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Failure to find aversive marking of toxic foods by Norway rats

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A series of five experiments were undertaken to determine whether Norway rats, *Rattus norvegicus*, that had learned to avoid eating an unfamiliar food (demonstrators) would mark the food (or its surroundings) in a way that reduced the probability that naïve conspecifics would eat the marked food. We found no evidence that demonstrators aversively marked foods that they had learned to avoid. To the contrary, naïve subjects ate more in areas soiled by demonstrators that had learned to avoid a food located there than they ate in unsoiled areas. Furthermore, when naïve rats were given a choice between two samples of an unfamiliar food, one in an area soiled by demonstrators that had learned to avoid the food, the other in an area soiled by demonstrators that had not learned to avoid the food, the naïve rats ate an equal amount in both areas. The data indicate that, as in social learning about food at a distance from a feeding site, residual cues deposited by rats around feeding sites directly affect where or what conspecifics eat, not where or what they avoid eating. We discuss possible ultimate explanations for this failure of naïve rats to learn socially to avoid foods in areas that conspecifics soil after becoming ill.

At least seven different kinds of social interaction affect choice of either foods or feeding sites by young Norway rats, *Rattus norvegicus*. (1) The milk of a lactating rat contains flavours of foods that she has eaten, and while weaning, pups show enhanced preferences for the flavours of foods that they experienced in maternal milk (Galef & Henderson 1972; Galef & Clark 1972; Galef & Sherry 1973; Martin & Alberts 1979). (2) At weaning, rat pups use visual cues to locate adult conspecifics feeding near their nest site, approach those adults and take their first meals of solid food when and where adults are eating (Galef & Clark 1971). (3) Young rats follow adults from their nest to feeding sites and ingest the foods at these sites (Galef et al. 1987). (4) While feeding, adult rats deposit as yet undefined residual cues both at feeding sites (Galef & Heiber 1976) and on foods (Beck & Galef 1989), and young rats prefer marked to unmarked locations. (5) When returning to a harbourage site from a feeding site, adult rats leave trails that juveniles follow to the feeding sites that adults are exploiting (Galef & Buckley 1996). (6) Young rats snatch food from tolerant elders and subsequently show an enhanced preference for the stolen food, but do not show a similar preference for the same food when they obtain it from inanimate substrate (Galef et al. 2001). (7) A naïve rat (an observer) that interacts briefly with a conspecific that has recently eaten a distinctively flavoured food (a demonstrator), subsequently shows an enhanced preference for whatever food its demonstrator ate (reviewed in Galef 1996). Last, circumstantial evidence suggests that flavours from foods a rat dam is eating may enter her amniotic fluid, and fetal exposure to such cues may enhance pups' preferences at weaning for the flavours of foods experienced in utero (Smotherman 1982; Hepper 1988).

The interaction between demonstrator rats and their observers while at a distance from a feeding site has been the subject of more intense study than any of the other processes involved in social learning about foods because it is both the most powerful and most sophisticated of known social influences on rats' food choices. Effects of demonstrator rats on their observers' food preferences are found under a wide range of experimental conditions (Galef et al. 1984) and last for weeks (Galef 1989; Galef & Whiskin 2003).

Common sense suggests that, after an observer rat interacts with a seriously ill demonstrator, the observer should show an aversion to (not a preference for) the foods that its demonstrator has eaten. However, the reverse is the case. Observer rats acquire enhanced preferences for (not aversions to) foods eaten by sick or unconscious demonstrators, and these preferences are at least as strong as those acquired for foods eaten by healthy

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demonstrators (Galef et al. 1983, 1990, 1999; Grover et al. 1988). Thus, the enhanced appetence of observer rats for foods eaten by demonstrators can have a potentially maladaptive consequence, increasing naïve rats' probability of ingesting toxic foods.

The failure of rats to communicate food avoidance to conspecifics is particularly surprising given that a wide range of species, from Pharaoh's ants, *Monomorium pharaonis* (Robinson et al. 2005), to red-winged blackbirds, *Agelaius phoeniceus* (Mason & Reidinger 1982), produce signals that cause conspecifics to avoid foods or feeding sites. Pharaoh's ants produce pheromones that repel nestmates from entering unrewarding routes, and blackbirds avoid coloured containers that they have seen others feed from before becoming ill.

The surprising failure of observer Norway rats to use information extracted from ill demonstrators to avoid ingesting the foods that those demonstrators have eaten has yet to be fully explained (but see Tuci et al. 2001). Here, we considered the possibility that rats have not evolved behavioural mechanisms that allow them to learn to avoid illness-inducing foods eaten by conspecifics with whom they interact because, in natural circumstances, socially communicated information at a feeding site that induces avoidance of noxious potential foods is more reliable than that obtained by interaction with a conspecific at a distance from a feeding site. In particular, we explore the possibility that, like Pharaoh's ants, Norway rats sent-mark unrewarding sites in such a way as to dissuade conspecifics from exploiting them.

A potential demonstrator rat may be ill for reasons unrelated to its ingestive history. It may have parasites in its gastrointestinal tract or have contracted an infectious virus causing gastrointestinal distress (Galef 1991). If ingestion of toxins is a relatively rare cause of illness, then information that a demonstrator rat is ill would not reliably indicate that it had ingested a toxic substance. Consequently, avoiding foods eaten by ill demonstrators might entail lost-opportunity costs outweighing the potential benefits of doing so.

Steiniger (1950), who was the first to provide evidence (albeit anecdotal) of social influences on the food choices of Norway rats, attributed young rats' avoidance of poison baits to their parents' marking, with urine and faeces, of bait that they had learned to avoid, thus making it unattractive to their offspring. If, as Steiniger (1950) suggested, rats mark poison baits so as to make them unattractive to others, there would be little need for observer rats to have evolved an ability to learn to avoid foods eaten by sick demonstrator rats after interacting with them at a distance from a feeding site. Information acquired from a sick demonstrator rat, at a distance from a feeding site, would be not only redundant with, but also less reliable than information available directly on a potential food that an experienced rat had eaten, learned to avoid and marked with a warning substance of some kind.

Results of previous studies have shown that adult rats soil both foods and feeding sites where they find safe foods, thus making them more attractive to young conspecifics than either unmarked foods or feeding sites (Galef & Heiber 1976; Galef & Beck 1990). The finding of increased attractiveness of foods soiled by rats indicates that urine and faeces do not, as Steiniger (1950) hypothesized, cause rats to reject foods. However, it remains possible that rats that have learned to avoid a food will mark that food or its surroundings in some special way, making a food or feeding site relatively unacceptable to naïve conspecifics.

We undertook the present series of experiments to examine the possibility that rats will mark a food that they have learned is toxic so as to increase the probability that naïve conspecifics will avoid it. In experiments 1–4, we examined the hypothesis that rats will aversively mark a food that they have learned to avoid wherever they encounter it. In experiment 5, we determined whether rats that had learned to avoid a food would aversively mark that food in the place where they first ate it.

EXPERIMENT 1: POISONED DEMONSTRATORS

In experiment 1, we made demonstrator rats ill by injecting them with a toxin immediately after they had eaten an unfamiliar food, thus training them to refuse to eat that food (Garcia & Koelling 1966). To permit demonstrator rats to mark the food that they had been trained to avoid, we confined two of them for 24 h with the food that they had learned to avoid in half of a large enclosure. The half of the enclosure from which demonstrators were excluded contained a feeding site identical to the one to which the demonstrators had access.

At the end of the 24-h period, we removed the demonstrators and the partition dividing the enclosure. We then introduced a naïve rat into the enclosure, and let it feed, for 24 h, from the two food bowls containing the food the demonstrators had learned to avoid, one in the soiled section of the enclosure, the other in the unsoiled area. To provide a baseline to examine effects of illness on any residual cues left in a cage by demonstrator rats, we also examined the effect on naïve rats' choice of feeding site of residual cues deposited by healthy demonstrators treated identically to the poisoned demonstrators described above, but injected with saline solution instead of a toxin.

In a second study, we directly compared the effects of residual cues deposited by healthy and ill demonstrators on the food choices of naïve rats by confining a pair of ill demonstrators on one side of the partition and a pair of healthy demonstrators on the other before allowing a naïve rat to choose between feeding sites.

Methods

Subjects

Twenty-eight experimentally naïve, 8-week-old, female Long–Evans rats obtained from Charles River Canada (St Constant, Quebec) served as subjects. Twenty served as subjects in study 1, 10 randomly assigned to the experimental condition and 10 to the control condition. The remaining eight naïve rats served as subjects in study 2 (see Procedure).

Seventy-two additional rats that had participated in other experiments, but that had no experience of the food that they were to eat in the present experiment, served here, in pairs, as demonstrators. We randomly assigned half the demonstrators and half the subjects to experimental and control conditions.

In previous similar studies of influences of residual cues on feeding-site preferences conducted in our laboratory (Galef & Heiber 1976), we used individual rats rather than pairs of rats as demonstrators and found that juveniles preferred marked sites to unmarked ones. In pilot experiments conducted in preparation for the present series of experiments, we found that, although naïve adult subjects preferred feeding sites marked by a single, unpoisoned demonstrator to unmarked feeding sites, the effect was not robust, and we had to collect data from an inordinate number of subjects to achieve statistical significance. In our earlier studies (Galef & Heiber 1976), we had used lactating female rats as demonstrators for young. Such females eat far more, and produce far more faeces (Galef & Muskus 1979), than do the virgin females that we intended to use as demonstrators in the present experiments. To increase the amount of scent deposited during 24 h, we used pairs of demonstrators in the present studies.

Apparatus

We housed all demonstrators individually in stainless-steel, hanging cages, measuring 35 × 18 × 21 cm (Wahmann Co., Baltimore, Maryland, U.S.A.), while we trained 36 of them to avoid an unfamiliar food.

During preparation of enclosures by demonstrators and testing of subjects, all animals resided in test enclosures, measuring 1 × 1 × 0.3 m, constructed of hardware cloth, angle iron and galvanized sheet metal. Each enclosure contained a water bottle, painted wooden nestbox and two ceramic food bowls (15.0 cm in diameter and 6 cm deep). The floor of the enclosure was covered to a depth of 2–3 cm with wood-chip bedding (Aspen shavings, Northeast products Co., Warrensburg, New York, U.S.A.), and the enclosure was divided into two equal parts, each measuring 0.5 × 1 × 0.3 m, by a galvanized sheet-metal partition (Fig. 1a, b).

Diets

We used two diets in the experiment: (1) powdered rat chow (Harlan-Teklad Rodent Laboratory Chow 8640, Madison, Wisconsin, U.S.A.; diet 8640) and (2) a palatable sucrose and coconut-oil-based diet (Harlan-Teklad Protein-free Basal Mix, catalogue number TD 86146) to which we added 17.5% high-protein casein (Harlan-Teklad catalogue number 160030) to create a protein-sufficient diet (diet PS). A food containing 12% protein by weight is considered adequate for young rats (Canadian Council on Animal Care 1980).

Procedure

Training demonstrators. We first placed each demonstrator rat, housed in an individual hanging cage, on a 23-h schedule of food deprivation, eating diet 8640 for 1 h/day for 2 consecutive days. Following a third 23-h period of food deprivation, we weighed each rat and provided it with a weighed sample of diet PS for 1 h. At the end of this 1-h feeding period, we injected the 36 demonstrators assigned to the experimental condition intraperitoneally with a 0.13-M lithium-chloride (LiCl) solution equivalent to 1.5% of the subject's body weight. When we injected demonstrators assigned to the experimental condition with lithium-chloride, we injected the 36 demonstrators assigned to the control condition with an equivalent amount of isotonic saline.

Preparing enclosures

Immediately following injection, we placed a food bowl containing diet PS on each side of the partition. In study 1, we placed a pair of poisoned (experimental condition) or a pair of saline-injected (control condition) demonstrators on one side of each partitioned test enclosure (Fig. 1a), counterbalancing across subjects the side on which demonstrators were placed. In study 2, we placed a pair of poisoned demonstrators on one side of each partitioned test enclosure and a pair of saline-injected demonstrators on the other (Fig. 1b), counterbalancing across subjects the side on which demonstrators of the two types were placed. Demonstrators then remained undisturbed for 24 h with access to a weighed sample of diet PS in a ceramic food bowl.

Testing naïve subjects

At the end of the 24-h occupancy of test enclosures by demonstrators, we (1) removed all demonstrators and the partition from each enclosure, (2) weighed the food bowls on both sides of each enclosure after ensuring that the unsoiled food bowl contained an amount of food roughly equivalent to that in the soiled food bowl, and (3) placed a single naïve subject in each enclosure. Twenty-four hours later, we weighed both food bowls and determined the amount of diet PS that subjects had eaten from each.

Cleaning

After each naïve subject was tested, we removed all bedding from the enclosure, and washed the enclosure with detergent at high temperature in a commercial cage washer.

Ethical note

The procedures used in these experiments were approved by the McMaster University Animal Research Ethics Board in June 2001 and June 2004 (Animal Utilization Proposals 01-06-28 and 04-06-28).

Results and Discussion

Demonstrators poisoned before being placed in an enclosure sometimes spilled food offered to them in the large enclosures, so it was not possible to be sure that they ate none of it. However, LiCl-injected pairs of demonstrators that did remove any food from the bowl available to

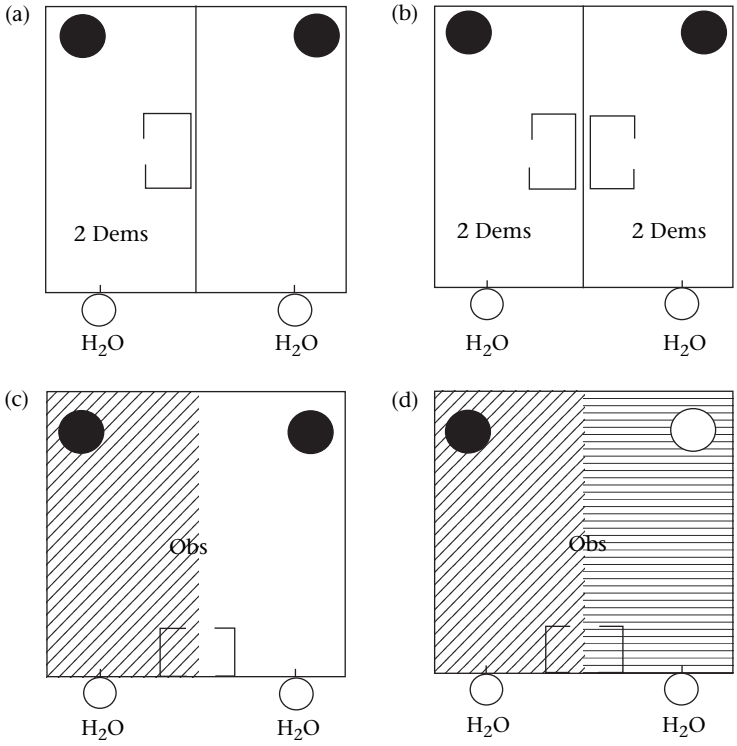

Figure 1. Overhead schematic of an enclosure configured as: (a) in experiment 1, study 1, (b) in experiment 1, study 2 for preparing enclosures, (c) in experiments 1, 2 and 3 for testing some naïve subjects, (d) in experiments 4 and 5 for testing observers. Dems = demonstrators; Obs = observer. Filled circles = food unfamiliar to observer. Open circles = diet 8640. Diagonal hatching = area soiled by ill demonstrators. Horizontal hatching = area soiled by healthy demonstrators.

them ($N = 4$) removed 1–6 g, far less than the 32 g or more that saline-injected pairs of demonstrators removed from the food bowl available to them.

Study 1

As we have found previously (Galef & Heiber 1976; Galef & Muskus 1979), subjects assigned to the control condition (those choosing between a food bowl in an area previously occupied by healthy demonstrators and a food bowl in a previously unoccupied area) preferred to eat from the food bowl in the soiled area (one-sample t test: $t_9 = 2.47$, $P < 0.04$; Fig. 2). Similarly, subjects assigned to the experimental condition that chose between a bowl of diet PS in an area soiled by a pair of demonstrators that we had trained to avoid ingesting diet PS and a food bowl containing diet PS in a previously unoccupied area ate significantly more from the food bowl in the soiled area than from the food bowl in the unsoiled area ($t_9 = 2.43$, $P < 0.04$; Fig. 2). The percentage of the total amount eaten taken from the soiled area did not differ as a function of whether subjects' demonstrators were poisoned or unpoisoned (Student's t test: $t_{18} = 0.40$, $P = 0.70$; Fig. 2).

Study 2

Naïve subjects offered a choice between food bowls in areas previously occupied by poisoned and unpoisoned demonstrators showed no preference for either feeding site ($t_7 = 0.15$, $P = 0.44$; Fig. 2).

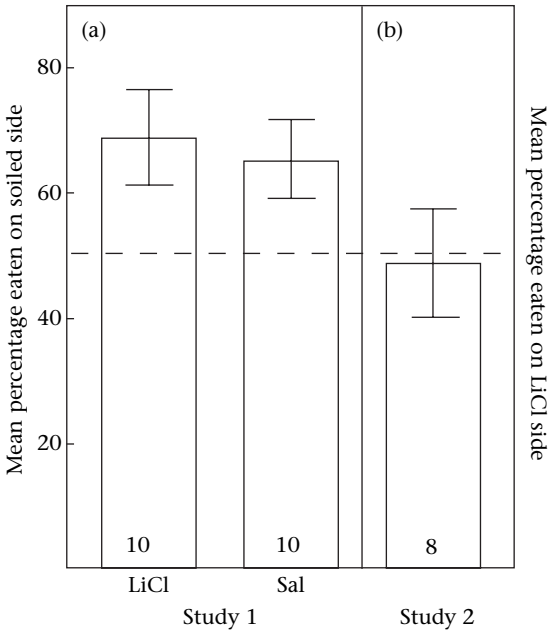

Figure 2. Experiment 1: (a) mean ± SE percentage of the total amount of food eaten by subjects during the 24-h test in study 1 that was from the side of the enclosure soiled by demonstrators injected with either lithium-chloride (LiCl) or saline (Sal) solution, (b) mean ± SE percentage of the total amount of food eaten by subjects during the 24-h test in study 2 that was from the side of the enclosure soiled by demonstrators injected with lithium-chloride (LiCl) solution. Numbers inside histograms = N per group.

The results of both studies in the present experiment provide no support for Steiniger's hypothesis that Norway rats that have learned to avoid a food will mark either the food or its environs so as to reduce the probability that naïve conspecifics will ingest it.

Failure to find a predicted effect of an independent variable is difficult to interpret because of the possibility that some small change in experimental design might reveal that effect. In the present experiment, this difficulty was somewhat ameliorated because we did find a reliable effect of ill as well as of healthy demonstrators on the feeding-site preferences of naïve subjects. That effect was, however, opposite to that predicted on the hypothesis that rats aversively mark foods that they have learned to avoid. Still, the possibility remains that, under conditions other than those prevailing in experiment 1, naïve rats might avoid a food in an area soiled by conspecifics that had learned to avoid eating it.

In experiments 2 and 3, we explored two possible causes of the failure to find aversive marking of a food by rats in experiment 1. In experiment 2, we determined whether rats that became ill after eating an unpalatable food (rather than the palatable diet CS used in experiment 1) would mark the unpalatable food so as to decrease the probability that naïve conspecifics would eat it. In experiment 3, we used a deficiency state, rather than a toxin, to induce an aversion to food, and examined its effects on marking of foods.

EXPERIMENT 2: POISONED DEMONSTRATORS, UNPALATABLE FOOD

Scott (1990) has argued that hedonic responses to flavours predict the nutritive value of foods. Sweet foods tend to be safe and nutritious; bitter foods tend to be toxic. If so, rats might have evolved to behave differently when they become ill after eating an unpalatable food that they are predisposed to treat as dangerous than if they become ill after eating a palatable food that would not, in itself, elicit defensive behaviours.

On such an argument, demonstrator rats might aversively mark unpalatable, but not palatable foods associated with illness, and naïve rats might treat unpalatable, aversively marked foods differently than palatable, aversively marked foods. We therefore repeated experiment 1, except that we taught demonstrators an aversion to an unpalatable rather than a palatable diet, and offered naïve subjects a choice between samples of that unpalatable diet soiled by poisoned demonstrator rats and either (1) clean samples of the unpalatable diet or (2) samples of the unpalatable diet soiled by demonstrators that had not been poisoned after eating it.

Methods

Subjects

Fifteen experimentally naïve, 7-week-old, female Long–Evans rats served as subjects (eight in study 1 and seven in study 2), and an additional 44 females that had served as subjects in other experiments served as demonstrators here.

Apparatus and diets

The apparatus and diets were those used in experiment 1, with one exception. To make the food to which demonstrators learned an aversion relatively unpalatable, we added 0.5% cayenne pepper (Smart Choice Ground Cayenne Pepper, Atlantic Signal Co., Toronto, Ontario) by weight to all samples of diet PS, thus creating unpalatable diet CP. In a pilot study, six female rats, like those used as naïve subjects in the present experiments, offered a choice between diet PS and diet CP ate a mean $\pm$ SE of $82.4 \pm 4.6\%$ of diet PS.

Procedure

The procedure was identical to that of experiment 1 with two exceptions. First, to reduce the number of subjects in the experiment, we examined only: (1) effects of poisoned demonstrators on naïve subjects' feeding-site preferences ($N = 8$ subjects) and (2) feeding-site preferences of naïve subjects choosing between two feeding sites, one marked by poisoned demonstrators and the other by unpoisoned demonstrators ($N = 7$ subjects). Second, we poisoned demonstrators with LiCl after they ate diet CP, and we allowed naïve subjects to choose between two weighed samples of diet CP.

Results and Discussion

Subjects choosing between two samples of unpalatable diet CP, one in an area soiled by demonstrators poisoned after eating diet CP and the other located in an unsoiled area (Fig. 1a, c), preferred to eat in the area that had contained the poisoned demonstrators (one-sample t test: $t_7 = 2.74$, $P < 0.03$; Fig. 3). Furthermore, subjects choosing between two samples of diet CP, one placed in an area previously occupied by poisoned demonstrators and the other in an area previously occupied by unpoisoned demonstrators, ate an equal amount of food from the two sides ($t_6 = 0.72$, $P = 0.50$; Fig. 3). Thus, like subjects in experiment 1 that fed on a palatable food, subjects in the present experiment offered samples of unpalatable food in areas previously soiled by demonstrators that had eaten the unpalatable food and had become ill did not avoid feeding sites in areas soiled by poisoned demonstrators.

EXPERIMENT 3: PROTEIN-DEFICIENT DEMONSTRATORS

Experiment 3, was identical to study 1 of experiment 1 except that we used a protein deficiency, rather than injection with a toxin, to induce demonstrators' learned aversion to a food.

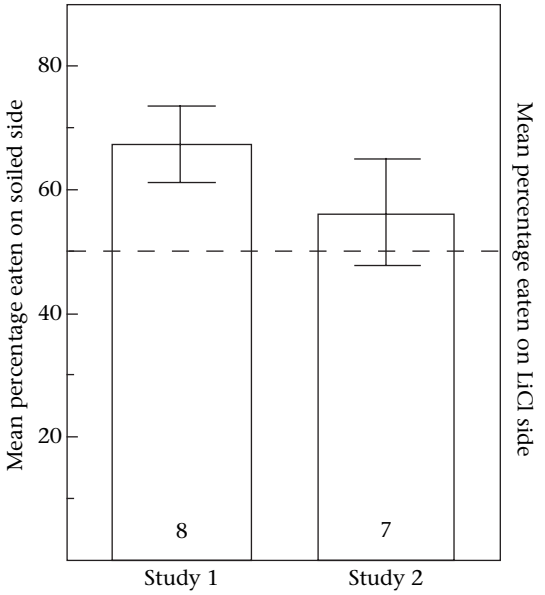

Figure 3. Experiment 2: mean ± SE percentage of the total amount of food eaten by subjects during the 24-h test in studies 1 and 2 that was from the side of the enclosure soiled by demonstrators injected with lithium-chloride (LiCl) solution. Numbers inside histograms = *N* per group.

Methods

Subjects

Twenty experimentally naïve, 7- or 8-week-old, female Long–Evans rats served as subjects and an additional 40 9- or 10-week-old rats that had served as subjects in earlier experiments served in demonstrator pairs. We randomly assigned half of the subjects and half of the demonstrators to experimental and control conditions.

Diets

In addition to powdered laboratory chow (diet 8640) and protein-sufficient diet PS, we also used a protein insufficient diet (diet PIS) prepared by mixing 4% by weight of high-protein casein to Harlan-Teklad Protein-free Basal Mix.

Apparatus

The apparatus was that used in previous experiments.

Procedure

The procedure was identical to that used in study 1 of experiment 1 (Fig. 1a, c) except that: (1) both 1 week before we placed demonstrator pairs in test enclosures, and during the 24 h that demonstrators spent in test enclosures, we fed them ad libitum on either diet PIS (experimental group) or diet PS (control group), (2) we did not inject demonstrators, and (3) during testing, the two food bowls available to naïve subjects contained the same food that their respective demonstrators had eaten. To determine whether our independent variable was effective, we also weighed all demonstrators both at the start of the experiment and 1 week later, just before we placed them in test enclosures.

Results and Discussion

Demonstrators assigned to the experimental condition, that ate diet PIS for 7 days, lost a mean ± SE of 7.3 ± 0.9% of their body weight, while demonstrators assigned to the control condition gained 11.2 ± 1.6% of their body weight. When offered a choice between diets PIS and PS, six females identical to those used as demonstrators in the experiment that had previously been maintained on diet PIS for 1 week ate an average of 4.3 ± 1.2% diet PIS.

As in experiments 1 and 2, observers assigned to both experimental and control conditions left residual cues in the test enclosure that rendered the previously occupied portion of the enclosure more attractive than the unmarked side (experimental condition: $t_9 = 1.92$, $P < 0.04$; control condition: $t_9 = 3.10$, $P < 0.01$; Fig. 4). Again, as in experiments 1 and 2, subjects assigned to experimental and control conditions in the present experiment did not differ significantly from one another in the percentage of food that they ate from the bowl in the soiled side of the enclosure ($t_{18} = 0.26$, $P = 0.40$).

EXPERIMENT 4: TWO FOODS IN TEST ENCLOSURES

The results of experiments 1, 2 and 3, in which subjects showed a preference for, rather than an aversion to, a food found in areas previously occupied by ill conspecifics, suggest that, contrary to Steiniger's (1950) hypothesis, Norway rats do not mark a food that they have learned to avoid so as to reduce the probability that conspecifics will eat it. However, the residual cues left by rats that have been poisoned after eating a food may be used by other rats to avoid a marked food, rather than to avoid a food that is in a marked location. Of course, in the world outside the laboratory, a food is unlikely to be toxic in one

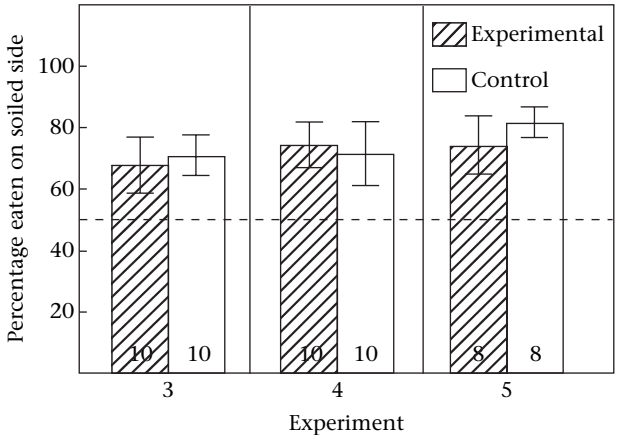

Figure 4. Experiments 3, 4 and 5: mean ± SE percentage of the total amount of food eaten by subjects in experimental and control groups that was from the side of the enclosure soiled by demonstrators. Numbers inside histograms = *N* per group.

location and safe in another. Consequently, rats might have evolved to learn to use social cues to avoid foods rather than locations. If so, then the first three experiments, in which the same food was available on both sides of the test enclosure, might, therefore, have failed to reveal effects of residual cues left by poisoned demonstrators on the food choices of naïve subjects.

Here, we taught demonstrator rats an aversion to a food and allowed them to mark an area containing that food before offering naïve subjects a choice between the soiled area containing the food that the demonstrators had eaten and a familiar, safe food in the unsoiled area of the enclosure. If naïve rats avoid a food rather than a location marked by ill conspecifics, then naïve rats choosing between an unfamiliar food marked by demonstrators avoiding it and an unmarked, familiar, safe food should eat less of the marked food than naïve rats choosing between the same two foods when the unfamiliar food is soiled by demonstrators not trained to avoid it.

Methods

Subjects

Twenty experimentally naïve, 7-week-old female Long–Evans rats served as subjects. A further 40 rats that had served in other experiments served as demonstrators here. We randomly assigned half of the subjects and half of the demonstrators to experimental and control conditions.

Apparatus

The apparatus was that used in experiments 1, 2 and 3.

Procedure

Preparation of the enclosure was similar to that of study 1 of experiment 1 except that there was a bowl of familiar, safe diet 8640 on the far side of the partition from demonstrators. During testing of naïve subjects, each had a choice for 24 h between an unfamiliar food (diet PS) on the soiled side of the enclosure and a familiar food (diet 8640) on the unsoiled side of the enclosure.

Results and Discussion

As in experiments 1, 2 and 3, observers assigned to both experimental and control conditions took a greater percentage of their 24-h intake from the soiled side than from the unsoiled side of the test enclosure (experimental condition: $t_9 = 3.24$, $P < 0.01$; control condition: $t_9 = 2.05$, $P = 0.04$, one-tailed; Fig. 4). Although the two-tailed P value was not significant for subjects in the control condition, results of the preceding three experiments, as well as those of Galef & Heiber (1976) and Galef & Muskus (1979), lead to the strong prediction that control subjects in the present experiment would prefer, rather than avoid, food in an area soiled by conspecifics.

Most important, subjects assigned to control and experimental conditions did not differ in the percentage of unfamiliar diet PS that they took from the soiled side of the enclosure ($t_9 = 0.21$, $P = 0.83$). Thus, even when naïve rats could choose between a familiar food and an unfamiliar food that had been soiled by conspecific demonstrators that had either learned or not learned to avoid that unfamiliar food, naïve subjects showed no effect of the aversion learned by their demonstrators on their own food choices.

EXPERIMENT 5: POISONING IN TEST ENCLOSURES

To speed the completion of experiments 1–4, we trained demonstrators to avoid a food while they resided in hanging cages, and then placed them in the large floor enclosures. We had space for only a limited number of such enclosures, and if we had both trained demonstrators and tested observers in them, the rate at which we could run the experiment would have been reduced by two-thirds. However, in natural situations, an individual would be most likely to aversively mark a food that it had learned was toxic in the same location where it had eaten that food. Consequently, our procedures in experiments 1–4, requiring that demonstrators mark a food that they had learned to avoid in an unfamiliar location, may have interfered with demonstrator rats marking a food so that others would avoid it. Here we repeated experiment 4, but we trained demonstrators in the same enclosures in which they were subsequently to mark rather than in their respective home cages.

Methods

Subjects

Sixteen experimentally naïve Norway rats served as subjects and an additional 32 rats that had served here as subjects in other experiments served as demonstrators.

Apparatus

The apparatus and diets were those used in experiment 1.

Procedure

The procedure was similar to that of experiment 4 except that, in the present experiment, we trained the 10 pairs of demonstrators randomly assigned to the experimental condition to avoid diet PS while in the same enclosure in which we subsequently tested naïve subjects. In brief, we moved each pair of demonstrators assigned to the experimental condition from their home cage (a wire-mesh hanging cage) into one side of an enclosure and gave them access to diet 8640 for 1 h/day for 2 consecutive days. Following a third 23-h period of food deprivation, we (1) moved the demonstrator pair to the opposite side of the enclosure, (2) cleaned the side of the enclosure that the demonstrators had previously occupied with detergent and water, and with alcohol, (3) offered the demonstrators diet PS for 1 h and (4) injected the demonstrators with LiCl solution. The demonstrators then remained undisturbed for 24 h on the side of the enclosure where they had been poisoned. Next, we removed the demonstrators and the partition dividing

the enclosure, and introduced a subject into the enclosure for 24 h where it chose between diet 8640 and diet PS.

We treated the remaining 16 demonstrators and eight subjects assigned to the control condition exactly as we treated those assigned to the experimental condition except that when we injected demonstrators assigned to the experimental condition with LiCl solution, we injected demonstrators assigned to the control condition with saline solution.

Results and Discussion

As in all four previous experiments, observers assigned to both experimental and control conditions ate more from the soiled side than from the unsoiled side of the test enclosure (experimental condition: $t_7 = 2.44$, $P < 0.05$; control condition: $t_7 = 6.34$, $P < 0.01$; Fig. 4). Most important, subjects assigned to the two groups did not differ significantly from one another in the percentage of total food eaten that was from the soiled side of the enclosure ($t_{14} = 0.26$, $P = 0.46$). Thus, even when demonstrator rats learned to avoid an unfamiliar food in the same location where they could subsequently mark it aversively, they failed to do so.

GENERAL DISCUSSION

The results of the present series of experiments are not consistent with Steiniger's (1950) proposal that Norway rats mark a food that they have learned to avoid in such a way as to reduce its acceptance by naïve conspecifics. To the contrary, results of the present studies, like those of previous studies of behavioural processes involved in social influence on the food choices of rats (Galef et al. 1983, 1990), indicate that rats learn from one another where and what to eat, not where and what to avoid eating. Many (but not all) other species that forage socially, similarly use positive, but not negative, recruitment signals even though formal models indicate that use of negative signals would increase foraging efficiency (Strickland 1999).

It is, of course, impossible to rule out the possibility that, under conditions other than those examined here, rats would place aversive marks on foods that they had learned to avoid. However, the apparent difficulty of producing evidence of aversive marking of foods by rats that have learned to avoid them, together with the ease of demonstrating the attractiveness of areas that contain such foods, and have been soiled by rats, suggests that aversive marking by rats may not occur.

Why might rats that have learned to avoid a food not mark it in a way that would dissuade conspecifics from ingesting it? In order for a rat that has learned to avoid a food to aversively mark it, the knowledgeable rat would have to return to a place where it no longer intended to eat and deposit on it some residual cue that conspecifics would avoid. Animals do not necessarily become ill instantly after eating a toxin, and a variety of animals have evolved exceptional abilities to tolerate long delays between eating a food and experiencing illness and still learn an aversion (Garcia & Kimeldorf 1957), suggesting that such delays between ingestion and illness are common in nature. If so, a return to a toxic food would be necessary, and leaving cues that inhibited others' ingestion of a potentially toxic food would be costly to the marker. If so, marking of aversive foods would be an instance of altruistic 'teaching' (sensu Caro & Hauser 1992; Galef et al. 2005). Perhaps because of its altruistic nature, teaching has rarely evolved and has proven difficult to demonstrate experimentally in animals generally, and in Norway rats in particular, even when conditions for teaching are optimized (Galef et al. 2005).

The failure to find evidence of aversive marking of toxic potential foods by rats leaves unanswered the question that motivated the present studies. Why do 'observer' rats that interact with ill conspecific demonstrators at a distance from a feeding site develop enhanced preferences for, rather than aversions to, foods eaten by ill demonstrators. Rats can both discriminate ill conspecifics from healthy ones (Coombes et al. 1980; Lavin et al. 1980; Galef & Whiskin 2001) and identify foods that ill conspecifics have eaten (Galef et al. 1983). Rats readily learn to avoid an unfamiliar food that they eat before interacting with an ill conspecific (Coombes et al. 1980; Lavin et al. 1980; Galef et al. 1983). Thus, all the elements needed for rats to learn aversions to foods that ill conspecifics have eaten are present. Yet, such learning does not seem to occur (Galef et al. 1983, 1990). The failure of rats to learn to avoid foods eaten by ill conspecifics, even though they have in place the behavioural substrate sufficient for such learning, suggests that there may have been selection against it.

Wild Norway rats are extremely hesitant to ingest unfamiliar foods (Barnett 1958), have congenital distaste for bitter flavours that are correlated with the presence of toxins (Garcia & Hankins 1975; Scott 1990) and learn rapidly to associate illness with previously sampled, unfamiliar foods (Garcia & Koelling 1966). Thus, the behavioural repertoires of individual rats may suffice to protect them from ingesting deleterious quantities of naturally occurring toxins.

Possibly, the frequency with which rats become ill from ingesting toxic substances is low relative to the frequency with which they become ill for reasons unrelated to ingestion of toxic substances (e.g. viral infections, organic malfunctions, etc.). Possibly, lost-opportunity costs of avoiding potential foods eaten by sick individuals after interacting with them are greater than the benefits of avoiding such potential foods (Galef 1991). If so, natural selection may have acted to inhibit naïve individuals' learning aversions to unfamiliar, potential foods after interacting with ill conspecifics that have eaten those foods (Tuci et al. 2001). Strickland (1999) provides similar arguments concerning the apparent absence of negative recruitment signals in ants (but see Robinson et al. 2005). More generally, evolution of learning processes in response to specific environmental demands has been well documented (e.g. Balda et al. 1998; Shettleworth 1998) and provides a possible explanation for the apparent failure of rats to integrate their abilities to learn from others about poison foods so as to avoid foods that more experienced conspecifics have marked.

Whatever the ultimate cause of the apparent failure of Norway rats to learn to avoid foods eaten by ill conspecifics, the results of the present studies indicate that it is not because such learning would be redundant with a more reliable type of socially acquired information about foods resulting from aversive marking of foods by conspecifics that have learned to avoid those foods.

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