OLFACTORY CUES IN FEEDING

THE ROLE OF RESIDUAL OLFACTORY CUES IN THE DETERMINATION OF THE FEEDING SITE SELECTION AND EXPLORATION PATTERNS OF DOMESTIC RATS

BY

LINDA M. HEIBER

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AUTHOR: Linda M. Heiber

SUPERVISOR: Dr. Bennett G. Galef, Jr.

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SCOPE AND CONTENTS: The results of the present series of experiments indicate that 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 can mark an area so as to induce pups to explore and feed in it. Residual cues continue to affect the feeding and exploratory behavior of pups to maturity.

Discrepancies between the results obtained in the olfactory discrimination apparatus (Leon & Moltz, 1971) and the present experiments are resolved and evidence presented for the existence of residual cues attractive to pups not contained in anal excreta. 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.

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During their first few days of feeding on solid food, weanling domesticated rat pups exhibit a strong preference for a relatively unpalatable diet which the adults of their colony have been trained to eat (Galef & Clark, 1971, 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, in press; 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 weeks postpartum, contain olfactory cues (the maternal pheromone) highly attractive to pups of weaning age (Leon, 1974; Leon & Moltz, 1971, 1972). This finding suggests the possible existence of a second mechanism whereby adult rats might 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 the 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 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[1] to influence the exploratory behavior and feeding site selection of their young.

Method

Apparatus

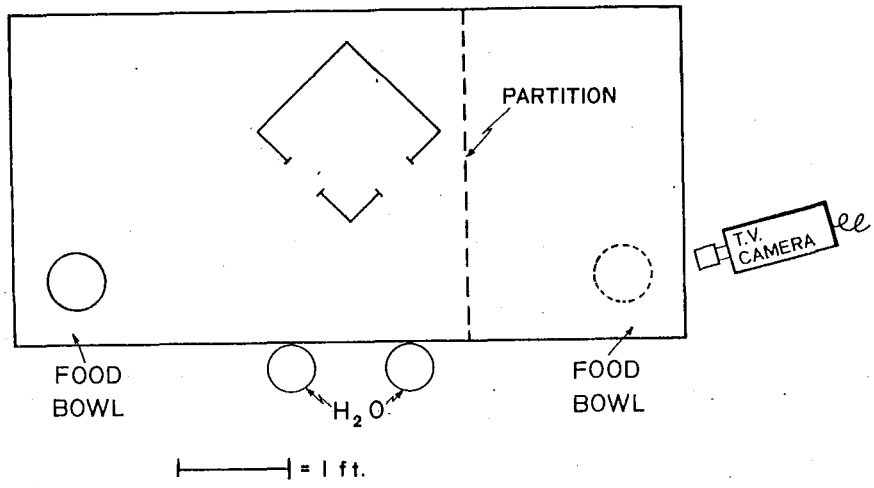
Three test enclosures identical to that illustrated in Figure 1a, were constructed of slotted angle-iron and hardware cloth. The galvanized sheet metal floor of each enclosure was lined with unused newsprint and a wooden nest box (12 x 12 x 7 in.), with two entrance holes (3 x 3 in.), was placed in its 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.

Insert Figure 1 about here

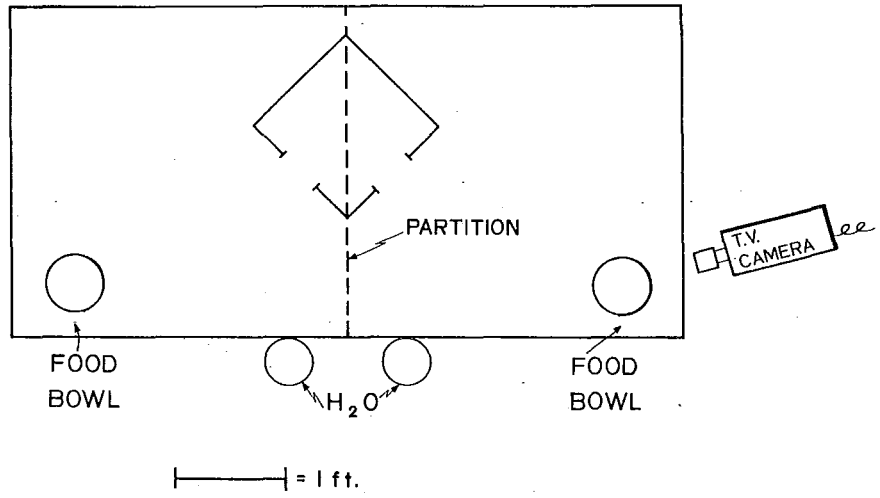
Procedure

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 marked and unmarked portions of the enclosure as experimental subjects. Experimental and apparatus subjects were treated differently, and the treatment of each group is,

a


PARTITION
T.V. CAMERA
FOOD BOWL
H_2O
FOOD BOWL
= 1 ft.

b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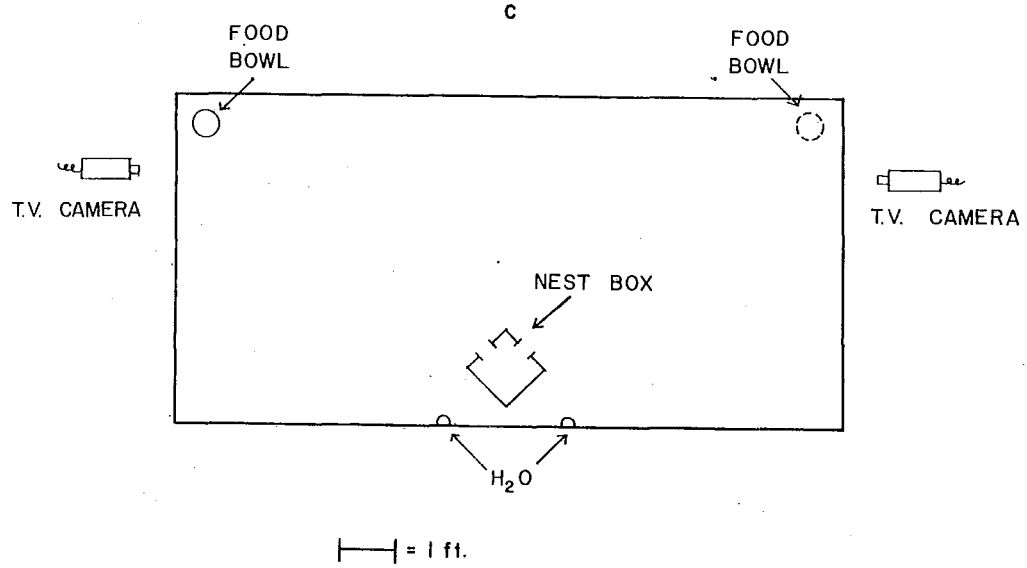
c


FOOD BOWL
FOOD BOWL
T.V. CAMERA
T.V. CAMERA
NEST BOX
H_2O
= 1 ft.

therefore, described separately below.

Apparatus Subjects

Prior to placement in the larger portion of each test enclosure pregnant apparatus subjects were maintained in 12 x 14 x 7 in. home cages on ad libitum Purina Laboratory Chow and water. On Day 3 postpartum one mother and her litter (culled to 6 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 in the present experiment.

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 12 x 14 x 7 in. cages. For the first 9 days postpartum, the dam of each experimental litter was fed Purina Laboratory Chow ad libitum 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 and 9-10 p.m.) to ensure that her young took their first meals of solid food in the test enclosure. Nine pups born to 3 Long-Evans females served as experimental subjects.

Beginning on Day 16 postpartum, pups from experimental litters were 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 taken from the enclosure, and, if necessary to equate food availability, spillage 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 and the amount of time spent feeding at each food bowl. At the end of each daily 3-hr. test session (after three individual experimental pups had been observed) the experimenter replaced the partition and the apparatus animals.

Results and Discussion

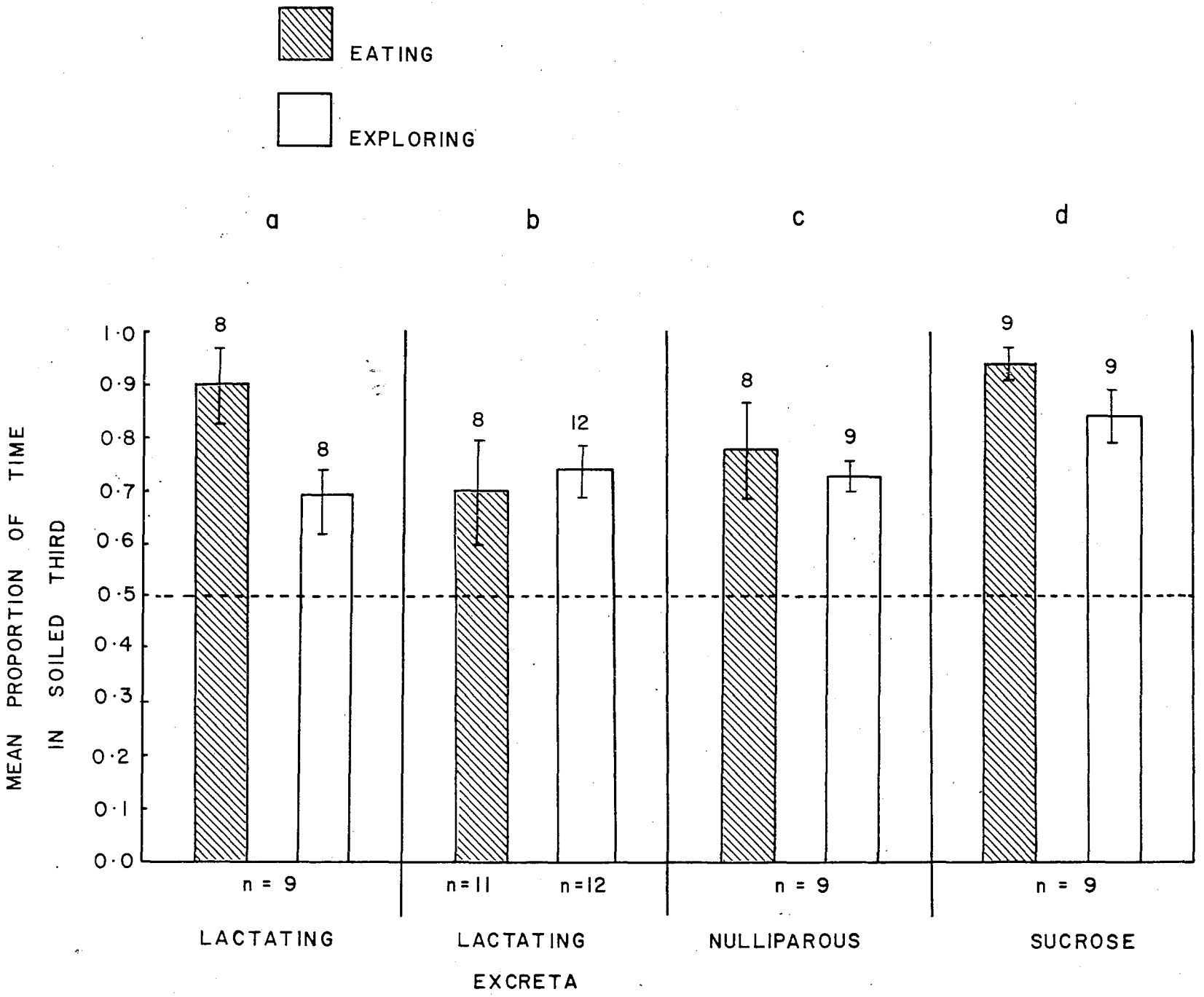
The main results of Experiment 1 are presented in Figure 2a, which indicates the amount of exploring and feeding time spent by pups in the soiled end third of the test apparatus as a proportion of total exploring and feeding time in both the soiled and unsoiled end thirds of the test enclosure. As would be expected, if residual cues deposited by adults are sufficient to influence the exploration and feeding of weanlings, the majority of pups ate and explored more in the soiled than in the unsoiled portion of the enclosure.

Insert Figure 2 about here

Experiment 2

The results of Experiment 1 indicate that lactating female rats will chemically mark an area in which they are confined so as to make it 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 & Moltz (1971) and Leon (1974) have interpreted the data from their extensive investigations of the maternal pheromone as demonstrating, (1) that it is contained in the anal excreta of lactating female rats, (2) that it is not emitted in sufficient quantity to be effective by nulliparous females, and (3) that it is not synthesized by lactating females maintained on a diet containing sucrose as its only constituent carbohydrate. The hypothesis tested in the present study, that the cues responsible for the biasing of the pups' behavior in Experiment 1 were the maternal


EATING
EXPLORING
a
b
c
d
MEAN PROPORTION OF TIME IN SOILED THIRD
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0·0
8
8
8
12
8
9
9
9
n = 9
n=11
n=12
n = 9
n = 9
LACTATING
LACTATING
NULLIPAROUS
SUCROSE
EXCRETA

pheromone, thus leads to predictions that pups will only feed and explore preferentially on that side of a cage marked by a lactating female rat (as shown in Experiment 1), or by her anal excreta, and 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 placing apparatus females and their litters in the test enclosure directly, 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 their urine and anal excretions placed in the test apparatus. Just prior to the introduction of the first experimental pup into each test enclosure on each test day, the urine and excretment taken from a metabolism cage was distributed around one of the two food bowls. One-third of the material was placed 4 in., 1/3 from 4 to 8 in., and 1/3 from 8 to 12 in. 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 were used as apparatus subjects, and 12 pups from 3 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 were used as apparatus subjects and 9 pups born to 2 females as experimental pups.

Lactating Female Sucrose Based Diet Study

The procedure was identical to that of Experiment 1 except that both lactating apparatus females and the dams of experimental pups were maintained on the sucrose-based diet described by Leon (1974). Three mothers and their litters were used as apparatus subjects and 9 pups from 3 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 4 recently parturient females and their young served as apparatus subjects. Twelve pups born to 4 additional females served as experimental subjects.

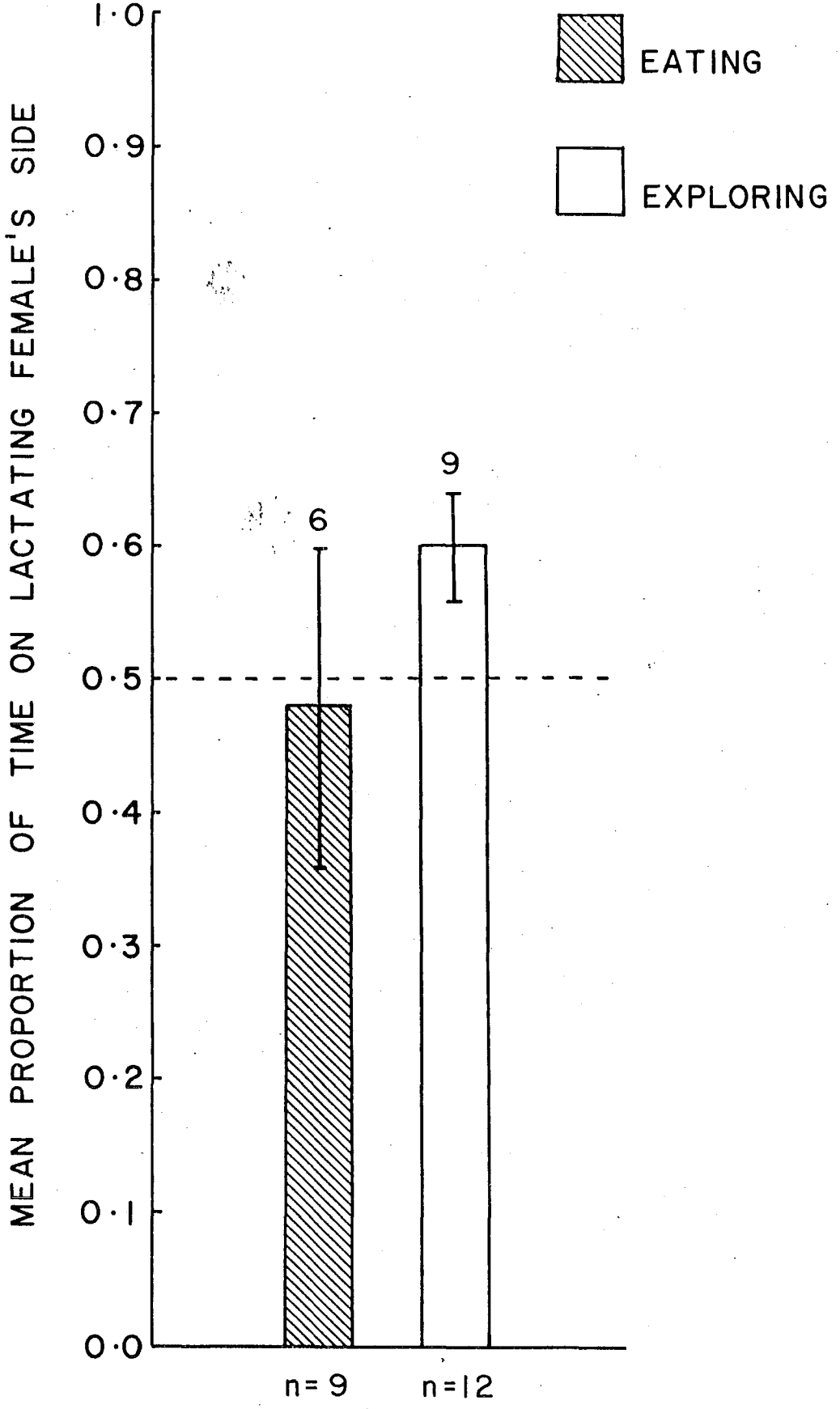
Results

The main results of Experiment 2 are presented in Figures 2b-d and 3. As is clear from examination of the figures: (1) The fresh excreta of a lactating female is sufficient to attract pups to a feeding site and cause them to eat there. (2) The residual cues deposited by a nulliparous female are sufficient to attract pups to an area and cause them to feed in it. (3) 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. (4) In a direct comparison of the attractiveness of the residual cues deposited by lactating and nulliparous females (Fig. 3), although the area marked by a lactating female was slightly more attractive to pups than that marked by a nulliparous female, there was no reliable difference in the time spent by pups exploring or eating in the two areas.

Insert Figure 3 about here

Discussion

The results of the first of the present series of experiments suggest that the attractive residual cues deposited by the lactating rats of Experiment 1 were contained in their excreta, as is the maternal pheromone. To the contrary, the results of the remaining studies 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).


EATING
EXPLORING
MEAN PROPORTION OF TIME ON LACTATING FEMALE'S SIDE
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0·0
6
9
n= 9
n=12

Before accepting the hypothesis that we are dealing in the present experiments 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 have used Wistar rats in their work. It is possible that the conditions of synthesis or excretion of the attractive material Leon (1974) has described differ between strains. Second, the testing procedures 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 in Leon (1974) (Leon, personal communication). A single target 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 there, consisted of a start box, leading to an 18 in. alley, ending in a 2 in. 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, through the alley and, hence, to the start box, delivered olfactory stimuli from the goal compartments to a subject.

As in the studies by Leon (1974), litters of pups (reduced to 6 pups/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, personal communication).

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

Lactating Female-Purina Diet (LF-P) Study

Anal excreta from 6 lactating females maintained on Purina Laboratory Chow were used as target material and their 36 pups 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 6 lactating females maintained for 25 days on 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 6 nulliparous females maintained on Purina Laboratory Chow were used as target material and 36 pups from 6 litters maintained on the same diet served as subjects. Six pups were exposed to the target material collected from each nulliparous female.

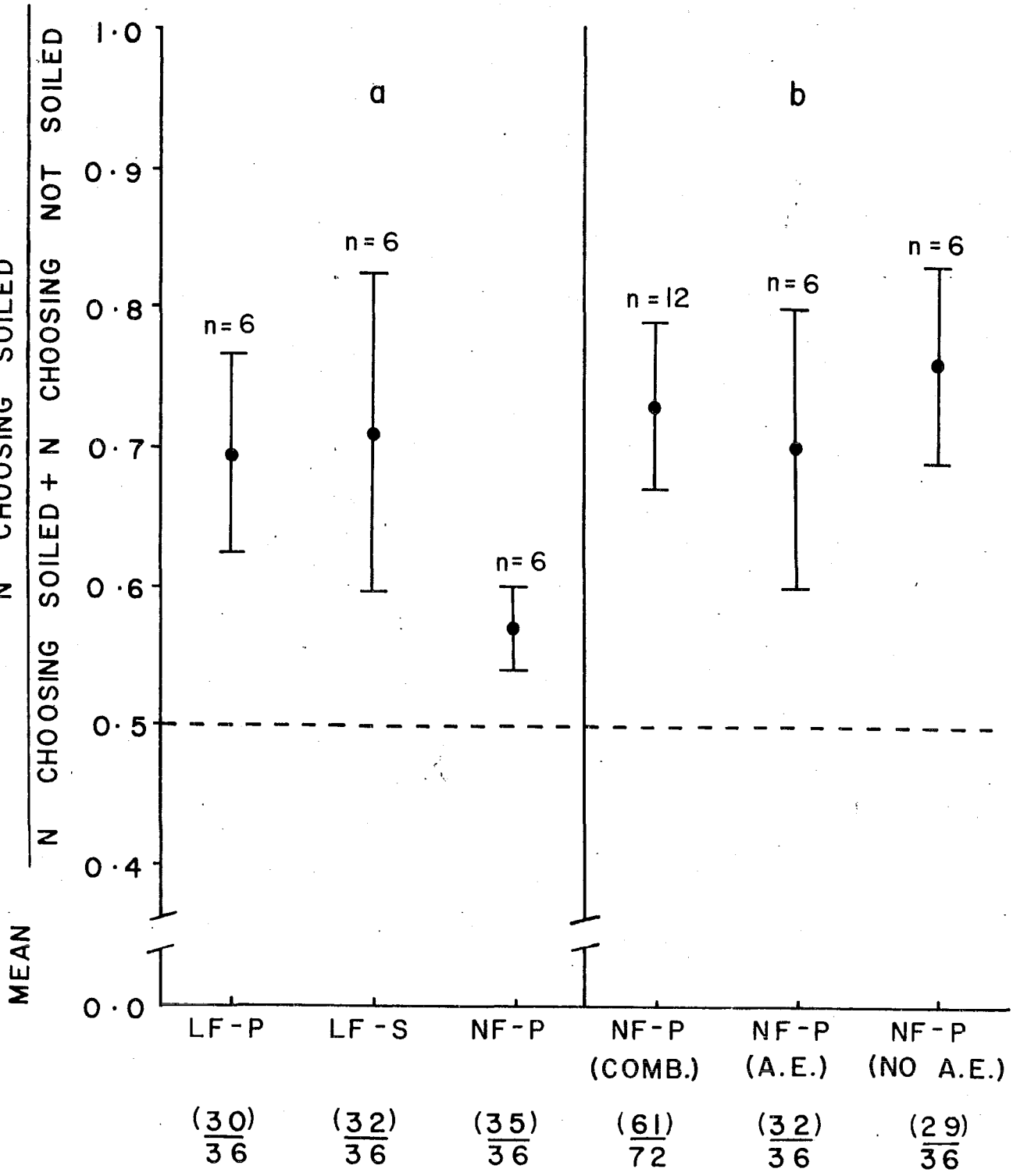
Results and Discussion

The main results of Experiment 3 are presented in Figure 4a, which describes the mean proportion of pups in each litter choosing either goal box, which chose the goal box containing soiled material. The numbers in paranthesis 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.

Insert Figure 4 about here

The results of the present 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 lactating females eating a sucrose-based diet in the present series of experiments and of females in the investigations of Leon (1974) is seen to be the result of differences in the strain of rat employed. Apparently Long-Evans rats synthesize attractive anal excreta when maintained on a sucrose-based diet, while Wistar rats do not.


MEAN
N CHOOSING SOILED / N CHOOSING SOILED + N CHOOSING NOT SOILED
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·0
a
b
n = 6
n = 6
n = 6
n = 12
n = 6
n = 6
LF - P
LF - S
NF - P
NF - P (COMB.)
NF - P (A.E.)
NF - P (NO A.E.)
(30/36)
(32/36)
(35/36)
(61/72)
(32/36)
(29/36)

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 that of Leon's Wistar nulliparae.

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

Experiment 4

If 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 defecate when confined for 3 hr. in a small cage (20/20), relatively few nulliparous females do so. Although one cannot specify the exact percentage of nulliparae 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 occurs is much higher for lactating than for virgin rats. During 3 hr. isolation, defecating virgins produced a mean of .81 g. wet weight of anal excreta (n=8, S.E.M.= ±.10 g.) and lactating females a mean of 7.7 g. wet weight of anal excreta (n=8, S.E.M.= ± 1.0 g.). It is therefore possible that, as Leon (1974) has suggested, the failure of pups to approach the anal excreta of nulliparae and their willingness to approach that of lactating females in the olfactory discrimination apparatus is due to the scarcity of excreta produced by nulliparae and the abundance of that produced by postparturates.

In the procedures described in Experiments 1 and 2 above all apparatus animals were left in the test enclosures for 21 hr/day and, of course, both postparturates

and nulliparae deposited considerable quantities of anal excreta in the apparatus, though the latter only half so much material as the former (Virgins, n = 4, $\overline{X}$ = 4.5 g/21 hr., S.E.M. =±.1 g.; Lactating females, n = 4, $\overline{X}$ = 9.3 g/21 hr., S.E.M. =±.7 g.; partially dry weights).

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

Alternatively, it is possible that nulliparae in Experiment 2 above 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 which is 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 both 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 nulliparae held in isolation for 3 hr. were used as a target in the olfactory discrimination apparatus to determine whether or not nulliparae deposit cues attractive to pups via some medium other than their anal excreta.

Method

Study 1

Fourteen nulliparous female Long-Evans rats and eight 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 8 cages in which nulliparae deposited visible quantities of anal excreta. The excreta

from three virgins ($\bar{X}$ = 1.58 g, S.E.M. = ± .24 g) was 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. The six 21 day old pups of the female contributing anal excreta were each allowed 15 min. to choose between goal boxes.

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, S.E.M. = ± 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 indicates 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: (1) when the soiled tray contained anal excreta (A.E.), (2) when the soiled tray contained no anal excreta (No A.E.) and (3) for both (1) and (2) considered together (Comb.).

The finding that the total excreta of nulliparous females collected over 3 hr. is attractive to 21 day old pups regardless of whether or not visible quantities of anal excreta are present suggests the existence of an attractive cue not contained in anal excreta. Comparison of the attractiveness of the anal excreta of nulliparae

(Figure 4a, Group JF-P) with the attractiveness of their total excretions (Figure 4b, Combined Group) supports the same conclusion.

Discussion

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

First, the finding of equal attractiveness of equal weights of anal excreta taken from lactating rats and nulliparae suggests that the difference in the behavior of pups in the olfactory discrimination apparatus exposed to anal excreta of post-parturates and virgins is due, as Leon (1974) has 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. isolation and very little during 3 hr. restraint in the olfactory discrimination procedure, the differential outcomes of the two test procedures under consideration becomes predictable.

Second, the finding in Study 2 of an attractive cue deposited by nulliparae 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 nulliparae using the procedures of Leon (1974) and those of Experiment 2. While the Leon (1974) studies employed anal excreta alone as target material, the total excreta deposited by nulliparae were presented to pups in Experiment 2. It seems reasonable to conclude that both factors indicated above played a role in producing the contradiction in apparent attractiveness of the excreta of nulliparae under discussion.

Experiment 5

The results of preceding experiments indicate that female rats can mark an area which they occupy with attractive residual cues, 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 paper, then it is necessary that rats not only 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 the present experiment apparatus subjects were free to distribute their excreta as they wished, and experimental subjects were used to determine whether or not the distribution achieved was such as to bias pups toward feeding at the location at which 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 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 3 mothers as experimental subjects.

Large Cage Study

The procedure was identical to that of the Small Cage Study except that the 72 sq. ft. enclosure illustrated in Figure 1c was employed. Four lactating females and their litters served as apparatus subjects and 24 pups born to 4 mothers as experimental animals.

Results and Discussion

The main results of Experiment 5 are presented in Figure 5, which indicates

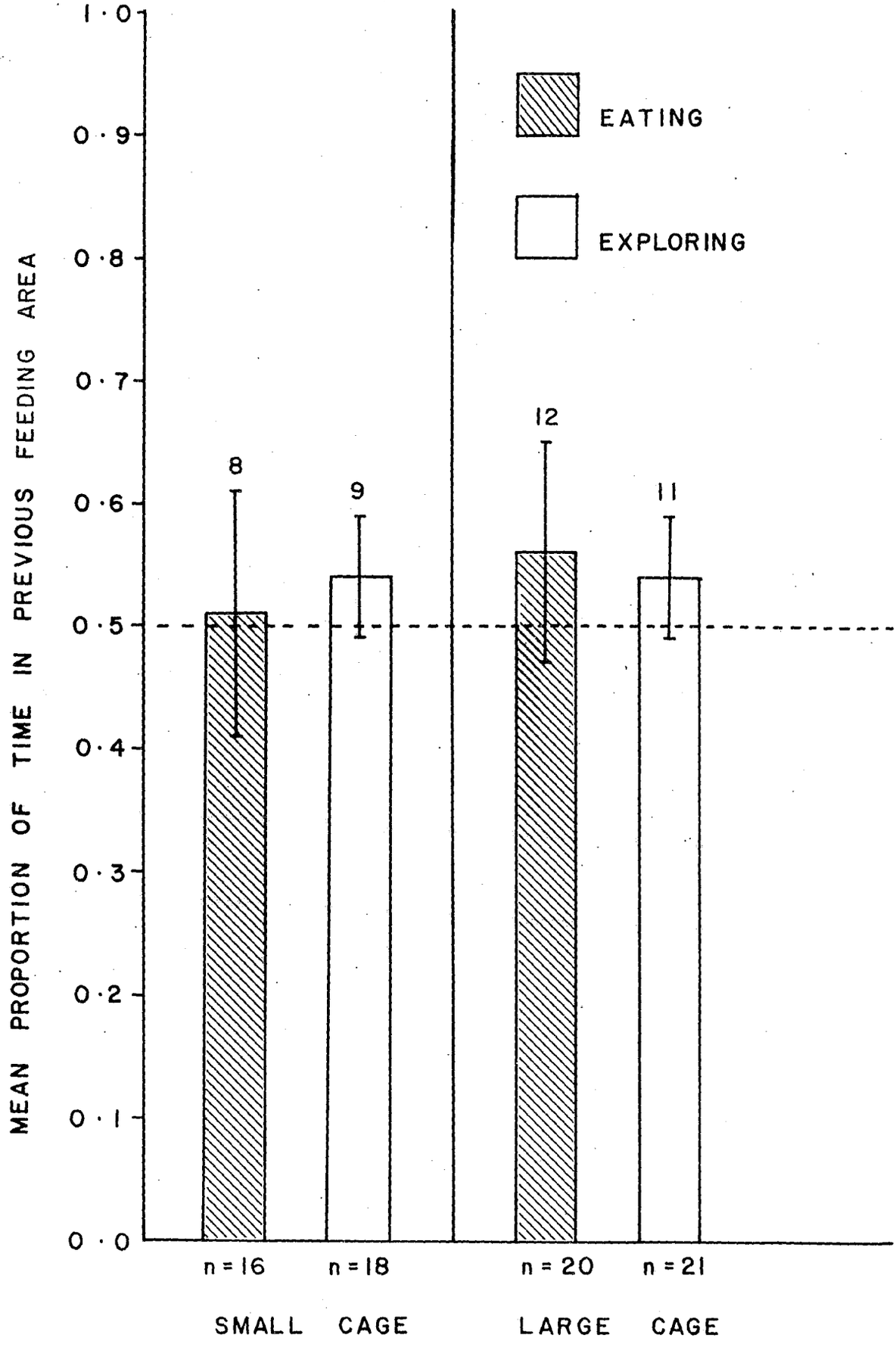
the percentage of time experimental pups spent exploring and feeding in the portion of the enclosure in which apparatus subjects had been fed. As is apparent from examination of the figure, there was a slight tendency for pups to explore and feed more than would be expected by chance in the sections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

Insert Figure 5 about here

Examination of the distribution of excreta in the test enclosures revealed a high concentration of boluses in the immediate vicinity of the food bowl at which the apparatus animals had fed. In the small enclosures an average of 26 percent of boluses were located within 6 in. of the food bowl and in the large enclosure an average of 73 percent were located within 12 in. of the food bowl (areas equaling, respectively, approximately 8 and 4 percent 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 above, 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 mark 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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MEAN PROPORTION OF TIME IN PREVIOUS FEEDING AREA
EATING
EXPLORING
8
9
12
11
n = 16
n = 18
n = 20
n = 21
SMALL CAGE
LARGE CAGE

distribution of residual olfactory cues may be difficult to observe in the laboratory because of the restricted space available and the high probability that all of it will be visited and marked 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 effect the movement of pups during the time when they begin to venture out of the burrow to seek solid food. Calhoun (1962) has 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 the present 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 or not residual cues effect the behavior of pups at an age when they normally explore alone in the environment and, thus, to determine whether or not 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 postpartum 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 10g. (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 female 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.

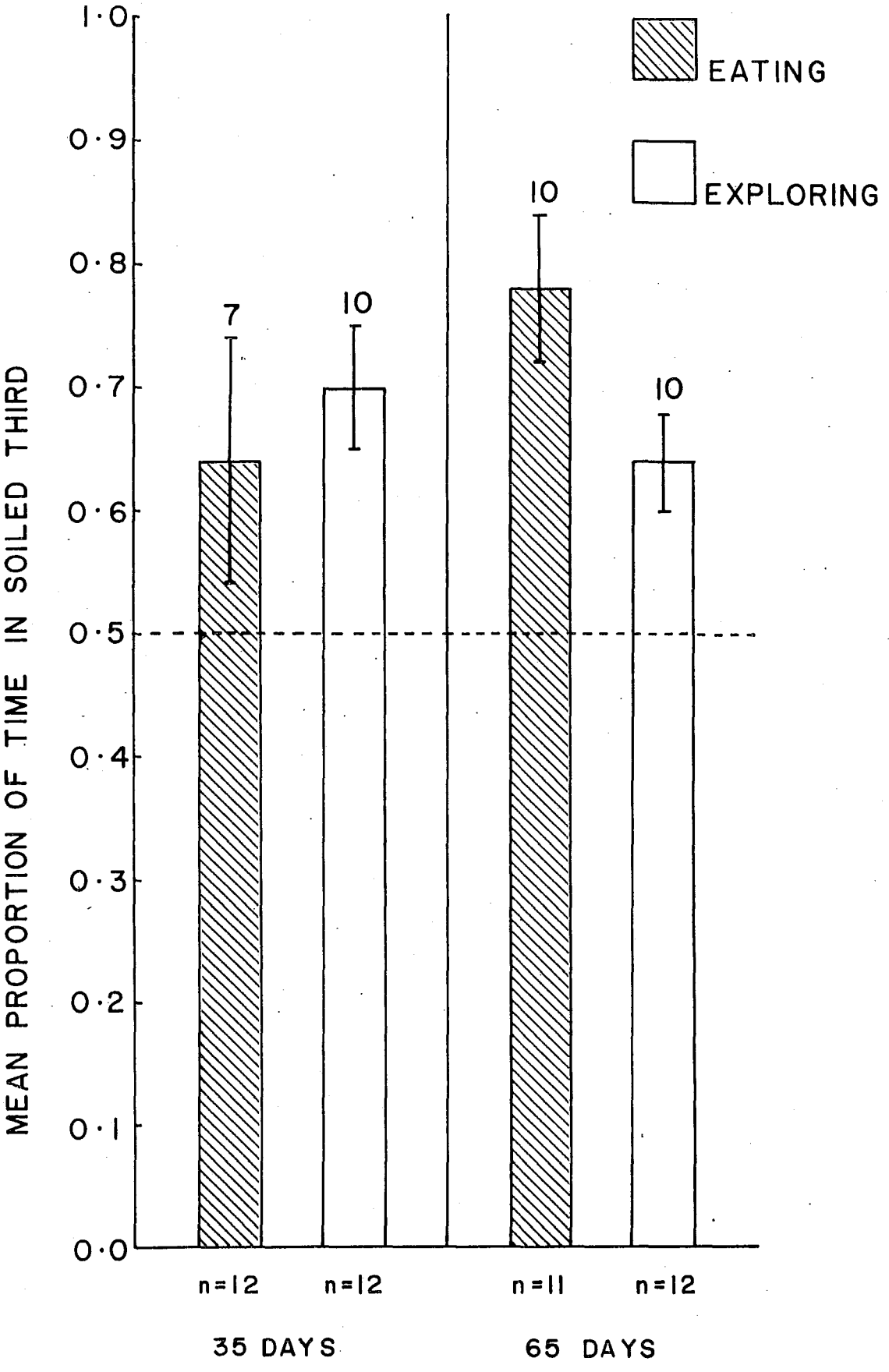
Results and Discussion

The main results of Experiment 6 are presented in Figure 6 which indicates 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 behaviour of experimental pups of either age as a function of sex. We do not understand the relative weakness of the effect of residual chemical cues on feeding site selection by 35 day old pups, given their strong effect on exploration.

Insert Figure 6 about here

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 non-lactating rats deposit attractive residual cues not present in anal excreta. While the tendency of pups to approach the former type of cue wanes as weaning progresses (Leon & Moltz, 1971; Moltz, Leidahl & Rowland, 1974) their tendency to approach the latter extends to maturity. Thus, although the large quantity of anal excreta produced by lactating females may serve to mediate mother-young interaction within the burrow, it is cues conveyed via some other vehicle which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MEAN PROPORTION OF TIME IN SOILED THIRD
EATING
EXPLORING
7
10
10
10
n=12
n=12
n=11
n=12
35 DAYS
65 DAYS

may function to influence patterns of movement and exploration of adolescent rats when they leave the protection of the natal site.

Although the data presented here are not sufficient to identify the source of this second type of cue, work by Von Reiff (1956) indicates that the trails of wild rats are scent marked with secretions of the uro-genital tract. In particular, the decomposition products of rat urine fermentation, resulting from the action of bacteria from the intercellular space of the connective tissue of the uro-genital tract, are strongly attractive to conspecifics. It is possible that these residual chemical cues, produced by bacterial action on the urine of rats, are, at least in part, responsible for the tendency of weaning pups to explore and eat in areas soiled by conspecifics in preference to unsoiled ones.

Figure Captions

Fig. 1. Enclosures in which experimental pups were observed.

Fig. 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 ± 1 S.E.M. and the numbers above the flags the number of pups preferring the soiled side. See text for explanations of panels a - d.

Fig. 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 indicated ± 1 S.E.M. and the numbers above the flags the number of pups preferring the side soiled by a lactating female.

Fig. 4. Mean proportion of pups choosing either goal box which chose the soiled goal box. Flags indicate ± 1 S.E.M. See text for explanation of the groups.

Fig. 5. Mean amount of time eating at the previously utilized food bowl as a proportion of total time eating at both food bowls and mean amount of time exploring in the end-third containing the utilized bowl as a proportion of total time spent exploring in both end-thirds. Flags indicate ± 1 S.E.M. and the numbers above the flags the number of pups exploring more on the side containing the previously utilized food bowl.

Fig. 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 indicate ± 1 S.E.M. and the number above the flags the number of pups preferring the soiled side. See text for explanation of panels (a) and (b).

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FOOTNOTES

1. All rats used in the present series of experiments were from the Long-Evans strain maintained by the Canadian Breeding Farms, St. Constant, Quebec, Canada.