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BRIEF COMMUNICATION

Ethanol Selection in the Rat Following Forced Acclimation[1]

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VEALE, W. L. *Ethanol selection in the rat following forced acclimation.* PHARMAC. BIOCHEM. BEHAV. 1(2) 233–235, 1973.–In the rat, forced drinking of ethanol does not produce greater drinking subsequently. The gradual acclimation to ethanol in the presence of an alternate solution has been shown to result in greater selection of ethanol in later tests [11]. Rats forced to drink a 30% concentration of ethanol for several months following an interval during which time they were forced to drink ethanol solutions in increasing concentrations from 3–30%, selected alcohol in a free-choice test situation in amounts in excess of 11 g/kg/day. This finding suggests that forced acclimation to ethanol does not necessarily lead to reduced subsequent ethanol selection may be an effective means of getting animals to select ethanol in relatively large amounts. It is suggested that the forced acclimation procedure combined with the method recently described by Falk *et al.* [3] may produce greater maintained volitional drinking of ethanol.

Ethanol selection Acclimation to ethanol Ethanol drinking

SEVERAL investigators have demonstrated that the forced consumption of ethanol solutions in concentrations ranging from 8 to 24 per cent does not result in an increase in subsequent drinking of ethanol [4,9]. However, a slight increase in the selection of ethanol in a concentration of 5% was observed following forced consumption of the same concentration for several days [5]. When forced to consume ethanol in solutions ranging from 5–20%, rats showed only a very slight increase in ethanol consumption even though they had been maintained on the test concentration for as long as 4 months [6]. Similarly, rats restricted to concentrations of ethanol from 2–16% for 6 months increased their preference only slightly for the lower concentrations (2 and 8%) but rejected the higher (16%) concentration [10]. More recently, Veale and Myers [11] showed that when rats were forced to drink a 15% solution of ethanol their subsequent preference for ethanol was reduced whereas animals that had been gradually acclimated to ethanol increased their consumption progressively. In an extension of this study Carey [1] showed that the forced drinking of 5% ethanol by fluid deprived rats for 30 min per day for 10 days did not increase the subsequent drinking this same solution whereas forced drinking of a 10% ethanol solution reduced subsequent drinking. On the other hand, Cicero *et al.* [2] demonstrated that rats restricted to 7% ethanol for 133 days showed an increase in ethanol consumption in a subsequent self-selection test.

Recently, Falk *et al.* [3] have specified several criteria which would facilitate the study of alcoholism using an animal model and have furnished impressive evidence that these criteria have been met. We present evidence in this paper that forced drinking of high concentrations of ethanol does not necessarily lead to reduced volitional intake and may even result in the selection of high levels of ethanol in a self-selection situation. We further suggest that this method, in combination with that described by Falk *et al.* [3], may result in the production of a more appropriate animal analogue of alcoholism than now exists.

METHOD

Twenty 120-day old male rats of the Long-Evans strain were housed individually and maintained on an ad lib diet in an air-conditioned laboratory room under constant illumination at 70–72° F.

Over a period of 5 months, 9 rats were gradually forced to drink increasing concentrations of ethanol solutions beginning with 3%. Every 14 days the concentration was increased by one step in the following sequence; 3, 4, 5, 7, 9, 10, 12, 15, 20, 25 and 30%. The rats were then maintained on 30% ethanol for the following 6 months before they were offered a choice between tap water and a solution of ethanol. At the beginning of the eleventh month ethanol solution and water were available simultaneously the ethanol solutions in concentrations beginning at 3% and increasing one step each day in the sequence described

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above. During the experiements, 3 Kimax drinking tubes were used and the preference tests employed the 3 bottle method of Myers and Holman [7].

The control group had no previous exposure to ethanol but where treated as those in the forced acclimation group. Water was available in 3 Kimax drinking tubes in each cage over the entire 11 month experimental period before they were offered a choice between ethanol and water in the sequence described above. Following the preference test a 2-day period elapsed and the same test was repeated in both groups.

Alcohol solutions were mixed in tap water (v/v) and replaced each day. Body weight was recorded at regular intervals and the animals were observed for unusual behavioral responses.

RESULTS AND DISCUSSION

The effect of forced consumption of a relatively high concentration of ethanol following previous gradual forced acclimation produces a significant increase in ethanol selection in a free-choice test. Figure 1 illustrates the intake of ethanol for both the control and forced acclimation groups in g per kg per day for each concentration of the test sequence. The intake record represented by the crosses (x x) illustrates the mean ethanol intake and twice the standard error for the forced acclimation group during the first test sequence. The intake for the control group is represented with twice the standard error by the circles (o o). It is evident that the animals that had ethanol as their sole fluid for 6 months following gradual forced acclimation consumed significantly more ethanol in the free-choice setting ($t = 7.92; df = 18; p<0.01$) than did the control animals. It is interesting to note that the 12% solution which was well above that normally selected by rats [8,11] was selected in the greatest amount. The mean weight of these rats was 480 g which means that the intake for the 12% concentration represents an intake of 11.2 ± 1.68 g per kg per day.

In the second ethanol solution-water test which was separated from the first by 2 days, the difference between the forced acclimation group and the control group was significantly different ($t = 7.70$; $df = 18$; $p<0.01$). The absolute amount of ethanol consumed by the forced acclimation group during the second test was less than that consumed by this same group during the first test. The intake records with twice the standard error are illustrated in Fig. 2 for both the forced acclimation (x – x) and control (o – o) groups.

The results of these experiments clearly indicate that prior exposure to ethanol is a significant factor in determining subsequent intake. It has been shown previously that if an animal is forced to drink ethanol in concentrations above that which the animal would normally select in a free-choice situation, the subsequent selection of ethanol in a free-choice test is reduced [11]. Even when the concentration the rats are forced to drink is relatively low (5%) the subsequent increase is very small [1,6]. Further, in the studies which forced consumption of a non-preferred solution produced lower subsequent ethanol drinking [11], the animals may not have been able to acclimate to the high concentration directly without previous exposure. In the experiments of this paper, 30% ethanol was forced upon a group of rats for a period of 6 months, but only after they had been gradually acclimated to this high

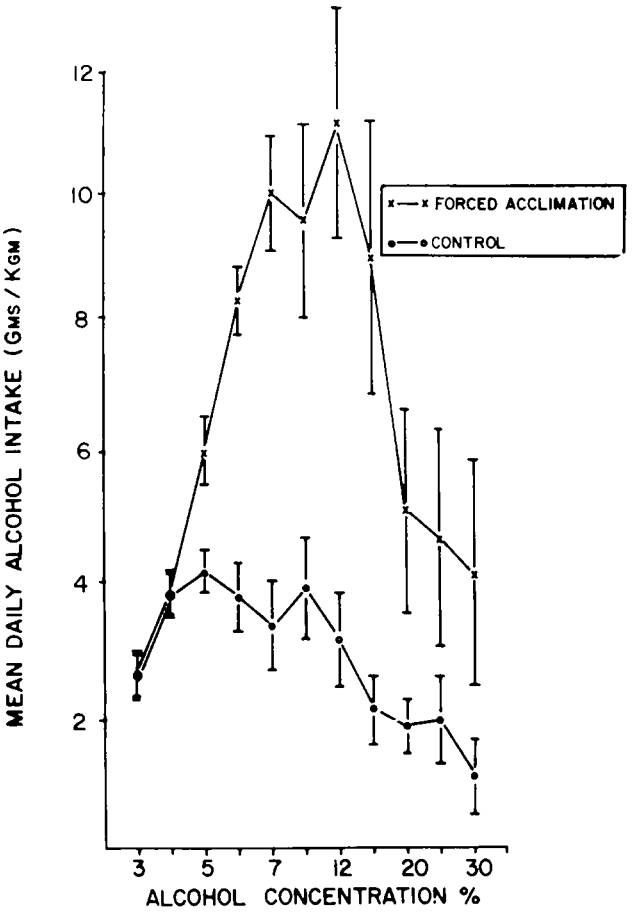

FIG. 1. Intake of alcohol (g/kg/day) for the specific concentration offered on each day of the alcohol solution–water choice sequence. The intake for the control group is represented by the circles (o – o) and that for the forced acclimation group is represented by the crosses (x – x). Each point represents a mean value and each vertical bar represents twice the standard error.

concentration over a period of 5 months. In a self-selection test at this time the forced acclimation animals consumed significantly larger quantities of ethanol than did control animals. Throughout the experimental period all animals showed a normal weight gain and displayed no unusual behavioural patterns. Following the termination of ethanol the rats did appear irritable upon handling but this behaviour disappeared over a period of 1–3 days.

Following forced acclimation the amount of alcohol selected by the rats was 3.1 to 11.2 g per kg per day depending on the concentration available. It is interesting to note that the 12% concentration was consumed to the greatest extent and that this concentration is well above that normally preferred by rats [11]. The fact that the lower concentrations of 2, 3, 4 and 5% were not selected in as great a quantity as the concentrations from 7–15 would indicate that the long-term forced acclimation changed the animals' normal selection of the lower concentration to higher ones. Carey [1] found that the effect on subsequent ethanol preference of previous experience with ethanol

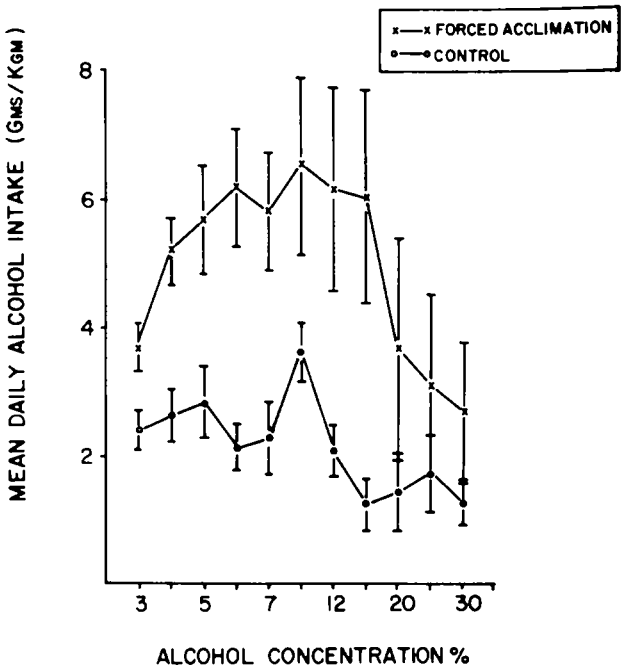

FIG. 2. Intake of alcohol (g/kg/day) for the specific concentration offered on each day of the alcohol solution--water choice sequence. The intake for the control group is represented by the circles (○ – ○) and that for the forced acclimation group is represented by the crosses (x – x). Each point represents a mean value and each vertical bar represents twice the standard error.

eventually did disappear and this is compatible with our results.

The results indicate the great need for careful interpretation of data involving ethanol intake in animals which have had previous exposure to the substance. The type of initial exposure, the length of forced consumption, the presence or absence of an alternative fluid, the interval between the last opportunity to consume ethanol and the concentration of the solutions themselves as well as the method of presentation are all extremely important variables which must be considered in experiments which involve the ingestion of ethanol. In addition, these results suggest that the problem of getting animals to select alcohol over an alternative solution in relatively large amounts may be one which can be overcome. Recently, Falk *et al.* [2] have demonstrated that an animal's intake of ethanol can be maintained at high levels for relatively extended periods of time using the technique of schedule-induced polydipsia. Perhaps the use of the schedule-induced polydipsia technique to maintain the intake of ethanol following a regimen of forced acclimation may lead to an even better animal analogue of alcoholism.

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