

Role of Residual Olfactory Cues in the Determination of Feeding Site Selection and Exploration Patterns of Domestic Rats

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Weanling domestic rat pups feed and explore in areas containing residual olfactory cues deposited by conspecific adults in preference to clean areas. Both nulliparous and lactating Long-Evans female rats deposit residual cues in an area that induce pups to explore and feed in it. Residual cues continue to affect the feeding and exploratory behavior of pups to maturity. Discrepancies between results obtained in the olfactory discrimination apparatus used by Leon and Moltz and those of the present experiments are resolved, and evidence is presented for the existence of residual cues not contained in anal excreta, which are attractive to pups. It is suggested that residual cues deposited by adult rats can play a role in directing weanlings to their first meals of solid food in the natural environment.

During their first few days of feeding on solid food, weanling domesticated rat pups exhibit a strong preference for a relatively unpalatable diet that the adults of their colony have been trained to eat (Galef & Clark, 1971b, 1972). Analysis of this transfer of an acquired feeding preference from adult to young rats has indicated the existence of two mechanisms, each sufficient to enable

adult rats to influence weanlings' choice of diet. First, the data support the hypothesis that gustatory cues incorporated in a lactating female's milk, reflecting the flavor of her diet, are sufficient to allow pups to recognize that diet and to cause them to preferentially ingest it during weaning (Bronstein, Levine, & Marcus, 1975; Galef & Clark, 1972; Galef & Henderson, 1972; Galef & Sherry, 1973). Second, the physical presence of adult rats at a feeding site has been found to attract pups visually to that site and, in situations in which dietary alternatives are spatially separate, to influence weanling selection of diet (Galef & Clark, 1971a, 1971b).

Recent studies of the interaction of rat dams and their young have provided evidence that the anal excreta of dams from 2 to 4 wk postpartum contain olfactory cues (the maternal pheromone) highly attractive to pups of weaning age (Leon, 1974; Leon & Moltz, 1971, 1972). This

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finding suggests the possible existence of a second mechanism whereby adult rats influence the feeding site selection of the young of their colonies and, thereby, their selection of diet. If recently parturient rats concentrate deposition of their excreta in areas in which they feed, then these residual cues could serve, as does the physical presence of adult rats at a feeding location, to attract weanlings to a food source utilized by conspecifics.

The experiments presented below examine four related issues concerning the possible influence of residual cues deposited by adult rats on the exploration and feeding site selection of conspecific weanlings. Experiment 1 demonstrates that rat pups will explore and feed in an area soiled by a lactating adult conspecific in preference to an unsoiled area. Experiments 2, 3, and 4 investigate the relationship between the cues effective in producing this bias in exploration and feeding site selection of weanlings and the maternal pheromone described in detail by Leon (1974). Experiment 5 examines, in two laboratory situations, the actual pattern of distribution by adult rats of their excreta, and Experiment 6 examines the range of ages at which pups can be influenced in their exploration and feeding site selection by residual cues deposited by adults.

EXPERIMENT 1

The present experiment was undertaken to determine the sufficiency of residual chemical cues deposited in an area by lactating female rats to influence the exploratory behavior and feeding site selection of their young.

Method

Apparatus. Three test enclosures identical to that illustrated in Figure 1a were constructed of slotted angle iron and hardware cloth. The galvanized sheet-metal floor of each enclosure was lined with unused newsprint, and a wooden nest box (.30 × .30 × .17 m) with two entrance holes (.08 × .08 m) was placed in its center. A removable galvanized sheet-metal partition was installed so as to prevent access to one third of each enclosure. Two food bowls, both containing powdered Purina Laboratory Chow, and water were continuously

present in the positions indicated. Closed-circuit television permitted monitoring of the enclosures from an adjacent room.

Subjects. Subjects depositing residual cues in the test apparatus are referred to below as *apparatus subjects*, and pups used to determine the relative attractiveness of the soiled and unsoiled portions of the enclosure as *experimental subjects*. Experimental and apparatus subjects were treated differently, and the treatment of each group is, therefore, described separately below.

All rats were from the Long-Evans strain maintained by the Canadian Breeding Farms, St. Constant, Quebec, Canada.

1. *Apparatus subjects.* Prior to placement in the larger portion of each test enclosure, pregnant apparatus subjects were maintained in .30 × .35 × .18 m home cages on ad lib Purina Laboratory Chow and water. On Day 3 postpartum, one mother and her litter (culled to six pups shortly after birth) were transferred to the larger portion of each test apparatus and left undisturbed for 13 days. On Days 16-24 postpartum, apparatus mothers and pups were removed to holding cages for 3 hr/day. Three Long-Evans female rats and their litters served as apparatus subjects.

2. *Experimental subjects.* Pups in litters to be used as experimental subjects were equated for day of birth (± 1 day) with that apparatus litter to which they were assigned, and were maintained in .30 × .35 × .18 m cages. For the first 9 days postpartum, the dam of each experimental litter was fed Purina Laboratory Chow ad lib in her home cage. For the subsequent 13 days she was fed the same diet, in a cage separate from her young, on a 3 hr/day feeding schedule (12-2 p.m. and 9-10 p.m.) to ensure that her young took their first meals of solid food in the test apparatus. Nine pups, randomly selected from the litters of three Long-Evans females, served as experimental subjects. Diagrams of the pelage markings of each experimental pup permitted the identification of individuals within a litter.

Beginning on Day 16 postnatally, each experimental pup was observed individually in a test apparatus for 50 min/day for seven consecutive days. Three experimental pups were observed consecutively in each apparatus each day.

Test procedure. At the beginning of a test session the apparatus animals were removed from the test enclosure, the sheet-metal barrier was taken from the enclosure, and if necessary to equate food availability, spillage was placed around the bowl in the previously unoccupied section of the test enclosure. An experimental pup of the same age as the removed apparatus pups was then placed in the nest box and left undisturbed for 50 min.

The experimenter recorded the amount of time each experimental pup spent in the previously occupied and unoccupied end thirds of the enclosure in which it was tested and the amount of

time it spent feeding at each food bowl. Data from each pup was summed across its seven test periods for purposes of analysis. At the end of each daily 3-hr test session (after three individual experimental pups had been observed in each apparatus), the experimenter replaced the partitions and the apparatus animals.

Results and Discussion

The main results of Experiment 1 are presented in Figure 2a, which shows the amount of exploring and feeding time spent by pups in the soiled end third of the test

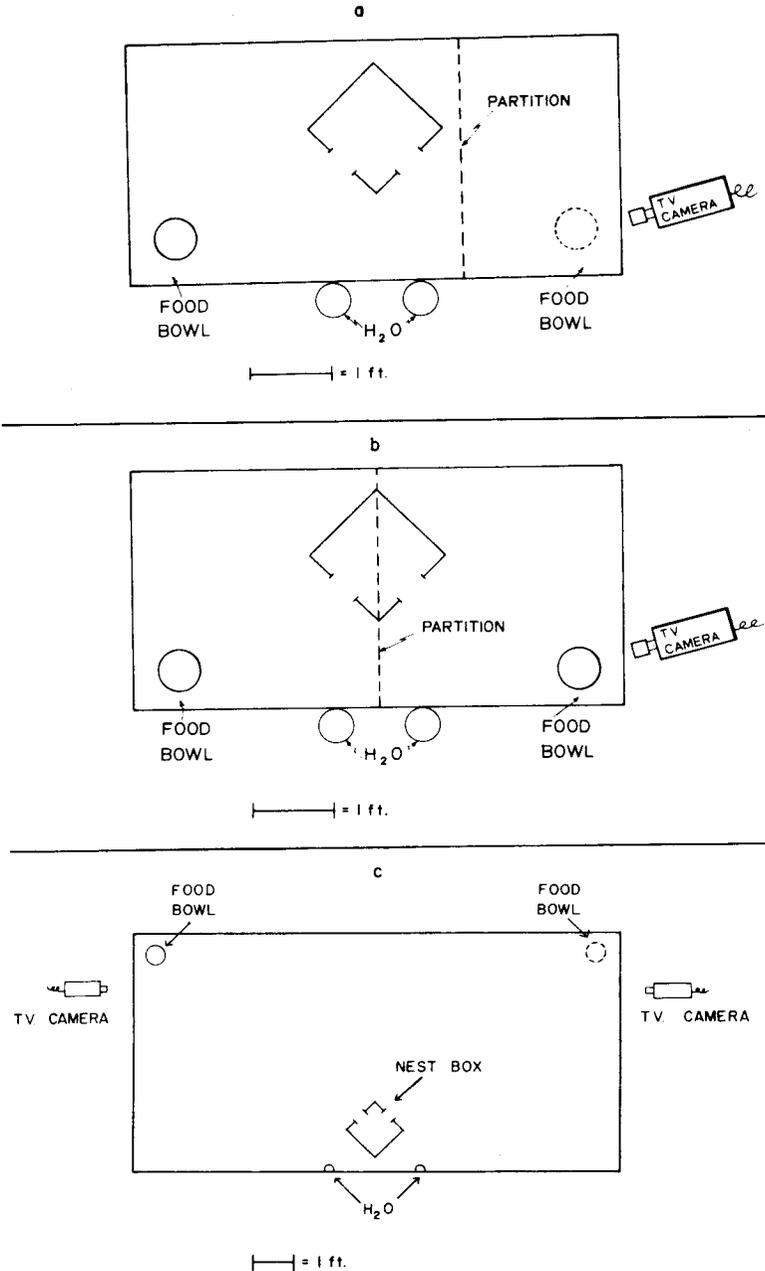


FIGURE 1. Enclosures in which experimental pups were observed.

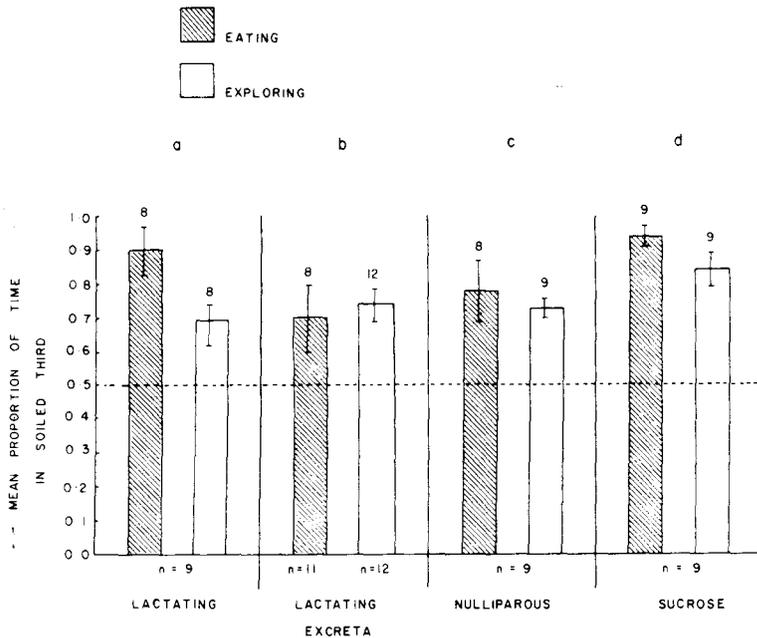


FIGURE 2. Mean amount of time spent by pups exploring in the soiled end third of enclosures as a proportion of total time spent exploring in both end thirds, and mean amount of time spent by pups eating in the soiled end third as a proportion of total time eating in both end thirds. (Flags represent $\pm 1 SE$. The numbers above the flags are the numbers of pups preferring the soiled side.)

apparatus as a proportion of total exploring and feeding time in both the soiled and unsoiled end thirds of the test enclosure. As would be expected if residual cues deposited by lactating female rats are sufficient to influence the exploration and feeding of weanlings, the majority of pups ate and explored more in the soiled than in the unsoiled portion of the enclosure.

EXPERIMENT 2

The results of Experiment 1 indicate that lactating female rats leave residual cues in an area in which they are confined, rendering that area attractive to weanlings. It cannot, however, be inferred from this observation that the residual cues affecting pups' patterns of exploration and feeding in Experiment 1 are those identified by Leon and Moltz (1971) as the maternal pheromone.

Leon and Moltz (1971) and Leon (1974) interpreted the data from their extensive investigations of the maternal pheromone as

demonstrating (a) that the pheromone is contained in the anal excreta of lactating female rats, (b) that it is not emitted by nulliparous females in sufficient quantity to be effective, and (c) that it is not synthesized by lactating females maintained on a diet containing sucrose as its only constituent carbohydrate. The hypothesis tested in this experiment, that the cues responsible for the biasing of the pups' behavior in Experiment 1 were the maternal pheromone, thus leads to predictions that pups will feed and explore preferentially on that side of a cage soiled by a lactating female rat (as shown in Experiment 1) or by her anal excreta, but not in an area marked by a nulliparous female or a lactating female eating a diet containing sucrose as its only carbohydrate.

Method

Excreta study. The procedure was identical to that of Experiment 1 except that instead of direct placement of apparatus females and their litters in the test enclosure, individual Long-Evans dams

and their young were confined in a metabolism cage for 21 hr/day (from 5:30 p.m. to 2:30 p.m.) from Days 16-24 postpartum, and the urine and excrement taken from a metabolism cage was distributed around one of the two food bowls. One third of the material was placed .10 m, one third from .10 to .20 m, and one third from .20 to .30 m from the rim of the bowl. The experimenter removed the soiled substrate at the end of each day's running and placed fresh urine and feces around the food bowl at the commencement of each subsequent experimental session. Four mothers and their litters served as apparatus subjects, and 12 pups from three litters as experimental subjects.

Nulliparous female-Purina diet study. The procedure was identical to that of Experiment 1 except that a nulliparous female maintained on Purina Laboratory Chow was placed in each test enclosure as an apparatus subject. Three nulliparous females served as apparatus subjects, and nine pups born to three females as experimental subjects.

Lactating female-sucrose-based diet study. The procedure was identical to that of Experiment 1 except that lactating females used as apparatus subjects and the dams of the experimental pups were maintained on the sucrose-based diet described by Leon (1974). Three mothers and their litters served as apparatus subjects, and nine pups from three litters as experimental subjects.

Lactating female-nulliparous female study. The method was identical to that of Experiment 1 except that the test enclosure used was that illustrated in Figure 1b and a lactating female and her young were confined on one side of the partition and a nulliparous female on the other side. Four nulliparous and four recently parturient females and their young served as apparatus subjects. Twelve pups born to four additional females served as experimental subjects.

Results

The main results of Experiment 2 are presented in Figures 2b-d and 3. As is clear from examination of the figures, (a) the fresh excreta of a lactating female are sufficient to attract pups to a feeding site and to increase the time the pups spend feeding there; (b) the residual cues deposited by a nulliparous female are sufficient to attract pups to an area and to cause them to feed in it; (c) the residual cues deposited by a lactating female maintained on a diet having sucrose as its only constituent carbohydrate are sufficient to attract pups and influence their feeding behavior; and (d) in a direct comparison of the attractiveness of the residual cues deposited by lactating

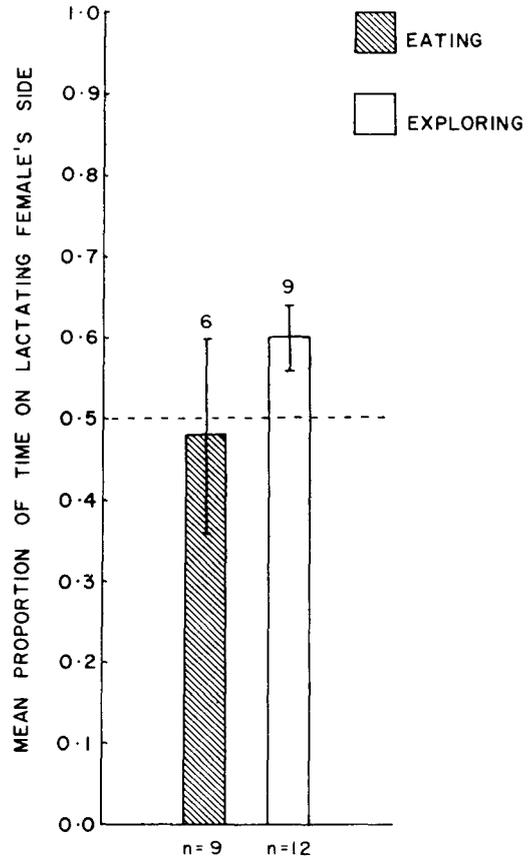


FIGURE 3. Mean amount of time spent by pups eating and exploring in the end third of the enclosure soiled by a lactating female as a proportion of total time spent eating and exploring in both end thirds. (Flags represent $\pm 1 SE$. The numbers above the flags are the numbers of pups preferring the side soiled by a lactating female.)

and by nulliparous females (Figure 3), although the area marked by a lactating female is slightly more attractive to pups than that marked by a nulliparous female, there is no reliable difference in the time spent by pups exploring or eating in the two areas.

Discussion

The results of the first study of the present experiment suggest that the attractive residual cues deposited by the lactating rats of Experiment 1 were contained in their excreta, as is the maternal pheromone. On the

other hand, the results of the remaining studies of Experiment 2 did not support the hypothesis that the cues influencing the exploration patterns and feeding site selection of pups were related to the maternal pheromone described by Leon (1974) and by Leon and Moltz (1971).

Before accepting the hypothesis that we are dealing with a second type of attractive chemical contained in the excreta of rats, it is necessary to consider some alternative explanations of the discrepancies in the data of Leon's studies and those described above. First, the present studies were conducted using Long-Evans rats as subjects, while Leon and Moltz used Wistar rats in their work. It is possible that the conditions of synthesis or of excretion of the attractive material described by Leon (1974) differ between strains. Second, the testing procedure used by Leon and Moltz in their work to determine the relative attractiveness of chemical cues excreted by rats differed considerably from our own, and it is possible that procedural differences are sufficient to account for the differences in observed outcome.

The most direct means of investigating the first of these alternatives was to replicate the procedures of Leon and Moltz, using Long-Evans rats as subjects rather than rats of the Wistar strain. Evidence gathered in the course of this replication offered, in addition, an opportunity to gather information bearing on the role of procedural differences in producing the observed discrepancies.

EXPERIMENT 3

In the present series of studies the excreta of female Long-Evans rats were examined as sources of attractive cues in the olfactory discrimination apparatus developed by Leon and Moltz (1971).

Method

The apparatus and procedures were exactly those described in detail by Leon (1974, Note 1). A single animal was isolated in a small stainless steel cage for 3 hr. Any anal excreta present at the end of the 3-hr period (target material) were

collected with clean glass slides and placed in a disposable plastic petri dish. The soiled petri dish was then placed in one of the two goal compartments of the olfactory discrimination apparatus described below, and a clean petri dish in the other.

The olfactory discrimination apparatus, the same piece of equipment used by Leon (1974) and by Leon and Moltz (1971) and described in detail by them, consisted of a start box leading to an .46-m alley and ending in a 5-cm cliff beyond which lay two visually concealed goal compartments. Forced air, passing from a central source through each goal compartment and from there up the cliff, though the alley, and hence to the start box, delivered olfactory stimuli from the goal compartments to the subject.

As in the studies by Leon (1974), litters of pups (reduced to six pups per litter shortly after birth) were isolated for 3 hr prior to introduction into the test apparatus. Following isolation, pups were placed individually in the start box of the olfactory discrimination device and allowed 15 min to choose between goal compartments. A choice was defined as a descent of the cliff to the entrance of one of the two goal compartments.

The only difference in our procedure and that of Leon (1974) was that we handled our pups for 2 min/day for the 5 days prior to olfactory discrimination testing. This alteration in procedure was necessitated by the tendency of unhandled Long-Evans pups to make very short latency random choices following placement in the start box. Unhandled Wistar pups do not behave in this fashion (Leon, Note 2).

In each of the studies described below, pups were reared by their natural mother, eating ad lib a diet identical to that of the animal providing excreta for use in the olfactory discrimination apparatus, and they were tested at 20-21 days of age.

Lactating female-Purina diet (LF-P) study. Anal excreta from six lactating females maintained on Purina Laboratory Chow were used as target material, and the 36 pups of these females served as subjects. Each pup had as target material the anal excreta of its own mother.

Lactating female-sucrose-based diet (LF-S) study. Anal excreta from six lactating females maintained for 25 days of the sucrose-based diet employed in Experiment 2 were used as target material, and the 36 pups of these females served as subjects. Again, each pup had as target material the anal excreta of its own mother.

Nulliparous female-Purina diet (NF-P) study. Anal excreta from six nulliparous females maintained on Purina Laboratory Chow were used as target material, and 36 pups from six litters maintained on the same diet served as subjects. Six pups were exposed to the target material collected from each nulliparous female.

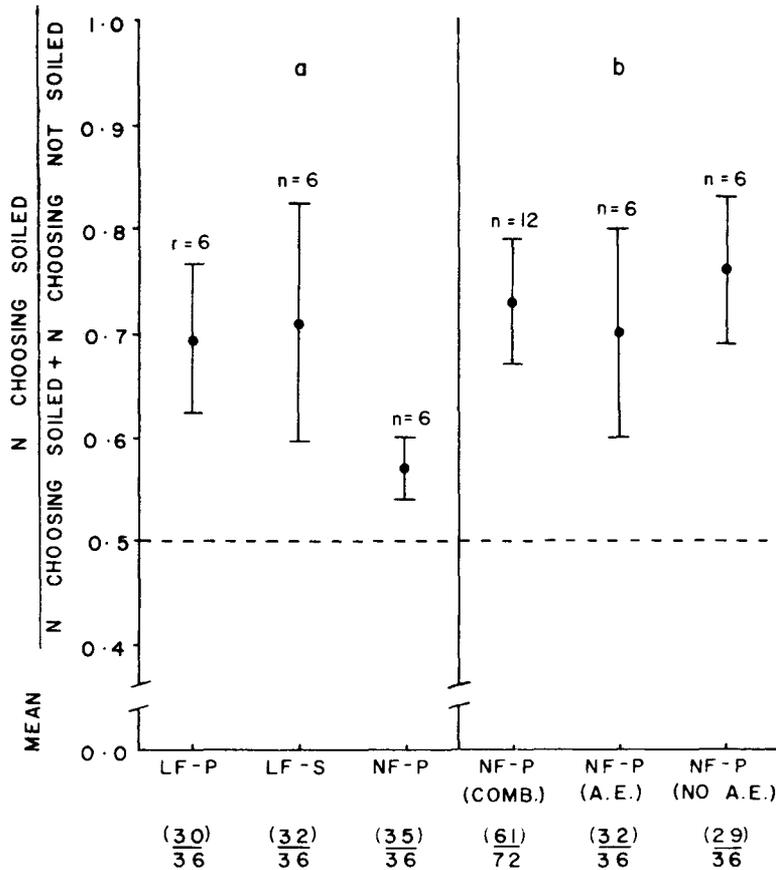


FIGURE 4. Mean proportion of pups choosing either goal box which chose the soiled goal box. (Flags represent ± 1 SE. The numbers above the flags are the number of litters in each group. Abbreviations for identification of groups: LF = lactating female; P = Purina diet; S = sucrose-based diet; NF = nulliparous female. A.E. = anal excreta.)

Results and Discussion

The main results of Experiment 3 are presented in Figure 4a, which shows the mean proportion of pups in each litter descending to either goal box which chose the goal box containing target material. The numbers in parentheses along the abscissa indicate the number of pups in each study making a choice. The remaining pups failed to descend the cliff during the 15-min test period.

As is apparent from examination of Figure 4a, the anal excreta of lactating Long-Evans rats eating either Purina chow or a sucrose-based diet were approached with equal probability, and both were approached

with higher probability than would be expected by chance and with higher probability than the anal excreta of nulliparous Long-Evans females.

The results of this experiment resolve one of the contradictions between the data collected by Leon (1974) in the olfactory discrimination apparatus and those presented in Experiment 2 above. The discrepancy in attractiveness of the residual olfactory cues deposited by lactating females eating a sucrose-based diet in the present series of experiments and of females in the investigations of Leon is seen to be the result of differences in the strain of rat used. Apparently Long-Evans rats

synthesize attractive anal excreta when maintained on a sucrose-based diet, while Wistar rats do not.

On the other hand, strain differences are not sufficient to explain the failure of the excreta of nulliparous females to attract pups in the studies by Leon and their ability to do so in Experiment 2 above. When placed in the olfactory discrimination apparatus, the anal excreta of nulliparous Long-Evans females failed to attract pups as did those of Leon's Wistar nulliparas.

The following experiment considers two alternative explanations of the discrepancy between the attractiveness of the excreta of nulliparous females in Experiment 2 above and their failure to attract in the olfactory discrimination apparatus.

EXPERIMENT 4

When one observes animals during the course of their participation in the olfactory discrimination procedures described by Leon and Moltz (1971), one is struck by the fact that although all lactating females (20/20) defecate when confined for 3 hr in a small cage, relatively few nulliparous females do so. Although one cannot specify the exact proportion of nulliparas producing visible quantities of anal excreta when isolated for 3 hr, as this varies with both strain and life history (Long-Evans handled, 3/9; Long-Evans unhandled, 10/13; Wistar unhandled, 5/15), it is clear in all cases examined that both the probability of production of detectable quantities of excreta and the amount of excreta produced when excretion does occur are much higher for lactating than for virgin rats. During 3 hr of isolation, defecating virgins produced a mean of .81 g wet weight of anal excreta ($n = 8$; $SE = \pm .10$ g), and lactating females a mean of 7.7 g wet weight of anal excreta ($n = 8$; $SE = \pm 1.0$ g). It is therefore possible, as Leon (1974) has suggested, that the failure of pups to approach the anal excreta of nulliparas and their willingness to approach that of lactating females in the olfactory discrimination apparatus are due to the scarcity of excreta produced by nulliparas and the abundance of that produced by postparturates.

In the procedures described in Experiments 1 and 2 all apparatus animals were left in the test enclosures for 21 hr/day, and of course, both postparturates and nulliparas deposited considerable quantities of anal excreta in the apparatus, though the latter only half as much material as the former (virgins: $n = 4$, $\bar{X} = 4.5$ g/21 hr, $SE = \pm .1$ g.; lactating females: $n = 4$, $\bar{X} = 9.3$ g/21 hr, $SE = \pm .7$ g.; partially dry weights).

Thus, the difference in the effects on behavior of the presence of the excreta of nulliparas in the Leon procedures and in Experiment 2 might simply result from differences in the amount of target material present in the two situations, as a result of differences in the time over which target material was deposited.

Alternatively, it is possible that nulliparas in Experiment 2 deposited chemical cues not contained in anal excreta, which are attractive to pups. The procedure of Leon (1974) precludes the discovery of evidence of such cues in the olfactory discrimination apparatus in that it is the anal excreta alone that are transferred from holding cage to olfactory discrimination apparatus goal box and to which the pups are exposed as target material.

The two studies described below investigate these hypotheses. In the first study, pups were tested in the olfactory discrimination apparatus using equal amounts of anal excreta from lactating and nulliparous females as simultaneously presented targets to determine the relative attractiveness of the two. In the second, the total excreta of nulliparas held in isolation for 3 hr were used as a target in the olfactory discrimination apparatus to determine whether nulliparas deposit cues attractive to pups via some medium other than their anal excreta.

Method

Study 1. Nulliparous female Long-Evans rats and Day 21 postpartum females of the same strain were individually confined for 3 hr in small stainless steel cages. Anal excreta were collected separately from each of the cages in which nulliparas deposited visible quantities of anal excreta. The

excreta from three virgins ($\bar{X} = 1.58$ g, $SE = \pm .24$ g) were then transferred to a petri dish, weighed, and placed in one of the goal boxes of the olfactory discrimination apparatus. An equal weight of anal excreta collected from a single lactating female was transferred to a second petri dish and placed in the other goal box of the olfactory discrimination apparatus. In eight replications, six 21-day-old pups of a lactating female contributing anal excreta were each allowed 15 min to choose between goal boxes, one containing the anal excreta of their own dam and the other containing those of three nulliparas.

Study 2. Twelve nulliparous Long-Evans rats maintained on Purina Laboratory Chow were individually isolated in small cages with removable Plexiglas floors for 3 hr. Each floor was individually placed in one goal box of the apparatus, and a clean Plexiglas floor in the other. Six 21-day-old pups from each of 12 litters maintained on Purina Laboratory Chow were allowed 15 min to choose between goal boxes.

Results

Study 1. The mean percentage of pups in each litter choosing the anal excreta of a lactating female in preference to that of an equal amount of anal excreta from a nulliparous female was 50 ($n = 8$; $SE = \pm 8$). Fifteen of the 48 pups used in the experiment failed to make a choice.

Study 2. The main results of Study 2 are presented in Figure 4b, which shows the mean proportion of pups in each litter choosing the soiled tray in preference to the clean one. The three points on the graph indicate separately the mean proportion of pups choosing the goal box containing the soiled Plexiglas floor (a) when the soiled tray contained visible quantities of anal excreta, (b) when the soiled tray contained no visible anal excreta, and (c) when both situations are considered together.

The finding that the total excreta of nulliparous females collected over 3 hr are attractive to 21-day-old pups regardless of whether visible quantities of anal excreta are present or not suggests the existence of an attractive cue not contained in anal excreta. Comparison of the attractiveness of the anal excreta of nulliparas (Figure 4a, Group NF-P) with the attractiveness of their total excretions (Figure 4b, combined group) supports the same conclusion.

Discussion

The results suggest two causes of the discrepancy in the apparent attractiveness of the excreta of nulliparous Long-Evans rats in the olfactory discrimination apparatus and in the test enclosures used in Experiment 2.

1. The finding of equal attractiveness of equal weights of anal excreta taken from lactating rats and nulliparas suggests that the difference in the behavior of pups in the olfactory discrimination apparatus exposed to anal excreta of postparturates and virgins is due, as Leon (1974) suggested, to differences in the quantity of material excreted by female rats in different reproductive states during 3 hr of isolation. Considered with the observation that virgins deposit large amounts of anal excreta in the test enclosures used in Experiment 2 during 21 hr of isolation and very little during 3 hr of restraint in the olfactory discrimination procedure, the differential outcome of the two test procedures under consideration becomes predictable.

2. The finding in Study 2 of an attractive cue deposited by nulliparas, but not contained in anal excreta, provides a further plausible explanation of the difference in outcome of measures of the attractiveness of the excreta of nulliparas, using the procedures of Leon and those of Experiment 2. While the Leon studies used anal excreta alone as target material, the total excreta deposited by nulliparas were presented to pups in Experiment 2. It seems reasonable to conclude that both factors identified above played a role in producing the contradiction in apparent attractiveness of the excreta of nulliparas under discussion.

EXPERIMENT 5

The results of the preceding experiments indicate that female rats deposit residual cues in areas that they occupy and that these cues can influence the exploratory and feeding behavior of pups. However, if such cues function to direct pups to specific feeding sites, as suggested in the introduction to the present article, then it is necessary not only that rats excrete attractive

residual cues but also that they concentrate excretion of them in the vicinity of feeding areas. In both Experiments 1 and 2, apparatus subjects were confined by the experimenter in the area in which they fed, thus forcing excretion near one potential feeding site rather than another. In this experiment, apparatus subjects were free to distribute their excreta as they wished, and experimental subjects were used to determine whether the distribution achieved was such as to bias pups toward feeding at the location where apparatus subjects fed.

In order to assess the generality of the observed results two separate studies were conducted in cages of differing size.

Method

Small-cage study. The procedure was identical to that employed in Experiment 1 except that no partition was introduced into the test enclosure illustrated in Figure 1a and that one of the food bowls was present in the test enclosure only while experimental subjects were in it. The experimenter, in addition to recording the amount of time spent by pups eating and moving in the end thirds of the enclosure, mapped the distribution of anal excreta in each test enclosure prior to placing an experimental pup in it. Six lactating females and their litters served as apparatus subjects, and 18 pups born to three mothers as experimental subjects.

Large-cage study. The procedure was identical to that of the small-cage study except that the 6.69-sq.-m enclosure illustrated in Figure 1c was employed. Four lactating females and their litters served as apparatus subjects, and 24 pups born to four mothers as experimental animals.

Results and Discussion

The main results of Experiment 5 are presented in Figure 5, which gives the percentage of time experimental pups spent exploring and feeding in the portion of the enclosure in which apparatus subjects had been fed. As is apparent from examination of the figure, there was a slight tendency for pups to explore and feed more than would be expected by chance in the sections of the test enclosures previously used for feeding by apparatus subjects. However, none of these differences were reliable.

Examination of the distribution of excreta in the test enclosures revealed a high concentration of boluses in the immediate

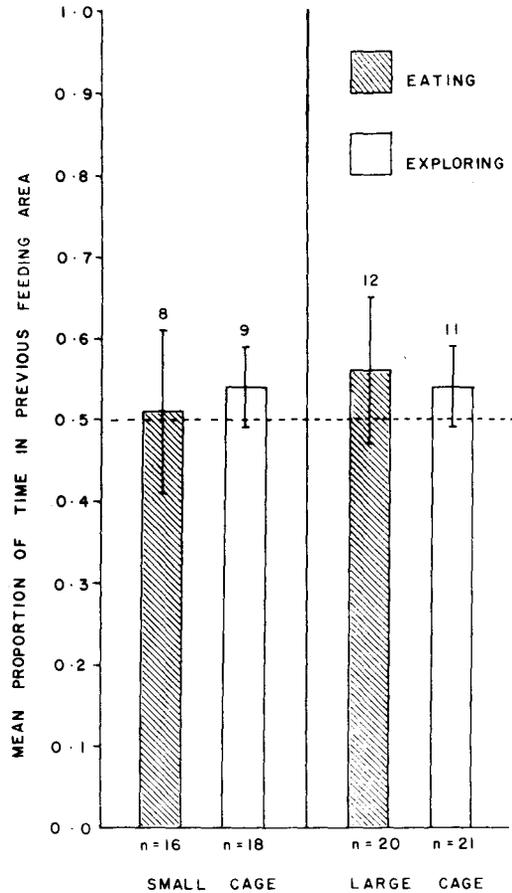


FIGURE 5. Mean amount of time spent by pups eating at the previously utilized food bowl as a proportion of total time eating at both food bowls, and mean amount of time spent exploring in the end third containing the utilized bowl as a proportion of total time spent exploring in both end thirds. (Flags represent ± 1 SE. The numbers above the flags are the numbers of pups preferring the side containing the previously utilized food bowl.)

vicinity of the food bowl at which the apparatus animals had fed. In the small enclosures an average of 26% of boluses were located within .15 m of the food bowl, and in the large enclosure an average of 73% were located within .30 m of the food bowl (areas equaling, respectively, approximately 8% and 4% of the total enclosure area) with an essentially random scattering of the remaining boluses with respect to feeding site location.

The data of the present experiment do not support the hypothesis that residual cues deposited by lactating rats are distributed in such a way as to lead pups to one feeding site in preference to another in experimental areas of convenient size for use in the laboratory. Pups will explore and eat as readily in areas of low excreta density as in those of high excreta density. This finding contrasts markedly with the data of Experiments 1 and 2, which indicate a strong tendency of pups to explore and eat in areas soiled by conspecifics in preference to totally unsoiled ones.

If one extrapolates from the present series of experiments to the natural situation, in which wild *R. norvegicus* do not visit large areas within their home ranges (Telle, 1966) and therefore cannot soil them, it seems reasonable to suggest that the naturally occurring distribution of conspecific residual olfactory cues might play an important role in influencing the exploration and feeding site selection of young rats in the natural habitat. Such effects of the natural distribution of residual olfactory cues may be difficult to observe in the laboratory because of the restricted space available and the consequent high probability that all of it will be visited and soiled by residents.

EXPERIMENT 6

For residual olfactory cues deposited by adult rats to function to direct weanlings to food sites in natural situations, they must affect the movement of pups during the time when they begin to venture out of the burrow to seek solid food. Calhoun (1962) reported that young wild *R. norvegicus* undertake their first extensive wanderings outside the burrow at approximately 34 days of age.

It has been demonstrated that by the time albino rat pups reach 27 days of age, their tendency to approach a lactating female in preference to a nulliparous one has already waned (Leon & Moltz, 1972; Moltz, Leidahl, & Rowland, 1974). Although these data do not bear directly on the question of the range of pup ages during

which residual cues deposited by adults are effective in influencing the movement of pups, they suggest that rat pups may lose their tendency to explore in areas soiled by conspecifics before they begin to explore on the surface in the natural habitat.

In this experiment, rats of 35 and 65 days of age were tested to determine the influence of residual olfactory cues deposited by adult conspecifics on the exploration patterns and feeding site preferences of younger animals. Its purpose was to determine whether residual cues affect the behavior of pups at an age when they normally explore alone in the environment and, thus, to determine whether they might actually play an important role in the weaning of rats in the natural habitat.

Method

The method was identical to that of Experiment 1 except that experimental subjects were 35 or 65 days of age at the commencement of testing. Experimental pups were weaned at 21 days of age and maintained on ad lib Purina Laboratory Chow until 24 hr prior to testing. On testing days they received either 5 g (35-day-old subjects) or 10 g (65-day-old subjects) of food per day immediately after each test session. Six male and six female experimental subjects from four litters were 35 days of age at the time of initiation of testing, and seven females and five males from four litters were 65 days old at the time of initiation of testing. Three nulliparous apparatus subjects maintained on ad lib Purina Laboratory Chow were placed in each test enclosure for the 21 hr preceding testing on each test day.

Results and Discussion

The main results of Experiment 6 are presented in Figure 6, which shows the amount of exploring and feeding time spent by pups in the soiled end third of the test apparatus as a proportion of total exploring and feeding time in both the soiled and unsoiled end thirds of the test enclosure. As would be expected if residual cues deposited by adults are sufficient to influence the exploration and feeding of 35- and 65-day-old rats, the majority of both groups ate and explored more in the soiled than in the unsoiled portion of the test enclosure. There was no difference in the behavior of experi-

mental pups of either age as a function of sex. We do not understand the relative weakness of the effect of residual chemical cues on time spent feeding at the soiled site by 35-day-old pups, given their strong effect on exploration patterns of animals of this age.

GENERAL DISCUSSION

The data reported above support the conclusion of Leon (1974) that the anal excreta of lactating rats are attractive to pups prior to weaning. In addition, they indicate that nonlactating rats deposit attractive residual cues some of which are not present in anal excreta. While the tendency of mothers to produce and of pups to ap-

proach the former type of cue may wane as weaning progresses (Leon & Moltz, 1971; Moltz et al., 1974), the pups' tendency to approach the latter type of cue, not dependent on reproductive state, extends to maturity. Thus, although the maternal pheromone produced by lactating females may serve to mediate mother-young interaction within the burrow, it is some other residual olfactory cue that functions to influence patterns of movement and exploration of adolescent rats when they leave the protection of the natal site.

Although we have collected little evidence as to the nature of these alternative residual olfactory cues, von Reiff (1956) reported that the fermenting urine of rats, produced by the action of bacteria from the intercellular space of the connective tissue of the urogenital tract on fresh rat urine, is highly attractive to conspecifics and plays a role in directing the movement of rats within their clan territories (Telle, 1966). It seems reasonable therefore, to hypothesize that deposits of fermenting urine may be, at least in part, responsible for the observed ability of adult rats to influence the exploration and feeding patterns of their young via residual olfactory cues.

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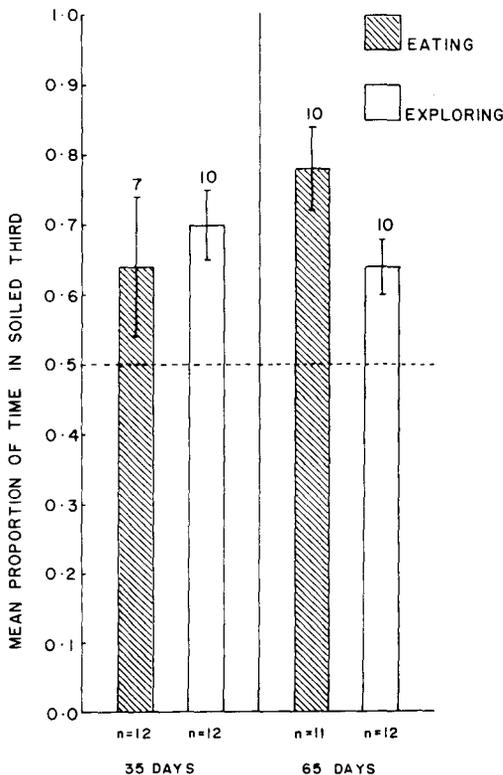


FIGURE 6. Mean amount of time spent by pups exploring in the soiled end third of enclosures as a proportion of total time spent exploring in both end thirds, and mean amount of time spent by pups eating in the soiled end third as a proportion of total time eating in both end thirds. (Flags represent $\pm 1 SE$. The numbers above the flags are the numbers of pups preferring the soiled side.)

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