BEHAVIOURAL PHARMACOLOGY
Persistent High Alcohol Consumption in Alcohol-Preferring (P) Rats Results from a Lack of Normal Aversion to Alcohol
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(Received 3 December 2009; in revised form 2 February 2010; accepted 15 March 2010)

ABSTRACT — Aims: In this study, we tested the impact of pretreatment with alcohol on subsequent alcohol drinking in outbred Sprague–Dawley and selectively bred alcohol-prefering (P) rats. Methods: As a pretreatment, male Sprague–Dawley and P rats were given a passive oral administration of either alcohol (1.0 g/kg) or tap water. Then, they were given free choice of drinking alcohol (5% v/v) or water in their home cages, which was measured over 4 weeks. Results: Without alcohol pretreatment, there was no significant strain difference in alcohol preference; both strains preferred 5% (v/v) alcohol solution. The strain difference was only apparent in the groups given alcohol pretreatment. This arose from the fact that alcohol pretreatment significantly reduced alcohol preference in the Sprague–Dawley rats to a level well below 50%, while it did not alter drinking behavior in P rats. The same effects were seen with total alcohol consumption (g/kg/day). These effects persisted throughout the 4 weeks of the study. Conclusions: The principal difference between the Sprague–Dawley and P rats was that the P rats did not show the normal aversion to alcohol after forced exposure to alcohol that the Sprague–Dawley rats showed. One of the potential contributors to high alcohol intake and preference in P rats may be lack of sensitivity to aversive effects of alcohol.

INTRODUCTION
Usually the first experience with drugs including alcohol is an important determinant factor in subsequent use. Experiencing aversion to the initial drug episode may discourage the use of a drug while experiencing reward and euphoria may encourage it. The first experience, reward or aversion, depends on several factors including the environment setting, the onset of the effect, prediction of the effects, psychological conditions of the subject at the time of the use and, very importantly, the genetic makeup of the subject. It is estimated that about 60% of alcoholism risk is genetic and about 40% stems from environmental factors. The genetic component of alcoholism has been modeled in rats by selectively breeding rats with high levels of alcohol preference (Lumeng et al., 1982a, b; Li et al., 1993). The rat models can be useful for determining the neurobehavioral bases for alcoholism to help direct development of new efficacious approaches to therapeutic treatment. These genetic models can help determine which aspects of the biobehavioral effects of alcohol are key in determining heavy consumption and alcoholism.

In humans, it has been conclusively demonstrated that a low level of initial behavioral or subjective response to alcohol ingestion predicts a higher rate of alcohol use disorder onset over time (Schuckit and Smith, 1996; Schuckit and Gold, 1998; Schuckit and Smith, 2000, 2001; Schuckit et al., 2005, 2006, 2009; Schuckit 2009; Trim et al., 2009). In all of these studies, a lower level of response early in life predicted later heavy drinking and alcohol use disorder. These findings suggest that low level of response to alcohol is a unique risk factor for alcohol use disorders across adulthood.

Similar findings have been reported in animal models of drinking behaviors (Croehlich et al., 1988; Kurtz et al., 1996; Stewart et al., 1992, 1996). It has also been shown that alcohol-naïve, alcohol-preferring (P) rats compared with selectively bred alcohol non-prefering (NP) rats are less sensitive to the sedative/hypnotic effect of alcohol. Compared with NP rats, P rats took longer to lose righting reflex, had a shorter recovery time and recovered at a higher blood alcohol concentration following a single sedative/hypnotic dose of alcohol given systemically (Kurtz et al., 1996). Using a conditioned taste aversion paradigm, Froehlich et al. (1988) demonstrated that repeated pairing of saccharin and alcohol produced stronger and prolonged taste aversion to saccharin in the NP rats compared with the P rats. These findings suggest that genetic factors are important in determining whether a drug will be perceived as aversive or rewarding, and consequently, among other factors, genetic factors will determine whether an organism will avoid a particular drug.

A number of studies have shown that both selectively bred P rats and non-selectively bred (i.e. Fawn-Hooded) rats P rats differ from Wistar and Sprague–Dawley rats substantially in the amount of alcohol consumed and preference for alcohol (Eriksson, 1968; Lumeng et al., 1982a, b; Mardones and Segovia-Riquelme, 1983; Rezvani et al., 1990; Li et al., 1993; Colombo, 1997), suggesting the influence of genetics in drinking behaviors. However, it is not clear if the main driving force underlying the increased alcohol consumption in P rats is that they find alcohol more rewarding or less aversive than other rats. The present study was designed to determine and compare the level of aversion to alcohol in P and Sprague–Dawley rats. It is hypothesized that P rats drink more alcohol than Sprague–Dawley rats because alcohol is less aversive in P rats than Sprague–Dawley rats.

METHODS
Animals
The animals used in these studies were adult male Sprague–Dawley rats (Taconic Farms, Germantown, NY, USA) and rats selected from 66 and 67 generations of selectively bred alcohol-prefering P line at Indiana University, Indianapolis. Rats were housed individually in a standard laboratory maintained at 21 ±
1°C and relative humidity at 50 ± 10% and reversed light cycle (lights off: 0700–1900). The P and Sprague–Dawley rats weighed an average of 373 ± 4.3 and 303 ± 4.3 g, respectively, at the beginning of the experiment. The treatment and care of the animals was carried out under an approved protocol of the Animal Care and Use Committee of Duke University in an Association for Assessment and Accreditation of Laboratory Animal Care-approved facility.

**Experimental protocol**

At about 80 days of age, the rats were handled briefly for 10 min/day for several days. Then, rats were given either one single dose of 1 g/kg alcohol (16% v/v) or an equal volume of tap water by gavage once and only at the beginning of the experiment. To avoid possible taste aversion, all solutions were directly delivered into the stomach and not into the mouth. Fifteen minutes later, rats were placed in specialized polycarbonate cages that were fitted with two 100-ml graduated Richter drinking tubes for the recording of water and alcohol (5% v/v). The reason for choosing 5% alcohol rather than a higher concentration was that alcohol at this concentration is more palatable for outbred strains of rats such as Sprague–Dawley. Furthermore, with this concentration of alcohol, there is less variability in drinking in outbred strains. Rats had a choice between water and alcohol for the remainder of the study. Rats had continual access to water and alcohol for four consecutive weeks, and their water and alcohol intake levels were measured during the day at approximately the same time. Procedures were similar to those routinely used in our laboratory (Rezvani and Grady, 1994; Rezvani et al., 1995; Overstreet et al., 1996; Rezvani et al., 1997, 1999, 2000, 2007, 2009).

**Preparation of alcohol solutions**

A solution of 5% (v/v) alcohol was prepared twice weekly from a solution of 100% ethanol mixed with tap water.

**Statistical analysis of data**

Alcohol intake was calculated as grams per kilogram from a volume of 5% (v/v) alcohol consumed. Alcohol preference was calculated as a percentage of alcohol consumed over total fluid intake (alcohol + water). Means were calculated and subjected to ANOVA with strain and pre-testing alcohol treatment as between-subjects factors and weekly averages of percent alcohol vs water preference and grams per kilogram per day of alcohol intake. Significant interaction effects were followed up by tests of the simple main effects of each strain at each pre-testing alcohol treatment condition to determine which individual strain and treatment groups differed from each other. A P-value of 0.05 (two-tailed) was used as the threshold for significance.

**RESULTS**

For amount of alcohol consumed (g/kg/day), there was not a significant main effect of strain (P = 0.098). However, there was clearly a significant interaction of strain × alcohol pretreatment (F(1.27) = 7.27, P < 0.025). As displayed in Fig. 1A (mean alcohol consumption over 4 weeks), the tests of the simple main effects showed significantly (P < 0.005) greater alcohol consumption in P rats than Sprague–Dawley rats when there was alcohol pretreatment, but not when the pretreatment was only water. This did not result from the induction of alcohol drinking by alcohol pretreatment in P rats. They did not significantly change their consumption with alcohol pretreatment. Rather, it was the Sprague–Dawley rats that showed a significant (P < 0.005) decrease in alcohol consumption after alcohol pretreatment by nearly 50%. Fig. 2A shows the detailed week-by-week levels of alcohol consumption. There was a significant main effect of week (F(3.81) = 13.30, P < 0.0005) with an overall increase in alcohol consumption over weeks. There was no significant interaction of weeks with either strain or alcohol pretreatment. The lower amount of alcohol consumption in Sprague–Dawley rats with pre-exposure vs Sprague–Dawley rats without alcohol pre-exposure as well as relative to P rats pre-exposed to alcohol was maintained throughout the 4-week study. Sprague–Dawley and P rats without alcohol pre-exposure did not differ in alcohol consumption at any point in the 4-week study.

The percent alcohol preference measure showed similar results. With percent preference, there was a significant main effect of strain (F(1.27) = 14.80, P < 0.001) with the P rats having a higher preference for alcohol than the Sprague–Dawley rats (81.1 ± 3.6% vs 52.0 ± 7.3%); however, this resulted completely from the lower alcohol preference in the Sprague–Dawley rats given alcohol pretreatment (Fig. 1B). There was no significant difference in the preference between the strains when no alcohol pretreatment was given. As with
alcohol consumption, there was a significant strain × alcohol pretreatment interaction ($F(1,27) = 7.41, P < 0.025$). Follow-up tests of the simple main effects showed that alcohol pretreatment significantly ($P < 0.005$) reduced alcohol preference in the Sprague–Dawley rats while the P rats did not show a change. There was a significantly ($P < 0.0005$) greater alcohol preference in the P rats pretreated with alcohol vs pretreated Sprague–Dawley rats. Sprague–Dawley rats showed aversion to alcohol with only 36.0% preference for the alcohol-containing bottle. This was well below the 50% chance level and the 68.1% level shown by Sprague–Dawley rats not given alcohol pretreatment. This is the same pattern of effects as shown with the grams per kilogram per day alcohol consumption measure.

Fig. 2B shows the week-by-week breakdown in alcohol preference by the groups. As with the consumption, the preference data showed a significant main effect of weeks ($F(3,81) = 14.03, P < 0.0005$) with overall decreased alcohol preference by the groups. As with the consumption, there was a significant strain × alcohol pretreatment interaction ($F(1,27) = 7.41, P < 0.025$) with overall reduced alcohol consumption and preference for 4 weeks after a single alcohol pretreatment. On the other hand, the P rats given the same alcohol pretreatment showed no such effect. Without alcohol pretreatment, there were no discernible differences between Sprague–Dawley and P rats in either the amount of alcohol consumed and the percent alcohol preference. The lack of difference in alcohol preference between Sprague–Dawley and P rats before being treated with alcohol is likely contributed to the palatability of 5% (v/v) alcohol. It is likely that at higher concentrations of alcohol, the P rats will drink significantly more alcohol than Sprague–Dawley rats. The increased alcohol consumption seen in P rats seems to be entirely due to a lack of the normal aversion to initial alcohol treatment rather than increased preference. It has previously been shown that the P rats self-administer significant quantities of alcohol intragastrically, indicating that post-absorptive effects of alcohol, rather than its taste or smell, are reinforcing for the P rats (Waller et al., 1983a). It has also been demonstrated that P rats find the post-ingestional effects of high dose of alcohol less aversive and low dose of alcohol more rewarding than their control counterpart, the NP rats (Froehlich et al., 1988). The possible effects of taste in the current experiment should be ruled out because alcohol solution was delivered directly into the stomach and not into the mouth. Genetic differences in sensitization and tolerance development to alcohol between the P and NP rats have also been reported. Kurtz and colleagues (1996) studied the initial sensitivity to alcohol and the development of tolerance comparing the P with the NP rats. They demonstrated that P rats were less sensitive to the behaviorally impairing effects of alcohol than were NP rats, as evidenced by longer latency to lose righting reflex and a shorter time to recover following an acute dose of alcohol. It has also been shown that within-session tolerance to alcohol is developed in P rats (Waller et al., 1983b). Thus, it can be speculated that the combination of lack or less sensitivity to alcohol, less aversion to alcohol and development of acute tolerance to alcohol may serve to increase alcohol intake in P rats. As mentioned in the Introduction, in the conditioned taste aversion (CTA) paradigm (Froehlich et al., 1988) and in the conditioned place preference (CPP) paradigm (Stewart et al., 1996), both P and NP rats showed aversion to alcohol. However, the magnitude of aversion to alcohol was significantly less in P rats compared with NP rats. In one study, no difference was found between P and NP rats using the CPP paradigm (Schechter, 1992). Our results should not be directly compared with CTA and CPP results because measuring drinking alcohol is the most appropriate test for testing aversion to alcohol in rats that have been selectively bred to drink alcohol.

The fact that one single intragastric administration of alcohol impaired acquisition of alcohol drinking in Sprague–Dawley rats but not in P rats suggests that this selectively bred line might have less aversion to alcohol than the outbred Sprague–Dawley line. One can speculate that the diminished aversion to alcohol may partly explain the high level of drinking in selectively bred P rats.

Interestingly, similar findings have been reported in humans regarding the importance of diminished aversive effects of alcohol in alcoholics. A lower level of response, i.e. lower sensitivity to subjective and intoxicating effects of alcohol,
has been repeatedly demonstrated in adult children of alcoholics (Schuckit and Smith, 1996; Schuckit and Gold, 1998; Schuckit and Smith, 2000, 2001; Schuckit et al., 2005, 2006, 2009; Trim et al., 2009). Indeed, low level of response to alcohol is shown to be a unique risk factor for future heavy drinking (Trim et al., 2009). Clearly, genetic factors play a major role in lower sensitivity to alcohol, which may lead to heavy drinking.

The current results clearly call for further research, especially regarding the relative roles of pharmacokinetic and pharmacodynamic effects underlying the diminished aversive effects of alcohol in P rats. However, since P rats and its control counterpart, NP rats, metabolize alcohol at the same rate (Waller et al., 1983b), it is unlikely that the lack of aversion to alcohol in P rats is of pharmacokinetic nature. Determining critical pharmacodynamic factors underlying this effect will be key not only for understanding the basic biology of addiction but also for developing new treatments to combat alcoholism. This study demonstrates the importance of aversion to initial alcohol exposure in the control of later voluntary consumption. The lack of normal aversion can be a key factor leading to heavy alcohol consumption and increased risk for alcoholism.

Acknowledgements — We are grateful to Drs Lawrence Lumeng and Richard Bell of the Indiana University School of Medicine for generously providing alcohol-prefering P rats (supported by NIAAA R24 AA015512-02).

Conflict of interest statement. None declared.

REFERENCES


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