Discrimination between deionized water and D₂O in a runway using olfaction in the rat

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This experiment tested whether rats could discriminate between D₂O and deionized water using olfaction alone or using both gustation and olfaction. These substances were used as cues for the presence of reward or nonreward in a runway and, if perceived, should have resulted in patterned responding. Twenty experimentally naive male albino rats were given 200 trials in a runway under a random-partial-reinforcement schedule. One-half of the rats could use both taste and smell of D₂O and deionized water as cues for reward and nonreward, while the other half of the subjects were allowed only to smell these substances. The results showed that, after 100 trials, patterned running developed equally well with smell alone as with both taste and smell as cues. Moreover, D₂O and deionized water were equally effective as cues for reward and for nonreward in both smell alone and taste/smell conditions.

It is a well-established phenomenon that animals can rapidly develop conditioned aversions to foods (Garcia & Koelling, 1966; Rozin & Kalat, 1971). The primary cue for rats in this conditioning process is gustation, with olfaction playing a secondary role. Researchers have shown that conditioned aversions are most readily established to gustatory stimuli, and only moderate aversions are developed to olfactory cues, while nongustatory exteroceptive cues (i.e., sounds, lights) are poorly associated with aversions (Garcia & Koelling, 1967; Lorden, Kenfield, & Braun, 1970). A wide range of taste and olfactory cues have been effectively used to develop aversions. Among these, recent research has shown the development of an aversion to heavy water, D₂O (Browder, Upchurch, & Kirby, 1974; Smith, 1968).

Deuterium is a poison, and chronic exposures to concentration above 40% can produce a number of symptoms: piloerection, spontaneous aggression, activity fluctuations, appetite changes, sterility, spasms, and ultimately, death (Bachner, McKay, & Rittenberg, 1964; Katz, Crespi, Czajka, & Finkel, 1962). Since the chemical compound D₂O is so similar to H₂O, it is interesting to speculate whether olfactory and/or gustatory cues serve as the basis for this learned aversion. In Browder et al., the positions of the tubes were changed daily, and since both D₂O and deionized water have identical appearance plus the fact that rats are notoriously poor at using visual cues to establish an aversion (Best, Best, & Mickley, 1973; Wilcoxon, Dragoin, & Kral, 1971), either gustatory or olfactory cues must be involved. Informal observations from our laboratory had suggested that the animals approached

and apparently avoided the D₂O tube without making contact with the solution, which suggests that olfaction was sufficient to mediate the aversion. Smith (1968) has suggested that the more stable bonds between deuterium and oxygen, compared to hydrogen and oxygen, result in differential stimulation of taste receptor sites, and it is this gustatory mechanism that is responsible for discrimination between these two substances.

The present study used a behavioral test to separate the possible cues that allow the development of an aversion to D₂O. The task was a running response in an alley under a random-partial-reinforcement schedule. Rats are capable of developing a patterned running response with a single-alternation reinforcement schedule, fast running on rewarded trials and slow running on nonrewarded trials, but this is not possible when a random-partial-reward schedule is used (Longnecker, Krauskopf, & Bitterman, 1952; Tyler, Wortz, & Bitterman, 1953). Two types of cues were correlated with the presence or absence of reward in the goalbox. Rats were either able to smell D₂O or deionized water or to both smell and taste these stimuli. If patterned running developed under one or both of these conditions, or if there were rate differences in its development, this would provide evidence of the rat's capacity to discriminate by taste or smell the difference between deionized water and D₂O. Further, it would assess the earlier report of olfactory avoidance of D₂O by the rat.

METHOD

Subjects

The subjects were 20 experimentally naive male albino rats obtained from Charles River Laboratories. All were approximately 90 days old at the beginning of the experiment and were individually housed.

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Figure 1. Mean running speed as a function of blocks of 10 trials for the smell only and taste/smell groups on both rewarded and nonrewarded trials.

Apparatus

The apparatus was a straight alley consisting of an 8-in. startbox, a 24-in. runway, and a 12-in. goalbox; alley dimensions were 4 in. wide and 5 in. high. The walls were constructed of unpainted plywood and the alley was covered by a hinged ¼-in. Plexiglas lid. The floor consisted of ¼-in. hardware cloth. One-quarter inch Plexiglas guillotine doors separated the start- and goalboxes from the runway. Two doors were used for the startbox, one with a 10-ml drinking bottle of D₂O, the other with an identical bottle of deionized water mounted on the runway side of the door. A 1-in. opening was cut into the doors to allow easy access to the bottles for those subjects who both drank and smelled the stimulus. For those which were allowed only to smell the cue, a screen of ¼-in. hardware cloth was attached in front of the opening. This prevented the subject from reaching the solutions. A tray was mounted under each bottle to prevent any fluid from dripping onto the runway floor. A photocell connected to a Hunter Photo Relay was attached 1 in. from the back of the goalbox. A timer was activated when the startdoor was lifted and stopped when the subject broke the photocell beam located over the foodcup (distance = 35 in.). The D₂O was 99.88% mole with a density of 1.105 g/ml and odor and turbidity rating of "none." Ten 45-mg Noyes pellets were used on the reinforced trials. Illumination was provided by a 25-W red bulb hung above the apparatus.

Procedure

The subjects received food ad lib for 1 day, and they were then placed on a 23-h deprivation schedule. They received a total of 12 g of nourishment daily, which included the amount of reinforcement eaten during the running trials and the powdered Purina lab chow to which they had access for 1 h in their home cages following the running trials. Ad-lib water was available in the home cages. The subjects were randomly assigned to one of four groups ($N = 5$): (1) subjects could smell and taste solutions, and D₂O was a cue for reward; (2) subjects could smell and taste solutions, and D₂O was a cue for nonreward; (3) subjects could only smell stimuli, and D₂O was a cue for reward; and (4) subjects could only smell stimuli, and D₂O predicted nonreward.

From Day 1 to Day 5, each subject was handled for 5 min, and on Days 6 and 7 each was placed in the apparatus with both doors opened with access to 10 reinforcement pellets in the goalbox for a period of 5 min. On Day 8, when the training trials began, each subject was placed in the startbox with both guillotine doors down and allowed to orient to the door. After 15 sec, both doors were raised, and when subject was inside the goalbox they were lowered. If reinforcement was present,

subject was left in the goalbox until he had eaten all 10 pellets; if no reinforcement was present, he was left in the goalbox for 15 sec. Ten trials were run each day for each subject until a total of 200 trials per subject was reached. The reinforcement schedule was established so that half of the trials in each block of 10 were rewarded (i.e., +00+0, 0++0+, +00++, 00+0+, 0++00, etc.) for each day was random. Each day the bottles were randomly shifted to a different startdoor within the appropriate condition.

RESULTS

All response latencies were converted to reciprocals for subsequent analysis. An analysis of variance was performed on the mean running speeds for the subjects over the first 10 trials. None of the variables approached significance, which indicates that the groups were identical at the beginning of training.

Over the first 90 trials of acquisition, there was no indication of patterned running (See Figure 1). Around Trials 90-100, patterned running began to develop. An analysis of variance was performed on mean speeds over the last 11 blocks of trials (Trials 90-200). Both between-group main effects were insignificant, comparison of speed with taste and smell stimuli vs. smell alone ($F = .09$, $df = 1/16$) and D₂O as the positive cue vs. H₂O as the positive cue ($F = 1.69$, $df = 1/16$). Thus, the lack of opportunity to taste the stimuli or the presence of D₂O as the positive stimulus did not affect the development of patterning. Simply, the discrimination of deionized water from D₂O can be made when the only possible cue is olfaction, and the addition of taste did not affect the establishment of this discrimination. Since the taste/smell animals did routinely lick at the solutions, this difference is not due to the subjects' not experiencing the cues.

There was a highly significant effect of reinforcement ($F = 45.27$, $df = 1/16$, $p < .0001$) with the subjects in both groups running faster on rewarded trials than nonrewarded trials. Also, there was a significant interaction between Reinforcement by Days ($F = 56.51$, $df = 10/160$, $p < .0001$), which reflected a major drop in running speeds on trials when the cues signaled nonreward, while speeds on rewarded trials remained stable.

The effects of Days and several other interactions with the days variable were significant, but since these F ratios accounted for very little of the total variance in the data, they are not presented here.

DISCUSSION

The major finding of this study was that rats would discriminate between D₂O and deionized H₂O on an olfactory basis. Smith (1968) has assumed that the basis for discrimination between D₂O and H₂O is gustatory, and has proposed a mechanism explaining how the two substances differentially stimulate the receptor site. The

results of the present experiment suggest that olfaction as well, and possibly predominantly, is responsible for the rat's capacity for making this discrimination.

Further, the development of patterned running was at approximately identical rates for the taste/smell and the smell-only groups. It is important to stress that on almost every trial the subjects were oriented toward the startdoor for several seconds prior to the start of that trial and that on most trials the taste/smell rats were observed to lick at the given solution. Therefore, the subjects were exposed to both potential cues and the failure to find any difference in rate of development of the behavior was not due to a failure of the rats to lick the solutions. It is significant that the rats were able to discriminate between these chemically similar stimuli by smell alone, since unpublished data from this laboratory using human subjects found that discriminations between D₂O and deionized H₂O could not be made when olfaction was the only cue available, but did occur when the subjects tasted the stimuli (Kirby, Pick, & Smith, Note 1).

It is surprising that the taste/smell group did not show an earlier patterning, since taste has been shown to be a potent stimulus for rats. However, this is primarily in the context of the establishment of food aversions, and it is possible that taste, especially with a subtle stimulus, is not as discriminable unless an illness is involved. It is conceivable that olfactory and gustatory cues from D₂O and H₂O are equally salient in animals which have consumed little or no D₂O, but that the gustatory cue becomes more salient after the animal has learned an aversion to D₂O. Therefore, the question of which sensory modality is most important for establishing the

preference for deionized water over D₂O is yet to be determined.

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NOTE

1. Kirby, R. H., Pick, D. F., & Smith, M. E. Discrimination of D₂O and deionized water using gustatory and olfactory cues in the human, in preparation.

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