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Genetic–environment analysis of sensitivity and acute tolerance to ethanol in mice

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Abstract

The purpose of this study was to characterize initial sensitivity (IS), acute functional tolerance (AFT), and rate of tolerance development to ethanol in lines of mice selected for aggression mice as well as to investigate the impact of isolate housing on these phenotypes. The results showed that for IS, there were no differences among treatment groups. For acute tolerance and rate of tolerance development, a Line × Sex × Housing interaction was present, with the response to housing being more pronounced in the low aggressive line than the high aggressive line, and the females being more affected than the males. Correlational analysis showed low to moderate associations between rate of tolerance development and IS, as well as between rate of tolerance and AFT. Housing condition significantly influenced female expression of ethanol phenotypes as compared to males. The line of the subject also influenced the magnitude of expression of these phenotypes. These findings suggest that environmental and genetic influences interact to influence acute tolerance and rate of tolerance development.

Keywords: Aggression; Mice; Initial sensitivity; Acute functional tolerance; Ethanol; Genetic–environment

1. Introduction

Acute tolerance to ethanol was first discussed by Mellanby (1919), who reported that subjects kept at the same blood ethanol concentration (BEC) over a period of time showed progressive recovery from motor impairment originally seen at the same BEC. More recently, this phenomenon has come to be called acute functional tolerance (AFT) (Erwin and McClearn, 1981). Mellanby asserted that acute tolerance develops in animals as well as in humans, and furthermore asserted that it plays a role in alcohol consumption. The major defining characteristic of AFT is that it develops rapidly during initial intoxication and therefore, is believed to reflect pharmacodynamic rather than pharmacokinetic processes. Since acute tolerance was first reported, numerous studies have been conducted to investigate possible mechanisms for this phenotype. These include different neurotransmitter systems (Allan and Harris, 1987; Grover et al., 1994; Littleton et al., 1980; Taberner, 1989), impact of learning on AFT (Hiltunen and Jarbe, 1992; Mayfield et al., 1992), the impact of individual differences (van Erp and Miczek, 1997), including genetic makeup (Erwin, 1985).

The interplay between alcohol and aggression has been the focus of numerous studies, in both humans and in animals. Previous studies have suggested that after ethanol administration, aggressive behavior can either increase or decrease, depending on the dose. Relatively high doses of ethanol have been reported to decrease aggressive behavior (Crowley et al., 1974; Mos and Olivier, 1988; Smoothy and Berry, 1983), while low to moderate doses of ethanol may increase aggressive behavior (Blanchard et al., 1987; Ellman et al., 1972; Lister and Hilakivi, 1988; Miczek and Barry, 1977). Moreover, van Erp and Miczek (1997) assert that individual differences play a significant role in the amount of aggression displayed after ethanol administration.

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With the use of genetic models, one can investigate the impact of genetically based individual differences, as well as genetic background on aggression or the effects of alcohol, including tolerance and initial sensitivity (IS). Indeed, researchers have studied the role of genetic makeup on tolerance and sensitivity to alcohol (Erwin and McClearn, 1981; Gallaher et al., 1996; Khanna et al., 1990; Littleton et al., 1980), as well as aggression (Berton et al., 1997; Cairns, 1996; Crusio, 1996; Schicknick et al., 1993), but not both. Therefore, we undertook this study to investigate whether having been selectively bred for high or low aggression might also impact sensitivity to and the development of acute tolerance to ethanol. Furthermore, the impact of environmental manipulations on these ethanol phenotypes was also investigated.

2. Methods

2.1. Selective breeding

Selective breeding was implemented by Cairns et al. (1983) at the University of North Carolina to produce three lines of mice that differed in their patterned social behavior, specifically isolation-induced intermale aggression. High-aggressive (NC900), low-aggressive (NC100), and mid-aggressive (NC500) were established from an outbred ICR foundation stock. Line differences in aggression were observed among males in the third generation (Cairns et al., 1983), and have persisted in each of the subsequent generations. Pregnant dams from the 12th generation of these lines were brought to the Pennsylvania State University in 1985. In each generation to the present (30th generation), selection has been based on the aggressive behavior of isolate-reared males assessed in the dyadic test ("standard opponents" test). This procedure was carried out at 42–50 days of age by placing the isolate-reared subject with a group-reared, same-sex conspecific in a neutral chamber. Males chosen for mating on the basis of their aggressive behavior were assigned to the sisters of other males selected within each line. Brother–sister matings were thus avoided. Females were not assessed for aggressive behavior during the selection process; however, the females of each selected line have been shown to differ in isolation-induced postpartum aggression as well as repeated intruder trials over the life span of group-reared animals (Hood and Cairns, 1988). Thus, selection for isolation-induced aggression in males apparently also influences aggressive behavior in females.

2.2. Subjects

The subjects consisted of male and female mice from the 30th generation of selection based on the criterion of low and high isolation-induced intermale aggression. The low (PSU100) and high (PSU900) aggressive lines were reared at the Centralized Biological Laboratories at the Pennsylvania State University. The ages of the mice on test day were between 60 and 80 days. All subjects had free access to food and water and were on a fixed light cycle of 12 h (on at 1300 h and off at 0100 h). Ten subjects from each line, sex, and housing condition were used in this experiment. All subjects were free of major pathogens such as mouse hepatitis virus.

2.3. Housing

Housing conditions for animals varied. Half of the subjects were isolated at day of weaning (23 days of age) or kept in unisex groups of three littermates per cage. All isolate-housed animals were tested 40 days post isolation.

2.4. Procedure

Before AFT testing, all subjects were taken from their cage and weighed. Animals were then trained to balance for 3 min on a 2.54-cm wooden dowel that was suspended 50 cm above the floor in a Plexiglas box (dimensions $70 \times 50 \times 50$ cm). Once the subjects were able to complete the task, they were returned to their home cage until testing at 1600 h. During the testing period, all subjects received intragastric administration of 3.0 g/kg ethanol (20% v/v in

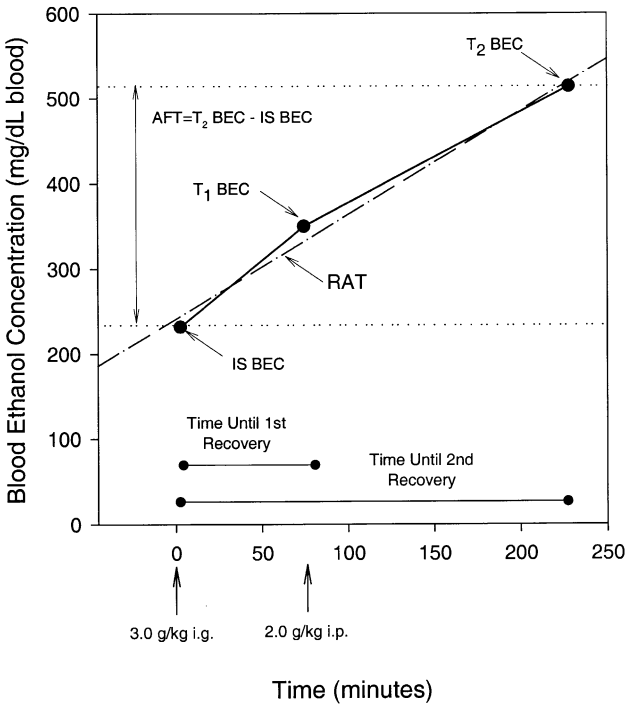

Fig. 1. Method of determining AFT and RAT: BEC as a function of time and ethanol dose. Values represent an individual subject for BEC in mg/dL at the times indicated after 3.0 and 2.0 g/kg ethanol. More details pertaining to determination of IS and regain of balance on the stationary dowel are described in the Methods section.

sterile saline), and then were placed on the dowel. When the subject lost its ability to balance on the dowel, the time was recorded and a 25-μl blood sample via the retro-orbital sinus was taken for BEC measurement. The BEC of this sample defined IS. The animals were then placed on the dowel every 5 min thereafter until they regained the ability to balance on the dowel for 1 min. Once this criterion was met, the time was recorded and another blood sample (25 μl) was taken. The BEC of this sample was labeled T1 and used in calculating the rate of acquisition of functional tolerance. After the blood sample was taken, the subject received an intraperitoneal injection of 2.0 g/kg ethanol (10% v/v in sterile saline). The subject was then repeatedly placed on the dowel until it again regained the ability to balance for 1 min on the dowel. When this criterion was achieved, the time was recorded and a third blood sample (25 μl) was taken. The BEC of this sample was labeled T2 and used to calculate rate of acquisition of tolerance (RAT) and to define total AFT.

2.5. Blood ethanol assay

The colorimetric method of Lundquist (1959) was used to determine BEC. All blood samples were stored at 4°C and analyzed 12 to 24 h after collection.

2.6. Calculations

AFT was calculated by subtracting the BEC for the second regain of balance time (T2) and the BEC of the IS.

The RAT was calculated in the data analysis by using linear regression to determine the slope of the tolerance

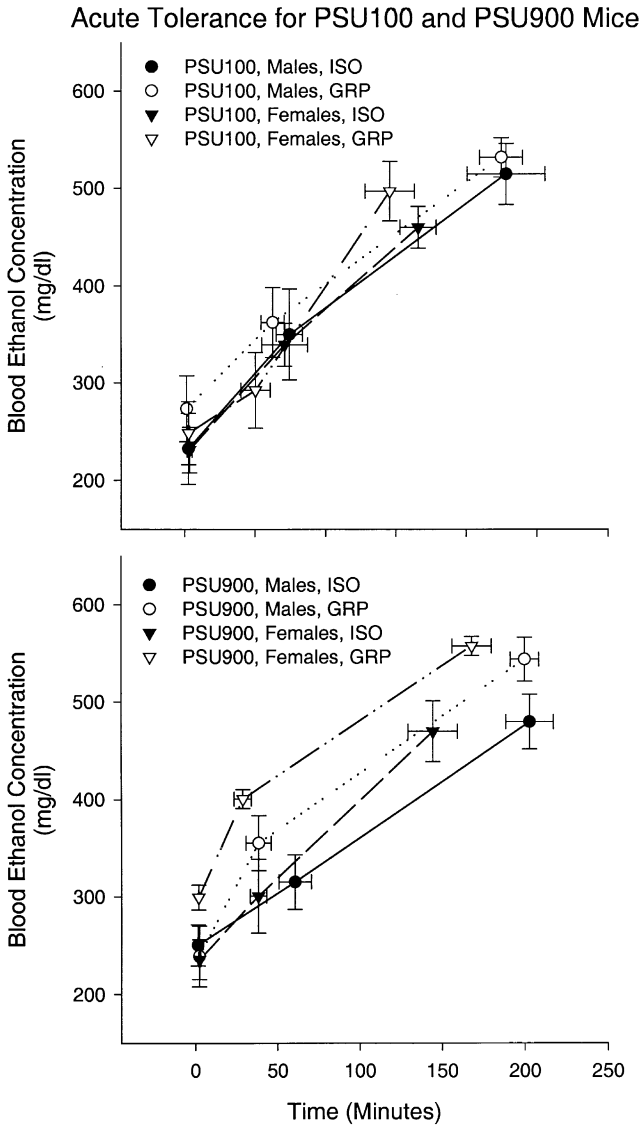

Fig. 2. Mean tolerance curve all treatment groups. Time point of onset of ataxia (IS) and regain of balancing on static dowel (T1 and T2) are plotted against BEC at that specific time point.

curve (BEC vs. time that each blood sample collected). This was performed for each subject. The slope of this regression line (mg dl^{-1} min^{-1}) defined RAT. More specifically, the time and BEC for IS, T1, and T2 were used to determine RAT. Much consideration was given to the use of linear regression for the measurement of RAT, and the authors agreed that when plotted (Fig. 1), the time vs. BEC for IS, T1, and T2 appeared to be linear in most cases, suggesting that equal tolerance was developed between IS and T1 as between T1 and T2.

2.7. Data analysis

Analysis of variance (ANOVA) for the between-subjects variables (line, sex, housing) experiment was used as primary data analysis. Pearson Product–Moment Correlation was used to index associations between phenotypes.

3. Results

The tolerance curve for all treatment groups are presented in Fig. 2.

3.1. Initial sensitivity

ANOVA revealed that there were no line, sex, or housing main effects on IS. Fig. 3 presents mean IS values for line, sex, and housing conditions.

3.2. Acute functional tolerance

ANOVA revealed a Line × Sex × Housing interaction ($F = 3.898$; $P < .05$; $df = 1,50$). This interaction is illustrated in Fig. 4. Isolation decreased AFT in PSU100 females compared to their group-housed cohorts. In the PSU900 females, isolation increased AFT compared with group-housed cohorts. In contrast, no effects of line or housing condition were observed in the males.

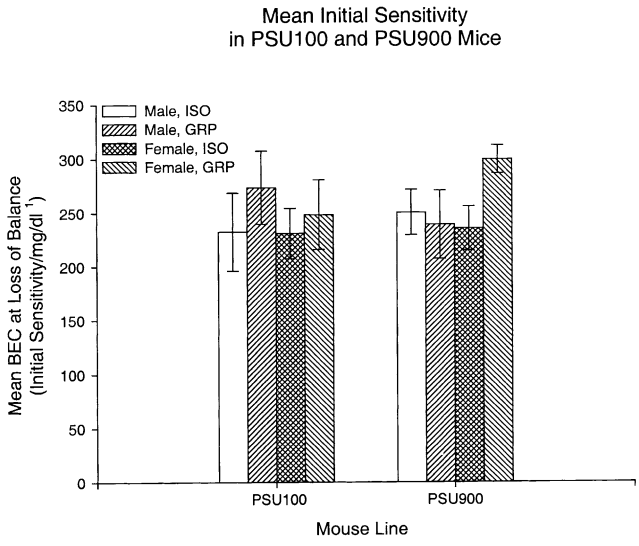

Fig. 3. Mean IS in high and low aggressive mice. IS was defined as BEC at the first fall from the static dowel.

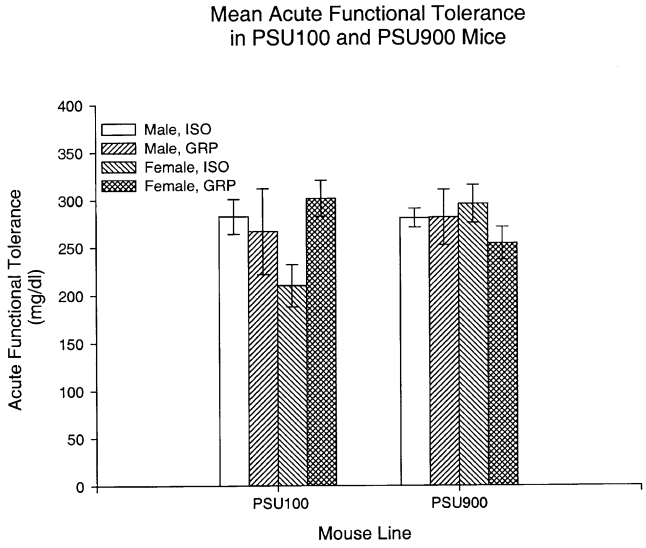

Fig. 4. Mean AFT in high and low aggressive mice. AFT was calculated by subtracting the BEC of IS and the BECs of the second regain of balance. Consult text for methodological details.

3.3. Rate of tolerance development

ANOVA revealed a sex main effect ($F = 5.662$; $P < .02$; $df = 1,59$), a Line × Housing interaction ($F = 10.639$,

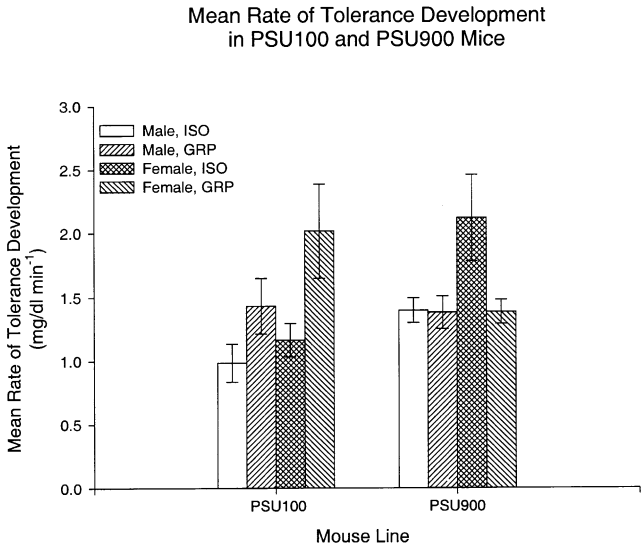

Fig. 5. Mean rate of tolerance development. Rate was calculated by the linear regression of ethanol concentration of each sample on the time the sample was collected. The slope of the regression line was used as the measurement of rate of acquisition of acute functional tolerance. Consult text for methodological details.

$P < .001$; $df = 1,59$), and a trend for a Line × Sex × Housing interaction ($F = 3.203$, $P < .07$; $df = 1,59$). Fig. 5 illustrates these findings. Group-housed PSU100 females evinced a higher rate of tolerance than both their isolate-housed cohorts, and males of both lines and treatment conditions. Additionally, group-housed PSU100 males evinced a trend of achieving a higher RAT development compared with isolate-housed cohorts. Conversely, isolate-housed females PSU900 evinced a higher RAT than both their group-housed cohorts, and males of both lines and treatment conditions.

3.4. Correlational analysis

IS to ethanol was negatively correlated with RAT development ($r = -.26$; $P < .05$; $df = 56$) and AFT ($r = -.57$; $P < .001$; $df = 56$). AFT and RAT were positively correlated ($r = .39$; $P < .005$; $df = 56$).

4. Discussion

The current study extends previous knowledge about IS, AFT, and RAT to ethanol in two ways. First, by intragastric administration of ethanol, we were able to better estimate IS because of slowed (compared to intraperitoneal administration) absorption and distribution, thus defining this phenotype in a unique way. This technique allows the measurement of IS by giving the researcher time to actually observe the phenotype and obtain a more accurate estimate of BEC. Second, in this study, we calculated both AFT and RAT. This work presents a novel metric that describes the process of tolerance development, i.e., RAT. Earlier work (Erwin and Deitrich, 1996) presented differences in rates of tolerance acquisition, however, these calculations were based upon group means of several dose groups. Our calculations are for individual animals tested repeatedly within session. Simultaneously calculating AFT and RAT, as well as IS, allows for exploration of possible relationships among these ethanol phenotypes.

The primary goal of this study was to determine if mice selected for differences in aggression also differ in their sensitivity and acute tolerance development to ethanol. The secondary goal of this study was to investigate the environmental impact on these ethanol-related phenotypes in these genetically defined mice. The environmental component of this study was included because the selected trait in these animals is a social isolation-induced phenotype. We observed that isolate housing influences the expression of AFT in the female mice but in opposite directions, depending on line. The PSU100 isolate-housed female mice displayed a decrease in AFT when compared to their group-housed cohorts, and PSU100 male mice. However, the PSU900 isolate-housed female mice displayed an increase in AFT when compared to their group-housed cohorts, and PSU900 male mice.

One remarkable finding from this study is the final BEC at regain of balance for many of our subjects. These BEC values often exceeded 500 mg dl^{-1}, a value where most naïve mice would be in deep sleep or even in a life-compromising situation. Perhaps the most proximal studies to ours are the selective breeding studies of Erwin and Deitrich (1996). In these studies, all of the ethanol was administered intraperitoneally at lower doses. Nevertheless, final BECs at regain often achieved 400–425 mg dl^{-1}. There are several possibilities for the differences in reported BEC at regain of balance. First, our dosing regimen is quite different from all of the others. We gave an initial dose of 3.0 g/kg intragastrically and a follow-up dose of 2.0 g/kg intraperitoneally. This dosing regimen would have produced both higher BEC values and a larger area under the BEC–time curve (AUC). Our results would thus lead to the hypothesis that AFT is affected by BEC peak and AUC. Moreover, absorption from the GI system may in itself influence both rate and extent of AFT and may more reliably reflect the acute tolerance in human alcohol consumption. Exactly what pharmacodynamic processes might underlie these phenomena is unclear at this time. The second possible reason for the discrepancy concerns the genetic starting material. The selected lines of Erwin and Deitrich were derived from the heterogeneous stocks from the Institute for Behavioral Genetics (HS/Ibg), derived from an eight-way diallel cross performed by McClearn and Kakihana (1981) over 25 years ago. The 100 and 900 selected lines of mice were derived from entirely different genetic material, viz. the outbred CD1 mice. Other than the present work, nothing is known about alcohol sensitivity in these animals.

Results from the correlational analysis suggest there is an inverse phenotypic relationship between IS to the effects of ethanol and the magnitude of tolerance development as well as the rate in which tolerance develops. More specifically, individuals with a low sensitivity to the effect of ethanol will display a higher extent of tolerance and at a faster rate than those that are more sensitive to ethanol's effects. Additionally, the correlational analysis suggested that there is a significant relationship between the amount of tolerance developed and the rate at which tolerance develops.

The most interesting finding, as well as the most perplexing, was the influence of housing condition on the rate of tolerance development. In the male mice, in contrast to the females, housing condition had little impact on the rate of tolerance development. Female PSU100 mice that were isolate-housed displayed a lower rate of tolerance development than their group-housed controls, while the isolate-housed PSU900 mice displayed a higher rate of tolerance development compared to their group-housed controls. This finding, coupled with the AFT findings, demonstrates a significant Genetic × Environment interaction.

Similar Genetic × Environment interactions pertaining to ethanol-related phenotypes have been reported in the liter-

ature. For example, inbred BALB/c mice that were handled early in life demonstrated an increase in ethanol consumption in adulthood compared C57BL/10, which showed no such handling-related effect (Jones et al., 1985). Additionally, Jones et al. (1990) reported that isolate housing for 21–22 days after weaning decreased hypnotic sensitivity to ethanol by 15% in long-sleep mice as compared to their group-housed cohorts. Our findings also indicate that housing may affect ethanol effects differentially, depending on genetic makeup. While it is clear that for AFT, both genotype and environment (and sex) are important, it remains to be seen whether the same Gene × Environment interaction would be predictive of alcohol consumption in these animals. Earlier work (Erwin and McClearn, 1981) has demonstrated a correlational link between AFT and alcohol consumption in mice. Moreover, there is a proposed link between genetics and IS to alcohol-related problems in humans (Schuckit, 1988) and evidence for environment and genetics to play a role in at least one type of alcohol-related disorder (Cloninger, 1987).

In conclusion, the data generated in this study suggest that there are genetic-based differences in acute tolerance to ethanol and that there are associations among IS, AFT, and RAT to ethanol. However, the degree to which these relationships are influenced by common genetic mechanisms is beyond the scope of this study but a worthwhile subject for further investigation. Finally, we have also demonstrated the impact of sex and environment interactions on important ethanol-related phenotypes.

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