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BRIEF COMMUNICATION

# Chronic Ethanol Tolerance Through Free-Choice Drinking in the P Line of Alcohol-Preferring Rats[1]

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GATTO, G. J., J. M. MURPHY, M. B. WALLER, W. J. McBRIDE, L. LUMENG AND T.-K. LI. *Chronic ethanol tolerance through free-choice drinking in the P line of alcohol-preferring rats.* PHARMACOL BIOCHEM BEHAV **28**(1) 111–115, 1987.—The objective of this study was to determine if the selectively bred P line of alcohol-preferring rats would develop behavioral (neuronal) tolerance with free-choice drinking of ethanol. Adult, male P rats were divided into four groups. One group (FCE) received food, water and a 10% (v/v) ethanol solution ad lib, while the control group (C) had only food and water. The other two groups received either a liquid diet containing 5% (v/v) ethanol (LDE) or a control liquid diet (LDC). All groups were kept on their respective feeding regimens for 14 days. The mean (±SEM) ethanol intakes for the FCE and LDE groups were 6.8±0.5 and 9.9±0.4 g ethanol/kg body wt./day, respectively. A shock-motivated jumping task was used to test for tolerance. Each rat received an IP injection of 2.5 g ethanol/kg and was tested every 15 minutes for recovery to a criterion of 75% of the performance level achieved with training. All rats were tested twice, once on the day before beginning their feeding regimens (day 0) and again 14 days later. Tolerance was assessed from differences in time of recovery to criterion performance and in blood alcohol concentrations (BACs) at recovery on day 0 vs. day 14. The mean recovery times for the C, FCE, LDC, and LDE groups on day 0 were 177±6, 170±6, 143±10 and 153±13 minutes, respectively, and the BACs were 219±6, 222±5, 220±19 and 214±6 mg%, respectively. On day 14, the FCE and LDE groups exhibited tolerance with shorter recovery times of 80±7 and 70±9 minutes and higher BACs at recovery of 273±5 and 286±14 mg%, respectively. No significant differences in time of recovery or BACs were observed between days 0 and 14 for the C and LDC groups. The results demonstrated that free-choice consumption of 10% ethanol by P rats is sufficient to produce behavioral/neuronal tolerance which is similar in magnitude to that seen for P rats given ethanol in a liquid diet.

Alcohol-preferring rats Free-choice drinking Behavioral tolerance Chronic ethanol tolerance Blood alcohol concentrations

THE development of tolerance to the intoxicating effects of ethanol is a prominent feature of alcoholism [12]. It is hypothesized that the development of chronic tolerance contributes to continued excessive intake of alcohol and to eventual dependence. Accordingly, among the criteria for an adequate animal model of alcoholism is the requirement that the chronic voluntary intake of ethanol should produce functional or behavioral (neuronal) tolerance [3,11].

Methods designed to make an animal tolerant to ethanol are well established, e.g., inhalation, intubation, and consumption in liquid diets [1,11]. However, the animal is made tolerant forcibly with these techniques. A more desirable approach and one analogous to human self-administration is the voluntary consumption of alcohol in the presence of food and water ad lib. The problem with this free-choice approach is that only a small percent of most common stock rats would voluntarily consume quantities of alcohol deemed necessary to induce tolerance (5–8 g ethanol/kg body weight/day). To overcome this problem, two alcohol-preferring rat lines have been independently raised by means of selective breeding.

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One developed by the Research Laboratories of the State Alcohol Monopoly in Helsinki, Finland (Alko), is called the AA line [2] and the other, raised in our laboratory, is the P line of alcohol-preferring rats [5,6].

Studies have shown that rats of the P line satisfy virtually all of the perceived criteria for an animal model of alcoholism. In a free-choice situation, with food, water and 10% (v/v) ethanol available, P rats consistently consume more than 5.0 g ethanol/kg/day [5–7], an amount which approaches their maximum capacity for ethanol elimination [4]. With operant responding in the presence of unlimited availability of food and water, the P rats work to obtain ethanol [10], and enhancement of ethanol intake occurs with bar-pressing for ethanol as reward when compared with ethanol intakes observed in the free-choice situation [9]. In addition, P rats have the capacity to develop acute tolerance to the depressant effects of ethanol [19], exhibit increased locomotor activity with low doses of ethanol [20], consume ethanol for its rewarding post-ingestive effects [16,17], and develop physical dependence when given the opportunity to drink ethanol over a prolonged period of time [18].

Some evidence suggestive of tolerance development had been obtained in the past for P rats given free-choice consumption of 10% ethanol [5]; however, the present study is the first rigorous demonstration of chronic tolerance. Since the development of tolerance with chronic consumption is an important criterion for an animal model of alcoholism and may be an important component of the genetic predisposition to alcoholism, the present study examined whether the genetically selected P rats would develop chronic tolerance through free-choice drinking, and whether this developed tolerance is similar in degree to that seen through forced consumption of ethanol in a liquid diet.

## METHOD

### *Animals*

Thirty-two adult, male rats of the alcohol-preferring P line [6,7] from the S-23 generation were used. The rats weighed 250–500 g at the beginning of the study. Animals were individually housed in a temperature- and humidity-controlled environment with a normal 12 hour day-night cycle beginning at 0600 hours. During a two-week acclimation period, the rats were given only food (Purina Lab Chow, No. 5001) and water ad lib.

### *Diets*

Following the acclimation period, the animals were divided into four groups. The control (C) and free-choice ethanol (FCE) groups (n=10/group) were matched for body weights, as were the liquid diet control (LDC) and liquid diet ethanol (LDE) groups (n=6/group). The C group continued on the normal diet of food and water ad lib. To assure that the FCE group would quickly attain stable peak intakes, these rats received 10% (v/v) ethanol as the sole source of fluid for four days, followed by 10 days of free-choice of the 10% ethanol and water. Food was provided ad lib throughout the 14 days. The LDE group was given ad lib a liquid diet containing 5% (v/v) ethanol (Bio-Serv, Inc.). The diet consisted of 36% of the total calories as ethanol, 18% as protein, 35% as fat and 11% as carbohydrates. The LDC group received a control liquid diet which had 47% of the total calories as carbohydrates, 18% as protein and 35% as fats. The caloric densities of both liquid diets were 1 kcal/ml. The LDC group was pair-fed to the LDE group, i.e., the LDC group received the volume of diet that the paired LDE animal consumed the previous day. If the entire ration was presented once daily, rats restricted to the LDC condition often would consume the total allotment within the first few hours of presentation. Accordingly, in order to more closely approximate the usual pattern of consumption of the LDE group, the allotted diets for the LDC rats were divided into three "portions." This feeding regimen consisted of presenting 25% of the diet at 1000 hours, 25% at 1600 hours, and the remaining 50% at 2200 hours.

It should be noted that the LDE group, in accordance with the recommended schedule for administering the liquid diet as suggested by Bio Serv, Inc., spent the first seven days in the process of being accustomed to the diet. In the first three days, the LDE rats were given a liquid diet consisting of $^2/_3$ control diet and $^1/_3$ ethanol diet. On days 4 through 7, the diet consisted of $^1/_3$ control and $^2/_3$ ethanol diet. Beginning on day 8, the rats were given the complete ethanol diet. The rationale for acclimating the rats to the ethanol diet was to avoid the possibility that the animal would refuse to consume the diet when presented in its undiluted form immediately.

### *Assessment of Tolerance*

A descending jumping platform similar to that described previously [8, 15, 19] was used to assess behavioral tolerance. Rats were individually placed on the grid floor of the apparatus and had two seconds to jump onto a platform to avoid a 0.5 mA constant-current AC scrambled footshock. After two seconds, the shock was activated and the motorized platform began to descend at a rate of one cm/sec. The rat could then escape shock by jumping onto the descending platform. The height jumped was recorded when the rat had grasped the top of the platform with at least three paws. Animals were trained in the apparatus during the two-week acclimation period. Each rat received daily sessions of five trials with one minute intertrial intervals. The platform was initially set at 10 cm, and was raised by 10 cm intervals each day until a height of 50 cm was attained. By the tenth day of training, all rats avoided the shock and jumped to a criterion of 50 cm on every trial. Training and subsequent testing on the apparatus were performed between 1200–1700 hr.

On day 0, food but not water was removed at 0600 hours, and control values for the assessment of tolerance were obtained by giving the trained rats a single intraperitoneal injection of 2.5 g ethanol/kg body wt. Following injection, the rats were tested every 15 minutes on the apparatus for recovery of the ability to jump 37.5 cm (75% of the pre-test height of 50 cm). At recovery to this criterion, blood was drawn from the retro-orbital sinus for the determination of alcohol content (BAC). Fourteen days later, the rats receiving ethanol had the ethanol removed at 0300 hours. Between 0300–0600 hours, both the LDC and LDE groups received equivalent volumes of the control diet (approximately 20 ml). Subsequently, all four groups were subjected to the same testing paradigm as on day 0. The rats were not exposed to the jumping apparatus between days 0 and 14. The injection of test doses of ethanol, the testing of jumping performance, and the chronic feeding of the animals were conducted in different rooms. The ethanol for injection was a 12% (w/v) solution in saline.

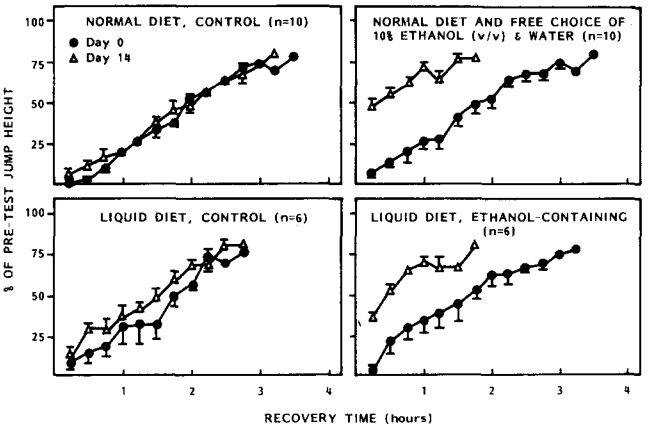


FIG. 1. Jumping performances for control (normal diet and liquid diet) and chronically ethanol-exposed P rats (free-choice and liquid diet containing ethanol) following an injection of 2.5 g ethanol/kg body weight. Data are the means±SEM of the number of animals indicated in parentheses, except for points approaching the 75% recovery since individual animals met the criterion at different times.

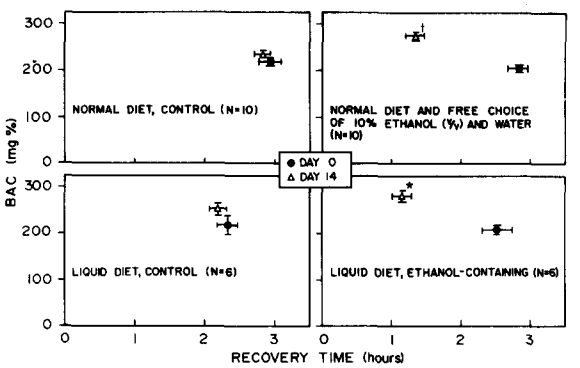


FIG. 2. Blood alcohol concentrations (BAC) at recovery and recovery times for control and chronically ethanol-exposed P rats on the jumping test after an IP injection of 2.5 g ethanol/kg body weight. Data are the means±SEM of number of animals indicated in parentheses. The indicated statistical differences for the data at day 14 versus day 0 refer to differences for both BAC and time of recovery. *$p<0.01$ for BAC and $p<0.005$ for recovery time of LDE group day 14 vs. day 0. †$p<0.005$ for recovery time and $p<0.001$ for BAC of FCE group day 14 vs. day 0.

*Blood Ethanol*

Blood samples were collected in heparinized capillary tubes. After centrifugation, the plasma fractions were sampled by direct injection into a Hewlett-Packard 5730A gas chromatograph equipped with a flame ionization detector as previously described [8,19].

*Statistical Analysis*

The results are expressed as mean values±SEM. Independent *t*-tests were used to compare separate groups, while a paired-*t* was used for within group comparisons. Where appropriate, analyses of variance and Newman-Keuls *post hoc* tests were employed for multiple comparisons.

## RESULTS

The mean body weights on day 0 for the normal diet (C), normal diet with free choice of 10% ethanol (FCE), liquid diet control (LDC) and the liquid diet containing 5% ethanol (LDE) groups were 429±18, 424±24, 341±16, and 341±17 grams, respectively. The rats in the C and FCE groups were significantly heavier than those in either of the liquid diet groups, $F(3,28)=12.41$, $p<0.001$; Newman Keuls, $p<0.05$. The lighter weight rats were assigned to the liquid diet conditions because smaller animals acclimate more easily to the diet. The mean body weights on day 14 for the C, FCE, LDC and LDE groups were 426±17, 420±13, 331±16 and 333±18, respectively. Body weights did not change significantly over the two-week period for any of the four groups.

The ethanol intakes for the FCE and LDE groups on day 13 were 7.0±0.4 and 9.3±0.6 g/kg/day, respectively. The mean intake of the LDE group was significantly higher than the intake of the FCE group, $t(14)=3.31$, $p<0.01$. The range of ethanol intake for the LDE group was 7.0–12.0 g/kg/day, while the range for the FCE group was 5.0–9.2 g/kg/day. On the first three days when the LDE rats were given a liquid diet consisting of 2/3 control diet and 1/3 ethanol diet, the mean ethanol intake was 5.2±0.2 g/kg/day. During the second phase of acclimation (days 4–7) when the diet consisted of 1/3 of the control diet and 2/3 of the ethanol diet, the mean intake of ethanol was 9.1±0.4 g/kg/day. From day 8–13, when the complete ethanol diet was presented, the mean ethanol intake was 9.9±0.4 g/kg/day. The FCE group received 10 days of free-choice between water and 10% ethanol after the initial four days, when the only source of fluid was 10% ethanol. The mean ethanol intake during the first four days was 8.2±0.7 g/kg/day, with a range of 6.2–10.6 g/kg/day. For the ensuing 10 days, the mean intake was 6.8±0.5 g/kg/day with a range of 5.0–8.7 g/kg/day.

The jumping performances of the four groups following IP administration of 2.5 g ethanol/kg body wt. are illustrated in Fig. 1. It is evident that the rats exposed to chronic ethanol, whether free-choice or liquid diet, showed better performance ($p<0.05$ by paired *t*-test for all points below 75% of pre-test jump height) on day 14 than on day 0 at all time points after the injection of 2.5 g ethanol/kg. Statistical analysis of the jumping performances of the C and LDC rats indicated that they were nearly identical on day 14 compared with day 0 (Fig. 1).

The relation between the mean recovery times and BACs at the time of recovery of days 0 and 14 for the four groups is shown in Fig. 2. Mean recovery times on day 0 for the C, FCE, LDC and LDE groups were 177±6, 170±6, 143±10 and 153±13 minutes, respectively, and the mean BACs at recovery were 219±6, 222±5, 220±19, and 214±6 mg%, respectively. However, the FCE group on day 14 exhibited a shorter recovery time of 80±7 min ($p<0.005$) and the BAC at recovery, 273±5 mg%, was higher ($p<0.001$) than on day 0. On day 14, the LDE group had shortened their recovery time to 70±9 min ($p<0.005$) and the BACs were increased to 286±14 mg% ($p<0.01$) in comparison with day 0.

The recovery times and BACs on day 14 were not significantly different for the FCE and the LDE groups. No significant differences were observed for either control group between days 0 and 14. On day 14, the mean recovery time for

the C group was 172±7 min and the BACs were 229±4 mg%. The LDC group on day 14 had a mean recovery time of 135±8 min and BACs of 238±11 mg%.

## DISCUSSION

The development of tolerance through chronic consumption of ethanol was one of the last requirements needed to establish the selectively bred P rats as an appropriate animal model of alcoholism [3,11]. As evidenced by the superior jumping performance on day 14 (Fig. 1) at higher BACs (Fig. 2), the P line of rats became tolerant with the forced administration of ethanol after a relatively short period of exposure. Although the method of inducing tolerance by forced administration in a liquid diet may be a suitable experimental approach to elucidate potential neurobiological concomitants of tolerance, it is not satisfactory in studies of an animal model of alcoholism. The present study demonstrated that the mean recovery times and mean BACs at recovery of the P rats following free-choice drinking of 10% ethanol (FCE) were nearly identical to those observed for the LDE group (Figs. 1 and 2). This finding indicates that ethanol tolerance develops to approximately the same degree in P rats that consume a 10% solution of alcohol voluntarily as it does in P rats under the condition of forced ethanol consumption. The present demonstration that P rats develop chronic tolerance through the volitional intake of ethanol established a convenient tool for the investigation of the genetically-controlled neurobiological mechanisms of chronic tolerance.

Learning has been suggested by a number of investigators as being a mechanism for the development of tolerance to ethanol [14,21]. Tabakoff and his co-workers have proposed two subcomponents of alcohol tolerance, one being "environment-dependent" and the other "environment-independent" [13]. The "environment-dependent" or "conditioned" tolerance arises from the association of environmental cues with the administration and testing of the drug. With repeated testing of the drug in the same environment, the animal develops environment-dependent tolerance and, as long as the testing regimen remains constant, tolerance will persist. On the other hand, if the tolerant animal is exposed to repeated testing under identical conditions, but receives the drug in a novel environment, any tolerance that develops is usually less than the environment-dependent tolerance. This supports the view of learning as being a significant mechanism of tolerance development. However, under chronic ethanol exposure, tolerance can occur regardless of the environmental conditions. Two requirements are associated with this "environment-independent" tolerance: (a) the route of ethanol administration during chronic exposure period must differ from the route used on the test day, and (b) the drug must be given in a novel environment on the test day. The present study was designed to satisfy these requirements. Environmental cues would have been totally eliminated had the exposure of the rat to the jumping apparatus been limited to one test session instead of the two sessions they received. However, the experience of the first session did not facilitate the performance of the paired control groups, whereas the FCE and LDE chronic ethanol groups performed significantly better with higher BACs in the second test session. Therefore, it can be concluded with reasonable certainty that learning cues were not the overriding factor in the tolerance exhibited by the two chronic ethanol groups in the present study.

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