Discrimination by Rats of Conspecific Odors of Reward and Nonreward

Abstract. *The hypothesis that, after receiving reward and nonreward, rats excrete differential odors perceptible to other rats was tested by making the correct turn in a T-maze contingent on discrimination of any such odors. Clear evidence for an "odor of nonreward or frustration" was obtained, and there was the suggestion of a transistory odor after early reward trials.*

Laboratory rats appear to excrete odorants, perceptible to other rats, as a function of certain kinds of experimental treatments. This observation is of relevance to the study of pheromones and animal behavior and is of considerable methodological importance for behavioral studies employing rats as subjects. Specifically, electric-shock stress versus nonshock (*1*) and reward versus nonreward (*2*) are differential treatments apparently capable of producing a differential excretion of odor in the rats which receive them. In the case of reward versus nonreward, however, the precise source of the differential in resulting odorants—whether from reward or nonreward, or both—has not been clear, although it seems likely from related data (*3*) that nonreward or, in general, "frustration" elicits a distinctive odor. Furthermore, it has not been conclusively demonstrated that the odorants resulting from nonreward or reward, or both, can acquire a cue function in relation to reinforcement contingencies. In most of the studies on this topic the acquired cue function was confounded with any capacity of the odorant to slow a running response through an unconditioned "avoidance" or "alarm" reaction or through novelty or stimulus change.

To clarify these matters we administered reward and nonreward to "odorant subjects" in the choice area of an enclosed T-maze and then determined whether other experimental subjects could utilize the odorants, presumed to have been excreted by the odorant subjects, as cues for correctly turning left or right in the maze. One group, group RN, received "odor of reward" as a cue for turning into one of the arms of the maze and "odor of nonreward" as a cue for turning into the other arm. A second group, RC, received odor of reward as one cue and clean floor paper (presumably a minimum-odor condition) as the other. Similarly, group NC received odor of nonreward and a clean floor as cues. A fourth group, CC, received clean flooring on all trials as a control for any extraneous discriminative odors not produced by the experimental manipulations, for example, odor produced by the food reward in the correct arm of the maze.

The walls and floor of the T-maze were constructed of pine and painted flat gray, and the top consisted of hinged sections of clear plexiglass. The stem of the T-maze, including the 19.0-cm start box, was 55.9 cm long, and the crossbar of the T, including the two 20.3-cm goal boxes, was 60.3 cm long. The maze was 11.4 cm wide and 20.3 cm deep throughout. The start and goal boxes were separated from the remainder of the maze by metal guillotine doors. The maze was hinged at the back so that kraft paper from a continuous roll could easily be pulled across the floor, providing a clean floor surface. Similarly, a roll of brown Scott paper toweling was affixed to the right of the start box in an upright position. This paper was threaded around the walls and could be pulled through, providing a clean surface for the interior maze walls. Goal cups at the ends of the goal boxes were sufficiently deep that experimental subjects could not see whether they were baited until directly over them.

To provide an odor of reward a hungry odorant subject was placed directly into the choice area with the goal box and start box doors closed. At the juncture of the stem and crossbar of the maze was an abundant supply of food pellets (45 mg) in a removable, circular food cup. The subject was allowed to remain in the area with the food for 30 seconds before being returned to its home cage. Similarly, odor of nonreward was created by leaving one of these same odorant subjects in the choice area without food for 30 seconds. A "clean odor" cue was created by pulling fresh paper across the floor of the maze prior to a trial.

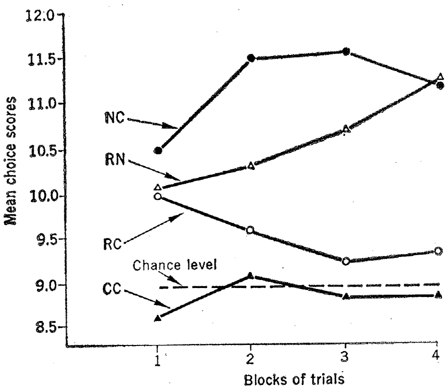

Fig. 1. Mean number of correct choices for the four groups over the last 12 days in blocks of 3 days or 18 trials.

Immediately after the setting up of one of these odor cues, the goal cup was removed from the choice area and a food-deprived experimental subject received a trial. The subject was placed into the start box, the start box door was opened, and the rat was permitted entrance into either arm of the maze. As soon as the subject entered a goal box the door was closed behind it. If the rat entered the correct arm, it was given eight food pellets (45 mg) and was then returned to its cage. If it entered the incorrect arm containing an empty goal cup, it was removed after 5 seconds.

In each of three replications there were 16 experimental and 8 odorant subjects—naive, female, albino rats. They were 90 days old when placed on a limited-amount, food-deprivation diet and 100 days old on the first day of the study. In each replication all 16 experimental animals (four from each group) received trial 1 before trial 2, and so forth. Subjects received six trials a day for 14 days for a total of 84 trials. The six trials each day consisted of three trials with each of the two odor conditions (cues), the order of which was random with the restriction that no condition could be presented more than twice in succession within a day. For half the animals in each group a given odor condition signaled reward in the right arm of the maze, and for the other half it signaled reward in the left arm. Although group CC had only one odor condition, the side on which the reward was located was randomized in the manner of the other groups. After each trial by an experimental subject the floor paper was replaced with clean paper. The paper lining against the walls was replaced with clean paper after all the experimental subjects completed a trial, that is, after every 16 trials or six times a day (*4*).

Figure 1 presents the mean number of correct choices for the groups over the last 12 days of the study. The scores for the first 2 days of training are not included in the figure. During these first days, the odorant animals were not well adapted to the experimental apparatus, and they tended to explore the choice area rather than eat on rewarded trials. For this reason it is doubtful that these animals released a characteristic reward odor for experimental subjects to utilize in their discrimination.

Groups RN and NC demonstrated a clear, though imperfect, discrimination which increased over trials. During the middle trials, group RC appeared to display a weak discrimination which then disappeared, and group CC showed no evidence of any discrimination. In a three-way analysis of variance of the data of Fig. 1, the odor of nonreward was significant ($F = 6.71$; d.f. $= 1/44$; $P < .025$), while odor of reward, trial blocks, and all interactions were nonsignificant.

As a consequence of nonreward, rats apparently excrete an odorant sufficiently discriminable to serve as a cue to another rat for an arbitrarily designated response. The source of the odor does not appear to be urine or feces because such excretion was not evident after the initial trials. Whether the odor is a "frustration odor" produced by nonreward or merely the animal's characteristic scent cannot be conclusively determined from these data, although the absence of any lasting odor of reward strongly suggests the former interpretation. Some caution regarding this interpretation arises from the fact that the animals behaved very differently on rewarded and nonrewarded trials, being rather still while eating and highly agitated when nonrewarded. However, why an animal's characteristic scent would be a much better cue when widely distributed on nonrewarded trials than when concentrated in one locus on rewarded trials is not clear, unless movement results in more scent being excreted.

Indirect support for the frustration-odor hypothesis comes from the observation that nonreward and electric shock appear to produce a common reaction in rats (*5*) and that shock produces a distinctive odor (*1*). An interpretation in terms of a frustration odor, which is actually a more general odor of emotionality, is further suggested by the facts that (i) the performance of group RC deteriorated while that of group RN improved, and (ii) the performance of group RN was initially inferior to that of group NC. This is precisely what would be expected if the early rewarded trials were a source of emotional odor resulting from incomplete habituation to the experiment and if this emotional odor were similar to an odor of nonreward.

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Simulation of the Mating Advantage in Mating of Rare Drosophila Males

Abstract. *In several species of* Drosophila, *certain males mate more frequently when they are less abundant than they do when they are in the majority. The mating advantage may be artificially induced by the use of a "double chamber" technique, even when there is no difference in the actual frequencies of the competing males.*

The relation between the relative frequencies of different kinds of *Drosophila* males in a confined space and their mating efficiencies has been studied (*1, 2*), and all indications point to the proportionately superior performance of minority males. Rare *Drosophila* males mate more than males which are common. This is so in the cases where the males differ in geographic origin, in karyotype, in mutant markers, in the presence or absence of mutant markers, and in the temperature at which these males are raised. Such an advantage may be obscured when additional males of the rare kind are nearby, although they do not have access to the females whose behavior is being scored (*3*). These experiments were conducted by placing two observation chambers (10 cm wide) one on top of the other, separated only by a cheesecloth partition which formed the ceiling of the lower and the floor of the upper chamber (Fig. 1). The minority males in the upper chamber had no advantage over the majority ones if the lower chamber contained an excess of the rare type above. This happened because the cheesecloth layer is no barrier to airborne or substrate vibrations or to olfactory cues. Furthermore, the flies in the bottom layer were attracted to the ceiling of their section because of the light coming from above. When so attracted, they could easily push legs or antennae or both through the cheesecloth and thus come into actual physical contact with the individuals confined to the upper chamber. In effect then, under these conditions, there was no rare type at all, and the females, who do almost all of the choosing during *Drosophila* courtship, behaved accordingly—they engaged in random mating.

My report concerns the creation of a rare type along with its associated mating advantage, with this same double chamber technique. Equal numbers (12 pairs each) of two kinds of *Drosophila pseudoobscura* aged virgin adults were introduced, without anesthetization, into the upper chamber. They differed in karyotype—half were homozygous for the Arrowhead gene arrangement and half were homozygous for the Chiricahua inversion. This combination mates at random when equal numbers of both types are present (*3, 4*; where they are referred to as the N or negative set of strains). When large numbers of one of these two types are confined just below the observation chamber but in physical contact with those above, the alternate karyotype becomes the rare one above, and randomness of mating is eliminated in favor of the rare male (Table 1).

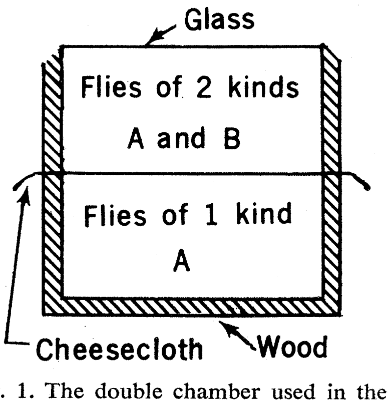

Fig. 1. The double chamber used in these tests of mating frequency in *Drosophila pseudoobscura.*