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The combined effects of dosage level and interstimulus interval on the formation of one-trial poison-based aversions in rats

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Adult male rats were allowed to drink a novel solution of sodium saccharin which was followed .5, 1.5, 4.5, 7.0, 13.5, or 24.0 h later by intubation of a .9, 2.7, 8.1, or 12.15% (w/v) solution of sodium chloride (NaCl). Three days after the single training trial, consumption of saccharin was again measured. Significant differences between groups were found. When consumption by the experimental groups at each CS-UCS delay was compared with that of the isotonic NaCl (.9%) control group, it was found that all groups showed aversions at delays of .5, 1.5, and 4.5 h. Animals intubated with 8.1% or 12.15% NaCl solution also showed aversions at a delay of 7.0 h, and those intubated with the 12.15% solution showed an aversion at a delay of 13.5 h. No NaCl concentration used produced aversions at a CS-UCS interval of 24.0. These results reflect differences in the effectiveness of a range of NaCl concentrations in producing one-trial aversions at long CS-UCS intervals.

The paradigm used to demonstrate poison-based avoidance learning is a simple one. Ingestion of a harmless food (CS) is followed, after some period of time, by sickness (UCS). Upon complete recovery from the sickness, subjects are allowed to consume the same food substance and, when comparisons are made with appropriate controls, it is typically found that they now avoid the food which preceded the induced illness. It is now well established that long delay poison-based aversions are a form of learning and not an artifact of the experimental situation (see Revusky & Garcia, 1970). Several explanations, all based in one form or another on some aspect of the relationship between the taste and the sickness, have been proposed to explain why it is possible to obtain associations with only one trial and with long delays between the CS and the UCS using the poison-based avoidance learning paradigm but not with more traditional ones. For example, the principle of stimulus relevance stated that "certain associations are formed more easily if the events to be associated are capable of being perceived as belonging together (Capretta, 1961, p. 241)." That is, certain events such as eating and illness are more easily perceived by the learner as belonging together than are other events such as eating and shock. As a result, eating and illness are readily associated and, since the events are "relevant" for the learner, they can be associated even if the delay between them is several minutes or hours (Revusky, 1971).

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The principle of relevance has been used as an explanation for one-trial long delay learning (Revusky & Garcia, 1970) in spite of the fact that the original procedure used to demonstrate the relevance principle employed neither long CS-UCS intervals nor a one-trial learning situation (Braveman & Capretta, 1965; Capretta, 1961). The technique used by Braveman and Capretta (1965), for example, involved preference testing rats with a sugar and sugar-saccharin solution for 5 days prior to training. On 5 of the training days, experimental subjects received a stomach preload of NaCl followed by access to their preferred solution, while on 5 alternate days they received a stomach preload of plain water and access to their nonpreferred solution. Preloading subjects in this manner insured that the shortest possible CS-UCS interval was used, i.e., the UCS immediately preceded the CS so that sickness was contiguous with consumption of the CS. Subjects were then given two more daily preference tests with the solutions.

In two pilot studies, the present investigators showed that long delay learning could in fact occur with intubated NaCl as the UCS, but the conditions of the studies did not allow an evaluation of the aversion after only one training trial. Also, the results of these studies were equivocal with respect to the finding of a delay of punishment gradient. That is, the findings from one study showed that aversions were stronger with a 1-h delay than with a 5-h delay, while, in the second study, animals made sick 7 h after consumption of their preferred solution formed aversions that were as strong as those formed by animals trained with a 1-h delay.

The inability to find a clear-cut gradient of punishment was of particular interest since the occurrence of these gradients has been one of the strongest arguments in support of the view that long

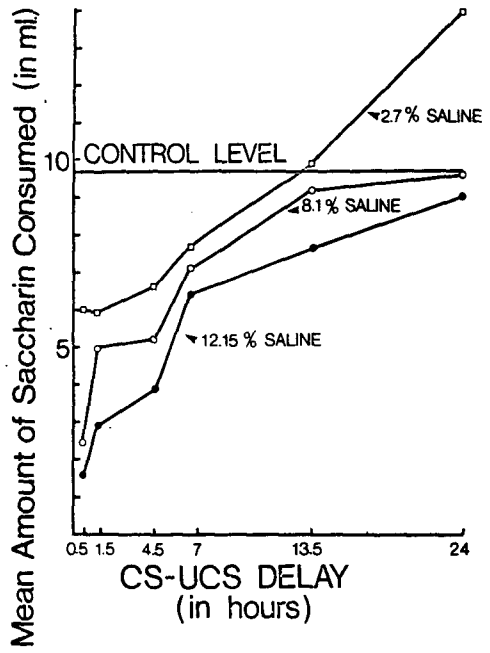

Figure 1. Mean saccharin consumption of all groups on posttraining preference test day.

delay aversions are not different from more typical forms of learning. Also, delay of punishment gradients have been shown to be a general characteristic of other toxins (see Revusky, 1971). Thus, while findings from experiments which use the NaCl intubation method have been used as the basis for a learning interpretation of poison-based aversions, it was not possible to obtain a clear-cut delay of punishment gradient when this method was used.

It is possible that floor effects may have obscured the delay of punishment gradient in the two preliminary studies. That is to say, the use of too many training trials, the use of CS-UCS intervals which were not long enough, or the use of a very intense UCS could have produced maximal aversions in all animals and thus would have obscured a gradient. For example, a toxin that might have produced weaker aversions with a 7-h CS-UCS interval than with a 1-h interval using one training trial may have produced aversions of equal strength if several training trials were used. It is also possible that sufficiently long CS-UCS intervals were not used in the preliminary studies so that maximal aversions were produced in all animals. Finally, the use of a very intense UCS could have led to floor effects. Pilot studies by the present investigators showed that intubation of a NaCl solution more concentrated than 13.0% (w/v) was fatal in water-deprived rats. Hence, the 10.0% (w/v) concentration used in the two preliminary studies was a fairly intense UCS and may have produced so severe an illness that, when combined with repeated training trials and CS-UCS intervals of only moderate duration, maximum aversions were obtained. To correct for these possible defects, the present study involved the use of one CS-UCS trial as well as factorial combinations of a number of NaCl concentrations and CS-UCS intervals.

METHOD

Subjects

The subjects were 180 experimentally naive male albino rats, 90 days old at the start of the experiment, obtained from the Canadian Breeding Farms. Animals were maintained in individual cages on ad-lib food throughout the experiment. Six subjects died during the experiment and were replaced in later replications so that there were between six and eight subjects in each experimental and control group.

Procedure

All subjects were adapted to a water-deprivation schedule on which they received water for 10 min each day in their home cages. After 6 days, all animals had reached a stable level of consumption. The single training trial took place on the next day, at which time all subjects received .5% (w/v) sodium saccharin during their 10 min drinking period. Then, after a CS-UCS interval of .5, 1.5, 4.5, 7.0, 13.5, or 24.0 h, treatment groups were intubated with a 1.5% body weight dose of 2.7, 8.1, or 12.15% (w/v) NaCl. As a control for the intubation procedure, groups of animals were intubated, after the same CS-UCS intervals, with a 1.5% body weight dose of a nontoxic isotonic (.9%) NaCl solution. Intubation was accomplished by holding the subject's mouth open with a rubber brace (cut from a one-hole hard-rubber stopper) and inserting a catheter directly into the subject's stomach. The tip of the catheter was first dipped in water to encourage the animal to swallow. Once the catheter was in the animal's stomach, the NaCl was injected slowly by means of a syringe attached to the catheter. Two hours after the NaCl intubation, all subjects received water for 6 h to facilitate recovery from the NaCl-induced illness. Forty-eight hours after animals had received saccharin on the training day, all subjects were returned to a 10-min/day water schedule. The following day, all subjects received a 10-min drinking trial with saccharin. The amount of water or saccharin consumed on each day was recorded to the nearest .5 ml.

RESULTS

A one-way analysis of variance (Winer, 1962) showed that saccharin consumption on the training day, prior to sickness, was equivalent for all groups ($F = .81$, $df = 23,156$; $p > .05$). The results of the posttraining phase of the study are summarized in Figure 1, which shows the mean saccharin consumption of all experimental groups on the posttraining test day. The posttraining test day consumption was analyzed using a 3 by 6 (concentration by delay) analysis of variance. Data from the .9% (isotonic) NaCl group was omitted from the analysis because, as expected, all subjects in this group increased their saccharin consumption between the training and posttraining measures. By including their data in the analysis, the effects would have been spuriously inflated. The results of this analysis are given in Table 1, and show that on the posttraining test day both NaCl concentration and CS-UCS delay were significant effects. Individual comparisons on the

amount consumed by the experimental groups and the pooled control (.9%) group at each delay were made using independent t tests. A summary of these comparisons is given in Table 2. From this table it can be seen that when the 12.15% NaCl solution was intubated, aversions to saccharin water were obtained at all but the 24-h delay. When an 8.1% NaCl solution was intubated, aversions were produced at delays up to 7 h but not at 13.5 or 24 h. Finally, when the weakest (2.7%) NaCl concentration was used, aversions were produced only at .5, 1.5, and 4.5 h but not at 7.0, 13.5, or 24 h.

Table 2
Individual Comparisons of Experimental Groups With the Pooled Control Group at All CS-UCS Intervals on the Posttraining Test Day

	CS-UCS Delay (in h)					
Comparison	.5	1.5	4.5	7.0	13.5	24.0
2.7%-.9%	**	**	*	n.s.	n.s.	*[a]
8.1%-.9%	**	**	**	*	n.s.	n.s.
12.15%-.9%	**	**	**	**	*	n.s.

[a] *2.7% mean greater* *n.s. = not significant* *$p < .05$* **$p < .01$*

DISCUSSION

These results show that intubation of NaCl produces effects that are similar to those found with other toxins. First of all, aversions were produced when only one CS-UCS pairing was used even though there was a delay. Secondly, there was a very orderly gradient of effectiveness which appeared to be dependent on the concentration of NaCl used and on the CS-UCS interval. For example, 2.7% NaCl produced aversions at delays up to 4.5 h, 8.1% at delays up to 7.0 h, and 12.15% at delays up to 13.5 h.

The present results are comparable with those reported by Kalat and Rozin (1971) as well as results from a study by Wright, Foshee, and McCleary (1971). Using intubation of .15 M lithium chloride as a UCS and one training trial Kalat and Rozin (1971) found aversions to sucrose at delays up to 3 h. A group tested at 7 h, however, did not show a reduced preference. This finding is similar to the results found in the present experiment with 2.7% NaCl. Wright, Foshee, and McCleary (1971) used cyclophosphamide as a toxin and three CS-UCS pairings at intervals up to 120 min. They found that dosage level, CS-UCS delay, and the Dosage by Trials interaction significantly influenced the strength of the aversion. That is to say, aversions to saccharin were strongest when the delay was 30 min and with a 75 mg/kg concentration of cyclophosphamide. Increases in delay interval and/or decreases in concentration served to weaken the aversion. Comparisons between aversions reported by Wright et al. and those reported in the present experiment indicate, however, that for comparable delay intervals, aversions disappeared more quickly with increases in CS-UCS interval and/or decreases in concentration when cyclophosphamide was used than when intubated NaCl was the UCS. It is possible that the steeper gradient with cyclophosphamide could have occurred because Wright et al. used three training trials. Alternatively, it is possible that with the concentrations used, cyclophosphamide is a less effective toxin than stomach intubated NaCl.

Table 1
Analysis of Variance on Saccharin Intake by Experimental Groups on the Posttraining Test Day

Source	SS	df	MS	F
Delay (D)	901.3	5	180.3	23.10**
Concentration (C)	227.2	2	113.6	14.55**
D by C	61.3	10	6.1	.79*
Error	913.1	117	7.8	

**Not significant* **$p < .00001$*

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