Conditioned taste aversions: Two-stimulus tests are more sensitive than one-stimulus tests*

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Weanling and mature rats were presented with saccharin or saline solutions for 1 h on alternate days. Following exposure to saccharin, rats were injected with 0, 21, or 37 mg/kg of cyclophosphamide. Injections had no significant effect on saccharin preference in one-stimulus tests, but had a highly significant effect in two-stimulus tests.

One-stimulus and two-stimulus tests have been used to measure food and taste preferences (Morgan, 1965). In one-stimulus tests, animals are presented with substances one at a time, as in a successive discrimination. Their intake of a test substance is compared either with their intake of another substance presented at a different time or with intake of the test substance by other animals. In two-stimulus tests, animals are presented with two substances, as in a simultaneous discrimination, and preference ratios are computed, usually by dividing intake of the test substance by total intake of both substances.

Both methods have been used to measure conditioned taste aversions in rats. These aversions are established by presenting rats with a novel-tasting test fluid and, following their drinking of the fluid, inducing nausea either by X-irradiation or injection of certain drugs. When again presented with the test fluid in one-stimulus tests, the rats typically drink less than they did initially and less than do controls, whereas their drinking of familiar fluids is relatively unaffected (Garcia & Koelling, 1967). In two-stimulus tests, the rats prefer the test fluid less than do controls (Rozin, 1969).

In one-stimulus tests, rats which have previously drunk lithium chloride (LiCl) solution, an initially palatable salt that induces illness, will subsequently avoid drinking both LiCl and sodium chloride (NaCl) (Nachman, 1963; Strom, Lingenfelter, & Brody, 1970). On the other hand, if two-stimulus tests are used, rats avoid LiCl but drink NaCl (Harriman, Nance, & Milner, 1968; Strom et al, 1970). These results suggest that two-stimulus tests are more sensitive in situations comparing two similar-tasting fluids. Further, Rozin (1969) found that two-stimulus tests detected effects of delayed conditioning of a taste aversion when one-stimulus tests did not. However, his tests were conducted under very high deprivation, which may have forced relatively high intake of the test fluid in one-stimulus tests (Grote & Brown, 1970).

The present study indicates that two-stimulus tests are more sensitive than one-stimulus tests, even when two highly discriminable fluids are presented under moderate deprivation, and for young as well as mature rats.

METHOD

Subjects

The Ss were 24 male Long-Evans hooded rats individually housed in standard laboratory cages with Purina Lab Chow freely available. Twelve rats were 20 days old and 12 were 70 days old at the start of the experiment.

The rats in each age group were assigned randomly to three treatment groups of four each. Rats in the 21-mg/kg and 37-mg/kg groups were injected with those doses of cyclophosphamide, and rats in the control group were injected with comparable amounts of isotonic saline following exposure to saccharin. During the experiment, several rats died, resulting in the final N shown in Table 1. All data from rats which died were discarded.

Procedure

On Days 1-3, the rats were adapted to a fluid-deprivation schedule. Water was presented for 1½ h in the morning (morning session) and for 5 h in the evening (evening session), approximately 5 h after the morning session. After the 3 adaptation days, the morning sessions were reduced to 1 h. Fluids were presented in 50-ml tubes attached to the front of the home cage, and the amount consumed was determined by weighing the tubes on a chemical balance before and after each session.

An 0.1% saccharin solution was presented to all rats in the morning session on Days 4, 6, 8, and 10. Approximately 30 min after the saccharin was removed on Days 4, 6, and 8, the 21-mg/kg and 37-mg/kg rats were injected intraperitoneally with cyclophosphamide, and control rats were injected with isotonic saline. No injections were given on Day 10. A 1.1% saline solution was presented during the morning sessions on Days 5, 7, 9, and 11, with no injections. Saline was used as the control fluid so that both fluids presented during the morning sessions in conditioning would be novel tasting.

Water was presented to the rats during the morning sessions on Days 12 and 13. Eight hours later each day, the rats were given two-stimulus tests, with the saccharin and saline solutions presented simultaneously for 1½ h. The position of the bottles was reversed after 45 min to control for position preferences.

Measures

Comparable sets of measures were computed for one-stimulus and two-stimulus tests. For one-stimulus measures, intake of saccharin on Day 10 and saline on Day 11 was used. A saccharin preference was computed for each rat by dividing its saccharin intake on Day 10 by its total intake of saccharin and saline on Days 10 and 11. For two-stimulus measures, mean saccharin and saline intake in the two two-stimulus tests was used, and a saccharin preference was computed by dividing each rat's saccharin intake in the two tests by its total saccharin and saline intake.

Each measure was analyzed with a 2 by 3 analysis of variance, using an exact solution for unequal numbers of Ss in each group.

RESULTS AND DISCUSSION

The mean scores for all groups on both one- and two-stimulus measures are shown in Table 1. Directly below the scores for each measure are the results of the relevant analysis of variance. In the one-stimulus tests, intake of both saccharin and saline decreased with increasing dosage of cyclophosphamide. The preference measure, however, showed no conditioning effect, and there is not

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Table 1
Mean Intake of Saccharin and Saline and Mean Saccharin Preference in Single-Stimulus and Two-Stimulus Tests, and Analysis of Variance for Each Measure

			Single-Stimulus			Two-Stimulus		
Age	Dosage	N	Saccharin	Saline	Preference	Saccharin	Saline	Preference
21 Days	Control	4	7.35	10.27	.41	9.11	2.41	.80
	21 mg/kg	4	5.27	7.88	.40	4.52	5.10	.55
	37 mg/kg	4	2.85	8.27	.20	1.89	8.14	.17
70 Days	Control	3	16.77	19.13	.48	12.58	4.55	.74
	21 mg/kg	3	9.33	8.80	.59	9.73	5.87	.70
	37 mg/kg	2	5.85	5.60	.49	5.08	6.82	.39

Analyses of Variance

Source	df	F Ratios					
Dosage	2,14	11.47†	5.17*	1.90	7.89**	2.09	7.40**
Age	1,14	21.67†	2.23	6.14*	7.10*	0.14	0.67
Interaction	2,14	2.67	3.32	0.76	0.18	0.30	0.55

*†p < .001, **p < .01, *p < .05*

even a trend apparent in the 70-day-old groups. Interestingly, the age effect was significant.

The two-stimulus preference test, on the other hand, shows a highly significant effect of dosage, with saccharin preference decreasing with increasing dosage at both ages. Saccharin intake decreased with increasing dosage, but saline intake showed an increasing trend, the opposite of that shown in the one-stimulus tests. There was no significant effect of age on the preference measure.

The present results when considered with those of Rozin (1969) and Strom et al (1970) indicate that two-stimulus tests are more sensitive than one-stimulus tests under a variety of conditions in detecting conditioned taste aversions. Furthermore, absolute intake in one-stimulus tests may be a misleading measure since under some circumstances decreased intake of the test fluid may reflect an effect of the drug on the rat's overall fluid intake rather than on intake of a specific fluid. Unfortunately, valid preference ratios cannot usually be computed from one-stimulus tests, since the test periods are typically the only time fluids are available. If intake of one fluid is low in a given test, intake of the other fluid in the next test may be increased as a function of greater thirst. A preference ratio computed across those two tests would not be a valid measure. Ratios were computed for the one-stimulus tests in the present study only because supplementary access to water was given.

Although two-stimulus tests are a sensitive measure of conditioned taste aversions, there are other situations where a different test may be a more appropriate measure of taste preferences. Indeed, the present results suggest, albeit very tentatively, that one-bottle tests may be prefereable for measuring developmental trends. Also, Deutsch and Jones (1960) found that rats drank more hypotonic saline than water in two-stimulus tests, but preferred water as a reinforcement in a learning situation.

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