Early Weaning Does Not Accelerate the Expression of Nursing-Related Taste Aversions

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Preweanling rat pups do not display an aversion to a flavor conditioned stimulus (CS) paired with illness if the CS is presented during the act of suckling. In contrast, 20-day-old pups do form such a conditioned taste aversion while suckling (Martin & Alberts, 1979). The dissolution of the nursing-related “blockade” of toxiphobia correlates with the onset of solid food intake. Moreover, prevention of weaning prolongs the blockade; ingestive experience with solid food is necessary for the expression of nursing-related taste aversions in 26-day-old “food naive” pups (Gubernick & Alberts, 1984). The present experiments tested the possibility that premature weaning to solid food might accelerate the onset of nursing-related toxiphobia. Pups were weaned at 13 days of age and ingested only food and water. These prematurely weaned pups received taste aversion conditioning while suckling on Day 16, but showed no aversion to the CS flavor during a later food test. Thus, conditions that lead to early weaning (ingestion of solid food) do not accelerate the onset of taste aversions to mother’s milk.

Infant rats can learn numerous kinds of associations, including toxiphobia following conditional pairing of taste or odor cues with illness (Campbell & Alberts, 1979; Rudy & Cheatle, 1977). However, rats less than 17 days of age do not display such learned aversions when they are trained during the act of suckling (Martin & Alberts, 1979). We have described the pup’s failure to acquire or display nursing-related toxiphobia as a “blockade” of the toxiphobia because it appears that specific aspects of the suckling context interfere with the typical consequence of such associative pairings (Martin & Alberts, 1979).

Older rats pups, those more than 20 days of age, learn taste aversions even when training occurs in the context of suckling (Martin & Alberts, 1979). We believe that the “blockade” of toxiphobia operates on the pup’s ability to express rather than to learn the aversion (Gubernick & Alberts, 1984; Martin & Alberts, 1979).

What developmental mechanisms dictate the transition from the stage when suckling interferes with conditioned aversions to the subsequent stage when it does not? We have examined this question from a viewpoint of feeding experience. Specifically, we noted

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that the dissolution of the blockade normally occurs at the age when pups wean, i.e.,
when they begin to ingest solid food but still suckle mother’s milk (Martin & Alberts,
1979).

Perhaps the experience of ingestion of solid food is a mechanism that mediates the
pup’s ability to express taste aversions to mother’s milk. To examine this idea, we
imposed a drastic delay in weaning by providing pups with continued access to mother’s
milk, but deprived them access to solid food. We found a blockade of toxiphobia (i.e.,
no aversions) in 26-day-old, delayed-weaning (i.e., food naive) pups that were trained
in the context of suckling (Gubernick & Alberts, 1984).

We further examined this ingestional hypothesis by introducing food to pups that
had been maintained solely on mother’s milk beyond the typical age for weaning. A
brief, 4-hour, exposure to food was sufficient to permit the subsequent expression of
taste aversions in these delayed-weaning pups (Gubernick & Alberts, 1984).

These findings implicate initial ingestive experience with solid food as a factor that
removes the nursing-related blockade of toxiphobia. Because delayed weaning appears
to maintain the blockade, early weaning might hasten its dissolution. The present research
was designed to determine whether premature weaning to solid food would prematurely
terminate the blockade and permit expression of taste aversions to nursing-related cues.

**Experiment I**

Gubernick and Alberts (1984) found that for weanling aged (26-day-old) pups, inges-
tive experience with solid food was necessary to permit expression of nursing-related
taste aversions. Experiment I was designed to determine whether early weaning to solid
food would permit expression of such learned aversions in 16-day-old pups, an age when
suckling-related aversions are normally not expressed (Martin & Alberts, 1979).

Under typical laboratory conditions, Norway rat pups do not ingest solid food until
about Days 16–18 (Galef, 1981; Thieles & Alberts, 1983). In Experiment I animals were
weaned on Day 13; they lived in social groups in a warm environment and ingested
powdered food and water. On Day 16 animals received a flavor-illness pairing or control
treatments in one of two contexts. Animals experienced flavored milk while attached to
the nipple of an anesthetized dam, or while in the presence of an anesthetized dam and
not suckling. Conditioned aversions were measured with a food test on Day 21. If
ingestive experience is important in permitting the onset of aversions to mother’s milk
then these prematurely weaned pups should express an aversion to a flavor paired with
illness in a suckling context.

**Method**

Subjects

A total of 87 Sprague-Dawley (S.P.F., Charles River) rat pups served as subjects.
All animals were born in our Animal Behavior Laboratory. Litters were reared with their
mothers in standard maternity cages (48 × 20 × 26 cm). Three days after birth (Day
0) each litter was reduced to eight pups.

Premature Weaning. On Day 13 litters were separated from their mother, weighed,
and marked individually. Groups of four littermates were placed into polypropylene
rearing cages (26 × 16 × 13 cm) within an incubator, maintained at 28° C. Powdered
Purina Rat Chow and water were available, ad libitum, in separate plastic dishes. We
abandoned earlier attempts to provide a wet mash diet because the young pups tended
to become covered with the damp food.
Food and water consumption were estimated by changes in pup body weight after weaning. Pup weights were recorded to the nearest .1 g on Days 13, 15, 16, 17, 19, and 21. Changes in pup weights were expressed as percentages of each pup's initial body weight. For instance, a pup's weight change between Days 13 and 15 was calculated as:

\[
\text{weight Day 15} - \text{weight Day 13} \div \text{weight Day 13} \times 100
\]

To confirm that the weaning manipulation successfully led to early ingestion of food, we compared body weight changes of pups weaned onto solid food on Day 13 with the weight changes of pups maintained under identical conditions but without food or water between Days 13 and 15 \((n = 24)\) or between Days 15 and 16 \((n = 32)\). Pups deprived between Days 15 and 16 were treated identically to test pups before Day 15.

**Milk CS.** The conditioned stimulus (CS) was Carnation Evaporated Milk flavored with McCormick pure almond extract (2 µl/ml milk). The flavored milk was warmed to room temperature before use.

**Cannulation.** A tongue cannula, designed and implanted according to the general procedure described by Hall and Rosenblatt (1977), was installed in each pup on Day 16. Cannulas consisted of polyethylene tubing (Clay Adams, P.E. 10, .11 mm i.d.) with one heat-flanged end. Pups were anesthetized with ether and a curved wire (high “E” guitar string, .013 mm diameter) was inserted into the ventral surface of the jaw, through the diagastric muscle and the tongue. The site of penetration was just anterior to the intraoral eminence. The wire was pushed out through the open mouth and the nonflanged end of the cannula was threaded onto the wire. The wire and the cannula were then pulled back through the tongue and the wire was removed from the cannula. Thus, the flanged end of the cannula remained on the back of the pup’s tongue and tubing extended about 2.5 cm below the pup’s jaw. Cannulation required 1–2 mins and pups became active within 5 mins after cannulation. Pups readily attached to a nipple during training 4–5 hr after surgery.

**Training.** After cannulation on Day 16, pups were deprived of food and water for 4–5 hr before training. In the ON-nipple (suckling) condition, a lactating rat was anesthetized (chloral hydrate, i.p., .1% body weight) and placed in a maternity cage on her back with nipples exposed. One noncannulated pup remained with the dam during training because this seemed to facilitate nipple attachment by cannulated pups. Pups were trained individually. Each pup’s cannula was attached to tubing leading to a 1-cc syringe filled with almond-flavored milk. After a pup was securely attached to a nipple, approximately .2 ml of flavored milk was delivered by an infusion pump (Harvard Model 600-900) in four or five discrete bursts (.393 ml/min). The pup was removed 15–30 sec after the last milk delivery and injected with lithium chloride or saline solution. Pups in the lithium group \((n = 16)\) received an injection of .15 M LiCl (i.p., 2% body weight) and pups in the saline group \((n = 16)\) received an identical dose of isotonic saline. An additional, “backward” (noncontingent) control group was injected with LiCl on Day 15, about 24 hr before exposure to the milk CS (group Li-B, \(n = 14\)).

Three OFF-nipple groups were trained with the lactating dam on her ventrum so that nipples were not exposed. Pups in the OFF-nipple groups (group LiCl, \(n = 16\); group Saline, \(n = 15\); group Li-B, \(n = 13\)) were not allowed to attach to the nipple during training. Otherwise the training procedures for the three OFF-nipple groups were the same as for the analogous ON-nipple groups. Powdered food and water were replaced in all rearing cages about an hour after training. Pups in Group Li-B were isolated from food and water on Day 15 for an hour after the injection. Cannulas were clipped at the pup’s jaw after training. No fewer than four of the six treatment conditions were represented in each litter.

**Testing.** Pups were deprived of food but not water for 1–2 hr before testing on Day 21. The testing apparatus consisted of a polypropylene cage (46.9 × 25.4 × 12.7 cm)
that contained two adjacent metal food trays (12.8 × 5.6 × 8.8 cm) at one end of the cage (see Fig. 1 in Gubernick & Alberts, 1984). A metal cup (5.7 × 5.6 cm) was taped in place at the rear of each food tray. Twelve grams of powdered Purina Rat Chow and 12 g of powdered Chow containing the almond CS (8 μl almond extract/gram powdered chow) were separately available. Pups were tested individually for 24 hr. Water was available throughout the testing session.

Intake of almond chow and unflavored chow were measured to the nearest .01 g. Percentage intake of almond chow was calculated for each animal in the following manner: (amount almond chow consumed/amount almond + unflavored chow consumed) × 100.

Results and Discussion

Pups prematurely weaned on Day 13 showed no aversion to a nursing-related flavor CS paired with illness. In contrast, prematurely weaned pups trained identically, but not while suckling, exhibited aversion to the almond-flavored CS during the food test.

Figure 1 shows the mean percentage intake of almond-flavored chow for the ON-nipple and OFF-nipple treatment groups. Among the three ON-nipple groups (left panel), there were no significant differences in intake of almond-flavored chow, $F(2, 43) = 1.36$, $p > .10$. Planned comparison analysis substantiated the statistical equivalence of all three groups: intake of almond-flavored chow by the LiCl and saline groups did not differ significantly, $F(1, 43) = 2.55$, $p > .10$. Similarly there was no significant difference between groups LiCl and Li-B in the ON-nipple condition. Thus, we found no evidence of toxiphobia to the almond-flavored CS in the ON-nipple condition.

In contrast, there were significant differences in intake of almond-flavored chow among the OFF-nipple groups, $F(2, 41) = 7.02$, $p < .01$. Specifically, pups that received a flavor-illness pairing OFF-nipple (group LiCl) showed less intake of almond-flavored chow than pups in the saline group, $F(1, 41) = 7.14$, $p < .02$ (Fig. 1, right panel). The LiCl group also showed less intake of almond-flavored chow than group Li-B (OFF-nipple), $F(1, 41) = 12.66$, $p < .001$.

Table 1 shows mean percentage weight changes of pups after weaning on Day 13. Deprived pups showed greater decreases in body weight than did test pups between Days 13–15, $t(93) = 9.33$, $p < .001$ and Days 15–16, $t(68) = 8.82$, $p < .001$. There were
clear increases in body weight after Day 16. These observations indicate that test pups ingested powdered food and water both before and after training (Day 16). The body weight changes shown in Table 1 suggest that pups did not ingest or utilize sufficient quantities of food and water to maintain normal growth trajectories. Nevertheless, their body weights differed significantly from the deprived controls, indicating that test pups were consuming food and water before training.

The results of Experiment I indicate that early weaning to solid food does not necessarily ameliorate the blockade of nursing-related toxiphobia. Sixteen-day old pups that received flavor aversion conditioning while suckling failed to show aversions to the CS in the food test. However, pups that were not attached to the nipple during taste aversion conditioning showed clear evidence of an aversion to the CS flavor, indicating that early weaning did not interfere with the pups’ basic abilities to acquire or express aversions.

It might be important to note that, under natural conditions, the weanling pup does not make the abrupt transition from mother’s milk to solid food that was imposed by our procedure. Weaning is typically a gradual process, during which the 15- to 30-day-old rat simultaneously utilizes two sources of nutrition—mother’s milk and solid food (Galef, 1981). It is possible that pups need to experience both milk and food during weaning in order to display food aversions to CSs presented during nursing. We have, however, also used an early weaning procedure in which pups ingested both solid food and mother’s milk each day. These 16-day-old pups experienced illness after receiving almond-flavored milk while suckling \((n = 10)\), but showed no aversion to the CS flavor relative to saline-injected animals \((n = 9)\) in a later food test (Mean Percentage Intake of Almond Chow = 30.0 and 29.7, respectively). These results, like those of Experiment I, suggest that premature weaning to an “alternative” food source, such as solid food, does not accelerate the onset of suckling-related toxiphobia.

It is conceivable that the opportunity to suckle during training produced an excitatory state in early-weened pups that interfered with their perception of the flavor CS. These pups then would have failed to show toxiphobia due to a failure to perceive the flavor CS. Indeed, the early-weaned pups appeared more aroused than normally weaned pups when given the opportunity to suckle during training. Their excitement did not, however, obscure the typical elements or temporal sequences of suckling (Hall & Rosenblatt, 1977); the behavior of early-weened pups was clearly modulated by milk delivery. In addition, pups in the ON- and OFF-nipple control groups (Saline and Li-B) showed no differences in intake off almond-flavored chow (Fig. 1). One might expect OFF-nipple pups to display enhanced intake of almond-flavored chow relative to the ON-nipple pups if the excited state of the ON-nipple pups truly interfered with perception of the CS and rendered them naive to the flavor stimulus. This difference did not appear in the data.
Experiment II

Martin and Alberts (1979) found that normally reared pups trained before Day 20 do not show suckling-related taste aversions and suggested that the onset of suckling-related taste aversions is dependent, in part, on maturational processes. This suggestion was modified to include the necessary role of experience with solid food (Gubernick & Alberts, 1984).

The results of Experiment I indicate that early experience with solid food does not accelerate the dissolution of the blockade of toxiphobia and permit the expression of suckling-related taste aversion learning in 16-day-old pups. It is possible that some aspects of the premature weaning procedures may have produced a learning deficit related to the nursing context. If this is the case, prematurely weaned pups should not show nursing-related flavor aversions when trained on Day 20, an age when such aversions normally emerge (Martin & Alberts, 1979). If, however, the persistence of the blockade found in the present research is due to intrinsic, maturational processes then prematurely weaned pups trained on Day 20 should show suckling-related taste aversions.

Method

Subjects

Twenty-four pups were subjects in Experiment II. Rearing and housing conditions were the same as described in Experiment I.

Procedure

The main procedural change from Experiment I was that training occurred on Day 20. Cannulation, training and testing were as described in Experiment I. All pups were trained ON-nipple. Four pups in each litter were assigned to group LiCl (n = 12) and the other four pups were assigned to group Saline (n = 12). One hour after training pups were returned to the rearing cages with powdered food and water. All pups were deprived of food for 1–2 hr before food testing on Day 22.

Results and Discussion

In the food preference tests, mean percentage intake of almond chow for group LiCl ON-nipple (M = 22.1, S.E. = 5.50) was significantly less than group Saline ON-nipple (M = 47.5, S.E. = 5.48), t(23) = 3.28, p < .005. These results are consistent with the hypothesis that the onset of suckling-related taste aversion learning is a maturation-related process. Twenty-day-old, prematurely weaned pups that received a flavor-illness pairing while suckling showed a significant aversion to the CS flavor relative to control pups in the food preference tests. This finding indicates that the premature weaning procedures of the present research did not produce a learning deficit related to the nursing context.

General Discussion

The early weaning procedures used in the present research did not hasten the onset of suckling-related taste aversion learning. Pups were weaned on Day 13 and ingested only solid food or solid food and mother’s milk before training on Day 16. These food-experienced pups received a flavor-illness pairing in a suckling context on Day 16, but
did not show an aversion for the CS flavor in a later food preference test. Similarly reared pups that were not suckling during training showed clear aversions to the CS flavor during the food tests. Thus, prematurely weaned pups show the same nursing-related blockade of toxiphobia exhibited by normally reared pups (Martin & Alberts, 1979).

If the manipulations in the present research had been effective in eliminating the blockade they would have allowed a definitive statement on the role of feeding experience in the onset of taste aversions to mother’s milk. One lesson to be learned from the negative outcome of the present study, however, is that an early-weaned animal is not necessarily a complete model of a normal weanling. Although our procedures were successful in inducing early ingestive behavior with solid food, the postdigestive consequences of such ingestion may not have been equivalent to those experienced by a weanling-aged animal.

In any case, the present findings contrast with the effects of food experience on suckling-related aversions in older pups raised solely on mother’s milk (i.e., delayed weaning). Gubernick and Alberts (1984) found that pups raised only on mother’s milk and trained on Day 26 did not show aversions to a CS experienced while suckling (blockade was extended). Pups trained under these delayed weaning conditions, but allowed brief access to solid food before or after training did show an aversion to the CS in a later food test (blockade was eliminated). Food experience appears to permit the expression of suckling-related taste aversions in these older animals.

Thus, the same experience—initial ingestion of solid food—has different behavioral outcomes at two stages in development. Solid food permits expression of a taste aversion to mother’s milk in 26-day-old but not in 16-day-old pups.

Notes
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