

# Preference for Novel Flavors in Adult Norway Rats (*Rattus norvegicus*)

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The authors fed rats 1 of 2 distinctively flavored, roughly equipalatable diets for 3 days then offered them an ad libitum choice between the 2 diets. For 3 days, subjects exhibited a reduced relative intake of whichever diet they had previously eaten (Experiment 1). Such reduction in relative intake was as effective as a toxicosis-induced conditioned aversion in determining subjects' food choices (Experiment 2). The strength of exposure-induced reduction in relative intake did not depend on similarity of the 2 diets offered for choice either to each other or to subjects' maintenance diet (Experiment 3) but did require continuous exposure to a diet (Experiment 4). These experiments provide the first evidence of a robust, exposure-induced decrease in food preference in rats lasting for days rather than minutes.

Familiarity of a food can be an important determinant of its acceptance by both wild and domesticated Norway rats. Genetically wild rats, even those reared in the laboratory, are extremely reluctant to ingest unfamiliar foods, and if only an unfamiliar food is available, they may go several days without eating (Barnett, 1958; Galef, 1970). Overcoming such "neophobic" responses (Barnett, 1958) is considered essential to success in poisoning rats (e.g., Meehan, 1984), and prebaiting (i.e., giving rats prolonged access to unpoisoned bait before poison is added) substantially enhances intake of a bait when poison is added (Chitty, 1954).

Unlike their wild forbears, domestic rats will sometimes prefer unfamiliar to familiar foods, and under special conditions, such enhanced ingestion of unfamiliar items can be quite pronounced. For example, thiamin-deficient rats prefer any unfamiliar food to familiar foods as a result of forming conditioned aversions to foods eaten while developing a thiamin deficiency (Rodgers & Rozin, 1966).

*Sensory-specific satiety*, which is opposite in effect to the "non-specific neophilia" described by Rodgers and Rozin (1966), refers to decreased acceptance of a food immediately following its ingestion (Rolls, 1986). In rats, sensory-specific satiety has been shown to result in both decreased intake of a recently ingested food and decreased motivation to work for that food (e.g., Balleine & Dickinson, 1998; Colwill & Rescorla, 1985). Although effects of sensory-specific satiety in animals are occasionally large (e.g., Balleine & Dickinson, 1998; Colwill & Rescorla, 1985; Morrison, 1974), they are invariably brief (on the order of minutes) and sometimes so small as to be statistically unreliable (e.g., Berridge, 1991).

Here, we first describe an exposure-induced effect on diet choice in domestic rats that results in reduced relative intake of a diet that lasts for days (Experiment 1) and is as potent as a conditioned flavor aversion (Garcia & Koelling, 1966) in reducing

relative intake of a food (Experiment 2). We then explore the range of conditions under which such exposure-induced effects on relative intake are expressed (Experiments 3 and 4).

## Experiment 1

In the course of studies of social influence on the food choices of Norway rats, we discovered, quite unexpectedly, that feeding a rat one of two diets for 3 consecutive days resulted in markedly reduced intake of that diet when both diets were subsequently offered to the subject. In Experiment 1, we provide formal evidence of such an effect of a recent history of ingesting a diet on subsequent relative intake of the diet.

## Method

*Subjects.* Fifty experimentally naive female Long-Evans rats (*Rattus norvegicus*) purchased from Charles River Canada (St. Constant, Quebec) at 42 days of age served as subjects. For 7 days after subjects arrival in the laboratory, we housed them in groups of 3 or 4 to permit recovery from any stress resulting from transportation. We then transferred each subject to an individual hanging cage, measuring 20 × 21 × 34 cm, where it remained for the duration of the experiment.

The rack of cages containing subjects was located in a temperature- and humidity-controlled colony room maintained on a 12:12-hr light-dark cycle. All subjects received ad libitum access to water and pellets of PMI Rodent Chow 5001 (PMI Nutrition International, Brentwood, Missouri) until the start of the experiment.

*Apparatus.* During the experiment, subjects ate powdered chow from semicircular, stainless-steel food cups, measuring 10 cm in diameter and 5 cm deep, that we attached to one wall of each rat's cage. To prevent spillage, we filled each food cup to less than half its depth with powdered food. A paper towel placed under each cup permitted monitoring of spillage, which was negligible.

*Diets.* We prepared a cocoa-flavored diet (diet coc) and a cinnamon-flavored diet (diet cin) by mixing, respectively, either 20 g of Hershey's Low Fat Cocoa (Hershey Canada Inc., Mississauga, Ontario) or 10 g of McCormick's Pure Ground Cinnamon (McCormick Canada, London, Ontario) with 1 kg of powdered PMI Rodent Diet 5001 (PMI Nutrition International, Brentwood, Missouri).

*Procedure.* To begin the experiment, we placed a single food cup containing either diet cin ( $n = 26$ ) or diet coc ( $n = 24$ ) in the home cage of each subject. We then left subjects undisturbed, except for daily replacement of ingested food, for either 3 ( $n = 25$ ) or 5 days ( $n = 25$ ). At the end of the prefeeding period, we removed the food cup from each subject's

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cage and replaced it with two weighed food cups, one containing diet cin and the other containing diet coc.

Every day for the next 4 days, we weighed, refilled, and reweighed each food cup and then calculated the percentage of each subject's total daily intake that was diet cin.

*Results and Discussion*

The main results of Experiment 1 are presented in Figure 1, which shows the mean percentage of subjects' intake that was diet cin. As is evident from inspection of Figure 1, during the 4-day choice test, subjects preexposed to diet coc for either 3 or 5 days showed a greater relative intake of diet cin than did subjects preexposed to diet cin. The effect of preexposure on diet preference was observable for 3 days in subjects preexposed to diet cin or diet coc for 3 days (Student's *t* test on Day 3 of testing),  $t(23) = 2.25, p < .04$ , and for 2 days in subjects preexposed to a diet for 5 days,  $t(23) = 2.45, p < .02$ . The results clearly demonstrate an effect of diet familiarity that lasted considerably longer than 24 hr.

Experiment 2

To explore the strength of the preexposure-induced food aversion demonstrated in Experiment 1, in Experiment 2 we compared effects of toxicosis-induced conditioned flavor aversions and preexposure-induced reduced intake of a food.

*Method*

*Subjects.* Sixty-seven 42-day-old female Long-Evans rats served as subjects in Experiment 2.

*Apparatus.* The apparatus was the same as that used in Experiment 1.

*Procedure.* After subjects had been allowed to recover from effects of transport to the laboratory, they were weighed, moved to individual hanging cages, and randomly assigned to one of six groups (see Figure 2).

We then placed all 67 subjects on a restricted feeding schedule, offering them unadulterated powdered PMI Rodent Chow 5001 for 1 hr per day for 2 successive days. On the third day of scheduled feeding for 1 hr, we fed 33 of the subjects diet cin and 34 diet coc.

On the third day of scheduled feeding after subjects had finished eating, we induced an aversion to either diet cin or diet coc in 23 of the 33 subjects fed diet cin and 25 of the 34 subjects fed diet coc (the 48 subjects assigned to the experimental condition) by injecting them (intraperitoneal) with 0.3% of body weight 0.13 mol lithium chloride solution. At the same time, we injected with 0.3% of body weight isotonic saline the remaining 10 subjects fed diet cin and 9 subjects fed diet coc that we had assigned to the poison control condition.

For the next 3 days, those subjects assigned to the experimental condition ( $n = 28$ ) that had been poisoned after eating diet coc ( $n = 15$ ) ate diet cin, and those subjects assigned to the experimental condition that had been poisoned after eating diet cin ( $n = 13$ ) ate diet coc. We treated the 20 subjects assigned to the exposure control condition exactly as we treated subjects assigned to the experimental condition with one exception: After we induced an aversion to either diet cin ( $n = 10$ ) or diet coc ( $n = 10$ ), we fed them unadulterated rather than flavored chow for 3 days. Thus, subjects assigned to the two experimental groups were taught a conditioned aversion to either diet cin or diet coc before they learned an exposure-induced aversion to the other diet. Subjects assigned to the two poison control groups were taught only a conditioned aversion to either diet cin or diet coc, and subjects assigned to the two exposure control groups learned only an exposure-induced aversion to either diet cin or diet coc.

Last, we gave each subject two weighed food cups, one containing diet cin and the other diet coc. Twenty-four hours later, we determined the amount of each diet eaten by each subject and the percentage of each subject's total intake that was diet cin.

*Results and Discussion*

The main results of Experiment 2 are presented in Figure 2, which shows the mean amount of diet cin eaten (as a percentage of total intake during 24 hr of testing) by subjects assigned to experimental, poison control, and exposure control conditions.

*Poison control condition.* Subjects assigned to the poison control condition that ate diet coc before they were poisoned ate far more diet cin than did subjects assigned to the poison control condition that ate diet cin before they were poisoned (Mann-Whitney *U* test,  $U[10, 10] = 0, p < .001$ ). Clearly, we were successful in inducing taste aversions in the 48 subjects that we injected with lithium chloride solution.

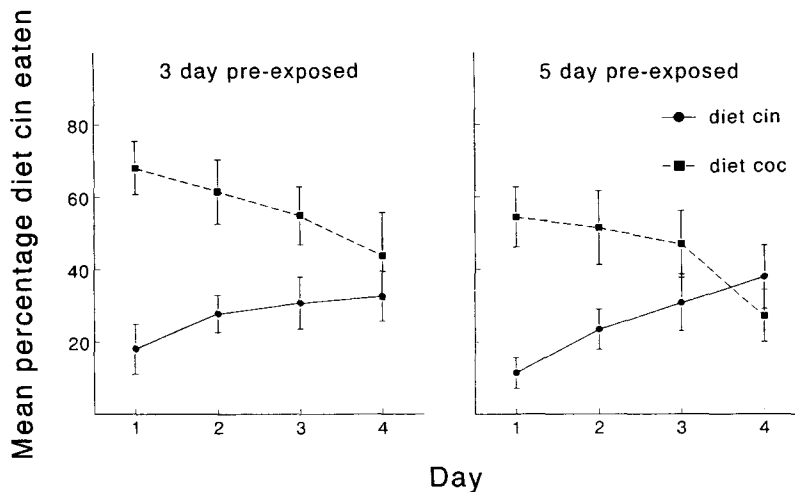
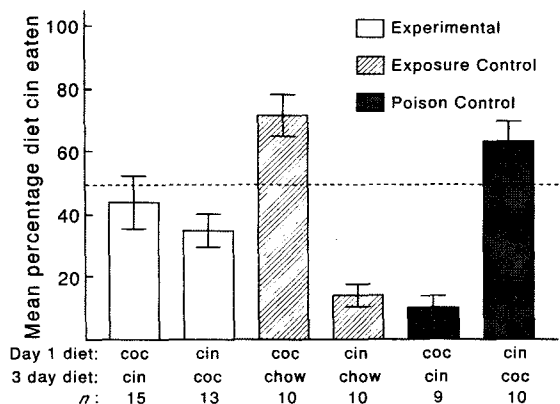


Figure 1. Mean amount of cinnamon-flavored diet (diet cin) eaten, as a percentage of total intake during 24 hr, by subjects prefed either diet cin or cocoa-flavored diet (diet coc) for 3 days (left) and 5 days (right) before testing. Error bars represent standard errors of the mean.



**Figure 2.** Mean amount of cinnamon-flavored diet (diet cin) eaten, as a percentage of total intake during 24 hr, by subjects assigned to experimental, poison control, and exposure control conditions. On Day 1, subjects in each condition received either cocoa-flavored diet (diet coc) or diet cin for 1 hr. All were then injected, and on Day 5, all received a choice between diet cin and diet coc for 24 hr. Subjects in the experimental group were poisoned after eating either diet cin or diet coc on Day 1 and then received the other diet for the next 3 days. Subjects in the exposure control condition were poisoned after eating either diet cin or diet coc on Day 1 and then received unflavored chow for the next 3 days. Subjects in the poison control condition were injected with saline after eating either diet cin or diet coc on Day 1 and then received either diet cin or diet coc for the next 3 days. Error bars represent standard errors of the mean.

**Exposure control condition.** Subjects assigned to the exposure control condition that ate diet coc for 1 hr, were injected with saline, and then ate diet cin for 3 days, ate a significantly smaller percentage of diet cin during testing than did subjects that ate diet cin for 1 hr, were injected with saline, and then ate diet coc for 3 days,  $U(10, 9) = 0, p < .001$ . Clearly, we were successful in creating exposure-induced aversions to diet cin and diet coc in those 47 subjects that ate either diet cin or diet coc for 3 days and were not poisoned.

**Experimental condition.** Subjects in which we first induced a conditioned aversion to diet cin (by injecting them with lithium chloride after they ate diet cin) and then induced an aversion to diet coc by feeding them diet coc for 3 days showed as great a preference for diet cin during testing as did subjects in which we first induced a toxicosis-based aversion to diet coc and then fed diet cin for 3 days,  $U(15, 13) = 83, ns$ . Assuming an additive interaction between effects of preexposure and toxicosis-based conditioned aversions, preexposure-induced food aversions were as important as were toxicosis-based conditioned aversions in determining food choice.

**Comparison of experimental and exposure control conditions.** A  $2 \times 2$  analysis of variance (ANOVA) revealed a main effect of diet fed before poisoning to subjects assigned to experimental groups and chow control groups,  $F(1, 44) = 23.15, p < .0001$ , no main effect of feeding subjects diet cin, diet coc, or unadulterated powdered chow for 3 days,  $F(1, 44) = 0.25, ns$ , and most important for present purposes, a highly significant interaction between effects of poisoning on one of two diets and whether one of those diets or unadulterated chow was eaten for the next 3 days,  $F(1, 44) = 12.28, p < .001$ . Eating diet cin after learning a toxicosis-based aversion to diet coc or eating diet coc after learning a

toxicosis-based aversion to diet cin had profound influence on subsequent preference between diets cin and coc that eating unflavored chow for 3 days did not.

### Experiment 3

Experiment 3 was undertaken to begin to explore the generality of the phenomenon described in Experiments 1 and 2. A large number of parameters, ranging from the relative palatability of diets to the time they are available to subjects, could be investigated. However, because the phenomenon under investigation appeared to be an effect of diet familiarity, we decided to begin by exploring effects of familiarity and similarity of preexposed diets on subsequent diet preference.

In both Experiments 1 and 2, the diets fed to subjects were based on subjects' maintenance diet and were quite similar to one another, differing only in an added flavorant. Possibly, the unusually large effects of exposure to a food on subsequent preference for that food seen in Experiments 1 and 2 are restricted to diets either similar to one another in taste, smell, and texture or having a familiar base diet as their main component. In Experiment 3, we determined effects on rats' food preferences of 3 days prior exposure to one of two markedly dissimilar diets, both quite different from the subjects' maintenance diet.

### Method

**Subjects.** Thirty-five experimentally naive 49-day-old female Long-Evans rats served as subjects. We used 16 similar animals to establish the relative palatability of Normal Protein Test Diet and Rodent Bacon Lover Treat (described below).

**Diets.** We fed subjects in Experiment 3 diet cin, diet coc (see Method of Experiment 1), powdered Normal Protein Test Diet: Rat (diet NPT; Harlan Teklad, Madison, WI, Catalogue No. 170590), and Rodent Bacon Lover Treat (diet RBLT; Bioserv, Frenchtown, NJ). Diet NPT is composed principally of casein and corn starch, whereas diet RBLT consists mainly of ground corn, soybean meal, and meat meal.

**Apparatus.** The apparatus was the same as that used in Experiments 1 and 2.

**Procedure.** To determine the relative palatability of diets NPT and RBLT to naive rats, we offered 16 subjects a choice between those diets for 24 hr.

To determine the effects of 3 days of preexposure to either diet NPT or diet RBLT on subsequent preference for those diets, we fed subjects either diet NPT ( $n = 9$ ) or diet RBLT ( $n = 8$ ) for 3 days then offered all 17 subjects a choice between those diets for 24 hr. Similarly, to determine effects of 3 days preexposure to diet cin or diet coc on subsequent preference for those diets, we fed subjects either diet cin ( $n = 9$ ) or diet coc ( $n = 9$ ) for 3 days then offered all 18 subjects a choice between diets cin and coc for 24 hr.

### Results and Discussion

Subjects maintained on powdered PMI Rodent Diet 5001 then offered a choice between diets NPT and RBLT for 24 hr ate an average ( $\pm 1 SEM$ )  $53.7\% \pm 5.0\%$  diet NPT. The two diets were roughly equipalatable.

The main results of Experiment 3 are presented in Figure 3, which shows the mean percent of diet cin and diet NPT ingested by subjects preexposed, respectively, to familiar-similar and unfamiliar-dissimilar diets. As can be seen in Figure 3 (left panel) and as in Experiments 1 and 2, subjects preexposed for 3 days to either

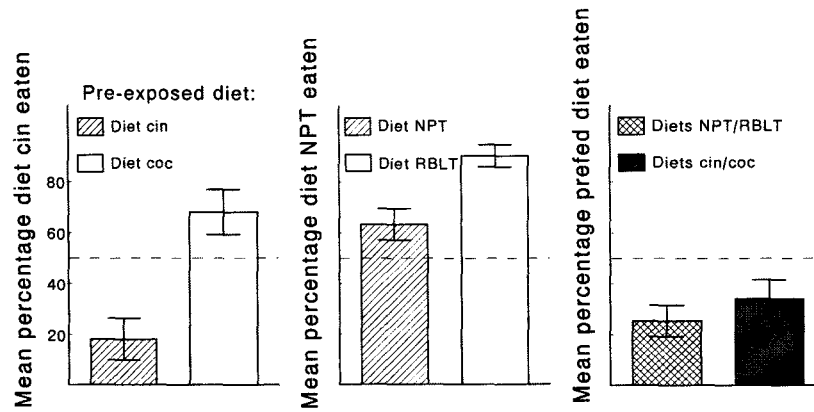


Figure 3. Left: Mean amount of cinnamon-flavored diet (diet cin) eaten, as a percentage of total intake during 24 hr, by subjects prefed either diet cin or cocoa-flavored diet (diet coc) for 3 days before testing. Center: Mean amount of Normal Protein Test Diet (diet NPT) eaten, as a percentage of total intake during 24 hr, by subjects prefed either diet NPT or Rodent Bacon Lovers Treat (diet RBLT) for 3 days before testing. Right: Mean percentage of prefed diet eaten by subjects whose data are presented in the other two panels of the figure. Error bars represent standard errors of the mean.

diet cin or diet coc and then offered a choice between these two relatively familiar and similar diets preferred the diet to which they were not preexposed,  $t(16) = 4.90, p < .001$ . Similarly (see the center panel of Figure 3), subjects preexposed to either diet NPT or diet RBLT for 3 days and then offered a choice between these two unfamiliar and quite distinctive diets also preferred the diet to which they were not preexposed,  $t(15) = 3.55, p < .003$ .

The right panel of Figure 3 shows the results of Experiment 3 recalculated as a percentage of nonpreexposed diet eaten by subjects preexposed to either diet cin or diet coc or to either diet NPT or diet RBLT. Such presentation permits direct comparison between effects of preexposure on food choice using relatively familiar-similar and unfamiliar-dissimilar diet pairs as stimuli. As can be seen in the right panel of Figure 3, we found no difference between the size of the effect of preexposure when relatively familiar-similar diets and relatively unfamiliar-dissimilar diets were used as stimuli,  $t(33) = 0.90, ns$ .

The results of Experiment 3 indicate that the relatively large and long-lasting effects of preexposure to a diet on preference for that diet seen in Experiments 1-3 are not restricted to relatively familiar or relatively similar diets.

#### Experiment 4

In all three preceding experiments, we have assumed that avoidance of ingestion of a familiar food depends on continuous access to that food for 3 consecutive days. It is, of course, possible that 3 days of exposure to a food reduces intake of a food regardless of the distribution of those 3 days of experience. In the present experiment, we compared the effect on rats' preference for a food of 3 consecutive days of eating that food and eating the same food every other day for 5 days.

#### Method

**Subjects.** Forty experimentally naive 49-day-old female Long-Evans rats served as subjects.

**Apparatus.** The apparatus was the same as that used in Experiment 1.

**Procedure.** We treated subjects assigned to the massed condition of the present experiment just as we had treated subjects assigned to the 3-day condition in Experiment 1. That is, we fed each subject either diet cin ( $n = 10$ ) or diet coc ( $n = 10$ ) for 3 consecutive days and, immediately after the third day of diet preexposure, offered all 20 subjects a choice between diets cin and coc for 24 hr. We treated the 20 subjects assigned to the spaced condition exactly as we had treated subjects assigned to the massed condition except that we fed subjects assigned to the spaced condition unflavored chow for 24 hr between both their first and second and second and third days of eating flavored chow.

#### Results and Discussion

The main results of Experiment 4 are presented in Figure 4, which shows the mean amount of diet cin eaten by subjects assigned to massed and spaced conditions as a percentage of their total intake of food during the 24-hr test period. As can be seen in Figure 4, we found no significant main effects of either preexposed

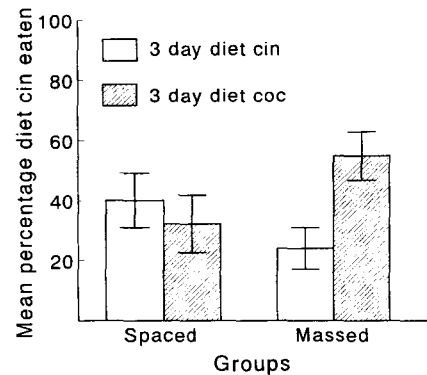


Figure 4. Mean amount of cinnamon-flavored diet (diet cin) eaten, as a percentage of total intake during 24 hr, by subjects prefed either diet cin or cocoa-flavored diet (diet coc) for 3 consecutive (massed group) or alternate (spaced group) days before testing. Error bars represent standard errors of the mean.

diet ( $2 \times 2$  ANOVA),  $F(1, 36) = 1.80$ , *ns*, or condition,  $F(1, 36) = 0.15$ , *ns*, on diet preference during testing but a significant interaction between main effects,  $F(1, 36) = 5.18$ ,  $p < .03$ , indicating that massed preexposure had a significantly greater effect on diet preference than did spaced preexposure. Student's *t* tests on the percentage of diet eaten by subjects assigned to spaced and massed conditions and preexposed to either diet cin or diet coc revealed that during testing diet choices of subjects assigned to the massed, but not the spaced, condition were affected by the flavor of the diet to which they were preexposed: massed condition,  $t(18) = 2.90$ ,  $p < .01$ ; spaced condition,  $t(18) = 0.60$ , *ns*.

### General Discussion

The results of the present series of experiments indicate that after a rat eats a food for 3 days in succession its relative intake of that food is depressed for the next 3 days. So far as we know, this is the first report of ingestion of a food producing such robust and long-lasting aversion in subsequent preference tests in rats. We were, of course, surprised to discover a previously undescribed, but robust, determinant of food choice in Norway rats, animals that have served as subjects in studies of ingestive behavior and food choice for more than 70 years.

In the interval between completion of these studies and acceptance for publication of the present article, DiBattista (2002) published similar findings from experiments in which golden hamsters served as subjects. In DiBattista's experiments, hamsters were given ad libitum access to either allspice- or marjoram-flavored powdered Purina Rodent Chow (enriched with oil) for 12 successive days and were then offered a choice between the two diets for 30 min. DiBattista found that hamsters preexposed to either diet for 12 days preferred the other diet during the choice test.

Not surprisingly, given the evidence in the literature at the time DiBattista (2002) published his studies, he interpreted the aversion to familiar diets he observed in hamsters in terms of differences in the presumed feeding ecology of golden hamsters and Norway rats. Of course, the present results indicate that rats, like hamsters, show a reduced relative intake of a preexposed diet.

Why did we find a long-lasting and profound reduced relative intake of a familiar diet, whereas others studying what has been called "sensory-specific satiety" (Hetherington & Rolls, 1996; Rolls, 1986) generally found ephemeral and weak effects? Of course, without conducting the relevant experiments we cannot know. One obvious difference between our experiments and those of most others who have looked at effects of familiarity on ingestion in domestic Norway rats is in choice of the dependent variable. We used relative intake in a choice situation as a measure, whereas most others have looked at effects of familiarity on either absolute intake of a single diet or willingness to work for a diet (e.g., Balleine & Dickinson, 1998; Berridge, 1991; Colwill & Rescorla, 1985). Morrison (1974), who found robust but transient effects of preexposure to a flavor on flavor preference in a choice situation, used a much briefer exposure period than we did in the present series of studies. We speculate that measures of choice are simply more sensitive to effects of diet familiarity than are other measures of preexposure effects and that long-lasting effects on diet choice require days rather than hours of preexposure.

Why, in an ultimate sense, should animals as different as Norway rats and golden hamsters both show a reduced relative acceptance of familiar foods? We can but speculate. Perhaps a behavioral mechanism that biases animals not to become dependent on a single food while it is readily available provides protection against the eventual disappearance of that food. Animals might be at risk if they were motivated to start looking for alternative sources of nutrition only when their current source of food has failed. Alternatively, dependence on a single food might increase the probability of an animal developing a micronutrient deficiency, so a mechanism producing increased avoidance of continuous intake of the same food might be beneficial.

Regardless of why rats respond to continuous exposure to foods by reducing their relative intake of that food, the present data suggest that care must be taken to guard against previously unexpected effects of diet exposure in design and interpretation of experiments in which animals are offered the same food for several days and the food is then provided as an alternative in a choice situation.

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