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Alcohol Dependence and Taste-Mediated Learning in the Rat[1]

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CRAWFORD, D. AND T. B. BAKER. *Alcohol dependence and taste-mediated learning in the rat.* PHARMAC. BIOCHEM. BEHAV. **16**(2) 253–261, 1982.—Alcohol dependence and taste-mediated learning were investigated in the rat using an intubation procedure to produce dependence, an experimenter-controlled conditioned stimulus (CS) flavor infusion procedure, and behavioral criteria to assign conditioning alcohol dosage. Stress produced by the experimental procedures (e.g., handling, stomach distention) produced flavor aversions, and these aversions were attenuated by small alcohol doses. When stress was reduced and made less associable with the flavor CS, alcohol dependence, by itself, did not protect against the development of alcohol-induced taste aversions. Alcohol withdrawal produced aversions for an associated flavor, but these aversions were attenuated by pairing the flavor with small doses of alcohol. Thus, the relief of alcohol withdrawal illness did not produce preferences for associated flavors, but it did protect against the development of taste aversions.

Alcohol Alcohol dependence Taste aversion Taste preference

IN attempting to develop an animal model of human alcoholism, researchers have been thwarted by animals' unwillingness to consume large alcohol quantities. A number of procedures have been developed to increase animals' alcohol consumption, including schedule induction [18], habituation to alcohol solutions [35], and intubation with large alcohol doses for several days [14,16]. However, even with these specialized procedures it has been difficult to obtain levels of free-choice, oral alcohol consumption sufficient to produce physical dependence, a hallmark of human alcoholism. Therefore, researchers have sought other methods to produce increased alcohol consumption. One such method is medicine effect, or taste preference, conditioning.

Medicine effect conditioning refers to development of preferences for flavors paired with recuperation from illness. Rats learn preferences for flavors paired with recovery from thiamine deficiency [34] and apomorphine [21] illnesses.

Human alcoholics drink in a fashion that might produce preferences for alcohol through medicine effect learning [24, 29, 30]. With repeated opportunities to relieve alcohol withdrawal illness by consuming more alcohol, these pairings of the flavor of alcohol with the relief of alcohol withdrawal provide an appropriate paradigm for medicine effect conditioning. This conditioning process would not explain development of initial, heavy drinking required to institute dependence, but it could account for heavy drinking in previously dependent persons.

The evidence from animal studies concerning medicine effect learning is mixed. Although most researchers [4,32] have found alcohol dependence per se does not affect subsequent preferences for alcohol, Deutsch and Walton [16] found that dependence induced a preference for flavors paired with the alcohol administration. Such results are not necessarily due to medicine effect conditioning, as they could be due to enhanced alcohol tolerance developed during dependence induction, or due to unconditioned stimulus preexposure effects [5,12].

Pairing a flavor with the relief of alcohol withdrawal has yielded inconsistent results. Marfaing-Jallat and Le Magnen [29] induced dependence with a liquid diet procedure [19]. They found little evidence of conditioned taste preferences, but their results did indicate that rats acquired aversions for flavors presented while rats exhibited withdrawal symptoms and that pairing a small alcohol dose (1.5 g/kg) with the flavor reduced both the withdrawal symptoms and aversion learning.

In a second study of medicine effect learning, Le Magnen *et al.* [26] gave rats exhibiting withdrawal symptoms a small alcohol dose (2 g/kg) paired with saccharin consumption after two, four, or eight days of alcohol administration. A subsequent saccharin drinking test revealed a significant, positive linear relationship between the number of days of alcohol administration and the percentage change in saccharin consumption. Le Magnen *et al.* [26] suggested these results were due to medicine effect conditioning; i.e., the rats receiving alcohol the longest (8 days) had the most severe withdrawal illnesses and thus, the 2 g/kg alcohol dose resulted in the greatest reduction in withdrawal symptoms and the greatest saccharin preferences. However, their data show differences in saccharin intake among groups at the conditioning session, presumably due to adipsia among the rats most severely withdrawn from alcohol. Furthermore, mean saccharin intake at the post-test was not linearly related to the number of days of infusion with alcohol. Thus,

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these results provide little evidence of medicine effect learning.

In the present series of experiments we investigated both medicine effect and taste aversion conditioning involving alcohol. Our procedures differed from previous research in this area in four major respects. First, we administered alcohol by gavage. While criticisms have been raised that this method is inadequate to control blood alcohol level [33] and is artificial in the sense that animals do not self-administer alcohol doses, it does afford a high degree of control over the level and temporal characteristics of alcohol doses [31]. Thus, we had considerable control over when rats entered withdrawal and how much alcohol they received prior to withdrawal. Furthermore, intubation results in an extremely high rate of alcohol dependence with appropriate dosing [2,28]. Second, because Marfaing-Jallat and Le Magnen's [29] and Le Magnen *et al.*'s [26] failures to find strong evidence of medicine effect conditioning may be due to their use of a single conditioning trial, we used multiple conditioning trials. Third, because we wanted to achieve a high degree of control over the presentation of the CS, and because withdrawing animals are often adipsic [22], we used a passive flavor exposure technique to administer the conditioned stimulus (CS). Fourth, we determined conditioning alcohol doses (the unconditioned stimuli, UCS's) according to behavioral criteria of intoxication and withdrawal. Thus, animals with severe withdrawal symptoms received more alcohol than rats with mild symptoms. We feel this method affords a more sensitive determination of appropriate alcohol dosages than methods used in previous research.

We conducted a preliminary experiment that is relevant to the interpretation of Experiments 1a, 1b and 2. This preliminary study will be described only briefly because it produced only a few meaningful results which are important merely because they set the stage for the later experiments.

The preliminary study was designed to determine if rats acquire preferences for flavors paired with a decrease in alcohol withdrawal symptoms. In this study, we used the procedures listed above; delivery of alcohol and nutrients via gavage, behavioral criteria for administration of alcohol, passive exposure to the saccharin CS, etc. In this study, we found that animals appeared to develop preferences for saccharin when it was paired with small doses of alcohol administered when animals displayed withdrawal symptoms. However, two anomalous results comprised interpretation of that study. First, rats that had large, dependence-producing doses of alcohol paired with saccharin failed to show saccharin aversions in subsequent saccharin preference tests. Second, nondependent rats that merely received saccharin exposure but no alcohol appeared to have acquired saccharin aversions. These findings cast doubt on the adequacy of the procedures used in this first study. Therefore, we conducted Experiments 1a and 1b in order to identify the cause of the anomalous findings.

Our first objective was to explain the lack of saccharin aversions among rats receiving large, dependence-producing doses of alcohol (1.5–6.0 g/kg) paired with saccharin. We felt that this result could not have been due to the inadequacy of the flavor (CS) exposure procedure. Our pilot research had shown this procedure to produce rapid taste aversion learning to a variety of flavor CS's. Since the flavor exposure procedure appeared adequate we speculated that animals might not have acquired saccharin aversions in the preliminary study because alcohol dependence may have blocked the development of aversions to alcohol-paired flavors. This finding is precedented as Deutsch and Walton [16] found that rats made dependent on alcohol failed to learn aversions to alcohol-paired flavors. Baker and Cannon [1] and Hunter *et al.* [23] also reported an absence of aversions for flavors paired with dependence-producing amounts of alcohol.

EXPERIMENT 1a

Alcohol dependence could attenuate aversions to alcohol-paired flavors for two reasons. First, alcohol doses may relieve withdrawal symptoms in dependent animals and this may make alcohol doses less aversive. Second, the temporal pattern of alcohol dosings may interfere with aversion learning in dependent animals. The frequent alcohol dosings necessary to produce dependence may result in a continual state of malaise in animals, and this may degrade the flavor-alcohol contingency. This notion is consistent with basic research showing that magnitude of aversion learning is positively correlated with the length of the intertrial interval [17].

In Experiment 1a we examined the separate effects of alcohol dependence and frequency of alcohol dosings on aversion learning with an alcohol UCS. Thus, one group of rats was made dependent with frequent large doses of alcohol paired with saccharin. Two groups of nondependent rats received the same total amount of alcohol as one another, however, one group received saccharin-alcohol pairings at frequent intervals, and the other at spaced intervals. The fourth group was a nondependent control group (cf. Table 1).

METHOD

Subjects

Thirty-six naive male Holtzman rats weighing 215–245 g were assigned randomly to four groups (n=9 each). Triads of rats in Groups 1, 3 and 4 were yoked on the basis of weight.

Apparatus and Materials

Rats were housed individually in standard wire mesh cages. Except as noted, they were given free access to lab chow (Wayne Lab-Blox, Allied Mills, Inc.) and water.

An ethanol solution (30%, v/v) was prepared from 95% ethanol and tap-water. A liquid diet solution was prepared by supplementing a nutritionally complete liquid diet (Sustacal, Mead Johnson and Co.) with a vitamin solution (Homicebrin, Lilly and Co., 2 ml/100 ml). A sucrose solution was prepared that was isocaloric to the ethanol solution.

Fluids were administered by gavage using Cutter-Resiflex infant feeding tubes (size 8FK, 38 cm long) attached to syringes. Rats' mouths were perfused with the end of a feeding tube attached to a 10 cm^3 syringe. A saccharin solution was prepared by dissolving saccharin in tapwater (2 g/l).

Procedure

Preconditioning phase (Days 1–7). To habituate rats to the intubation procedure, sham intubations were performed twice daily. All animals were allowed access to fluids for only 20 min/day and were given the liquid diet and tapwater at these times. Rats were allowed to drink the liquid diet in order to hinder the subsequent development of associations between the flavor of the diet and the effects of alcohol [7].

First conditioning phase (Days 8–11). Beginning at 1600

TABLE 1
REGIMENS FOR EXPERIMENT 1a AND 1b

Group	Sessions 1, 4 and 7	All remaining sessions
1	Dependence: Saccharin + large alcohol dose	Dependence: Saccharin + large alcohol dose
2	Continual malaise: Saccharin + small alcohol dose	Continual malaise: Saccharin + small alcohol dose
3	Aversion learning: Saccharin + large alcohol dose	Control: Water + sucrose dose
4	Control: Saccharin + sucrose dose	Control: Saccharin + sucrose dose

hr, nine conditioning sessions occurred at 8-hr intervals. Lab chow and water were not available during this phase of the experiment.

All animals were weighed at the beginning of each conditioning session. They were then fed the liquid diet by gavage according to weight loss criteria. All rats received 20 ml at the first session. Thereafter, volume of diet administered was determined by an animal's weight loss since the first session. Rats losing 5 g or less received 10 ml of liquid diet, those losing 6–10 g received 15 ml, and those losing more than 10 g received 20 ml. Approximately 1 hr after feeding, rats' mouths were perfused with either tapwater or saccharin solution using the passive exposure procedure. Rats received 2 ml of tapwater or saccharin over a 2 min period. Immediately afterwards rats were intubated either with ethanol or sucrose.

In Sessions 1, 4, and 7 rats in Group 1 received a 4 g/kg alcohol dose. At all other conditioning sessions their doses were determined by behavioral criteria of intoxication. Animals which were ambulatory with little or no ataxia and which had immediate righting reflexes received 4 g/kg; rats able to stand but ataxic, with delayed righting reflexes, received 3 g/kg; rats which were conscious but unable to stand received 1.5 g/kg; and animals which did not respond to a tail pinch received no alcohol. The maximum alcohol dose was 4 g/kg to ensure that animals in Group 1 received no single dose larger than those given to rats in Group 3, since larger alcohol doses produce greater aversions [13]. Rats in Group 1 were exposed to the saccharin flavor before every alcohol dose. Rats in Group 2 were also exposed to the saccharin flavor at every conditioning session. They were then intubated with a 1.33 g/kg alcohol dose. In Sessions 1, 4 and 7 animals in Group 3 were exposed to the saccharin flavor and then given a 4 g/kg alcohol dose. In the six remaining sessions they were exposed to tapwater and intubated with the sucrose solution; their sucrose doses were equivolume and isocaloric with the alcohol doses of their yoked partners in Group 1. Rats in Group 4 received sucrose doses paired with the saccharin flavor at every conditioning session. Their sucrose doses were also equivolume and isocaloric with the alcohol doses of their yoked partners in Group 1.

First assessment phase (Day 11). Behavioral ratings of ethanol withdrawal were conducted by two independent observers, blind to group membership, using criteria developed by Hunter *et al.* [22]. These criteria included extensor rigidity, hyperreflexia, and tremulousness, which can be scored reliably by independent observers [1]. Seizures were not induced because they can be fatal. All animals in Group 1 and a randomly selected sample of those in Groups 2–4 were observed. Ratings occurred 8 hr after the last conditioning session.

Second conditioning phase (Days 31–34). Procedures during this phase of the experiment were identical to those used during the first conditioning phase, except in Session 1 the first alcohol dose for rats in Group 1 was increased from 4 g/kg to 6 g/kg. This change was made because not all rats in Group 1 showed withdrawal signs following the first conditioning phase.

Second assessment phase (Days 34–40). Behavioral ratings of alcohol withdrawal were conducted on Day 34, 8 hr after the last conditioning session, using the same procedure employed in the first assessment phase. A series of three two-bottle preference tests, using tapwater and the saccharin solution, was conducted at 24-hr intervals on Days 37–39. Animals were allowed access to the two solutions for 20 min; bottle positions were reversed after 10 min. A one-bottle test, during which rats were allowed to drink the saccharin solution for 20 min, was conducted on Day 40, 24 hr after the last two-bottle test. Animals were deprived of fluid for 24 hr preceding the first test and between tests.

RESULTS AND DISCUSSION

Because three animals in Group 1 died, all analyses were computed using unweighted means corrections.

Figure 1 illustrates mean intake of the saccharin solution for all groups during Tests 1–3, the two-bottle tests, and Test 4, the one-bottle test. A 4 (group) × 3 (test day) mixed analysis of variance [36] for Tests 1–3 showed a significant main effect of group, $F(3,29)=4.95$, $p<0.05$. An a posteriori test of simple main effects [36] indicated that the groups differed significantly only at Test 3 ($p<0.05$); a subsequent Newman-Keuls test [36] showed that rats in Group 2 consumed significantly more of the saccharin solution at this test than rats in all other group ($p<0.05$) and that there were no other significant differences among groups.

A one-way analysis of variance for Test 4 showed significant differences in saccharin consumption, $F(3,29)=4.48$, $p<0.05$. A Newman-Keuls a posteriori test indicated that rats in Group 2 again consumed more of the saccharin solution than those in Groups 1, 3, and 4 ($p<0.05$) and that there were no other significant differences among groups.

The alcohol administration data showed that Group 1 received 11.6 and 9.2 g/kg/day alcohol doses for the first and second dependence induction cycles respectively. Of course, Groups 2 and 3 received 4 g/kg/day/cycle.

The kappa coefficient [3] for interobserver reliability of ratings of alcohol dependence was 1.00. Rats in Group 1, made dependent on alcohol, showed an average of 1.70 signs of withdrawal. For rats in Groups 2 and 3, which received alcohol but were not made dependent, the mean number of withdrawal signs was 0.10; rats in Group 4, which did not receive alcohol, showed an average of 0.30 signs of withdrawal.

Experiment 1a was designed to test two explanations of the failure of dependent rats in our preliminary study to de-

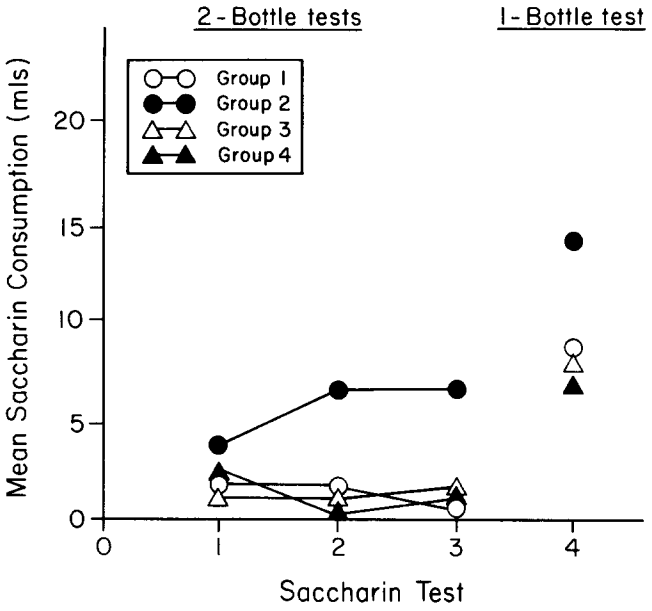

FIG. 1. Mean saccharin consumption by Groups 1–4 during the two-bottle Saccharin Tests 1–3 and during the one-bottle Saccharin Test 4 following the second conditioning phase.

velop aversions to saccharin, relative to nondependent controls. We proposed that the lack of aversion learning might occur because dependence and/or intrinsic withdrawal-relief attenuates the development of aversions [16]. Alternatively, the temporal pattern of alcohol dosings used to produce dependence might produce a continual state of malaise, and this might attenuate the development of aversions due to a reduced correlation between the CS and US.

The results of Experiment 1a do not provide strong support for either of these hypotheses. Neither frequent alcohol dosings, nor alcohol dependence appeared to reduce saccharin aversions among Group 1 animals. However, there was a striking similarity between Experiment 1a and the preliminary study: Animals given saccharin paired with small doses of alcohol (e.g., Group 2) consumed more saccharin than control animals given saccharin exposure but no alcohol (e.g., Group 4). In Experiment 1a this relationship held even though Group 2 animals were not alcohol-dependent. Thus, their increased saccharin intake cannot be explained by the relief of withdrawal symptoms.

Nondependent control rats in Experiment 1a and in the preliminary research had strong saccharing aversions (cf. Fig. 1). We hypothesized that these aversions occurred because rats associated saccharin with stress induced by handling and fluid administrations (e.g., gastric distention, see [15]). We also hypothesized that the relative preferences for saccharin shown by animals having small doses of alcohol paired with saccharin (e.g., Group 2, Fig. 1) was due to stress reduction by the small alcohol doses. Thus, although alcohol may not have produced learned taste preferences through medicine effect conditioning, it may have been rewarding because it ameliorated stress induced by handling and sundry experimental procedures.

EXPERIMENT 1b

Experiment 1b differed from Experiment 1a only in the flavor, alcohol and liquid diet administration procedures. The saccharin-alcohol contingencies were unchanged (see Table 1).

The results of Experiment 1a suggested that stress caused by the experimental procedures produced saccharin aversions in the nondependent controls in Experiment 1a and in the preliminary study. Therefore, in Experiment 1b procedures were modified in order to reduce both stress and the associability of stress with the saccharin flavor. Therefore, rats were habituated to fluid and diet administration procedures more extensively during the preconditioning phase. Animals were restrained for a briefer time during exposure to saccharin. Because the volume of liquid diet administered in a single dosing may have produced aversive abdominal distention in the previous studies, the maximum volume per dosing was reduced and the number of dosings per day increased. To reduce further the likelihood that administration of the liquid diet was associated with the saccharin flavor, all saccharin-intubation pairings were separated from feedings by a minimum of 1 hr. Thus, changes made in the experimental procedures were intended to reduce the level of stress or make it less associable with the CS.

METHOD

Subjects

Thirty-six naive male Holtzman rats weighing 290–360 g were assigned randomly to five groups (n=9 each). Triads of rats in Groups 1, 3 and 4 were yoked on the basis of weight.

Apparatus and Materials

All apparatus and materials were identical to those used in Experiment 1a.

Procedure

Preconditioning phase (Days 1–9). On Days 1–7 rats were allowed access to fluid for only 20 min/day and were given two water bottles at these times. This was intended to maintain rats in a state of fluid deprivation, thus rendering the perfusion procedure less aversive. On Days 1–9 rats' mouths were perfused with water twice daily; rats were restrained by hand and given 3 ml tapwater over approximately 1 min. To habituate rats to the intubation procedure, on Days 1–5 animals were given sham intubations twice daily; a tube was introduced orally into rats' stomachs, but no fluid was intubated. In addition, on Days 6–9 animals were intubated with 5 ml water twice daily.

To habituate rats to the liquid diet regimen, rats were maintained on the liquid diet for 2 days preceding the first conditioning phase. To reduce the aversiveness of this procedure and the likelihood that rats would form associations between its aversive aspects and the saccharin flavor during the conditioning phases, liquid diet doses were decreased from those used in previous studies; to ensure that animals received adequate nutrition, the number of feedings was increased from 3/day to 4/day. On Days 8–9 rats were weighed and then fed the liquid diet by gavage at 1000, 1400, 1800 and 2200 hr daily. At the first feeding all rats received 10 ml. Later doses were determined by an animal's weight loss since the first feeding. Rats losing more than 5 g received 10 ml; all others received 5 ml. Lab chow was not available on

these days. This feeding schedule was continued throughout the first conditioning phase.

First conditioning phase (Days 10–12). Beginning at 0800 hr, nine conditioning sessions occurred at 8-hr intervals. Procedures were identical to those used in the first conditioning phase of Experiment 1a (cf. Table 1) except for three changes. Animals in Group 1 received a 6 g/kg alcohol dose in Session 1, in order to more reliably produce alcohol dependence. The feeding schedule and dosage were altered, as described above. In addition, all feedings were separated from flavor-alcohol pairings by a minimum of 1 hr. This change was made to decrease the likelihood that aversive aspects of the feeding procedure would be associated with the saccharin flavor [15].

First assessment phase (Days 13–17). Behavioral ratings of alcohol withdrawal were conducted on Day 13, 8 hr after the last conditioning session, using the same procedure as in Experiment 1a. A two-bottle preference test, using tapwater and the saccharin solution, was conducted on Day 17. Animals were allowed to drink for 75 min, and bottle positions were reversed after 15 and 45 min. Animals were deprived of fluid for 24 hr preceding the test.

Second conditioning phase (Days 18–26). On Days 18–21 rats were allowed access to fluid for only 20 min/day and were given two water bottles at these times. On Days 22–23 animals were fed the liquid diet by gavage, using the same feeding schedule and dosage employed on Days 8–9. Beginning at 0800 hr on Day 24, nine conditioning sessions occurred at 8-hr intervals; procedures were identical to those used in the first conditioning phase.

Second assessment phase (Days 27–35). Behavioral ratings of alcohol withdrawal were conducted on Day 27, 8 hr after the last conditioning session, using the same procedure as in the first assessment phase. A two-bottle preference test, identical to that done on Day 17, was conducted on Day 32. A series of three one-bottle tests, using the saccharin solution, was conducted at 24-hr intervals on Days 33–35. Animals were allowed to drink the saccharin solution for 75 min at each test and were not allowed fluid at other times. One-bottle tests were instituted to discover whether Group 1 or 3 would show more rapid extinction of saccharin aversions.

RESULTS AND DISCUSSION

One animal in Group 1 died during the experiment. All analyses in which group sizes differed were computed using unweighted means corrections.

Figure 2 illustrates mean intake of the saccharin solution for all groups during Test 1, following the first conditioning phase, and Tests 2–5, following the second conditioning phase. A one-way analysis of variance showed significant differences in saccharin consumption during Test 1, $F(3,32)=65.48$, $p<0.05$. A Newman-Keuls a posteriori test indicated that rats in Group 4 consumed more of the saccharin solution than rats in all other group ($p<0.05$), that rats in Group 2 consumed more than those in Groups 1 and 3 ($p<0.05$), and that Groups 1 and 3 did not differ significantly.

A one-way analysis of variance of saccharin consumption during Test 2 also showed significant differences among groups, $F(3,31)=5.59$, $p<0.05$. A Newman-Keuls a posteriori test showed that rats in Groups 2 and 4 consumed significantly more than those in Groups 1 and 3 ($p<0.05$); there were no other significant differences.

A 4 (group) × 3 (test day) mixed analysis of variance of saccharin consumption during Tests 3–5 indicated a significant main effect of group, $F(3,31)=100.00$, $p<0.05$; a significant main effect of test day, $F(2,62)=3.26$, $p<0.05$; and a significant interaction, $F(6,62)=9.91$, $p<0.05$. An a posteriori test of simple main effects showed significant differences among groups at Tests 3, 4 and 5 ($ps<0.05$). Subsequent Newman-Keuls test showed that at Tests 3 and 4 Groups 3 and 1 differed significantly from Groups 2 and 4 ($ps<0.05$) and that there were no other significant differences at these tests. At Test 5, Group 1 differed significantly from all others, Group 3 was significantly different from Groups 2 and 4 ($ps<0.05$), and Groups 2 and 4 did not differ.

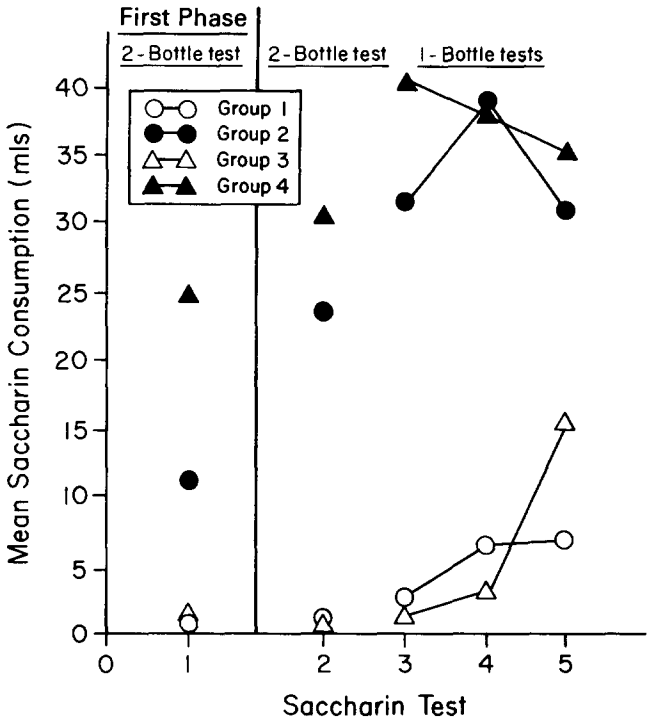

FIG. 2. Mean saccharin consumption by Groups 1–4 during the two-bottle Saccharin Test following the first conditioning phase, and during the two- and one-bottle Saccharin Tests 2–5 following the second conditioning phase.

One-way analyses of variance showed no significant differences in body weight before the first conditioning phase, at the first and last conditioning sessions of the first and second conditioning phases, and at the first and second preference tests (all $Fs<2.54$, $ps>0.05$). There were significant differences in body weight at Test 5, $F(3,31)=9.50$, $p<0.05$. Newman-Keuls a posteriori tests showed that Groups 2 and 4 were heavier than Groups 1 and 3.

The alcohol administration data revealed that Group1 rats were given 11.4 and 10.8 g/kg/day alcohol doses for the first and second dependence induction cycles. Groups 2 and 3, of course, received 4/kg/day/cycle.

As in Experiment 1a, the blind, independent observers achieved high reliability in rating withdrawal signs ($r_k>0.90$). Mean withdrawal ratings following the first conditioning phase were 3.33, 0.30, and 0.0 for Groups 1–3 respectively,

and 2.6, 0.40, and 0.30 for the same groups after the second conditioning phase.

In Experiment 1b, Group 4, the nondependent controls, consumed more saccharin than other groups. This suggests that the changes in the experimental procedure from Experiment 1a reduced the association between the CS and aversive aspects of the diet and flavor administrations, such as stress due to handling.

Group 1, which received saccharin-alcohol pairings and was made dependent on alcohol, did not show an attenuated aversion relative to Group 3, which received large doses of alcohol paired with saccharin but was not made dependent. This contradicts the hypothesis that alcohol dependence attenuates the development of aversions to alcohol-paired flavors. It appears that the dependent rats in the preliminary study and in Experiment 1a did not show attenuated aversions; instead, their failure to show aversions relative to nondependent controls probably reflects saccharin aversions in controls.

In general, the saccharin consumption of Groups 2 and 3 was quite consistent with basic principles of taste-mediated learning. Group 2 had the weakest aversion to saccharin; these animals received saccharin paired with a small dose of alcohol. Group 3 eventually consumed more saccharin than Group 1, and this was undoubtedly because Group 3 received fewer pairings of saccharin with the large alcohol dose. Thus, the results of Experiment 1b were very consistent with predictions derived from basic learning principles and did not appear to reflect the operation of dependence per se.

EXPERIMENT 2

In Experiment 2, as in the preliminary study, we again investigated whether rats learn taste preferences for a flavor paired with the relief of alcohol withdrawal illness. In order to reduce the influence of stress, we used the fluid and diet administration procedures developed in Experiment 1b. Two other changes were made in this study relative to the preliminary study. Rats were kept dependent for a longer period ot time in Experiment 2 before pairing saccharin with withdrawal-relief (small doses of alcohol). This was done to insure that rats would have severe withdrawal symptoms prior to withdrawal-relief. In addition, the number of possible flavor-alcohol pairings per withdrawal treatment session was increased from three to four.

METHOD

Subjects

Thirty-six naive male Holtzman rats weighing 225–285 g were assigned randomly to four groups (n=11 each). Pairs of rats in Groups 1 and 4 were yoked on the basis of weight.

Apparatus and Materials

All apparatus and materials were similar to those used in Experiments 1a and 1b, except the sucrose solution was replaced by tapwater and a 3.5 g/l saccharin solution was used. A more concentrated saccharin solution was used in order to decrease rats' natural preference for the flavor, thereby decreasing the likelihood that a ceiling effect might mask acquired taste preferences.

TABLE 2
REGIMENS FOR EXPERIMENT 2

Group	Dependence Induction (Sessions 1–6, 8–9, 11–12)	Withdrawal Treatment (Sessions 7,10)
1	Dependence: Large alcohol dose	Withdrawal relief: Saccharin + small alcohol dose
2	Dependence: Large alcohol dose	Alcohol control: Water + small alcohol dose
3	Dependence: Large alcohol dose	Saccharin control: Saccharin + untreated withdrawal
4	Nondependent control: Water dose	Nondependent control: Saccharin + water dose

Procedure

Preconditioning phase (Days 1–9). Procedures used during this phase of the experiment were similar to those used during the preconditioning phase of Experiment 1b, except animals were intubated with the liquid diet at 0900, 1300, 1700 and 2100 hr on Days 8–9. This change was made in order to maintain intervals of at least 1 hr between feedings and other procedures once the conditioning phase began. This feeding schedule was continued during the conditioning phase. Also, Group 2 rats were given exposure to the saccharin solution during the 20 min drinking periods on Days 1–7 to habituate any saccharin neophobia prior to the saccharin preference tests.

Conditioning phase (Days 10–13). Table 2 presents the experimental regimens for all groups. Beginning at 0800 hr, 12 alcohol administration sessions occurred at 8-hr intervals. Lab chow and water were not available during this phase of the experiment. During Sessions 1–6, 8–9, and 11–12, the dependence induction sessions, all rats except those in Group 4 were intubated with large alcohol doses to produce dependence; rats in Group 4 were intubated with water during the dependence induction sessions. Sessions 7 and 10 were withdrawal treatment sessions.

All animals were weighed at the beginning of each alcohol administration session. In Session 1, rats in Groups 1–3 received a 6 g/kg ethanol dose. In all other dependence induction sessions, their doses were determined by the behavioral criteria of intoxication used in Experiments 1a and 1b. Rats in Group 4 were intubated with 5 ml water in each dependence induction session. All alcohol and water intubations were separated from feedings by at least 1 hr.

The first withdrawal treatment session, Session 7, began 8 hr after alcohol administration Session 6. Group 1 rats were rated with the behavioral criteria of alcohol withdrawal [22] at the beginning of Session 7 as well as 2, 4, and 6 hr after the start of that session. If rats showed at least two withdrawal signs, including extensor rigidity, tremulousness, and tail stiffening, they received 1 g/kg alcohol (7.5%, v/v) those showing one sign of withdrawal and no signs of intoxication

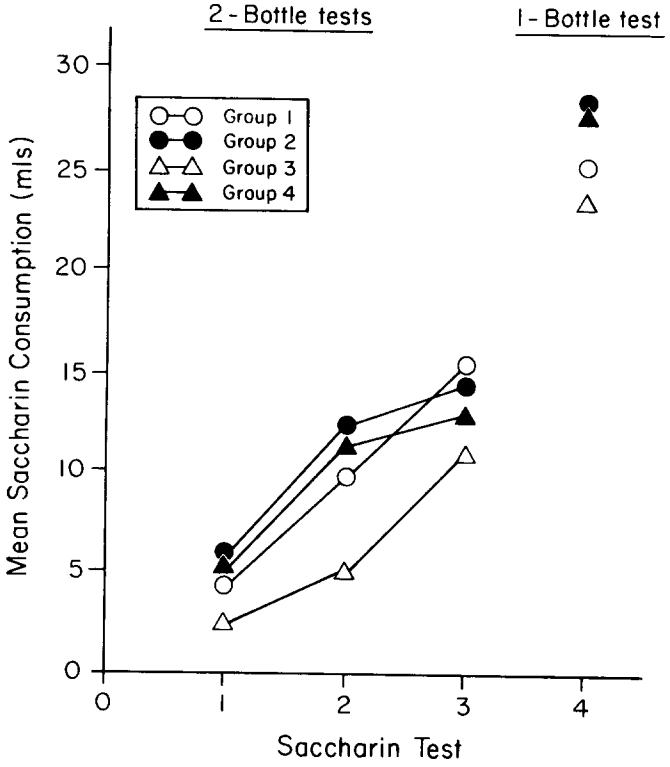

FIG. 3. Mean saccharin consumption by Groups 1–4 during two-bottle Saccharin Tests 1–3 and the one-bottle Saccharin Test 4.

received 0.5 g/kg; and rats showing any signs of intoxication received no ethanol. If Group 1 rats satisfied the behavioral criteria for the 0.5 or 1.0 g/kg doses, they were perfused with saccharin just prior to receiving alcohol. Rats were not given saccharin if they did not receive an alcohol dose. Group 2 animals were treated the same as Group 1 rats during withdrawal treatment sessions, except Group 2 animals were never exposed to the saccharin flavor even if they were given a withdrawal treatment alcohol dose. Group 4 rats were treated the same as Group 1 animals except they were given tapwater in lieu of the alcohol dose. These rats received saccharin exposure at the same times as their respective Group 1 partners. Rats in Group 3 were perfused with saccharin and intubated with water (5 ml) 2, 3, 4, and 6 hr after the start of Session 7. Group 3 rats' first exposure to saccharin was delayed 2 hr in order to insure that saccharin was paired with withdrawal symptoms and not intoxication. Since Group 3 rats did not receive alcohol doses paired with saccharin exposures, these rats were given 2.5 g/kg ethanol at the start of Session 7 to equate them with Groups 1 and 2 for total alcohol dose, and to keep them from withdrawing completely during Session 7. Our previous research suggested that Group 1 and 2 animals would receive approximately 2.5 g/kg ethanol over an 8 hr withdrawal treatment session.

The second withdrawal treatment session, Session 10, began 8 hr after alcohol administration Session 9. The procedures used in this session were identical to those used in the first withdrawal treatment session.

Assessment phase (Days 14–21). Behavioral ratings of alcohol withdrawal were conducted on Day 14, 8 hr after the last alcohol dose, using the same criteria employed in Experiments 1a and 1b. All rats in Groups 1–3 and a randomly selected sample of those in Group 4 were observed.

A series of three two-bottle preference tests, using tapwater and the saccharin solution, was conducted at 24-hr intervals on Days 18–20. Each test lasted 60 min, and bottle positions were reversed after 15 and 45 min. A one-bottle test was conducted on Day 21, using the saccharin solution; rats were allowed to drink for 90 min. Animals were deprived of fluid for 24 hr preceding the first test and between tests.

RESULTS AND DISCUSSION

Three Group 3 rats died during the experiment; therefore all analyses were conducted with unweighted means corrections. Two deaths appeared to be due to aspiration of intubated fluids.

Figure 3 depicts mean consumption of the saccharin solution for all groups during Tests 1–3, the two-bottle tests, and Test 4, the one-bottle test. Analyses of variance statistics revealed significant differences among groups at Tests 1, 2, and 4 (all $Fs \geq 3.66$, $ps < 0.05$). Newman-Keuls a posteriori tests showed that Group 3 consumed less saccharin during these tests than Groups 2 and 4 ($ps < 0.05$); the comparison of Groups 1 and 3 merely approached significance ($p < 0.10$).

Groups received almost identical amounts of alcohol during dependence induction (10.4–10.8 g/kg/day). A 3 (group) $\times$ 4 (day) mixed analysis of variance showed no group effect, $F(2,21) < 1.0$, but did reveal a day effect, $F(3,63) = 22.03$, $p < 0.01$, as all groups received decreasing amounts of alcohol over the course of the experiment. Groups did not differ on the basis of weight at any point in the experiment ($ps > 0.05$).

Groups 1 and 2 did not differ on the basis of amount of alcohol administered during withdrawal treatment sessions ($p > 0.10$). The mean amount of alcohol administered to relieve withdrawal signs during Sessions 7 and 10 was 2.10 and 2.17 g/kg respectively. Data revealed that most rats manifested withdrawal signs during the two withdrawal treatment sessions as animals received a mean of 6.1 alcohol doses over the eight possible dose delivery occasions.

The kappa coefficient for interobserver reliability of ratings of alcohol withdrawal was .63. Rats in Groups 1–3 showed an average of 1.30 signs of withdrawal eight hours after the last alcohol dose, while Group 4 rats showed a mean of 0.5 signs. Because some animals in Groups 1–3 appeared to still be intoxicated during the eight-hour withdrawal rating period, animals were rated again at eleven hours post-withdrawal. This rating revealed multiple withdrawal signs in all but two Group 1–3 rats (>2 signs/rat).

The results of Experiment 2 fail to demonstrate medicine effect conditioning. Group 1, which received saccharin paired with the relief of alcohol withdrawal, did not show a preference for saccharin, relative to any of the control groups, in any of the preference tests. It is tempting to speculate that Group 1 rats might have acquired taste preferences had they received larger or smaller withdrawal-relief alcohol doses. However, extensive pilot research in our laboratory, and research by others [9, 27, 29] shows that slightly higher doses routinely produce aversions while smaller doses usually have no effect.

Group 3, which received saccharin paired with untreated alcohol withdrawal, showed an aversion to saccharin relative to Group 4, the nondependent controls. This systematically

replicates Marfaing-Jallat and Le Magnen's [29] finding that rats learned an aversion to a flavor paired with untreated alcohol withdrawal. While Group 3 consistently displayed saccharin aversions across the preference tests, Group 1 did not. This suggests that withdrawing animals will not acquire aversions for flavors paired with alcohol if doses are carefully titrated to reduce withdrawal malaise, and yet not produce profound intoxication. This may explain a lack of taste aversions for flavors paired with alcohol solutions following oral self-administration of alcohol to the point of dependence [1].

As in Experiment 1b, the nondependent control group showed no aversion to saccharin. This lends additional support to the hypothesis that stress induced by the experimental procedures led to saccharin aversions in the nondependent controls in Experiment 1a and in the preliminary study; the procedural changes made in Experiment 1b apparently reduced the level of stress or its associability with the CS. Although Group 4 consumed less of the saccharin solution in the preference tests than the nondependent controls in Experiment 1b, this undoubtedly is due to the use of a more concentrated saccharin solution in Experiment 2 rather than to a learned aversion.

GENERAL DISCUSSION

Several major conclusions emerge from these studies. First, there was no evidence of medicine effect conditioning involving the relief of alcohol withdrawal. This finding is consistent with results obtained by Marfaing-Jallat and Le Magnen [29]. Moreover, our method provided a more sensitive test of taste-mediated learning than previous research. In our experiments rats received multiple conditioning trials, rather than a single trial, as Marfaing-Jallat and Le Magnen [29] and Le Magnen *et al.* [26] used. In addition, the conditioning doses of alcohol were administered according to criteria of withdrawal severity. This permitted idiosyncratic dosing to relieve alcohol withdrawal. The feeding regimen we employed prevented serious weight loss and ensured that nutritional status was not confounded with experimental manipulations. Our procedures for presenting saccharin (CS) and alcohol (US) permitted us to equate appropriate comparison groups on timing and amount of CS and US exposure. Finally, the extensive habituation used in Experiments 1b and 2 latently inhibited incidental procedural cues.

Despite these methodological improvements, we found no evidence of medicine effect conditioning. While our results do not preclude the possibility that such learning occurs, they do suggest that, at least in rats, such learning is not robust or of large magnitude. One reason that alcohol-induced taste preferences may be so difficult to demonstrate in rats is that rats are extremely sensitive to changes in their internal milieu, and almost any internal changes result in aversions for associated flavor cues [20]. Perhaps humans are not as likely to develop aversions for flavors paired with internal changes. There certainly is evidence, though, that humans can acquire strong taste aversions [1, 6, 10, 11].

While medicine effect conditioning may not play an important role in the development of alcoholic drinking, the relief of alcohol withdrawal symptoms may, nonetheless, exert an important effect. Our research, like that of Marfaing-Jallat and Le Magnen [29], suggests that alcohol withdrawal is aversive and that rats acquire aversions to flavors paired with withdrawal. However, if flavors are paired with small doses of alcohol that relieve withdrawal symptomatology, aversion learning does not occur.

The preliminary study and Experiment 1a highlighted two important phenomena relevant to taste-mediated learning and alcohol dependence research. In these studies, it appeared that stress related to either handling or the diet administration produced aversions to saccharin. This finding is congruent with Krane and Wagner's [25] observation that prolonged (shock-induced) stress can result in taste aversions. These aversions were significantly attenuated by small doses of alcohol, consistent with the hypothesis of Black *et al.* [8] that small alcohol doses reduce stress induced by handling. Thus, although it is unlikely that taste preferences can develop through medicine effect conditioning involving the relief of alcohol withdrawal, it is possible that, at least in the rat, small preferences can be acquired due to the ability of alcohol to mitigate certain types of stress. This could explain some reports of preferences for alcohol-paired flavors in which animals were subjected to high levels of stress concomitant with alcohol delivery (e.g., restraint, [16]).

One other finding is relevant to future research on alcohol dependence and taste-mediated learning. We obtained no evidence that alcohol dependence per se attenuated aversion learning to flavors paired with dependence-producing doses of alcohol (Experiment 1b). Dependence did appear to be a factor in aversion learning, but only if animals were first made dependent and then had a novel flavor paired with small doses of alcohol.

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