

## MOTHER'S MILK: A DETERMINANT OF THE FEEDING PREFERENCES OF WEANING RAT PUPS<sup>1</sup>

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Cues associated with the diet eaten by a lactating female rat are transmitted to her young, probably via her milk, and are sufficient to markedly influence the food preferences of her young during weaning. For their first meals of solid food, the weanlings actively seek and preferentially ingest the diet their mother has been eating during the nursing period, even if that diet is relatively unpalatable.

Females of all mammalian species can nurse their offspring and are thus able to provide neonates with necessary nutrients during a period when the young are incapable of foraging for themselves. As lactation on the part of the female wanes, the young must gradually make the transition from a pure milk diet to one of foodstuffs obtained directly from the environment, if they are to achieve physical independence from their mother and survive.

Although it has been generally assumed that weaning rats have to find appropriate solid foods in the environment without assistance from the adults of their colony (Barnett, 1956; Scott, 1958), the results of recent investigations (Galef, 1971; Galef & Clark, 1971a, 1971b, 1972) indicate that rat pups are aided in their search for appropriate substances for ingestion during weaning by a tendency to approach adults of their species at a distance from the nest site and to begin feeding in their vicinity. The present series of experiments investigates the possibility that a second mech-

anism facilitates the pups' transition to feeding on solid food.

As Ewer (1968) has observed in a somewhat different context, the prolonged period of interaction between mother and young during the nursing period may have profound psychological consequences as well as the more evident physiological ones. The data reported below indicate that during the nursing period young rats, while receiving milk from their mother, are provided with cues which cause them to seek out and preferentially ingest the foodstuff their mother has been ingesting during lactation.

### EXPERIMENT 1

Experiment 1 was designed to determine whether or not the diet ingested by a lactating female rat during the nursing period could influence the dietary preference of her nurslings at weaning.

#### *Method*

*Subjects.* The subjects were four female hooded rats obtained from the Quebec Breeding Farm and 32 pups born to the females.

*Procedure.* Individual recently parturite female rats and their litters were maintained in plastic home cages (12 × 14 × 6½ in.) on a 12-hr. day-night cycle (light onset 8 A.M.) with water available ad lib. The females were removed from their home cages to separate enclosures for three 1-hr. feeding periods daily. During the three 1-hr. feeding periods two of the females were fed powdered Purina Lab Chow and the other two Turtlox "fat-sufficient diet," a powdered sucrose and casein based diet, highly preferred to the Purina diet, and differing from it in taste, smell, color, and texture.

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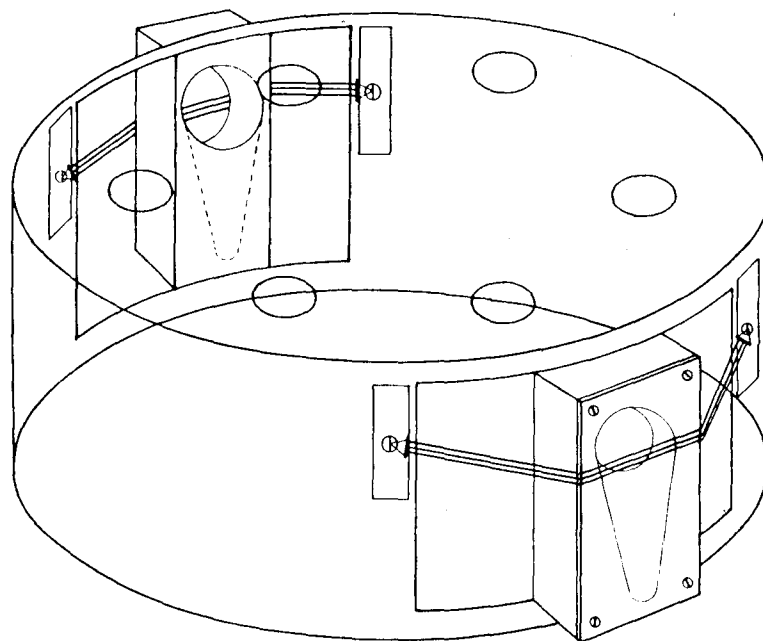


FIG. 1. Schematic diagram of pup-testing apparatus.

Litters of pups were reduced to eight pups/litter on the day of birth and half of each litter cross-fostered to a mother feeding on the opposite diet from that of its natural mother at 5 days of age (counting the day of birth as the first day of age). Pups whose nursing mother ate the Turtox and Purina diets will be referred to below, respectively, as Turtox and Purina groups. Pups were individually identified by tail painting until they reached 8 days of age and subsequently by reference to pelage markings.

**Apparatus.** The testing apparatus is illustrated in Figure 1. It consisted of a clear, circular, plastic laboratory dish 10 in. in diameter  $\times$  3 in. deep, closed with a clear plastic lid into which air holes were drilled. Two circular holes  $1\frac{1}{4}$  in. in diameter with centers 2 in. above the floor of the enclosure and  $180^\circ$  apart were opened in the vertical wall of the dish. Attached externally to each of these holes by metal springs was a detachable food cup designed so as to minimize spillage by weanling rats. Each cup consisted of a curved plastic section subtending  $47^\circ$  of an arc cut from the wall of a second 10-in.-diam. plastic laboratory dish. A hole  $\frac{7}{8}$  in. in diameter was drilled in this section. Attached to the convex surface of the section was a  $1\frac{1}{2} \times 2\frac{1}{2} \times 1$  in. block of clear plastic hollowed out by tangentially joining a  $\frac{7}{8}$ -in.-diam. hole (top) with a  $\frac{3}{8}$ -in.-diam. hole (bottom),  $1\frac{1}{2}$  in. center to center, drilled through the largest face of the plastic block. The back of the block was closed with a  $1\frac{1}{2} \times 2\frac{1}{2}$  in. plastic plate and the plastic block fixed against the  $47^\circ$  plastic section so that

the  $\frac{7}{8}$ -in. hole in each corresponded. Powdered food was placed in the food cups so that the upper surface of the food was slightly below the lower edge of the  $\frac{7}{8}$ -in. hole in the plastic shield.

**Testing.** On Days 15 and 16 of age pups were habituated to the apparatus by placing entire litters in it, with the food cups empty, for two 1-hr. sessions/day. On Days 17-23 each pup was placed individually in the testing apparatus for 20 min/day during its mother's 3-hr. feeding period and offered the Turtox diet in one food cup and the Purina diet in the other. The location of the two food cups was reversed and the test enclosure rotated  $60^\circ$  between pups.

The experimenter weighed each cup before and after each pup's feeding period on a Mettler balance accurate to .0001 gm. Any spillage was returned to its appropriate cup before weighing.

### Results and Discussion

The main results of Experiment 1 are presented in Figures 2 and 3, which show, respectively, the mean amount of Purina diet and the mean amount of Purina as a proportion of the total amount eaten by pups in the Purina and Turtox groups on Days 18-23. Data from Day 17 are not presented because too few animals ate any food at all to give meaningful data. Only animals which ate more than .01 gm. of

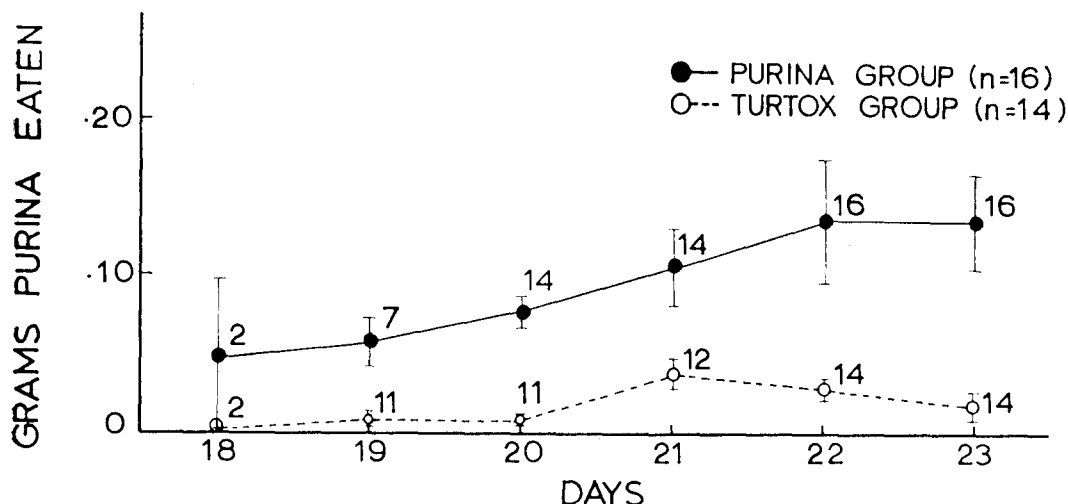


FIG. 2. Mean amount of Purina diet eaten by pups in Experiment 1. (Flags indicate  $\pm 1\sigma$ .)

either diet on any day are included in Figures 2 and 3. It is clear from inspection of the figures that pups in the Purina group ate significantly more of the normally nonpreferred Purina diet than did those in the Turtox group. Comparison of intakes of Purina and Turtox diets of pups cross-fostered and reared by their natural mothers showed no difference between groups, indicating the absence of prepartum and first 5-day postpartum effects of maternal diet on the food preference of pups at weaning. This last result might be predicted on the basis of data suggesting an absence of differential responses to tastes (Jacobs & Sharma, 1969) and odors (Salas, Schapiro, & Guzman-Floris, 1970) in rat pups during the first 5 days postpartum.

The results indicate that mother's diet during the nursing period can have a major effect on pups' early food preference even under conditions where mothers and pups are not allowed to interact directly in the presence of solid food.

#### EXPERIMENT 2

The results of Experiment 1 indicate that the diet of a lactating mother can play a major role in determining the dietary preferences of her pups at weaning. The remainder of this article is concerned

with the determination of the means by which information concerning the mother's diet is transmitted to the pups during the nursing period. Three alternative means of transmission suggest themselves. First, it is possible that the young gain familiarity with the diet their mother is eating as a result of ingesting samples of her feces in the home cage (unpublished observations of pre-weaning rat pups by the authors indicate that from 14 days of age pups often nibble feces lying about their home cage floor). Second, it is reasonable to assume that particles of food cling to the mother's fur and vibrissae while she is feeding and that the pups may gain familiarity with the diet their mother is eating by ingesting these residual particles. Third, it is possible that the flavor or odor of the mother's diet is in some way incorporated into her milk and that the young become familiar with her diet as a direct consequence of the ingestion of mammary secretions. The present experiment is concerned with the first of these alternatives.

If the young rats are becoming familiar with the diet their mother is eating by ingesting feces that they find in their home cage, then the presence in the home cage of feces from an animal eating another diet should disrupt the preference for the mother's diet observed in Experiment 1.

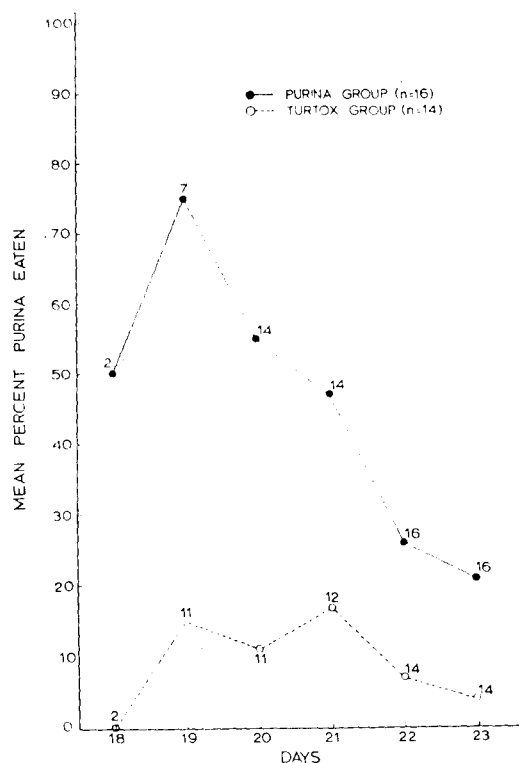


FIG. 3. Mean amount of Purina diet eaten, as a percentage of total intake, by pups in Experiment 1.

### Method

**Subjects.** Subjects were two female hooded rats obtained from the Quebec Breeding Farm and litters of their offspring which were reduced to eight pups/litter on the day of birth. Two additional female hooded rats of the same strain were also used.

**Procedure.** With the exception of the conditions under which lactating females and their young were maintained in their home cage, the procedure for the present experiment is identical to that of Experiment 1. In this experiment each litter and its nursing mother was kept in the lower half of a duplex cage made by placing two  $12 \times 14 \times 6\frac{1}{2}$  in. plastic cages one atop the other. A  $10 \times 8\frac{1}{2}$  in. piece of the floor of the upper cage was removed (leaving a small ledge on which the resident of the upper cage could stand to avoid having its feet bitten) and replaced with  $\frac{1}{2}$ -in. hardware cloth through which urine and feces could easily fall to the cage below. One of the nursing females residing in the lower cage was fed the Purina diet and the other the Turtox diet during the three daily 1-hr. feeding periods. The resident of the upper half of each cage was removed from it to a separate cage and fed the opposite diet from her down-

stairs neighbor for 3 hr/day. Pups were tested for dietary preference as in Experiment 1.

### Results and Discussion

The results of Experiment 2 are presented in Figure 4. The solid lines in this figure represent the amount of Purina eaten, as a percentage of total intake, by pups whose mothers were fed Turtox and Purina in the present experiment. Comparison with the data presented in Figure 3 indicates that the presence of foreign feces does not appreciably effect the food preferences of the pups.

### EXPERIMENT 3

The present experiment examines the possible role of food particles clinging to the coat of the mother in determining the dietary preference of the pups at weaning observed in Experiment 1.



FIG. 4. Mean amount of Purina diet eaten, as a percentage of total intake, by pups in Experiments 2 and 3.

### Method

**Subjects.** Subjects were four female hooded rats obtained from the Quebec Breeding Farm and their litters.

**Procedure.** The procedure used here was identical to that of Experiment 1 with two exceptions. First, each litter was reduced to 12 rather than 8 pups at birth. Second, before being returned to her home cage after each feeding period, each mother was wiped clean with a paper towel and then brushed on her chest, forelegs, and vibrissae with .1 gm. of food particles prior to being returned to her home cage after each feeding period. One Turtox and one Purina fed mother were brushed with Turtox diet and one of each with Purina diet before being returned to their home cages.

### Results

The results of Experiment 3 are presented in Figure 4. The dotted lines represent the percentage of Purina eaten by pups whose mothers ate Turtox and Purina and were brushed with the opposite diet. Data are not presented for the control conditions (Turtox fed, Turtox brushed and Purina fed, Purina brushed) as the data from these groups were essentially identical to the data of the Turtox and Purina groups shown in Figure 3. Comparison with Figure 3 indicates that placing particles of a diet other than that on which a mother is feeding on the anterior portion of her body does not affect the pups' dietary preferences at weaning. It is, therefore, highly unlikely that such particles are the means by which the pups become familiar with their mother's diet.

#### EXPERIMENT 4

The results of Experiments 2 and 3 suggest that neither the mother's feces nor particles of food clinging to the mother's coat are responsible for the observed effect of the mother's diet on the food preferences of her young. The present experiment examines the role of mother's milk in the determination of this food preference.

### Method

The method was similar to that of Experiment 1. Subjects were eight recently parturite female hooded rats obtained from the Quebec Breeding Farm, their litters reduced to eight pups/litter on the day of birth, and four virgin female rats of the same strain. Pups were cross-fostered at 5 days

of age so that each recently parturite female raised two of her own pups and two from each of three other litters. Each pup had a lactating mother with whom it nursed 14 hr/day (major mother) and a female with whom it spent 6 hr/day (minor mother) from Days 5-23 postpartum, when the experiment was terminated. Pups were tested and mothers fed during the remaining 4 hr. of each day. Minor mothers were of two kinds, either lactating females or virgin females in whom maternal behavior but not lactation had been induced as a result of exposure to neonatal rat pups (Rosenblatt, 1967). There were eight groups of pups in all which can be described in terms of the diets of their major and minor mother (either Turtox [T] or Purina [P]) and the reproductive condition of their minor mothers (either lactating [L] or virgin [V]). If the notation LP-VT is used to describe a group of pups whose major mother was fed Purina and whose minor mother was a virgin female eating Turtox, the eight groups can be described as follows: LP-LP, LP-LT, LT-LT, LT-LP, LP-VP, LP-VT, LT-VT, LT-VP. The first four groups are those with lactating minor mothers and the last four those with virgin minor mothers. Pups were tested as in Experiment 1.

### Results and Discussion

All virgin females showed a full range of maternal behaviors directed toward the pups placed in their care (nest building, retrieval, crouching over the young, etc.). The main results of Experiment 4 are presented in Figures 5 and 6, which indicate the mean amount of the Purina diet eaten by the pups in the various groups as a percentage of total intake. Data for pups with virgin and lactating minor mothers are given in Figures 5 and 6, respectively.

Inspection of Figure 5 reveals that exposing pups to a nonlactating minor mother showing extensive maternal behavior for 6 hr/day has no effect on their food preferences at weaning. There is no difference in dietary preference between the LP-VP group and the LP-VT group or between the LT-VT group and the LT-VP group. To the contrary, as shown in Figure 6, exposing pups to a lactating minor mother can have a major effect on the pups' food preferences during their first few days of ingesting solid food. On Days 18, 19, and 20 there are clear differences between LP-LP and LP-LT groups and between LT-LT and LT-LP groups in the proportion

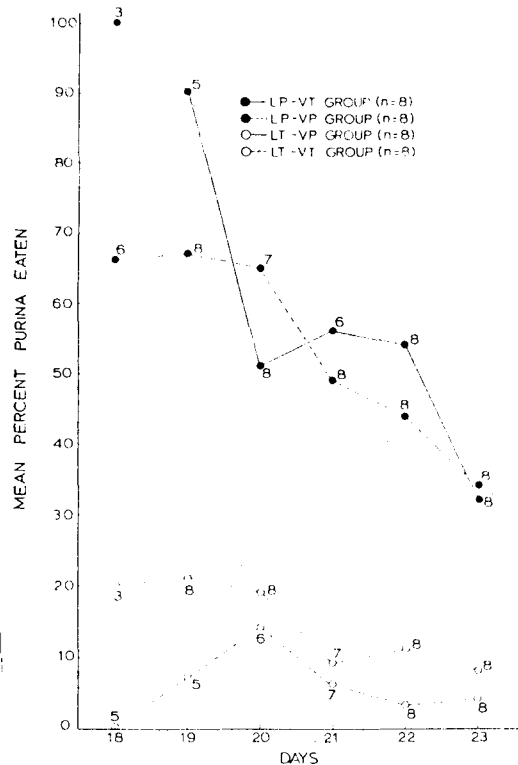


FIG. 5. Mean amount of Purina diet eaten, as a percentage of total intake, by pups with virgin minor mothers.

of Purina they eat. The interpretation of these data most consistent with the results of Experiments 2 and 3 is that the cues causing pups to preferentially ingest the diet their mother has been eating during the nursing period are contained in the pups' mothers' milk.

#### GENERAL DISCUSSION

The evidence presented here indicates that young rat pups become familiar with cues associated with the diet their mother is eating as a result of ingesting her milk. More important, these cues appear to markedly affect the initial dietary preference of the young. Although these mother's-milk-determined preferences are quite transitory in the experimental situation described here, rat pups living under more natural conditions show far more lasting effects of cues associated with mother's milk in their choice of food for ingestion

(Galef & Clark, 1972) as do rats tested at later ages (Le Magnen, 1969).

At first glance the proposal that complex long chain molecules associated with dietary flavor or smell can pass intact from the mother's gastrointestinal tract into her milk might seem untenable. However, in spite of the general paucity of information concerning the excretion of ingested chemicals in breast milk (Arena, 1970), evidence derived from a variety of sources indicates that such transfer can readily occur. Clinical literature indicates that a wide variety of substances (including antibiotics, sulfonamides, most alkaloids, salicylates, bromides, quinine, alcohol (Eastman & Hillman, 1961), nicotine, marijuana, DDT, oral contraceptives, and amphetamines (Arena, 1970; Knowles, 1965), when ingested by lactating human females, pass intact into the nursing in-

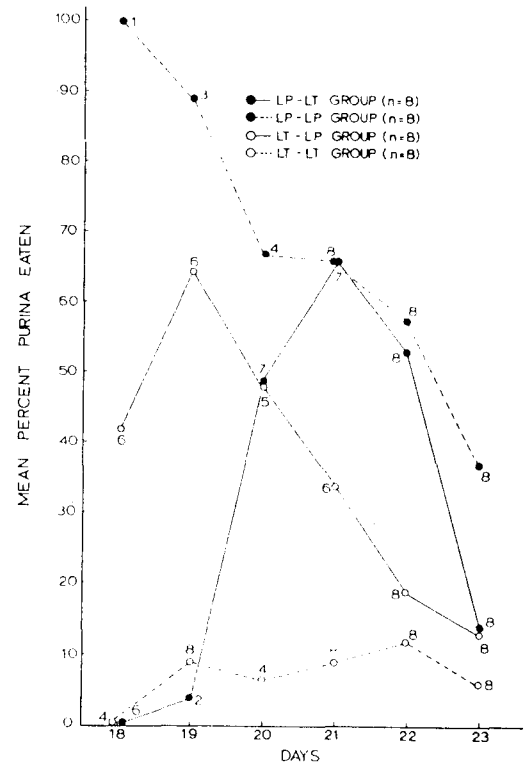


FIG. 6. Mean amount of Purina diet eaten, as a percentage of total intake, by pups with lactating minor mothers.

fant. Perhaps more relevant to the present discussion, Ling, Kon, and Porter (1961) have described changes in the flavor of cows milk associated with the ingestion of certain natural foodstuffs. The evidence, thus, suggests that physiological mechanisms exist whereby complex substances ingested by the mother can become incorporated into her milk and transferred intact to her young.

Of course, even if the young can become familiar with taste or olfactory cues associated with their mother's diet via her milk, this does not mean that they will seek out and preferentially ingest food substances possessing these cues. An important question remains as to the causes of the observed appetitive behavior of the young in the series of investigations described above. Unfortunately our present data do not permit elucidation of this question.

It is clear, however, that the cues provided by the composition of mother's milk may be sufficient to provide the young rat with sure knowledge of its mother's diet, thus providing the weanling rat with an alternative method to that described in Galef (1971) and Galef and Clark (1971a) of locating needed safe nutrients in its general environment.

The tendency of the young to ingest the same diet as the adults of their colony is, thus, seen to be determined by two separate complementary behavioral tendencies. Weanling rats both follow the adults of their colony to a food source and seek a diet having gustatory or olfactory cues similar to those found in their mother's milk.

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