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Biphasic Effects of Ethanol Tested With Drug Discrimination in HAD and LAD Rats

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KRIMMER, E. C. *Biphasic effects of ethanol tested with drug discrimination in HAD and LAD rats.* PHARMACOL BIOCHEM BEHAV **43**(4) 1233–1240, 1992.—Seventh-generation selectively bred high-alcohol-drinking (HAD) and low-alcohol-drinking (LAD) rats were trained to make differential responses for ethanol (0.75 g/kg, IP) and saline vehicle, following postadministration intervals (PI) of 2 min (HAD-2 and LAD-2 animals) and 30 min (HAD-30 and LAD-30 animals). ED_{50} values of 0.395 and 0.352 g/kg, respectively, for HAD-2 and LAD-2 animals and 0.269 and 0.314 g/kg, respectively, for HAD-30 and LAD-30 animals reflect the absence of any phenotypic difference for the discriminative stimulus effects of ethanol. HAD-2 animals were more responsive than LAD-2 animals to the stimulating effects of ethanol as measured by total response rates during training sessions. The differential ethanol response generalized to pentobarbital in all four groups but not to morphine, an alternative CNS depressant. The specific antagonist of 5-hydroxytryptamine$_3$ receptors, 3-tropanyl-3,5-dichlorobenzoate (MDL 72222), up to doses of 14.0 mg/kg failed to antagonize the discriminative effects of ethanol. Ethanol sleep times did not differ between groups.

Ethanol Drug discrimination Selective breeding HAD LAD Alcohol Sleep time
Stimulus properties of drugs Pentobarbital MDL 72222

ETHANOL has biphasic actions on activity that are both time and dose related. Low and high doses can produce the opposite responses of stimulation and depression, respectively. A high dose of ethanol can also produce a time-related change from stimulation to depression (14).

Ethanol-induced motor stimulation has been reported to have a positive relationship with ethanol drinking preference in ethanol-preferring (P) but not in nonpreferring (NP) rats (20). Increased activity was observed during the first 6 min postadministration of low IP ethanol doses (0.12 and 0.25 g/kg) to P rats. NP rats failed to show increased spontaneous motor activity (SMA) at any tested ethanol dose (0.12–1.5 g/kg). These investigators suggested that the low-dose increased activity and stimulation observed in P rats is an expression of the positive reinforcing or rewarding features of ethanol consumption and that some attribute of ethanol perceived as reinforcing by P rats is closely related to the ethanol-induced increase of SMA.

Drug discrimination (DD) is a paradigm used to study perceived effects of many drugs including ethanol (1,2,15). Recently, DD was used to compare the HAS and LAS rat lines, which are selectively bred for differential sensitivity to the hypnotic effects of ethanol (6,7). These studies reported that when compared to LAS animals HAS animals had a slightly greater but transient sensitivity for the discriminative effects of ethanol when trained with a low dose of ethanol (0.6 g/kg) at a postadministration interval (PI) of 10 min (6). The difference was reinstated and persisted after the training dose was increased to 1.0 g/kg ethanol (7). Other lines [high alcohol drinking (HAD)/low alcohol drinking (LAD)] of rats have been selectively bred for differential ethanol drinking preference. The HAD and LAD lines voluntarily consume ethanol at daily rates of approximately 5.5 and 1.1 g/kg, respectively (9). The HAD and LAD rat lines have also been tested in the DD paradigm (8). ED_{50} values for DD were nearly identical for these two lines when discriminative responses were trained 2 min following the exceptionally low dose of 0.5 g/kg ethanol and saline (8). Response rates during DD training sessions indicated that the HAD line was more responsive than the LAD line to a stimulating effect of ethanol. Thus, the pattern of differential stimulation for these animals is similar to that reported for other selectively bred P and NP animals when tested for SMA at short intervals after low doses of ethanol (20).

The present study continues the earlier report in which the HAD/LAD lines were trained to discriminate ethanol at 2 min (8) and broadens the overall study by including additional HAD and LAD animals that received the same discrimination training but at a 30-min PI. The longer PI of 30 min was used to emphasize the later depressant effects of ethanol and minimize or perhaps avoid the stimulation effects that occur at short intervals after ethanol. As in our earlier report, re-

sponse rates during training sessions and tests with novel drug conditions were used as a measure of the effects of ethanol on activity levels.

The brain serotonin [5-hydroxytryptamine (5-HT)] system has been implicated in the reinforcing properties of ethanol. NP animals have a lower receptor density and/or metabolic functioning within some brain 5-HT systems (12) and pharmacological manipulation of these systems with fluoxetine produces a robust reduction of ethanol self-infusion by P rats. The selective 5HT receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) reduced ethanol consumption in P animals (18). Clinically, fluoxetine, a serotonin uptake inhibitor, reduces alcohol intake in problem drinkers (13). The role of the 5-HT system in the mediation of the discriminative effects of ethanol was suggested when 1-(3-trifluromethylphenyl)piperazine (TFMPP), a 5-HT_{1B} agonist, elicited the drug response in animals trained to discriminate ethanol from saline (16). More recently, it has been reported that MDL 72222 [3-tropanyl-3,5-dichlorobenzoate (MDL)], a 5-HT_3 receptor antagonist, shifted the discriminative stimulus effects of ethanol doses to the right in pigeons (5) and in pigeons and rats (4). These results suggest a surmountable antagonism of the discriminable stimulus effects of ethanol. The present study tested for the generality of the antagonism by MDL in the HAD/LAD lines as part of the ongoing effort to define behavioral differences or similarities for the HAD and LAD selectively bred lines that coexist with their differential drinking preferences.

METHOD

Animals

Twenty-four male rats were received from Indiana University School of Medicine. The animals represented the seventh-generations of selective breeding for HAD and LAD preferences (9). The original animal stock was the N/Nih Heterogeneous Strain (HS) presently maintained by the National Institute of Health (NIH) Animal Resource Center. Animals (12 of each line) were alcohol naive, approximately 11 weeks old at the start of the initially reported experiments (8), and approximately 37 weeks old at the beginning of this phase of investigation. They were individually housed in suspended metal wire cages with free access to water throughout the experiment. Daily food rations of a 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. The average weights and ranges at the beginning of this phase of training were 221 g (174–250) for 12 HAD animals and 211 g (199–235) for 12 LAD animals. Vivarium facilities had an ambient temperature of 20–22°C and lights were on from 0600 to 1800 h daily.

Drug Discrimination Training

The experimental environment 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 chamber was contained in a ventilated, sound-attenuated cubicle equipped with a houselight. All test parameters and data collection used a modified version of the computer software package described by Emmett-Oglesby et al. (3) and Spencer and Emmett-Oglesby (17).

Animals were initially trained to press one lever after receiving 0.5 g/kg ethanol (15% w/v in saline) and press the alternative lever after receiving an equal volume (3.3 ml/kg) of saline (8). Either ethanol or saline was administered intraperitoneally prior to a 6-min training session. The 6-min training session followed a 2-min PI for six HAD (HAD-2 group) and six LAD (LAD-2 group) animals and followed a 30-min PI for remaining animals (HAD-30 and LAD-30 groups). Initially, a reinforcement (45-mg Noyes food pellet) was delivered following each correct lever press [fixed ratio 1 (FR1)]. An FR2 schedule was subsequently introduced after six training sessions and the reinforcement requirement was gradually increased to FR10 over the next 24 training sessions. Incorrect responses were recorded but produced no programmed consequence.

The animal was returned to its home cage during the PI. Following the assigned (2 or 30 min) PI, the animal was removed to the test chamber and the houselight turned on to signal the beginning of the daily 6-min training session. Reinforcements were obtained by pressing either the designated "ethanol lever" or "saline lever" depending upon whether the animal had respectively received ethanol or saline. For all training sessions, the schedule of administration randomly alternated between ethanol and saline with the restriction that the same condition was not administered on more than two consecutive training sessions. To control for possible position preference, lever assignments were counterbalanced for ethanol and saline and for HAD and LAD animals. The assigned lever conditions for each rat remained constant throughout the experimentation. Animals were trained between 0800 and 1100 h on 5 days each week.

At the completion of 38 training sessions, each 6-min training session began with an extinction period of either 0, 15, 30, or 60 s. During an extinction period, lever presses were recorded but no reinforcements were delivered. The four extinction periods were randomly alternated and imposed with equal frequency during both ethanol and saline training conditions. A reinforcement was delivered on the first and each subsequent completion of the FR10 schedule that occurred following any initial extinction period. The purpose of imposing extinction periods of varying lengths (0, 15, 30, 60) during training sessions was to acclimate animals to the eventual test day conditions. Following 50 training sessions (25 with each condition), tests with novel ethanol doses (0.062, 0.125, 0.25, 0.75 g/kg) were interspersed with ongoing maintenance training sessions. A test session followed the appropriate PI and consisted of allowing the animal 60 s access to both levers under conditions of extinction. The animal was immediately removed from the chamber and returned to its home cage following the 60-s period without receiving reinforcement. Initial data for animals trained with a 2-min PI have been previously reported (8). Animals trained following the longer PI of 30 min were not included in that earlier report because their drug discrimination performance was below that of 2-min trained animals.

Beginning with training session 97 and the second phase now reported here, the training dose was increased by 50% to 0.75 g/kg for all animals and additional training sessions were given prior to tests with novel conditions. The new results are reported here for both 2- and 30-min PI groups. Except for the different PIs, which remained constant during all phases for both 2- and 30-min animals, the training and testing proce-

dures were the same for all groups. Two animals died prior to this second phase so that $n = 6$ remained so for the HAD-2 and LAD-2 groups and $n = 5$ for the HAD-30 and LAD-30 groups.

Test of Novel Drug Conditions

Following the additional 20 training sessions (10 with each condition) which established discrimination for the higher ethanol dose (0.75 g/kg), tests with ethanol doses and PIs that differ from those during training, as well as tests with two other CNS depressants (pentobarbital and morphine), were interspersed with maintenance training sessions. A test session, as described above, was a 60-s extinction period followed by immediate removal of the animal to its home cage.

Ethanol (0.125, 0.25, 0.5, 0.75, and 1.0 g/kg) was tested at the usual PI for each group (dose-response determination) and 0.75 g/kg ethanol was tested at PIs of 1, 2, 5, 30, 60, and 90 min (time course determination). Pentobarbital doses of 0.625, 1.25, 2.5, 5, and 7.5 mg/kg and morphine 2.5 mg/kg were tested at 10 min for generalization with ethanol. MDL doses of 3.5, 7.0, and 14.0 mg/kg were administered intraperitoneally in a volume of 1 ml/kg 30 min prior to 0.75 g/kg ethanol as a test for antagonism. MDL (7.0 mg/kg) was also administered 30 min prior to saline. Test sessions were conducted at the usual 2- or 30-min PI after ethanol and saline administration. The concentrations were based upon the salt weights and administered in a volume of 1 ml/kg. Pentobarbital Na (Sigma Chemical Co., St. Louis, MO) and morphine sulfate (Mallinckrodt, Paris, KY) were prepared in 0.9% saline and administered intraperitoneally and subcutaneously, respectively. MDL, from Research Biochemicals, Inc. (Natick, MA), was dissolved with a drop of glacial acetic acid, taken to volume with saline, and the solution adjusted to pH 5.5 with NaOH.

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 body weight. Similar doses and concentrations are used during the selective breeding procedures for HAS/LAS animals and have been used with these animals during similar studies from this laboratory (6,7). The moment of loss of righting reflex (LORR) 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.

Data Analyses

Drug lever choice was expressed as the percentage of total responses made on the animals' designated ethanol lever during the 60-s extinction periods. Combined presses on both levers during these 60-s periods, as well as total lever presses during 6-min training sessions, were also assessed. Both percent drug choice and response rates 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 for a particular animal, 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 ANOVA was used to analyze dose effects for each phenotype separately, followed by posthoc Scheffe's test for comparisons with saline control results when appropriate. A $p < 0.05$ was taken to indicate a significant difference. A computer-generated formulation of Litchfield-Wilcoxon analysis (19) yielded ED_{50} values and confidence levels for ethanol dose-response curves.

RESULTS

Baseline data was obtained from six sessions (three saline and three ethanol) with 60-s extinction periods during training sessions 146–168. Average percent drug choices, calculated respectively for saline and 0.75 g/kg ethanol, were 16.1 and 78.7% for the HAD-2 group, 14.8 and 82.0% for the LAD-2 group, 22.6 and 90.8% for the HAD-30 group, and 32.0 and 78.8% for the LAD-30 group. Figure 1 (upper half) depicts these data together with results of tests with novel ethanol

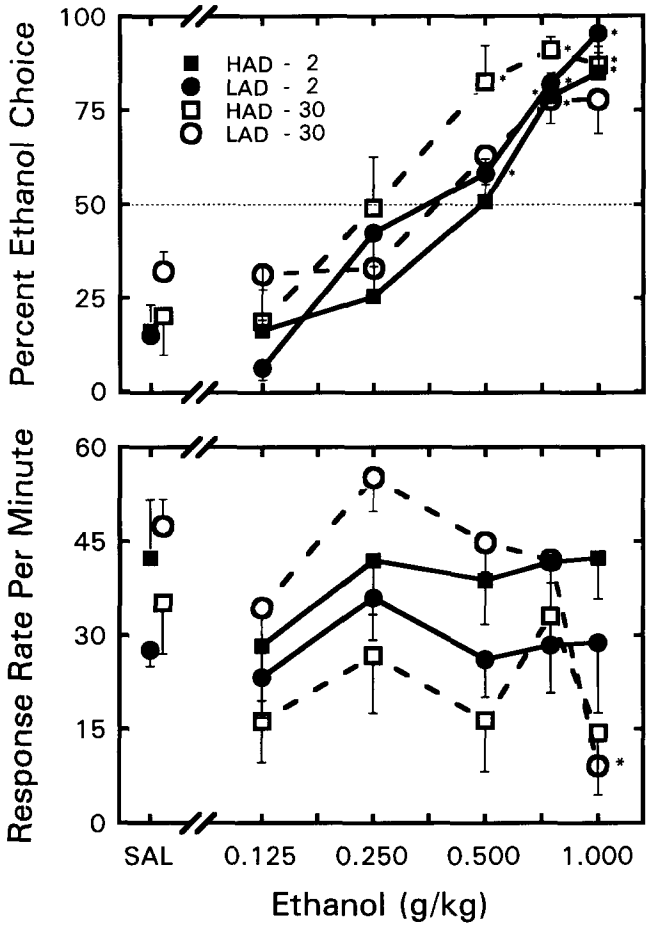

FIG. 1. Percent ethanol choice (top) and response rate per minute (bottom) for various ethanol doses tested at the usual 2- or 30-minute postadministration interval. The four saline (SAL) points and the ethanol points for 0.75 g/kg are baseline values obtained during training. Points are means for high-alcohol-drinking (HAD)-2 [■ (6)], low-alcohol-drinking (LAD)-2 [● (6)], HAD-30 [□ (5)], and LAD-30 [○ (6)] animals. Vertical lines indicate positive or negative half of SEM. *Significant differences ($p < 0.05$) from the saline baseline for percent ethanol choice and differences ($p < 0.001$) from the saline baseline for ethanol response rates.

doses (0.125, 0.25, 0.5, and 1.0 g/kg), interspersed among the same series of training sessions. There is a strong linear relationship for dose and percent drug choice across all animals, $F(4, 72) = 43.609, p < 0.0001$, but no interaction with lines (HAD and LAD), $F(4, 72) = 1.268, p < 0.290$, or with training times (2 and 30 min), $F(4, 72) = 0.153, p = 0.961$. There was no dose × group interaction, $F(12, 72) = 0.977$, $p = 0.479$. Random response choice (50%) would be the expected result in the absence of any learning and is a more conservative basis of comparison than testing for differences between saline and drug results. The above percent drug choices following both the saline and ethanol (0.75 g/kg) training conditions differ significantly from random responding for all four groups.

Figure 1 (lower half) shows the total response rates obtained during the same 60-s extinction periods of baseline and test sessions. There is a nonlinear dose to response rate effect, $F(4, 72) = 7.875, p < 0.0001$, and an interaction with lines, $F(4, 72) = 2.911, p = 0.027$, and with training times, $F(4, 72) = 4.973, p = 0.0013$. There is also a dose × group interaction, $F(12,72) = 3.169, p = 0.0011$. Average group response rates following various ethanol doses differ from the saline response rate only when the highest dose (1.0 g/kg) significantly depressed the response rate of the LAD-30 group ($p < 0.0005$).

Tests with novel ethanol doses enabled calculating ED_{50} values of 0.395 g/kg for HAD-2 animals, 0.352 g/kg for LAD-2 animals, 0.269 g/kg for HAD-30 animals, and 0.314 g/kg for the LAD-30 animals (Table 1). The ED_{50} values do not differ significantly, although a value of 0.269 suggests slightly greater sensitivity to the ethanol discriminative effects for the HAD-30 group.

Figure 2 utilizes the same format as Fig. 1 to depict the results of tests with various pentobarbital doses (0.625, 1.25, 2.5, 5.0, and 7.5 g/kg). The ethanol discriminative response generalized in a dose-related manner to pentobarbital in each of the four groups (upper half). Across all animals, there is a linear relationship between dose and percent drug choice, $F(4, 72) = 18.281, p < 0.0001$, but no interaction with the two training times, $F(4, 72) = 0.219, p = 0.927$, or with line, $F(4, 72) = 1.804, p = 0.137$, and no training dose × group interaction, $F(12, 72) = 0.937, p = 0.516$. Although the ED_{50} values for pentobarbital (Table 1) do not differ, a slightly greater sensitivity for the discriminative effect by the HAD-30 group is again suggested by the relatively low ED_{50} value of 0.44 mg/kg.

Figure 2 (lower half) shows the response rates for the various doses of pentobarbital. There is a nonlinear relationship for dose and response rate, $F(4, 72) = 20.456, p < 0.0001$, but no interaction with training time, $F(4, 72) = 2.238, p = 0.0732$, or with line, $F(4, 72) = 0.770, p = 0.548$.

TABLE 1

ED_{50} VALUES AND 95% CONFIDENCE LIMIT FOR ETHANOL AND PENTOBARBITAL CALCULATED FOR EACH OF FOUR GROUPS

Group	Ethanol (g/kg)	Pentobarbital (mg/kg)
HAD-2	0.395 (0.227–0.683)	2.03 (0.801–5.128)
LAD-2	0.352 (0.200–0.620)	2.40 (0.953–6.056)
HAD-30	0.269 (0.134–0.544)	0.44 (0.070–2.715)
LAD-30	0.314 (0.142–0.692)	1.69 (0.689–4.146)

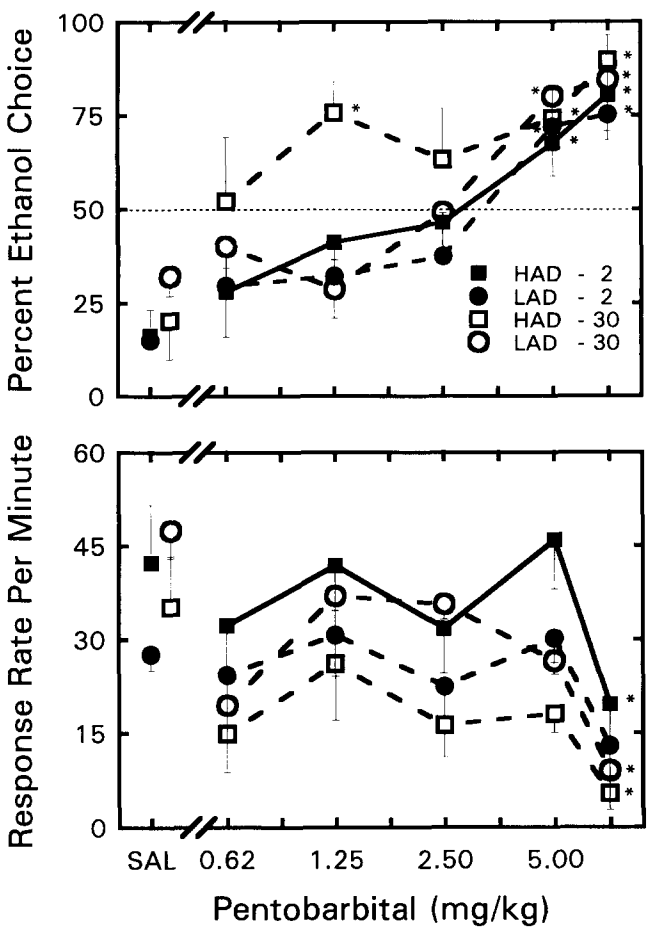

FIG. 2. Percent ethanol choice (top) and response rate per minute (bottom) made during generalization tests with various doses of pentobarbital administered 10 min before each test. The four saline (SAL) points are baseline values obtained during training. Points are means for high-alcohol-drinking (HAD)-2 [■ (6)], low-alcohol-drinking (LAD)-2 [● (6)], HAD-30 [□ (5)], and LAD-30 [○ (5)] animals. Vertical lines indicate positive or negative half of SEM. *Significant differences ($p < 0.05$) from the saline baseline for percent ethanol choice and differences ($p < 0.001$) from the saline baseline for ethanol response rates.

Figure 3 shows the results of tests when the ethanol training dose (0.75 g/kg) was tested using various PIs of 1–90 min. The abscissa of Fig. 3 is represented as a log scale rather than a linear scale to portray the full time course (1–90 min) and provide separation of data points for the shorter PIs of 1, 2, and 5 min. All groups in general made the drug choice during PI times of 2–30 min; however, only animals trained at 30 min continued to make the ethanol choice more frequently than chance (50%) when tested at 60 min (upper half). There is a nonlinear relationship of percent drug choice with time for the averages of all animals, $F(5, 90) = 10.696, p < 0.0001$, but no interaction with training time, $F(5, 90) = 1.514, p = 0.193$, or with line $F(5, 90) = 1.962, p = 0.092$.

Response rates (Fig. 3, lower half) during the time course experiment show a relationship of response rates with time for the averages of all animals, $F(5, 90) = 6.717, p < 0.0001$, and there is an interaction with the training time, $F(5, 90) = 4.828, p < 0.001$, but not with line, $F(5, 90) = 0.601, p =$

0.699. Response rates at the various times for each group in general do not differ from the saline rates for the respective groups.

Response on both levers for the entire 6-min training session were also totaled and these represent a measure of overall directed activity following saline or ethanol administration. Average response rates over blocks of eight sessions (four saline, four ethanol) were used to calculate ratios of ethanol to saline response rates for each rat. Group averages are plotted in Fig. 4 beginning with session 97, the training point when the ethanol training dose for all groups was increased to 0.75 g/kg. Previously, we reported that the calculated ratios of ethanol to saline response rates for the HAD-2 and LAD-2 groups differed during sessions 31-54 (8). The HAD-2 group responded at higher rates following the ethanol training dose of 0.5 g/kg than they did during saline sessions. It was also noted that the difference between the HAD-2 ratio and the LAD-2 ratio decreased after session 54 (8). Figure 4 shows that the ratio difference was reinstated for HAD and LAD animals trained at 2 min when the training dose was increased to 0.75 g/kg in this series of sessions. ANOVA shows that the HAD-2 group again differed from the LAD-2 group, $F(1, 10) = 7.708, p = 0.020$, and from other HAD animals trained at 30 min, $F(1, 9) = 6.137, p = 0.035$. Ratios for HAD-30 group did not differ from the LAD-30 group, $F(1, 9) = 0.1400, p = 0.717$. All differences disappeared by session 129.

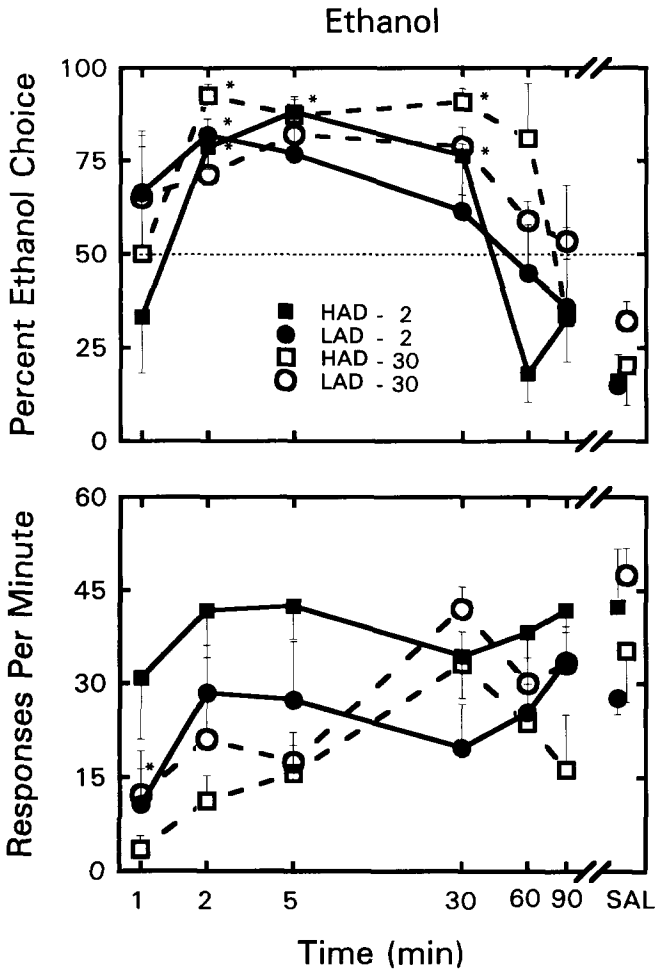

FIG. 3. Percent ethanol choice (top portion) and response rate per minute (bottom portion) made during various times following 0.75 g/kg ethanol. The four saline (SAL) points are baseline values obtained during training and are included for reference. Points are means for high-alcohol-drinking (HAD)-2 [■ (6)], low-alcohol-drinking (LAD)-2 [● (6)], HAD-30 [□ (5)], and LAD-30 [○ (5)] lines. Vertical lines indicate positive or negative half of SEM. *Significant differences ($p < 0.05$) from the saline baseline for percent ethanol choice and ethanol response rates.

Tests With Novel Test Conditions

Table 2 shows results of tests when various doses of MDL were administered in combination with ethanol and saline. In general, all MDL doses (3.5, 7.0, and 14.0) were disruptive and produced moderate to severe depression of response rates. The lowest dose of MDL did not block the discriminative effects of ethanol (0.75 g/kg) but still reduced responding in all groups. Morphine was tested as a general nonhypnotic depressant. A subcutaneous dose of morphine (2.5 mg/kg) elicited the saline response in all groups while severely depressing response rates in all groups.

Sleep-Time Tests

Ethanol (3.0 g/kg, IP) caused sleep for 20 of 22 animals. The remaining two animals were identified and noted as having received a portion of the fluid injected into the intestinal lumen and therefore were eliminated from these calculations. The sleep times were 103.7, 110.2, 83.4, and 54.2 min, respectively, for HAD-2, LAD-2, HAD-30, and LAD-30 groups. An ANOVA indicated that the sleep times of the four groups did not differ, $F(3, 19) = 2.262, p = 0.120$. A difference was found when an average sleep time of 106.3 min for the combined HAD-2 and LAD-2 animals was compared with an average of 68.8 min for the HAD-30 and LAD-30 animals, $F(1, 18) = 5.255, p = 0.034$.

DISCUSSION

Drug discrimination training enables a high degree of specificity for responding to a particular drug effect. The drug effect is by definition a discriminable or perceived drug attribute. The experiments reported here are part of a continuing effort that employs the DD paradigm to further describe animals in which the primary phenotypes are the result of selective breeding for secondary properties of ethanol. A further consideration of studies from this laboratory has been an emphasis on low training doses so as to approach doses used during other reports of effects on activity (20).

Previously, we reported that in addition to their differential drinking preferences, for which they are selectively bred, animals of the HAD but not the LAD line were temporarily responsive to the stimulating action of ethanol during ethanol discriminative training. The lines, however, did not differ in their sensitivity to the discriminative effects of ethanol when training occurred at 2 min postadministration (8). The exceptionally low ethanol dose (0.5 g/kg) used in the earlier report was chosen so as to optimally test for differential phenotype sensitivity, which was subsequently not detected. The earlier phase of investigation with these selectively bred lines also involved unreported HAD and LAD animals that received training following a 30-min PI. These 30-min trained animals did not achieve an acceptable level of performance even after extensive training, thus suggesting that the discriminative task

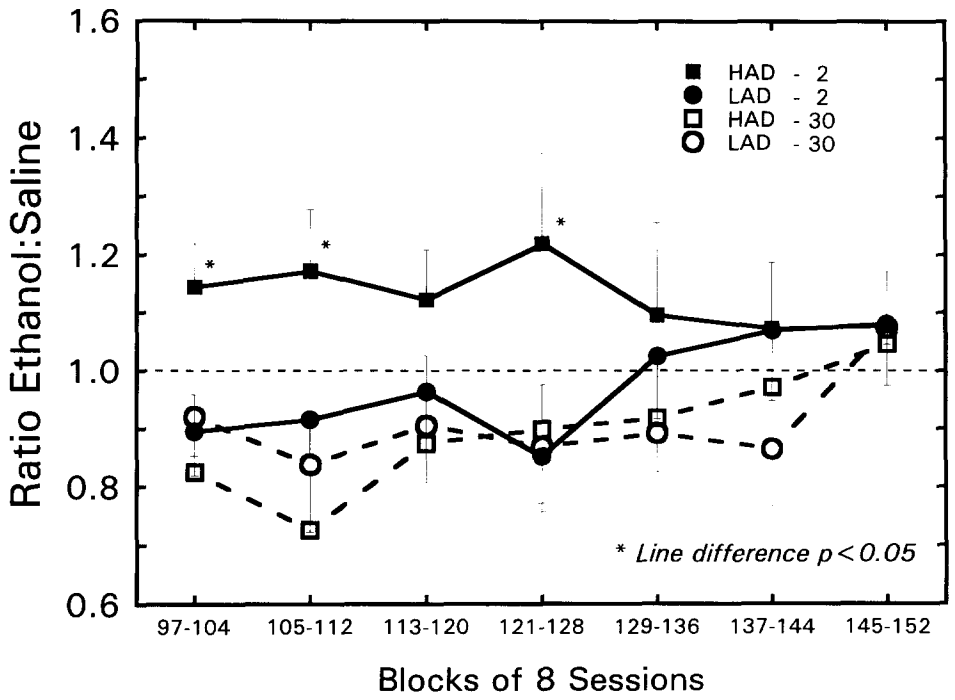

FIG. 4. The abscissa represents those training sessions reported here (97-152) and grouped as blocks of eight sessions. The points represent an average of the response ratio calculated for each animal. The ratio was obtained by dividing total responses made during four ethanol training sessions by total responses made during four saline training sessions. *Significant line differences ($p < 0.05$) for animals trained with the same postadministration interval.

employing 0.5 g/kg ethanol was more readily learned by animals of both lines following a 2-min PI than by animals trained with a 30-min PI. Stable discriminative responding occurred subsequently for all groups when the training dose was increased by 50% to 0.75 g/kg and the results of that phase are now reported here.

The ED_{50} values following training with 0.75 g/kg are essentially the same for all four groups. Animals trained at 30 min, however, show a slight tendency toward greater sensitivity for the discriminative effects of ethanol and pentobarbital (Table 1). Other reports have shown that brain ethanol concentrations determined in Wistar rats peaks at about 6 min and are about twice the concentrations found at 24 min (20); thus, an ethanol discrimination cue at 30 min would be expected to be weaker than a cue at 6 min. Animals trained at the longer interval would necessarily acquire greater sensitivity for the discriminative effects and thus yield slightly lower ED_{50} values (1). The generalization of the ethanol discriminative response to pentobarbital but not to morphine, which also caused a substantial suppression of responding, indicates that the depressant effects of ethanol are not a major component of the ethanol stimulus complex.

During this later phase with the slightly higher ethanol training dose, differential phenotypic sensitivity for the dis-

TABLE 2

PERCENT ETHANOL CHOICE AND RESPONSES/min FOR TESTS WITH MDL 72222 ALONE AND IN COMBINATION WITH VARIOUS DOSES OF ETHANOL

Ethanol (g/kg)	MDL (mg/kg)	% Ethanol Choice				Response Rate/min			
		HAD-2	LAD-2	HAD-30	LAD-30	HAD-2	LAD-2	HAD-30	LAD-30
0*	0	16.1 (6)	14.8 (6)	20.1 (5)	32.1 (5)	42.3	27.7	35.2	47.4
0.75*	0	78.7 (6)	82.0 (6)	90.8 (5)	77.7 (5)	41.7	28.4	33.0	42.0
0	7.0	0.0 (2)	– (0)	3.8 (2)	40.2 (4)	4.3	0	4.8	6.0
0.75	3.5	66.7 (5)	83.1 (5)	82.9 (4)	70.0 (3)	35.7	22.8	12.2	13.6
0.75	7.0	40.0 (3)	100.0 (1)	57.6 (3)	96.3 (3)	5.2	1.2	4.6	5.0
0.75	14.0	– (0)	100.0 (1)	0.0 (1)	100.0 (1)	0	16.7	0.2	0.2

Numbers in parentheses are numbers of animals responding.
*Training condition.

criminative effects of ethanol was not evident for training following either a 2- or 30-min PI. A transient (sessions 97–128) but significant sensitivity for the stimulating action of ethanol reported in our earlier study (8), however, reoccurred for the HAD-2 group during this phase. The transient nature of this response-rate-stimulating effect would suggest that behavioral adaptation or tolerance developed after repeated exposure to ethanol during training and testing sessions. Other studies have reported that a lower dose of ethanol (0.25 g/kg) increased SMA during the period 0–6 min postadministration when tested in another line of ethanol-preferring (the P line) rats but not in the NP line (20). That same study reported tolerance was not observed to either the stimulant or depressant action of a higher dose of ethanol (1.5 g/kg) when the chronic treatments were spaced at least 4 days apart. In the present study, an irregular spacing of five administrations of ethanol (0.75 g/kg) averaged over a period of 14 days did induce an apparent tolerance to the stimulant action in the HAD-2 group. The convergence of ratio curves (Fig. 4) might suggest that some tolerance also developed to the depressant action of ethanol. Another possibility, of course, is that learned compensatory responses developed for both actions. There were no apparent changes of sensitivity for the discriminative effects of ethanol during the same time that ethanol-saline response ratios were changing. The temporal nature of the stimulating effect of ethanol was also evident in the present study by the failure to demonstrate a similar stimulation of responding when HAD-30 and LAD-30 animals were trained at 30 min.

Results of this study using MDL (3.5–14.0 mg/kg) do not agree with earlier reports that MDL (3.0–17.0 mg/kg) shifted the dose–effect function for ethanol discrimination to the right (5). Indeed doses of 7.0 mg/kg MDL administered alone and 14.0 mg/kg administered together with the ethanol training dose (0.75 g/kg) were found extremely disruptive to responding by all animal groups in this study. The lowest 2 MDL doses (3.5 and 7.0) were mildly disruptive when administered with ethanol and also provided no clear evidence of antagonism. This discrepancy with previous results might be attributed to a species difference (pigeons vs. rats) and/or a training dose of ethanol that was twice the dose used in the current study. The training dose (1.0 g/kg) for a second study (4) reporting results of MDL with rats, however, was only slightly higher than the dose used in this study and thus allows no explanation for the different results.

More recently, it has been reported that there were no differences in brain 5-HT_3 receptor site densities of P and NP rats (10) and that MDL did not significantly alter ethanol intake in the P line of alcohol-preferring rats (11), thus suggesting no evidence for involvement of 5-HT_3 receptors in ethanol drinking behavior. The present study also suggests that 5-HT_3 receptors are not involved in the discriminative effects of ethanol, at least not in the HAD/LAD rat lines.

The sleep-time test permits further measures of these animals using yet another ethanol effect for which they were not selectively bred. Overall, the sleep times for the present HAD and LAD lines showed no phenotype differences but, instead, their sleep times were intermediate with anticipated and quite divergent sleep times demonstrated for the HAS and LAS lines when tested in this laboratory (6). These relationships should be expected based upon the selective breeding criterion for animals. A somewhat puzzling finding, however, was that animals trained at 2 min sleep longer than those trained at 30 min. Perhaps animals that were required to be active during the later depressant phase of ethanol while in the discrimination paradigm also acquired behavioral tolerance to the sedative effects.

Results of the present study are consistent with previous reports from this laboratory that indicate the discriminative properties of ethanol, that is, the perceived attributes, are dissociated from secondary ethanol attributes such as the hypnotic effects and the voluntary consumption effects for which the tested animals were selectively bred. The failure to find similar relative sensitivities to various effects of ethanol indicates that more than one set of genes controls the effects of ethanol. These differences, however, will continue to provide a powerful analytic tool for determining mechanisms of drug action.

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