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Genetic and Environmental Factors in Ethanol Self-Administration

FRANK R. GEORGE[1]

Behavior Genetics Laboratory, Preclinical Pharmacology Branch, National Institute on Drug Abuse
Addiction Research Center, Box 5180, Baltimore, MD 21224
U.S. Department of Health and Human Services, Public Health Service
Alcohol, Drug Abuse and Mental Health Administration
and Department of Pharmacology and Toxicology, School of Pharmacy
University of Maryland, Baltimore, MD 21201

GEORGE, F. R. *Genetic and environmental factors in ethanol self-administration.* PHARMACOL BIOCHEM BEHAV 27(2) 379–384, 1987.—Findings presented in this paper from pharmacogenetic studies of oral ethanol self-administration suggest a correlation between ethanol preference and self-administration and indicate that there are important genetic as well as environmental determinants of ethanol reinforced behavior. AA (alcohol accepting) and ANA (alcohol non-accepting) rats, animals bred selectively for differential ethanol preference, showed large differences in operant responding for ethanol. AA rats drank significantly more ethanol than water, and their intake varied as a function of etahnol concentration. Intake of water and ethanol solutions did not differ in the ANA rats. In two inbred strains of rats, F344 and LEWIS, ethanol maintained higher response rates and was consumed in larger volumes than the water vehicle. In a third series of studies, C57BL/6J mice, which exhibit high ethanol preference and low sensitivity, readily self-administered ethanol in an operant situation. Conversely, BALB/cJ mice, which exhibit low preference and high sensitivity, were not positively reinforced by ethanol. The results demonstrate the experimental control possible by the utilization of genetically defined animals, even when complex learned behavioral sequences are being measured, and indicate that genotype and environment interact in a complex but definable way to determine the degree to which ethanol comes to function as a positive reinforcer.

Ethanol self-administration Operant behavior AA and ANA rats LEWIS and F344 rats
C57BL/6J and BALB/cJ mice Behavior genetics

MOST animal studies of ethanol drinking have used the two-bottle choice technique developed by Richter and Campbell in 1940 [30]. Volitional choice in this particular paradigm has been likened to voluntary ethanol consumption in humans [13]. In general, many rats and mice will consume ethanol in concentrations under 8% (w/v) [2,22], but intoxication is not reliably observed, and the sustained blood ethanol levels that are necessary for physiological dependence do not occur [4]. An important step in determining the substrates of ethanol abuse has been examination of genetic factors in ethanol-seeking behaviors. The two-bottle choice method was introduced into the area of experimental pharmacogenetics in the early nineteen sixties [18,19]. A common example is that C57BL/6J mice prefer ethanol, whereas BALB/cJ mice avoid ethanol [18,19]. Typically, controversy has been a characteristic of debates about the relationship between two-bottle choice preference for a drug and drug-reinforced behavior, since in the preference studies there is little convincing evidence that animals are administering ethanol for its post-absorption interoceptive properties. However, intravenously administered ethanol has been shown to decrease free-choice ethanol consumptiom in P (preferring) rats [36,37], suggesting that ethanol drinking behavior is controlled by blood ethanol levels and CNS effects. One difficulty encountered in preference studies is that the methods, while relatively simple, are not readily modified to examine in detail the various environmental factors which may contribute to the establishment and maintenance of ethanol preferring behaviors.

A different approach to conceptualizing and measuring ethanol drinking grew out of studies of drug reinforced behavior. In the 1960s procedures were devised so that laboratory animals could intravenously inject drugs into themselves [3, 35, 38]. In general, animals self-administer the same drugs that humans abuse, and they do not self-administer drugs that humans do not abuse [11]. In these operant designs, drug-seeking behavior is conceptualized as a specific instance of operant behavior. Under these conditions, ethanol as well as drugs from several other pharmacological classes have been shown to act as positive reinforcers for animals [1, 10, 24, 25, 29, 39].

Two problems in establishing ethanol as a reinforcer when it is taken orally are the aversive taste of ethanol concentrations above 6% (w/v) and the delay between drinking

[1]Requests for reprints should be addressed to Dr. Frank R. George, NIDA Addiction Research Center, Box 5180, Baltimore, MD 21224.

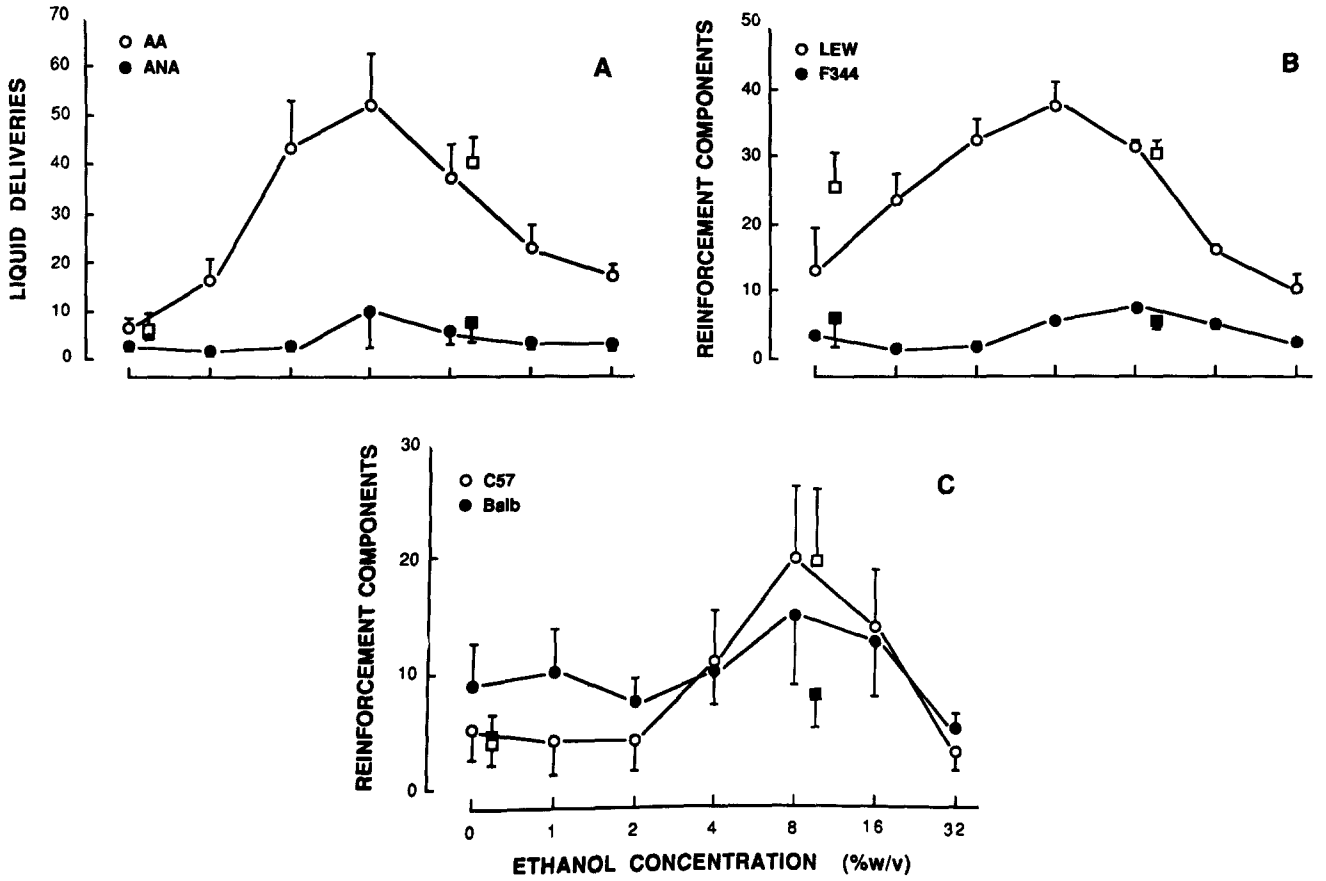

FIG. 1. (A) Mean liquid deliveries obtained by AA and ANA rats on a FR 1 schedule of reinforcement as a function of ethanol concentration available during one-hour test sessions. Each point represents the condition mean (±S.E.M.) for seven rats. Repeated measures ANOVA: F(Genotype)=14.82, $p<0.002$; F(Concentration)=14.27, $p<0.0001$; ANA F=1.09, n.s.; AA F=16.39, $p<0.0001$. The ANA rats did not increase their level of responding above baseline levels at any of the concentrations tested. (B) Number of lever press responses per 1 hr sessions as a function of ethanol concentration for LEW and F344 rats. One reinforcement component represents one lever press activating the spout for 20 licks of 5.0 μl liquid. Each point represents the condition mean (±S.E.M.) of four animals. Brackets indicate the S.E.M. Across concentrations the LEW rats consumed more ethanol than the F344 rats, F(strain)=49.83, $p<0.0005$. Blood ethanol levels were a biphasic function of ethanol concentration in both strains, but were significantly higher in the LEW rats than in the F344 rats, F(strain)=305.37, $p<0.0001$. (C) Reinforcement components as a function of increasing ethanol concentration. One reinforcement component represents one lever press activating the spout for 10 licks of 2.0 μl liquid. Each point represents the condition mean (±S.E.M.) of ten C57BL/6J and nine BALB/cJ animals over a minimum of four sessions. If no standard error bar is present, S.E.M. fell within the confines of the symbol. Dunnett's t (one-tailed), C57BL/6J, $df=11$: 0% vs. 4%=2.09, n.s.; 0% vs. 8%=5.30, $p<0.01$; 0% vs. 16%=3.07, $p<0.05$; 0% vs. 32%=0.70, n.s.; 0% vs. 0%(retest)=0.33, n.s.; 8% vs. 8%(retest)=0.02, n.s. A significantly greater number of lever presses were made by the C57 mice at 4%, 8%, and 16%. The number of lever presses then decreased, as ethanol concentration was further increased. Post-session blood samples contained the highest BEL at 8%. (C) also shows the pattern of responding in the BALB/cJ mice. These mice did not increase their level of liquid intake above baseline levels at any of the concentrations tested. BALB/cJ, $df=9$; 0% vs. 4%=0.33, n.s.; 0% vs. 8%=2.15, n.s.; 0% vs. 16%=1.22, n.s.; 0% vs. 32%=1.61, n.s.; 0% vs. 0%(retest)=1.65, n.s.; 8% vs. 8%(retest)=2.38, $(0.10>p>0.05$.

ethanol and the onset of the interoceptive effects that follow absorption [27,28]. These problems are obstacles for both 2-bottle choice and operant self-administration studies. However, after appropriate training [20], animals from several species will drink ethanol in concentrations as high as 32% (w/v) in preference to water [12,23]. The use of these methods is not limited to ethanol, since other drugs can be established as orally effective reinforcers with these procedures [1, 24, 25].

Oral self-administration procedures utilizing the operant paradigm combine several key criteria necessary for an effective animal model of alcohol abuse, including an oral route of administration, precise manipulation of environmental factors and the possibility of maintaining adequate blood levels to produce tolerance and physical dependence. However, the focus in self-administration studies has been to determine the environmental conditions important in the initiation, persistence and pattern of drug-seeking behaviors. These experiments generally have used a limited number of genetically undefined subjects, and experimental conditions are often varied independently across subjects. Individual differences found in these studies have generally been attributed to differences in training and subject history, or often ignored. It is possible that individual differences in ethanol sensitivity, in metabolism of ethanol, and in the reinforcing effects of ethanol may have given rise to this variability in results, decreasing the predictive value of these studies and limiting the potential for defining possible mech-

anisms of action. By utilizing the principles of both operant conditioning and pharmacogenetic analysis, our laboratory has been involved in systematic investigations into the genetic and environmental conditions under which ethanol comes to serve as a positive reinforcer.

The purpose of the present paper is to report results which demonstrate the differences between several genetically distinct rodent stocks in the reward efficacy of ethanol over a wide range of ethanol concentrations. Six genetically distinct rodent lines were used in these experiments, including the ALKO AA (alcohol-accepting) and ANA (alcohol non-accepting) rat lines, the LEWIS (LEW) and Fischer 344 (F344) inbred rat strains, and the C57BL/6J (C57) and BALB/cJ (BALB) inbred mouse strains.

The AA and ANA rat lines were derived from a heterogeneous foundation stock in a two-way selection for high and low ethanol drinking. The selection criterion was the preference ratio of 10% (v/v) ethanol to total daily liquid intake, adjusted for body weight and total daily caloric intake [6,7]. The AA rat preferring lines consume almost as much ethanol in a free choice situation as they can metabolize, while the non-preferring animals drink very small amounts of ethanol. The LEW and F344 rat strains were selected because they have a long history of genetic divergence and are readily available from commercial vendors. In addition, much is now known about alcohol related effects in these strains [8, 15, 17]. Similarly, the C57BL/6J and BALB/cJ mouse strains have a genetically divergent history as well as documented differences in ethanol preference and sensitivity [18].

In contrast to most previous studies of genetic effects on ethanol drinking, the environmental control and manipulations made possible through the use of operant conditioning procedures were used, and in contrast to most previous operant studies, genotype was controlled and included as an independent variable. Ethanol intake was compared across a broad range of ethanol concentrations. In addition, ethanol consumption was verified by determining blood ethanol levels.

METHOD

Animals

Eight each AA and ANA adult male rats (ALKO Laboratories, Helsinki), fourteen weeks old at the start of their training were used. Ten adult male C57BL/6J mice and ten BALB/cJ mice (Jackson Laboratories), six months old at the start of their training were used. Adult (11 weeks) LEW (LEW/CRLBR) and F344 (CDF(F-344)CRLBR) male rats were obtained from Charles River Laboratories, Wilmington, MA. All 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

The operant chambers were constructed from aluminum and clear Plexiglas with the floor comprised of a stainless steel mesh. Each cage was enclosed in a sound proof chamber. A small muffin type fan provided internal ventilation. During sessions, a white house light was continually lit.

In this system a spout was used to deliver a minute amount of liquid in response to a lick. 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 (2.0 μl/lick for mice, 5.0 μl/lick for rats) directly onto the tongue. A reservoir was mounted on the outside of the chamber.

Procedure

Daily training and testing sessions were one hour in length for rats and 30 min for mice. All animals were run on a Fixed Ratio 1 (FR 1) schedule such that one lever press resulted in the presentation of liquid containing water or an ethanol solution. For each lever press, AA and ANA rats received a 0.11 ml dipper of liquid, LEW and F344 rats were allowed 20 licks from a spout which delivered 5.0 μl per lick, and C57BL/6J and BALB/cJ mice were allowed 10 licks from a spout which delivered 2.0 μl per lick. The rats and mice were initially reduced to 75% and 80%, respectively, of their free-feeding weights and ethanol was gradually introduced to the animals postprandially.

The procedures used to initiate lever pressing and drinking have been fully described elsewhere [5,29]. Session duration was 30 min for the mice and 1 hour for the rats. To induce drinking, water bottles were removed and the animals were given their food prior to or during the experimental session. Liquid deliveries were available on a continuous reinforcement schedule. That is, a single lever press either activated a dipper in the case of the AA and ANA rats, or allowed a fixed number of reinforced spout contacts in the case of the other animals. The illumination of stimulus lights above the delivery system signaled that liquid was available. After this training period, all animals had free home cage access to water for the remainder of the experiment.

Once food-induced responding became stable, a series of increasing ethanol solutions, in the order 1%, 2%, 4% and 5.7% (w/v) (8% for the mice), replaced water. Each solution was present for at least 4 stable sessions. Within each genotype no significant differences were seen in the volumes of ethanol solutions and water consumed (μl/g body weight). Thus, the absolute amount of ethanol consumed (g/kg) increased as the concentration of ethanol was increased.

To determine if ethanol had come to function as a reinforcer, ethanol drinking behavior was next tested in the absence of food-inducement, and the animals received all of their daily food allowance after the sessions. After baseline levels of responding for water were established, concentration-response curves for these animals were determined by substituting 1%, 2%, 4%, 8%, 16% and 32% (w/v) solutions of ethanol for water. Each concentration was available in sequence, for at least four consecutive daily sessions. Retest conditions at 8% and 0% were also performed. On the last day of each treatment condition replicate 10 μl tail blood samples were obtained at the end of the experimental session and blood ethanol concentrations (BEC) were determined as previously described [15].

RESULTS

Figure 1A indicates that the ethanol-reinforced behavior exhibited by the AA rats was an inverted U-shaped function of concentration, consistent with ethanol serving as an effective reinforcer. These animals maintained BEC of approximately 100 mg/dl when given access to 4%, 8%, 16% or 32% ethanol in the test sessions. Response levels for retest con-

ditions were not different from those for initial test conditions indicating no changes in baseline levels of responding and no development of tolerance. The ANA rats did not increase their level of responding above baseline levels at any of the concentrations tested, suggesting that ethanol did not function as a reinforcer for this genetic stock.

Figure 1B shows that the number of ethanol deliveries in both the LEW and F344 was an inverted U-shaped function of ethanol concentration, although the F344 data is less robust. Across concentrations the LEW rats consumed more ethanol than the F344 rats, $F(\text{strain})=49.83$, $p<0.0005$. For both strains the quantity consumed (mg per 100 g body weight) increased with increases in the ethanol concentration. Blood ethanol levels were a biphasic function of ethanol concentration in both strains, but were significantly higher in the LEW rats than in the F344 rats, $F(\text{strain})=305.37$, $p<0.0001$.

The pattern of intake exhibited by the C57BL/6J mice is also an inverted U-shaped curve, as depicted in Fig. 1C. Liquid intake (μl/g body weight) increased as ethanol concentration increased until 8%, after which intake declined slightly. These animals consumed a significantly larger amount of liquid at 4%, 8% and 16% versus vehicle (0%). A significantly greater number of lever presses were made at 4%, 8%, and 16%. The number of lever presses then decreased, as ethanol concentration was further increased. Post-session blood samples contained the highest BEL at 8%. The amount of liquid consumed per gram body weight during retest conditions was not different from those found in the initial test conditions at both 8% and 0% as measured by volume consumed or BEL.

Figure 1C also shows the pattern of intake in the BALB/cJ mice. The BALB/cJ mice did not increase their level of liquid intake above baseline levels at any of the concentrations tested. As a consequence, ethanol intake (g EtOH/kg), did increase as ethanol concentration was increased. The highest session intake (g EtOH/kg) occurred at 16%. At 32%, the amount of liquid consumed per gram body weight was significantly lower than baseline level. The amount of liquid consumed per gram body weight during retest conditions was not different from that found in the initial test conditions at both 8% and 0% for volume.

DISCUSSION

An important consequence of the food-induced drinking procedure was that sufficient ethanol was ingested so that all animals were exposed to the interoceptive effects of ethanol. The blood ethanol levels confirmed that substantial intake of ethanol occurred, and the levels obtained are consistent with other acute studies showing behavioral changes, for example open field activation, at these ethanol levels [14,34]. Significantly, it was possible to induce ethanol drinking in the ANA rats and BALB mice. These animals drank equal volumes of ethanol solutions and water postprandially, suggesting that ethanol is not aversive to these animals due to the drug's taste or smell.

The results from the present experiment demonstrate large strain differences in ethanol self-administration. Ethanol has been established as a reinforcer in the AA rats but not in the ANA rats. Also, ethanol was established as a strong positive reinforcer in the Lewis rats but only as a weak reinforcer in the F344 strain. Finally ethanol came to serve as a reinforcer for C57 mice but not for BALB mice. These findings complement earlier data obtained with preference procedures and extend the range of conditions over which ethanol intake has been shown to be controlled to a significant extent by genetic factors.

A consistent finding in drug self-administration studies is an inverted U-shaped function between number of drug injections or deliveries and the size of the drug dose or concentration [21, 26, 32, 33]. As drug dose or concentration is increased, the number of drug deliveries first increases and then decreases. In the present work, the pattern of intake across concentrations for those rodents in which ethanol was established as a positive reinforcer was also a typical inverted U-shaped curve. An inverted U-shaped function was not obtained with the F344 rats, although the quantity of ethanol consumed (mg/100 g body weight/hr) increased with increases in the ethanol concentration. The slope of the functions for quantity consumed and for blood ethanol levels were greater for the LEW and AA rats as well as C57 mice than for the F344 rats. These results suggest that ethanol functions as a weak reinforcer only under certain conditions for the F344 rats.

Many findings in the present study support the concept that genetic factors are important determinants of ethanol reinforced behavior. The measurement of ethanol drinking over a range of conditions makes it very unlikely that the differences are due to using conditions that are optimal for one genetic stock but not for another. These findings of a strong role for genetic factors in ethanol-reinforced behavior indicate that genetic factors may also be important determinants of behavior reinforced by other drugs.

ANA animals did not consume ethanol under conditions of food deprivation in a manner similar to the AA rats, which experienced identical treatment histories. Even food deprivation to maintain reduced body weight did not facilitate the establishment of ethanol as a reinforcer for these animals. The present data indicate that genotypes known to differ in preference ratios for ethanol in a two-bottle choice paradigm also show large differences in operant behavior maintained by ethanol. These data, taken together with similar evidence from the P and NP rats [35], as well as other data from the AA and ANA rats [31], the C57 and BALB mice [5] and the LEW and F344 rats [34], indicate that ethanol preference in the two-bottle choice situation and ethanol self-administration under operant conditions have at least some common underlying mechanisms. Most other studies examining genetic influences in response to ethanol have measured simple phenotypic or physiological measures, thus the present work is important because it extends previous preference data to more complex ethanol related phenotypes. Furthermore, the current data suggest that the animal preference model of ethanol drinking provides some information, within theoretical limits, about the reward efficacy of ethanol. However, since the preference model may describe ethanol drinking behavior only under somewhat specific environmental conditions, it may not be analogous to or predictive of the tendency for humans to abuse ethanol. Operant methodology, though not without some experimental problems, may better facilitate manipulation of the many environmental factors which influence the rewarding properties of ethanol.

The results of these studies are also significant in that it is commonly accepted that food deprivation increases intake of ethanol and other drugs, including amphetamine, pentobarbital and etonitazine [1,21]. However, these genetic studies suggest that food deprivation will enhance drug intake only in those animals genetically predisposed to accept a particular

drug as a reinforcer [9]. The importance of these findings is that the increase in ethanol intake during food deprivation cannot be attributed simply to caloric factors, since animals genetically selected for low ethanol intake did not increase intake under the condition of food deprivation.

In conclusion, the fact that complex, learned operant behaviors are sensitive to genetic factors suggests that future studies on drug reinforced behavior either control for or utilize genetic influences on behavior. Elucidation of the biological substrates which are responsible for genetic differences in the reinforcing effects of ethanol would greatly contribute toward our understanding of alcohol and drug abuse.

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