

# Acquisition and Waning of Exposure-Induced Attraction to a Nonnatural Odor in Rat Pups

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The present experiments examine acquisition and waning of exposure-induced attraction to a nonnatural odor in rat pups. I found that (1) acquisition of attraction induced by experience of an odor associated with the mother (M-E) and of attraction induced by experience of an odor in the general environment (S-E) had similar time courses and were of similar magnitude in pups < 20 days of age; (2) waning of S-E-induced attraction to a nonnatural odor was far more rapid than waning of M-E-induced attraction; (3) M-E and S-E to an odor were less effective in pups > 20 days of age than in pups < 20 days of age; and (4) although pups > 20 days of age receiving M-E to an odor exhibited enhanced attraction to that odor, pups of equal age receiving S-E to the same odor did not.

Results of recent studies conducted in a number of laboratories indicate that each of 2 types of early experience is sufficient to render nonnatural odors attractive to weanling rat pups. Pups tested at 19 days of age in a variety of situations exhibit a preference for odors regularly experienced either in direct association with the dam or in the general environment during the 1st 19 days of life (Alberts, 1981; Galef, 1981; Galef & Kaner, 1980; Leon, Galef, & Behse, 1979).

Although both simple exposure (S-E) and maternal exposure (M-E) to a nonnatural odor are sufficient to establish a preference for that odor in 19-day-old pups, the effects of S-E and M-E differ in important ways (Alberts, 1981; Galef, 1981; Galef & Kaner, 1980). For example, Galef and Kaner (1980) have found that continuous simple exposure (S-E) of pups to the odor of peppermint extract for 30 days failed to cause 31-day-old pups to prefer the odor of peppermint to clean air, while exposure of pups to the odor of peppermint painted on their dam (M-E) for the same period resulted in preference for peppermint odor in 31-day-olds. Similarly, Galef (1981) found that continuous S-E to peppermint extract for 24 days prior to testing failed to cause 25-day-old pups to prefer a feeding site marked with the odor of peppermint to a clean feeding site, while similar exposure of pups to the odor of peppermint painted on their dams (M-E) resulted in preference for a peppermint-marked feeding site in 25-day-olds.

The finding that M-E to an odor is sufficient to maintain an attraction to that odor at least throughout weaning, while S-E to the same odor has effects limited to a shorter period during ontogeny, indicates the existence of important differences in either the acquisition or waning of M-E- and S-E-induced attractiveness of odors. The

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present series of studies was undertaken to examine these differences in detail. The results provide an explanation of (1) the success of both S-E and M-E in establishing preference for an odor and (2) the failure of S-E, but not M-E, to maintain that preference.

## General Method

### Subjects

Subjects were litters of Long-Evans rat pups born in the McMaster colony to stock animals obtained from the Canadian Breeding Farms, St. Constant, Quebec. Each litter was culled to 6-9 pups (depending on the experiment in which it was used) and each subject was toe-clipped at 2 days of age for individual recognition within its litter. All litters were left undisturbed with their dams (maintained on ad lib Purina Laboratory Chow and water in 35 × 30 × 15-cm polypropylene cages) until completion of the experiment, except as noted in the Procedure sections of individual experiments described below.

### Procedure

At parturition, each litter and dam were assigned to 1 of 4 conditions. Litters assigned to either of 2 Experimental Conditions were kept in the main colony room, where they were isolated from exposure to the inherently aversive (Galef & Kaner, 1980) olfactory stimulus employed as an independent variable ("Pure Mint and Peppermint Extract," Club House Foods Ltd., London, Ontario). Litters and dams assigned to serve as Foster Families for pups in Experimental litters were maintained in a separate colony room.

Within 24 hr of parturition, each Experimental litter was assigned a Foster Family, the young of which were born within 24 hr of the time of birth of that Experimental litter. Depending on whether an Experimental litter was to receive simple exposure to peppermint extract (S-E) or exposure to peppermint extract in association with a dam (M-E), its Foster Family was treated in 1 of 2 ways.

On the day following birth of a Foster Family assigned to an Experimental litter in the S-E condition, two 100-ml capacity glass jars, lidded with hardware cloth and filled with cotton batting, were placed in the home cage of the Foster Family. Two ml of peppermint extract were added daily to each jar until completion of the experiment, 1 ml at 9:00 a.m. and 1 ml at 4:30 p.m.

Foster Families assigned to Experimental litters in the M-E condition were treated identically to Foster Families assigned to Experimental litters in the S-E condition except that the experimenter painted 2 ml of peppermint extract onto the dorsal surface of the dam of each Foster Family twice daily instead of placing the peppermint extract in bottles in that Foster Family's home cage.

### Apparatus

Attractiveness of peppermint extract to subjects was measured in 11 × 21 × 17-cm test enclosures constructed of transparent Plexiglas (see Fig. 1). Each test enclosure was housed in a light- and sound-attenuating freezer chest.

Two 2.5-cm-diameter circular openings (6 cm center-to-center) were located in one 11 × 17-cm wall of each test enclosure. Each opening provided access to a stimulus

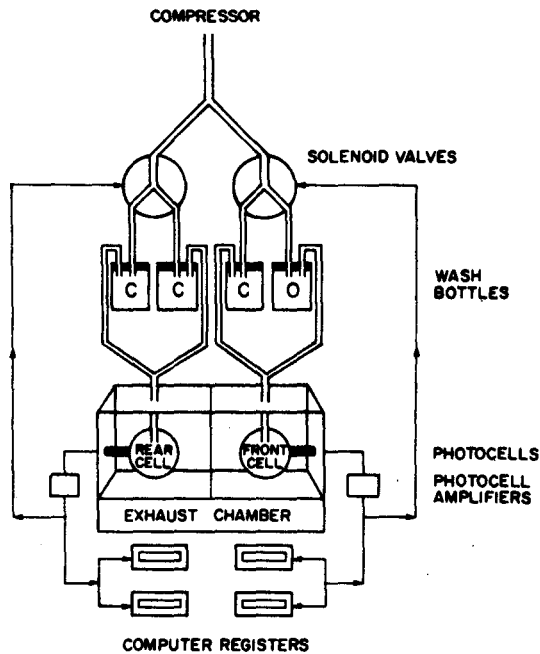


Fig. 1. Schematic diagram of the olfactory preference apparatus. C = Clean. O = Odorant.

chamber through which flowed an airstream. A light source mounted between the 2 openings activated 2 photocells, 1 at the point on each opening opposite the light source.

Insertion by a subject of its nose into an opening both exposed the subject to the airstream flowing behind that opening and interrupted the beam of light activating the photocell mounted on the edge of that opening. Deactivation of a photocell activated 2 computer registers (Apple II plus). One register recorded the duration of each photocell deactivation, and the other recorded a single event for each photocell deactivation.

Deactivation of a photocell also caused a solenoid valve (Skinner Precision Industries, New Briton, Connecticut, Model V52DB2100) to close and remain closed until its associated photocell was reactivated. Valve closure redirected the airstream entering a stimulus chamber from 1 channel to another. Throughout most of any test session, filtered airstreams entered both stimulus chambers after passing through clean wash bottles. When the beam of light falling on a photocell was interrupted, the airstream entering the stimulus chamber associated with that photocell was redirected by a solenoid valve either through a 2nd clean wash bottle (rear stimulus chamber) or through a wash bottle containing a sample of peppermint extract (front stimulus chamber). After passing through a stimulus chamber, each airstream exited through an exhaust chamber to the building exhaust system. [See Galef & Kaner (1980) for a more detailed description of the apparatus and procedure.]

### Test Procedure

A 2-ml sample of peppermint extract was placed in 1 of the wash bottles connected to the front stimulus chamber of an apparatus. After the wash bottle was placed in position, a rat pup was introduced into the enclosure and left undisturbed for 1 hr.

Upon completion of a test session, the experimenter recorded the number of times the subject had sampled from each stimulus chamber and the total number of seconds the subject had spent with its nose in each stimulus chamber, and placed a fresh sample of peppermint extract in the appropriate wash bottle connected to the front stimulus chamber.

### Data Analysis

To determine the relative attractiveness of the odor of peppermint extract, I calculated an *odor preference ratio*, defined as the number of seconds spent by a subject with its nose in the front stimulus chamber (through which an odor-bearing airstream passed) divided by the total number of seconds spent by that subject with its nose in either stimulus chamber. The data of any subject failing to sample from each stimulus chamber at least 10 times during the course of its 1-hr test period were excluded from analysis.

## Experiment I

As mentioned in the introduction, Galef and Kaner (1980) have found that both 18 days of simple exposure to an odor and 18 days of exposure to the same olfactory stimulus painted on a dam produce preference for that odor in 19-day-old rat pups. It cannot, however, be inferred from the finding that extensive exposure to an odor in 2 different circumstances results in preference for that odor that the details of preference acquisition are similar in the 2 cases. It might, for example, be the case that acquisition of an M-E-induced attractiveness is a gradual, continuous process, while S-E-induced attractiveness is the result of an acute process acting during a restricted sensitive period during ontogeny. If such a difference were found in the pattern of acquisition of S-E- and M-E-induced odor preference in 19-day-old pups, it might provide an avenue of approach to understanding of the observed difference in S-E- and M-E-induced odor preferences of 31-day-old pups.

The present experiment was composed of 2 studies. In each, parameters of odor exposure were varied systematically in S-E- and M-E-treated pups to determine the effects of different ages at exposure, and durations of exposure to an odor, on the attractiveness of that odor to 19-day-old rat pups. In the 1st study, I examined the effects of duration of exposure to an odor in the period immediately preceding testing at 19 days of age on its attractiveness. In the 2nd study, I examined the effects of pup age when experiencing an odor for 7 days on that odor's attractiveness to 19-day-old pups. Such procedures should permit identification of any marked differences between pups receiving S-E and M-E treatments prior to weaning in their pattern of acquisition of odor attraction.

### Study 1: Duration of Exposure

#### Subjects

Subjects were 40 litters of rat pups (culled to 9 pups/litter on their day of birth) and their dams. Twenty litters were assigned to serve as Experimental subjects and 20 to serve as Foster Families.

**Procedure**

Each member of each of the 20 Experimental litters was left undisturbed in its home cage except for a period of days during which it was housed with its Foster Family. Table 1 describes the fostering schedule for pups in each Experimental litter. Experimental pups in 10 litters were placed in Foster Families whose dams were painted with peppermint extract (M-E Condition) while those in the remaining 10 litters were placed with Foster Families whose home cages contained peppermint extract in 100-ml bottles (S-E Condition).

To control for possible litter effects, only 1 pup from each Experimental litter was assigned to any one of the treatment conditions described in Table 1.

**Testing**

When 19 days of age, each pup was tested, using the procedure described in General Method, to determine the attractiveness to that pup of an airstream bearing peppermint extract in comparison to a clean airstream.

**Study 2: Age at Exposure**

**Subjects**

Subjects were 40 litters of Long-Evans rat pups (culled to 7 pups/litter on the day of their birth) and their dams. Twenty litters served as Experimental subjects and 20 dams and litters served as Foster Families.

**Procedure and Testing**

Procedure and testing were identical to those of Study 1 except with respect to the age at which pups received S-E or M-E to peppermint extract. Pups from each Experimental litter in the present study received 7 days of S-E or M-E, as indicated in Table 2.

*TABLE 1. Age of Pups and Number of Days Pups in Experimental Litters were Placed with Foster Families for S-E or M-E.*

Condition	Fostering		Age of Pup at Testing
	Age of Pup (Days)	No. of Days	
1	null	0	19
2	18-19	2	19
3	17-19	3	19
4	16-19	4	19
5	15-19	5	19
6	13-19	7	19
7	8-19	12	19
8	4-19	16	19
9	2-19	18	19

TABLE 2. Age of Pups and Number of Days Pups in Experimental Litters were Placed with Foster Families for S-E or M-E.

Condition	Fostering		Age of Pup at Testing
	Age of Pup	No. of Days	
1	null	0	19
2	2-8	7	19
3	5-11	7	19
4	8-14	7	19
5	11-17	7	19
6	13-19	7	19
7	2-19	18	19

## Results and Discussion

The main results of Studies 1 and 2 are presented in Figures 2 and 3, respectively. These figures show the mean odor preference ratios of pups in the 16 groups, treatment of which is described in Tables 1 and 2.

As we have found previously (Galef & Kaner, 1980), when tested at 19 days of age, (1) pups reared without exposure to peppermint extract tended to avoid contact with a peppermint-odor-bearing airstream (Condition 1, Fig. 2; Condition 1, Fig. 3; Sign Test,  $x = 1$ ,  $p < .001$ ); (2) pups either simply exposed to peppermint extract continuously from Days 2-19 postpartum and pups receiving similar exposure to peppermint extract painted on their dam both exhibited preference for the odor of peppermint extract at 19 days of age (Fig. 2, Condition 9; Fig. 3, Condition 7; Sign tests, both  $p$ 's  $< .02$ ).

Reference to Figure 2 also reveals that, as a general rule, attraction to peppermint odor increased with increasing duration of pretest peppermint exposure (Spearman rank correlation coefficients: M-E groups,  $r = .77$ ,  $p < .05$ ; S-E groups,  $r = .87$ ,  $p < .01$ ).

As can be seen in Figure 3, 7 days of exposure to peppermint extract in the 2 periods of exposure immediately preceding testing were more effective in enhancing peppermint preference than 7 days of exposure at ages more distant from the test date

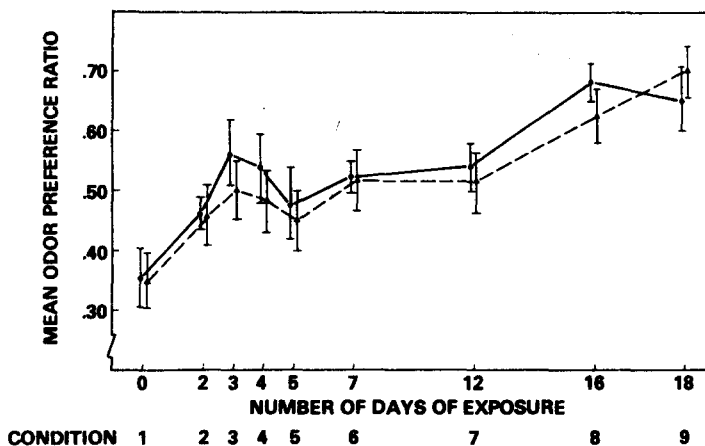


Fig. 2. Mean odor preference ratios of pups experiencing S-E or M-E to peppermint extract for 0-18 days prior to testing at 19 days. See Table 1 for description of Group treatments. Flags indicate  $\pm 1$  S.E.M. M-E,  $\bullet - \bullet$ . S-E,  $\blacktriangle - \blacktriangle$ .

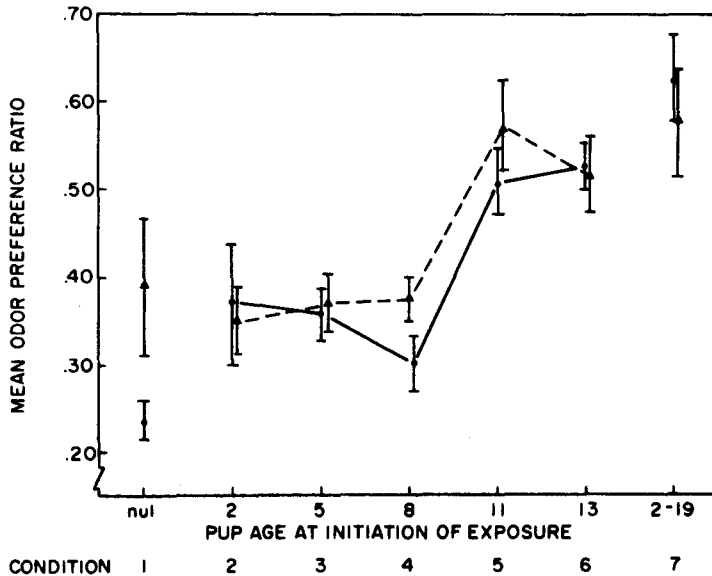


Fig. 3. Mean odor preference ratios of 19-day-old pups at testing experiencing S-E or M-E to peppermint extract for 7 days at various ages. See Table 2 for description of Group treatments. Flags indicate  $\pm 1$  S.E.M. Symbols as in Figure 2.

(M-E groups,  $X^2_4 = 22.2, p < .001$ ; S-E Groups,  $X^2_4 = 11.8, p < .02$ ). Most important, relative to the question of whether there exist significant differences in the pattern of attraction-acquisition in pups receiving S-E and M-E to a nonnatural odor prior to weaning, the effects of parametric variations in periods of odor exposure had very similar effects on pups receiving M-E and S-E to that odor.

Analysis of the mean number of times pups inspected both ports during a 1-hr test period (Study 1: S-E Groups,  $\bar{X} = 88.2, S.E.M. = 6.7$ ; M-E Groups,  $\bar{X} = 91.5, S.E.M. = 9.2$ ; Study 2: S-E Groups,  $\bar{X} = 111.1, S.E.M. = 12.3$ ; M-E Groups,  $\bar{X} = 111.7, S.E.M. = 6.9$ ) revealed no consistent differences in the frequency of inspection of ports by subjects as a function of pretest treatment. Similarly, pups subjected to various treatments did not differ in the mean duration of their inspections of ports (Study 1: S-E Groups,  $\bar{X} = 1.8$  sec,  $S.E.M. = .07$  sec; M-E Groups,  $\bar{X} = 1.8$  sec,  $S.E.M. = .09$  sec; Study 2: S-E Groups,  $\bar{X} = 1.7$  sec,  $S.E.M. = .10$  sec; M-E Groups,  $\bar{X} = 1.6$  sec,  $S.E.M. = .04$  sec).

The data of the present experiment, thus, offer no support for the hypothesis that the pattern of acquisition of S-E- and M-E-induced odor attractiveness in preweaning pups differs in important ways. The present results, therefore, offer no insight into the causes of previously observed differences in the odor preference of S-E- and M-E-treated pups in the period after weaning (Galef & Kaner, 1980).

### Experiment II

Although S-E- and M-E-induced odor attraction both appear to be of equal magnitude and similar in their patterns of acquisition in 19-day-old pups, it is possible that the duration of the effects of S-E- and M-E-induced odor attraction differs significantly.

In the present experiment pups received either S-E or M-E to peppermint extract from Days 2-19 postpartum and were then removed from contact with peppermint extract

for varying periods of time prior to testing for attraction to the odor of peppermint extract relative to a clean airstream.

## Method

### Subjects

Subjects were 30 litters of Long-Evans rat pups, culled to 6 pups/litter on their day of birth.

### Procedure

Each of 20 litters and their dams were removed from the main colony room on Day 2 postpartum and assigned to S-E or M-E treatment conditions. The dams of litters assigned to the M-E group were painted on their dorsal surfaces with 2 ml of peppermint extract twice daily (9 a.m. and 4:30 p.m.). Litters assigned to the S-E Group had two 100-ml-capacity, cotton-batting-filled jars placed in their home cages on Day 2 postpartum. One ml of peppermint extract was added to each jar whenever dams in the M-E group had peppermint extract painted onto their backs. The remaining 10 litters (Control Group) were left undisturbed in the colony room and received no exposure to peppermint extract.

At 19 days of age all 30 subject litters were washed, dried, and weaned to cages (1 litter/cage) in the main colony room, where they were isolated from exposure to peppermint extract. On Days 19, 20, 26, 34, 50, and 65 postpartum, 1 pup from each of the 30 litters was tested, as described in General Method, for their attraction to the odor of peppermint extract.

## Results and Discussion

The main results of Experiment II are presented in Figure 4, which shows mean odor preference ratios of pups in M-E, S-E, and Control Groups. As is evident from inspection of the figure, subjects in the M-E group continued to exhibit an enhanced attraction to the odor of peppermint extract relative to subjects in the Control Group until they reached 50 days of age (Mann-Whitney  $U$  test:  $U = 9, p < .002$ ), while subjects in the S-E condition exhibited rapid attenuation of their exposure-induced attraction to the odor of peppermint extract following removal from exposure to peppermint extract at 19 days of age.

### Experiment III

The results of Experiment II provide a partial explanation (see General Discussion) of the attraction to the odor of peppermint extract exhibited by 31-day-old rat pups that receive M-E to peppermint odor from 2 to 31 days of age (Galef & Kaner, 1980). For pups in the M-E condition, exposure to peppermint odor from 2 to 19 days of age is sufficient to produce enhanced attraction to that odor at 31 days of age.

The results of Experiment II also provide a partial explanation of the failure of 31-day-old pups that have received S-E to that odor from 2 to 31 days of age to exhibit enhanced attraction to it. Pups in the S-E condition, when 31 days of age, exhibit no



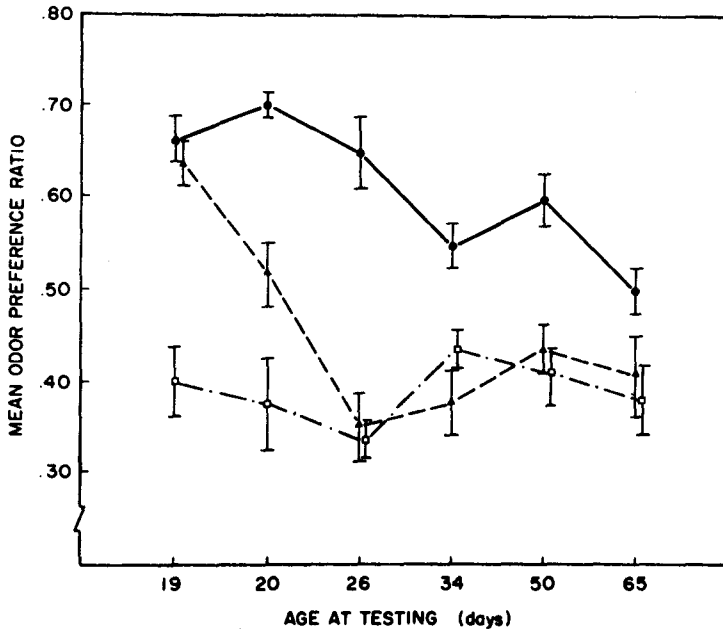


Fig. 4. Mean odor preference ratios of pups experiencing S-E or M-E to peppermint extract from 2 to 19 days of age or no experience of peppermint extract, tested at various ages for peppermint odor preference. Flags indicate  $\pm 1$  S.E.M. Symbols as in Figure 2. Control,  $\square$ — $\square$ .

residual effects of exposure to peppermint odor experienced during the 1st 19 days of life. If S-E to peppermint odor were found to be ineffective in enhancing the attractiveness of the odor of peppermint extract to pups more than 19 days of age, then the failure of pups receiving S-E to peppermint odor from 2 to 31 days of age to exhibit an enhanced attraction to peppermint odor at 31 days of age would be explained. The present experiment was, therefore, undertaken to explore the efficacy of S-E and M-E to a nonnatural odor in enhancing the attractiveness of that odor to rat pups 21-30 days of age.

**Method**

**Subjects**

Subjects were 40 litters of Long-Evans rat pups (culled to 8 pups/litter on the day of their birth) and their dams. Twenty dams and litters served as Foster Families and 20 litters of pups served as Experimental subjects.

**Procedure**

Pups from 10 Experimental litters were placed in Foster Families in which peppermint extract was painted on their dam (M-E condition), while those in the remaining ten litters were placed with Foster Families, the home cages of which contained peppermint extract in 100-ml bottles (S-E condition).

Table 3 describes the fostering and testing schedules employed. Two pups from each of the 10 litters receiving M-E and 2 pups from each of the 10 litters receiving S-E were assigned to each of the 4 conditions described in Table 3.

TABLE 3. Age of Pups and Number of Days Pups in Experimental Litters were Placed with Foster Families for S-E or M-E.

Condition	Fostering		Age of Pup at Testing
	Age of Pup	No. of Days	
1	10-19	10	19
2	21-30	10	30
3	null	0	19
4	null	0	30

### Results and Discussion

The main results of Experiment III are presented in Figure 5, which shows the mean odor preference ratios at testing of the 8 groups (4 S-E and 4 M-E), treatment of which is described in Table 3.

First, the data presented in Figure 5 show, and statistical tests confirm, that 10 days of exposure to a nonnatural odor were more effective in enhancing attraction to that odor in pups less than 20 days of age than in pups greater than 20 days of age. This generalization was true of pups in both the M-E exposure condition (Mann-Whitney  $U$  test:  $U = 9, p < .02$ ) and the S-E exposure condition (Mann-Whitney  $U$  test:  $U = 16, p < .02$ ).

Second, the data presented in Figure 5 indicate that whereas exposure of 21- to 30-day-old pups to an odorant painted on the dam (M-E) significantly enhanced the attraction of that odor relative to nonexposed controls (Mann-Whitney  $U$  test:  $U = 9, p < .02$ ), similar S-E to an odorant had no effect on attraction (Mann-Whitney  $U$  test:  $U = 29, p > .20$ ).

The results of the present experiment provide a 2nd partial explanation of (1) the failure of pups receiving S-E to an odorant from 2 to 31 days of age to exhibit enhanced

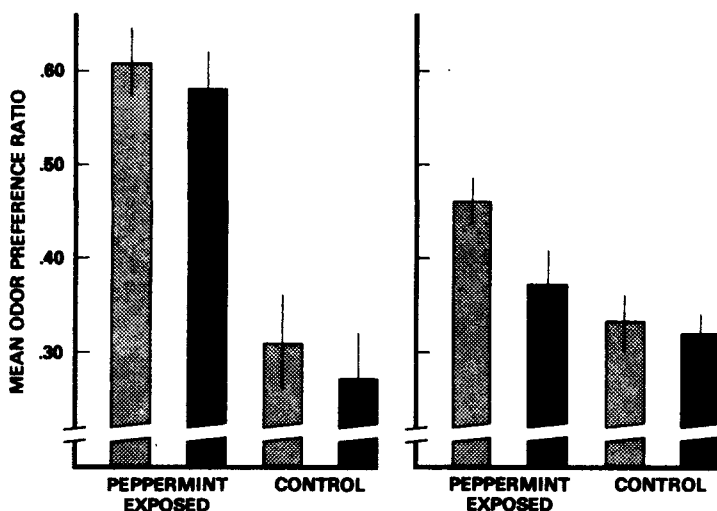


Fig. 5. Mean odor preference ratios of pups experiencing S-E or M-E to peppermint extract for 10 days prior to testing at 19 (left-hand panel) or 30 (right-hand panel) days of age. See Table 3 for description of Group treatments. Flags indicate  $\pm 1$  S.E.M. M-E, stippled, S-E, dotted, Control, solid black.

attraction to that odorant at 31 days of age, and (2) the maintenance of enhanced attraction to an odorant in pups receiving M-E to that odorant from 2 to 31 days of age. Pups receiving M-E continued to be influenced by exposure to the odorant after they reached 20 days of age, while pups receiving S-E were not.

### General Discussion

The results of the present studies provide an explanation of the failure of pups continuously and simply exposed to a nonnatural odor to exhibit a preference for that odor during the postweaning period. The present data also provide an explanation of the continued preference of pups for a nonnatural odor experienced in conjunction with a conspecific. Subjects receiving S-E both rapidly lose any olfactory preference acquired prior to reaching 19 days of age and are not sensitive to S-E at later ages. Subjects receiving M-E continue both to exhibit the effects of M-E to an odor occurring prior to 19 days of age and to be responsive to M-E as they grow older.

The data presented above do not provide any insight into the reasons why S-E and M-E to an odorant differ in the durations of their effects on odor attractiveness. The cause of such differences might be psychologically trivial or might reflect important differences in the processes underlying S-E- and M-E-induced odor preferences. It is possible, for example, that because pups spend many hours each day in close proximity to their dam, odors painted on a dam are experienced by pups with greater frequency or at greater intensity than odors in the general environment. Such differences in exposure to the olfactory stimulus experienced by pups in M-E and S-E conditions might be reflected in differential rates of waning of exposure-induced enhanced attraction to the odorant. Alternatively, odors experienced by pups in direct association with a dam may come to function primarily as signals for the presence of primary reinforcers, while odors experienced in the general environment may orient behavior as a result of effects of familiarity on preference [Zajonc (1968); see Galef (1981) for further discussion].

Regardless of the behavioral processes underlying the findings described above, the results of the present experiments suggest that both simple exposure to odors and exposure to odors associated with conspecifics play central roles in the development of olfactory preference in preweaning rat pups (see also Alberts, 1981). After weaning, the role of simple exposure in determining olfactory preference is minimal, while that of exposure in association with conspecifics remains significant.

### Notes

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