Pharmacology Biochemistry & Behavior, Vol. 15, pp. 33–36, 1981. Printed in the U.S.A.

Task-Dependent Ethanol Effects on Escape in Rats Bred for Ethanol Sensitivity

M. B. BASS AND D. LESTER

Center of Alcohol Studies and Department of Psychology, Rutgers University
New Brunswick, NJ 08903

Received 10 November 1980

BASS, M. B. AND D. LESTER. *Task-dependent ethanol effects on escape in rats bred for ethanol sensitivity.* PHARMAC. BIOCHEM. BEHAV. **15**(1) 33–36, 1981.—Lines of rats selectively bred for differences in degree of locomotor depression by ethanol were tested for ethanol-induced impairment of jumping to a descending platform to escape 0.3 mA shock. The MA ("most affected") line showed greater decreases in height jumped than the LA ("least affected") line at IP doses of 1.25, 1.75, and 2.25, but not at 0.75 g ethanol/kg. MA rats also showed greater increases in latency to first jump (at 1.75 and 2.25 g/kg) which largely accounted for the line difference in decrease in height jumped. Males showed greater impairment than females on both measures. While extending the greater ethanol sensitivity of MA than LA rats to impairment of an escape response, the results contrast with previous studies of water escape where the LA line showed greater impairment than the MA line.

Ethanol sensitivity Task dependency Escape Selective breeding Pharmacogenetics Sex differences

THE ataxia seen after treatment of laboratory animals with ethanol has suggested the use of motor impairment in studies of ethanol intoxication [1, 2, 4, 7, 8, 11, 13, 22, 25] and tolerance [5, 9, 14, 15, 19, 25]. Rats have been selectively bred for sensitivity to ethanol-induced motor depression [20]. The resulting selected lines (LA="least affected"; MA="most affected") differ in the degree of ethanol-induced depression in behavioral situations besides that used for selection [27] as well as in sensitivity to the soporific effect of ethanol [21]. The differential responsiveness of these lines appears to be due to differences in CNS sensitivity since no differences have been found in blood [21] or brain [5] ethanol levels, or in rates of ethanol clearance [16].

Recent studies [4,5] have shown that LA and MA rats differ in sensitivity to ethanol-induced impairment of swimming, but that the line difference is in the opposite direction: the LA line shows greater impairment. This reversal, observed in four different generations of rats, is not abolished by chronic ethanol treatment [5].

Motor activity, the phenotype under selection, is without reinforcement contingencies, whereas swimming is a motivated, instrumental escape response. The present research investigated the generality of the reversed order of sensitivity seen on the water escape task by studying ethanol's effects on a different kind of escape situation.

METHOD

Animals

Sixteen rats from second litters of the 17th selected generation of LA and MA rats (four per line and sex) were used. Rats were housed four per 44×21×20 (height) cm cages under a 12-hr light (0700–1900), 12-hr dark cycle. Water was available ad lib; food (Purina Lab Chow) was removed 18 hr prior to testing. Rats were approximately 250 days of age at the start of testing.

Apparatus

A modification of the apparatus described by Tullis *et al.* [25] was used. It consists of a motor-driven platform (23×17 cm) which descends from an initial height of 50 cm at 0.75 cm/sec to a minimum height of 7.1 cm above an adjacent 29×23 cm Grason-Stadler grid floor. Scrambled footshock was delivered through the grid floor by a Grason-Stadler E1064GS shock generator. A rubber mat glued to the top surface of the platform facilitates a secure grip for the subject jumping up to escape shock. The platform and grid floor are enclosed on all sides and above in a 48×23×76 (height) cm Plexiglas compartment. A meter stick, mounted vertically on the outside of the compartment, indicates platform height.

Procedure

Rats first received two 10-min exposures to the apparatus (one per day) with the platform down, for familiarization. They were then trained to escape scrambled footshock (0.3 mA) which began upon placement on the grid floor and was of 60 sec maximum duration. Platform descent began with shock onset. Initial platform height was gradually increased from 10 to 50 cm during the first two sessions, which each consisted of 10 trials. Rats received eight additional sessions of 5 trials/day at 1-min intertrial intervals during the three-week training period.

Testing consisted of three pretreatment trials, followed by IP injection and subsequent trials; means of the second and third preinjection trials were taken as baseline performance. Rats were tested two consecutive times (results of which were averaged) beginning at 5, 10, 15, and 25 min after injec-

tion. The platform was stopped at the time of escape (three or more paws on it); platform height was measured [25]. In addition, latency to first jump (both hind paws off the grid), measured by stopwatch, was recorded to the nearest 0.1 sec. Any rat failing to jump within 60 sec received a 60 sec latency score and a 7.1 cm height score.

Three days prior to the start of the ethanol experiment, rats were given a saline (0.9% NaCl) pretest; the volume injected was that of the highest ethanol dose (22.5 ml/kg). The ethanol experiment consisted of four 4×4 Latin squares (one square for each line and sex) with rows as rats, columns as days, and treatments corresponding to ethanol doses of 0.75, 1.25, 1.75, and 2.25 g/kg. Thus, each rat received each dose in a random order. Ethanol was given as a 10% w/v-0.9% saline solution. Four days separated each successive test day.

RESULTS

Both absolute data and changes from baseline were used in the analysis of the saline pretest. Changes from baseline were used to quantify impairment in the ethanol experiment.

Saline Pretest

Small but significant differences in mean absolute height jumped (LA: 48.99 cm; MA: 47.63 cm) were observed, $F(1,12)=6.13$, $p=0.029$. LA rats also showed a shorter mean latency to first jump than MA rats (0.61 versus 1.65 sec), $F(1,12)=6.46$, $p=0.026$. Neither line, however, showed any changes in jump latency or height jumped after saline injection, and there were no line differences in either decrease in height jumped, $F(1,12)=0.83$, $p=0.38$, or increase in jump latency, $F(1,12)=1.73$, $p=0.21$. There were no sex differences (both $p>0.64$), or changes over trials (both $p>0.10$) for either measure.

Ethanol Experiment

As in the saline pretest, baseline jump height of LA rats was greater than that of MA rats, $F(1,12)=9.79$, $p=0.0087$. Baseline heights were therefore used as covariates for decreases in jump height. Baseline jump latencies of LA rats were again shorter than those of MA rats, $F(1,12)=10.13$, $p=0.0079$; baseline latencies were used as covariates for increases in jump latency. There were no sex differences or line×sex interactions for either measure of baseline performance (all $p>0.31$).

One rat died between the third and fourth ethanol test days; missing values for a Latin square design were estimated and degrees of freedom were subtracted from the appropriate error terms [6].

Body Weight

Mean body weights (averaged over the ethanol experiment) were 436.5±21.9 (SEM) g for LA males, 234.4±7.1 for LA females, 425.1±14.0 for MA males, and 252.0±5.9 for MA females. Males weighed more than females ($p<0.0001$), but there was no line difference, $F(1,12)=0.05$, $p=0.83$, or line×sex interaction ($p=0.31$).

Jump Height

Mean decreases in jump height (averaged over trials) are presented in Fig. 1. MA rats showed greater decreases than

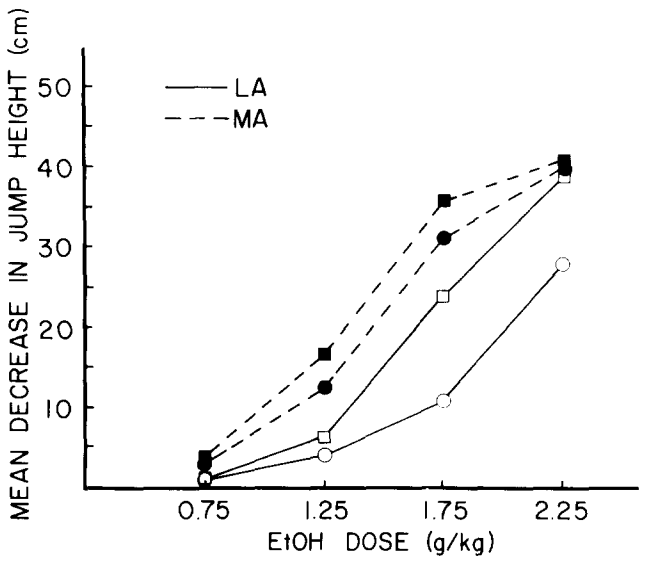

FIG. 1. Mean decrease in height jumped as a function of ethanol dose in rats of each line and sex. Squares represent males; circles, females. Each point is the mean of 4 rats, averaged over trials beginning at 5, 10, 15, and 25 min after treatment.

LA rats, $F(1,12)=24.50$, $p=0.0003$, and males showed greater decreases than females, $F(1,12)=7.45$, $p=0.0183$. Duncan's multiple range test (alpha=0.05) indicated significant line differences at 1.25, 1.75, and 2.25, but not at 0.75 g/kg. A line difference was evident when baseline jump height was used as a covariate, $F(1,11)=12.38$, $p=0.0048$, as was a sex difference, $F(1,11)=6.25$, $p=0.029$. No line×sex interaction was observed in either analysis (both $p>0.28$). Decreases in jump height were dose-dependent, $F(3,23)=141.87$, $p<0.0001$, and a line×dose interaction was observed, $F(3,23)=4.42$, $p=0.0135$. The line×dose interaction was also significant in the analysis of covariance, $F(3,22)=4.62$, $p=0.0119$.

There was no days effect ($p=0.082$), nor did days interact with line, sex, or line×sex (all $p>0.12$). There were also no sex×dose, or line×sex×dose interactions (both $p>0.15$). Consistent with other observations [4,25], there were decrements in impairment over trials, $F(3,36)=46.33$, $p<0.0001$. A marginal line×trials interaction was obtained, $F(3,36)=2.86$, $p=0.0505$. In addition, there were significant dose×trials, $F(9,69)=2.58$, $p=0.013$, and line×dose×trials, $F(9,69)=2.71$, $p=0.01$, interactions. The latter appears to reflect sustained rather than diminishing impairment of MA rats at 2.25 g/kg. No other interactions approached significance (all $p>0.16$).

Jump Latency

Mean increases in latencies to first jump (averaged over trials) are presented in Fig. 2. MA rats showed greater increases in latency than LA rats, $F(1,12)=37.02$, $p<0.0001$, and males showed greater increases than females, $F(1,12)=13.60$, $p=0.0031$. Duncan's multiple range test indicated line differences at 1.75 and 2.25 g/kg, but not at the lower doses. Line and sex effects were also significant in analysis of covariance: $F(1,11)=14.48$, $p=0.0029$ for line,

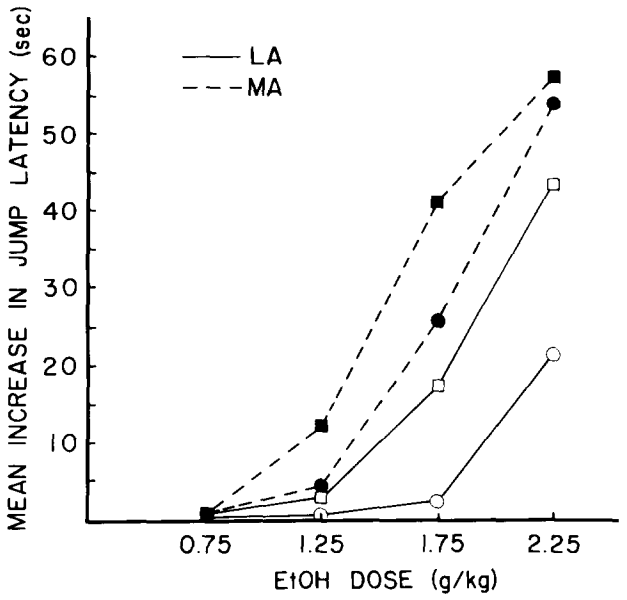

FIG. 2. Mean increase in latency to first jump as a function of ethanol dose in rats of each line and sex. Squares represent males; circles, females. Each point is the mean of 4 rats, averaged over trials beginning at 5, 10, 15, and 25 min after treatment.

and $F(1,11)=14.40$, $p=0.003$ for sex. There was no line×sex interaction in either analysis (both $p>0.48$).

With the exception of a sex×dose interaction, $F(3,23)=4.11$, $p=0.018$, conclusions were similar to those reached on the basis of decrease in jump height. There was a significant dose effect, $F(3,23)=126.39$, $p<0.0001$, and a line×dose interaction, $F(3,23)=11.30$, $p<0.0001$. This interaction was significant in the analysis of covariance, $F(3,22)=14.22$, $p<0.0001$. There was no days effect, $F(3,23)=2.46$, $p=0.088$, and no interactions with days (all $p>0.20$). The significant trials effect, $F(3,36)=34.37$, $p<0.0001$ did not interact with line, sex, or line×sex (all $p>0.13$), but did interact with dose, $F(9,69)=4.88$, $p<0.0001$, line×dose, $F(9,69)=5.13$, $p<0.0001$, and line×sex×dose, $F(9,69)=2.89$, $p=0.0058$.

Height-Latency Correlation

Changes in height and latency reflect similar, but not identical, aspects of intoxication. The degree of a linear relation between the two depends on the extent to which the first jump is a successful escape. Changes in latency to the first jump may reflect sensory (i.e., analgesic) effects [3, 10, 18, 24, 26], as well as motoric effects of ethanol. Changes in height jumped may include an additional motoric component beyond the constraints imposed by latency. An analysis of covariance of mean decrease in jump height correcting for the influence of mean increase in jump latency found no line, $F(1,11)=0.01$, $p=0.94$, or sex, $F(1,11)=0.14$, $p=0.71$, effects. This suggests that line and sex differences in increase in jump latency account for much of the differences in decrease in jump height.

DISCUSSION

The MA line was more sensitive than the LA line to impairment by ethanol on the jump escape task. A previous study of water escape, which used siblings of the rats of the present study [5], found a line difference in the opposite direction: LA rats showed greater impairment. The present findings, in conjunction with previous ones [4,5], indicate task-dependent genotypic differences in ethanol's effects on escape and thus do not support the hypothesis that the reversed line difference seen in impairment of swimming is due to an interaction of genotype with escape contingencies and ethanol.

Differences between the two escape paradigms, however, should not be overlooked. The jump task involves escape from painful stimulation whereas the swim task presumably does not. Previous findings [10] indicate that while undrugged LA and MA rats do not differ in either startle amplitude or incidence of vocalization to a range of intensities of noncontingent, intermittent shock, the MA line shows greater ethanol-induced analgesia. Such a difference in ethanol's effects on sensory processes may contribute to the present results. The greater increase in jump latency of MA rats and its relation to the line difference in decrease in jump height is consistent with this hypothesis. It is unlikely, however, that differential analgesia would totally account for the present findings, since the MA line is also more sensitive than the LA line to ethanol-induced impairment of active avoidance [23].

There were no sex differences in baseline performance, but males showed greater impairment than females. In contrast with the line difference, the direction of the sex difference is consistent with the water escape findings [4,5], and with a hypothesis that, at least in these lines, males are more sensitive to ethanol's effects on escape than are females. In view of the lack of sex (or line) differences in concentrations of ethanol in brains of LA and MA rats [5], an explanation in terms of differential disposition of ethanol is unlikely.

Results of this study extend the greater ethanol sensitivity of MA than LA rats [10, 16, 20, 21, 23, 27] to impairment of an escape response. Since other factors such as sensory processes, as well as attention and memory, are also involved in performing the task, these findings should not necessarily be taken as evidence for greater ethanol-induced incoordination in MA than in LA rats. Moreover, lack of a line difference in intoxication as measured by the moving belt test [11] has previously been reported [16].

Although there are probably differences to some extent in the musculature involved in performing the two escape tasks, both appear to involve primarily the hind legs. While peripheral effects may contribute to impairment, they would not be expected to be task-dependent. The observed task specificity, therefore, supports the notion of line differences in CNS sensitivity to ethanol.

Task-dependent genotypic differences in ethanol-induced impairment of escape emphasize the role of the behavioral situation as a determinant of intoxication. They also suggest that different mechanisms could subserve what might appear to be similar effects of ethanol. Evidence that drugs differentially alter various acute effects of ethanol [8, 12, 13, 17] is consistent with this hypothesis.

ACKNOWLEDGEMENTS

This research was supported in part by USPHS grants AA-00216 and AA-01849 to D. L., a Biomedical Research Support Grant from Rutgers University, and the Charles and Johanna Busch Memorial Fund of Rutgers University. Elizabeth Haugen carried out the selective breeding.

REFERENCES

1. Arvola, A., L. Sammalisto and H. Wallgren. A test for level of alcohol intoxication in the rat. *Q. Jl Stud. Alcohol* **19:** 563–572, 1958.
2. Bacopoulos, N. G., I. Bize, J. Levine and L. S. Van Orden, III. Modification of ethanol intoxication by dopamine agonists and antagonists. *Psychopharmacology* **60:** 195–201, 1979.
3. Bass, M. B., H. J. Friedman and D. Lester. Antagonism of naloxone hyperalgesia by ethanol. *Life Sci.* **22:** 1939–1946, 1978.
4. Bass, M. B. and D. Lester. Rats bred for ethanol sensitivity: impairment of swimming by ethanol and pentobarbital. *Psychopharmacology* **63:** 161–167, 1979.
5. Bass, M. B. and D. Lester. Tolerance to ethanol-induced impairment of water escape in rats bred for ethanol sensitivity. *Psychopharmacology* **71:** 153–158, 1980.
6. Cochran, W. G. and G. M. Cox. *Experimental Designs*, 2nd ed. New York: John Wiley and Sons, 1957, pp. 125–126.
7. Erickson, C. K., T. D. Tyler and R. A. Harris. Ethanol: modification of acute intoxication by divalent cations. *Science* **199:** 1219–1221, 1978.
8. Frankel, D., H. Kalant, J. M. Khanna and A. E. LeBlanc. Interaction of propranolol and phentolamine with ethanol in the rat. *Can. J. Physiol. Pharmac.* **54:** 622–625, 1976.
9. Frankel, D., J. M. Khanna, A. E. LeBlanc and H. Kalant. Effect of *p*-chlorophenylalanine on the acquisition of tolerance to ethanol and pentobarbital. *Psychopharmacologia* **44:** 247–252, 1975.
10. Friedman, H. J., M. B. Bass and D. Lester. Ethanol-induced analgesia in rats selectively bred for ethanol sensitivity. *Pharmac. Biochem. Behav.* **13:** 773–776, 1980.
11. Gibbins, R. J., H. Kalant and A. E. LeBlanc. A technique for accurate measurement of moderate degrees of alcohol intoxication in small animals. *J. Pharmac. exp. Ther.* **159:** 236–242, 1968.
12. Harris, R. A. Alteration of alcohol effects by calcium and other inorganic cations. *Pharmac. Biochem. Behav.* **10:** 527–534, 1979.
13. Kiianmaa, K. Alcohol intake and ethanol intoxication in the rat: effect of a 6-OHDA-induced lesion of the ascending noradrenaline pathways. *Eur. J. Pharmac.* **64:** 9–19, 1980.
14. Lê, A. D., J. M. Khanna, H. Kalant and A. E. LeBlanc. Effect of *l*-tryptophan on the acquisition of tolerance to ethanol-induced motor impairment and hypothermia. *Psychopharmacology* **61:** 125–129, 1979.
15. LeBlanc, A. E., H. Kalant and R. J. Gibbins. Acute tolerance to ethanol in the rat. *Psychopharmacologia* **41:** 43–46, 1975.
16. Lester, D., G. W-J. Lin, N. Anandam, E. P. Riley and E. X. Freed. Selective breeding of rats for differences in reactivity to alcohol. IV. Some behavioral and chemical measures. In: *Alcohol and Aldehyde Metabolizing Systems, Vol. III. Intermediary metabolism and neurochemistry*, edited by R. G. Thurman, J. G. Williamson, H. Drott and B. Chance. New York: Academic Press, 1977, pp. 635–642.
17. Matchett, J. A. and C. K. Erickson. Alteration of ethanol-induced changes in locomotor activity by adrenergic blockers in mice. *Psychopharmacology* **52:** 201–206, 1977.
18. Montoya, G. and L. Bardisa. Influencia de la reserpina, nialamida, l-dopa y dopamina en el efecto analgesico del etanol (Influence of reserpine, nialamide, l-dopa and dopamine on the analgesic effect of ethanol). *Arch. Biol. Med. exp.* **7:** 8–10, 1970.
19. Nikander, P. and L. Pekkanen. An inborn alcohol tolerance in alcohol-preferring rats. The lack of a relationship between tolerance to ethanol and the brain microsomal (Na^+-K^+) ATPase activity. *Psychopharmacology* **51:** 219–223, 1977.
20. Riley, E. P., E. X. Freed and D. Lester. Selective breeding of rats for differences in reactivity to alcohol. I. General Procedures. *J. Stud. Alcohol* **37:** 1535–1547, 1976.
21. Riley, E. P., E. D. Worsham, D. Lester and E. X. Freed. Selective breeding of rats for differences in reactivity to alcohol. II. Behavioral measures in MA and LA rats. *J. Stud. Alcohol* **38:** 1705–1717, 1977.
22. Samson, H. H. and J. L. Falk. Ethanol and discriminative motor control: effects on normal and dependent animals. *Pharmac. Biochem. Behav.* **2:** 791–801, 1974.
23. Shapiro, N. R. and E. P. Riley. Avoidance behavior in rats selectively bred for differential alcohol sensitivity. *Psychopharmacology* **72:** 79–83, 1980.
24. Stanton, H. C. and H. H. Keasling. Simultaneous blood levels and toothpulp threshold changes following intravenous ethanol administration to rabbits. *Q. Jl Stud. Alcohol* **18:** 205–211, 1957.
25. Tullis, K. V., W. Q. Sargeant, J. R. Simpson and J. D. Beard. An animal model for the measurement of acute tolerance to ethanol. *Life Sci.* **20:** 875–882, 1977.
26. Wolff, H. G., J. D. Hardy and H. Goodell. Studies on pain: measurement of the effect of ethanol on the pain threshold and on the "alarm" reaction. *J. Pharmac. exp. Ther.* **75:** 38–49, 1942.
27. Worsham, E. D., E. X. Freed, E. A. Lochry and E. P. Riley. Generality of differential sensitivity to alcohol in selectively bred rats. *Physiol. Psychol.* **5:** 429–432, 1977.