Taste Aversions to Mother’s Milk: The Age-Related Role of Nursing in Acquisition and Expression of a Learned Association

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The development of taste aversion learning to novel cues contained in mother’s milk was examined in rat pups. Pups receiving distinctive milk by experimenter-delivered oral infusions followed by toxicosis formed an aversion to the dam’s diet. Robust aversions were learned as early as Day 10 and were retained for at least 11 days. When the same distinctive milk was obtained directly from a foster mother through nursing, however, only weanling-age pups (over 20 days) formed an aversion. X-ray analysis of nipple location in the mouths of suckling pups suggested that pups between the ages of 10 and 21 days receive milk at a similar tongue locus. Flavored milk was then delivered at specific time intervals in controlled quantities through tongue cannulas implanted at loci corresponding to the nipple position shown by the X-rays. Cannulated preweanling pups that were attached to a nipple during milk delivery failed to associate the taste cue with illness, whereas both preweanlings off the nipple and weanlings on the nipple acquired aversions to the taste cue in the milk. The evidence obtained in these experiments suggests that pups of all ages are incapable of expressing a taste aversion in a nursing situation and that preweanling pups in particular are also deficient in acquiring aversions within a suckling context. The inability of preweanling pups to acquire taste aversions in a nursing situation appears to result from a failure to associate taste cues with illness rather than a failure to detect taste cues obtained from a nipple.

The early ontogenesis of learning has evaded vigorous empirical pursuit in the past, in part because traditional laboratory methods are generally inappropriate for training and testing neonates. More recent successful demonstrations of learning in preweanling animals have used procedures that incorporate stimuli and response measures tailored to the neonate. It is not surprising that many of these techniques focus on learning abilities relevant to suckling and other aspects of early behavior related to ingestion. Learning has been demonstrated in nipple selection by kittens (Rosenblatt, 1971), control of suckling behavior in rat pups (Teicher & Blass, 1976), and the development of anticipatory conditioned reactions to experimenter-provided handling cues that preceded feeding (Thoman, Wetzel, & Levine, 1968). With nonnutritive suckling as reinforcement, evidence has also been obtained that 7-day-old rat pups are capable of learning a spatial discrimination task (Kenny & Blass, 1977) and that 10-day-old pups can learn to anticipate the absence of a previously present event (Amsel, Burdette, & Letz, 1976).

Several recent studies have investigated the acquisition and retention of conditioned taste aversions in neonates and this paradigm appears to hold much promise for studying the development of both learning
and memory. It has been demonstrated that 10-day-old rat pups can acquire conditioned taste aversions to grape juice (Campbell & Alberts, 1979) and that 21–22-day-old rat pups can acquire one-trial conditioned taste aversions to cues present in mother’s milk (Galef & Sherry, 1973). Both of these studies utilized a technique of oral infusion of the conditioned stimulus (CS) directly into the mouth of the pups. The combined results of these studies provide a basis for an alternative strategy in investigating learning and memory abilities in immature organisms. If rat pups younger than 21 days of age are also capable of discriminating taste cues in mother’s milk, then it might prove possible to obtain evidence of early associative learning mechanisms both during and after the nursing period. By presenting the CS (a novel taste) through the milk of a foster mother, an attempt can be made to condition and measure an aversion in preweanling subjects. Retention studies can also be performed after weaning with food preference tests that include the diet from which the cues in the milk were derived.

Experiment 1: Taste Aversion Conditioning with Oral Infusion of Milk and a Food Preference Test at Weaning

The purpose of this experiment was to determine whether preweanling rat pups are capable of discriminating specific taste cues added to milk and avoiding these cues when they appear in solid-food substances. Pups between the ages of 10 and 21 days received an initial pairing of flavored milk with poisoning, followed by a food preference test at weaning. Procedurally, Experiment 1 resembled Galef and Sherry’s (1973) study in which milk, taken from the teats of rat dams maintained on a distinctively tasting food, was placed in the mouth of weanling-age rat pups and was followed with an ip injection of lithium chloride (LiCl). Rat pups made ill with LiCl (the unconditioned stimulus; US) avoided ingesting the diet eaten by the donor dams, whereas subjects in the various control conditions displayed no such avoidance. This indicates the ability of weanling-age pups to detect food-related taste cues in mother’s milk and to reject or select solid foods on that basis. The pups used in Galef and Sherry’s study were 21 days old when given the taste–illness experience; solid-food tests were administered 24 hr later. Because the altricially born rat undergoes vast maturational changes during the first 3 postnatal weeks, these results may not be directly applicable to younger animals. It was therefore necessary to explore carefully the parameters of taste aversion training and testing for rat pups of preweanling ages.

Experiment 1 consisted of two experimental “preparations” for conditioning taste aversions in rat pups. An initial investigation, conducted with 15- and 20-day-old pups, included three control conditions to determine whether the injection procedure, exposure to the CS alone, or unpaired presentations of the CS and US would, themselves, produce a suppression of Diet G (geraniol) preference. On the basis of the results of the initial investigation, the main investigation included a broader range of ages (10–18 days) and only the conditioning and injection-control conditions. The CS was geraniol-flavored milk in both the initial and main investigations. In the main investigation the flavored milk was taken directly from the teats of lactating rat dams maintained on a geraniol-flavored diet. However, milking rats is an arduous and time-consuming practice, so the initial investigations were performed with cow’s milk to which geraniol was added. Geraniol in rat’s milk and in cow’s milk is distinctive to human taste. In both investigations the pups were given a three-choice food preference test at weaning.

Method

Experiment 1A: Oral Injection of Flavored Cow’s Milk and a Food Preference Test at Weaning

Subjects. A total of 64 rat pups served as subjects in this experiment. All subjects were born in the Indiana University colony, outbred from stock purchased from Laboratory Supply (Indianapolis), and reared in standard polypropylene maternity cages (48 X 20 X 26 cm). The colony was maintained on a 16:8 hr light/dark cycle. Three days after birth (Day 0) litters were reduced to eight pups each. Until the day of experimentation, all subjects remained with and nursed from their natural
mothers which were being fed a diet of the usual Purina Rat Chow pellets.

**Milk source.** The CS utilized in this experiment was prepared by adding .1 ml of geraniol to 100 ml of Carnation evaporated milk, which produced a flavored milk highly distinctive to a human observer. The milk was warmed to room temperature (21–22 °C) prior to use.

**Training.** Training occurred on either Day 15 or Day 20 (32 pups at each age). On the day of training, litters were removed from the mother and placed for 3.5 hr in an incubator maintained at 32 °C. At the end of the deprivation period, pups were marked, weighed, and assigned to one of the conditions described below. Training began with oral infusion of the flavored cow’s milk. Pups were hand-held while approximately .1 ml was slowly infused into the mouth by a 1-ml syringe with a 2-cm length of polyethylene tubing over the needle tip. Swallowing and tongue movements were elicited at both ages. In two of the treatment conditions (described below), the first oral infusion was followed by ip injection of LiCl or isotonic saline. All pups received two exposures to the flavored milk. Thus a total of .2 ml were infused over a 3-min interval.

On the day of training, two pups from each of eight litters were assigned to one of the following treatment conditions: (a) conditioning (G-Li)—oral infusion of the CS followed immediately by an ip injection of .15 M LiCl (2% body weight) and a second exposure to the CS; (b) injection control (G-Sal)—same as above except pups were injected with an equal volume of isotonic saline; (c) flavor control (G)—two discrete oral infusions of the CS; and (d) temporal control (Li-G)—two oral infusions of the CS and an ip injection of .15 M LiCl 24 hr prior to or subsequent to training.

After training, pups were replaced in the incubator for 2 hr and then returned to their litter cages.

**Testing.** The testing apparatus consisted of a standard wire mesh cage (26 X 22 X 20 cm), containing an 8 X 25 cm metal food tray. Three plastic food cups (2.5 X 4.5 cm) were set 4 cm apart in the tray. Subjects were given access to three powdered diets: unflavored Purina Rat Chow (Diet P), Purina Rat Chow flavored with geraniol—the cue contained in the milk CS (Diet G)—or acetophenone—a novel, distinctive taste (Diet A). The flavors were added at a concentration of 4 μl/g. Approximately 10 g of each diet were placed in the separate food cups, and water was provided ad lib.

Testing took place on Day 21 or Day 22 for both age-groups. Following a 4-hr deprivation period, the subjects were placed in individual cages and allowed a 4-hr period of access to the three diets. Intake of each diet was determined by weighing food cups on an analytical balance accurate to .01 g. Spillage was collected and returned to the proper cup for weighing. A preference ratio was calculated for the diet containing taste cues present in the flavored milk (Diet G) by the following formula: amount of Diet G consumed/total amount consumed (Diets A + P + G) X 100.

**Experiment 1B: Oral Injection of Flavored Rat’s Milk and a Food Preference Test at Weaning**

**Subjects.** A total of 106 pups served as subjects.
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CS alone (Group G) or unpaired presentations of the CS–US (Group Li–G) do not significantly alter the subjects’ behavior in a food preference test at weaning. Rat pups as young as 15 days of age are clearly capable of discriminating taste cues in cow’s milk and acquire and express significant aversions to these taste cues.

**Experiment 1B**

The mean percentage preference for Diet G was greatly reduced in the conditioning group compared with the injection-control group. An overall analysis of variance revealed a significant treatment effect, \( F(1, 26) = 9.7, p < .005 \). Figure 2 shows the percentage preferences for Diet G in the three age-groups. Although a significant difference between experimental and control pups was obtained in each group, suppression of Diet G became more pronounced with age. Diet G preference scores were 28.5%, 19.3%, and 9.26% for pups receiving the taste–illness pairing in the 10–12, 13–15, and 16–18 day age-groups, respectively.

**Discussion**

The results of Experiments 1A and 1B suggest that 10- to 20-day-old rat pups can detect the taste of geraniol in milk, associate that taste with LiCl-induced illness, and avoid ingesting geraniol even when it is contained in a safe, familiar base diet (Purina Rat Chow). Geraniol survives metabolic degradation and passes through the rat dams’ lactational system in clearly detectable quantities, as evidenced by the performance of pups in Experiment 1B. The suppression of Diet G intake by pups in the conditioning group appears to be due to the formation of an association between the taste of geraniol and illness and cannot be accounted for by supposed aversive characteristics of the injection procedure or to some nonspecific effect of LiCl. Moreover, the use of a three-choice test allowed the inclusion of a novel taste (Diet A), in addition to the pups’ safe, familiar food (Diet P) and the test taste (Diet G). Intake of the novel taste did not differ between treatment groups, which indicates that experimental pups were not avoiding Diet G simply because of a general toxicosis-induced neophobia. There was, however, great variability in the subjects’ reactions to the novel diet. The majority of pups consumed only small quantities of Diet A regardless of the initial treatment received, but 10%–15% expressed a preference for Diet A.

The present experiment thus confirms the basic finding of Galef and Sherry (1973) that rat pups can recognize cues in solid food on the basis of chemical constituents in a milk diet, and it extends the phenomenon to pups as young as 10 days of age. The results also
support the finding of Campbell and Alberts (1979) that rat pups as young as 10 days of age can rapidly form conditioned taste aversions. The 11-day retention of the aversion by 10-day-old pups exceeds even that reported by Campbell and Alberts and underscores their finding that memory for conditioned taste aversions is well developed in the altricial rat at ages when other associations are rapidly forgotten (see Campbell & Coulter, 1976; Campbell & Spear, 1972). It was noted in the present results that the magnitude of the conditioned aversion increased with age (see Figure 2). However, because all pups were tested with a solid-food test at weaning, age of training was confounded with retention intervals. To eliminate this retention-interval confound, it might be possible to use the same milk-borne CS in a nursing test with preweanling subjects and thereby test pups at a constant posttreatment interval.

Experiment 2: Taste Aversion Conditioning and Testing in a Nursing Situation

The present experiment was designed to determine whether rat pups will readily form an aversion to distinctively tasting milk obtained from a foster mother (as in Experiment 1B) when both training and testing are performed in the nursing situation. This procedural adaptation would facilitate the study of learning and memory in preweanling subjects, too immature to be used in food preference tests. The subjects in the present study ranged from 3 to 21 days of age, in order to include age-groups younger than those previously tested as well as age-groups previously shown to be capable of rapid taste aversion conditioning. Pups received distinctively tasting milk by nursing from a foster mother and were tested 1 day later in the same nursing situation.

Method

Subjects

Ninety-six Sprague-Dawley rat pups (from 12 litters) were subjects. Litter size and housing conditions were the same as in Experiment 1. Subjects were offspring of mothers on flavored diets (Diet G: two litters; Diet A: one litter) or an unflavored diet (Diet P: nine litters). The females on flavored diets were removed prepartum and maintained in separate colony rooms. The flavors were added to the base diet of Purina Rat Chow at a concentration of 4 μl/g.

Training

On the day of training, each litter was randomly divided into a conditioning group (CS-Li) and an injection-control group (CS-Sal). Subjects were deprived for 3.5 hr in an incubator (32 °C), marked, weighed, and placed with a foster mother for a 1-hr nursing period. In each case the foster mother was maintained on a diet different from that of the pups’ natural mother, i.e., offspring of Diet G females were placed with a Diet A or Diet P foster mother. The conditions under which training occurred are summarized in Table 1.

Testing

Taste aversions were measured by the pups’ intake of distinctively tasting milk from the foster mother from which they had nursed during training. Body weight gain after a 1-hr test was used as the measure of intake by nursing. Testing occurred 24 hr after training in order to minimize possible problems associated with rapid forgetting in the neonates. All subjects appeared to have recuperated, as evidenced by normal overnight weight gain. Pups were removed from their natural mother and deprived for a 3.5-hr period in an incubator. Prior to recording initial weight, the subjects’ bladders were voided by gently stroking the anogenital area with a Q-tip in order to eliminate the confound of excretion during the test period. Litters were placed with the foster mother for 1 hr and then reweighed on an analytical balance accurate to .01 g.

Table 1

<table>
<thead>
<tr>
<th>Age at training (in days)</th>
<th>Diet of natural mother</th>
<th>Diet of foster mother</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>P</td>
<td>G</td>
</tr>
<tr>
<td>3</td>
<td>G</td>
<td>A</td>
</tr>
<tr>
<td>7</td>
<td>P</td>
<td>G</td>
</tr>
<tr>
<td>8</td>
<td>G</td>
<td>P</td>
</tr>
<tr>
<td>9</td>
<td>P</td>
<td>G</td>
</tr>
<tr>
<td>10</td>
<td>A</td>
<td>G</td>
</tr>
<tr>
<td>11</td>
<td>P</td>
<td>A</td>
</tr>
<tr>
<td>13</td>
<td>P</td>
<td>G</td>
</tr>
<tr>
<td>21 (4 litters)</td>
<td>P</td>
<td>G</td>
</tr>
</tbody>
</table>

Note. Diets utilized in this experiment consisted of unflavored Purina Rat Chow (Diet P) and Purina Rat Chow flavored with geraniol (Diet G) or acetophenone (Diet A) at a concentration of 4 μl/g.
Results and Discussion

Rat pups 3–21 days of age failed to display a taste aversion to the milk of foster mothers on distinctive diets when training and testing occurred in the nursing situation. Figure 3 shows the mean total intake of milk during the test period for pups trained at the ages of 3–7 days (n = 24), 8–10 days (n = 24), 11–13 days (n = 16), and 21–22 days (n = 32). It is clear from Figure 3 that experimental and control pups in each age-group consumed equivalent amounts. Since there were no age-related differences in taste aversion learning, the data were collapsed across ages for statistical analysis. The results of a t test for related measures (r = .92) revealed no significant differences between groups, t(23) = .783, p > .25.

The failure of pups to acquire an aversion to the milk of foster mothers on distinctive diets cannot be attributed to the qualities of the CS since at least Diet G rat milk was found to be a highly adequate taste cue for aversive conditioning in rat pups (Experiment 1B) and 9 of the 12 litters in this experiment were exposed to Diet G foster mothers. The results of both the previous experiment and related work (Campbell & Alberts, 1979; Gregg, Kittrell, Domjan, & Amsel, 1978) demonstrate that the pre-weanling pup, at least down to 10 days of age, is capable of such conditioning. These considerations suggested that some aspect(s) of the training or testing regimen used in the present study obviated the acquisition or expression of taste aversions. We must therefore undertake a more detailed analysis of the nursing situation and our experimental protocols in order to determine why the milk cues received through suckling or used during nursing tests do not demonstrate taste aversion conditioning whereas the same cues applied directly to the pups' mouth and used in food tests work reliably. Experiments 3–5 are addressed to features of nursing behavior and conditioning parameters that are of potential importance for learning and memory studies.

Experiment 3: Taste Aversion Conditioning in a Nursing Situation and a Food Preference Test at Weaning

Oral infusion of milk containing a distinctive flavor CS, when paired with LiCl poisoning, produces an aversion to eating solid food containing the flavor CS present in the milk (Experiment 1). Paradoxically, however, the same flavored milk CS ingested

![Figure 3. Mean weight gain (±SE) by pups trained and tested in a nursing situation.](image-url)
by rat pups through suckling and similarly paired with LiCl poisoning does not lead to evident taste aversions in subsequent nursing tests (Experiment 2). One possible explanation for these discrepant results is that pups cannot “refuse” the teat, i.e., the immature rat may be unable to express a taste aversion by inhibition of nipple attachment or suckling. According to this logic, taste aversions were found in Experiment 1 because alternative foods were available in the food preference test and the pups did not have to inhibit ingestion per se. Another explanation is that suckling cues are stronger incentive stimuli for pups than are food cues. In other words, the performance demands of the nursing test might have obscured a conditioned taste aversion formed after exposure to the CS during nursing.

Experiment 3 was designed to determine whether pups that were poisoned after nursing from a foster mother eating a diet distinctive from that of their own mother would avoid that diet in a food preference test at weaning. As in Experiment 1, the present study consists of an initial investigation (Experiment 3A) with three control conditions, using 21- and 22-day-old pups. The main study (Experiment 3B) involved a more extensive developmental analysis, using pups from 7 to 22 days of age.

Method

Subjects

Subjects were 155 Sprague-Dawley rat pups born and reared under the same conditions described previously. Six lactating female rats maintained in a separate room and fed a diet of Purina Rat Chow flavored with geraniol served as sources of the CS for aversion training.

Training

Experiment 3A. Four litters (eight pups in each litter) were trained on Day 21 or Day 22, and two pups from each litter were assigned to one of the four conditions described in Experiment 1: conditioning (G-Li), injection control (G-Sal), flavor control (G), or temporal control (Li-G). As in Experiment 2, pups were given access to a foster mother and allowed to nurse for 1 hr (in the present experiment, all foster mothers were maintained on Diet G). Nursing was verified by observation and pre- and postnursing weights.

Experiment 3B. On the day of training, three or four pups from each litter were randomly assigned to either the conditioning group (G-Li) or the injection-control group (G-Sal). Training with a foster mother on Diet G occurred at Days 7–13 (n = 44), 14–17 (n = 43), or 21–22 (n = 36).

Testing

All pups were given a three-choice food preference test on Days 21, 22, or 23. The testing procedure and apparatus were identical to those used in Experiment 1. On the day of testing, pups were removed from the mother and kept in an incubator at 32 °C during a 4-hr deprivation period. Pups were then placed in individual cages and allowed 4 hr of access to the three diets (Diets G, A, and P; see Experiment 1). Preference ratios were computed for the diet of the foster mother from which the subjects had nursed (Diet G).

Results

Experiment 3A

The mean Diet G intake of subjects in the conditioning group was 4.6%, whereas in the three control conditions Diet G intake ranged from 35.3% to 54.1% for the three-choice test, as shown in Figure 4. A planned comparison analysis that contrasted the conditioning treatment with the three control conditions revealed a significant effect of treatment, F(1, 9) = 18.9, p < .002.

Experiment 3B

The only subjects to display a significantly reduced preference for Diet G were the 21–22-day-old pups in the conditioning group, whose Diet G preference was 24.3%. The two younger age-groups (7–13 and 15–17
days of age) that received the taste–illness pairing displayed a 54.1% and 50.7% preference for Diet G, respectively, as shown in Figure 5. An overall analysis of variance on the combined results for all ages revealed no significant effect of treatment, $F(1, 27) = .11$, $p = .74$, but did imply an age effect, $F(2, 27) = 2.47$, $p < .1$. We therefore analyzed the Treatment $\times$ Age interaction separately for the three age-groups; the results are provided in Table 2.

Inspection of Table 2 reveals a significant treatment effect for the 15-17-day group, which represents a statistically significant increase in preference for Diet G, compared with their littermate controls. Figure 5 shows that the Diet G preference of the 15-17-day-old conditioning group was 50.7% whereas their littermate controls expressed only a 39% preference for Diet G during the test. The same trend was observed in the 7-13-day group. In contrast, the significant effect obtained with 21-22-day-olds reflects an aversion to Diet G.

### Discussion

Although the results of Experiment 3A suggested that pups can associate taste cues in a milk CS received from the mother with immediate postigestional illness and will express appropriate taste aversions toward solid food containing the CS, the developmental analysis of Experiment 3B nonetheless indicates that this ability emerges only after Day 20. There is an obvious discrepancy between the results obtained here and the results of Experiment 1. Pups as young as 10 days of age express significant aversions to taste cues present in mother’s milk received through oral infusions, whereas 17-day-olds provide no evidence of having formed such associations when the flavored milk is obtained in a nursing situation.

It was noted in Experiment 3B that 7- to 17-day-olds displayed an increased preference for Diet G, relative to controls, after nursing from a Diet G mother and succumbing to LiCl-induced malaise. Although we have no definitive explanation for this surprising result, it was a statistically reliable effect in the present experiment. In an attempt to summarize the evidence obtained so far, Table 3 shows group preferences for Diet G after nursing training with a Diet G dam (Experiment 3) as well as the Diet G preferences after oral-infusion training (Experiment 1B), organized into comparable age-groups.

Comparison of the preference values in Table 3, along with other results presented in this article, indicates that taste aversions can be conditioned at early ages if flavored milk is delivered to the pups by oral infusions but that conditioning will not occur if the flavored milk is delivered by the nipples during suckling. After Day 20, however, pups readily learn to avoid food containing chemical cues on the basis of a single cue–illness pairing after suckling exposure.

The dramatic difference between aversion learning to nipple-delivered and orally in-
Table 3

<table>
<thead>
<tr>
<th>Age</th>
<th>Milk received by oral infusion (Experiment 1)</th>
<th>Milk received from nipple (Experiment 3)</th>
</tr>
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<tbody>
<tr>
<td>7-17</td>
<td>50.4%</td>
<td>18.8%</td>
</tr>
<tr>
<td></td>
<td>Conditioning group Injection control</td>
<td>Injection control</td>
</tr>
<tr>
<td>21-22</td>
<td>43.7%</td>
<td>37.0%</td>
</tr>
<tr>
<td></td>
<td>Conditioning group Injection control</td>
<td>Injection control</td>
</tr>
<tr>
<td></td>
<td>25.1%</td>
<td>5.5%</td>
</tr>
<tr>
<td></td>
<td>50.3%</td>
<td>35.2%</td>
</tr>
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</table>

Fused taste CSs led us to a more critical examination of differences between the two procedures. Information regarding the necessary and sufficient conditions for both acquisition and expression of taste aversion learning may be important in the interpretation of early learning studies.

Experiment 4: Determination of Nipple Position During Milk Letdown

In Experiment 3, it was found that older rat pups (20 days or more) learn taste aversions based on milk-borne cues detected during nursing whereas younger pups do not. However, the younger pups are demonstrably capable of learning taste aversions to the same milk-borne cues if the milk CS enters their mouth by an experimenter’s blunt syringe rather than as a consequence of nursing (Experiment 1). It is possible that the younger pups suckle in a way that prevents milk from sufficiently stimulating their taste receptors. Hall and Rosenblatt (1977) recently showed that during the nursing phase of life, the “profile” of suckling behavior in rat pups changes considerably. Although the suckling changes described in their work did not involve nipple position, their observations are not inconsistent with the idea that older pups apprehend and hold nipples differently than do their younger counterparts. Specifically, the diminution of the “stretch reflex” and the increasing tendency to leave or switch nipples suggest the possibility that older pups may hold the nipple less deeply than do the infants and, together with the overall growth of the pups’ head, this could result in stimulation of different tongue regions by the milk as it leaves the nipple.

Experiment 4 investigated the position of the dam’s nipple in the pups’ mouth during nursing. Nipple position was verified through X-rays of pups suckling on nipples that contained small metallic implants.

Method

Subjects

Six rat pups, 10, 13, and 21 days of age, served as subjects (n = 2 at each age).

Procedure

In preparation for this investigation, a lactating rat was anesthetized (Equi-Thesin, ip, 2 ml/kg) and equipped with small metallic implants to permit visualization of nipples in X-ray photographs. A 25-ga. needle was inserted directly into the distal end of the nipple, penetrating the length of the teat to a distance of about 7 mm, and then clipped where it exited the nipple. The elasticity of a lactating nipple is considerable, and this procedure left a radiopaque implant in the distal end of the teat. Two nipples were implanted.

With the facilities and generous assistance available at a local veterinary clinic, a series of X-ray photographs were taken while rat pups suckled vigorously on one of the implanted teats. Pups were deprived of food, water, and maternal contact for 6 hr before testing. Attachment to an implanted nipple was induced by the method described in Drewett, Statham, and Wakerley (1974). To stimulate maximally vigorous nursing episodes, we injected the anesthetized dam with oxytocin (Syntocinon) after the pups had attached. Oxytocin injection typically produces energetic suckling activity associated with milk release (Drewett et al., 1974; Hall & Rosenblatt, 1977).

Results and Discussion

Figure 6 consists of two representative X-rays from the series (reproduced as positives), showing a 10-day-old (upper panel) and a 21-day-old (lower panel) suckling on implanted nipples. The arrows superimposed on the photographs point to the needle fragments contained in the suckled teats. Both X-rays were taken shortly after exogenous oxytocin was applied to the dam. Although we do not know whether the im-
planted nipple delivered milk, the pups were displaying vigorous suckling postures and activity associated with milk letdown.

By using anatomical landmarks discernible in the X-rays shown in Figure 6 and in the others in this series, it was possible to estimate the depth to which nipples are apprehended and held by pups under these nursing conditions. The nasal fossae can be seen in Figure 6 (both panels) with a nearly complete dorsal view, which confirms that the pups’ head is horizontally oriented. Thus, the nipple is, in fact, positioned deep in the oral cavity; its apparent position is not an artifact of viewing an anterior placement through the caudal aspect of the cranium. It is difficult to ascertain the precise location of the nipple in these X-rays, but the metal fragment was reliably located posterior to the orbit, at least to the level of the squamosal bone. Reconstructing this anterior–posterior site in the sagittal plane (see Greene, 1935), the nipples would be positioned, by a conservative estimate, at the posterior of the tongue and soft palate. All rat pups tested (10–21 days of age) displayed the same, deep-throated nipple position during suckling.

Although definite conclusions cannot be drawn from these data about age-related differences in nipple position during suckling under natural conditions, these observations suggest that older pups do not characteristically hold nipples in a different, more an-

Figure 6. X-rays of a 21-day-old pup (lower panel) and a 10-day-old pup (upper panel) suckling on an implanted nipple. (Arrows point to the needle fragment contained in the tip of the nipple. In both X-rays the second implant is visible as a tangential dark bar near the snout of the suckling pup.)
terior oral site than do infants. During milk letdown when the critical taste stimuli would be introduced into the mouth, pups of all ages tested appeared to utilize similar modes of nipple apprehension. These observations do not support the view that taste aversions are formed by 20-day-old pups on the basis of milk cues from nursing because they hold nipples in a different site on the oral sensorium than do younger pups. Instead, the results of the present investigation suggest that suckling rats of all ages tested receive taste input from the same topographical region of the tongue. Since the literature on taste-bud development in the rat indicates that all receptors are completely functional by approximately Day 12 (Farbman, 1965), these results suggest a developmental shift in the integration of olfactory and gustatory cues received through nursing. The following experiment pursues this suggestion and entertains a more detailed consideration of the results.

**Experiment 5: Controlled Milk Delivery While Attached or Unattached to a Nipple**

One of the major implications of the preceding experiments is that taste experiences derived from suckling are relatively immune to taste aversion conditioning in young rat pups. Before proceeding to detailed consideration of such a proposition, however, we felt that an additional investigation of learning in suckling and nonsuckling situations would aid understanding of the processes at work in this early learning task.

The major purpose of Experiment 5 was to control the site of the taste receptors initially stimulated by the flavored milk. Two groups of pups received experimenter-delivered milk through a cannula located at a specific tongue locus. Pups in both groups were in the presence of a dam at the time of the controlled milk delivery and received identical quantities of the flavored milk, but one group was attached to a nipple during delivery whereas the other group was not. The behavior of both preweanling and weanling-age subjects that received the milk while attached to a nipple was carefully observed in order to determine whether behavioral aspects of suckling (other than nipple position) could account for the previously demonstrated age-related difference in learning ability.

**Method**

**Subjects**

A total of 126 Sprague-Dawley rat pups served as subjects. The mothers of these subjects were fed an ad lib diet of unadulterated Purina Rat Chow. Rearing conditions were the same as in previous experiments.

**Cannulation Procedure**

Each subject except in one control group was equipped with a "tongue cannula" similar to that used by Hall and Rosenblatt (1977), which permitted the experimenter to deliver liquids (flavored cow's milk in this study) to a predetermined locus on the tongue of a freely moving rat pup. The cannulas were prepared and implanted according to the general procedures described by Hall and Rosenblatt. Briefly, the cannula was fashioned from polyethylene tubing (PE 10, Clay-Adams) with a flanged end. While the subject was under ether anesthesia, a curved 8-cm piece of wire (a high "E" guitar string, .23 mm in diameter) was inserted into the ventral surface of the jaw and up through the digastric muscle and the tongue. The site of penetration of the wire on the surface of the tongue could be tested with light probing under visual guidance, but the desired locus was just caudal to the deepest part of the pup's tongue that could be seen through the open mouth. The wire was then pushed to the front of the mouth, and the nonflanged end of the cannula was fitted over the tip of the wire, which was then used to thread the cannula back through the tongue and out the skin of the jaw, with the flange of the cannula then pulled flat against the tongue. Postmortem inspection showed that this procedure allowed reliable placement of the cannula to correspond to the nipple position during suckling, as shown in Figure 6. The implantation procedure required less than 1 min, and the pups were able to be used for experimentation later in the same day.

**Training**

On the day of training, subjects were removed from the mother rat and deprived in an incubator (32 °C) for a minimum of 8 hr (10–11- and 15–16-day-olds) or 12 hr (21–22-day-olds). The variable periods of deprivation were required to induce pups to attach reliably to a nipple of an anesthetized mother (Hall, Cramer, & Blass, 1977). Pups were cannulated and marked at least 2 hr prior to training and returned to individual compartments in the incubator.

At the start of each training session a deprived, noncannulated pup was placed in the maternity cage with the mother rat and allowed to initiate nursing.
Once nursing began, the dam was removed from the cage, anesthetized (Equi-Thesin, 2.3 ml/kg, ip), and replaced so that the noncannulated pup could resume suckling. Cannulated pups were then introduced into the maternity cage. The presence of the noncannulated sibling nursing from the anesthetized dam seemed to facilitate approach and nipple attachment from the appropriate cannulated subjects.

Cannulated pups attached to the nipples of the anesthetized dam were reliably observed to suckle despite the blockage of milk letdown caused by the dose and recency of anesthesia (see Lincoln et al., 1973). Thirty seconds after attachment to a nipple, a series of milk deliveries was initiated through the tongue cannula. Approximately .2 ml of the flavored milk was delivered at a rate of .393 ml/min with the aid of an infusion-withdrawal pump (Harvard Model 600-900). Milk composed of Carnation evaporated milk flavored with geraniol at a concentration of 1 ml/l was maintained at 32 °C prior to loading for infusion. Subjects were assigned to one of four conditions: (a) ON(nipple):G-Li—milk delivery while attached to a nipple, followed by injection of .15 M LiCl (ip, 26 body weight); (b) ON(nipple):G-Sal—same as above except injected with an equal volume of isotonic saline; (c) OFF(nipple):G-Li—milk delivery while not attached to a nipple, followed by injection of LiCl as described above; and (d) Li— injection of LiCl alone. Subjects in the last condition (Li) were the noncannulated pups that remained attached to the dam's nipple during the experiment but were never exposed to the flavored milk. All subjects were replaced in the incubator for a 1-hr period prior to being returned to the maternity cage. After milk infusion, the canulas were clipped off under the pups' jaw, with the small flanged portion left in the tongue.

Testing

All subjects received the same three-choice food preference test utilized in previous experiments. Pups that were cannulated on Days 10–11 or 15–16 were tested on Day 22, and those cannulated on Days 21–22 were tested 4 days after training. In pilot work it was found that cannulated pups tended to avoid all flavored diets when tested 1–2 days after training. During the interval between training and testing, subjects in the oldest age-group were deprived of access to a solid-food diet (mothers were fed in isolation from their offspring for a 4-hr period each day). These precautions were found necessary to obtain reliable sampling of the three diets.

Data Analysis

The data from the food preference test in this experiment were analyzed differently than in the previous experiments. Variability in attachment to a nipple of the anesthetized mother, along with attrition of cannulated pups before testing, resulted in an unequal number of subjects from each litter under the four treatment conditions. Collapsing data for littermates under each condition, as done earlier, would have required an extremely large N, and we therefore decided to treat each subject as an individual observation. In no case, however, were there fewer than two of the four treatment conditions represented within a litter. The number of subjects successfully tested in each group was as follows: 10–11-day-olds—ON:G-Li (n = 11), ON: G-Sal (n = 11), OFF:G-Li (n = 10), Li (n = 9); 15–16-day-olds—ON:G-Li (n = 11), ON:G-Sal (n = 13), OFF:G-Li (n = 10), Li (n = 5); 21–22-day-olds—ON: G-Li (n = 13), ON:G-Sal (n = 13), OFF:G-Li (n = 12), Li (n = 8).

Results and Discussion

The results of the present experiment support the basic findings and corroborate the interpretation of the earlier studies in this series. Rat pups under the age of 20 days receiving flavored milk through a tongue cannula learned to avoid the chemical cue in the milk when milk delivery occurred while they were not attached to a nipple; littermates receiving the same milk CS while attached to a nipple failed to form the aver- sion. These results are shown in the left and center panels of Figure 7. In contrast, nipple attachment or nursing activity did not have a similar effect on taste aversion conditioning in somewhat older pups, 21–22 days at training. The right panel of Figure 7 shows that the 21–22-day-olds formed clear taste aversions to the geraniol cue in can- nula-delivered milk whether they were "on" or "off" a nipple during the milk delivery. Planned comparisons were used to analyze the treatment effects. Table 4 presents a summary of the results of tests, contrasting the conditioning groups (ON:G-Li or OFF: G-Li) with their littermate controls (G-Sal and Li).

Behavioral observations also failed to reveal any major changes in suckling activity between Day 10 and Day 22. The possibility that olfactory input might vary with age because of nostril position or force of suckling was not confirmed. Pups of all ages appeared to grasp and maintain contact with the nipple with the same vigor, once milk delivery was initiated. The only consistent difference observed was in the extent of the stretch reflex, with older pups (21–22 days old) tending to have the stretch confined to the anterior portion of the body. The results of the present experiment thus demonstrate that when the same milk cue is delivered to the same place on the tongue, in equal
amounts and on the same time schedule, taste aversions are not formed if the recipient pup is less than 20 days of age and has a nipple in its mouth. Pups older than 20 days acquire robust taste aversions with equal rapidity without regard to the suckling contingencies that so definitely affect pups only a few days younger.

In light of the well-documented importance of temporal parameters in conditioning young animals (Caldwell & Werboff, 1962; Gray, Yates, & McNeal, 1967) and especially taste/odor aversion conditioning in rat pups (Gregg et al., 1978; Rudy & Cheatle, 1977), it is appropriate to describe an additional experiment that was run to equate CS and US delivery, using orally infused (OI) versus on-the-nipple (N) presentations of novel-tasting rat's milk (Martin, Note 1).

A total of 78 rat pups (11-18 days of age) were deprived of maternal contact for 3.5 hr in a warm incubator. For Group N, following deprivation, pups were marked, weighed, and given access to a Diet G foster dam. An observer timed and recorded three events: time to first milk delivery, time to second milk delivery, and termination of the second nursing bout. The pups' stretch reflex was used to recognize milk delivery (Lincoln et al., 1973). Immediately after the first milk letdown, we injected pups with either .15 M LiCl or equimolar NaCl (ip, 2% body weight), using preloaded syringes to minimize the interruption of nursing. After the second nursing bout, which ended when the dam left the nesting area, the litter was placed in the incubator for 2 hr before being returned to the home cage. If any pups of a litter did not reattach to a nipple following injection, the entire litter was eliminated from consideration; 7 of 12 litters were thus discarded.

Two hours after training for Group N, the same foster mother was anesthetized, and her milk was manually expressed in accordance with the method of Experiment 1. Following deprivation, subjects in Group OI (matched approximately for age with Group

Table 4
Treatment Within Age Contrasts:
Comparison of Mean Percentage Preference for Diet G by Pups Exposed to Taste-Illness Pairing While Attached (ON:G-Li) or Not Attached to a Nipple (OFF:G-Li) With Littermate Controls (Experiment 5)

<table>
<thead>
<tr>
<th>Age</th>
<th>Treatment group</th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-11</td>
<td>ON:G-Li</td>
<td>1,114</td>
<td>.03</td>
<td>.860</td>
</tr>
<tr>
<td></td>
<td>OFF:G-Li</td>
<td>1,114</td>
<td>4.43</td>
<td>.038</td>
</tr>
<tr>
<td>15-16</td>
<td>ON:G-Li</td>
<td>1,114</td>
<td>.19</td>
<td>.660</td>
</tr>
<tr>
<td></td>
<td>OFF:G-Li</td>
<td>1,114</td>
<td>5.92</td>
<td>.016</td>
</tr>
<tr>
<td>21-22</td>
<td>ON:G-Li</td>
<td>1,114</td>
<td>6.46</td>
<td>.012</td>
</tr>
<tr>
<td></td>
<td>OFF:G-Li</td>
<td>1,114</td>
<td>9.34</td>
<td>.003</td>
</tr>
</tbody>
</table>

Figure 7. Mean percentage preference (±SE) for Diet G by pups receiving flavored cow's milk through a tongue cannula while attached or not attached to a nipple and given a food preference test at weaning.
N pups) were exposed to the same CS–US–CS intervals as those experienced by Group N subjects. The Diet G rat milk was orally infused by the experimenter according to the temporal patterns for the two sequential bouts experienced on the nipple by Group N pups. Thus, the procedure constituted a “yoked” control, using novel-tasting rat’s milk and varying mode of CS delivery.

The three-choice food preference test revealed significant aversions to Diet G only in the pups receiving the flavored milk by oral infusion followed by toxicosis (Group OI:G-Li). The Diet G preference of these pups was only 17.4%, compared with a 46.8% preference in their littermate controls (Group OI:G-Na). In contrast, the pups receiving milk of the same distinctive flavor directly from the teat (Group N:G-Li) subsequently exhibited a 40.2% preference for Diet G which was, in fact, a slightly greater preference than the 34.8% intake of their littermate controls (Group N:G-Na). These results are in agreement with the data of Experiment 5 and again show the apparent blockade of taste aversion conditioning in preweanlings when CS delivery is coincident with suckling activities. Moreover, this demonstration extends the phenomenon to include taste and temporal parameters characteristic of the natural nursing situation.

General Discussion

The present five experiments, considered together, help to clarify the parameters of taste aversion conditioning in preweanling rats. Experiments 1–3 were designed to determine the age and the conditions under which taste aversions can be learned by rat pups. The results of these experiments instigated further investigation into aspects of the nursing situation which might affect acquisition of taste aversions and thus prove to be of general importance for learning and memory studies in neonatal subjects (Experiments 4–5).

Acquisition and Long-Term Retention of Taste Aversions in Rat Pups

We found that pups as young as 10 days of age can detect a specific chemical cue in mother’s milk and cow’s milk, associate the cue with a single toxic experience, and subsequently avoid an otherwise acceptable food that contains the test cue. In addition to the ability to acquire taste aversions with a single taste–illness pairing, even the youngest pups displayed long-term retention of the conditioned aversions in our experiments. There are other reports of long-term memory for conditioned taste aversions by rat pups (Campbell & Alberts, 1979; Gregg et al., 1978), but the present study reports the most dramatic results for 10-day-olds found thus far.

The long-term retention of taste aversions by pups contrasts sharply with numerous reports that juvenile rats, like the young of other altricial species, forget very rapidly. Campbell and Alberts (1979) proposed that memory for taste aversions may develop before memory for other learned tasks. The present results constitute an even more compelling demonstration of excellent taste aversion learning and retention in the infant rat. Moreover, the three-choice food test used in the present investigation provides good indication that the pups’ aversions were not general neophobic reactions to a novel or distinctive food.

Although the present experiments did not seek to define the lower limits of taste aversion learning, or the durability of the pups’ memory, the performance of the 10-day-olds in Experiments 1 and 5 extends significantly the age and retention limits demonstrated in previous studies.

Nursing and Acquisition of Taste Aversions

One of the more startling results of the present investigation was the discovery that rat pups less than 20 days of age do not form taste aversions to a CS obtained while nursing whereas older pups readily condition to such cues (Experiments 3 and 5). Are the younger subjects in fact detecting taste cues while nursing? We believe that sufficient evidence has been obtained to justify an affirmative answer. It was clear from Experiment 1 that the taste stimulus itself was perceptible to all pups since milk manually expressed from Diet G dams was an entirely
sufficient CS for conditioning when infused into the mouth (Figure 2). Moreover, the younger pups receiving various kinds of taste—illness pairings in a nursing situation consistently displayed an increased preference for the CS associated with poisoning. This result appeared as a trend or with statistically significant nonrandomness in several experiments (see Tables 2 and 3; Figure 5; Martin, Note 1). One possibility is that this paradoxical effect is simply due to an increase in arousal following injection of LiCl. The unexpected event of illness may lead to priming of the taste representation present in short-term memory and result in an enhanced "learned familiarity" effect (Best & Barker, 1977). Although we cannot draw definite conclusions at present, this "illness-induced preference" implies that the cue was detected during suckling.

We have not identified any major changes in the suckling behavior of pups between Day 10 and Day 21 that can account for the ability of 21-day-olds to acquire aversions on the nipple. X-ray analysis of suckling associated with milk release (Experiment 4) indicated that analogous populations of tongue receptors are stimulated by the nipple tip in both younger and older groups (Figure 6). The scant literature on taste-bud development in rats suggests that receptor maturation is complete by Day 12 (Farbman, 1965).

Use of the tongue cannula (Experiment 5) permitted control over quantity, timing, and placement of milk delivery (flavored cow's milk) for pups younger and older than 20 days in on-nipple and off-nipple situations. Again, 21-day-olds formed strong aversions after training in a nursing situation, whereas the younger pups did not. Application of a temporal yoking procedure to match milk delivery (flavored rat's milk) between nursing and orally infused subjects (Martin, Note 1, described in Experiment 5) also failed to produce taste aversion conditioning in preweanling subjects in a nursing situation. One is led to conclude that some aspect of suckling behavior interferes with the ability of the younger subjects to associate taste cues with aversion-producing consequences.

Nursing as a Reinforcing and Nonpunishable Activity

Suckling behavior, even without nutritive consequences, is reinforcing to rat pups. It is possible that suckling reinforcement can override or neutralize the flavor—toxicosis association in young pups. To explain taste aversion learning by 21-day-old pups after obtaining a CS by nursing, however, one would have to predict that nipple cues are less reinforcing to the weanling-aged animal. Relevant evidence is less consistent on this point. Kenny and Blass (1977) found the rewards of nonnutritive suckling sufficient to train spatial choices by 21-day-olds in a T-maze, but they used a rigorous, 24-hr deprivation condition; indeed, weanling pups do not reliably attach to nipples of anesthetized dams unless they are substantially deprived (Hall & Rosenblatt, 1977), and pups between the ages of 18 and 24 days given dry suckling rewards frequently show an increase in attachment latency with repeated trials (Amsel & Stanton, Note 2). Specific investigations must be designed to evaluate the rewarding aspects of suckling throughout the nursing period and potential changes in the rewards of suckling during weaning.

Although suckling can act as a reinforcing stimulus, it is not known if suckling itself can be reinforced or punished in neonatal rodents as it can be in human infants (Lipsitt, Kaye, & Bosack, 1966). In fact, we noted in Experiment 2 that pups may be reluctant or incapable of expressing an aversion to perceptible cues that are presented within a larger context of normal maternal stimuli. It is interesting to note that in certain mutant strains of Mus musculus, the mothers produce "toxic" or "lethal" milk (lacking necessary trace metals). Despite the disastrous effects of the milk, infant offspring and even older, foster pups continue to nurse until they succumb (Piletz & Ganschow, 1978). We have found (unpublished observations, 1978) that pups poisoned after oral infusion of geraniol-flavored milk readily nurse from a Diet G dam but later refuse to eat geraniol-flavored food. Thus, the nursing situation presents two problems...
of potential importance to some analyses of early learning and memory. First, young animals may form aversive associations to nursing-related cues only with great difficulty. Second, suppression of nursing as a response measure for learning may be beyond the capabilities of some altricial neonates.

We know of no evidence to contradict our results and conclusion that neonatal rodents do not form aversive associations to stimuli presented in concert with suckling stimuli, although the infant can readily acquire and remember both positive and negative associations experienced outside the nursing sequence. It is as if there is a sanctity to maternal cues, making them immune to aversive conditioning. Another more testable explanation is that the mechanisms involved in the lessening of reflex components of nursing are related to the emergence of processes that permit the weanling to make learned associations during suckling.

Reference Notes


References


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