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A Simplified Procedure for Producing Ethanol Self-Selection in Rats

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STEWART, R. B. AND L. A. GRUPP. *A simplified procedure for producing ethanol self-selection in rats.* PHARMACOL BIOCHEM BEHAV 21(2) 255–258, 1984.—Procedures almost exclusively involving the use of operant conditioning equipment have been successful in training rats to drink ethanol and establishing ethanol as a reinforcer. The present report describes a variation of this procedure which, in a relatively short period of time also establishes ethanol as a reinforcer, results in significant blood ethanol levels, but does not require extensive programming or recording equipment and is therefore more amenable to studies in which large numbers of animals are required.

Ethanol self-selection Blood ethanol levels Operant conditioning

A commonly used method in studies which attempt to evaluate determinants of ethanol consumption is that of giving rats continuous access to drinking tubes containing water and solutions of ethanol and water. Concentrations above six percent (weight/volume, w/v) are generally not preferred [15] and typical daily drug consumption is usually well below the rat's metabolic capacity [16]. Since this low level of consumption is insufficient to produce overt intoxication or even pharmacologically meaningful blood ethanol concentrations, this method has been considered to be inadequate as a model of human alcoholism [2].

Procedures utilizing operant conditioning techniques have been developed which produce higher levels of oral ethanol consumption in rats [3,14]. However, these methods have a practical limitation in that they involve the use of one operant conditioning chamber per animal and require extensive programming equipment. Thus studies which require a large number of subjects are constrained by the availability and expense of such equipment.

The purpose of this report is to describe a procedure adapted from the techniques of Meisch and others [5, 11, 12] which achieves substantial and selective ethanol intake and results in pharmacologically relevant blood ethanol levels during relatively short experimental sessions. Blood ethanol data, obtained in this study, has heretofore not been reported by Meisch and co-workers or by Macdonall and Marcucella [9] who also used similar manipulations to increase drug consumption by rats. Furthermore, since equipment requirements are minimal, this method is suitable for and could be used in experiments where group size makes the use of operant conditioning apparatus impractical.

METHOD

Subjects

Six male Wistar rats (Charles River, Montreal) weighing 312–433 g were housed individually and kept on a 12/12 hr light/dark cycle with lights off at 7:00 a.m. Water was continuously available. The animals' feeding conditions will be described below.

Drug Preparation

Solutions of 2, 4 and 8% ethanol (w/v) were prepared in tap water. For example, the 8% solution was made by adding 10.14 ml of absolute ethanol to a volumetric flask with sufficient tap water to make a total volume of 100 ml.

Apparatus

The apparatus consisted solely of standard hanging wire "drinking" cages, 30×20×15 cm, equipped with a single 100 ml graduated drinking tube (BioServ, Frenchtown, NJ) in the front and a small glass food cup inside.

Training Procedure

The training procedure takes advantage of the rat's propensity to eat and drink at the same time. Thus, if water is offered to food-deprived rats in conjunction with a measured amount of food, substantial amounts of the fluid will be consumed in a relatively brief period of time. This pattern is not disrupted if solutions with increasingly higher concentrations of ethanol are substituted for the water, even when they are of the order which animals would otherwise avoid. When ethanol is later offered in the absence of food, drinking remains elevated.

At the start of the procedure all animals were first reduced to 80% of their free-feeding weights and maintained at this weight by home cage feeding of Purina lab chow. Daily drinking sessions then took place at approximately the same time. A drinking session consisted of removing the rats from their home cages and placing them individually into separate drinking cages for 60 min. Readings from the filled graduated drinking tubes were taken at the beginning and at the end of each session. During the initial stage of training (27 ses-

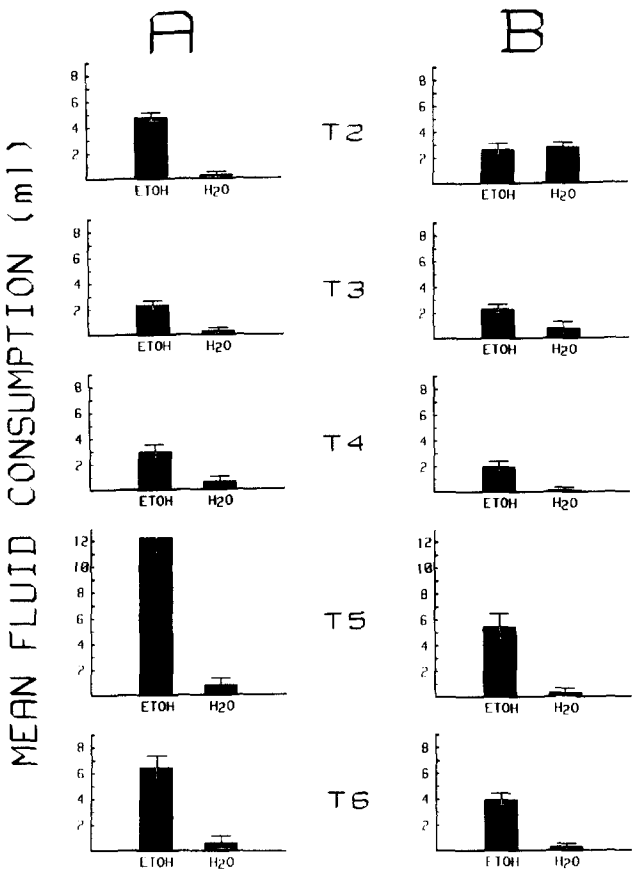

FIG. 1. Mean amount of 8% ethanol solution and water self-administered during 60 min sessions for each rat averaged over 6 ethanol and 6 water sessions (A) when the animals were food deprived and maintained at 80% of their free-feeding weights and (B) when the rats had free access to food and returned to normal body weights.

sions), 5 g of Purina lab chow was always placed in the food cups of the drinking cages. For the first 10 of these sessions water was the available liquid, to be replaced by 2% ethanol for two sessions, then 4% ethanol for four sessions and finally 8% ethanol for 11 sessions. The in-session feedings were then discontinued and sessions with 8% ethanol and no food were given for an additional 14 days.

Evaluation of Ethanol Drinking

Eighty percent feeding condition. Animals continued to be maintained at 80% of their free-feeding weights. Following the training procedure, 8% ethanol and water were alternated daily as the available liquid in order to determine whether the drinking was specific to the drug. This alternation continued for 12 days and was immediately followed by eight sessions during which ethanol was the only available liquid and readings from the drinking tubes were taken at 0, 15, 30 and 60 min. In this way the temporal distribution of drinking could be examined. Finally, daily 8% ethanol drinking sessions continued and alternate days were designated as blood sampling sessions during which the rats were placed in their drinking cages in the usual manner and allowed to drink for either 15, 30 or 60 min. They were then removed from the drinking cages and had 50 μl samples of blood drawn from the cut tip of the tail. The amount of ethanol consumed during that session was noted. Two different samples for 15, 30 and 60 min were collected from each rat during six different sessions.

Free-feeding condition. The rats were then given ad lib access to food in their home cages. Daily 60 min sessions with 8% ethanol continued until both the increase in body weight and amount consumed had stabilized (40 sessions). At this time the determination of drug-specific drinking, temporal distribution of drinking and blood ethanol concentrations was carried on in a manner identical to that in the 80% feeding condition.

TABLE 1

TEMPORAL DISTRIBUTION OF 8% ETHANOL SOLUTION INTAKE (ML)

Time	0–15 min	15–30 min	30–45 min	45–60 min
80% Feeding Condition	5.0 ±0.59	0.67±0.16	0.15±0.06	0.10±0.06
Free-Feeding Condition	2.37±0.21	0.55±0.13	0.13±0.05	0.18±0.07

The numbers represent the mean (±S.E.) of five animals across 8 different drinking sessions.

Blood Analysis

Samples were prepared and analyzed using gas-liquid chromatography according to the method of LeBlanc [6].

RESULTS AND DISCUSSION

One animal, rat T1, developed an infection and died early in the experiment, but the other five rats successfully acquired and maintained ethanol drinking.

Eighty percent Feeding Condition

Figure 1A shows the mean amount of 8% ethanol solution and water consumed by each rat during the 12 sessions in which ethanol and water alternated. Ethanol consumption was consistently and significantly greater than water consumption (rat T2: $t(5)=20.12$, $p<0.01$; rat T3: $t(5)=4.47$, $p<0.01$; rat T4: $t(5)=3.5$, $p<0.02$; rat T5: $t(5)=12.99$, $p<0.01$; rat T6: $t(5)=7.78$, $p<0.01$) and ranged from a mean of 2.3 ml (0.57 g/kg) for rat T3 to a mean of 12.3 ml (2.85 g/kg) for rat T5.

Table 1 shows the temporal distribution of ethanol drinking during the eight sessions in which readings were taken every 15 min. While some drinking occurred throughout the entire 60 min session, the majority of it took place during the first 15 min of the session. This pattern is consistent with that observed in experiments where rats press a lever to gain access to the drug [10].

Figure 2A shows the blood ethanol concentrations from samples obtained 15, 30 and 60 min after the initiation of drinking plotted as a function of dose (ml 8% ethanol consumed converted to g/kg). Systematic increases in blood ethanol levels with increases in 8% ethanol consumption were indicated by significant positive correlations (two-tailed, $df=8$) for the 15 min ($r=0.75$, $p<0.02$) and 60 min ($r=0.86$, $p<0.01$) sampling times, and by a non-significant

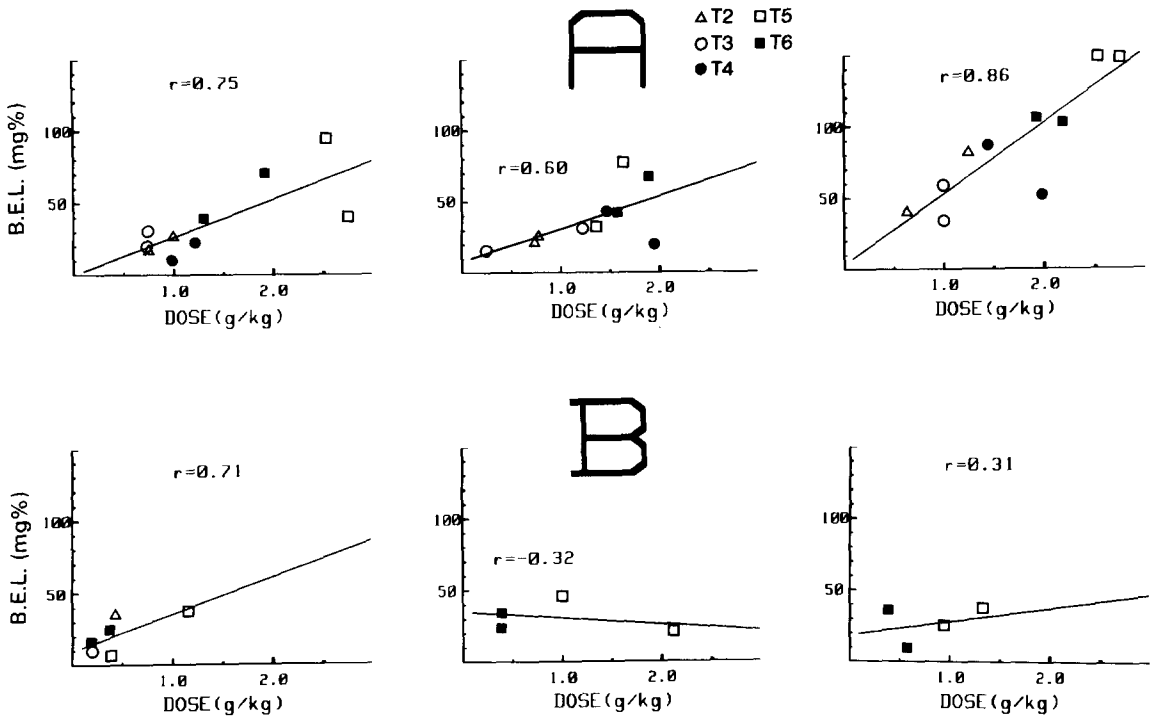

FIG. 2. Far left, middle, and far right panels represent blood ethanol concentrations (mg%) from sample obtained 15, 30 and 60 min respectively after the initiation of drinking plotted as a function of dose (ml 8% ethanol solution consumed converted to g/kg). Two different determinations for each of the three sampling times were made for each of the five animals (rats T2–T6), (A) during the 80% feeding condition and (B) during the free-feeding condition.

tendency for the 30 min sampling time (r=0.60, N.S.). Although behavioural tests were not conducted, previous work has shown that motor impairment does result with blood ethanol levels equal to the highest levels achieved in the present experiment (~150 mg%) [7]. Furthermore, all of the blood ethanol levels presently measured were probably sufficient to produce significant central nervous system effects since, for example, a similar range of blood ethanol levels altered the spontaneous firing rates of hippocampal neurons [4].

Taken together, these findings indicate that the amounts of ethanol consumed under the 80% feeding condition were sufficient to presume that a pharmacologically significant drug effect had resulted.

Free-Feeding Condition

Figure 1B shows the mean amount of 8% ethanol solution and water consumed by each rat during 12 sessions in which ethanol and water were alternated. Ethanol consumption continued to be significantly greater than water for rats T4, T5 and T6 ($t(5)=10.95, p<0.01$; $t(5)=4.79, p<0.01$; $t(5)=5.9, p<0.01$). However the ethanol and water consumption did not differ for rats T2 and T3 ($t(5)=0.28$, N.S.; $t(5)=2.39$, N.S.). An analysis of variance comparing the amounts of ethanol consumed during the 80% and the free-feeding conditions indicated that allowing the animals to regain body weight resulted in a significant reduction in ethanol consumption, $F(1,48)=8.26$, $p<0.01$. However Table 1 shows that the temporal distribution of drinking nevertheless remained similar to that in the 80% feeding condition, i.e., with most of the drinking taking place at the beginning of the drinking session.

The reduced drinking in the free-feeding condition was reflected in the blood ethanol levels obtained 15, 30 and 60 min after the initiation of drinking (Fig. 2B). Over half of the samples yielded no detectable ethanol concentrations, while those that did were low and only the 15 min sampling time showed a non-significant tendency when correlated with dose (15 min: r=0.71, N.S.; 30 min: r=−0.32, N.S.; 60 min: r=0.31, N.S.). Similar reductions in ethanol consumption following return to free-feeding body weights have been shown in experiments in which animals lever-press for the drug [12,13]. However, the notion that this phenomenon constitutes evidence that rats consume ethanol for its caloric value rather than for its pharmacological effects is still a matter for debate [1,8].

CONCLUSIONS

The results obtained indicate that this procedure can reliably establish ethanol as a reinforcer for rats. The amounts of drug self-administered are large enough to produce pharmacologically relevant blood ethanol levels and these levels can correlate significantly with the doses achieved.

The characteristics of the drinking behavior observed were very similar to those seen in the operant conditioning models from which this methodology was derived [10]. Thus, the temporal distribution of drinking was such that most of the ethanol was consumed at the beginning of the session and the return to free-feeding conditions brought about a reduction in the amount of ethanol consumed.

Since only a cage, a drinking tube, and a food cup are required as equipment, this method is very amenable to re-

search using large numbers or groups of animals. A study has recently been completed in our laboratory in which 30 animals were simultaneously trained to drink ethanol using this technique (Stewart and Grupp, manuscript in preparation). It is suggested that this method may be a viable alternative to the standard 24-hr access 2-bottle choice procedure in common usage.

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