Rats Selectively Bred for Ethanol Preference or Nonpreference Have Altered Working Memory

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ABSTRACT

To examine whether the cognitive deficit observed in chronic alcoholics is because of the chronic exposure to alcohol or to a factor that contributes to the chronic alcohol use, working memory was evaluated under a delayed matching-to-position task in rats selectively bred for ethanol preference or nonpreference (iP/iNP, iHAD1/iLAD1, and iHAD2/iLAD2). Before the study on working memory, rats were studied under a progressive ratio schedule of food presentation to determine whether differences in motivation to respond for food existed between the alcohol-preferring and -nonpreferring strains. No such differences were observed. Under the delayed matching-to-position schedule, the length of the delay was titrated such that accuracy was maintained at approximately 80%, and the mean length of the delay for each experimental session provided a

measure of working memory function. In two (iP/iNP and iHAD1/iLAD1) of the three pairs of selectively bred rats, nonpreference to ethanol was associated with better working memory performance. In the third pair of selectively bred rats (iHAD2/iLAD2), the relationship was reversed, with increased ethanol preference associated with better working memory function after saline administration. After ethanol administration, both the mean delay and the rate of responding were decreased in all six strains. Based upon an ED₅₀ analysis, there was little evidence of strain difference in sensitivity to ethanol on either parameter. Additional studies are needed to better understand the relationship between working memory and ethanol preference in these rats.

Several studies have shown altered cognitive function, including memory disturbances, in humans who abuse ethanol and other drugs of abuse (Bowden et al., 2001; Rogers and Robbins, 2001; Block et al., 2002; Mintzer and Stitzer, 2002). However, it is not clear whether these differences existed before the abuse or whether the differences are the result of the abuse. One approach to answering this question is to examine working memory in alcohol-preferring (P) and alcohol-nonpreferring (NP) rats in both the absence and presence of ethanol.

In 1974, Li and Lumeng began studies on an outbred strain of Wistar rats from the Walter Reed Army Institute of Research, Silver Spring, MD (Lumeng et al., 1977; Li et al., 1979, 1981). These studies on gene-specific behavioral traits led to the development of the P and NP rat lines. Rats that

were selected for breeding in the P line consumed in excess of 5 g/kg/day ethanol and demonstrated a better than 2:1 preference for ethanol over water. Rats selected for breeding in the NP line consumed less than 1.5 g/kg/day ethanol and did not exceed an ethanol-to-water preference of greater than 0.5:1. After approximately 30 generations of breeding, voluntary ethanol consumption of P rats averaged 9.2 ± 0.4 g/kg/day, whereas the NP rats consumed 1.9 ± 0.7 g/kg/day. In addition, when given free access to food along with a choice of 10% ethanol or water, P rats consume 20 to 30% of their total calories per day as ethanol and maintained body weights equivalent to P rats given food plus water alone (Lumeng et al., 1977; Li et al., 1981).

In an attempt to develop replicate lines of rats showing high levels of ethanol preference and nonpreference, these investigators initiated a second selective breeding program in the mid-1980s. Because the P/NP lines originated from a limited genetic base (Wistar), this second selective breeding started with a foundation stock of the genetically heterogeneous N/Nih rat. The heterogeneous N/Nih rat was developed from the systematic crossing of eight separate inbred strains. Using the same breeding criteria as used in the development of the P and NP rats, the Indiana University high and low

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ABBREVIATIONS: P, preferring; NP, nonpreferring; HAD, high alcohol-drinking; LAD, low alcohol-drinking; FR, fixed ratio; ANOVA, analysis of variance; CI, confidence interval.

alcohol-drinking replicate lines (HAD1/LAD1 and HAD2/LAD2) were developed (Lumeng et al., 1986; Li et al., 1993). In 2002, these lines were reported to be in their 35th generation, with the HAD rats consuming approximately 9.5 g/kg/day ethanol and the LAD rats consuming approximately 0.5 g/kg/day ethanol when given free access to 10% ethanol, water, and food (Murphy et al., 2002). Together, the three rat lines (P/NP, HAD1/LAD1, and HAD2/LAD2) are ideal for this study because although they represent different genetic backgrounds, and the same criteria were used to direct the bidirectional breeding. Since these early studies, true inbred strains have been developed for each of the three pairs of selectively bred rats.

Although there have been many studies examining alcohol preference in these lines, there are few if any studies that have examined any measure of cognitive function in these rats. Furthermore, there are few if any studies that have examined preference for food or reinforcers other than ethanol. Thus, this study had two goals. The first was to determine whether the differences in ethanol preference extended to the reinforcing efficacy of food pellets because the planned test of working memory involved the use of food-maintained operant responding. The reinforcing efficacy of the food pellets was determined using a progressive ratio schedule of food-pellet presentation (Hodos, 1961; Hodos and Kalman, 1963; Skjoldager et al., 1993; Grasing et al., 2003). The second goal was to examine working memory and the effects of ethanol on memory function in the alcohol-preferring and nonpreferring rats. Working memory was measured using a delayed matching-to-position schedule of food presentation (Dunnett, 1985; Dunnett, 1993). If differences in working memory can be demonstrated and shown to be associated with alcohol preference apart from any differences in food preference, it would suggest two possibilities: 1) the memory deficits observed in chronic alcoholics may have existed before the chronic alcohol consumption, and 2) there is a possible genetic association between altered memory and alcohol preference. Such findings could provide insight into the possible role and causes of cognitive deficits observed in humans that abuse ethanol.

Materials and Methods

Subjects. Young adult male rats of the six inbred strains (iP, iNP, iHAD1, iLAD1, iHAD2, and iLAD2) were obtained from the Indiana University-Purdue University Indianapolis Alcohol Research Center breeding colony (Indianapolis, IN). In addition, as a control for the genetic background of the iP and iNP rats, young adult male Wistar rats were obtained from Charles River Canada (St. Constant, QC, Canada). At the beginning of the experiment, the rats were food deprived to 85% of their free feeding weight and maintained at this weight by postsession feedings for the duration of the experiments. Water was freely available in the home cage. A 12-h light/dark cycle was maintained with lights on from 6:30 AM to 6:30 PM, and all rats were tested 5 days/week (Monday-Friday) between 7:30 AM and 5:30 PM. All experimental protocols were approved by the University of Arkansas for Medical Sciences Animal Care and Use Committee, and all experimental procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health (Bethesda, MD).

Apparatus. Rats were tested in standard rat chambers (MED Associates, St. Albans, VT). The chamber could be illuminated by a 28-V d.c. bulb (house light) mounted in the ceiling of the chamber.

Each chamber was equipped with two retractable levers mounted on the front wall of the chamber. A third lever was mounted on the rear wall of the chamber. All three levers were approximately 8 cm above a grid floor. A 28-V d.c. bulb was mounted directly above each lever. On the front wall, between the two retractable levers, a rectangular pellet receptacle was mounted providing access to 94-mg food pellets (Bio-Serve, Inc., Frenchtown, NJ). During pellet delivery, the pellet receptacle was illuminated with a 28-V d.c. bulb. Each chamber was housed in a sound- and light-attenuating chamber, and a cooling fan provided a low-level masking noise inside the chamber.

Progressive Ratio Schedule (First Determination). After initial response training, experimentally naive rats of each of the seven strains with no prior exposure to ethanol were trained to respond on the lever mounted on the front wall and to the right of the pellet receptacle (the active lever) under a progressive ratio schedule. Responses on either of the other two levers in the chamber were not counted and had no consequence. The start of the session was signaled by the illumination of the house light and the stimulus above the active lever. The completion of five responses [fixed ratio (FR)5] on the active lever extinguished the house light and stimulus light above the active lever, and initiated a 5-s delay during which a 94-mg food pellet was delivered to the illuminated pellet receptacle. At the end of the 5-s food delivery period, the light illuminating the food receptacle was extinguished, and the house light and stimulus light above the active lever were illuminated. After each food pellet delivery, the FR value was increased by five responses (FR5, FR10, FR15, FR20, FR25, etc.). The session terminated after 3600 s or after the rat failed to respond for 120 s. The breakpoint was defined as the ratio value at which the rat failed to respond for 120 s or the last ratio value completed when the session terminated after 3600 s.

Progressive Ratio Schedule (Second Determination). After stability of the breakpoint was achieved (a 2-week period with no significant trends in the value of the breakpoint), a second determination of the progressive ratio breakpoint was conducted to control for satiation. During this second determination, the starting FR value for the session for each rat was determined to be 60% of each rat's breakpoint value achieved during the first progressive ratio determination rounded up to the next multiple of five. Thus, if during the first determination the breakpoint value for a given rat was determined to be 110, the starting ratio value for that rat during the second progressive ratio determination was 70. All other aspects of the schedule remained the same.

Progressive Ratio Schedule (Third Determination). After stability of the breakpoint was obtained during the second determination (no significant trends in the breakpoint over a 2-week period), the starting ratio value for each session was returned to FR5 as in the first determination. After stability of responding was achieved with a starting ratio value of FR5, a dose-response curve for the effect of ethanol on the food-maintained progressive ratio performance was determined.

Matching-to-Position Schedule. A second group of experimentally naive, young adult male rats consisting of six to eight rats of each of the seven strains with no prior exposure to ethanol, was trained to respond under a delayed matching-to-position schedule of food presentation. After the initial response training, the rats were trained to respond on each of the three levers in the chamber under an FR5 schedule of food presentation. Once an equal reinforcement history had been established on all three levers, the rats were trained to respond under the delayed matching-to-position schedule. The first trial of each session under this schedule was signaled by either the right or left lever mounted on the front wall of the chamber being extended into the chamber, and the house light and stimulus light above the extended lever being illuminated. Which lever was extended at the start of each trial was determined randomly for each trial. Upon the completion of an FR5 on the extended lever, the lever was retracted, the stimulus light above the lever was extinguished, a 3-s delay was started, and the stimulus light mounted above the lever on the rear wall of the chamber was illuminated. Upon the first

response on the back lever after the completion of the 3-s delay, the stimulus light above the back lever was extinguished, both levers on the front wall were extended, and the stimulus lights above each extended lever were illuminated. A response on the same lever as was extended before the delay was defined as a correct matching response. A correct matching response retracted both front levers, extinguished the stimulus lights above each lever and the house light, and initiated a 5-s period during which a 94-mg food pellet was delivered to the pellet receptacle. An incorrect matching response, a response on the lever not extended before the delay, resulted in the retraction of both levers, the initiation of a 5-s time-out period, and the extinguishing of all lights in the chamber. At the end of the 5-s food delivery period or the 5-s time out, the start of the next trial was signaled by the extension of one of the two front levers, illumination of the stimulus light above the extended lever and illumination of the house light, as on the previous trial. The session terminated after 60 trials or 3600 s, whichever occurred first.

To minimize differences in reinforcement density and to standardize task difficulty across animals, the length of the delay was titrated based upon the rat's performance in an identical manner to that used in this laboratory in studies of working memory in pigeons (Wenger et al., 1993). In brief, during the first five trials of each session, the length of the delay was fixed at 3 s. On the sixth and all subsequent trials, the length of the delay was adjusted based upon the previous five trials. If the correct matching response was observed on five of the previous five trials, the delay increased in length by 3 s. If only four correct matching responses were made on the previous five trials, the delay length stayed the same. Finally, if only three or fewer correct matching responses were made on the previous five trials, the delay decreased by 3 s down to a minimal value of 3 s. Working memory was then measured by determining the average length of the delay on all trials completed during the session rather than comparing percentage of accuracy. After responding stabilized (no significant trends in the mean delay achieved for the session or rate of responding over a 2-week period), baseline performance values were determined, and a dose-response curve was determined for the effect of ethanol on responding under the delayed matching schedule.

Ethanol. Both ethanol and control saline injections were administered by the intraperitoneal route 30 min before the start of the session. For the progressive ratio experiments, ethanol was delivered as a 10% (w/v) solution, whereas in the matching-to-position experiments; ethanol was delivered as a 15% (w/v) solution. In all experiments, 100% ethanol was diluted to the appropriate concentration with physiological saline (0.9%). Typically, ethanol was administered before the start of the session on Tuesday and Friday, and saline was administered before the start of the session on Thursday. Ethanol doses were administered in a mixed sequence such that on a given test day no more than two subjects in a given strain received the same dose. Thus, some subjects received the lowest ethanol dose first, some received intermediate doses first, and some received the highest ethanol dose first.

Data Collection. Under the progressive ratio schedule, the breakpoint value was determined for each daily session for each rat. After responding stabilized following each change in schedule parameter, the breakpoint value was determined for each rat in the strain by averaging the breakpoint for an additional 10-consecutive daily session. A mean $\pm$ S.E. for each strain was determined from these 10-day averages.

Under the matching-to-position schedule, for each daily session the rates of responding during the FR5 on the sample lever before the delay, and the mean delay length for the session were collected. The mean delay length equaled the sum of the delay lengths in each trial in the session divided by the number of trials in the session. In cases where a rat did not complete at least 10 trials (e.g., after high doses of ethanol), the data for that rat was not included in the group average for the mean delay value.

Statistical Analysis. To compare control performance of the Wistar rats with the iP or iNP strains under the progressive ratio schedule or under the matching-to-position schedule, we used an ANOVA followed by a Bonferroni post hoc test. To compare the control performance under both schedules between alcohol-prefering and -nonpreferring strain pairs (iP versus iNP, iHAD1 versus iLAD1, or iHAD2 versus iLAD2), we used a *t* test. Statistical difference was defined in each case as $p < 0.05$.

When studying the effects of ethanol in each of the seven strains, statistical differences between ethanol doses and saline within a given strain were determined using a repeated measures ANOVA followed by a Bonferroni post hoc test. When the data failed to pass the test of normality or equal variance, Friedman's repeated measures ANOVA on ranks was used followed by Dunn's post hoc test. Statistical difference was defined in each case as $p < 0.05$. To examine differences across strains in response to ethanol on the progressive ratio breakpoint, and on the rate of responding and mean delay values for the matching-to-position schedule, the ED₅₀ value for ethanol was determined using all doses on the descending leg of the dose-response curve for each rat. In cases where a rat completed less than 10 trials under the matching-to-position schedule and no mean delay was calculated, these doses were excluded in the calculation of the ED₅₀ value. From these data, a group mean and 95% confidence interval (CI) were determined for each strain. The lack of overlap in the 95% CI was interpreted as a statistically significant difference.

Results

Because the tests of working memory used in this study involved operant responding maintained by food presentation, it was necessary to determine whether the differences in alcohol preference among the strains extended to differences in the reinforcing properties of food. Thus, the rats were trained to respond under a progressive ratio schedule of food presentation. During the initial breakpoint determination, the starting FR value for each session was set at FR5. Under these conditions, it can be seen in Fig. 1 that the breakpoint value (first determination) was significantly higher in the Wistar rats than in the iP or iNP inbred strains [$F_{2,17} = 9.28$; $p = 0.002$]. There were no significant differences in the breakpoint values between any of the ethanol-preferring and -nonpreferring pairs of the inbred strains. To control for possible satiation during these experiments, the breakpoint was determined a second time with the starting FR value determined for

Fig. 1. Progressive ratio breakpoint for food presentation in the seven strains. Abscissa, the seven rat strains; W, Wistar. Ordinate, progressive ratio breakpoint (the ratio value at which responding ceased for 120 s). Filled bars (first determination) represent the breakpoint when the starting ratio value was FR5. Cross-hatched bars (second determination) represent the breakpoint when the starting ratio was 60% of each subject's breakpoint value determined when the starting ratio was set at FR5. Bars and brackets indicate the mean $\pm$ S.E. (*n*) = the number of subjects included in each group mean.

each rat to be 60% of its breakpoint value achieved during the first determination. As shown in Fig. 1, a significant strain difference was again observed between the Wistar rats and iP or iNP rat strains [$F_{2,18} = 13.41$; $p = 0.001$]. As with the first determination, no differences were observed in the breakpoint between any of the ethanol-preferring and -nonpreferring rat strains. In addition, there was no difference in the breakpoint value as a function of the starting FR value in any strain, suggesting that the breakpoint measured was a valid measure of the reinforcing properties of the food and was not a function of satiation.

To determine the effects of ethanol on the progressive ratio breakpoint values, the rats were retrained with a starting FR value of FR5. Once the breakpoints had stabilized, a dose-response curve for the effects of ethanol was determined (Fig. 2). As seen in the first two determinations of the breakpoint, Wistar rats had a higher breakpoint after saline than any of the alcohol-preferring or -nonpreferring strains. Ethanol decreased the breakpoint in all strains studied. An ED_{50} analysis of the descending limb of the ethanol dose-response curve (Table 1) showed that the ED_{50} for the breakpoint decreasing effect of ethanol was lower in the iNP strain than in the Wistar or iP strains of rats. The iNP rats were also more sensitive

to ethanol than the iHAD1 rats. No other significant differences were observed between the strains.

When experimentally naive Wistar rats were trained under the delayed matching-to-position task of working memory, the mean delay value achieved after saline administration was 21.5 ± 2.9 s, and the rate of responding during the completion of the FR5 before the delay was 0.36 ± 0.04 responses/s (Fig. 3A). After ethanol administration, the mean delay value was decreased as a function of increasing dose, and a decrease compared with saline in working memory was observed at 1.5 g/kg [$F_{6,39} = 6.99$; $p < 0.05$]. Ethanol decreased rates of responding in Wistar rats compared with saline at doses of 1.5 and 1.75 g/kg [$F_{6,42} = 11.99$; $p < 0.05$]. The ED_{50} for the effect of ethanol on mean delay and rate of responding were 1.42 and 1.22 g/kg, respectively (Table 1).

Experimentally naive iP and iNP rats were trained to respond under the delayed matching-to-position schedule in a manner identical to the Wistar rats. After saline administration, the mean delay (Fig. 3B) achieved during the session in the iP rats (20.7 ± 2.4) was nearly identical to that observed for the Wistar rats (Fig. 3A). In contrast, the mean delay achieved by the iNP rats for the session after saline (31.0 ± 2.3 s) was longer ($t = 3.14$, $p = 0.01$). It is interesting to note that there was no difference between the iP and iNP

Fig. 2. Effect of ethanol on the progressive ratio breakpoint for food presentation. Abscissa, dose of ethanol in grams per kilogram given by the intraperitoneal route. Ordinate, progressive ratio breakpoint value. Data points above ethanol doses represent the mean $\pm$ S.E. of individual determinations in each subject. Points and brackets above S represent the mean $\pm$ S.E. after saline administration. The asterisk (*) indicates a significant difference from saline control.

TABLE 1

ED_{50} values for effects of ethanol

Numbers in parentheses (n) following the ED_{50} value represent the number of rats included in the calculation of the mean ED_{50} value. Note that for the mean delay value under the matching-to-position schedule, no descending leg of the dose-response curve was observed over the dose range tested in one of seven iLAD1 rats. Thus, the ED_{50} and CI values shown for mean delay are from six rats.

Strain	Progressive Ratio Breakpoint		Matching-to-Position Mean Delay		Matching-to-Position Responses/Second	
	ED_{50}	95% CI	ED_{50}	95% CI	ED_{50}	95% CI
	g/kg		g/kg		g/kg	
Wistar	1.19 (7)	1.05–1.34	1.42 (8)	1.24–1.60	1.22 (8)	1.13–1.31
iP	1.10 (8)	1.05–1.15	1.11 (6)	1.03–1.19	1.12 (6)	1.06–1.18
iNP	1.00 (6)	0.97–1.03	1.55 (6)	1.13–1.97	1.21 (6)	1.06–1.36
iHAD1	1.18 (8)	1.09–1.27	1.55 (7)	0.96–2.14	1.02 (7)	0.94–1.10
iLAD1	1.01 (5)	0.93–1.09	1.08 (6)	0.80–1.36	1.03 (7)	0.78–1.28
iHAD2	1.01 (8)	0.93–1.09	1.15 (7)	1.06–1.24	1.09 (7)	1.05–1.13
iLAD2	1.20 (8)	1.02–1.38	1.13 (7)	0.81–1.45	1.06 (7)	0.94–1.18

Fig. 3. Effect of ethanol in rats responding under the matching-to-position schedule. Abscissa (A–D), dose of ethanol in grams per kilogram given by the intraperitoneal route. Ordinate (A–D, top), mean delay achieved for the session in seconds; ordinate (A–D, bottom), rate of responding on the sample lever before the delay as responses per second. Data points and brackets above ethanol doses represent the mean $\pm$ S.E. of single determinations in each subject. Points and brackets above S represent the mean $\pm$ S.E. of saline in each subject. Asterisk (*) indicates a significant difference from saline control. In the top panel, (n) represents the number of subjects completing at least 10 trials during the session.

rats in the rate of responding (Fig. 3B) after saline administration. Likewise, no difference was observed in the rate of responding after saline between either the iP or iNP strains and the Wistar rats. Compared with saline, ethanol decreased the mean delay for the session in the iNP rats at 1.75 g/kg and the rate of responding at 1.25 g/kg and above (Fig. 3B). Similarly, ethanol decreased the rate of responding in the iP rats at doses of 1 g/kg and higher. This decrease in responding resulted in too few rats completing 10 or more trials to show significant decreases in the mean delay compared with saline. An ED₅₀ analysis of the descending leg of the curve for mean delay showed that there were no differ-

ences in the doses of ethanol required to decrease the mean delay or rate of responding in the iP and iNP rats (Table 1).

Experimentally naive iHAD1 and iLAD1 rats were trained to respond under the delayed matching-to-position schedule (Fig. 3C). After saline administration, iLAD1 rats performed at longer delay values than the iHAD1 rats ($t = 3.12$, $p = 0.009$), and there was a nonsignificant ($t = 2.14$, $p = 0.054$) trend toward a higher response rate in the iHAD1 rats compared with the iLAD1 rats. Compared with saline, ethanol decreased the rate of responding in the iHAD1 rats at 1 g/kg and higher, and this decrease resulted in a reduction in the number of rats completing at least 10 trials (Fig. 3C). Ethanol

not also decreased the rate of responding in the iLAD1 rats at a dose of 1.75 g/kg and decreased the mean delay in the iLAD1 rats at 1 and 1.75 g/kg (Fig. 3C). An ED_{50} analysis of the descending leg of the dose-response curve shows that ethanol decreased the rate of responding and mean delay in both strains and that there was no difference in the ED_{50} doses between the two strains (Table 1).

The relationship between ethanol preference and working memory function after saline in the iHAD2 and iLAD2 rats (Fig. 3D) was opposite that observed in the iP/iNP and iHAD1/iLAD1 rats. The mean delay observed for the session in the iHAD2 rats was longer ($t = 2.45$, $p = 0.03$) than that observed in the iLAD2 rats after saline administration, but there was no difference between the two strains in the rate of responding after saline. As in the other strains, ethanol decreased both the mean delay and rate of responding in these strains relative to saline. Doses of 1.25 g/kg and higher decreased the rate of responding in iHAD2 and iLAD2 rats, whereas a dose of 1.5 g/kg decreased the mean delay of both strains relative to saline. An ED_{50} analysis on the effect of ethanol showed no difference between the two strains in their sensitivity to ethanol (Table 1).

Discussion

The results of the present study show that under control conditions, the reinforcing properties of dry food, as measured by the breakpoint under a progressive ratio schedule, is greater in the Wistar rats than in any of the alcohol-preferring strains, but there is no difference among the alcohol-preferring and -nonpreferring strains as a function of alcohol preference. Thus, the difference in ethanol preference in these strains does not extend to differences in the reinforcing properties of dry food. Although there are significant differences between the body weights of the seven strains included in this study, the differences in body weight do not appear to be the explanation for the larger breakpoint in the Wistar rats. For example, there is little difference in body weight between the Wistar rats and iP rats, and a fairly large difference between the iP rats and iLAD2 rats. However, there is a large difference in the breakpoints observed for the Wistar and iP strains of rats, but no difference in the breakpoints was observed between the iP and iLAD2 rats. It also is unlikely that satiation is the explanation because increasing the starting FR value to that of 60% of the breakpoint value obtained when a starting FR value of 5 was used did not alter the breakpoint in any of the seven strains.

Ethanol decreased the breakpoint in all strains, and no difference was observed in the ED_{50} for ethanol in six of the seven strains. The iNP strain did have a lower ED_{50} value for ethanol than the Wistar, iP and iHAD1 strains, but there was no difference in the ED_{50} values for ethanol between Wistar, iP, iHAD1, iLAD1, iHAD2, and iLAD2 strains. Thus, these results do not appear to be the explanation for the differences observed in working memory function observed between the iP and iNP, iHAD1 and iLAD1, or iHAD2 and iLAD2 strains.

In both the iP/iNP and iHAD1/iLAD1 strains, baseline working memory performance was greater in the alcohol-nonpreferring strains compared with the alcohol-preferring strains. It is important to note that the performance of the iP rats was similar to that of the Wistar rats, and it is the iNP

rats that are different from the Wistar rats. It is also interesting that the reverse was true in the iHAD2/iLAD2 strains, with better working memory performance associated with the alcohol-preferring strain iHAD2. Why the opposite pattern was observed in the iHAD2/iLAD2 rats is not clear at this point.

When a dose-response curve for ethanol was determined in the seven strains of rats, the ED_{50} analysis of the effects of ethanol on the mean delay or rate of responding failed to show any differences as a function of alcohol preference in the three alcohol-preferring and -nonpreferring pairs. The only differences noted between the seven strains were between the Wistar rats and the iP rats for the effect on mean delay and between the Wistar rats and iHAD1 rats for the effect on rates of responding. Thus, what significant strain differences in response to ethanol that do exist between the alcohol-preferring and -nonpreferring strains are a function of baseline differences, and there is little evidence of differences in sensitivity to ethanol.

To our knowledge, this is the first study that has examined working memory in these inbred strains. It is interesting to note that a preliminary study from this laboratory reported a similar difference in working memory function in outbred P and NP rats by using the same delayed matching-to-position schedule as used in the present study (Wenger, 2002). In that study, the NP rats performed at approximately 80% accuracy at a delay that was more than twice as long that observed in the P rats. This indicates that the differences observed in the present study are not limited to the inbred strains. Taken together, these results suggest that the selective breeding that resulted in the well documented alcohol preferences in these rats also altered working memory function. However, at least in the iP and iNP rats and the outbred P and NP rats, the breeding program that resulted in the decreased alcohol preference increased the memory capacity compared with the outbred Wistar rats. Because the heterogenous N/NiH rat was not included in this study as a control for the genetic background of the iHAD1/iLAD1 and iHAD2/iLAD2 rats, a similar comparison cannot be made at the present time. The relationship between alcohol preference and baseline working memory performance observed in the iHAD1/iLAD1 pair of strains is opposite to that observed in the iHAD2/iLAD2 pair of strains. Thus, it is clear that the relationship between the parent strain and these alcohol-preferring and -nonpreferring strains is not the same as that observed for the Wistar and iP/iNP strains.

We are not aware of any systematic studies on neurochemical differences between the inbred strains that could be used to help understand the mechanism for the differences in baseline memory function. However, there are some neurochemical studies reporting differences in neurotransmitter systems using the outbred rat lines P/NP, HAD1/LAD1, and HAD2/LAD2. Several studies have reported differences in GABA systems in P/NP rats (McBride et al., 1990) and HAD/LAD rats (Hwang et al., 1990). Differences have also been reported in the dopamine systems of P/NP and HAD1/LAD1 rats (Gongwer et al., 1989; McBride et al., 1990; Katner and Weiss, 2001). Finally, differences have been reported in the serotonin systems of P rats compared with NP rats and in HAD1 rats compared with LAD1 rats (for reviews, see McBride et al., 1989, 1990). However, the limited number of studies and the direction of differences reported would not

appear to be consistent with the reports of differences in working memory reported in this study, and the question of what cellular mechanisms may mediate the differences in working memory must rely on future studies.

Finally, with respect to the issue of deficits in memory function in human alcoholics, the poorer memory function in two of the three alcohol-preferring strains relative to the nonpreferring strains is interesting, but it must be remembered that relative to the genetic background (Wistar), there is no deficit in working memory in the ethanol-preferring iP rats. Rather, it is the improvement in working memory observed in the nonpreferring iNP rat compared with the Wistar rat that accounts for the difference in working memory function between the iP and iNP rats. Thus, if there is a relationship between ethanol abuse and any preexisting cognitive function, it is not a simple relationship, and further study is needed to fully understand the role that cognitive function plays in ethanol abuse in humans.

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