MOTHER'S MILK:
A MEDIUM FOR TRANSMISSION OF CUES REFLECTING THE FLAVOR OF MOTHER'S DIET

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After being poisoned following ingestion of 1/2 cc of milk manually expressed from a female rat eating a diet different from that of their mother, nursing rat pups show an aversion to that female's diet during weaning. Pups fed three 1/2-cc meals of milk manually expressed from a female eating a diet different from that of their mother and which are not poisoned show a slightly enhanced preference for that female's diet at weaning. These results are interpreted as offering additional evidence (a) that a mother's milk contains gustatory cues reflecting the flavor of the mother's diet and (b) that these cues are sufficient to influence dietary preference at weaning.

Adult rats are capable of influencing the early dietary preferences of their young in at least two ways. First, pups who take their first meals of solid food in enclosures with adults of their colony are strongly attracted to feeding sites by the physical presence of adults in the vicinity of those sites. As a result of this attraction, pups will, with high probability, ingest their first meals of solid food in an area where adults are feeding and will preferentially ingest the same foodstuffs which the adults of their colony are eating if available diets are spatially separated from one another (Galef, 1971; Galef & Clark, 1971a, 1971b, 1972). Furthermore, rat pups who take their first meals of solid food in isolation from conspecifics still will actively seek and preferentially ingest the diet which the female from whom they have been nursing has been eating during the nursing period (Galef & Clark, 1972; Galef & Henderson, 1972). Initial studies of this second mode of influence suggest that the diet which a lactating female rat ingests during the nursing period directly affects the flavor of her milk, enabling pups to recognize the diet she has been eating when they wean to solid food and causing them to ingest that diet preferentially (Galef & Henderson, 1972; Le Magnen & Talon, 1968).

The evidence implicating mother's milk as the medium by which a lactating female rat transmits cues reflecting the flavor of her diet to her young is indirect and not entirely convincing (Galef & Henderson, 1972). Furthermore, while it has been shown in a few instances that the flavor of ingesta can affect the flavor of mammalian milk (Dougherty, Shipe, Gudnason, Leford, Peterson, & Scarpellino, 1962; Shipe, Leford, Peterson, Scanlan, Geerken, Dougherty, & Morgan, 1962), the accuracy with which the flavor of a lactating female's diet is reflected in the flavor of her milk and the effects of milk flavor on the early feeding preferences of the young remain unknown. The first experiment reported below provides a direct experimental test of the possibility that mother's milk serves as a medium for the transmission of flavor cues directly reflecting the flavor of the mother's diet from lactating rats to their nurslings. The second experiment examines the possibility that these cues are sufficient to affect pups' food preferences during weaning.

**EXPERIMENT 1**

Rats will rapidly learn to avoid ingesting a novel-flavored solution if ingestion of that
solution is followed by aversive gastrointestinal events (Garcia & Ervin, 1968). It is our hypothesis that the milk of a lactating female rat contains gustatory cues reflecting the flavor of her diet with sufficient accuracy to permit pups to recognize that diet during weaning to solid food. If this hypothesis is correct, then pups which have been poisoned immediately following the ingestion of milk from a female eating a diet different from that of their mother should show an aversion to the second female's diet at weaning.

**Method**

**Subjects**

The subjects were seven recently parturate female hooded rats obtained prepartum from the Canadian Breeding Farms and the seven experimental foster litters (eight pups/litter) which these females nursed. Twenty-four additional recently parturate rats (referred to below as nurses) obtained from the same supplier served as the source of pups for experimental litters and as the source of small quantities of milk to be fed to pups in the seven experimental litters. Each experimental litter was composed of two pups taken on their day of birth from each of four of the nurses.

**Procedure**

All 31 females and their litters were housed in plastic home cages (12 × 14 × 6½ in.) with food (Purina Laboratory Chow) and water available ad lib. On Day 5 postpartum each of the seven females whose litters were to be experimental subjects was placed on a 3 hr/day (1:30-3:30 p.m., 10:30-11:30 p.m.) feeding schedule. These females were removed to individual cages separate from their foster litters for daily feeding periods and fed one of two diets. Four of these females were fed Purina Laboratory Chow (to be referred to below as Diet A) during daily feeding periods, and three were fed Tutrix "fat-sufficient diet" (to be referred to below as Diet B), a powdered casein- and sucrose-based diet highly preferred by rats to Diet A. Twelve of the nurses were fed Diet A and 12 were fed Diet B ad lib in their home cages.

The experiment proper began when pups in experimental litters reached 21 days of age. At this time, the pups were assigned to one of the four conditions described below. Eight pups whose foster mothers were eating Diet A (Diet A pups) and eight whose foster mothers were eating Diet B (Diet B pups) were used in each of the first three conditions. Eight Diet A pups were used in the fourth condition.

**Experimental group.** Each pup in this group was fed, from a 1-cc Tuberculin syringe, ½ cc of milk expressed manually from a nurse eating the diet opposite that of its own foster mother and was immediately injected with .12 M LiCl (ip, 2% volume/body weight). Twenty-four hours following manual feeding and injection, pups were tested for dietary preference as described below. During the 24 hr. intervening between manual feeding and testing, pups were returned to their foster mothers.

**Flavor control.** Pups in this group were treated identically to those in the experimental condition except that they were manually fed ⅓ cc of milk expressed from a nurse eating the same diet as their own foster mother before poisoning.

**Temporal control.** Pups in this group were treated identically to those in the experimental condition except that (a) they were injected with .12 M LiCl (ip, 2% volume/body weight) on Day 20 postpartum. 24 hr. prior to receiving expressed milk from a female eating the opposite diet from that of their own foster mother, and (b) they were not poisoned following manual feeding on Day 21 postpartum.

**Control condition.** Pups in this group were treated identically to those in the flavor control condition but were never poisoned.

**Testing**

Twenty-four hours after receiving its meal of expressed milk, each pup was tested individually to determine its food preferences during its first hours of feeding on solid food. The testing apparatus, described in detail in Gale and Henderson (1972), consisted of a 10-in.-diam. plastic laboratory dish with two externally mounted detachable food cups. Diet A was placed in one food cup and Diet B in the other. The pups were left in the testing apparatus for 24 consecutive hr. Water was provided in a shallow dish placed in the center of the enclosure from Hour 9 of testing on. The experimenter determined 3-, 9-, and 24-hr. intakes of each diet by weighing food cups on an analytical balance accurate to .0001 gm.

**Milking**

Twenty-four hours prior to the time a given nurse was to be milked, she was separated from her litter and housed in a separate cage with her usual food and water available ad lib. Immediately prior to milking, the nurse was anesthetized (2.2 cc/kg ip injection of Equi-Thenin), and the fur surrounding each nipple was then clipped. Expression of milk was achieved by gentle kneading of the nipple and surrounding tissue. Milk droplets were collected in a Pasteur pipette and transferred to a small test tube. Most females yielded 2 cc or more of milk in a 10-40 min. period. The expressed milk was fed to each pup from a 1-ce Tuberculin syringe while the pup was lightly held in the experimenter's hand.

**Results and Discussion**

The results of Experiment 1 are presented in Figure 1, which indicates the mean
amount of Diet B as a percentage of total intake eaten by pups during their 24 hr. in the test apparatus. Data are presented for 9- and 24-hr. measures only because too few pups ate measurable quantities of either diet during their first 3 hr. in the test apparatus to give meaningful data.

Comparison of Diet A flavor and temporal controls with the corresponding Diet B groups reveals the previously described (Galef & Henderson, 1972) effects of mother's diet on pups' food preferences. Rat pups are clearly influenced in their choice of diet for early ingestion by the diet of the female from whom they nurse.

Furthermore, it is clear from examination of the lower portion of Figure 1 that Diet A pups in the experimental condition ate far less Diet B than pups in any control condition. Although a ceiling effect limited the magnitude of differences in percentage of Diet B intake between Diet B experimental and control groups, these differences were still in the predicted direction.

The results of the present experiment indicate that cues incorporated in the milk of
a lactating rat are sufficient to permit identification by pups of the diet eaten by a lactating female whose milk they have ingested. Pups poisoned after ingesting samples of milk expressed from a lactating female eating a diet different from that of their mother avoid that different diet during weaning.

Of course, the fact that cues in a lactating female rat’s milk are sufficient to allow pups to identify the diet she has been eating during lactation does not imply that the pups will actually use those cues in selecting a diet for ingestion during normal weaning.

**Experiment 2**

The present experiment was designed to determine whether or not the ingestion of rat milk containing cues reflecting the taste of a given diet is sufficient to cause pups to increase their preference for that diet.

**Method**

**Procedure**

The subjects were three recently parturient female hooded rats, obtained prepartum from the Canadian Breeding Farms, and the experimental foster litters which these females nursed. Eighteen additional lactating females obtained from the same supplier served as nurses.

**Procedure**

Litter composition, maintenance, and milking procedures were the same as those used in Experiment 1. In the present experiment all three females whose pups were to serve in experimental litters were fed Diet A in enclosures separate from those young for 3 hr/day (1:30-3:30 p.m., 10:30-11:30 p.m.). On Days 18, 19, and 20 postpartum, each pup in two of the experimental litters was fed 1/2 cc/day of milk manually expressed from the same nurse served as a control. In order to speed ingestion, pups in these litters were removed from their foster mothers for 3 hr prior to each feeding of expressed milk. Pups in one litter (experimental group) received three 1/2-cc meals of milk expressed from a nurse eating Diet B; pups in a second litter (control group), three 1/2-cc meals of milk expressed from a nurse eating Diet A; and the remaining eight pups (baseline group), no manually expressed milk at all.

**Results and Discussion**

The results of Experiment 2 are presented in Figure 2. The data indicate that pups who have ingested milk expressed from a female eating Diet B show a slightly enhanced preference for Diet B during the first 24 hr of weaning. At both 9 and 24 hr of weaning, pups in the experimental litter eat more Diet B than those in either control. Unfortunately, it is only after 24 hr of testing that the differences between groups approach acceptable levels of significance (Mann-Whitney U test: control vs. experimental at 24 hr., U = 13.5, p = .032; baseline vs. experimental at 24 hr., U = 19, p = .097). It is important to remember, however, that experimental pups received only 1 1/2 cc of milk from a nurse ingesting Diet B, and it is at least possible that with experience of greater quantities of Diet B milk, more robust effects would be observable. Unfortunately, practical difficulties precluded the delivery of greater quantities of milk to the pups.

Comparison of Figures 1 and 2 indicates that pups in the baseline and control conditions of the present experiment show a somewhat greater preference for Diet B than pups in any of the three Diet A control conditions. The cause of these differences is not known.

**General Discussion**

Comparison of Diets A and B temporal and flavor controls in Experiment 1 revealed the effects of the diet of a lactating
female rat on the food preferences of her pups at weaning. Diet A control pups ate far less Diet B than their Diet B counterparts. Previous experiments (Galef & Henderson, 1972) have implicated mother's milk as the medium by which cues concerning the mother's diet are transmitted from mothers to young. The present experiments provide further evidence that mother's milk contains cues sufficient to enable pups to recognize their mother's diet at weaning and weaker evidence that those cues are sufficient to produce a preference for their mother's diet.

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