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Ethanol Reinforced Responding in the Rat: Relation of Ethanol Introduction to Later Ethanol Responding[1]

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SAMSON, H. H., G. A. TOLLIVER AND T. A. ROEHRS. *Ethanol reinforced responding in the rat: Relation of ethanol introduction to later ethanol responding.* PHARMACOL BIOCHEM BEHAV 18(6) 895–900, 1983.—Rats, maintained on ad lib food and water, were trained to lever press on a concurrent schedule using water and sucrose (10% w/v) as the fluids presented contingent on responding. Each fluid was associated with a lever and available on a fixed ratio eight (FR8) response requirement. The lever with which each fluid was paired was alternated daily. When stable responding occurred, ethanol was substituted for water, first at low concentrations, and then slowly increased to 5% (v/v). When stable sucrose-ethanol responding was reached, the response requirement for sucrose was altered to fixed ratio requirements of either FR32 or FR64. Moderate, but unstable increments in the number of ethanol responses occurred during the increased sucrose response requirements. Following the sucrose response requirement manipulation, water was substituted for the sucrose. Ethanol responding was found to fall to or below prior baseline levels. Weight reduction by food restriction failed to increase ethanol responding. The levels of ethanol responding resulting from this introduction procedure were always much lower independent of body weight than previously reported levels found in rats trained to respond for ethanol using a different procedure of initial ethanol introduction.

Body weight Ethanol responding Schedule responding

STUDIES have shown that access to oral ethanol will maintain leverpress responding in the rat (see [6] for a review). While different manipulations have been used to establish oral-ethanol reinforced responding in rats [5, 7, 9], one factor common to these procedures is food restriction prior to the introduction of ethanol. Thus, either the pharmacological, caloric, or perhaps other properties of ethanol (or a combination of all) could provide the bases for reinforcement.

In two studies, in which body weight was allowed to return to (and/or above) the original free feeding levels after ethanol reinforcement had been established, ethanol responding markedly decreased as weight increased [8,10]. While a small amount of ethanol responding remained when body weights returned to their original values, a major proportion of the ethanol responding was lost. This change in responding could be interpreted in terms of the caloric properties of ethanol, but such an interpretation must be guarded, since experiments utilizing non-caloric drugs have found that weight reduction also produces marked increases in their self-administration [1, 2, 3]. It is possible that what is critical for the establishment of ethanol as a reinforcer, is the behavioral manipulations (i.e., the schedules, available substances, etc.) used during the original induction procedure and not the food restriction per se. It has been suggested that for human drug taking, environmental and social factors occurring at the time of original drug exposure may be instrumental in both the instigation and maintenance of drug taking [4]. Further work is needed to clarify the function of these original exposure conditions in regard to ethanol's self-administration in the rat.

Recently, we found that when ethanol and sucrose were simultaneously available on a concurrent schedule, using a sucrose concentration highly preferred to ethanol, large increases in ethanol intake occurred when the schedule controlling sucrose availability was increased from a response requirement of 8 lever presses (FR8) to 64 presses (FR64) with ethanol remaining at FR8 [11]. These rats were maintained at 80% of their free-feeding body weight using a restricted food regimen, and ethanol had been established as an oral reinforcer with water as the alternate choice before the sucrose was introduced. The augmentation (double in some cases) of ethanol intake suggested to us that this procedure of varying the response requirements of alternate reinforcers to ethanol in a concurrent schedule might induce ethanol maintained responding without using body weight manipulation or food restriction. Such a demonstration

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might help to clarify the function of food restriction on ethanol self-administration. The following experiments were designed to test this hypothesis.

EXPERIMENT 1

METHOD

Animals

Four experimentally-naive, 120 day old male rats (Long-Evans strain) obtained from the Department of Psychology, University of Washington breeding facilities were individually housed in hanging rodent cages on a standard cage rack with food (Purina Lab Chow) and water available at all times except as noted below. Artificial lighting was on from 8:00 to 20:00 hours daily. Room temperature and humidity were maintained within the standards proposed by the National Institutes of Health.

Operant Chambers

The test chambers were the same as those previously described [6,7]. They included two operant response levers (LVE/BRS rodent levers) and two dipper reinforcement delivery systems (Gebrands No. G56000) with a 0.1 ml dipper. The chambers were housed in sound attenuated chambers. Events were controlled with a combination of electromechanical and solid state programming equipment. At the beginning of each session, a light was illuminated in the chamber and, at the end of the session, it was turned off.

Initial Training Procedures

In order to shape lever pressing, the animals were water deprived from 20:00 hours until 10:00 hours the next day (i.e., a total of 14 hours). They were trained to press the lever to obtain a 10% sucrose solution (w/v). If the animals failed to show leverpressing within one hour, they were returned to their home cage, given water until 20:00 hours, and the procedure was repeated. Animals that failed to learn to press the lever within two sessions were not used. (Only one animal failed to meet this criteria and another was substituted). Following the two days of shaping, all animals were returned to ad lib water. At no time was food restricted.

Daily sessions of 30 minutes in length and five days a week were then conducted for the remainder of the study. Initially, only one lever was present in the chamber, always in the same position. As soon as the animals were showing consistent lever pressing (150–200 responses/session) on a continuous reinforcement schedule (CRF) for the sucrose solution (10%), the response requirement was increased over days until a stable baseline responding at FR8 was reached. At that time, the second lever was introduced at the other location in the box, and the initial lever removed. The animals were taken through the same procedure until stable responding at FR8 was reached on the second lever using the other dipper for sucrose (10%) presentation. Then both levers were placed in the chamber and a concurrent FR8 and FR8 with sucrose (10%) available on both levers was instituted, with the right dipper associated with the right lever, and the left dipper with the left lever. At all times in the concurrent condition, a 3-second changeover delay was in effect to maintain lever independence (see [9] for a more detailed explanation of apparatus and schedules).

TABLE 1

EXPERIMENTAL PROTOCOL FOLLOWED IN EXPERIMENT ONE

1. Sucrose (10%) FR8-Sucrose (10%) FR8 Concurrent Schedule (10 sessions)
2. Sucrose (10%) FR8-Ethanol (2%) FR8 Concurrent Schedule (3 sessions)
3. Sucrose (10%) FR8-Ethanol (3%) FR8 Concurrent Schedule (3 sessions)
4. Sucrose (10%) FR8-Ethanol (5%) FR8 Concurrent Schedule (4 sessions)
5. Ethanol (5%) FR8-Water FR8 Concurrent Schedule (5 sessions)
6. Sucrose (10%) FR8-Water FR8 Concurrent Schedule (5 sessions)
7. Sucrose (10%) FR8-Ethanol (5%) FR8 Concurrent Schedule (3 sessions)
8. Sucrose (10%) FR32-Ethanol (5%) FR8 Concurrent Schedule (3 sessions)
9. Sucrose (10%) FR8-Ethanol (5%) FR8 Concurrent Schedule (5 sessions)
10. Sucrose (10%) FR64-Ethanol (5%) FR8 Concurrent Schedule (5 sessions)
11. Sucrose (10%) FR8-Ethanol (5%) FR8 Concurrent Schedule (5 sessions)
12. Ethanol (5%) FR8-Water FR8 Concurrent Schedule (14 sessions)

Experimental Procedure

When stable responding on the sucrose-sucrose FR8 FR8 concurrent condition was reached, ethanol was introduced. For the next 10 sessions, ethanol and sucrose were concurrently available on the FR8 FR8 schedule. The position and lever association of ethanol and sucrose solutions alternated each session. For the first three sessions, a 2% ethanol solution (v/v in water) was concurrently available with a 10% sucrose solution. Then for the following three sessions, the ethanol concentration was increased to 3%. After these three sessions, the ethanol concentration was increased to 5% where it remained for the rest of the experiment. Following four sessions of the 5% ethanol -10% sucrose FR8 FR8 concurrent schedule, five sessions were conducted in which 5% ethanol was concurrently available with water on the FR8 FR8 schedule. As before, the position of each solution was alternated each session. Following this initial water-ethanol concurrent test, five sessions of a 10% sucrose-water FR8 FR8 concurrent were run, again with the sucrose and water position alternating each session.

Following the sucrose-water test, sucrose and ethanol were again concurrently available for three sessions. Then for three sessions, the FR requirement for the sucrose was increased to 32 while that for ethanol remained at 8. An additional five sessions of ethanol-sucrose were run with the FR requirement returned to 8 for both solutions. Next, five sessions were run with the response requirement associated with the sucrose lever increased to 64, and the ethanol lever remaining at 8. After these five sessions, five more sessions with sucrose and ethanol were run with the FR requirements at 8 for both levers. After this last sucrose-ethanol concurrent condition, the animals were run for 14 sessions with ethanol and water as the two available fluids, both available on a FR8 schedule, and alternating in position from session to session. (See Table 1 for the experimental design).

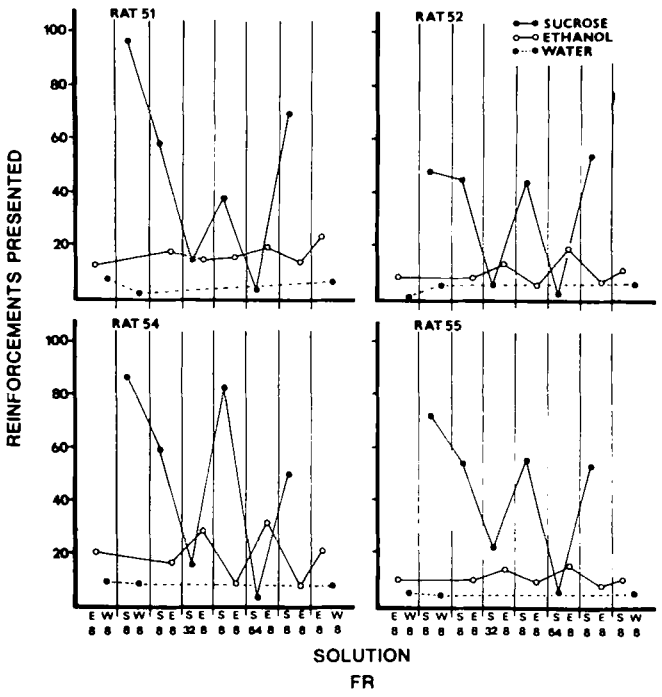

FIG. 1. Reinforcements presented for each concurrent pair condition. (E=ethanol (5% v/v); W=water; S=sucrose (10% w/v); FR=Fixed ratio response requirement.)

Data Recording

Daily body weights were recorded as were number of responses on both levers, number of reinforcements delivered, and the change in fluid volume of the respective fluid reservoirs. On some days, cumulative records were taken.

RESULTS

At the beginning of the experiment the animals had a mean body weight of 366 g (SD±46 g), while at the end of the experiment they had a mean body weight of 494 g (SD±27 g).

Figure 1 presents the average number of reinforcements presented for each of the concurrent fluids available in each condition. As can be seen, the schedule changes from FR 32 and FR 64 for sucrose greatly reduced the number of sucrose reinforcements presented, as would be expected. In two of the four animals, when the sucrose FR was increased in value, ethanol reinforcements increased (No. 52 and No. 54). However, in the remaining two animals, ethanol reinforcements increased very little as a function of change in the sucrose response requirement. In only one of the four animals (No. 51) did ethanol-water concurrent responding following the ethanol-sucrose manipulations show any change (an increase). In all the other animals, the mean ethanol reinforcements presented were not increased from the baseline condition observed prior to the ethanol-sucrose response manipulation. Inspection of the individual session responding for each animal (Fig. 2) indicated that for the animal which showed an average increase in ethanol responding (No. 51), a clear lever preference accounted for the result. This animal failed to follow ethanol as its availability alternated from lever to lever over sessions, showing high

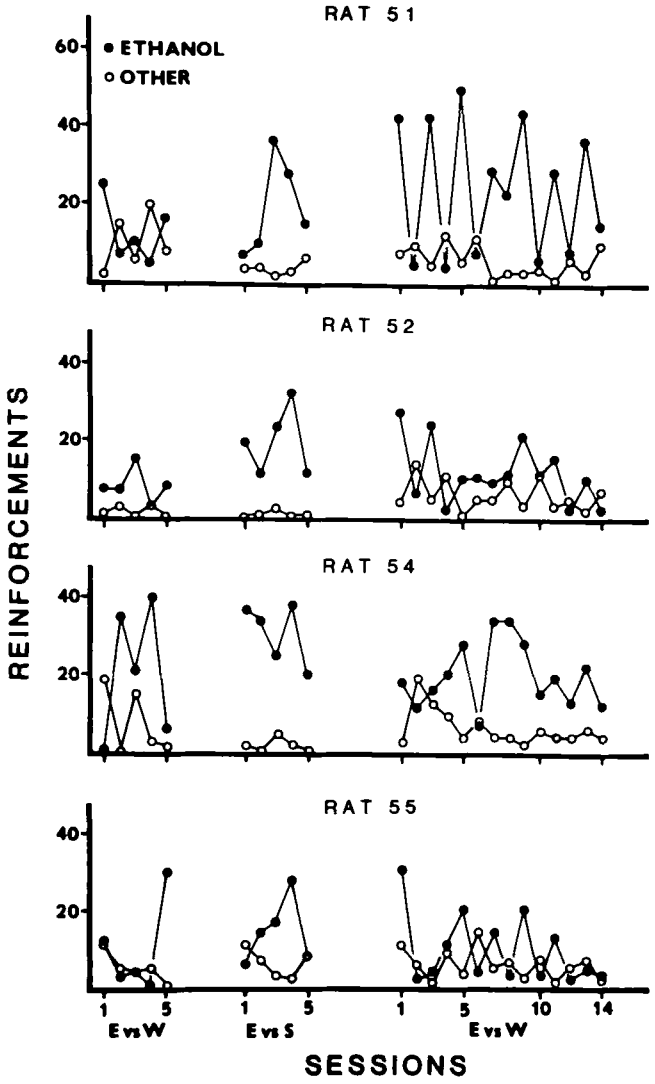

FIG. 2. Reinforcements presented prior, during, and after sucrose response requirement manipulation. (E=ethanol, W=water, S=sucrose.) In the E vs. S, the response requirement for sucrose was 64. For all other conditions it was 8.

ethanol responding only on the sessions in which ethanol was associated with the preferred lever. All of the other animals showed either no change in ethanol responding or minor fluctuating changes (i.e., animal No. 54).

DISCUSSION

The failure to produce either a marked ethanol increase during the schedule manipulation in the ethanol-sucrose FR8 FR64 concurrent condition, or changes in ethanol responding during the ethanol-water FR8 FR8 concurrent condition that followed, suggests that the specific procedures followed in this experiment failed to establish ethanol as a reinforcer. That the animals neither responded very much for ethanol in the ethanol-water condition or followed ethanol as it alternated across sessions might have been predicted, as there was very little "induced" ethanol intake as a result of the schedule manipulation during the sucrose-ethanol manipula-

tions. Thus, the failure to observe ethanol maintained behavior can be ascribed to the failure to effectively establish ethanol responding in the first place. However, if one compares absolute ethanol responding of the animals in this experiment with that of animals in our previous work that were also not weight limited [10], very similar total levels of ethanol responding are found. Therefore, it might be possible that ethanol had become a reinforcer, but because of the present body weight of the animal only minimal ethanol responding occurred. To determine if this was the case, Experiment 2 was performed.

EXPERIMENT 2

METHOD

The same four animals used in Experiment 1 were continued into this experiment. There were no changes in any of the daily procedures except that the animals were placed on a restricted-food ration which was given to them daily after their session in the operant chamber. The amount of food given was determined on an individual basis so that each animal slowly approached a body weight that was 80% of its current ad lib body weight.

Daily operant sessions were identical to those at the end of Experiment 1; i.e., an ethanol (5%)–water FR8 FR8 concurrent schedule. As before, the ethanol (5% v/v) and water position in the chamber was alternated each session (i.e., ethanol was associated with the right lever every other session). The same measures as in Experiment 1 were taken.

RESULTS

Figure 3 presents ethanol reinforcements and % ad lib body weight for each animal. In three of the four animals (No. 51, 52 and 55) the number of ethanol reinforcements per session was sometimes greater during weight reduction than at the ad lib level. These periodic increases always occurred when ethanol was associated with the same lever, suggesting that an animal's lever preference dominated over the reinforcing properties of ethanol. No similar changes in water responding occurred. When ethanol was associated with the preferred lever, it could maintain a moderate level of lever pressing behavior on many occasions (i.e., animals No. 51 and 52 with ethanol maintained responding on the preferred lever and minimal responding for ethanol on the nonpreferred lever (Fig. 3). Thus, the animals that did respond to ethanol failed to follow ethanol as it alternated each session. By the last 7–10 sessions, ethanol responding was at or below that seen prior to food restriction for all animals except animal No. 55, who continued throughout the experiment to respond for ethanol, but showed a marked lever preference. Animal No. 54, except for a brief period late into weight reduction, showed little change in behavior and by the end of the experiment was not responding for ethanol at all. It should be noted that this animal also did not respond for water. As well, the other three animals did not respond for water, even if it was on the preferred lever.

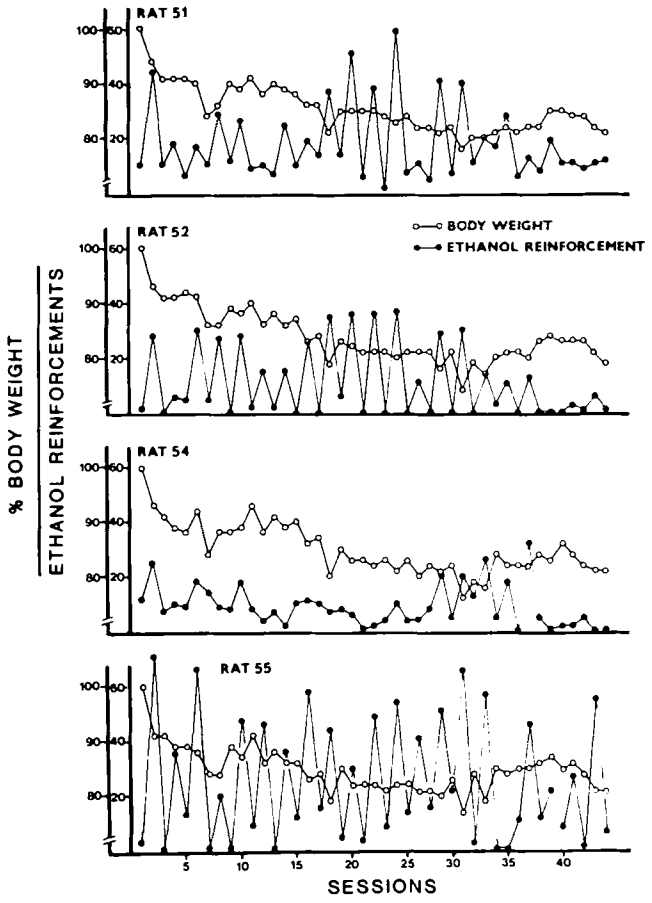

FIG. 3. Relation of body weight to number of ethanol reinforcements presented in the ethanol-water concurrent situation.

DISCUSSION

While on any given day, a particular animal might have shown an increase in ethanol intake as body weight decreased, the pattern of ethanol responding was markedly different from that seen in the prior studies using different procedures to induce ethanol as a reinforcer in weight reduced animals [6, 7, 9, 10]. In these prior studies in which ethanol-maintained responding was observed, not only was stability of responding found, but as well, those animals in the concurrent schedule studies [7, 9, 10, 11] followed ethanol as it alternated from lever to lever across sessions. At no time in this experiment was this following of ethanol observed. It is possible that the failure to observe this following was due to prior development of lever preferences that overshadowed the ethanol effect. While this cannot be ruled out, immediately prior to the ethanol-water concurrent conditions of Experiment 2 and the final phase of Experiment 1, the animals were tested on sucrose-ethanol concurrent conditions, in which they showed marked following of the sucrose solution as it alternated over sessions and no lever preference. Thus the failure to follow ethanol and the development of the lever preference resulted in the later ethanol-water condition. It is possible that had the animals been taken to 80% body weight directly, without any operant sessions during the weight loss period, the lever preference might not have developed. Only further experiments can clarify this result, but it is clear that given the specific experimental conditions of Experiments 1 and 2, ethanol did not maintain responding in any manner which could be considered as similar to those seen in previous studies using other induction procedures [6, 7, 9, 10].

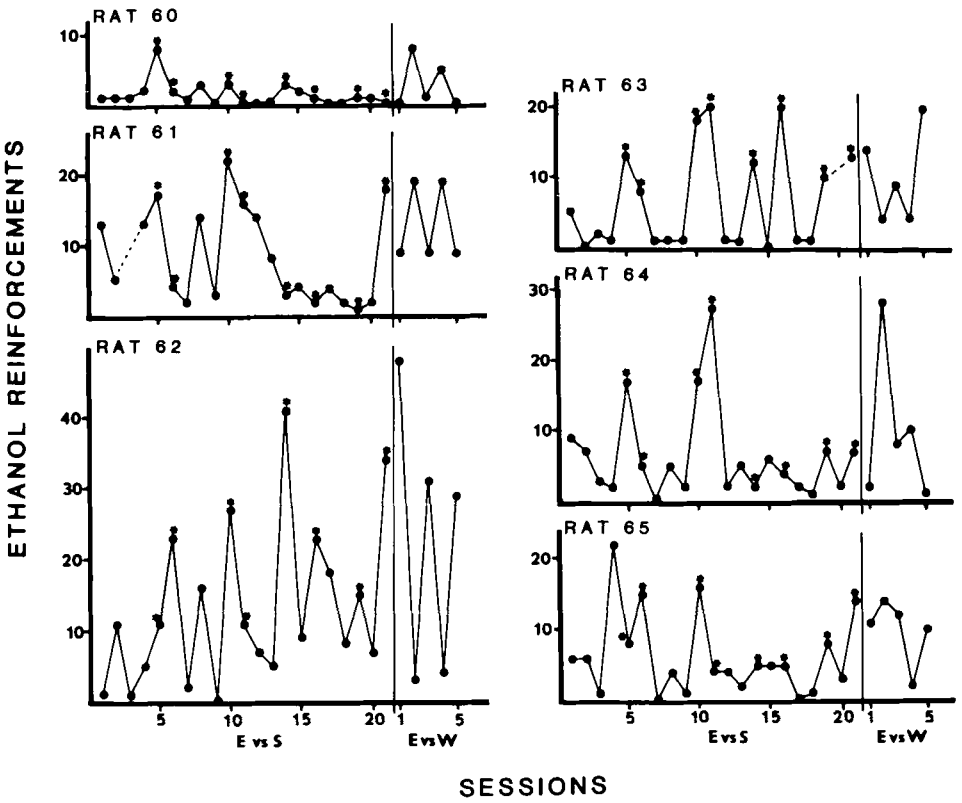

Fig. 4. Ethanol reinforcements presented in an ethanol-sucrose concurrent pairing. (Denotes session in which sucrose response requirement was increased from 8 to 64; ethanol was always at a response requirement of 8. E=ethanol; W=water; S=sucrose.)

EXPERIMENT 3

In order to determine whether the effects observed in Experiment 1 were representative, an additional 6 animals were examined using a similar procedure.

METHOD

Housing and lever-pressing training were identical to those of Experiment 1. When all animals had reached baseline performance on a 10% sucrose (w/v) 5% ethanol (v/v) FR8 FR8 concurrent as in Experiment 1, they were then given two sessions in which the FR requirement for sucrose was elevated to 64. They then received three sessions with the FR requirements for both fluids at 8, followed by a second set of two sessions with the sucrose FR at 64.

After two more sessions at equal response requirements for sucrose and ethanol (FR8 FR8), a block of eight sessions was conducted in which the sucrose FR requirement was alternated each session from 8 to 64. Following this last schedule manipulation, the animals were given five sessions in which ethanol was now concurrently paired with water, both on FR8 contingencies.

RESULTS

Figure 4 presents the number of ethanol dipper presentations under the various experimental conditions for each animal. In all but one animal (No. 60), the change in the sucrose response requirement from 8 to 64 and back to 8 produced changes in the number of ethanol dipper presentations. However, these changes were inconsistent both within and between animals. Responding in the subsequent water-ethanol concurrent schedule resulted in no changes in the ethanol presentations from baseline for 4 of the 6 animals. Two animals (No. 61 and 62) showed a slight overall increase in ethanol responding. However, their day-to-day variability in responding and bar preferences were strong influences as to the number of ethanol presentations that occurred.

DISCUSSION

The results from this experiment partially confirm those of Experiment 1 and suggest that schedule manipulation of a preferred solution does not result in ethanol becoming a strong reinforcer for the ad lib animal.

GENERAL DISCUSSION

From previous work in our laboratory [11], food deprived rats that had been originally trained on an ethanol-water concurrent paradigm that resulted in ethanol maintained behavior, greatly increased their ethanol responding when placed on a sucrose (5%)-ethanol concurrent in which the sucrose availability was programmed with a response requirement of 64 and ethanol at a response requirement of 8. In Experiments 1 and 3, a similar schedule manipulation with sucrose and ethanol, in animals not previously trained with a procedure that resulted in ethanol maintained responding,

failed to show the increase in ethanol responding. As well, reduction in body weight (Experiment 2) also did not increase ethanol responding following the sucrose-ethanol manipulation. It would therefore appear that in order for the schedule manipulation to be effective in increasing ethanol responding, ethanol must first be maintaining responding in this situation. It would also appear that the schedule manipulations used in these experiments do not result in ethanol maintained behavior.

We can only speculate about what produced the differences in ethanol maintained responding between our various studies at this time. In the method used in our prior work [9,10], the animals were first weight reduced, then trained to lever press for water, and finally introduced to ethanol by placing ethanol and water available concurrently in the chamber at the same time with the daily food ration. After a few sessions, the animals showed ethanol maintained behavior by responding on the appropriate lever as ethanol alternated session to session. Within ten sessions, marked ethanol responding (an average of 40 ethanol dipper presentations on a FR8 contingency) per session was observed in the majority of animals. Clearly, in the present experiments, there was an opportunity for this type of ethanol responding to develop, particularly in Experiment 2. What prevented this from occurring is of major importance for understanding the development of ethanol reinforced behavior.

Since the animals in the experiments reported here did in many cases ingest as much ethanol on a given session as seen in previous work, absolute ethanol intake would not seem to be the major factor involved. Second, since the animals in Experiment 2 were weight decreased to the same extent as in the prior work, weight reduction alone can also not account for the difference in results.

The one major difference between the methods for developing ethanol maintained behavior in our studies (the present study vs. [9,10] is the placement of the food ration into the operant chamber at the time that ethanol reinforcement is established. In one condition [9,10], the first exposure to ethanol was at the same time of food availability. Thus the ingestion and subsequent consequences of ethanol were paired with the reinforcement effects of the daily food ration. In the present study, the first experiences with ethanol did not occur at the same time as presentation of another reinforcer. In fact, during the phase of attempted ethanol induction, while paired with the availability of a strong reinforcer (i.e., the sucrose), ethanol was not positively but rather negatively paired (i.e., at the time when the sucrose response requirement was increased). Therefore, even though ethanol intakes slightly increased, they were not increased in conjunction with the availability of another strong reinforcer. The importance of this difference remains to be determined, but its implications suggest that events associated with the original induction of oral ethanol reinforcement could alter later ethanol maintained behavior.

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