and the observers have been offered two diets to eat, the duration of the effect is influenced by the number of hours per day that observers have the choice between diets available. The more hours per day that observers have to sample two diets, the fewer the days that socially induced preferences last (Galef and Whiskin, 2001). Socially induced enhanced preferences can be maintained for weeks, even when observers are exposed to the choice between diets 24 hr/day, if observers are given repeated exposures to a demonstrator (or demonstrators) that has eaten one of the diets (Galef, 1989; Galef and Whiskin, 2001).

Time Considerations

Once a pair of roughly equipalatable diets have been identified, a single technician working, 2 to 3 hr per day, can produce evidence of socially induced diet preferences in a cohort of 24 observers in 4 days.

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Demonstrates that changes in rats' diet preferences require simultaneous exposure to both the odor of a diet and carbon disulfide, a normal part of rat breath.

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