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Different Sensitivities to Ethanol in Alcohol-Preferring and -Nonpreferring Rats[1]

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LUMENG, L., M. B. WALLER, W. J. McBRIDE AND T.-K. LI. *Different sensitivities to ethanol in alcohol-preferring and -nonpreferring rats.* PHARMAC. BIOCHEM. BEHAV. **16**(1) 125–130, 1982.—The sensitivity of the P and NP rats to ethanol was determined by the jumping test [23]. Proper interpretation of this test requires knowledge of the regional differences in the distribution of ethanol as a function of time after ethanol injection. Ethanol concentration in brain was higher than those in tail blood and skeletal muscle within the initial 30 min following the intraperitoneal injection of ethanol and was also higher than that in cerebral blood in the first 15 min. However, after 60 min, ethanol concentrations in brain and tail blood were identical. After ethanol injection (2 g/kg), the P rats jumped 88, 78, 85, 54 and 19% higher than the NP rats at 30, 60, 120, 180 and 240 min, respectively. The tail blood ethanol concentrations did not differ between the P and NP rats after 60 min. The P rats also jumped higher than the NP rats after injection of 1.5 and 2.5 g/kg ethanol. These results indicate that the P rats are innately less sensitive to the effects of ethanol than the NP rats.

Ethanol Alcohol preference Sensitivity to alcohol Distribution of ethanol P and NP rats

THE voluntary consumption of ethanol by mice and rats has been demonstrated to be under genetic influence [4, 5, 10, 11, 13]. By selective breeding, the Research Laboratories of the State Alcohol Monopoly (Alko), Finland, [5] and our laboratory [11] have independently developed lines of rats that differ greatly in their voluntary alcohol-drinking behavior. The Alko AA line and our P line prefer to drink a 10% ethanol solution in a free-choice situation with food and water available ad lib while the Alko ANA line and our NP line exhibit alcohol aversion under the same testing condition. The P and NP lines, derived from a randomly bred, closed colony of Wistar rats (Wrm:WRC (WI) BR), have been raised to the S-16 generation in our laboratory and there is only minimal overlap in the voluntary alcohol consumption scores of these two lines.

Efforts have been made to identify the physiologic and biochemical correlates of alcohol drinking preference [10, 15, 19]. Studies on the AA and ANA lines have shown that the male AA rats are innately less sensitive to the pharmacologic effect of ethanol than the male ANA rats, as measured by sleep time [19] and by impairment of activity on the tilting plane [15]. Similarly, studies on the P and NP lines have indicated that male P rats are less affected than the male NP rats, when tested for the hypothermic effect of ethanol [10]; however, the difference between lines is small. Accordingly, we have performed additional studies to more clearly delineate the difference between P and NP rats in their innate sensitivity to alcohol. As here reported, the test utilizes the jumping apparatus [23]. The interpretation of this test is critically dependent upon a knowledge of the pharmacokinetics of ethanol distribution and elimination in rats after the intraperitoneal injection of ethanol. For this reason, studies on the regional differences in the distribution of ethanol as a function of time after injection were also performed.

METHOD

Animals

Male Wistar (Harlan Industries Inc., Indianapolis, IN) and P and NP rats, weighing 300–450 g, were fed ad lib standard laboratory chow (Wayne Lab-Blox, Allied Mills, Inc., Chicago, IL). They were housed individually in wire-bottomed cages and a controlled temperature and humidity environment with fixed light-dark cycles (7 a.m. to 7 p.m., light and 7 p.m. to 7 a.m. dark). The Wistar rats were used in the studies of alcohol distribution and metabolism. P and NP rats of the S-16 generation were employed to study the effect

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of alcohol in the jumping test. The drinking preferences of the P and NP rats were tested when they were 9-weeks old. The testing procedure has been described previously [11]. With food provided ad lib, the free-choice (10% (v/v) ethanol versus water) drinking scores of the P and NP rats in the S-16 generation were 5.3±0.2 (±SEM) and 0.9±0.1 g ethanol/kg/day, respectively. The P and NP rats were taken off ethanol for at least 2 months before testing in the jumping apparatus.

Measurement of Ethanol Distribution and Elimination Rate in Vivo

Ethanol, 2 g/kg body weight, was injected intraperitoneally as a 12% (g/v) solution in saline. At various time intervals after injection, the rats were lightly anesthetized with methoxyflurane (2,2-dichloro-1,1-difluoroethyl methyl ether). In one group of Wistar rats, brain, cerebral blood and tail blood samples were obtained almost simultaneously (within 1 min) for ethanol determinations, and in another group, skeletal muscle and tail blood samples were similarly collected.

The brains were freeze-stopped by means of the copper tubing method [21] and cerebral blood samples were collected from the blood that welled into the cerebral cavity made upon withdrawal of the copper tubing. The skeletal muscle samples were obtained by freeze-clamping the gastrocnemius muscle. The frozen brain and muscle samples were pulverized in mortars cooled with liquid nitrogen and 0.2 g of the tissue powder was added to 6 ml of 3 M perchloric acid. After homogenization in a Polytron PT20 tissue grinder, the samples were centrifuged at 18,000 G for 15 min at 4° and 0.5 ml of the supernatant fraction was used for ethanol determination. Measurement of the hemoglobin content of brain samples and the ratio of ethanol/hemoglobin concentrations in the corresponding cerebral blood samples were employed to correct for the extent of contamination of brain tissue samples by blood ethanol. The ethanol levels in brain and muscle were expressed as mg/ml of tissue water by assuming the water content of brain and muscle to be 80.6 and 75.8% (v/g), respectively [2].

Blood samples from tail and brain were collected in heparinized tubes and added to equal volumes of 3 M perchloric acid. After centrifugation, the supernatant fractions were used for ethanol determination.

Ethanol was measured by the head-space method in a Hewlett-Packard 5700 gas chromatograph with n-propanol as internal standard. Aliquots of the supernatant fractions from tissue and blood extracts were sealed in 25-ml serum vials. The vials were incubated at 65° for 30 min and four headspace samples were obtained from each vial for analysis. The glass columns were packed with 50% Porapak Q and 50% Porapak R (100/120 mesh) and the oven temperature was 150°.

Performance on the Jumping Test

The jumping apparatus was constructed as described [23]. Briefly, the apparatus is a Plexiglas compartment, 43 cm long, 20 cm wide and 76 cm high. It contains a grid floor and a motor-driven movable platform covered with a rubber mat. The grid floor upon activation is electrified with 500 μA of scrambled shock delivered by a model 700 Grason-Stadler shock generator. The movable platform is motorized to descend at a speed of 1 cm per second. The apparatus is constructed such that a rat placed on the grid floor must jump to the descending platform to avoid or escape electric shock.

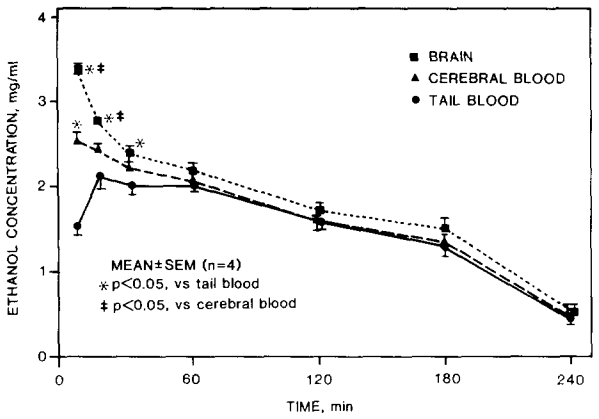

FIG. 1. Relationship of ethanol concentrations in brain, cerebral blood and tail blood after an intraperitoneal injection of ethanol (2 g/kg). ‡Significant difference when compared with the ethanol concentration in cerebral blood. *Significant difference when compared with the alcohol concentration in tail blood.

The P and NP rats usually required 10 days of training on the jumping apparatus in order to reach criterion. The height jumped, measured by a permanently mounted tape measure from the top of the grid floor, was assessed using as end-point the attainment of at least 3 paws grasping the top of the platform. By this criterion, all the rats jumped at least 46 cm on every trial at the end of the training period.

Testing in the jumping apparatus was performed between 8 a.m. and 2 p.m. On the test day, each rat received an intraperitoneal injection of either the 12 g percent ethanol solution or an equivalent volume of saline. After the injection of ethanol or saline, jumping performance at 5, 15, 30, 60, 120, 180 and 240 min was measured in each test session. Each animal was tested after the injection of ethanol in four separate sessions and after the injection of saline in four other sessions. The test sessions after ethanol injection and those after saline injection were performed in alternating sequence. They were also scheduled at least 2 days apart and training of the animals without injection of either saline or ethanol was continued between test days.

To determine the dose-response curves, the dosage of ethanol injected was varied from 1.5 to 2.5 g/kg. In the experiments to correlate tail blood ethanol concentrations with jump heights, blood samples were collected immediately after the jump trials at 60, 120, 180 and 240 min. The tails of both the ethanol- and saline-injected rats were cut in order to control the effect of this surgical procedure. In the experiments to determine the precision of the jumping test, jumping performance after the injection of ethanol (in this instance, 2g/kg) was measured 3 or 4 times one min apart at and immediately after the time points, 5, 15, 30, 60, 120, 180 and 240 min.

RESULTS

Regional Distribution of Ethanol after Intraperitoneal Injection

The concentrations of ethanol in brain, cerebral blood and

TABLE 1

WITHIN-SESSION AND BETWEEN-SESSION PRECISIONS OF THE JUMPING TEST DETERMINED IN REPRESENTATIVE P AND NP RATS AFTER THE INTRAPERITONEAL INJECTION OF ETHANOL, 2 g/kg

Time After Ethanol Injection min	P			NP		
	Mean Height cm*	Within-Session CV %†	Between-Session CV %*	Mean Height cm*	Within-Session CV %†	Between-Session CV %*
5	4	95	74	1	0	0
15	35	18	13	11	103	78
30	41	12	2	24	43	20
60	42	6	6	30	28	12
120	46	6	1	32	18	11
180	48	3	1	37	23	11
240	49	3	1	37	9	10

*Calculated from the mean height for each of the four sessions.

†Mean of the coefficients of variation (CV) for 4 separate sessions, 3–4 trials at each time point.

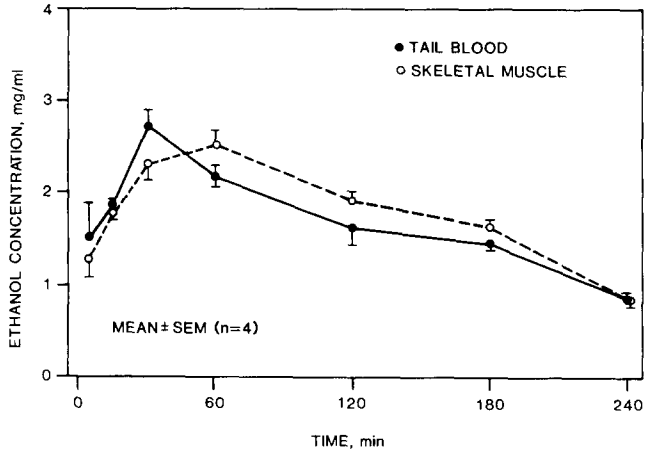

FIG. 2. Comparison of ethanol concentrations in tail blood and skeletal muscle after an intraperitoneal injection of ethanol (2 g/kg).

tail blood were measured in the Wistar rats as a function of time after the intraperitoneal injection of ethanol, 2 g/kg body weight (Fig. 1). The brain ethanol concentrations were significantly higher than those in tail blood at 5, 15 and 30 min after ethanol injection and were also higher than those in cerebral blood at 5 and 15 min. At 5 min, ethanol concentration of cerebral blood was also higher than that in tail blood. After these initial time points, the ethanol concentrations in these regions converged and then followed an almost linear decline as a function of time. Importantly, the curve of brain ethanol concentrations plotted as a function of time is concave between the 5 and 60 min time points whereas that for tail blood ethanol concentrations is convex, i.e., with an initial ascending and then a descending limb.

It is generally recongnized [18] that the distribution dynamics of drugs is dependent on rapid uptake into well-perfused tissues (e.g., the brain and liver) and slower entry into less well-perfused tissues (e.g., muscle and viscera). Because skeletal muscle has considerable mass, this factor also has important influence upon the overall distribution of drugs. In order to verify the applicability of these pharmacokinetic principles to ethanol distribution, the relationship between ethanol concentrations in muscle and tail blood was examined (Fig. 2). Ethanol concentrations in muscle and tail blood were virtually identical at all time points after the intraperitoneal injection of ethanol. Both curves were convex, exhibiting an ascending and then a descending limb.

From the data shown in Figs. 1 and 2, the ethanol elimination rates were estimated by extrapolating the linear portions of the curves to the time when the 2 g/kg dose was completely eliminated. The rates were 8.1 mmol/hr/kg and 7.4 mmol/hr/kg, respectively, and were similar to the elimination rate of unanesthetized Wistar rats, 7.6 mmol/hr/kg.

Precision of the Jumping Test

Table 1 depicts the within-session and between-session precisions of the jumping test as a measure of the depressant action of ethanol on neuromuscular function. Ethanol, 2 g/kg, was injected into each of the P and NP rats and the results shown are based on four separate sessions. The data indicate that the coefficients of variation of the jump heights were largest in the first 30 min after the injection of ethanol into the P and NP rats and they tended to be larger in the NP than in the P rat.

Effect of Ethanol on the P and NP Rats in the Jumping Test

The effect of ethanol, 2 g/kg, and an equivalent volume of saline given intraperitoneally on the performance of the P and NP rats in the jumping apparatus is shown in Fig. 3. After training, the P rats jumped 49 to 50 cm while the NP rats jumped 46 to 48 cm ($p<0.05$, P vs NP). The jump heights tended to decrease with time during the trial in both the P

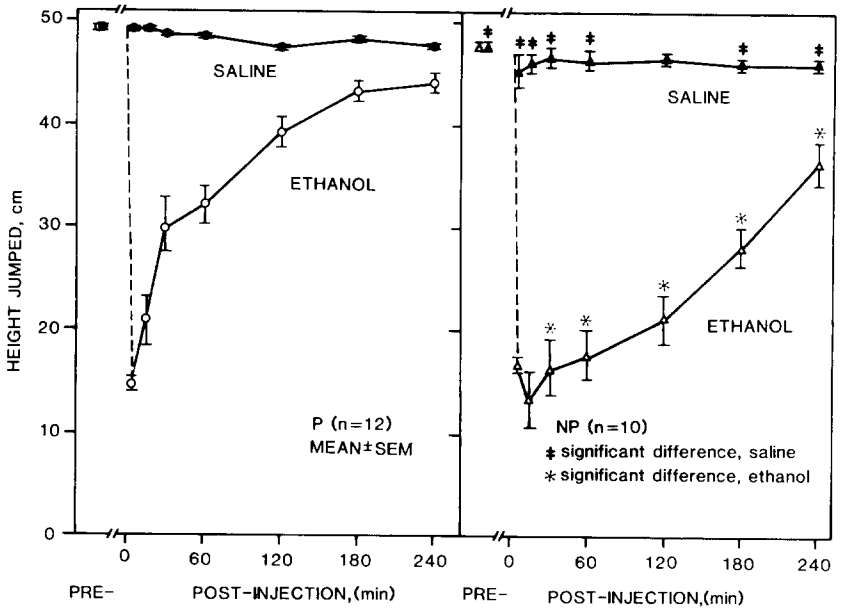

FIG. 3. Effect of saline and ethanol (2 g/kg) injection on the performance of the P and NP rats on the jumping test. ‡Significant difference between the P and NP rats before and after the injection of saline. *Significant difference between the P and NP rats after the injection of ethanol.

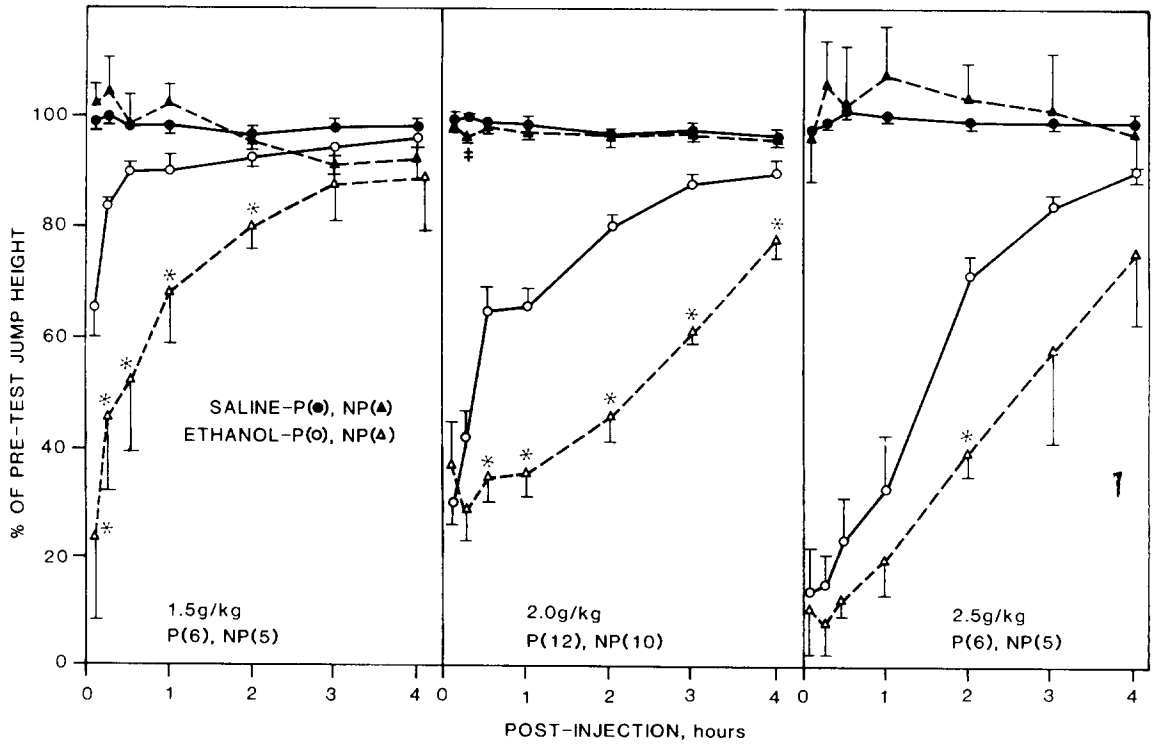

FIG. 4. Effect of saline and varying doses of ethanol injected on the performance of the P and NP rats on the jumping test (Mean±SEM). ‡Significant difference between the P and NP rats after the injection of saline. *Significant difference between the P and NP rats after the injection of ethanol.

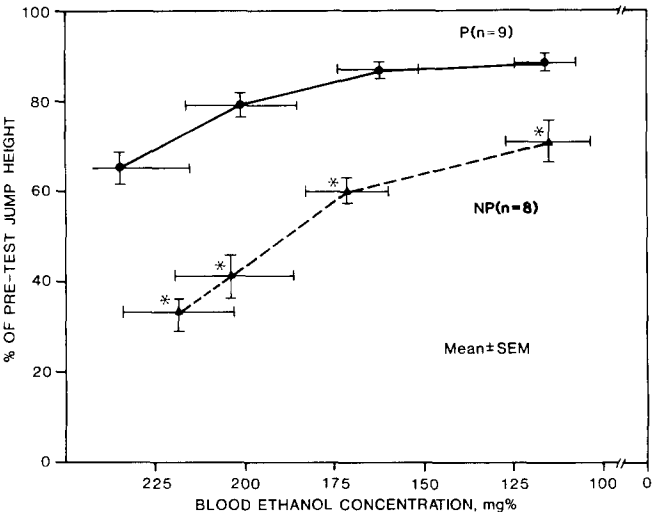

FIG. 5. Relationship of blood ethanol concentrations and performance of the P and NP rats on the jumping test after ethanol injection (2 g of ethanol/kg). *Significant difference in jumping performance between the P and NP rats.

and NP rats after saline injection, but these changes were small. Although the P animals jumped slightly higher than the NP animals at all time points after saline injection, the largest differences between the jump heights of the P and NP rats were elicited after ethanol injection. The jump heights of the P rats were less affected by ethanol than those of the NP rats at 30, 60, 120, 180 and 240 min.

The effect of varying doses of ethanol on the jumping performance of the P and NP rats is shown in Fig. 4. For ease of comparison, the data were normalized and expressed as percent of pre-test jump heights. Except for one data point, the P and NP rats did not differ in their normalized jumping scores after saline injection. At all doses of ethanol, 1.5, 2.0 and 2.5 g/kg body weight, the jumping ability of the P rats was less impaired than that of the NP rats. The largest differences were observed with the 1.5 and 2.0 g/kg doses.

The relationship between tail blood ethanol concentration and the performance of the P and NP rats in the jumping test is depicted in Fig. 5. Ethanol, 2 g/kg, was injected and tail blood samples were collected at 60 min and then at hourly intervals thereafter. Based on the data shown in Fig. 1, tail blood ethanol concentrations should accurately reflect those in the brain at these time points after the injection of ethanol. The tail blood concentrations of the P and NP rats at 1, 2, 3, and 4 h did not differ significantly. However, for the same blood ethanol concentration, the impairment of jumping performance in the P rats was considerably less than that in the NP rats. These results indicate that the P line of rats is innately less sensitive than the NP rats to the pharmacologic effect of ethanol.

DISCUSSION

The interpretation of the jumping test requires a clear understanding of the regional differences in the distribution of ethanol as a function of time after ethanol administration. Immediately after intraperitoneal injection, the volume of distribution for ethanol is smaller than that at equilibrium because of the low rate of perfusion of skeletal muscle by blood and large tissue mass of skeletal muscle. Thus, within the first 30 min after injection, brain ethanol concentrations are inordinately higher than those in skeletal muscle and tail blood. The distribution of ethanol does not achieve equilibrium until 60 min after injection and, consequently, tail blood ethanol concentrations do not accurately reflect those in brain until after this period. Somewhat similar results have been reported by other investigators in the mouse [22] and in the rat [3]. However, in the latter report, ethanol was administered by gastric intubation and the slow absorption by the gastric route significantly blunted the uneven distribution of ethanol in the different regions immediately after its administration. Additionally, in both the aforementioned studies, the relationship between ethanol concentrations in muscle and tail blood was not examined. The results reported here on ethanol distribution demonstrate that the jumping test cannot be used to assess acute tolerance by the administration of a single intraperitoneal injection of ethanol and the measurement of tail blood ethanol concentrations as previously thought [23]. The jumping ability is indeed more impaired during the ascending than the descending portion of the blood ethanol curve obtained by tail blood sampling, but brain ethanol concentration is higher during the former than the latter phase.

The jumping test, however, can be used as a measure of the acute depressant effect of ethanol. As shown in Table 1, precision is good except during the first 30 min after ethanol injection. A higher degree of variation is expected in the initial 30 min because the volume of distribution of ethanol and the concentration of ethanol in brain are changing rapidly. At the present, the precise neuronal pathway responsible for the impairment of jumping performance produced by ethanol is not known. It may involve the central and/or the peripheral nervous system and perhaps even the neuromuscular junction.

Studies on the rat lines genetically selected for alcohol preference and aversion (nonpreference) indicate that those with high alcohol drinking preference are relatively insensitive or more tolerant to the effects of ethanol. The higher innate tolerance to ethanol in these lines has been demonstrated previously in the AA line by sleep time [19] and activity on the tilting plane [15] and in the P-line by the hypothermic effects of ethanol [10]. In this report, the P-line is shown to exhibit greater innate tolerance to ethanol than the NP-line by the jumping test. Both the difference between the AA and ANA rats as measured by sleep time and that between the P and NP rats as measured by hypothermia were small. However, with the tilting plane in the AA and ANA rats and with the jumping test in the P and NP rats, larger differences are demonstrated. The disparity in sensitivity between these tests of the pharmacologic effects of ethanol is not unexpected, since the dose-response relationships are test-dependent. Furthermore, the sensitivity of each of the tests varies with dose, as evidenced by the decreased level of discrimination between the P and NP lines at the 2.5 g/kg dose as compared to the 1.5 and 2.0 g/kg doses.

Alcohol drinking preference as a phenotypic trait probably has multiple genetic determinants [12]. Additionally, certain environmental factors can influence the ultimate expression of this phenotypic trait [7]. Because innate sensitivity to ethanol is also inherited [6, 14, 17], particular emphasis has been directed toward the definition of the relationship

between alcohol sensitivity and alcohol drinking preference. It is of interest that even in inbred strains of mice, the alcohol-preferring, C57BL strain exhibits a shorter sleeping time following the administration of an anesthetic dose of ethanol than do the nonpreferring, DBA and BALB strains [9, 16, 20]. Although this relationship in inbred strains of mice can be a fortuitous association, it is probably less likely to be so in animals that have been raised by selective breeding for disparate alcohol drinking behavior, the alcohol-preferring, AA and P lines of rats and the alcohol-nonpreferring, ANA and NP lines of rats. Conversely, the alcohol preference of several lines of rodents selectively bred for differences in innate sensitivity to alcohol is noteworthy. These include the alcohol tolerant (AT) and alcohol non-tolerant (ANT) rats [6], the least-affected (LA) and most-affected (MA) rats [17], and the short-sleep (SS) and long-sleep (LS) mice [14]. It has been reported that animals of the AT and the SS lines without caloric restriction exhibit mild to moderate ethanol preference under certain conditions [1,8]. However the LA and MA rats apparently do not differ in free-choice drinking of 5.6% ethanol solution and water [24]. Collectively, these observations suggest a weak genetic correlation between alcohol preference and innate tolerance to alcohol, accountable either by genetic linkage or by overlap in genes that control both of these behaviors.

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