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Learned Taste Aversion to Saccharin Produced by Orally Consumed d-Amphetamine

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SANGER, D. J., A. J. GREENSHAW, I. P. THOMPSON AND J. D. MERCER. *Learned taste aversion to saccharin produced by orally consumed d-amphetamine.* PHARMAC. BIOCHEM. BEHAV. 13(1) 31–36, 1980.—Rats were presented with solutions containing both saccharin and d-amphetamine and the development of taste aversions to solutions of either or both of these substances was studied. In Experiment 1 it was found that taste aversions developed to solutions of saccharin (1 mg/ml) which contained amphetamine at concentrations of 0.01, 0.03 and 0.1 mg/ml. Experiment 2 showed that a taste aversion conditioned to a solution of saccharin (2 mg/ml) and amphetamine (0.2 mg/ml) generalised to solutions containing saccharin at concentrations between 0.625 and 20 mg/ml but not to a solution containing only amphetamine. In the third experiment it was found that the degree of generalisation of a taste aversion to lower saccharin concentrations depended upon the concentration used during conditioning trials. When the conditioning concentration was 0.625 mg/ml the aversion generalised to concentrations as low as 0.075 mg/ml but when a 10 mg/ml solution was used for conditioning the aversion did not generalise to concentrations below 2 mg/ml. The characteristics of taste aversions conditioned with orally consumed amphetamine are similar to those of conditioning involving injections of the drug.

Conditioned taste aversion Amphetamine Oral administration Rats

IN recent years a number of experimental reports have shown that injections of a variety of psychoactive drugs can produce aversions in rats or other animals to novel tasting solutions which are paired with drug administrations. One of the most remarkable aspects of this phenomenon is that in many cases the doses of drugs which lead to such conditioned taste aversions are very similar to the dose levels of the same drugs which will, in other situations, act as reinforcers thus maintaining instrumental responding. Cappell and Le Blanc [2] have discussed this apparent paradox and have described a number of factors which may contribute to the reinforcing and aversive effects of the same pharmacological agents.

One such factor is the route by which a drug is administered. Typically, in experiments concerned with taste aversions conditioned by drug administration, the drugs are given by intraperitoneal injection. In studies of the reinforcing properties of drugs subjects are usually trained to emit responses which will lead to drug self administration by the intravenous [7] or oral [8] routes. There is evidence suggesting that route of administration may not be a critical factor in determining whether a particular drug exerts reinforcing or aversive effects as several studies have reported that both reinforcing and aversive effects of the same drug administration may occur [13, 19, 20]. However, few studies have investigated the importance of route of drug administration in the conditioning of taste aversions [4].

The purpose of the experiments reported here was to investigate whether the consumption of solutions of d-amphetamine sulfate would lead to the development of aversions to an associated taste. It has previously been found [17] that rats will reject solutions of amphetamine when they are presented regularly and Carey [3] has shown that the degree of aversion to such solutions will increase with repeated presentations. It is difficult, however to directly compare these studies with experiments which have produced conditioned taste aversions with the drug given by injection, for several reasons. In previous studies of oral amphetamine consumption it has not always been clear to what extent aversions have been due to the noxious tastes of the drug solutions rather than to conditioned effects produced by the formation of associations between the taste of the solution and drug effects, and interpretations are also complicated by adipsia and anorexia produced by the drug. The present experiments demonstrate that it is possible to produce learned taste aversions in rats with orally consumed d-amphetamine using a procedure very similar to that used in studies involving drug injections. Similar effects have been reported previously with ethanol [6]. The results of these experiments also show that a taste aversion developed to a

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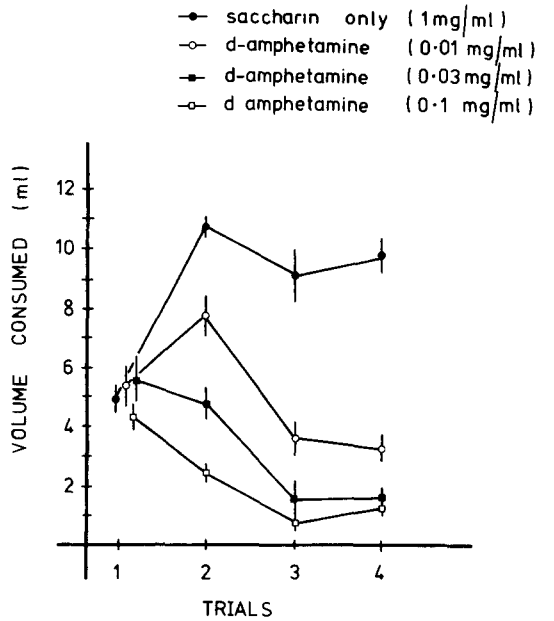

FIG. 1. Mean volumes of fluid consumed by rats which were presented with solutions containing saccharin at 1 mg/ml and different concentrations of d-amphetamine. Trials were 5 min in duration and were given on alternate days.

particular concentration of saccharin will generalise to a wide range of concentrations of this substance but that the extent of this generalisation depends upon the saccharin concentration used during conditioning.

EXPERIMENT 1

METHOD

Animals

Thirty-two experimentally naive, female Wistar rats with an average body weight of 350 g were used. The animals were individually housed with food freely available and water available as described in the Procedure.

Procedure

The rats were adapted to a regime of water availability which consisted of the presentation of water bottles for 5 min each morning (at approximately 11.00 a.m.) and for 30 min each afternoon (at approximately 4.00 p.m.). The short morning trial durations were used to ensure that when amphetamine was presented during these trials its consumption would not produce adipsia. Several days were allowed for the rats to adapt to this procedure and to establish stable rates of drinking before the experiment was begun. The animals were divided into four groups so that the average volume of water consumed during the morning drinking trials was similar in each group. Then on the first experimental day all animals were presented during the morning drinking period with a solution of sodium saccharin in water at a concentration of 1 mg/ml. In three of the groups the solutions also contained d-amphetamine sulfate at concentrations of 0.01, 0.03 and 0.1 mg/ml. The presentation of these solutions was repeated on alternate days for four trials. On intervening days and during afternoon drinking periods water was always presented to all animals.

TABLE 1

MEAN DOSES OF D-AMPHETAMINE CONSUMED DURING THE FOUR CONDITIONING TRIALS

Group	Concentration of d-amphetamine mg/ml	Mean dose of d-amphetamine mg/kg trials 1	2	3	4
1	0	—	—	—	—
2	0.1	1.5	0.7	0.3	0.3
3	0.03	0.5	0.4	0.2	0.2
4	0.01	0.1	0.2	0.1	0.1

values are rounded to the nearest 0.1 mg/kg.

RESULTS AND DISCUSSION

The mean volumes of solutions consumed by the four groups of animals over the four trials are presented in Fig. 1. This figure shows that comsumption of saccharin in the control group increased greatly on the second trial and remained at this higher level on subsequent trials. Such an effect is commonly observed in taste aversion experiments and is presumably due to the novelty of the saccharin solution on the first trial (neophobia). The animals which consumed the two higher concentrations of d-amphetamine showed decreases in the volumes of solutions consumed over the course of the experiment while the animals with the lowest d-amphetamine concentration showed an increase between the first and second trials which was followed by a decrease in volumes consumed. Statistical analysis using a repeated measures analysis of variance showed that the effect of amphetamine concentration was significant ($p<0.01$) as were the effects of trials and the interaction ($p<0.01$ in both cases).

These data show that learned taste aversions developed to solutions of saccharin and d-amphetamine. The average doses of d-amphetamine consumed by the rats are shown in Table 1. This table shows that the doses taken on the first trial were similar to doses which have been shown to produce taste aversions when given by intraperitoneal injection [1,2]. It should be noted, however, that in the present study the doses of drug taken during each trial necessarily declined across trials as the aversion developed and smaller volumes were consumed.

EXPERIMENT 2

There has been much discussion as to the extent to which taste aversion learning is governed by the same principles of learning which have been shown to operate in other situations. It has been suggested that taste aversion learning may be fundamentally different from other forms of learning [15] although recent reviews have pointed out that a close comparison between taste aversion and other forms of conditioning reveals that similar principles may be operating [9, 14, 16].

It is well known that when a conditioned stimulus has come to exert an effect on behavior that effect generalises to

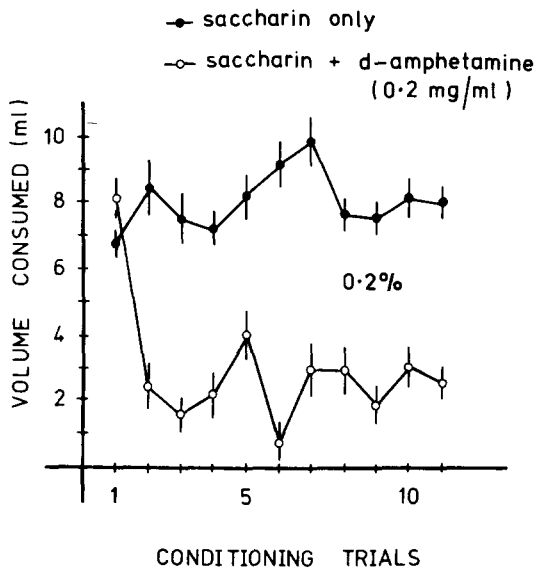

FIG. 2. Mean volumes of fluid consumed by rats presented with a solution containing either saccharin (2 mg/ml) or both saccharin (2 mg/ml) and d-amphetamine (0.2 mg/ml).

other stimuli which are similar to the conditioned stimulus, and that the extent of this generalisation depends upon the similarity between the stimuli. Although there have been many studies concerned with the nature of such generalisation gradients using visual and auditory stimuli [10] relatively little research has investigated inter- and intra-chemical generalisation of taste aversions [5, 11, 12, 18]. The present experiment, therefore, studied the generalisation of a saccharin taste aversion produced by orally consumed d-amphetamine to concentrations of saccharin other than that used for conditioning.

METHOD

Animals

Twenty experimentally naive male Wistar rats with an average body weight of 390 g were used. Housing conditions were as described for Experiment 1.

Procedure

The general procedure was as described for Experiment 1. After adaptation to the watering procedure the rats were divided into two groups and all animals were given a solution of sodium saccharin (2 mg/ml) during the 5 min morning drinking period. This initial presentation of saccharin was used to eliminate the complication of neophobia as was seen to occur in Experiment 1. Two days later and on alternate days throughout the experiment the animals in the control group continued to receive a solution of saccharin at this concentration while the members of the experimental group received the conditioning solution which contained both saccharin (2 mg/ml) and d-amphetamine sulfate (0.2 mg/ml).

For the first two morning drinking periods intervening between conditioning days water was presented to all animals. On subsequent intervening days the rats in both groups were presented with saccharin solutions at concentrations other than that used on conditioning days. These solutions used for testing generalisation did not contain amphetamine. The concentrations of saccharin used and the order in which they were presented were 1.0, 5.0, 10, 20, 0.1, 0.5, 0.75 and 0.625 mg/ml. On the last day of the experiment, which followed a conditioning day, all rats were given a solution containing d-amphetamine sulfate at a concentration of 0.2 mg/ml but without saccharin. Water was presented during the afternoon drinking periods throughout the experiment.

RESULTS AND DISCUSSION

The volumes of saccharin solution and saccharin and amphetamine solution consumed on conditioning trials are shown in Fig. 2. As in Experiment 1 the rats receiving the drug solution developed a strong aversion to this solution which was maintained for 11 conditioning trials even though after the first trial the volumes of solution, and thus the doses of amphetamine, consumed were relatively small. A repeated measures analysis of variance showed significant effects of trials and groups and also a significant interaction ($p<0.01$ in all cases).

The volumes of the different concentration saccharin solutions consumed by the experimental and control animals are presented in Table 2. It can be seen from this table that the control animals consumed similar quantities of all but the highest concentration of saccharin (20 mg/ml) where there was a decline in consumption. The taste aversion, as indicated by a significant difference in volumes consumed between the experimental and control groups, generalised to the concentrations of saccharin higher than the conditioning concentration and also to lower concentrations down to 0.625 mg/ml. These data were first analysed using a repeated measures analysis of variance which showed significant across trials and between groups effects and also a significant interaction ($p<0.01$ in all cases). The results of comparisons between the experimental and control groups, using the Newman-Keuls test, at each concentration are shown in Table 2.

On the last day of the experiment the volumes of d-amphetamine solution consumed were 8.5 ml for the con-

TABLE 2

MEAN (± SEM) VOLUMES OF FLUID CONSUMED AT DIFFERENT SACCHARIN CONCENTRATIONS

Group	Concentration of saccharin mg/ml							
	0.1	0.5	0.625	0.75	1.0	5.0	10	20
Control	7.1 ± 0.3	6.9 ± 0.3	8.7 ± 0.3	7.9 ± 0.2	8.9 ± 0.3	8.2 ± 0.4	8.5 ± 0.3	4.9 ± 0.3
Exptl	7.0 ± 0.7	6.5 ± 1.1	4.6 ± 0.6 *	2.0 ± 0.5 *	3.0 ± 0.6 *	2.1 ± 0.4 *	1.9 ± 0.3 *	1.8 ± 0.2 *

*$p<0.01$ difference between groups.
Conditioning concentration was 2 mg/ml saccharin + 0.2 mg/ml d-amphetamine.

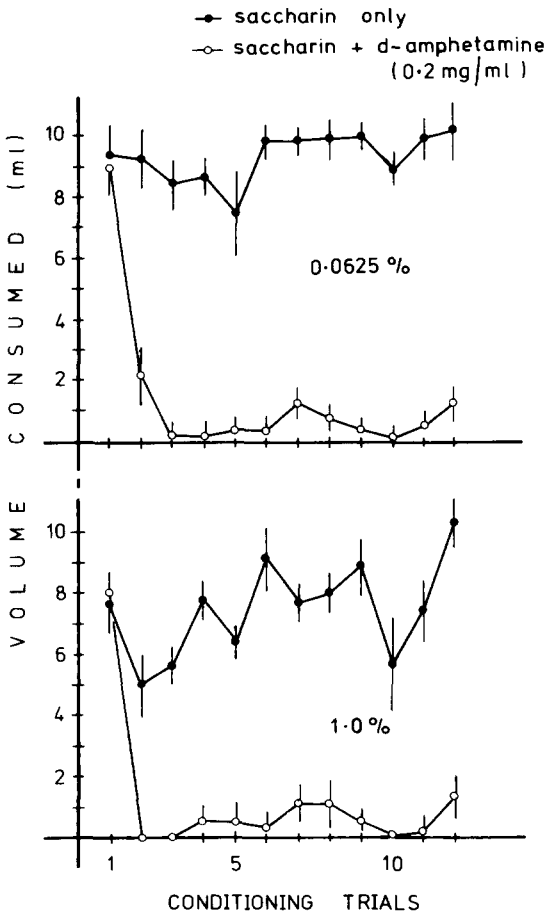

FIG. 3. Mean volumes of fluid consumed by rats presented with either saccharin or saccharin and d-amphetamine. The saccharin concentration was either 0.625 mg/ml or 10 mg/ml. In both cases the amphetamine concentration was 0.2 mg/ml.

trol group and 7.7 ml for the experimental group. The difference between these values was not statistically significant indicating that an aversion had not developed to the taste of amphetamine itself in the experimental animals.

EXPERIMENT 3

The results of Experiment 2 demonstrated that a taste aversion conditioned to a solution of d-amphetamine and saccharin at 2 mg/ml generalised across a wide range of concentrations of saccharin from 0.625 mg/ml to 20 mg/ml where the solution began to exert unconditioned aversive effects as indicated by the reduction in consumption in the control animals. A possible explanation for this result is that the aversion generalised to all saccharin solutions in which the taste of saccharin could be detected by the rats. In order to test this possibility a further experiment was carried out in which amphetamine-induced taste aversions were conditioned to either a high or a low concentration solution of saccharin in separate groups of rats. Generalisation to a range of concentrations of saccharin was then tested in both groups.

METHOD

Animals

Twenty, experimentally naive, female Wistar rats with average body weight of 250 g were used. Housing was as described previously.

Procedure

The procedure was similar to that described for Experiment 2. After adaptation to the watering regime the animals were divided into four groups of 5 rats each. For Groups 1 and 2 a solution of sodium saccharin at 0.625 mg/ml was presented during the 5 min drinking periods on alternate days and for Group 2 the solution also contained d-amphetamine sulfate at 0.2 mg/ml. For Groups 3 and 4 the saccharin concentration was 10 mg/ml and for Group 4 the solution also contained amphetamine at 0.2 mg/ml.

As in Experiment 2 a range of concentrations of saccharin was presented on days intervening between conditioning days. For Groups 1 and 2 the saccharin concentrations and the order of presentation were 2.0, 10, 0.1, 0.05, 0.01, 0.25, 0.025, 0.75, 0.625 and 0.075 mg/ml and for Groups 3 and 4 they were 0.625, 5.0, 2.0, 7.5, 0.1, 15, 0.3, 20 and 10 mg/ml. All animals were presented with a solution of d-amphetamine sulfate at a concentration of 0.2 mg/ml without saccharin on the last day of the experiment. Throughout the experiment water was presented during the afternoon drinking periods.

RESULTS AND DISCUSSION

The volumes of saccharin solution consumed during conditioning trials are presented in Fig. 3. It can be seen from this figure that strong aversions developed with both the high and the low saccharin concentrations. In both cases repeated measures analyses of variance showed significant between groups effects ($p<0.01$), significant across trials effects ($p<0.01$) and significant interactions ($p<0.01$).

The volumes of the diffferent concentration saccharin solutions consumed are presented in Tables 3 and 4. These data were first analysed using repeated measures analyses of variance which showed significant effects between groups and across trials and also significant interactions (all $p<0.01$). With both conditioning concentrations of saccharin the taste aversion generalised to all higher saccharin concentrations as indicated by the significant differences between the experimental and control groups with Newman-Keuls tests. When 0.625 mg/ml was the conditioning concentration the aversion generalised to lower concentrations of saccharin down to 0.075 mg/ml. There was no significant aversion, however, to concentrations of 0.01, 0.025 and 0.05 mg/ml. When the conditioning concentration was 10 mg/ml the aversion generalised to 7.5 and 2 mg/ml but not 0.1, 0.3 and 0.625 mg/ml. At 5 mg/ml the difference between the experimental and control group did not reach an acceptable level of statistical significance but there was also no statistically significant difference between the volume of this concentration solution consumed by the experimental group and the volume of the 10 mg/ml solution consumed by these animals, indicating that the aversion probably did generalise to this 5 mg/ml concentration. Had greater numbers than five animals been used in each group a statistically significant difference between the groups would probably have become apparent.

When the amphetamine solution (0.2 mg/ml) was presented without saccharin at the end of the experiment the mean volumes consumed were 7.6 ml for Group 1, 7.4 ml for

TABLE 3

MEAN (± SEM) VOLUMES OF FLUID CONSUMED AT DIFFERENT SACCHARIN CONCENTRATIONS

Group	Concentration of saccharin mg/ml									
	0.01	0.025	0.05	0.075	0.1	0.025	0.625	0.75	2.0	10
Control	9.6 ± 0.4	9.2 ± 1.5	9.2 ± 0.5	7.4 ± 0.4	7.4 ± 1.3	8.2 ± 0.4	8.8 ± 0.5	10.8 ± 0.4	9.2 ± 0.2	7.0 ± 0.8
Exptl	8.4 ± 0.9	10 ± 0.7	7.4 ± 0.7	2.0 ± 0.3 *	2.4 ± 0.9 *	3.0 ± 0.8 *	2.2 ± 0.5 *	4.0 ± 0.6 *	1.2 ± 0.2 *	1.6 ± 0.4 *

*$p<0.01$ difference between groups.
Conditioning concentration was 0.625 mg/ml saccharin + 0.2 mg/ml d-amphetamine.

TABLE 4

MEAN (± SEM) VOLUMES OF FLUID CONSUMED AT DIFFERENT SACCHARIN CONCENTRATIONS

Group	Concentration of Saccharin mg/ml								
	0.1	0.3	0.625	2.0	5.0	7.5	10	15	20
Control	7.2 ± 0.4	10.8 ± 0.3	7.4 ± 0.4	8.6 ± 1.2	8.0 ± 1.5	8.0 ± 0.6	8.4 ± 0.9	6.4 ± 1.1	8.4 ± 0.7
Exptl	8.6 ± 0.7	9.8 ± 0.8	5.4 ± 0.6	3.2 ± 0.4 †	4.2 ± 1.2	2.0 ± 0.3 †	2.8 ± 0.2 †	1.4 ± 0.4 †	0.8 ± 0.2 *

*$p<0.01$; †$p<0.05$ difference between groups.
Conditioning concentration was 10 mg/ml saccharin + 0.2 mg/ml d-amphetamine.

Group 2, 7.0 ml for Group 3 and 7.0 ml for Group 4. Thus, as in Experiment 2, the taste aversion did not generalise to the taste of the drug solution alone.

GENERAL DISCUSSION

As was pointed out earlier, one of the major differences between drug self-administration procedures and gustatory avoidance procedures is the route of drug administration. Previous studies have indicated, however, that the route of administration is not a critical factor in determining whether drugs will exert rewarding or aversive actions [4]. The present study confirms and extends previous work by demonstrating that d-amphetamine taken by the oral route can produce an aversion to saccharin when presented in the same solution. The fact that aversions were not shown to the taste of a solution containing amphetamine alone demonstrated that the saccharin had effectively masked the taste of amphetamine and that the effects observed were not due to any noxious taste of amphetamine.

The degree of aversion observed in the rats in Experiment 1 was a function of the concentration of amphetamine in the drinking fluid, higher concentrations producing stronger aversions. This result is consistent with a number of studies which have shown dose-related taste aversions conditioned with injections of amphetamine [1,2]. It is of some interest to note that the doses of drug consumed in the present experiment were similar to doses found effective in other studies when given by intraperitoneal injection. This may be contrasted to results reported by Eckardt [6] which indicated that ethanol was considerably less potent in producing learned taste aversions when taken orally than when given by injection. Average doses of amphetamine as low as 0.1 mg/kg produced clear taste aversions in the present study. Of course the doses taken depended upon the volumes of fluid consumed which varied between animals and across trials and thus could not be precisely controlled. Furthermore, as the taste aversion developed smaller volumes of fluid were consumed and thus lower doses of the drug were taken. Nevertheless, the data show that strong and reliable aversions were maintained throughout each of the three experiments.

Experiment 2 demonstrated that an aversion conditioned to a saccharin concentration of 2 mg/ml would generalise to all higher concentrations of saccharin presented to the animals (up to 20 mg/ml) and also to concentrations down to 0.625 mg/ml but not to lower concentrations. This result is consistent with previous studies of stimulus generalisation in learned taste aversion [5, 11, 12, 18] and indicates a similarity between such learning and other forms of learning [10]. The possibility arose, however, that a generalised aversion had developed to all concentrations of saccharin which the rats could detect. This explanation was tested in Experiment 3 in which generalisation gradients to different saccharin concentrations were obtained after conditioning with either a high or a low concentration. The results of this experiment showed that the concentrations to which the aversion generalised depended upon the concentration used in conditioning. Thus when a concentration of 0.625 mg/ml was used on conditioning trials the aversion generalised to concentrations as low as 0.075 mg/ml. However, when 10 mg/ml was used for conditioning the aversion did not generalise to concentrations lower than 2 mg/ml. In both cases, though, the aversion generalised to all concentrations of saccharin presented which were higher than the concentration used for conditioning.

Several conclusions may usefully be drawn from the results of the present study. It is clear that orally consumed amphetamine can produce strong taste aversions to saccharin in rats and the extent of the aversion depends upon the dose of drug consumed. Also, the doses shown to be effective in these studies were similar to doses found effective in

producing learned taste aversions when given by injection. Finally, it was found that generalisation gradients relating the concentration of saccharin to the degree of aversion can be plotted and the extent of the generalisation depends upon the concentration of saccharin used in conditioning. Since similar effects are observed in studies of stimulus generalisation using other stimulus modalities and different conditioning techniques, the present results are consistent with the view that taste aversion learning is governed by similar principles as are other forms of learning [9, 14, 16].

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