Pharmacology Biochemistry and Behavior, Vol. 43, pp. 625–629, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

BRIEF COMMUNICATION

Comparative Effects of Ethanol on Motor Activity and Operant Behavior

LAWRENCE D. MIDDAUGH,[1] KEXIN BAO AND CINDY L. SHEPHERD

Medical University of South Carolina,
Department of Psychiatry & Behavioral Sciences, Charleston, SC 29425-0742

Received 16 August 1991

MIDDAUGH, L. D., K. BAO AND C. L. SHEPHERD. *Comparative effects of ethanol on motor activity and operant behavior.* PHARMACOL BIOCHEM BEHAV 43(2) 625–629, 1992.—Ethanol effects on two types of motor activity and on lever responding for food delivered on a fixed-ratio 20 (FR 20) reinforcement schedule were compared using C57BL/6 (C57) mice. Low doses of ethanol (1–2 g/kg) transiently elevated horizontal activity and high doses (2.5 and 3.0 g/kg) reduced this behavior throughout testing with a slight recovery toward the end of a 16-min test period. In contrast, similar ethanol doses produced a monotonic reduction in both vertical activity and lever responding for food under the FR 20 schedule. The ethanol-induced reduction in FR 20 lever responding was less prolonged than the reduction in vertical activity but was more prolonged than the reduction in horizontal activity. Because vertical activity and lever responding for food delivered on the FR 20 schedule were never elevated, were reduced at ethanol doses that either stimulated or depressed horizontal activity, and were unaffected by low ethanol doses that did not affect horizontal activity, it is unlikely that either are sensitive to the stimulatory effects of ethanol. Accountable mechanisms for the different effects of ethanol on the three behaviors are unknown; however, the present study eliminates ethanol dose, postinjection time, testing time, and food deprivation condition as possible reasons for the differences.

C57 mice Ethanol Operant behavior Motor activity

A number of studies indicate that low doses of ethanol increase motor activity and that higher doses can either increase or decrease motor activity depending upon the time after injection and presumably the brain concentrations of the drug (17). Previous work from our laboratory using C57BL/6 (C57) mice is consistent with this literature, indicating that ethanol doses of 1.0–1.5 g/kg elevate locomotor activity whereas doses of 2.0 g/kg or higher have a biphasic effect, initially increasing then decreasing activity (14–16). Several reports also indicate that operant behavior (generally lever responding) maintained by various schedules of positive reinforcement (food or water) is increased and decreased by low and high doses of ethanol, respectively, for rats (8), pigeons (2,10), and humans (19). Some reports, however, note only a rate-decreasing effect of ethanol (5). Whether ethanol increased or decreased response rates in these studies depended upon the particular reinforcement schedule and/or the basal response rate, as well as the ethanol dose. Experiments from our laboratory (11,12) indicated that ethanol doses of 1.0 and 1.5 g/kg disrupted lever responding by C57 mice during generalization tests in a drug discrimination paradigm. Lower doses ranging from 0.25–0.75 g/kg had no influence on response rates during these tests and none of the doses elevated lever responding.

A reduction in lever responding produced by various drugs has sometimes been interpreted as indicative of a depressive effect (5,13). Studies in our laboratory, however, suggest that lever responding can be reduced by ethanol doses that either elevated (stimulated) or reduced (depressed) various measures of motor activity in other experiments. Differences in age of subjects, ethanol doses, interval between ethanol injection and behavioral assessment, and food deprivation conditions confounded a comparison between the motor activity and operant studies. In addition, the particular operant task we used was designed to assess the ethanol discrimination rather than response rate changes to ethanol. The present study was conducted to assess the effect of ethanol on lever responding for food delivered on a fixed ratio 20 (FR 20) schedule and on motor activity using similar dosing and time parameters as well as food deprivation conditions. Both vertical activity

[1] Requests for reprints should be addressed to Lawrence D. Middaugh, Ph.D., Medical University of South Carolina, Department of Psychiatry & Behavioral Sciences, Room 803 Research Building, 171 Ashley Avenue, Charleston, SC 29425-0742.

(rearing responses) and horizontal activity was measured to more thoroughly compare the effect of ethanol on motor activity and the FR 20 operant behavior.

METHOD

Motor Activity Studies

Subjects. Ten male C57, drug-naive mice obtained from Jackson Laboratory (Bar Harbor, ME) were used. They were housed singly in a colony room adjacent to the laboratory. The colony room was maintained on a 12 L : 12 D cycle with lights on at 0700 h, and temperature was regulated at 70 ± 3°F. The study began when mice were approximately 6 months old. One of the mice died as a result of an injection error, and data collected on this animal were eliminated from all statistical analysis.

Apparatus. Motor activity was assessed with a Digiscan Animal Activity Monitor system, Model RXYZCM(8) TAO, with a two-animal option (Omnitech Electronics, Columbus, OH). Two activity chambers were located in a 7 × 7-ft. cubicle. A fluorescent light provided 46 ft-c at the level of the activity chamber, and a fan provided ventilation and masking noise. Each activity chamber contained 16 photobeams positioned 5 cm apart: 8 on the *x*-axis and 8 on the *y*-axis. Photocells located on the wall directly opposite each photobeam were activated when the beam was interrupted by the animal. By recording which beams were interrupted, the distance the animal traveled (cm) during testing could be determined to provide a measure of horizontal activity. An additional eight photobeam/photocell arrangements located 7 cm above the floor of the chamber detected vertical movements. In this case, only the frequency of beam interruptions was determined. Each chamber was partitioned with acrylic into 20 × 20-cm quadrants. Mice were tested in one quadrant of each unit (i.e., two mice per test). The photocells of the activity chambers were connected to a Digiscan Analyzer located in an adjacent room. Data were gathered from the analyzer and stored on an IBM XT computer using ILAM software (Coulbourn Instruments, Lehigh Valley, PA). Two types of activity were assessed: horizontal activity, recorded as total cm traveled, and vertical activity, recorded as the total number of photobeams interrupted on the elevated sensors.

Procedures. Animals were weighed and deprived of all food for 24 h at the start of the experiment. They were then weighed daily throughout the experiment and given appropriate amounts of food to maintain body weight at 80 ± 5% of ad lib levels. After stabilizing at 80% of ad lib body weights, mice were tested twice a week (Monday and Thursday) between 1100 and 1400 h. Each mouse was tested 10 times. The first test was to habituate mice to the testing procedures and its particular activity chamber. The second test was also a familiarization test and began 5 min after IP injections of the water vehicle (0.02 ml/g body weight). The final eight tests assessed the effect of ethanol on motor activity. Ethanol was injected IP in doses of 0 (water vehicle, twice), 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 g/kg. Each mouse received each dose. The order of the doses was different for each animal to control for possible confounding due to the residual effects of prior ethanol exposure or experience with the testing environment. Five minutes after injections, mice were placed into their designated activity chamber and activity was recorded for 16 min. After completion of the test, mice were returned to their home cages. The activity chambers were wiped clean of urine and fecal boli after each test and disinfected at the end of the experimental day.

Operant Studies

Subjects. Five male C57, drug-naive mice obtained from Jackson Laboratories were used. They were housed singly in the colony room described above. The study began when mice were 3 months old.

Apparatus. Three operant chambers enclosed in sound- and light-controlled boxes were used. The operant chambers (16 × 16 × 11.4 cm) were constructed of grey Plexiglas with stainless steel grid floors. A food tray with a 1.9 × 2.5-cm opening was centrally located on one wall at floor level. Food pellets (20 mg, Noyes Co., Lancaster, NH) were delivered by Gerbrands Model D1 pellet dispensers (Arlington, MA). Light was provided by a miniature lamp (GTE 18-19) located directly above the food tray. Rodent levers (Model SRL-003, BRS/LVE, Laurel, MD) were located 4 cm to one side of the food tray and 3 cm above the floor. The levers required 8 g dead weight to close a switch, and subsequent release of the lever defined a response. The units were interfaced via LabLinc (Coulbourn Instruments) with an Apple IIe computer program that controlled house lights, timed the experimental session, delivered food pellets according to the FR 20 schedule, and recorded responses at intervals during the testing session.

Procedures.

Food deprivation and lever-response acquisition. Mice were food deprived as described above and water was given ad lib. For lever response acquisition, animals were placed in the chamber with five pellets available in the food tray at the start of each session. An additional pellet was dispensed for each lever press. This procedure was continued for four sessions, allowing a maximum of 10 responses or 15 min per session. After all animals had learned the lever press response (day 4), they were run 16 min per day 5 days a week for the rest of the study. Initially, they were placed on an FR 5 reinforcement schedule. After 5 days, the schedule was changed to FR 20. After 1 week of responding on the FR 20 reinforcement schedule, animals were injected IP daily with water (0.02 ml/kg) 5 min prior to testing. After 1 week of vehicle injections, they were tested weekly (Wednesday) under ethanol doses of 0.25, 0.50, 1.0, 1.5, 2.0, and 3.0 g/kg. In these tests, ethanol or its water vehicle were injected 5 min prior to testing and each animal was injected one time with each dose.

RESULTS

The data for the two experiments are summarized in Fig. 1 as dose–response functions for the three measures of behavior during 4-min intervals of the 16-min tests. Data for all three parameters were analyzed with repeated-measures analyses of variance (ANOVA) with dose and time as primary factors. The open symbols on each function indicate ethanol doses that significantly altered behavior from vehicle control levels. These analyses and inspection of the graphs indicate that the effects of ethanol depended upon the particular measure (i.e., horizontal activity, vertical activity, FR 20 lever responding) and the interval of testing, as well as the ethanol dose.

Horizontal activity varied as a function of ethanol dose, $F(6, 48) = 27.146, p < 0.001$; however, the effect of the drug interacted with time, dose × time, $F(18, 144) = 5.288, p < 0.01$. Because of the significant interaction, data were further analyzed for the simple main effects and differences between means within each time period were assessed with Duncan's multiple *t*-tests. The results of these analyses indicated that the 1.0-, 1.5-, and 2.0-g/kg doses of ethanol elevated horizontal activity above control levels by 47, 47, and 115%, respectively,

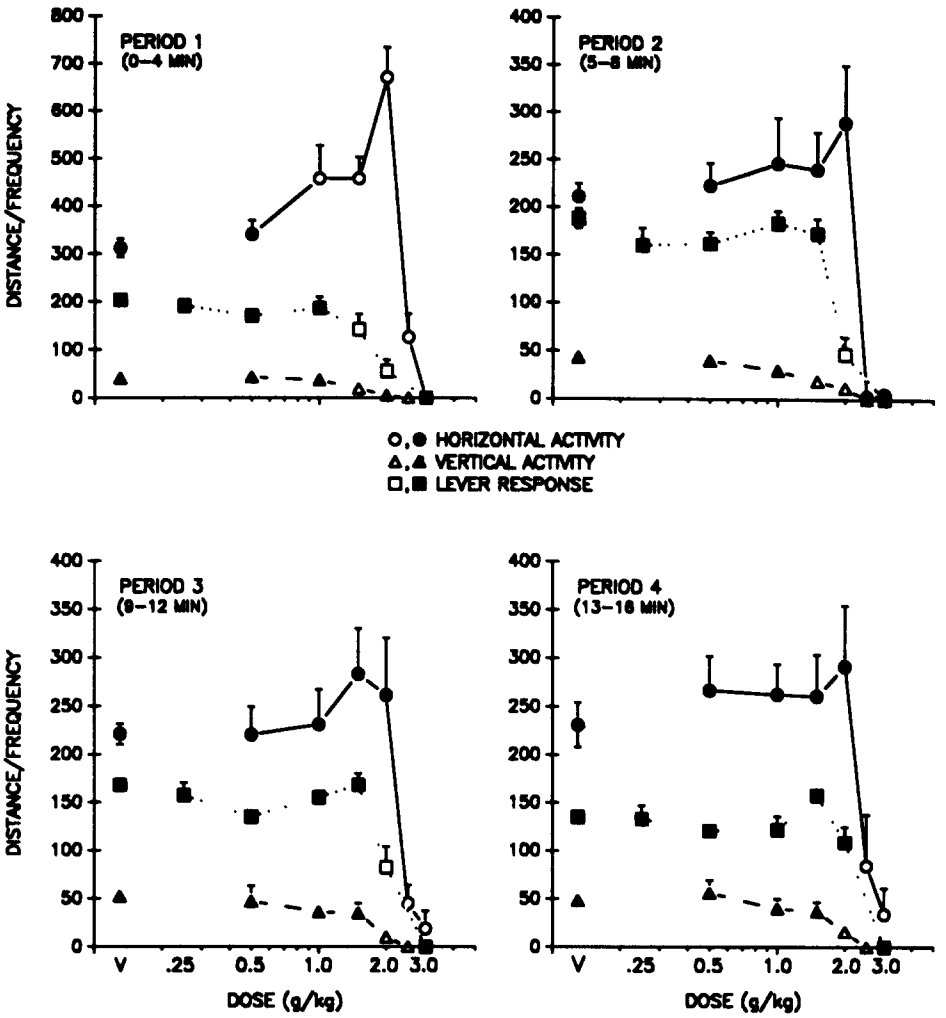

FIG. 1. Effects of ethanol on horizontal activity (distance in cm), vertical activity (frequency of beam interruptions), and lever responses (frequency) during 4-min periods of testing beginning 5 min after injection. Data points are mean ± SE based upon 9 or 5 C57 mice for the activity measures (Experiment 1) and lever responses (Experiment 2), respectively. Data points with open symbols differ significantly from their respective vehicle values (Duncan's multiple *t*-tests).

during the first 4-min period after injections. Horizontal activity after these lower ethanol doses did not differ significantly from control levels after the first period of testing. The higher doses of ethanol (2.5 and 3.0 g/kg) reduced horizontal activity significantly below control levels throughout the entire 16 min of testing. Both doses virtually eliminated this behavior during the second period of testing; however, recovery from these severe depressive effects was evident during the last 4-min period.

Vertical activity was reduced monotonically with increasing ethanol doses, which contrasts with the bidirectional effect of the drug on horizontal activity. The two highest ethanol doses (2.5 and 3.0 g/kg) completely eliminated vertical activity throughout the entire 16-min test period; hence, data for these doses were excluded from ANOVA. Vertical activity was reduced below control levels, $F(4, 32) = 5.932, p < 0.001$, with significant reductions below control levels beginning at the 1.5-g/kg dose. Unlike its effect on horizontal activity, the effect of ethanol on vertical activity did not interact with time, $F(12, 96) = 0.860$, and there was very little recovery of this behavior over the 16-min test.

Lever responding under the FR 20 schedule of food reinforcement was also monotonically reduced with increasing ethanol doses, $F(5, 20) = 17.057, p < 0.001$, as was noted for vertical activity. The highest ethanol dose (3.0 g/kg) completely eliminated lever responses; hence, data for this dose were not included in ANOVA. In contrast to the ethanol effect on vertical activity, however, the effect on lever responding interacted with time, $F(15, 60) = 7.077, p < 0.001$. After being substantially disrupted in the early time periods, lever responding recovered substantially over the course of the 16-min test period. After injections of the 1.5-g/kg dose, lever responding was reduced only during the first time period of testing (30% below control levels). After injections of the 2.0-g/kg dose, lever responding was reduced 72 and 75% below controls during the first and second period of testing but did not differ significantly from control levels during the fourth period.

DISCUSSION

In the present study, low doses of ethanol briefly elevated and higher doses reduced horizontal activity (locomotion) for prolonged periods. This pattern of brief elevation at low ethanol doses and more prolonged reduction at higher doses is consistent with our previous reports of ethanol effects on motor activity for this mouse strain (14-16), as well as with reports on other mouse strains (9,18) and rats (17). Although some reports indicate that ethanol does not stimulate motor activity of C57 mice (3,6,9,18), elevated activity after lower doses of ethanol has been observed in our laboratory utilizing four different methods of assessment and has also been reported by another laboratory (4). Because the duration of ethanol stimulation is shorter in C57 than most other mouse strains, its stimulatory effect is likely to be missed by studies that omit the early periods after ethanol administration.

Vertical activity, in contrast to horizontal activity, was not elevated by any of the ethanol doses but was reduced by doses of 1.0 g/kg or higher. Because this behavior was unaffected by the 0.5-g/kg dose at any time period, it is unlikely that it would be altered by doses lower than those used in this experiment. In addition, ethanol reduced vertical activity at doses and times when horizontal activity was either elevated (period 1; doses 1.5 or 2.0 g/kg), unaffected (periods 2-4; doses 1.5 and 2.0 g/kg), or reduced (periods 1-4; doses 2.5 and 3.0 g/kg). Because vertical and horizontal activity were not inversely affected by the midrange ethanol doses, behavioral incompatibility cannot account for the different effects of ethanol on these two behaviors. It is also unlikely that the different effect of ethanol on horizontal and vertical activity is due to a greater sensitivity of the latter to drug effects, as suggested in earlier reports for other drugs (20,21). Vertical activity in the present study was more sensitive than horizontal activity to ethanol effects only if ethanol-induced stimulation is ignored. If both the stimulatory and depressive effects of ethanol on horizontal activity are considered, the two measures appear to be equally sensitive to the drug. Neither of the measures were affected by the lowest dose, but both were altered by doses of 1.0 g/kg or higher.

The effect of ethanol on lever responding for food delivered on an FR 20 schedule of reinforcement closely paralleled its effect on vertical activity. As noted above for vertical activity, lever responding was not increased by any dose of ethanol, was disrupted by ethanol doses roughly equivalent to those that disrupted vertical activity, and was reduced at ethanol doses and at times after injection during which horizontal activity was elevated (period 1; doses 1.5 and 2.0 g/kg), unaffected (periods 2 and 3; dose 2.0 g/kg), or reduced (periods 1-4; dose 3.0 g/kg). The reduction of FR 20 lever responding produced by ethanol was less prolonged than the reduction of vertical activity but more prolonged than the reduction of horizontal activity. The greater and more prolonged ethanol-induced reductions in FR 20 operant behavior and vertical activity compared to horizontal activity may be because they involve more complex and coordinated movements, which are more susceptible to ethanol (7). The more rapid recovery of lever responding for food under the FR 20 schedule than of vertical activity might be related to the more significant consequences of lever responding in this experiment. The present experiment differs from what might be expected from reports of response rate increases following low ethanol doses in rats (0.2, 0.4 g/kg) maintained on an FR 10 schedule (8) and in pigeons (0.5-1.5 g/kg) maintained on a multiple fixed interval/FR schedule (2). However, the rate increases in these studies were not large, and the reinforcing conditions that facilitate ethanol-induced increases operant responding remain obscure.

It is unlikely that either vertical activity or lever responding on an FR 20 schedule are sensitive to the stimulatory effects of ethanol because they were both reduced at ethanol doses that either stimulated or depressed horizontal activity and were unaffected by ethanol doses too low to affect horizontal activity. If drug effects on horizontal activity, or locomotion, reflect their stimulatory and depressive (i.e., arousal) properties as commonly assumed (1,22), the present study suggests that neither vertical activity nor lever responding on an FR 20 schedule are useful for assessing the stimulatory dimension of ethanol effects. Mechanisms for the different effects of ethanol on the three behaviors are unknown; however, the present study eliminates ethanol dose, postinjection time, testing time, and food deprivation condition as possibilities.

ACKNOWLEDGEMENTS

The authors appreciate the word processing assistance of Marilyn Ruotolo. This work was supported by Grant AA06611 from NIAAA to L. D. M.

REFERENCES

1. Ahlenius, S.; Carlsson, A.; Engel, J.; Svensson, T.; Per Sodersten, F. K. Antagonism by alpha methyltyrosine of the ethanol-induced stimulation and euphoria in man. Clin. Pharmacol. Ther. 14:586-591; 1973.
2. Barrett, J. E.; Stanley, J. A. Effects of ethanol on multiple fixed-interval fixed-ratio schedule performances: Dynamic interactions at different fixed-ratio values. J. Exp. Anal. Behav. 34:185-198; 1980.
3. Becker, H. C.; Anton, R. F.; De Trana, C.; Randall, C. L. Sensitivity to ethanol in female mice: Effects of ovariectomy and strain. Life Sci. 37:1293-1300; 1985.
4. Crabbe, J. C., Jr.; Johnson, N. A.; Gray, D. K.; Kosobud, A.; Young, E. R. Biphasic effects of ethanol on open-field activity: Sensitivity and tolerance in C57BL/6N and DBA/2N mice. J. Comp. Physiol. Psychol. 96:440-451; 1982.
5. Duncan, P. M.; Cook, N. J. Ethanol-amphetamine interaction effects on spontaneous motor activity and fixed-interval responding. Psychopharmacology (Berl.) 74:256-259; 1981.
6. Frye, G. D.; Breese, G. R. An evaluation of the locomotor stimulating action of ethanol in rats and mice. Psychopharmacology (Berl.) 75:372-379; 1981.
7. Haubenreisser, T.; Vogel-Sprott, M. Reinforcement reduces behavioural impairment under an acute dose of alcohol. Pharmacol. Biochem. Behav. 26:29-33; 1987.
8. Holloway, F. A.; Vardiman, D. R. Dose-response effects of ethanol on appetitive behaviors. Psychonom. Sci. 24:218-220; 1971.
9. Kiianmaa, K.; Hoffman, P. L.; Tabakoff, B. Antagonism of the behavioral effects of ethanol by naltrexone in BALB/c, C57-BL/6, and DBA/2 mice. Psychopharmacology (Berl.) 79:291-294; 1983.
10. Leander, J. D.; McMillan, D. E.; Ellis, F. W. Ethanol and isopropanol effects on schedule-controlled responding. Psychopharmacologia 47:157-164; 1976.
11. Middaugh, L. D.; Ayers, K. L. Effects of ethanol on mature offspring of mice given ethanol during pregnancy. Alcohol. Clin. Exp. Res. 12:388-393; 1988.
12. Middaugh, L. D.; Bao, K.; Becker, H. C.; Sergent-Daniel, S. Effects of Ro 15-4513 on ethanol discrimination in C57BL/6 mice. Pharmacol. Biochem. Behav. 38:763-767; 1991.

13. Middaugh, L. D.; Blackwell, L. A.; Boggan, W. O.; Zemp, J. W. Brain concentrations of phenobarbital and behavioral activation or depression. Pharmacol. Biochem. Behav. 15:723–728; 1981.
14. Middaugh, L. D.; Boggan, W. O.; Randall, C. L. Stimulatory effects of ethanol in C57BL/6 mice. Pharmacol. Biochem. Behav. 27:421–424; 1987.
15. Middaugh, L. D.; Favara, J. P.; Boggan, W. O. Ethanol stimulation after chronic exposure in C57 mice. Pharmacol. Biochem. Behav. 34:331–335; 1989.
16. Middaugh, L. D.; Read, E.; Boggan, W. O. Effects of naloxone on ethanol induced alterations of locomotor activity in C57BL/6 mice. Pharmacol. Biochem. Behav. 9:157–160; 1978.
17. Pohorecky, L. A. Biphasic action of ethanol. Biobehav. Rev. 1: 231–240; 1977.
18. Randall, C. L.; Carpenter, J. A.; Lester, D.; Friedman, H. J. Ethanol-induced mouse strain differences in locomotor activity. Pharmacol. Biochem. Behav. 3:533–535; 1975.
19. Rumbold, G. R.; White, J. M. Effects of repeated alcohol administration on human operant behaviour. Psychopharmacology (Berl.) 92:186–191; 1987.
20. Sanberg, P. R.; Henault, M. A.; Hagenmeyer-Houser, S. H.; Russell, K. H. The topography of amphetamine and scopolamine-induced hyperactivity: Toward an activity print. Behav. Neurosci. 101:131–133; 1987.
21. Russell, K. H.; Giordano, M.; Sanberg, P. R. Amphetamine-induced on- and off-wall rearing in adult laboratory rats. Pharmacol. Biochem. Behav. 26:7–10; 1987.
22. Strömbom, U.; Svensson, T. H.; Carlsson, A. Antagonism of ethanol's central stimulation in mice by small doses of catecholamine-receptor agonists. Psychopharmacology (Berl.) 51:293–299; 1977.