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# HAS and LAS Rats Respond Differentially to Behavioral Effects of Ethanol, Pentobarbital, Chlorpromazine and Chlordiazepoxide[1]

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KRIMMER, E. C. *HAS and LAS rats respond differentially to behavioral effects of ethanol, pentobarbital, chlorpromazine and chlordiazepoxide*. PHARMACOL BIOCHEM BEHAV **39**(1) 5–13, 1991.—The drug discrimination paradigm (DD) was used to evaluate differences in performance of rats selectively bred for differential sensitivity to the hypnotic effects of ethanol. Tenth generation high-alcohol sensitive (HAS) and low-alcohol sensitive (LAS) rats were trained to discriminate between ethanol (1.0 g/kg, IP) and saline vehicle on a VR-5 schedule of reinforcement. The HAS strain was more sensitive to the discriminative effects of ethanol than the LAS strain, but the magnitude of difference was much smaller than the differential sleep-time differences. The biphasic action of ethanol was differentially seen when the LAS animals exhibited increased activity during both DD and spontaneous motor activity measures and the HAS exhibited decreased activity during DD only. Pentobarbital and chlordiazepoxide but not chlorpromazine elicited the ethanol discriminative choice in both HAS and LAS strains. Response rates during DD indicated a dissociation of rate depressant effects and discriminative performance following ethanol. These findings are discussed in relationship to some current and future uses of selectively bred animal strains and DD for studying the effects of alcohol.

Ethanol Drug discrimination Selective breeding HAS LAS Alcohol Sleep-time Stimulus properties of drugs

ETHANOL is primarily considered a central nervous system depressant capable of impairing, retarding and/or disrupting normal function. A moderate to high dose (>1 g/kg) is usually required to be able to measure decrements in performance, which is likely to reflect nonspecific toxicity (1).

Some effects of ethanol are under genetic influence and, therefore, may be inherited in humans (7,19). Animals have been selectively bred for differences in sensitivity to the depressant effect of ethanol (23). Sleep-time, measured by the interval from loss-to-recovery of the righting response following an hypnotic dose of ethanol, is an accepted method for determining sensitivity to ethanol. High-alcohol sensitive (HAS) and low-alcohol sensitive (LAS) strains of rats have been selectively bred for differential sensitivity to the hypnotic effect of ethanol. This measured effect is based on what may be considered untoward side effects rather than any subjectively perceived effect, i.e., an action of ethanol sought by humans during recreational use.

The subjectively perceived effects of ethanol were recently described for the HAS and LAS strains in a report from this laboratory (11). Pharmacological effects of ethanol served as differential stimuli in an operant conditioning task. Animals learned to make alternative responses solely on the basis of ethanol (0.6 g/kg, IP)-induced interoceptive cues. The results showed that, whereas the HAS and LAS strains clearly differed in sleep-time duration, there was only a marginal difference in their ability to discriminate ethanol from saline. A small difference was observed early in their training (40 sessions) but disappeared after 50 or 60 training sessions. Ethanol doses up to 0.9 g/kg did not differentially affect the response rates of the two strains. The results thus lend support to earlier suggestions that the discrimination of ethanol is not based upon its effect on response rates (3,25).

Frequent consumption of alcoholic beverages in many societies suggests that the effects of alcohol may have special attraction for many people. This might be related to the discriminative stimulus attributes of this drug (1). Alcohol has frequently been used in the drug discrimination paradigm (2, 11, 17). Drugs, however, are well known to have multiple actions and it has been suggested that the multiplicity of actions of centrally acting drugs may contribute to their effectiveness as discriminative

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stimuli (20). When drug conditions function as differential signals, it seems probable that the differential responses are based on distinctive rather than strong reinforcing or aversive effects of the drug (1). The advantages in using this behavioral paradigm to study the pharmacological effects of ethanol are that: a) drug discrimination does not presuppose either the reinforcing properties as proposed to be essential for self-administration (SA) or the aversive properties suggested to function to allow for conditioned taste aversion (CTA), and b) drug doses found capable of controlling differential responding in drug discrimination studies are low, so as not to rely on measured decrements to normal performance which result from nonspecific toxic effects. The discrimination protocol being reported here, however, includes the advantage of being able to determine the effects of ethanol upon response rates at the same time that it measures discriminative performance. Additionally, tests were conducted with three other CNS depressant drugs, pentobarbital, chlorpromazine, and chlordiazepoxide, in order to correlate the relationships between drug discrimination effects and response rate changes in the HAS/LAS rats.

## METHOD

### *Animals*

The eighteen male rats for this study were received from the Alcohol Research Center, University of Colorado Health Science Center and were the result of selective breeding to develop separate phenotypes with high-alcohol sensitivity (HAS) and low-alcohol sensitivity (LAS). These rats were the third through fifth littermates as ranked by an alcohol sleep-time screening test conducted by the supplier. (The first and second littermates were retained as the best phenotype representatives for continued breeding purposes.) Sensitivity was determined by the duration of loss of righting reflex (LRR) following parenteral administration of 3.0 g/kg ethanol (23). The original animal stock was the N/Nih heterogeneous strain (HS) that is presently maintained by the National Institutes of Health (NIH) Animal Resource Center (8). The animals (9 HAS and 9 LAS) represent the tenth generation of selective breeding and were previously trained to discriminate ethanol (0.6 g/kg) from saline (11).

The animals were individually housed in suspended metal wire cages and allowed free access to water. Daily food rations of commercial rat chow necessary to maintain their weights at 85–90% of their free-feeding weight were made available at approximately 1100 h each day following daily test/training sessions. Vivarium facilities had an ambient temperature of 20–22°C and were maintained on a 12:12 light:dark cycle with lights on at 0600 h.

### *Drug Discrimination Training*

The experimental space consisted of standard rodent operant test cages and computer interface (Med Associates Inc., East Fairfield, VT). Each chamber was equipped with two operant levers and a food receptacle located equidistant between the two levers. The test environment was contained in a ventilated, sound-attenuated cubicle equipped with a house light. All test parameters and data collection used a modified version of the software package described by Emmett-Oglesby et al. (5) and Spencer and Emmett-Oglesby (22).

The initial training procedures that were employed to establish discriminative response control with 0.6 g/kg ethanol and its saline vehicle are described in detail elsewhere (11). Briefly, the animals were trained to press one lever after receiving 0.6 g/kg ethanol (10% w/v in saline) and to press the alternative lever after receiving an equal volume (6 ml/kg) of saline. The drug condition (ethanol or saline) was administered intraperitoneally 10 min prior to a 10-min training session. Initially a reinforcement (a 45 mg Noyes food pellet) was delivered following each correct lever press (FR-1). A variable ratio of 2 (VR-2) schedule was introduced after 6 training sessions and increased to VR-5 (SD = 1). This final schedule (VR-5) defines a variable ratio where the mean number of correct responses needed for reinforcements is 5 with 95% of all reinforcements occurring between 3 and 7 responses. Incorrect responses were recorded but produced no programming consequence.

Starting with the 75th training session, the ethanol training dose was increased from 0.6 g/kg to 1.0 g/kg (10% w/v in saline solution). The initial lower ethanol training dose was specifically selected so that learning would occur over a moderately extended period of time. This permitted dose functions to be obtained at several stages of learning, i.e., after 40, 50 and 60 training sessions. In addition, the low dose selected for training allowed for performance to improve over the series of training sessions and provided latitude for differential responding to lower and higher ethanol doses. The dose was subsequently increased for the present study to optimize the discriminability within a food-rewarded task and to be comparable to the dose employed in other published studies (1). After administration of either ethanol or saline the animal was returned to its home cage and following a 10-min period for drug absorption it was placed into the test chamber and the house light turned on to signal the beginning of a 10-min training session. Depending on whether the animal had received ethanol or saline, it obtained reinforcements by pressing either the designated "ethanol lever" or "saline lever," respectively. In all training sessions the drug conditions randomly alternated between ethanol and saline with the restriction that the same drug condition not be administered on more than 2 consecutive training sessions. To control for possible position preference, lever assignments were counterbalanced for ethanol and saline and for HAS and LAS animals. These condition-lever assignments remained constant throughout the experimentation.

During the initial training period, a variable ratio schedule of VR-5 (SD = 1) was achieved and that schedule continued throughout this study. Subsequently, extinction periods of 0, 15 and 60 seconds, at the beginning of the 10-min training session, were introduced into the initial training and continued to be employed. During an extinction period lever presses were recorded but no reinforcements were delivered. The extinction durations of 0, 15, and 60 s, were randomly alternated and imposed with equal frequency under each training condition. A reinforcement was delivered on the first and each subsequent completions of the VR-5 schedule that occurred following the extinction period. The animals were trained between 0800 and 1100 h on 5 days each week.

### *Test of Novel Drug Conditions*

Following 24 training sessions (12 with each condition) that established discrimination with the higher ethanol dose (1 g/kg), tests with ethanol doses and administration times that differ from the training dose/time, as well as tests with novel drugs, were interspersed with maintenance training sessions. A test session began with an initial 60-s extinction period. However, the animal was immediately removed from the test chamber and returned to its home cage following the 60-s extinction period without receiving reinforcement.

Ethanol (at doses of 0.3, 0.6, 1.2 and 1.5 g/kg) was tested 10 min after injection (dose-response experiment) as well as 2.5,

40, 60, 90, 180 and 240 minutes after 1.0 g/kg administration (time-course experiment). Pentobarbital doses of 2.5, 5, 10 and 12 mg/kg were tested at 10 min and pentobarbital 10 mg/kg was tested at 30, 60 and 120 min after administration. Chlorpromazine (1, 3, and 4 mg/kg) was tested at 30 minutes and 1 mg/kg at 30, 60 and 120 minutes. Chlordiazepoxide doses of 2.5, 5, 10, 12, and 15 mg/kg were tested at 30 minutes and 10 mg/kg chlordiazepoxide was tested at 10, 30, 60, 120 and 180 min after its administration. The dose used for each time-course determination was selected on the basis of frequently used training doses in other drug discrimination studies (1). Pentobarbital Na and chlorpromazine HCl (Sigma Chemical Co., St. Louis, MO) and chlordiazepoxide HCl (Hoffmann-La Roche Inc., Nutley, NJ) were all prepared in 0.9% saline and administered intraperitoneally. The concentrations were based on the salt weight and administered in a volume of 1 ml/kg.

*Sleep-Time Tests*

For the purposes of sleep-time testing, ethanol (15% w/v solution in saline) was administered intraperitoneally in a dose of 3.0 g/kg of body weight. The moment of loss of righting reflex (LRR) was taken as zero time and the time until the animal regained the righting reflex (RR) was recorded. The criterion for regaining the righting reflex was met when the animal recovered from lying on its back on a flat surface and placed all four feet under it, three times in 60 s. Identical sleep-time tests were conducted with pentobarbital at a dose of 60 mg/kg (30 mg/ml solution in saline). Animals (1 HAS, 2 LAS) that had not regained RR after 7 hours were given a sleep-time score of 420 min.

The first ethanol sleep-time test with ethanol was conducted 14 days following the completion of dose- and time-function determinations with ethanol, pentobarbital, and chlorpromazine. The pentobarbital sleep-time tests were conducted 15 days later and the second ethanol sleep-time 14 days following the pentobarbital sleep-time test.

*Spontaneous Motor Activity (SMA)*

The activity units were identical Plexiglas cages ($L \times W \times D = 45.5 \times 35.5 \times 20.5$ cm) covered by a wire top. Four photo sensors and light sources were located 5.5 cm above the floor and 9.5 cm apart on the walls of the longer sides. Each interruption of a photo unit constituted one activity unit which were cumulated during 6 five-minute periods and recorded on a Commodore 64 computer. Room lights remained on during the entire procedure.

A series of 6 tests for SMA were started 4 days after the second ethanol sleep-time test. Tests following injections of either saline (S) or various doses of ethanol were conducted over a period of 9 days using the sequence S, 0.6, 1.0, 0.3, S, and 1.2 g/kg. Ethanol (15% w/v in saline) was administered intraperitoneally and the animal returned to its home cage for 10 minutes prior to being placed into an activity measuring unit for a 30-minute period.

*Data Analyses*

Drug lever choice is expressed as the percentage of total responses made on the ethanol designated lever during 60-s extinction periods. Total responding (combined presses on either lever) was also assessed. Both percent drug choice and total responding were analyzed using repeated measures analysis of variance (ANOVA) across doses for each animal with phenotype as the independent grouping factor. In those cases when ethanol completely suppressed responding, that animal contributed a score of zero to the response rate average. A percent drug choice score cannot be calculated in that instance and thus the animal does not contribute a score to the analysis with ANOVA. One-way analysis of variance was used to analyze dose-effects for each phenotype separately and was followed, when appropriate, by post hoc Scheffe's tests for comparisons with the saline control results. A $p<0.05$ was taken to indicate a significant difference. A computer-generated formulation of Litchfield-Wilcoxon analysis (24) yielded $ED_{50}$ values and 95% confidence limits for ethanol dose-response curves.

## RESULTS

*Drug Discrimination*

Figures 1, 2, 3 and 4 graphically portray results for ethanol, pentobarbital, chlorpromazine and chlordiazepoxide respectively. The top portion in each figure represents the percent drug choice for the dose-function determination (left side) and time function determination (right side). The lower portion of each figure shows the corresponding response rates generated during the same dose (lower left) and time (lower right) function tests. Scores for discrimination and response rates after saline (SAL or 0.0 dose) tests are included as reference points in each graph.

*Ethanol Tests*

The percentage of total responses made on the drug lever during the 60-s extinction periods that preceded 6 training sessions were averaged for each animal to provide saline and ethanol (1.0 g/kg) baseline rates. These 6 sessions were part of the ongoing maintenance sessions interspersed between tests with novel conditions. The mean percentage ethanol lever choices were 79.9% after 1.0 g/kg ethanol and 18.5% after saline (or 81.5% on the saline-appropriate lever) by the HAS animals and 72.2% after ethanol and 21.2% for saline by the LAS animals.

The selection of the drug lever was generally dose related for larger and smaller ethanol doses for both HAS and LAS animals (Fig. 1, upper left). A significant effect of phenotype on the overall response to ethanol doses was found, $F(1,16) = 12.72$, $p = 0.003$, thus indicating that HAS animals were more sensitive to the discriminative effects of ethanol. No interaction between dose and phenotype was found, $F(4,64) = 0.50$, $p = 0.74$. The upper right hand side of Fig. 1 shows percentage of ethanol lever choices during discrimination tests with 1 g/kg ethanol at novel time intervals. There was no overall effect of phenotype on percentage drug lever choice over the intervals of 2.5 to 240 min, $F(1,16) = 0.036$, $p = 0.852$.

The response rates (Fig. 1, lower half) subjected to the repeated measures ANOVA indicate no effect of phenotype on the response rates, $F(1,16) = 1.48$, $p = 0.241$, for ethanol doses 0.3 to 1.5 g/kg (lower left). A phenotype-dose interaction also did not occur, $F(4,64) = 0.93$, $p = 0.453$. There was no overall effect of phenotype on response rates over the full range (2.5 to 240 min; lower right) of test intervals, $F(1,16) = 2.70$, $p = 0.1198$. A separate analysis of response rates was made for those novel time intervals greater than the training interval of 10 minutes and during the times when percent drug choice differed from the saline baseline percentage, i.e., at 40, 60, and 90 min postinjection. A phenotype effect on response rate was found, $F(1,16) = 6.267$, $p = 0.024$. The HAS and LAS response rates differed significantly at the 60-min interval ($p<0.05$) with LAS rats showing less response suppression.

One-way ANOVA with subsequent Scheffe tests were used to test for significant differences from the saline baseline scores.

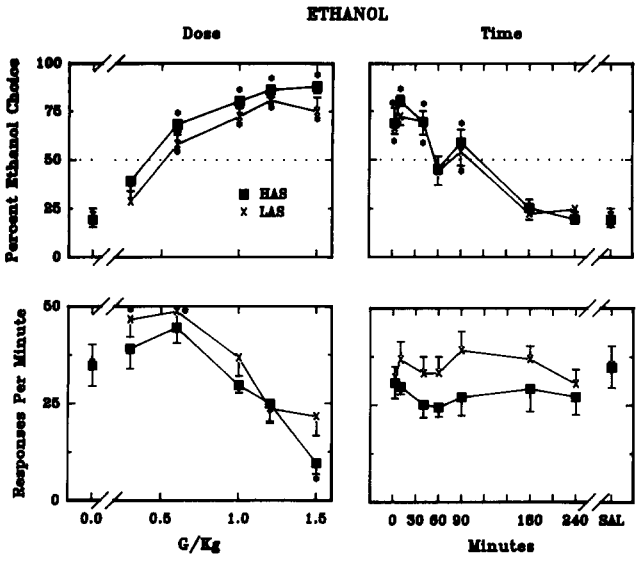


FIG. 1. Percent ethanol choice (top portion) and responses per minute (bottom portion) for various ethanol doses (left-hand side) and various times following 1.0 g/kg ethanol (right-hand side). The zero doses are baseline values for saline. Points are means for HAS ■ (n = 9) and LAS X (n = 9) strains. Vertical lines indicate positive or negative half of S.E.M. (*) indicates significant difference between ethanol result and saline baseline ($p<0.02$).

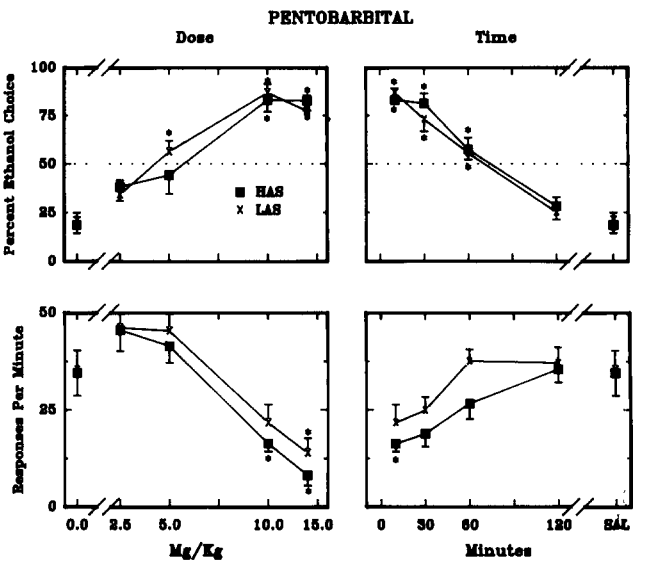


FIG. 2. Percent ethanol choice (top portion) and responses per minute (bottom portion) for various doses of pentobarbital (left-hand side) and various times following 10 mg/kg pentobarbital (right-hand side). The zero dose are baseline values for saline. Points are means for HAS ■ (n = 9) and LAS X (n = 9) strains. Vertical lines indicate positive or negative half of S.E.M. (*) indicates significant difference between the pentobarbital results and saline baseline ($p<0.02$).

The percent drug choice differed from the saline results for both HAS and LAS animals following doses of 0.6–1.5 g/kg ethanol ($p<0.003$). Response rates during the dose function determination (Fig. 1, lower left) showed trends for an increased rate following 0.3 and 0.6 g/kg and a subsequent depressed rate for higher doses. The increased response rates were significant ($p<0.02$)
following 0.3 and 0.6 g/kg ethanol for the LAS animals. The change from saline was significant only for the HAS animals ($p<0.003$) following ethanol 1.5 g/kg. The percent of ethanol choice responding for HAS and LAS animals following 1.0 g/kg ethanol (Fig. 1, upper right) differed from that of saline during tests at 2.5 to 90 min ($p<0.02$). The response rates for HAS and LAS animals did not differ from their respective saline rates at any time interval.

Ethanol $ED_{50}$ values (with 95% confidence limits) were 380 (211–684) mg/kg for the HAS animals and 519 (322–837) mg/kg for the LAS animals. These $ED_{50}$ values reflect the overall phenotype difference found with ANOVA for the two groups, but the $ED_{50}$ values do not significantly differ.

*Pentobarbital Tests*

Figure 2 graphically presents the results of discrimination tests and response rates with various doses of pentobarbital (left side) and tests at various times following 10 mg/kg pentobarbital (right side). Pentobarbital elicited the ethanol response in a dose-related manner for both the HAS and LAS groups (upper left). There was no effect of phenotype on the overall response to pentobarbital doses of 2.5 to 12 mg/kg, $F(1,16) = 0.0944$, $p = 0.763$. The effects of 10 mg/kg pentobarbital dissipated by 120 min (upper right) and there was no influence of phenotype on the generalization of the ethanol response to 10 mg/kg pentobarbital between 10 and 120 min, $F(1,16) = 0.425$, $p = 0.524$. Response rates for the doses of 2.5 to 12 mg/kg (lower left) also did not differ between the HAS and LAS rats, $F(1,16) = 1.245$, $p = 0.281$. The response rates following pentobarbital 10 mg/kg returned to the saline level at the 120-min test (lower right). There was a phenotype difference overall for the interval of 10 to 60 min, $F(1,16) = 5.435$, $p = 0.033$, which were those time intervals that percent ethanol choice responses for both HAS and LAS animals differed from the saline baseline percentages (above).

The $ED_{50}$ values (with 95% confidence limits) for pentobarbital were 4.15 (2.43–7.10) mg/kg for the HAS animals and 3.95 (2.33–6.68) mg/kg for the LAS animals (Table 1). These $ED_{50}$ values do not significantly differ.

One-way ANOVA with subsequent Scheffe tests were used to test for significant differences from the saline baseline scores. The percent drug choice differed from the percent drug choice following saline for both HAS and LAS animals following 10 and 12 mg/kg pentobarbital ($p<0.0001$) and following 5 mg/kg for the LAS animals ($p<0.005$). The response rates with pento-

TABLE 1

SLEEP-TIME AVERAGES (MIN) AND RANGES FOR HAS AND LAS ANIMALS ADMINISTERED ETHANOL (3.0 g/kg) OR PENTOBARBITAL (60 mg/kg) INTRAPERITONEALLY

| | HAS | | LAS | |
|---|---|---|---|---|
| Test No. | Mean | Range | Mean | Range |
| Ethanol* | 194.0 | 102–306 | 36.8 | 10–120 |
| Pent | 331.0 | 251–420 | 320.1 | 156–420 |
| Ethanol* | 237.0 | 183–327 | 53.3 | 0–90 |

*$p<0.0001$ for phenotype differences.

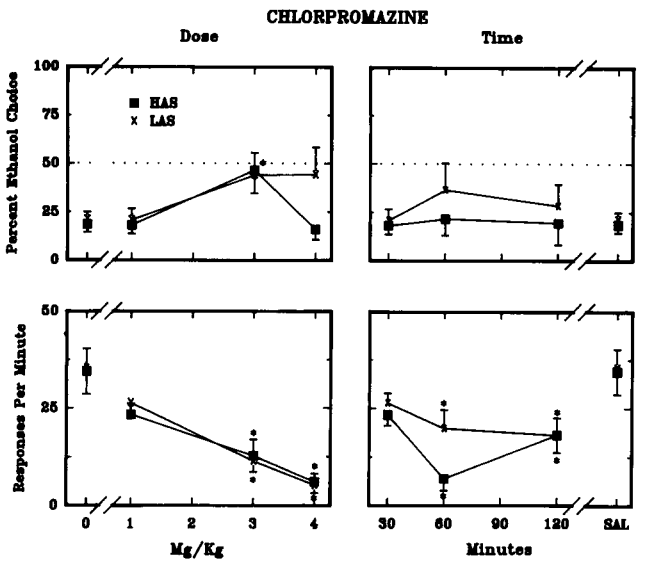


FIG. 3. Percent ethanol choice (top portion) and responses per minute (bottom portion) for various doses of chlorpromazine (left-hand side) and various times following 10 mg/kg chlorpromazine (right-hand side). The zero doses are baseline values for saline. Points are means for HAS ■ (n=9) and LAS X (n=9) strains. Vertical lines indicate positive or negative half of S.E.M. (*) indicates significant difference between the chlorpromazine results and saline baseline ($p<0.02$).

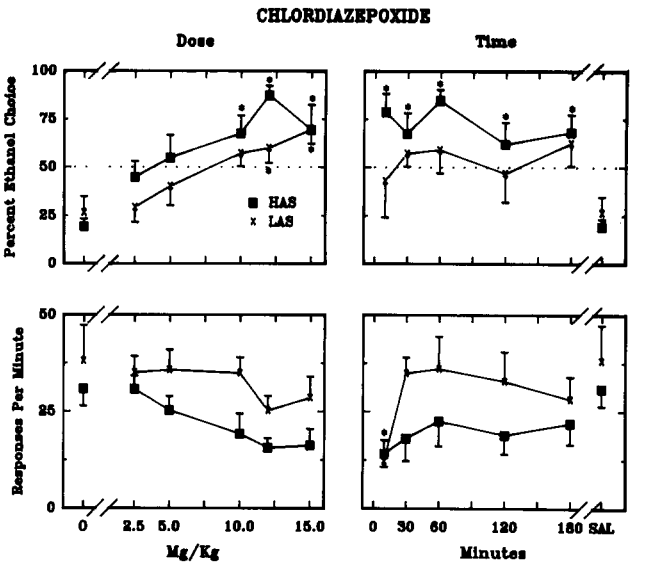


FIG. 4. Percent ethanol choice (top portion) and responses per minute (bottom portion) for various doses of chlordiazepoxide (left-hand side) and various times following 10 mg/kg chlordiazepoxide (right-hand side). The zero doses are baseline values for saline. Points are means for HAS ■ (n=7) and LAS X (n=6) strains. Vertical lines indicate positive or negative half of S.E.M. (*) indicates significant difference between the chlordiazepoxide results and saline baseline ($p<0.05$).

barbital test doses differed from the response rates following saline for both HAS and LAS animals following 12 mg/kg pentobarbital ($p<0.02$) and following 10 mg/kg for the HAS animals ($p<0.03$). Response rates during the pentobarbital dose function determinations (Fig. 2, lower left) showed a trend similar to that seen for ethanol with an increased rate following 2.5 and 5 mg/kg followed by a subsequent depressed rate for higher pentobarbital doses. The higher response rate, however, did not significantly differ from the saline rates. The percent of ethanol choice responding for both HAS and LAS animals following 10 mg/kg pentobarbital (Fig. 2, upper right) differed from that for saline during tests at 10 to 60 min ($p<0.0001$). The response rates for only the HAS animals differ from their saline rates at the 10-minute test interval ($p<0.02$).

*Chlorpromazine Tests*

Figure 3 presents the results of tests with various doses of chlorpromazine (left side) and tests at various times after 1 mg/kg of chlorpromazine (right side). Chlorpromazine at doses of 1, 3, and 4 mg/kg (upper right) did not elicit the ethanol discriminative response. These same doses, however, produced a marked depression of response rates for both HAS and LAS animals which were significantly different from the saline rates for both groups at 3 and 4 mg/kg ($p\leq0.001$). Chlorpromazine 1 mg/kg did not elicit the ethanol choice at any times between 30 and 120 minutes (upper right) but still depressed responding below the saline rates at 60 and 120 minutes for both HAS and LAS animals ($p<0.02$).

*Chlordiazepoxide Tests*

Figure 4 presents the results of tests with various doses of chlordiazepoxide (left side) and tests at several times following 10 mg/kg of chlordiazepoxide (right side). Between the chlorpromazine tests and the tests with chlordiazepoxide, the animals underwent sleep-time tests with pentobarbital and ethanol and the tests for spontaneous motor activity. During this same interval 2 HAS and 3 LAS animals died of unknown causes. The changes are reflected in the baseline results of Fig. 4. Chlordiazepoxide elicited the ethanol response in a dose-related manner for both the HAS and LAS groups (upper left). There was no effect of phenotype on the overall response to chlordiazepoxide doses of 2.5 to 15 mg/kg, $F(1,11)=1.9002$, $p=0.195$. The effects of 10 mg/kg chlordiazepoxide during the times of 10–180 minutes differed for the two groups, $F(1,11)=8.21$, $p=0.015$, indicating that the HAS rats were more sensitive to the discriminative effects of chlordiazepoxide over the tested time course. Response rates for doses of 2.5 to 15 mg/kg (lower left) also differ for the HAS and LAS groups, $F(1,11)=7.506$, $p=0.019$. An overall phenotype difference was not found for the interval of 10 to 180 min, $F(1,11)=2.681$, $p=0.130$. The LAS animals showed a marked and unexplained depression of response rate for 10 mg/kg chlordiazepoxide at ten minutes ($p=0.03$).

The $ED_{50}$ values (with 95% confidence limits) for chlordiazepoxide were 3.13 (1.10–8.92) mg/kg for the HAS animals and 7.04 (2.90–17.06) mg/kg for the LAS animals (Table 1). These $ED_{50}$ values do not differ significantly.

One-way ANOVA with subsequent Scheffe tests showed the percent ethanol lever choice differed from the same choice following saline for LAS animals given 12 and 15 mg/kg chlordiazepoxide ($p<0.05$) and following 10–15 mg/kg for the HAS animals ($p<0.04$). Response rates during the chlordiazepoxide dose function determinations (Fig. 4, lower left) did not show trends similar to those seen for ethanol and pentobarbital with increased responding following low doses and subsequent depressed responding following higher doses. While both HAS and LAS animals showed depressed response rates at the 10 minute

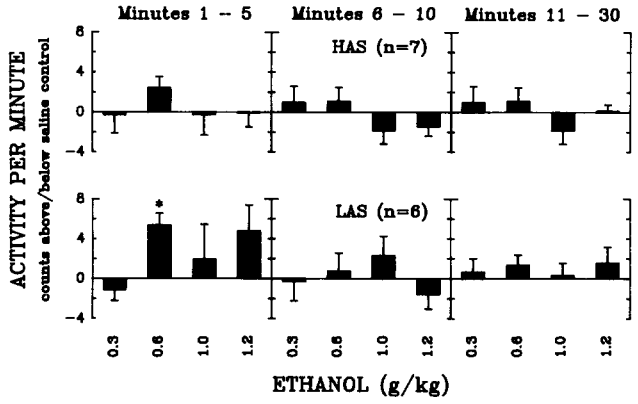


FIG. 5. Spontaneous motor activity shown as counts above or below saline control values for various doses of ethanol. Minutes 1–5 is the period 10–15 minutes post ethanol administration, minutes 6–10 is the period 16–20 minutes post ethanol and 11–30 is the period 21–30 minutes post ethanol. *Indicates activity significantly different than saline activity ($p<0.05$).

interval (Fig. 4, left) only the marked decrease for the LAS group was significant ($p=0.027$).

*Sleep-Time Tests*

The group averages for sleep-time tests are presented in Table 1. The sleep-time averages for the first ethanol test were 194 min for the HAS group and 36.8 min for the LAS group. A second ethanol sleep-time determination showed mean sleep-times of 237.0 and 53.3 min for the HAS and LAS lines, respectively. The overall results for ethanol are quite similar for averages and ranges of both lines and agree with earlier results obtained for these animals (11). HAS- and LAS-selected animals continued to be two very distinct groups of animals with no overlap of sleep-time measurements scores for ethanol. A sleep-time test with pentobarbital conducted between the first and second ethanol test, however, produced average sleep-times of 331.0 and 320.1 for the HAS and LAS line, respectively, suggesting that sensitivity to the depressant effects of ethanol does not generalize to all depressants.

*Spontaneous Motor Activity*

Figure 5 graphically presents the results of four doses (0.3, 0.6, 1.0, 1.2) of ethanol on activity. The activity rates for the 2 saline tests were averaged for each rat to establish a baseline activity level. Changes from the baseline caused by each ethanol dose were calculated (ethanol activity − baseline activity) separately for each animal. The results are given as activity rates per minute. The bars represent the averages for either the HAS (upper) or LAS (lower) animals. The left portion of Fig. 5 shows results of the first 5 minutes (minutes 1–5), the middle portion shows the second 5 minutes (minutes 6–10) and the last 20 minutes are presented in the right panels (minutes 11–20). There was a trend for increased activity by the LAS animals during the first 5 minutes in tests with ethanol doses of 0.6–1.2 g/kg and the increase was significant ($p<0.05$) for the 0.6 g/kg ethanol dose.

## DISCUSSION

The initial investigation of drug discriminative learning for these HAS/LAS rat strains used 0.6 g/kg ethanol for their training and reported that a difference between strains existed only during the early phase of training, i.e., following 40 sessions (11). The HAS line was more sensitive to the discriminative effects of ethanol at that earlier phase of discrimination learning but differential sensitivity was not detected in subsequent tests, i.e., after 50 or 60 training sessions. The present report extends these findings by including training sessions 75 through 139 in these same rats and, furthermore, employed the higher dose of 1.0 g/kg ethanol for this continued training. The purpose for increasing the dose during this phase of training was intended both to enhance the overall discriminative performance and to provide a dose that would be capable of inducing a mild response rate depression which had not been detected during the earlier training with 0.6 g/kg ethanol. The strain differences for the discriminative effects of ethanol indicating slightly greater sensitivity for the HAS line was restored at the higher ethanol dose and persisted throughout this later phase of training.

The $ED_{50}$ quantifies the potency the discriminative effects of a test drug for comparisons. Any $ED_{50}$ calculated for the training drug, however, is also a function of the training dose, i.e., the $ED_{50}$ is proportional to the training dose (1). Ratios of the $ED_{50}$ to the training dose (0.6 g/kg) were calculated from previous tests with these animals (11). The ratios were 0.32 and 0.59, respectively, for the HAS and LAS strains. These ratios are quite similar to those of 0.38 and 0.52 calculated, respectively, for the same animals in the present study following training with 1.0 g/kg ethanol. It is worth noting that the lower ratios for the HAS rats indicate a trend for greater sensitivity of that strain and that the ratios for the LAS rats are quite similar to those ratios reported for heterogeneous rats strains (1). These comparisons suggest that the outcome of selective breeding seems to have resulted in the HAS strain differing more from the heterogeneous strains than the LAS strains, at least, as reflected by differential sensitivity for the discriminative effects of ethanol.

Figure 1 (lower left) indicates that the two lowest novel doses of ethanol (0.3 and 0.6 g/kg) produced trends of increased responding. The rates differed from the saline rate for the LAS line at both doses ($p<0.02$). An independent measure of spontaneous motor activity (Fig. 5) also reflects the stimulant effects of 0.6 g/kg ethanol for the LAS line. Minutes 1 to 5 of SMA correspond to the first 5 minutes of discriminative drug training. Thus the short interval after the 10-min drug onset period also showed a significant increase of activity for the LAS animals in both the discrimination paradigm and the SMA tests. Apparently, the depressant actions of ethanol become a factor during minutes 6 to 10 (16–20 minutes postadministration) when no differences of SMA are apparent. The two test doses of 1.2 and 1.5 g/kg produced trends of decreased responding. The response rates differed significantly from the saline rate only for the HAS strain following the 1.5 g/kg ethanol dose. Thus the LAS animals were sensitive to the rate-increasing stimulant effects of a low ethanol dose, presumably because they are less sensitive to the depressant actions, whereas the HAS were sensitive to a rate depressant effect of a moderate ethanol dose. In spite of some differences between their respective saline response rates and rates following individual ethanol doses, the LAS and HAS animals do not show an overall effect of phenotype on response rate. A biphasic action of ethanol has also been reported with similar doses in other appetitive behavior with heterogeneous rat strains (9,13).

The discriminative drug effects were clearly dissociated from the depressant effects of ethanol as revealed during the time course experiment (Fig. 1, right side). The percent ethanol lever choice during the time course of 2.5 to 240 minutes was nearly identical for the HAS and LAS strains. The rapid onset of the

discriminable effects of ethanol within 2.5 minutes of administration agrees well with results obtained in heterogeneous rat strains trained with 1.0 g/kg ethanol at 20 min (10). However, the response rates for the HAS animals were significantly depressed compared to the LAS strain during those novel test times (40–90 min) when the percent ethanol choices following 1 g/kg ethanol significantly differed from the choice following saline for the respective strains. These results reflect a greater susceptibility of the HAS strain to the depressant effects while at the same time the HAS strain did not differ from the LAS strain in perception of and response to the discriminative effects of ethanol.

The significance of the biphasic effects of ethanol has previously been investigated in the drug discrimination paradigm when separate groups of rats were trained to discriminate 1.0 g/kg ethanol at 6 or 30 minutes after administration (21). A symmetrical inability to generalize to ethanol occurred when it was tested at the alternative onset times. This study also provided information suggesting the neurochemical mechanism of the biphasic effects of ethanol. Naloxone antagonized the early phase (excitatory) but not the later phase (sedation), thus suggesting a link between ethanol and endogenous opioids. Schechter (18) used the lower dose of 0.6 g/kg ethanol with the same onset times of 6 and 30 minutes. He reported an asymmetrical generalization in which only the animals trained at 30 minutes generalized to the alternative test interval. Both reports suggest that training at the short interval emphasizes the "excitatory" properties of ethanol whereas the longer interval emphasized the "sedative" properties. Schechter (18) suggests that animals trained at 30 minutes experienced stimuli at both 6 and 30 minutes, whereas animals trained at 6 minutes have experienced only stimuli produced from 0 to 6 minutes.

The significance of distinguishing the biphasic effects of ethanol has been shown in a clinical study involving normal (nonalcoholic) males (4). Those subjects with a positive family history of alcoholism (FHP) did not differ from those with a negative family history of alcoholism (FHN) on most measures of subjective and behavioral responses to ethanol. Nonalcoholic FHP males, however, did report a faster onset of ethanol effects than did the FHN group. The authors suggest that the differential onset times may "contribute to differential reinforcing effects, and relatedly, risk for excessive use."

Application of these suggestions to the present study would imply that the animals may have experienced a conflicting stimulus complex during the training period. That is the stimuli upon entering the training chamber (at 10 minutes post drug administration) may still be excitatory (euphoric) in nature; an effect which then dissipates to the early stage of depression as the 10-minute session progressed. This proposed ambiguity may account for the slightly less than optimal learning that occurred in both HAS and LAS animals. This consideration has played a major role in designing a study currently underway using the high alcohol drinking/low alcohol drinking (HAD/LAD) strains of rats. The drug onset time for discriminative training with ethanol has been shortened to 2 minutes and training duration to 6 minutes in order to emphasize the excitatory stage. Other animals of these strains are being trained during the period 30 to 36 minutes postadministration.

In the present study a sufficient dose of pentobarbital elicited the ethanol choice response, an effect reported previously for heterogeneous rat strains (1,2). Unlike the results with ethanol, the percent ethanol choice over a wide range of pentobarbital doses does not show a phenotype difference. When a dose of pentobarbital (10 mg/kg) was tested at various times after administration, the results were quite similar to those observed after ethanol, i.e., the percent ethanol response choice made following pentobarbital differed from the percent choice following saline for both the HAS and the LAS strains at 10, 30, and 60 minutes. However, there was no overall strain difference. The corresponding response rates for these same times reflected a significantly lower response rate for the HAS strain similar to the results with ethanol.

Differential effects for the depressant effects of pentobarbital have been reported during sleep-time tests with the HAS and LAS strains (23). Riley and co-workers (14,15) reported similar differential sensitivity to various depressants in the MA and LA lines, i.e., rats selectively bred for sensitivity to motor impairment measured in a stabilimeter following 1.5 g/kg ethanol. In contrast to these findings, no differences were observed with respect to pentobarbital-induced hypothermia in the MA and LA rats (12), thus emphasizing the point that selective breeding for the depressant/sedative properties of ethanol does not ensure differential sensitivity to other effects of ethanol.

The present study did not find differential sleep-times for the HAS and LAS animals in a test with 60 mg/kg pentobarbital (Table 1) unlike a report by Spuhler et al. (23) who used the same strains and pentobarbital dose and obtained much shorter sleep-times for both strains. It should be noted that the animals of this study had undergone considerable exposure to ethanol and mild exposure to pentobarbital prior to the sleep-time test with pentobarbital. In spite of these exposures the results of a subsequent sleep-time test with ethanol, however, were quite similar to previous results that found very clear strain effects in relatively drug-naive animals. Thus it appears that differential sleep-time sensitivity of the HAS/LAS animals to pentobarbital, unlike that for ethanol, is susceptible to changes produced by repeated exposure to ethanol and/or pentobarbital. Some interesting comparisons can be made in the long-sleep (LS) and short-sleep (SS) mice. The SS mice were more active in an open-field test after ethanol than were LS mice. The lines did not differ in performance on a rotating-rod apparatus after the same ethanol doses. A similar difference in the open-field activity of the selected lines was observed with pentobarbital (16). The selected lines, however, do not differ in sleep-time to 60 mg/kg pentobarbital (6).

The discriminative effects of ethanol did not generalize to several doses of chlorpromazine that were sufficient to depress response rates below the saline rates. Similar results have been found in heterogeneous rats (10) and provide additional evidence that sedation or depressant effects of ethanol are not a crucial component of the ethanol discriminative stimulus complex.

A sufficiently high dose of chlordiazepoxide, however, did elicit the ethanol response in both the HAS and LAS strains. Similar to the results for pentobarbital, there was no strain difference over the dose range of 2.5 to 15 mg/kg chlordiazepoxide. There was an effect of strain on the corresponding response rates during the dose-response determination ($p<0.02$) suggesting that the animals were reacting differentially to the sedative/depressant properties of chlordiazepoxide. The two strains responded differentially when 10 mg/kg chlordiazepoxide was tested over the time range of 10 to 180 minutes ($p<0.02$), but there was not a corresponding difference for a comparison of response rates.

The small but significant strain difference of the present study might be expected considering that the higher ethanol training dose (1.0 g/kg), although only slightly greater than the original training dose (0.6 g/kg), was capable of depressing behavior here, as in other studies (13). The saliency of the multiple actions that contribute to the discriminative stimulus complex of ethanol may have shifted with this change in dose. Thus the emphasis of ethanol actions in this paradigm may have

moved closer to the basis for selective breeding of these two lines, i.e., closer to the hypnotic effects induced by 3–3.6 g/kg. The results of this study suggest that ethanol's discriminative effect, rate depressant effect and hypnotic effect are not simply on a single continuum and, therefore, different doses of ethanol are not merely different magnitudes of the same stimulus.

Alcohol is known to have a multiplicity of actions. It is also accepted that many factors are involved in the development of alcoholism, including genetic factors. The present study in two of several strains (HAS/LAS) of rats selectively bred for a specific action of ethanol was undertaken in order to compare perceived effects of ethanol, with genetically determined differential ethanol induced sleep-times in the same rats. More relevant results may be forthcoming from a current study with the HAD/LAD strains because these animals, having been selectively bred for high- and low-ethanol drinking preferences, provide a major step towards a behavioral animal model for alcoholism.

Humans consume ethanol not for its hypnotic effects but for perceived effects and although the desired results are referred to as "reinforcing" or "rewarding," the effects are poorly understood and possibly poorly defined. When drugs function as discriminative stimuli they probably do so on the basis of distinctive rather than strong reinforcing or aversive effects. Unless the study currently underway with HAD/LAD animals finds a substantial correlation between drinking preference and differential sensitivity to the discriminative effects of ethanol there is still no selectively bred animal model that specifically measures the perceived/subjective effects of ethanol.

The present study, while showing significant differences between HAS and LAS strains in the DD paradigm, showed the differences were far from the magnitude found for sleep-times in the same animals. Much lower ethanol doses are needed during the selective breeding process in order to study the subtle behavioral effects involved in developing alcoholism. Such doses are practical with drug discrimination (11, 18, 25). It seems that an important and logical next step is to selectively breed animals for their differential discrimination of ethanol. That is to develop strains that are either highly sensitive or less sensitive to the perceived effects of ethanol. The resulting lines could be a powerful tool for correlational studies with such behaviors as sleep-time, volitional ethanol consumption, conditioned place preference and self-administration. These correlative studies might, in turn, provide some insight as to the nature of the stimulus properties of ethanol. Selective breeding for differential ethanol discrimination may also be useful for testing of other drugs with abuse liability. One purpose would be to investigate common biological factors for alcohol and other drugs of abuse that might suggest a general increased risk of substance abuse disorders based upon genetic inheritance. The present knowledge of alcoholism and more specifically the involvement of genetic factors in alcoholism does not yet allow the reliance on any one or two animal models.

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