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Oral Ethanol Reinforced Behavior in Inbred Mice

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ELMER, G. I., R. A. MEISCH AND F. R. GEORGE. *Oral ethanol reinforced behavior in inbred mice*. PHARMACOL BIOCHEM BEHAV **24**(5) 1417–1421, 1986.—The use of genetically defined animals in many areas of alcohol research provides valuable information about the contribution of genetic factors to ethanol-related behaviors. Utilizing the principles of operant conditioning, we determined whether mice which are known for high ethanol preference, C57BL/6J males, would orally self-administer this substance. Strategies used with other species were successful in inducing pharmacologically significant blood alcohol levels and in establishing ethanol as a reinforcer in this mouse strain. Responding for and consumption of 8% (w/v) ethanol exceeded baseline levels of responding for water. This species and method may prove useful in determining the genetic relationship among various ethanol-related behaviors and their mechanisms of action and in studies of behavior reinforced by drugs.

Behavior genetics Ethanol self-administration C57BL/6J mice Operant behavior Alcohol Inbred strains Ethanol preference

THE use of genetically defined animals in many areas of alcohol research has produced valuable information about the contribution of genetic factors to ethanol-related behaviors. A common example is that C57BL/6 mice prefer ethanol whereas DBA/2 mice avoid ethanol [5,17]. These studies provided the rationale for selectively breeding animals for ethanol related phenotyes. Several breeding programs to select for high and low ethanol intake have been successfully conducted in different laboratories [8, 15, 27]. Lines have also been selectively bred for high and low sensitivity to ethanol administered as an acute injection [1, 9, 18]. These studies give not only convincing evidence of the importance of genetic factors in response to ethanol but show that genetically defined animals are extremely valuable research tools for investigators interested in biological substrates of ethanol-related behaviors.

Studies utilizing animals selectively bred for ethanol-related phenotypes suggest that correlations exist among these behaviors. For example, high preference ratios and low sensitivity to acute doses of ethanol are highly correlated. The ALKO Alcohol Accepting (AA) and Alcohol Non-Accepting (ANA) rats, bred for differences in ethanol preference, also differ in acute sensitivity to doses of ethanol producing motor impairment and hypnotic effects [14, 16, 26]. Similar differences are obtained in sensitivity assessments using inbred mice ranked according to preference, such as the C57BL/6J and DBA/2J [6]. However, one limitation of many of these studies is the use of two bottle choice measures of ethanol preference. There is doubt concerning the validity of preference designs in measuring ethanol seeking behavior [4,7].

Another line of research using operant-techniques has demonstrated that ethanol, self-administered orally, can serve as a powerful reinforcer in rats, rhesus monkeys, and baboons [12, 13, 21]. As also seen in the preference studies, there are two problems in establishing ethanol as a reinforcer when it is taken orally. These are the aversive taste of ethanol concentrations above 6% (wt./vol.), and the delay between drinking ethanol and the onset of the interoceptive effects that follow absorption [24]. To overcome these difficulties, training procedures have been used which facilitate the establishment of ethanol as a reinforcer [19,21]. After these initial training procedures, animals will drink intoxicating amounts of ethanol in concentrations up to and including 32% (wt./vol.) in preference to water [11,20]. Importantly, self-administration techniques have also been successfully used to establish other orally delivered drugs as reinforcers for rats and rhesus monkeys [3, 22, 23].

Most studies of drug self-administration, in contrast to preference studies, have used within subject designs where little attention is given to the genotype of the subjects. Independent variables are often manipulated independently across animals. The need for a consistent population base is important and becomes more so if one is interested in elucidating the relationships among various drug-related behaviors and their biological substrates.

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The purpose of the present research was to initiate in mice the strategies successfully used in other species to establish ethanol as a reinforcer. In addition, by utilizing the principles of operant conditioning and pharmacogenetic analysis, to initiate a systematic investigation of the relationship between genetic factors and the conditions under which ethanol comes to serve as a positive reinforcer.

METHOD

Animals

Ten adult male C57BL/6J (Jackson Laboratories) mice, six months old and weighing approximately 27 g at the start of their training, were used. These animals were experimentally naive, housed individually in a temperature controlled room (26°C) with a 12-hr light-dark cycle (0700–1900 lights on), and given free access to Purina laboratory chow and tap water prior to initiation of the experiments.

Apparatus

Five mouse operant chambers were constructed from aluminum and clear Plexiglas with the floor comprised of a stainless steel mesh. Inside dimensions measured 165 mm L × 152 mm W × 127 mm H. Each cage was enclosed in a sound proof chamber constructed of plywood. The reservoir was mounted on the outside of the chamber. A small muffin type fan provided internal ventilation. The lever consisted of a balanced rocker arm designed to break an infrared photo beam with 0.5 g of force. A spout was used to deliver a minute amount of liquid in response to a lick. In this system, an electronic circuit senses the small current (resistance adjusted to 5.0 megohms) traveling from the brass spout, through the animal's body to the grounded cage floor. As the tongue contacts the spout tip, a solenoid valve is opened momentarily to deliver a droplet of liquid (approx. 2.0 μl/lick) directly onto the tongue. This delivery system was adapted from a system developed by Beardsley [2]. During sessions, the white house light was continually lit. Liquid deliveries were available on a heterogenous chain Fixed Ratio 1 (lever press) Fixed Ratio 1 (10) (spout contact) schedule of reinforcement; a single lever press allowed 10 reinforced spout contacts. The onset of stimulus lights above the spout signaled the second component of the chain during which spout contacts resulted in liquid delivery. Following the 10th lick the stimulus lights were turned off signaling the completion of the chain. A complete description of this system is provided elsewhere (George *et al.*, in preparation). System control and data aquisition were by solid state programmable modules (Coulbourn Instruments) located in an adjacent room.

Procedure

The procedure used to initiate lever pressing and drinking was similar to that successfully used in other species [21]. Mice were reduced to 80% of their free feeding weights by rationing their daily food allotment. Body weights were maintained at this level throughout the experiments. Sessions were run 7 days/week with 5 animals run per session in two successive 30 min periods. To induce drinking during the training procedure, water bottles were removed and the mice were given their food one hour prior to the start of the 30 min session. After the training period, all animals had free access to water in their home cage throughout the experiment. All sessions were run at Fixed Ratio size of 1. Once responding became stable, a series of increasing ethanol solutions (0.5%, 1.0%, 2.0%, 4.0% and 8.0% (w/v)) replaced water. Each solution was present for at least 5 consecutive sessions. Having established this concentration response curve, the ethanol concentration was maintained at 8.0%, and the amount of food before session was gradually decreased. Presession food initially averaged 2.3 g. Food was decreased, in order, to 1.0, 0.6, 0.3, 0.1 and 0 grams before session, the remainder being given after session. Each food condition was presented for five days. To determine if ethanol had come to function as a reinforcer 8.0% ethanol was again tested, followed by 0%(water), 8.0%, and 0% in that order.

TABLE 1

ETHANOL INTAKE (g/kg) AND POST-SESSION BLOOD ETHANOL LEVELS IN C57BL/6J MICE FOLLOWING SELF-ADMINISTRATION OF ETHANOL (n=10)

	8.0% (w/v) Food before session	8.0% (w/v) Food after session	RETEST 8.0% (w/v) Food after session
BEL	269 ± 21	199 ± 27	189 ± 17
Intake	5.60 ± 0.52	2.45 ± 0.32	2.40 ± 0.28

Blood ethanol levels (BEL) expressed in mg% (mean ± S.E.M.).

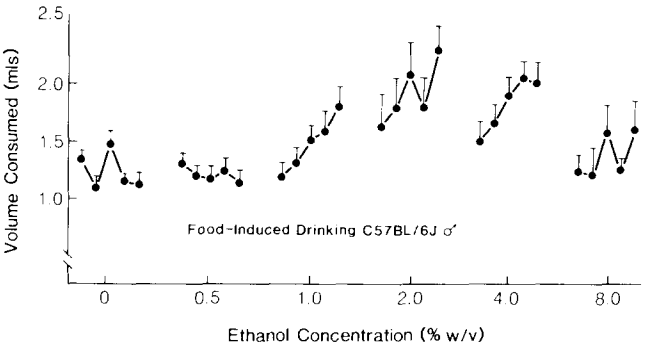

FIG. 1. Volume consumed as a function of increasing ethanol concentration during food-induced drinking phase in C57BL/6J male mice. Each point represents the daily session mean of 10 animals (±S.E.M.). Points represent consecutive test days within each concentration.

Blood Ethanol Assay

On the last or next to last day of each treatment condition duplicate 10 μl blood samples were obtained from the tail of each mouse after completion of the experimental session. An enzymatic assay based on the conversion of NAD to NADH during the oxidation of ethanol to acetaldehyde by the enzyme alcohol dehydrogenase was used. The blood samples were placed in 190 μl cold 0.55 M perchlorate, shaken, then centrifuged at 700 × g for five min. One hundred sevently μl of supernatant was removed and placed in a separate test tube to which 30 μl deionized water and 200 μl 0.222 M K_2CO_3 were added. The tubes were vortexed and

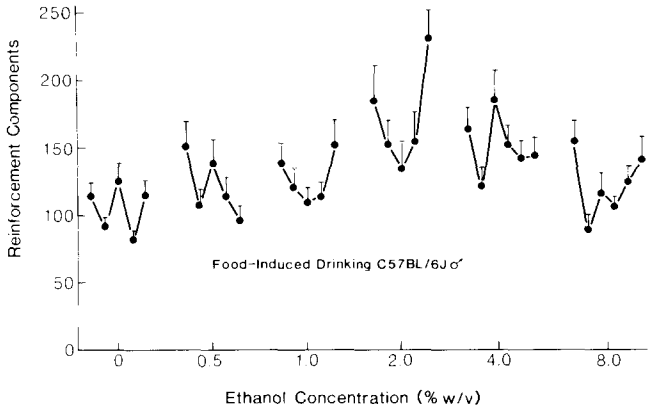

FIG. 2. Reinforcement components as a function of increasing ethanol concentration for food-induced drinking phase. One reinforcement component represents a lever press plus 10 licks in this hetetogeneous two component chain schedule of reinforcement. Volume of liquid consumed was linearly related to number of lever presses, r=.92. Each point represents the daily session mean of 10 animals (±S.E.M.). Points represent consecutive test days within each concentration.

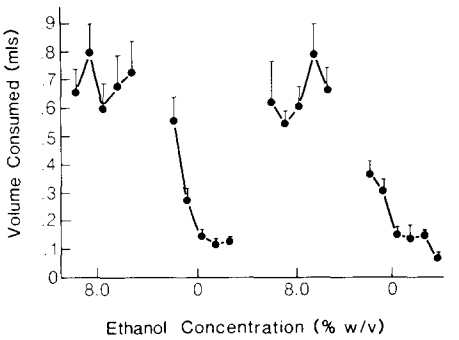

FIG. 3. Volume consumed as a function of the presence or absence of ethanol without food-inducement (daily food allotment given post-session). Repeated measures analysis of variance (ANOVA): Volume consumed, $F(3,27)=21.54$; $p<0.0001$. Dunnett's t, $df=16$: 8.0% vs. 0%=5.73, $p<0.01$; 8.0% vs. 8.0% retest=0.12, n.s.; 8.0% retest vs. 0% retest=5.48, $p<0.01$; 0% vs. 0% retest=0.37, n.s.

centrifuged for two min. Aliquots of 80 μl were removed in replicate and added to 640 μl cold 0.50 M Tris buffer at pH 8.8. Forty μl of 50 mM NAD^+ were next added, followed by 40 μl ADH (Sigma, 500 Units/ml). The samples were vortexed and then incubated at room temperature for at least one hour, then analyzed for the formation of NADH by measuring spectrophotometric absorbance at 340 nm. Samples were compared to standards which were made and analyzed concurrently with the samples.

RESULTS

Figure 1 shows the results of the food-induced drinking phase of this experiment. The training procedure was successful in obtaining the consumption of significant amounts of ethanol. As the ethanol concentration was progressively increased, the volume of liquid consumed did not significantly change. Thus, the absolute amount of ethanol consumed (g/kg) increased. The amount of ethanol consumed should have been sufficient to produce strong interoceptive effects, as indicated by the blood ethanol levels (BEL) and ethanol intakes seen during the presession food 8.0% condition (Table 1). At the higher ethanol concentrations the mice were often severely intoxicated, however this response was not quantified. Figure 2 shows the reinforcement components for the same food-induced drinking phase. One reinforcement component represents a lever press plus 10 licks in this heterogenous two component chain schedule of reinforcement. As the ethanol concentration was progressively increased, the number of responses remained stable. Volume of liquid consumed was linearly related to number of lever presses, r=.92.

When food was moved to post-session availability, the mice continued to respond for 8.0% ethanol, but at a decreased level, as seen in Figs. 3 and 4. The number of responses and volume consumed were significantly higher than the subsequent 0% (water) condition. Most responding for ethanol occurred at the beginning of the session, creating a negatively accelerated response pattern as shown by the cummulative records in Fig. 5. Additional retests at 8.0% and

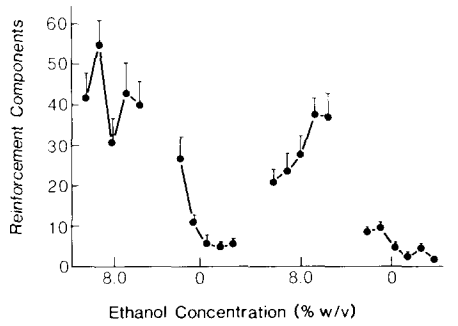

FIG. 4. Reinforcement components as a function of ethanol concentration without food-inducement (daily food allotment given post-session). Volume of liquid consumed was linearly related to number of lever presses, r=.93. Repeated measures analysis of variance (ANOVA): Reinforcement components, $F(3,27)=52.7$; $p<0.0001$. Dunnett's t, $df=16$: 8.0% vs. 0%=9.38, $p<0.01$; 8.0% vs. 8.0% retest=2.53, n.s.; 8.0% retest vs. 0% retest=7.96, $p<0.01$; 0% vs. 0% retest=1.11, n.s.

0% confirmed the previous findings. Responding for and consumption of 8% ethanol exceeded baseline levels of responding for water.

DISCUSSION

The present results indicate that the strategies successfully used in other species [12, 13, 21] to establish ethanol as a reinforcer were also effective in male C57BL/6J mice. Post-prandial drinking procedures were utilized in order to expose the animals to the interoceptive effects of ethanol. This involves gradually increasing the concentration of ethanol in order to avoid aversive reactions to the higher ethanol concentrations. Under these conditions of food-induced drinking, responding did not significantly change as the concentration of ethanol was increased from 0% to 8.0%. As a consequence the amount of ethanol (g/kg) consumed increased as ethanol concentration was increased. At 8% ethanol with food presession, the mice had blood ethanol concentrations in excess of 250 mg% and showed obvious

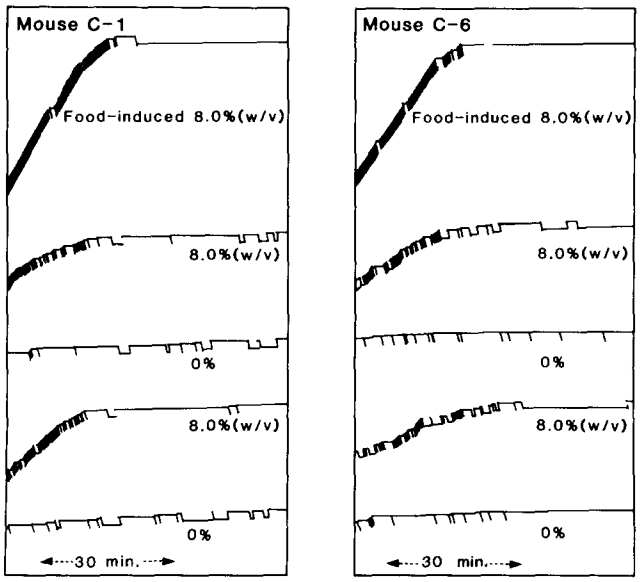

FIG. 5. Cumulative records of two mice, C-1 and C-6, across food-induced drinking period and post-session food availability at 8.0 and 0 (% w/v) ethanol concentrations. Most responding for ethanol occurred at the beginning of the session. Note typical negatively accelerated response curves.

signs of ataxia, indicating that these animals had become exposed to the interoceptive effects of ethanol without aversive consequences. When drinking was no longer induced by presession food, consumption of 8.0% ethanol decreased significantly. However ethanol drinking did persist and substantially exceeded water levels. Moreover, pharmacologically relevant post-session blood ethanol levels were obtained. Since most ethanol drinking occurred at the start of the 30 min sessions these samples provided a conservative estimate of circulating ethanol levels. When water replaced ethanol, classical extinction curves were noted as well as reaquisition curves when ethanol was once again reintroduced. These findings are consistent with ethanol serving as a postivie reinforcer. Since blood ethanol levels were not elevated for extended periods of time physical dependence was not a factor in maintaining responding. These results complement data obtained with preference procedures and extend the range of conditions over which C57BL/6J mice will consume ethanol.

Throughout this experiment, the animals were maintained at reduced body weights. It is commonly accepted that food deprivation increases intake of drugs such as ethanol and other drugs without caloric value such as cocaine, amphetamine, pentobarbital and etonitazine [3,21]. However, recent work suggests that while food deprivation enhances drug intake in animals genetically predisposed to accept a particular drug as a reinforcer, in animals which are not predisposed toward this effect, food deprivation does not increase drug intake [10,25]. Therefore, the increase in ethanol intake during food deprivation cannot be attributed simply to caloric factors, since animals genetically selected for low ethanol intake do not increase intake under the sole condition of food deprivation.

Few studies of ethanol drinking have correlated blood ethanol levels with the amount of ethanol consumed. Ethanol intake is usually inferred by measuring the liquid volume change in a reservoir. Many factors can affect the relationship between ethanol ingestion and blood or brain ethanol levels, including the pattern of intake as well as the concentration of the solution, route of administration, genotype, sex, age, feeding condition and history of the subject. Thus, determination of actual circulating ethanol levels is critical to an accurate understanding of the conditions under which ethanol comes to serve as a reinforcer. The blood ethanol levels seen in the present study following food-induced drinking, and following testing of ethanol's reinforcing efficacy were significantly elevated and are consistent with strong interoceptive effects.

The relationship between self-administration of a drug and preference for that substance in a choice situation is unclear. Preference studies have generally shown that animals will not consistantly consume intoxicating levels of ethanol. Another concern with preference studies is that if the pharmacological actions of ethanol are reinforcing, the temporal pattern of drinking should be related to maintenance of elevated BEL. Even high-preferring strains such as the C57BL/6J mice distribute their ethanol intake over time such that they usually avoid achieving BEL in excess of 150 mg% [7]. However, when given the opportunity to work for ethanol within a limited period of time, our results show that these mice will consistently drink enough ethanol to experience interoceptive effects.

The genetic correlations seen between ethanol preference and various acute measures of ethanol sensitivity and the successful incorporation of selective breeding methods in producing several divergent populations with regards to ethanol-related phenotypes provides not only evidence for genetic control of these characters but also suggests that there are some common underlying mechanisms which mediate an organism's overall response to ethanol. Utilizing the data base which genetically defined animals provide is important in operant paradigms because it places self-administration studies within a conceptual framework important to the development of a complete understanding of the factors involved in ethanol drinking. We have currently shown that mice which show a high ethanol preference ratio will self-administer ethanol and that it will come to serve as a positive reinforcer. This is an important step in interpreting preference data and will become increasingly useful as more strains are involved in operant paradigms.

This study demonstrates the experimental control possible with the use of genetically defined animals, even when complex operant behaviors are being measured. The use of an inbred mouse strain is valuable in establishing a data base so that factors relating to ethanol self-administration can be systematically investigated within the constraints of specified genetic and environmental conditions. The study of ethanol intake in operant situations using genetically defined animals whose preference for and sensitivity to ethanol are known should serve to integrate findings from other areas of alcohol research with findings from the area of ethanol reinforced behavior. In addition, this study also suggests that mice can be appropriate subjects for use in studies of behavior reinforced by drugs and that inbred mouse strains may aid in the analysis of genetic determinants of drug reinforced behavior.

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