Pharmacology Biochemistry & Behavior, Vol 27, pp 421–424 © Pergamon Journals Ltd , 1987 Printed in the U S A 0091-3057/87 $3 00 + 00

Stimulatory Effects of Ethanol in C57BL/6 Mice

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Received 22 December 1986

MIDDAUGH, L D , W O BOGGAN AND C L RANDALL *Stimulatory effects of ethanol in C57BL/6 mice* PHARMACOL BIOCHEM BEHAV 27(3) 421–424, 1987 —Although ethanol stimulation is well documented in several species including humans, there is some controversy about whether the stimulation occurs in the highly inbred mouse strain, C57BL/6 Since inbred mouse strains are frequently used to elucidate mechanisms for individual differences in reaction to alcohol, the present study was undertaken to more completely characterize the behavioral effects of ethanol and to help resolve some of the controversy regarding the drug's stimulatory effect on C57 mice Activity of female C57BL/6cr mice was assessed in either a lighted or dark environment for 20 min after injections of water or ethanol at doses of 0 5, 1 0, 2 0, 4 0 g/kg Elevated activity (stimulation) was observed in mice injected with relatively low ethanol doses and tested in the light The 2 0 g/kg dose produced a transient elevation in activity which declined rapidly across time Animals tested under the dark condition were not stimulated by the drug but had activity reductions to high doses of ethanol The detection of ethanol-induced stimulation appears to be related to the performance of control mice rather than a light-related difference in ethanol sensitivity

Ethanol stimulation Motor activity Inbred mice C57 mice

ALTHOUGH a biphasic action of ethanol on behavior (stimulation-depression) is well documented (see [8]), the literature is inconsistent regarding the stimulatory effects of the drug on C57BL/6 (C57) mice, an inbred strain Some reports indicate that ethanol does not stimulate C57 mice [1, 2, 4, 9, 10] Other reports [3,6] indicate that C57 mice are either stimulated or depressed by alcohol depending upon the dose injected, the time after injection, and the duration of the activity measure In our previously reported study [6], activity stimulation was observed under conditions when the activity level of control mice was relatively low (during the later stages of a one hour test period) and ethanol concentrations in brains of drug-injected mice were presumed to be relatively low (i e , lower doses) A recent report by Crabbe, Johnson, Gray, Kosobud and Young [3] also described a transient stimulatory effect of ethanol (2 g/kg) in C57 mice which began shortly after injection The review by Pohorecky [8] suggested that the large reported differences in the effects of ethanol on different species and strains might be partially accounted for by differing experimental conditions and methods of behavioral assessment Since the ambient lighting condition and the time or duration of activity assessment both influence the amount of activity recorded in activity test, these parameters might well influence the results obtained when assessing the influence of various drugs To help elucidate the apparent contradictory reports regarding ethanol effects on activity of C57 mice, we completed the present experiments assessing the influence of several ethanol doses on activity under two different lighting conditions

METHOD

Female C57BL/6cr mice 90 days of age were used They were maintained 4–6/cage in a colony room with lights on and off at 0700 and 1900 hr Food and water were available ad lib Activity of individual mice was assessed between 1000 and 1500 hr in a laboratory contiguous with the colony room using a 3-channel Stoelting Electronic Activity Monitor (Model 31409, Stoelting Co , Chicago, IL) This system is identical to that employed in two previous ethanol studies [5,10] It essentially consists of an oscillator which generates radio-frequency fields over the surface of three individual sensing platforms, and electronics to detect and process voltage changes resulting from perturbations of the fields The degree of voltage change is proportional to the degree of movement within the field such that large and/or rapid movements cause rapid voltage changes The resulting analog information can be processed to provide a digital out-put which, in our system, was counted across time by interfacing the system with an Apple IIe computer using a LabLinc interface (Coulbourn Instruments, Lehigh Valley, PA) The 3 sensors, remote from the oscillator and signal processing equipment, were located in a 5×6×8 ft testing

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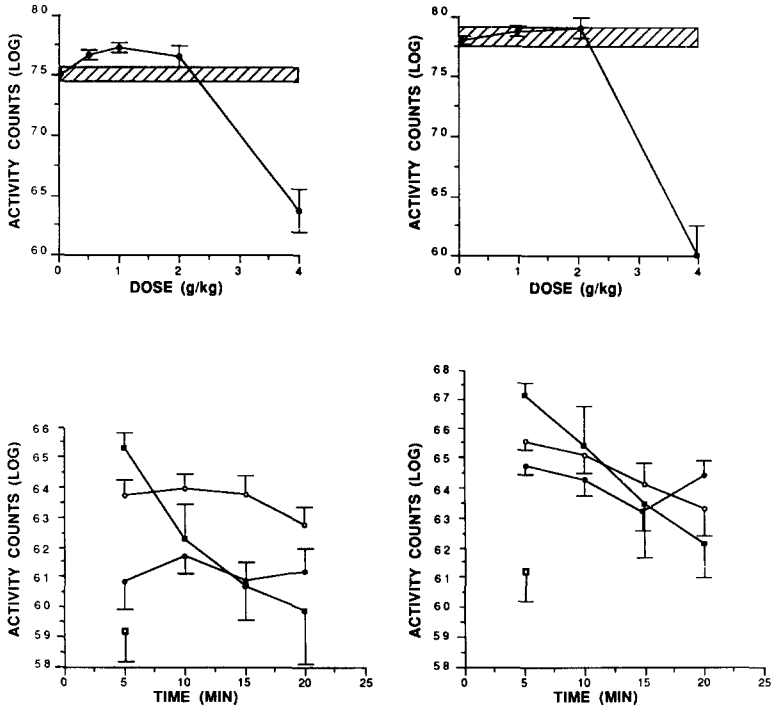

FIG 1 Effects of ethanol on motor activity of C57 mice measured under light (left graphs) or dark (right graphs) conditions The upper graphs reflect activity generated during a 20-min test after injections of water or the various ethanol doses The lower graphs show the distribution of activity at 5-min intervals across the 20-min test for mice injected with vehicle (solid circles) or ethanol 1 g/kg (open circles) or 2 g/kg (squares) Data for the 4 0 g/kg dose (open squares) are shown for only the first 5 min Data are expressed as means±standard errors of natural log transformed data

room adjacent to the laboratory The testing room contained an exhaust fan which effectively masked sound from the laboratory For the present study, the sensors were cross-calibrated for sensitivity (Activity Level settings 15, 22, and 24) and threshold reset time was set in the "Normal" mode

Animals were tested with the testing room lights either on or off which resulted in illuminations of 46 vs <1 ft candle at the level of the platform On the day of testing, mice were brought to the test room in groups of three and injected intraperitoneally (0 02 ml/g body weight) with either the vehicle (water) or ethanol at doses of 0 5, 1, 2, or 4 g/kg Ethanol concentrations ranged from 3% to 25% The mice were placed immediately into 32×21×13 cm polypropylene mouse cages (one per cage) with clear Plexiglas tops centered on the sensing platforms Activity was recorded for 20 min and the data were printed out at 5-min intervals The treatment conditions were distributed proportionally across the three activity sensors All tests occurred in clean cages to eliminate the influence of odors from the previous animal The effect of ethanol on the distribution of activity across time under the light condition utilized 65 mice, 16 per group except for the 0 5 g and the 4 0 g/kg dose groups which had 9 and 8 mice respectively The influence of ethanol on activity under the dark condition was determined using thirty-two additional mice injected at doses of 0, 1, 2, and 4 g/kg (8/group) Data from two animals injected with the 4 0 g/kg dose were lost due to equipment malfunction Activity tests in the dark were interspersed with tests in the light under similar dosing conditions

RESULTS

The effect of ethanol on activity is summarized in Fig 1 To reduce the differences in variability of activity scores within each group for statistical analysis, the raw activity scores were transformed to their natural log The means and standard errors of the log scores are shown in the figure The upper graphs summarize mean log scores for activity generated over the entire 20-min test period and the lower graphs summarize mean log scores for activity at 5-min intervals across the 20-min period Data collected under the light condition are shown on the left and those collected in the dark are shown on the right

Light Condition

The upper left graph of Fig 1 indicates that activity measured over the 20-min period in the light was elevated by injections of the lower doses of ethanol but reduced by the 4 0 g/kg dose Because the latter dose completely eliminated activity at later time periods of measurement, these data were eliminated from statistical analysis of the total activity scores Activity of mice injected with the 0 5, 1 0 and 2 0 g/kg doses of ethanol was 16%, 34% and 24% above that of controls An analysis of variance (ANOVA) of these data and subsequent comparison of the ethanol group means to the control mean via Dunnett's Test supported a significant increase for the 1 g/kg group, $t(53)=2\ 244$, $p<0\ 05$ This dose appeared to be maximally stimulatory under conditions of this experiment since the 2 0 g/kg dose produced no further

stimulation The 4 0 g/kg dose reduced activity by 65% compared to controls and completely eliminated activity in some mice during the later periods of activity assessment

Activity at 5-min intervals during the 20-min testing session in the light is summarized on the lower left graph of Fig 1 The single data point at T-1 reflects activity of mice injected with the 4 0 g/kg dose during the first five minutes (prior to the severe ataxia) Data for the lower doses were analyzed across time using a 3 (Dose) × 4 (Time Period) ANOVA The distribution of activity across time was clearly different for the three dose groups, $F(6,123)=4\ 761$, $p<0\ 001$, and data were further analyzed to compare each ethanol dose with the vehicle Inspection of the graph suggests that activity of the 1 g/kg group was higher but that its distribution across the test period was similar to that of water controls This observation was supported by a significant Dose effect, $F(1,30)=9\ 689$, $p=0\ 004$ The activity distribution of animals injected with the 2 g/kg ethanol dose differed from that of controls [Dose × Time interaction, $F(3,90)=5\ 537$, $p<0\ 001$] Subsequent analysis of the simple main effects indicated a significant elevation of activity for ethanol injected animals during the initial 5 min of measurement [Dose at T-1, $F(1,60)=7\ 745$, $p<0\ 01$] In addition, activity of ethanol injected mice declined across time, $F(3,90)=5\ 040$, $p<0\ 01$, whereas activity of control mice did not

The data summarized at T-1 of the graph were subjected to a separate ANOVA to include the data for the 4 0 g/kg group This analysis and post-hoc comparisons (Dunnett's Tests) of ethanol group means with the vehicle control indicate that both of the lower ethanol doses elevated activity [$t(53)=2\ 800$, $p<0\ 025$, $t(53)=4\ 400$, $p<0\ 005$], whereas the apparent reduction produced by the 4 0 g/kg dose was not supported statistically

Dark Condition

The activity of mice tested over the 20-min interval in the dark is summarized on the upper right graph and was analyzed as described above for similar data collected under the light condition As noted for animals tested in the light, the 4 0 g/kg dose severely reduced activity (81% reduction from controls) and the data were excluded from analysis of total activity scores In contrast to the results obtained under the light condition, however, neither the ANOVA nor the Dunnett's tests revealed a stimulatory effect of the 1 g or 2 g/kg doses of ethanol when activity was measured in the dark

The distribution of activity at 5-min intervals under the dark condition is shown on the lower right graph and also differs from the distribution obtained under the light condition The data points and analyses are as described above The 3 (Dose) × 4 (Time) ANOVA completed on data from the lower doses indicated that the distribution of activity across time differed according to ethanol dose, $F(6,63)=3\ 458$, $p<0\ 01$ Subsequent analyses indicated that the activity of mice injected with either the 1 g/kg, $F(3,42)=2\ 833$, $p=0\ 048$, or the 2 g/kg dose, $F(3,42)=5\ 536$, $p<0\ 01$, was distributed differently than that of water controls with activity of ethanol injected mice declining and that of control mice not changing significantly across time

The ANOVA and comparison of group means for data at T-1 indicated only a significant reduction in activity produced by the 4 g/kg dose of ethanol, $t(26)=2\ 915$, $p<0\ 01$, with no statistically supported differences for the 1 g and 2 g/kg doses

Light-Dark Comparison

To provide a direct comparison of the effect of ethanol on activity when assessed in the light or dark, data for the water, the 1 g and the 2 g/kg ethanol groups were subjected to a 2 (Light Condition) × 3 (Dose) × 4 (Time) ANOVA This analysis indicated that activity varied as a function of Lighting Condition, $F(1,66)=3\ 058$, $p=0\ 003$, and Time, $F(3,198)=13\ 444$, $p<0\ 001$ As anticipated from the above analyses, the activity levels depended on the particular dose of ethanol given and the time of assessment, $F(6,198)=5\ 481$, $p<0\ 001$ The analysis, however, provided no indication that the influence of ethanol interacted with the lighting condition An ANOVA of the T-1 scores, which included data for the 4 0 g/kg group, also indicated the influence of Lighting Condition, $F(1,78)=11\ 989$, $p<0\ 001$, and ethanol dose, $F(3,78)=13\ 857$, $p<0\ 001$, on activity but did not indicate an ethanol-lighting condition interaction

DISCUSSION

The major factors influencing the effect of ethanol on motor activity of C57 mice in the present study were the dose administered and the time after injection or time of activity measurement In addition, mice were more active in the dark than in the light which in turn can influence the conclusions about the stimulatory or depressive effects of ethanol In general, activity of ethanol-injected mice was elevated at times when brain concentrations of the compound were assumed to be relatively low (i e , doses of 0 5 to 1 0 g/kg or early after a 2 0 g/kg dose when maximum brain concentrations had probably not been attained) and when the activity of vehicle control mice was relatively low (i e , the Light Condition) The stimulatory effect of ethanol on C57 mice in this study differs from several previous reports [1, 2, 4, 9, 10], however, is in agreement with our previous report [6] and that of Crabbe *et al* [3] The present study demonstrates that ethanol can stimulate activity of female C57 mice as well as that of males which have been used in previous studies The stimulation of motor activity by the 2 0 g/kg dose of ethanol during the first 5 min of testing confirms the report [3] that locomotor activity of C57 mice was stimulated by this dose for a brief period after injection Thus, the present study indicates that ethanol can stimulate the general motor activity recorded by the radio-frequency field interruption technique in a relatively small enclosure as well as locomotor activity The present data also suggest that studies which begin testing several minutes after injection [1], which exclude data during early testing, or which combine data over long periods of time, will likely miss the stimulatory effects of ethanol in C57 mice

The relationship of the activity of ethanol-injected mice to that of vehicle-injected mice determines whether one concludes that ethanol stimulates or depresses activity The present experiment indicates that this relationship is influenced by the lighting condition under which activity is assessed The stimulatory effect of ethanol was statistically confirmed only when activity was assessed in a lighted (45 ft candles) environment The influence of the lighting condition on the relationship between vehicle and ethanol activity levels is clearly demonstrated during the first 5 min of testing When tested in the light, activity of mice injected with ethanol at the two lower doses was elevated compared to controls but that of animals injected with the 4 0 g/kg dose was not different from controls In contrast, when tested in the dark, activity of mice injected with the two lower doses

did not differ from controls, however that of animals injected with the 4 0 g/kg dose was below control levels Thus, the stimulatory effect of ethanol on C57 mice is more likely to be detected when activity is assessed in the light whereas its depressive effects are more likely to be detected when activity is measured in the dark

The activity levels of vehicle control mice under the different lighting conditions heavily influenced their relationship to activity of ethanol-injected mice The activity of vehicle-injected mice during the first 5 min of testing was 32% less when measured in the light than in the dark Since rodents are nocturnal animals, increased activity in a dark environment is not surprising Indeed, several studies (see [11]) document this phenomenon The higher activity level in the dark, compared to the light environment observed in the present study, confirms a previous report on activity of C57 mice in an open-field arena [7] The direct comparison of ethanol-injected and control mice tested under the two lighting conditions via ANOVA's revealed that activity was influenced by the lighting condition and the ethanol dose, however, did not indicate an interaction of these two main factors Thus, the effects of ethanol appear to be uninfluenced by the lighting condition per se Rather, the higher activity of the control mice measured in the dark appears to mask the detection of ethanol-induced stimulation The detection of ethanol stimulation when control response rates were relatively low in the present study was also noted in our previous report [6], however, low activity of controls in that study occurred late during testing after locomotion had habituated It is also of interest that many of the studies indicating no stimulatory effect of ethanol on C57 mice have generally included other strains with control activity levels substantially below that of control C57 mice Thus, it is possible that some of the reported strain difference in reaction to ethanol might be due to difference in control level activity

In summary, the present study confirms two previous reports that ethanol can stimulate activity of C57 mice and helps establish some conditions which favor the detection of ethanol stimulation in this strain Stimulation can be observed at low ethanol doses (1 0 g/kg) or briefly after higher doses (2 0 g/kg) when conditions favor low activity of control mice Manipulation of the lighting conditions, under which activity was measured, was sufficient to mask any ethanol induced stimulation

ACKNOWLEDGEMENTS

We acknowledge William H Frackelton for interfacing our equipment with a computer and his assistance with data analysis and Lynn Meeko for her assistance with manuscript preparation This work was supported by Grant No AA06611 from NIAAA to L D M

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