Alleyway paper flooring as a locus of nondifferential components of reward and nonreward odors

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The locus of discriminable differences in conspecific odors arising from rewarded (R) and non-rewarded (N) trials administered to rats in a straight alleyway was examined by collecting the paper flooring from the alleyway after a squad of donor rats had received R or N. Paper flooring was also collected after a different squad of donors, with no history of alleyway R and N, merely walked on the paper. Different test rats were trained to discriminate between one of these kinds of alleyway paper and clean paper, and then each test rat was successively shifted through two more discriminations involving each of the other kinds of alleyway paper and clean paper. Results indicate that all of the alleyway papers retained olfactory components, which were discriminable from clean paper, and that interchanging the kinds of alleyway paper produced little disruption of discrimination. Implications for the volatility and nondifferential nature of components captured on the paper are discussed.

Studies utilizing straight alleyways and T-mazes, modified to maximize the retention of rat odors, have provided support for the inference that laboratory rats leave odors in their environment when subjected to rewarded (R-odor) and nonrewarded (N-odor) goal events (e.g., Collerain & Ludvigson, 1977; Ludvigson, 1969; Mellgren, Fouts, & Martin, 1973; Seago, Ludvigson, & Remley, 1970). Furthermore, as evinced by such diverse indices as escape and approach responses (Collerain & Ludvigson, 1977; Mellgren et al., 1973), conditioned run speeds (e.g., Davis, Whiteside, Bramlett, & Petersen, 1981; Prytula, Davis, & Fanning, 1981; Taylor & Ludvigson, 1983), and mitral cell activity (Voorhees & Remley, 1981), R-odor and N-odor are discriminatively different to conspecifics.

It is likely, however, that these treatment-engendered odors do not exist in isolation from other olfactory components exuded by the rat. Even in the absence of explicit experimental treatments, odors have been shown to provide a basis for individual recognition in rats (Krames, 1970) and mice (Bowers & Alexander, 1967; Hahn & Simmel, 1968). Thus, some rat odors will likely exist for a period of time in an environment where a rat has been present, and experimental treatments such as reward and nonreward may simply add olfactory components to those which are already present but are not treatment related. In fact, studies showing that rats have more difficulty discriminating between N-odor and R-odor than between N-odor and a clean alleyway (Taylor & Ludvigson, 1980b) suggest that, although discriminably different, R- and N-odors share some common components.

Smith (1977) indicated that in social insects, alarm pheromones are more volatile than are trail pheromones or pheromones which serve to identify members of a species or group; thus, alarm pheromones diffuse faster and further. Although the status of R- and N-odors as pheromones is presently unclear, an understanding of the characteristics of these odors, such as their volatility and their behavioral/ecological significance, would perhaps be facilitated by first learning something about their environmental locus when encountered by conspecifics. A previous study by Taylor and Ludvigson (1980a) explored this issue with N-odor only. Rats, trained to discriminate between a clean alleyway and one in which donor rats had been nonrewarded, maintained their discriminations, with some diminution, when the alleyway was cleaned between donors by either changing the paper flooring or exhausting the air. When the alleyway was cleaned using both methods simultaneously, the rats' discriminations were eliminated. Although this finding was interpreted as evidence that some amount of N-odor existed in the air and on the floor of the alleyway, it may have been the case that olfactory components unrelated to the nonreward of donors provided a basis for the test rats' discriminations. Thus, it remains unclear whether N-odor

is relatively low in volatility and accumulates on the floor of the alleyway, or whether it is relatively high in volatility and vaporizes rather rapidly to fill the air space of the alleyway.

The present study focused on one environmental locus, the floor of the alleyway. Paper flooring was extracted from an alleyway in which a donor squad of 3 rats had either been rewarded or nonrewarded, or in which another squad of donors, having no history of reward and non-reward (animal odor) roamed the alleyway. If rat odors are retained on such papers, then conspecifics should be able to discriminate their presence. Furthermore, if R-odor, N-odor, and animal odor (A-odor) are different odors, then a rat trained to discriminate between the presence and absence of one of these odors should be disrupted when that odor is replaced by one of the other odors. If, on the other hand, the odors are largely the same across these treatments, then interchanging paper samples should not disrupt discriminative performance.

METHOD

Subjects

The subjects were 30 male albino rats obtained from the Holtzman Company at 99 days of age. The rats were individually housed in wire-mesh cages and provided free access to food and water until 131 days of age, at which time they were food deprived and gradually reduced in body weight to 80%-85% of free-feeding weight. Six of the rats were randomly selected to serve as test rats, and the remaining 24 rats were randomly divided into two donor groups of 12 rats each.

Apparatus

Two forms of apparatus were utilized: a donor alleyway and an olfactometer. The donor alleyway, constructed of stainless steel, measured 72 cm long × 7.62 cm wide × 20.3 cm high. The alleyway was equipped with a glass top, an air-exhaust system, and a means of completely covering the floor with adding machine paper. Further details of the alleyway may be obtained by referring to a previously published report (Taylor & Ludvigson, 1983), in which the behavior of the donor rats was discussed.

The olfactometer consisted of an air pump and purification system, an odor-presentation component, and a test chamber into which the olfactory stimulus was delivered. Room air was pumped (DeVilbiss Suction Pump No. 701), via a polyethylene hose, through three separate glass columns containing, in order, anhydrous calcium sulfate, charcoal granules, and distilled water. These three columns served to dehydrate, deodorize, and then partially rehydrate the air, respectively. The air was then carried, via glass tubing, through a standard needle valve and Dwyer flowmeter (RMA 16) which regulated the air flow at a rate of 5 liters per minute.

The odor-presentation component consisted of glass tubing, two three-way solenoid valves, two 8-oz glass sample jars, and a glass odor-delivery tube which protruded into the test chamber. The solenoid valves, controlled by programming equipment, allowed the air to flow through one sample jar at a time and then into the test chamber via the odor-delivery tube.

The test chamber consisted of a modified Lehigh Valley Electronics operant-conditioning chamber situated inside a sound-attenuating enclosure. A single lever, a food tray, and a chamber light were mounted on the front wall. Centered 3.81 cm above the lever was the odor-delivery tube, which protruded 1.27 cm into the chamber and terminated in a 3-mm wide opening in the bottom of the tube. The length of the chamber was 22.86 cm, and the width was restricted to 10.8 cm by exchangeable glass plates that extended the length and height of the chamber. On the back wall, a round portal, 3.81 cm in diameter, allowed the chamber air to be constantly evacuated with the aid of an attached hose and

exhaust fan. The sound-attenuating enclosure was equipped with a speaker through which white noise was emitted. Together, the speaker and exhaust fan provided a constant masking sound inside the chamber.

Procedure

Donors. Odor donor rats were of two types. Group NR, composed of 12 rats, received four rewarded (R) and four nonrewarded (N) trials in the alleyway during a day of training. They were run in squads of 3, where squad composition and ordinal position of rats in the squad remained constant within a day, but varied randomly across days. Each member of a squad was administered a trial, in succession, with all members receiving the same goal event on a particular trial. The sequence of R and N trials was randomly determined each day, with the restriction that not more than three R or three N events occur in succession. On R trials, 15 45-mg Noyes pellets were provided as a reward, with access limited to 30 sec. On N trials, the rat was merely confined to the goalbox, with an empty food cup, for 30 sec. The alleyway was left undisturbed until after the 3rd member of a squad was removed from the goalbox. At this time, the paper covering the floor was extracted, placed in a closed 8-oz glass jar, and saved until time to be used with the test rats. Group NR rats received 16 days of alleyway training before the paper flooring from their treatments was used as stimuli for the test rats.

Group A, composed of 12 rats, was divided into squads of 3 rats each, run in such squads, and the paper flooring was collected in the same manner as for Group NR. These rats, however, received only four trials in a day of training and were never rewarded with food pellets in the alleyway. Thus, to the extent that production of R- and N-odors requires some experience with the receipt of reward, these rats were not expected to produce such odors. Rather, any odors deposited in the alleyway would be part of their characteristic scent (A-odor). At the end of a day of training, these rats were provided with 60 45-mg Noyes pellets in the home cage after being returned to the animal colony. This was done in order to equate the diets of Group A and Group NR rats.

Test rats. Initially the 6 test rats were trained to leverpress on a VI 20-sec extinction multiple schedule, where the VI and extinction components were signaled by the houselight onset and offset, respectively. Once all 6 rats were consistently responding during light-on periods, and showing little, if any, responding during light-off periods, the olfactory discrimination procedure was initiated.

The olfactory discrimination procedure was accomplished by imposing a discrete-trial, "go, no-go" task onto the light-on period of the multiple schedule. To administer a trial, a test rat was placed in the chamber with the light off. Following a 5-sec delay, the light came on and air flowed through Sample Jar 1, which contained a strip of clean paper flooring, and into the chamber for 3 sec. The solenoid valves then switched and directed air through Sample Jar 2, which contained the paper providing the discriminative cue. Following a 2-sec delay, in which responses were not reinforced, the differential reinforcement period began. On S+ trials, the test rat was reinforced for the first response, following the lapse of a variable interval which could range from 3.75 to 36.25 sec. Reinforcement consisted of 15 45-mg Noyes pellets, dispensed at a rate of 2 pellets per second. As reinforcement began, the light went off and air delivered into the chamber was once again directed through Sample Jar 1. The rat was allowed 45 sec to consume the pellets before being removed to a waiting room while preparations were made for the next test rat to receive a trial. On S− trials, the procedure was the same as on S+ trials, except that no pellets were delivered at the end of the interval; instead, the rat was merely confined to the chamber for a 45-sec period. Each test rat received four S+ and four S− trials per day, with the order randomly determined. Each test rat received its first trial before any test rat received its second trial, and so on.

Over the course of the experiment, discrimination training for each test rat proceeded in three phases, where each phase involved discriminating between clean paper flooring and donor-treated paper flooring extracted from the alleyway. Two test rats were randomly selected to receive training in the three phases in the sequence R-odor, A-odor, N-odor; 2 received the sequence A-odor, N-odor, R-odor; and 2 received the sequence N-odor, R-odor, A-odor. For each of these pairs of test rats, 1 rat always received the donor-treated paper as the S+ cue and the clean paper as the S− cue, whereas the other rat received the clean

paper as the S+ cue and donor-treated paper as the S− cue. In each phase, each test rat was trained on the respective discrimination until demonstrating reliable differential responding or until it was concluded that a discrimination was unlikely to develop. Between trials, the olfactometer was purged with room air for 2 min and the test chamber was cleaned.

RESULTS AND DISCUSSION

The total exposure time and total responses across four S+ trials and across four S− trials in each daily session were computed for each test rat. These values were used to calculate a discrimination index (Millenson, 1967) for each test rat. This index was formed by dividing the rate of responding on S+ trials by the rate on S+ trials plus the rate on S− trials. The index reflects the percentage of total daily rate which occurred in the presence of the S+ cue, and permits a comparison of discriminative performance across conditions despite fluctuations in absolute response rates. Values of the index can vary between 0.0 and 1.0, with an index of 0.5 representing equal rates of responding in the presence of S+ and S−, and hence, no discrimination. Values between 0.5 and 1.0 represent proportionally higher rates in S+, compared with S−, and thus, differing levels of discrimination.

During each phase, the responding of each test rat was assessed for evidence of a reliable discrimination by using the Binomial Test and the Wilcoxon Sign-Ranks Test (Siegel, 1956). Using the Binomial Test, the daily discrimination indices over a run of 8 successive days within a phase were examined for values above 0.5. If no more than 1 of these days involved a value less than 0.5 (values of 0.5 had to be dropped from the analysis), then according to a one-tailed test, the binomial probability is less than 0.1 that the discriminative performance occurred by chance. Achieving this criterion was construed as indica-

tive of a discrimination, and then the discrimination was evaluated for stability using the Wilcoxon Test. Utilizing the six most recent discrimination indices, a set of five deviation scores was computed by taking the differences in the discrimination indices between successive days as deviation scores and ranking the five deviation scores from one (smallest) to five (largest). If the deviation score involved an increase in the discrimination index between successive days, a positive sign was attached to the corresponding rank; decreases in the index between successive days required a negative sign to be attached to the rank. If the sum of the ranks of the positive deviations was less than the critical value of T ($p < .40$), then the rat was considered to be performing at a stable level of discrimination. If the sum of the positive ranks was greater than the critical value, then training was continued, and each day the five most recent deviation scores were used to assess stability until the criterion was achieved. Once the stability criterion was achieved, the test rat was shifted to the next phase of the experiment.

The discrimination indices achieved by the test rats across all sessions and phases are shown in Figure 1. Five of the 6 test rats achieved the discrimination and stability criterion in every phase, although substantial differences occurred across rats in the magnitude of discrimination and the amount of training required to achieve a stable level of discrimination. Despite considerable training on the R-odor versus clean paper discrimination, Rat 4 never acquired this discrimination or any of the subsequent discriminations. However, based upon the performance of the 5 rats that did discriminate, several observations are noteworthy.

First, paper flooring taken from the alleyway following R, N, and neutral treatments all retained some olfactory components which enabled the test rats to dis-
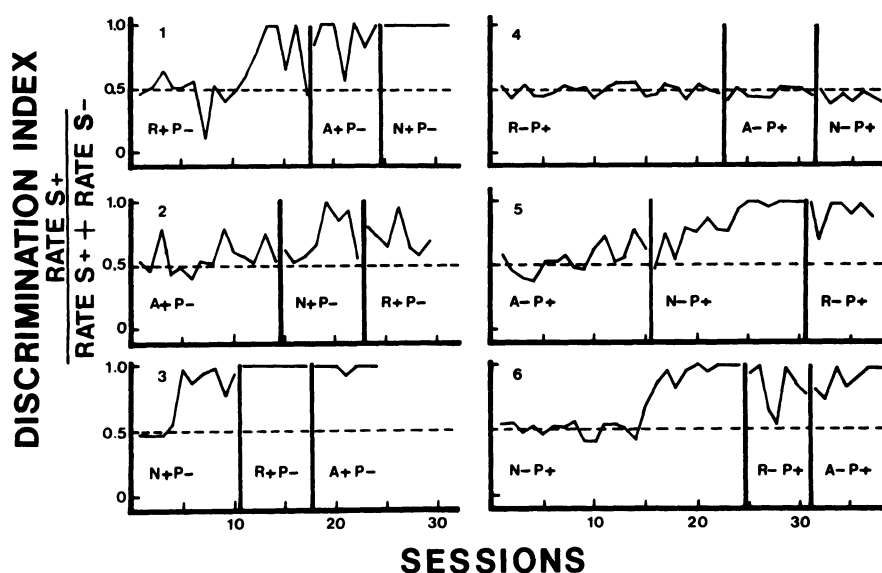

Figure 1. Discrimination indices for each test rat as a function of reward odor (R), nonreward odor (N), animal odor (A), and paper odor (P), where + = reinforcement for responding in the presence of the odor, and − = nonreinforcement of responding in the presence of the odor.

criminate these papers from clean ones. These olfactory components must have remained on the paper long enough to be captured in the sample jars, and hence do not appear to be highly volatile. Second, once the initial discrimination had been acquired, whether that discrimination involved papers from R, N, or neutral trials, interchanging these papers produced no evidence of substantial disruption in discriminative performance. All 5 rats met the discrimination criterion in Phases 2 and 3 within the minimum of 8 days required to achieve that criterion. Rat 5 required somewhat more extended training in Phase 2 in order to meet the stability criterion. Thus, transfer of discriminative control by the olfactory cues present on paper from R, N, and neutral trials was readily accomplished.

Third, although these data do not reveal the hypothetical perceptual categories used by the test rats in making these discriminations, the relative ease of transfer suggests that the rats treated the olfactory cues captured on paper as much the same across R, N, and neutral trials. Since a substantial number of studies clearly indicate that odors arising from R and N trials are discriminably different from one another, the present data seem to suggest that the differential olfactory components which typically mediate the R versus N discrimination are either not deposited on the paper, not retained on the paper, or if they are deposited and retained, are less salient, as defined by their ability to gain discriminative control, than are olfactory components which are common to R and N treatments. Since the test rats showed transfer whether going from neutral-treatment papers to N-treatment papers or from R-treatment papers to neutral-treatment papers, such papers appear to share the relatively salient olfactory component(s) of "rat odor."

Voorhees and Remley (1981) provided some evidence that an air stream directed into the naris of a rat after being passed over R, as opposed to N, paper samples elicits differential responsiveness in mitral cells of the rat's olfactory bulbs. Differential components may be present and detectable with a special preparation such as this, but may not appear in sufficient quantity to control differential responding in a behavioral task such as that utilized in the present experiment. Rather, the present study suggests that olfactory components retained on the paper are low in volatility and are primarily part of the rat's characteristic scent. Perhaps these components serve to identify individuals or group membership, as Smith (1977) has sug-

gested for social insects. Hence, future studies that attempt to isolate the differential, and behaviorally controlling, features of R- and N-odors should look for relatively volatile components which may best be collected from the air space in which donor rats receive these treatments.

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