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Preference for Natural Odors in Rat Pups: Implications of a Failure to Replicate[1]

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GALEF, B. G., JR. *Preference for natural odors in rat pups: Implications of a failure to replicate.* PHYSIOL. BEHAV. **26**(5) 783–786, 1981.—The results of the present experiment indicate that very minor changes in the diet on which a rat dam is maintained can have profound effects on the attractiveness of her feces to her pups. Rat pups exhibited a strong preference for the odor of feces taken from dams maintained on one sample of Purina Rodent Laboratory Chow No. 5001, but no preference for the odor of the feces of dams maintained on a second sample of the same diet. These data point to the need for very great care in the design of experiments employing, as independent variables, stimuli which are not under direct experimenter control. The results also suggest that experience of a natural odor during ontogeny may not be sufficient for the development of a preference for that odor in young rats.

Olfaction Preference development Maternal pheromone

FOR the past several years my co-workers and I have been studying the development of preference for olfactory stimuli in rat pups of weaning age [2, 3, 4]. In our test situation (see [2] or [3] for details of method and apparatus) an individual pup is simultaneously presented with two airstreams, one clean and one bearing an odor. To determine each subject's preference for, or aversion to, an odor we measure the number of seconds the subject spends in contact with each airstream during each of three 1-hr tests conducted at 24 hr intervals, when the subject is 19, 20, and 21 days of age. On each test day we calculate an "odor preference ratio," equal to the number of seconds the subject spends in contact with the odor-bearing airstream divided by the total number of seconds the subject spends in contact with either airstream. We use the median odor preference ratio for each subject's 3 days of testing to characterize that subject's preference for, or aversion to, the odor presented.

In the course of our experiments over the last 3 years we have tested one pup from each of 60 litters reared by dams eating Purina Rodent Laboratory Chow No. 5001 (Ralston Purina Co., St. Louis, Mo.) for its preference for the odor of 6–8 g samples of anal excreta taken from dams maintained on the same diet as the pup's own mother and nursing young of the same age (±24 hr) as the test pup. These six replications (10 pups/replication) have served as controls against which to measure the effects of various manipulations applied to sibs of pups in control groups. As can be seen in the bars labelled 1 to 6 in Fig. 1, our methods have produced remarkably consistent results over a 3-yr period, with mean odor preference ratios for six independent groups of ten control pups ranging from 0.65 to 0.62.

It, therefore, came as a considerable shock when, in September of 1980, using our standard procedures, we obtained a mean odor preference ratio for maternal anal excreta of 0.51 for a group of ten control pups (see bar 7 of Fig. 1).

Three weeks of cleaning of apparatus, checking of protocols and computer programs, examination of pups and dams, etc. provided no insight into the source of our difficulties.

Eventually we discovered that a new shipment of our standard rat diet had been received at McMaster in August of 1980. Search of the storeroom revealed several bags of the old shipment of chow and we were, therefore, able to determine whether pups reared by dams maintained on pellets from new and old shipments of Purina Rodent Laboratory Chow No. 5001 differed in their response to maternal excreta.

METHOD

Twelve late-pregnant Long-Evans rats born in the McMaster colony (descended from stock purchased from Canadian Breeding Farms, St. Constant, Quebec) were randomly assigned to one of two groups differing in the source of their rations. Six females and their litters (culled to 6 pups/litter on the day of parturition) were maintained on Purina Chow from the early shipment and six on Purina Chow from the shipment received in August.

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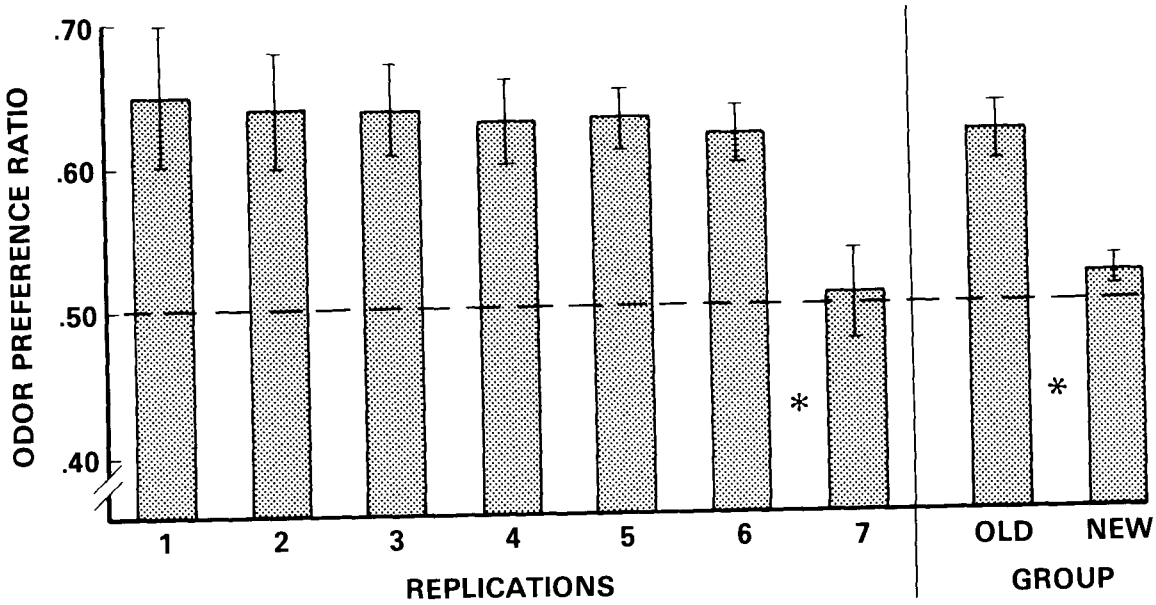

FIG. 1. Mean odor preference ratios of groups of 19 to 21-day-old rat pups simultaneously presented with a clean air stream and one bearing the odor of maternal anal excreta. Bars 1 through 7 are replications under identical conditions; bars "old" and "new" report results of the present experiment. Flags indicate ±1 SE. Bars to the left and right of an asterisk differ significantly from one another ($p<0.01$).

Each group of subjects was housed in a separate colony room and each individual female and her litter were left undisturbed until completion of the experiment, with ad lib access to the appropriate diet and water, in a 35×30×15 cm polypropylene cage carpeted with hardwood-chip bedding (Betachip Hardwood Laboratory Bedding, Northeastern Products Corp., Warrensburg, NY).

Each of the six pups in any litter was tested individually when 19, 20, and 21 days old (1-hr/day) to determine its odor preference ratio for fresh 6–8 g samples of anal excreta. These samples were taken from 19 to 21 day postpartum dams (other than the subject's own mother) eating rations from the same shipment of Purina Chow that the subject's mother had been eating.

To control for any possible litter effects, the mean of the odor preference ratios for the 6 pups in each subject litter was used as a single data point in all data analyses.

Lee and Moltz [6] have recently provided evidence of changes in the pH of maternal feces correlated with the attractiveness of that feces to pups. We, therefore, measured (using a Radiometer-Copenhagen pH meter, Model pHm 62, with a combination electrode) the acidity of the feces of 20, 19-day postpartum females maintained on old and new samples of Purina Chow to see if we could find any difference in the acidity of feces as a function of the diet sample on which dams were maintained.

RESULTS

The main results of the present experiment are presented in the bars labelled "old" and "new" in Fig. 1. As is clear from examination of the figure, and as statistical test confirmed, the anal excreta of dams fed Purina Chow from the old shipment was significantly more attractive to pups reared by dams eating that diet than was the anal excreta of dams fed Purina Chow from the new shipment to pups reared by dams eating that diet (Mann-Whitney U test, U=3, $p<0.008$).

The mean pH of feces of 19-day postpartum female rats maintained on old and new samples of Purina Rodent Laboratory Chow measured, respectively, 7.39±0.10 and 7.41±0.04 (SE).

DISCUSSION

The study in mammals of response to naturally produced odors provides unique challenges in experimental design. Because the experimenter can neither directly control the production of stimuli serving as his independent variable nor detect alterations in those stimuli, procedures are required which may be unnecessary in studies employing stimuli under more precise experimenter control. The design of experiments employing natural odors as stimuli must be arranged so that the behavior of subjects can serve as a biodetector of any relevant alterations in odors produced by animals maintained under apparently constant conditions.

The ability to monitor uncontrolled variation in naturally produced odors requires, at the very least: (1) the use of a large sample of subjects maintained under "constant" conditions as sources of an odor to control for inter- and intra-individual variation in stimulus production [1], (2) the use of designs controlling for possible inter-litter differences in response to odors [3], (3) the assignment of some subjects from each group examined in any experiment to replication of previous results to assure that olfactory stimuli have, in fact, remained constant over time, and (4) the calculation of measures of both central tendency and variance in subject preference for odors so that the experimenter can determine

if replications do, in fact, replicate previous results. If these four conditions are not met, alterations in a presumably constant natural odor are not detectable. If the experimenter does not have available a means of detecting uncontrolled variation in his test stimulus, observed differences in behavior between groups of subjects examined following different experimental treatments cannot be unambiguously attributed to experimenter manipulation of the independent variable.

The results of the present experiment, in which such controls for olfactory stimulus variation were employed, have rather disquieting implications for the study of the development of response to naturally occurring olfactory cues.

First, the present data suggest that it cannot be assumed that even apparently precise replication of work in the area of olfactory preference in young mammals will produce the same results or lead to the same conclusions. The results of the present experiment indicate that the anal excreta of 19- to 21-day postpartum female rats fed Purina Laboratory Chow will be either attractive or not attractive to their pups as a function of the particular sample of Purina diet used to maintain subjects and their dams.

Second, the present data indicate that experience of a natural odor during ontogeny does not necessarily result in development of preference for that odor [7]. If a pup's experience during ontogeny of the maternal excreta produced by its dam (maintained on a given diet) were sufficient to establish pup preference for the anal excreta of other lactating females eating that diet, then one would expect pups in *both* the "old" and "new" groups of the present experiment to exhibit robust preferences for samples of anal excreta with which they were tested (see also [2,3]).

Third, the failure to find significant differences in the acidity of the feces collected from 19- to 21-day postpartum dams producing attractive and not attractive feces, indicates that there is, as yet, no reliable physical indicator of the attractiveness of maternal anal excreta.

Previously reported contradictions in the outcome of experiments performed in various laboratories studying response to natural odors in rat pups [4] may have resulted, at least in part, from differences in the effectiveness of the means used to control for possible variability in presumably constant olfactory stimuli.

APPENDIX

While adequate experimental design offers the opportunity to detect uncontrolled variation in naturally produced olfactory stimuli, it would clearly be more efficient if experimental conditions could be arranged so as to prevent unintentional variation in stimulus production from occurring. Standard laboratory chows vary in their composition over time, reflecting changing market costs of their many ingredients as well as attempts to improve diets. According to the manufacturer's label, the two samples of Purina Rodent Laboratory Chow No. 5001 which we used differed from one another in a variety of ways. (The paragraphs below list the constituents of "new" and "old" samples of Purina Laboratory Rodent Chow No. 5001 as recorded on their labels. Ingredients unique to one sample have been italicized.)

"New" Purina Rodent Laboratory Chow No. 5001

Ground extruded corn, soybean meal, ground oat groats, dried beet pulp, wheat germ meal, fish meal, brewers' dried yeast, dehydrated alfalfa meal, cane molasses, dried milk products, meal and bone meal, wheat middlings, animal fat preserved with BHA, calcium carbonate, dicalcium phosphate, salt, calcium iodate, vitamin B-12 supplement, *DL methionine,* calcium pantothenate, choline chloride, folic acid, riboflavin supplement, thiamin, niacin, pyridoxine hydrochloride, ferrous sulfate, vitamin A supplement, D activated animal sterol, vitamin E supplement, *ferrous carbonate,* manganous oxide, cobalt carbonate, *copper sulfate, zinc sulfate,* zinc oxide.

"Old" Purina Rodent Laboratory Chow No. 5001

Ground extruded corn, soybean meal, ground oat groats, dried beet pulp, wheat germ meal, fish meal, brewers' dried yeast, dehydrated alfalfa meal, cane molasses, dried milk products, meat and bone meal, wheat middlings, animal fat preserved with BHA, calcium carbonate, dicalcium phosphate, salt, *animal livermeal,* calcium iodate, vitamin B-12 supplement, *methionine hydroxy analogue calcium,* calcium pantothenate, choline chloride, folic acid, riboflavin supplement, thiamin, niacin, pyridoxine hydrochloride, ferrous sulfate, vitamin A supplement, D activated animal sterol, vitamin E supplement, *iron oxide,* manganous oxide, cobalt carbonate, *copper oxide,* zinc oxide.

More precisely formulated diets might avoid the problem of diet induced changes in olfactory-cue production. Unfortunately, even diets which are compounded using a constant formula consist of natural ingredients which are themselves variable. For example, casein, a frequently used source of protein in rat diets, is not a uniform substance; it consists of four distinct proteins each of which has a number of variants of distinct chemical composition [8]. Casein from cattle of different breeds differs in its protein structure [5]. It is not known whether the attractiveness of feces of dams maintained on diets differing in the protein structure of their casein varies, but the assumption that diets of precise formulation are constant from year to year is unwarranted.

Further, it is not necessarily the case that the difference in attractiveness of olfactory cues emitted by females eating "old" and "new" samples of Purina Laboratory Chow observed in the present experiment reflected differences in their manufacture. Differences in batch handling after manufacture (i.e. differences in amounts of time in storage prior to utilization or differences in exposure of shipments to heat or moisture) might alter diets in important ways.

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