

# Establishment of Pheromonal Bonds and Diet Choice in Young Rats by Odor Pre-Exposure<sup>1</sup>

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LEON, M., B. G. GALEF JR. AND J. H. BEHSE. *Establishment of pheromonal bonds and diet choice in young rats by odor pre-exposure.* *PHYSIOL. BEHAV.* 18(3) 387-391, 1977. — Rat pups reared by mothers maintained on a diet inhibiting maternal pheromone production do not exhibit a tendency to approach the pheromone excreted by normally maintained lactating females. The results of the present experiments indicate that following exposure in isolation to the smell of maternal pheromone, rat pups reared by dams not producing maternal pheromone exhibit a strong preference for the odor of maternal pheromone. Furthermore, pups reared by non-pheromone producing dams, and exposed in isolation to an arbitrary odor, exhibit both a preference for that odor and a preference for a diet with which that odor is associated. These results are discussed as demonstrating the importance of stimulus familiarity, rather than association learning, in the development of pheromonal bonds and socially transmitted diet preferences.

Maternal pheromone    Diet preference    Social transmission    Weaning    Behavior development  
Stimulus novelty    Mother-young interaction

PARTURIENT rats typically emit an odor highly attractive to their 14 to 27 day old young [18]. This attractant, which has been termed maternal pheromone, is synthesized by fecal bacteria and emitted in the anal excreta of lactating female rats during the third and fourth weeks following parturition [15,18].

Although the causes of the initiation and termination of production of maternal pheromone by the parturient dam have been studied in some detail, relatively little is known about the development of response to this pheromone in young rats. Available information strongly suggests that the development of pups' tendency to approach the smell of their mother is dependent on exposure to maternal odor during early life. For example, pups reared by dams maintained on a diet which fails to provide a substrate sufficient for normal microbial function and which, therefore, suppresses maternal pheromone synthesis, do not exhibit any tendency to approach the odor of mothers ingesting a normal diet which is highly attractive to their own young [15]. Furthermore, pups reared by mothers not emitting maternal pheromone, when housed in a colony room containing females emitting maternal pheromone, will subsequently approach the anal excreta of pheromone producing animals [16]. It has also been suggested that pheromone producing mother rats emit qualitatively different attractants as a function of their diet and that pups show a strong preference only for the anal excreta of their dam or other similarly fed lactating females [16].

While the above findings suggest an important role of individual experience in the ontogeny of the attraction of pups to maternal odor, a question remains as to the nature of the experience necessary for normal approach responses to develop. Under normal circumstances, the young rat experiences the odor of its mother in close temporal contiguity with reinforcement resulting from contact with her. That is, approach to the smell of the mother is normally soon followed by reinforcing food, warmth, and bodily contact [13]. However, it is possible that neither experience of maternal contact following approach to the odor of the mother nor the association of maternal odor with the presence of the mother are necessary for the development of the tendency of pups to approach the smell of their dam.

## EXPERIMENT 1

The first experiment examines the possibility that simple exposure to the odor of a dam in the absence of any primary reinforcement is sufficient to produce a tendency in pups to approach that odor. To control pup exposure to maternal odors, pups were reared by a dam that did not produce an attractive maternal odor. Dams lacking maternal pheromone can be readily obtained by feeding parturient females a diet in which sucrose is the only carbohydrate. Pups reared by a mother maintained on such a diet are

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normally neither attracted to her odor nor to the odor of other mothers maintained on a diet sufficient to permit maternal pheromone production [15,16].

#### METHOD

##### *Animals and Procedure*

Twelve pregnant Wistar rats were placed in individual sealed containers (30.5 cm dia., 14 cm height; Tupperware) several days prior to parturition and maintained on ad lib water and a diet containing sucrose as its only constituent carbohydrate (diet composition in g/kg: sucrose 584.4, casein 211.0, cellulose 104.5, corn oil 50.0, salt mix 40.0, vitamin mix 10.0, Teklad Mills, Madison, Wisconsin). Filtered air (30 ml/min) was passed into each container through a 1.5 cm hole and allowed to exit via 3 similar holes to maintain a constant air flow. Bedding (Sani-chips; Animal Laboratory Diets, Derby, N.Y.) was changed and the container washed on Day 20 of gestation. After parturition each litter was culled to 8 pups.

From Day 1 to Day 19 postpartum pups were taken from their mother for 3 hr/day and placed in an exposure apparatus, similar to their home unit, but divided into 8 wedge-shaped sections each of which held a single, isolated pup. Pups from 5 litters received filtered air while in the exposure apparatus, while those from 7 other litters received filtered air which had passed through a sealed container holding a 16–21 day postpartum Wistar female maintained on Purina Laboratory Chow. Such females have previously been shown to produce anal excreta highly attractive to pups which they rear [15,17]. On Day 20 postpartum, the mother of each litter was removed from its home unit, and 3 hr later her pups were tested in an olfactory discrimination apparatus described below to determine their response to the odor of the anal excreta of Day 16–21 postpartum lactating Wistar rats maintained on Purina Laboratory Chow.

The olfactory discrimination apparatus, described in detail elsewhere [17], consists of a start box, leading to a triangular open field, ending in a shallow cliff beyond which lies two visually concealed goal compartments. Filtered air (55 liters/min) passing from a central source through each goal compartment permits delivery of olfactory stimuli from the goal compartment to the subject and provides a 72 db masking noise. Individual pups were placed 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. In the present experiment, anal excreta were collected from a 16–21 day postpartum female maintained on Purina Laboratory Chow after the female had been isolated in a stainless steel cage for 3 hr. The material was then transferred to a disposable plastic weighing dish and placed in one goal compartment; an empty plastic weighing dish was placed in the other.

For purposes of statistical analysis, the proportion of each litter choosing the compartment containing anal excreta was calculated and these proportions formed the basis for comparisons between groups of litters [1].

#### RESULTS AND DISCUSSION

Figure 1 describes the percentage of pups choosing the goal box containing maternal excreta, those choosing the

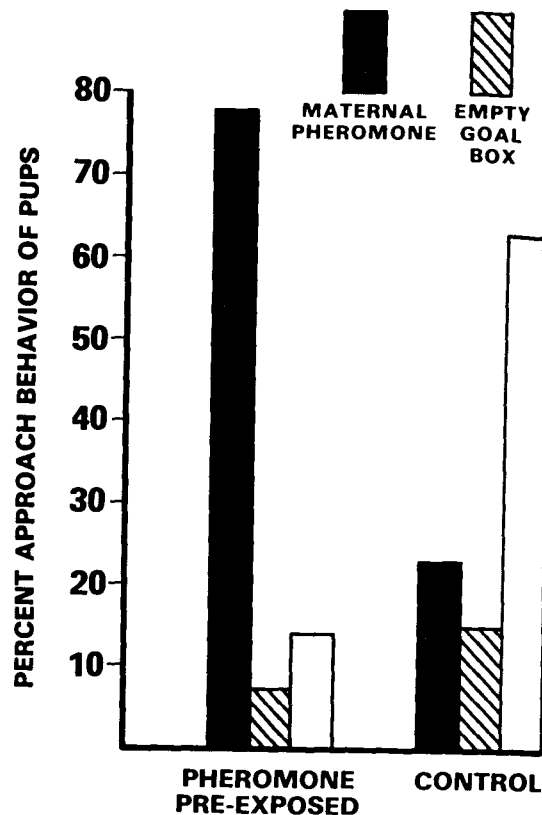


FIG. 1. Percent of pups pre-exposed to the odor of maternal pheromone or pre-exposed to a clean air stream choosing maternal pheromone or an empty goal box in the olfactory discrimination apparatus.

empty goal box, and those making no choice in the olfactory discrimination apparatus. As is clear from examination of the figure, pups which had been pre-exposed to the odor of a lactating female were far more likely to choose the goal box containing maternal excreta than pups lacking such pre-exposure (Mann-Whitney U Test,  $U = 10$ ,  $p < 0.005$ ). The proportion of pups exposed to maternal pheromone in isolation which approached the odor of anal excreta of a lactating female in the olfactory discrimination apparatus (0.79) was comparable to the proportion of pups (0.83) actually reared by dams maintained on Purina Laboratory Chow, previously found to choose the odor of the anal excreta of a lactating female in the same situation (cf. [15]). These data suggest that simple exposure of pups to the maternal pheromone in the absence of any cues related to the mother are sufficient to support development of normal responsiveness to maternal odor.

#### EXPERIMENT 2

While the naturally occurring odors synthesized by mammals are limited to the odors produced by caecal microorganisms, the possibility exists that the weanling rat responds to any pre-exposed odor by approaching it. Previous investigators have shown that the young of a large number of altricial and precocial rodent species will exhibit increased responsiveness to non-biologically significant odors following early exposure [4, 5, 7, 19, 20, 21, 22].



ever, in these experiments, exposure of altricial rodents to an odorant has typically occurred in the presence of the dam and siblings and, therefore, the possibility exists that olfactory learning was responsible for observed increases in approach behavior following odor exposure. Also, it is possible that the mechanism responsible for increased attraction of pre-exposed artificial odors may differ from that underlying the development of approach to those odors synthesized by the dam. In the present study, isolated rat pups were exposed to an arbitrary odor (peppermint extract) to determine whether the development of attraction as a result of simple exposure is limited to the class of odors produced in the caecum of the maternal rat.

METHOD

The method of the present experiment was identical to that of Experiment 1 except: (1) 6 litters of pups were exposed to the odor of peppermint extract (Reckitt and Coleman Ltd., Lachine, Quebec) while in the exposure apparatus, (by placing a 500 cc Nalgene bottle containing 6 cc of extract into the airflow) and (2) these peppermint pre-exposed pups and 5 litters exposed to a clean filtered air flow in the exposure apparatus were tested in the olfactory discrimination apparatus with 10 cc peppermint extract in one goal box and nothing in the other.

RESULTS AND DISCUSSION

Figure 2 describes the percentage of pups choosing the goal box containing peppermint extract, those choosing the empty goal box, and those making no choice in the olfactory discrimination apparatus. As is clear from examination of the figure, those pups which had been pre-exposed in isolation to peppermint extract odor were far more likely to choose the goal box containing peppermint extract than those lacking such pre-exposure (Mann-Whitney U Test,  $U = 0$ ;  $p < 0.005$ ). Furthermore, it is of interest to note that pups experiencing peppermint extract for the first time in the olfactory discrimination apparatus exhibited a significant tendency to avoid that odor by choosing the empty goal box, ( $\chi^2 = 5.0$ ,  $d.f. = 1$ ,  $p < 0.02$ ).

These data demonstrate that simple exposure to an arbitrary odor, even one not produced by a lactating dam, is sufficient to render that odor attractive to young rats. However, the data suggest that even inherently aversive olfactory stimuli may become attractive as a result of familiarity with them.

EXPERIMENT 3

The results of the preceding experiments indicate that exposure to an arbitrary odor early in life is sufficient to markedly enhance the attractiveness of that odor to rat pups. As discussed above, such familiarity with an odor's attractiveness is sufficient to account for the observed tendency of rat pups to approach either the odor of their own mother or that of other lactating conspecifics reared on her diet.

When pups undertake the transition from mother's milk to a solid source of nutrition to the independent acquisition of solid food, the tendency of pups to approach either their own dam or other familiar conspecific adults results in the young pups developing a clear preference for the diet

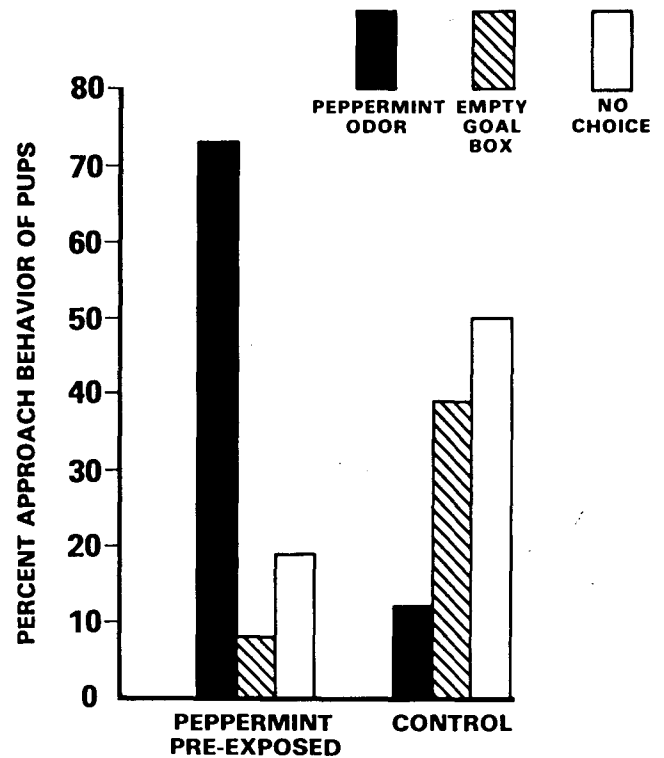


FIG. 2. Percent of pups pre-exposed to the odor of clean air stream choosing peppermint odor or an empty goal box in the olfactory discrimination apparatus.

which those conspecific adults have been eating [9, 10, 11]. Galef and Clark [10] have previously proposed a three-stage process by which such socially induced feeding preferences are hypothesized to develop. On this model: (1) the pups tend to approach adults at a feeding site, (2) become familiar with the taste and smell of the diet to which the adults lead them, and (3) subsequently avoid alternative diets because of the relative novelty of those diets. The proposed mechanism of the second stage in the transmission of a food preference from adult to young rats is very similar to the mechanism discussed in the experiments reported above as resulting in a tendency of pups to approach odors emanating from their dam. It is possible that the critical factor producing a particular diet preference in weaning rats is exposure of pups to the cues associated with a diet, just as the critical factor in producing approach to the smell of a lactating female is exposure to her odor. If this is the case, then it should be possible to produce a preference for a particular diet in pups by pre-exposing them to cues associated with that diet. In the present experiment pups were exposed to an arbitrary odor, that odor was associated with a diet, and the effects of odor exposure on food preference assessed.

METHOD

Animals and Procedure

Four pups were randomly selected from each of the 11 litters utilized in Experiment 2. All these pups were reared by dams maintained on sucrose based diet, and all had been tested on Day 20 in the olfactory discrimination apparatus.

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Pups from 6 litters had been exposed, in isolation, to the odor of peppermint extract 3 hr/day in the exposure apparatus and those from 5 litters to a clean airstream under the same conditions.

On Day 21 postpartum 24 pups which had been exposed to an air-stream containing peppermint odor and 20 pups previously exposed to the control air-stream were individually offered samples of lemon-flavored and peppermint-flavored sucrose based diet in a test apparatus for 24 hr.

Lemon flavored diet was prepared by spraying a solution of 12 ml pure lemon extract (Bowes Co. Ltd., Toronto) in 12 ml of ethyl alcohol into 1 kg sucrose based diet with an atomizer and thoroughly mixing the combined ingredients. Peppermint flavored diet was similarly prepared by mixing a solution of 15 ml peppermint extract (Reckitt and Coleman Ltd., Lachine, Quebec) in 12 ml of alcohol with 1 kg of sucrose based diet.

The test apparatus, designed so as to minimize diet spillage, consisted of a plastic dish, 22 cm dia., 6.6 cm deep, with two detachable food cups mounted 180° apart on the exterior of the plastic dish. Detailed description of the test chamber is to be found in Galef and Henderson [12]. Water was available throughout testing in a shallow dish placed in the center of the apparatus. Intake of each diet was determined by weighing food cups.

To control for any litter effects, data from all four pups in each litter were combined and statistical analyses carried out using the total intake of each litter as a single data point [1].

#### RESULTS AND DISCUSSION

The main results of Experiment 3 are presented in Fig. 3. As is evident from examination of the figure, litters of pups pre-exposed to the smell of peppermint extract showed an enhanced intake of peppermint-flavored sucrose-based diet in comparison with control animals (Mann-Whitney U Test,  $U = 0$ ,  $p < 0.005$ ). All 6 litters of pups pre-exposed to the smell of peppermint exhibited a preference for peppermint flavored food during a 24-hr diet selection test, while all five control litters preferred the lemon flavored diet. Thus, simple exposure to the smell of a diet is sufficient to produce a strong preference for that diet at weaning.

#### GENERAL DISCUSSION

A problem of long standing in the study of behavioral development concerns the mechanisms underlying the ontogeny of the approach and avoidance responses which young organisms exhibit with respect to external sources of stimulation. As in the present studies, particular attention has been focussed on the development of approach responses to conspecifics, a possibly necessary precursor to the occurrence of normal social behavior in vertebrates. Previous reviews of the field have indicated the importance in the emergence of approach behavior in the neonate, of phylogenetically evolved sensory-affective systems, of embryonic sensory stimulation, and of postnatal associative and perceptual learning of various kinds [3, 14, 27, 28, 29].

The results of the present series of experiments indicate that simple postnatal exposure of young mammals to either a naturally occurring or arbitrary stimulus, in the absence of any obvious reinforcement, is sufficient to enhance

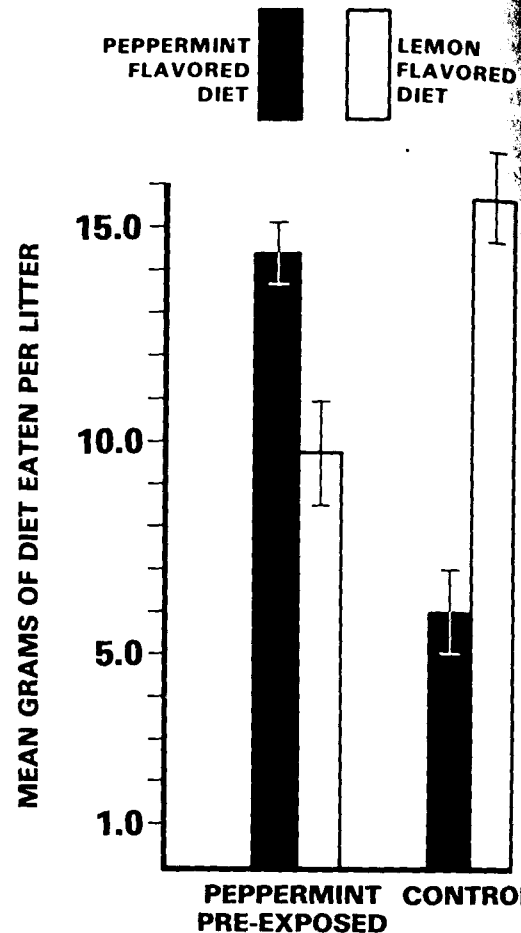


FIG. 3. Mean grams of peppermint- and lemon-flavored diet eaten by litters of pups pre-exposed to peppermint odor or a clean stream.

markedly the attractiveness of that stimulus in both affiliative and feeding situations. Extrapolation from the findings suggests a simple non-associative mechanism supporting the development of approach responses in juvenile mammals. We would propose that, as a general rule, familiarity with a given stimulus will enhance the relative attractiveness of that stimulus to a young animal.

Similar generalizations have been proposed previously both to explain neonatal behavior and certain aspects of diet selection by adults (See for example, [8, 28, 29]). In the case of neonatal altricial mammals, at least, this has received little direct experimental study. In the usual paradigm for the investigation of the formation of attention to stimuli as a result of exposure, stimuli are presented to the neonate in the presence of the dam and litter mates. Thus, the possibility of conditioned positive affective responses to an introduced stimulus typically have not been excluded [6].

The results of the present study support the contention that exposure to a stimulus in the absence of any reinforcement is sufficient to enhance the relative attractiveness of that stimulus. Described in these terms, the observed behavior of our subjects is not easily interpreted within the framework of conventional models of behavior.

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Alternatively, it is possible that for young mammals, as for [2,22], novel stimuli are inherently aversive. Placement of a subject in a test situation, to which it has not previously exposed, introduces that individual to a portion of novel, and therefore, aversive stimuli. In such a situation, that portion of the test environment containing familiar cues would be least novel and consequently least aversive. The young organism might be expected to seek out the source of familiar cues in the novel environment of the test situation to reduce its exposure to unfamiliar and, hence, aversive exteroceptive stimulation. In terms of this model, the apparent attractiveness of the novel stimulus is reinterpreted as a reduced unattractiveness of that stimulus in an environment composed of familiar, and therefore aversive elements. Such reduced

aversiveness resulting from exposure might be attributed either to reduced salience of the pre-exposed stimulus [24] or to a "learned safety" process [26]. Though admittedly speculative, the preceding account of the mechanism underlying neonatal approach to familiar stimuli is readily testable.

In conclusion, we would propose that the tendency of young mammals to approach familiar stimuli in an unfamiliar environment is a basic element in the organization of the response patterns of young mammals to external stimulation. As Rozin [25] has succinctly stated in a different context, "familiarity breeds content", and such content may serve to bring the young organism into contact with familiar conspecifics or familiar foods in an otherwise novel and therefore threatening environment.

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