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Lack of Tolerance to Ethanol-Induced Motor Impairment on Accelerod Performance in Rats

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UZBAY, I. T. AND C. J. WALLIS. *Lack of tolerance to ethanol-induced motor impairment on accelerod performance in rats.* PHARMACOL BIOCHEM BEHAV **63**(4) 607–611, 1999.—The effect of ethanol on rats was investigated at increasing rates of acceleration for bar rotation speed. Ethanol was given to rats by a liquid diet starting with 2.4% ethanol (v/v) for 3 days. Then the ethanol concentration was increased to 4.8% (v/v) for 3 days and finally to 7.2% (v/v) for 15 days. Accelerod performance was recorded before and throughout 20 days of ethanol intake. Mean blood ethanol levels were 266.34 ± 13.11 and 285.20 ± 9.77 mg/dl on the 7th and 15th days of ethanol (7.2% v/v) consumption, respectively, as measured in a parallel group of animals. Ethanol produced significant concentration-dependent impairments in the accelerod performance of rats. The motor impairment effect of ethanol was most prominent in the test using the greatest rate of acceleration (from 0 to 79 rpm within 2 min). The impairment effect of ethanol on accelerod performance occurred throughout the period of ethanol exposure. Our results indicate that motor impairment on the accelerod performance test produced by an ethanol liquid diet depends on the concentration of ethanol and the rate of acceleration. In addition, under free-access conditions accelerod performance may not be a suitable behavioral test for detecting tolerance development to ethanol in rats. © 1999 Elsevier Science Inc.

Ehanol Motor coordination Tolerance Rat(s)

THE measurements of locomotor activity (2,5,14), rotarod (2,3,22), and accelerod performance tests (3,22,24) have been widely employed to assess the sensorimotor deficits produced by ethanol and similar drugs. Ethanol exerts various dose-dependent behavioral effects in rodents, varying from stimulation of locomotor activity after low doses to motor impairment, hypothermia, loss of righting reflex, and coma caused by higher doses of ethanol (13,17).

Chronic exposure of animals and humans to ethanol results in decreased sensitivity, or tolerance, to most of its central effects (18). Tolerance to ethanol-induced motor incoordination (ataxia) and to its hypothermic, sedative, anesthetic, and anxiolytic effects has been shown in experimental animals exposed repeatedly to ethanol (19). The development of tolerance was considered evidence of central nervous system adaptation during the time when ethanol was present in the brain (20), but the mechanism of this phenomenon remains to be determined (4). Although the development of tolerance to ataxic and sedative effects of ethanol has been well documented in animals by a large number of behavioral tests including rotarod performance (11), the studies investigating tolerance developed to motor impairment effects of ethanol by an accelerod performance test are limited.

A previous study from our laboratory indicated that rotarod and accelerod performance tests seem to have differential characteristics in the context of tolerance development to chronic ethanol given by liquid diets in rats (22). In that study, we observed development of tolerance to the motor impairment effect of ethanol on rotarod performance, but not on accelerod performance. However, only one very fast rate of acceleration (from 0 to 80 rpm within 50 s) was used in the study, and the effect of ethanol on accelerod performance of the rats was not repeatedly evaluated during exposure to ethanol.

The present study was designed to determine the characteristics of the motor impairment produced by acute and chronic ethanol given by a liquid diet on accelerod perfor-

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mance in rats. This was done by measurement of accelerod performance of the rats exposed to three concentrations of ethanol, which increased over time. We also used four rates of acceleration for determining the effects of ethanol.

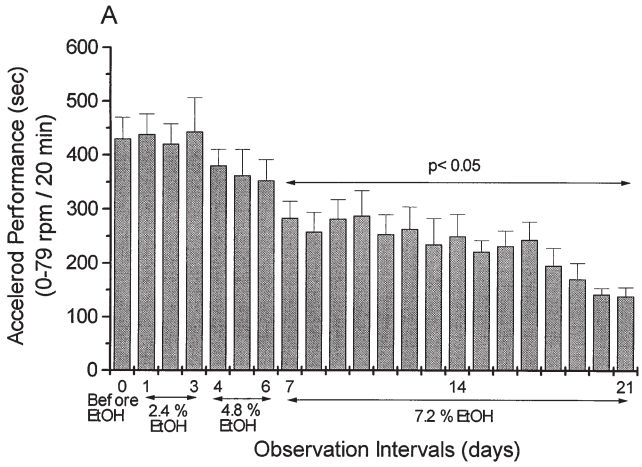
METHOD

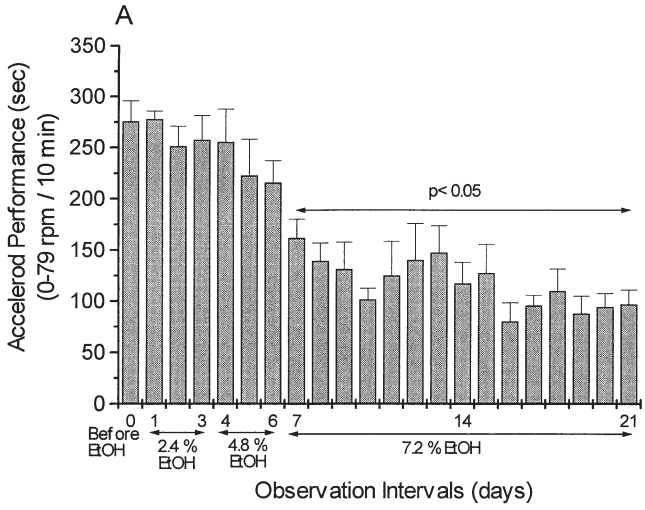
Animals and Laboratory

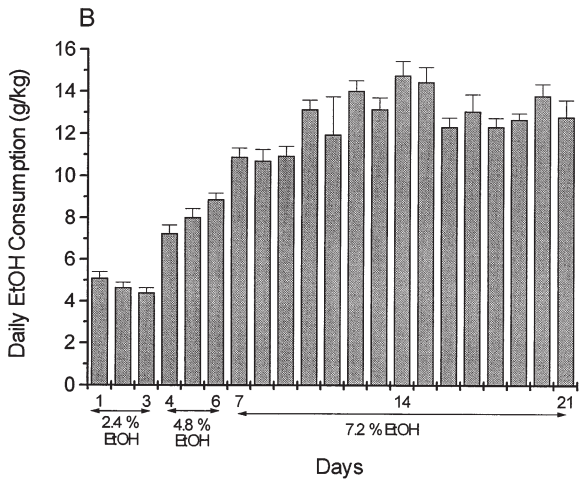
All procedures in this study are in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health. Adult male Wistar rats (212–237 g at the beginning of the experiments) were housed individually in metal cages in a quiet temperature-, and humidity-controlled room (22 ± 3°C and 62 ± 5%, respectively). A 12 L:12 D cycle was maintained (0800–2000 h light).

Procedure

Rats were assigned to six groups ($n = 8$ per group). They were allowed to acclimate to the laboratory conditions, and were given a modified liquid diet without ethanol for 7 days before beginning the experiments. Then ethanol was given ad lib to all rats in the modified liquid diet, offered as the only source of food and water as described previously (23). At the beginning of ethanol exposure, the liquid diet with 2.4% ethanol (v/v) was administered for 3 days. Then the ethanol concentration was increased to 4.8% (v/v) for 3 days and finally to 7.2% (v/v) for 15 days. As the ethanol concentration was increased, the diet was kept isocaloric by reducing sucrose. Liquid diet was freshly prepared daily and presented at the same time of the day (1000 h). Ethanol consumption was recorded daily and expressed as g/kg/day.







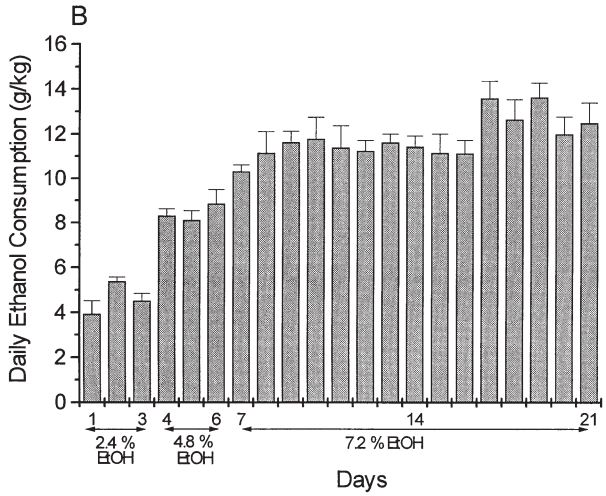


FIG. 1. Effects of ethanol on accelerod performance in the test from 0 to 79 rpm within 20 min (A) and daily ethanol consumption of the rats (B) ($n = 8, p < 0.05$ significantly different from preethanol baseline day).

FIG. 2. Effects of ethanol on accelerod performance in the test from 0 to 79 rpm within 10 min (A) and daily ethanol consumption of the rats (B) ($n = 8, p < 0.05$ significantly different from preethanol baseline day).

Accelerod performance (Rotamex V-EE/85, Columbus, OH) of rats in groups 1–4 was recorded prior to and during exposure to ethanol. Accelerod performance of the rats was measured as previously described (3). Training for rotarod and accelerod tests was performed during the week when ethanol-free diet was given. Rotational velocity of the rod was linearly increased from 0 to 79 rpm within 2, 4, 10, and 20 min, respectively. Groups were tested in different rotational velocities from each other. The time (seconds) at which the animal fell off the accelerating rods was automatically measured by the built-in timer of the apparatus.

Blood ethanol levels were determined in two groups of ethanol-receiving rats run in parallel to the behavioral test groups. These animals were not tested behaviorally. The blood samples were taken by cardiac puncture in anesthetized rats. Ether was used as an anesthetic agent during this procedure. The samples were taken 1 h before giving the fresh ethanol containing diet. Concentrations were determined by a fluorescence polarization immunoassay method (8) (TDX autoanalyzer Abbott Laboratories, TX) on the 7th and 15th days of ethanol (7.2%) administration.

All experiments were done at the same time of the day in the light cycle (1030 h).

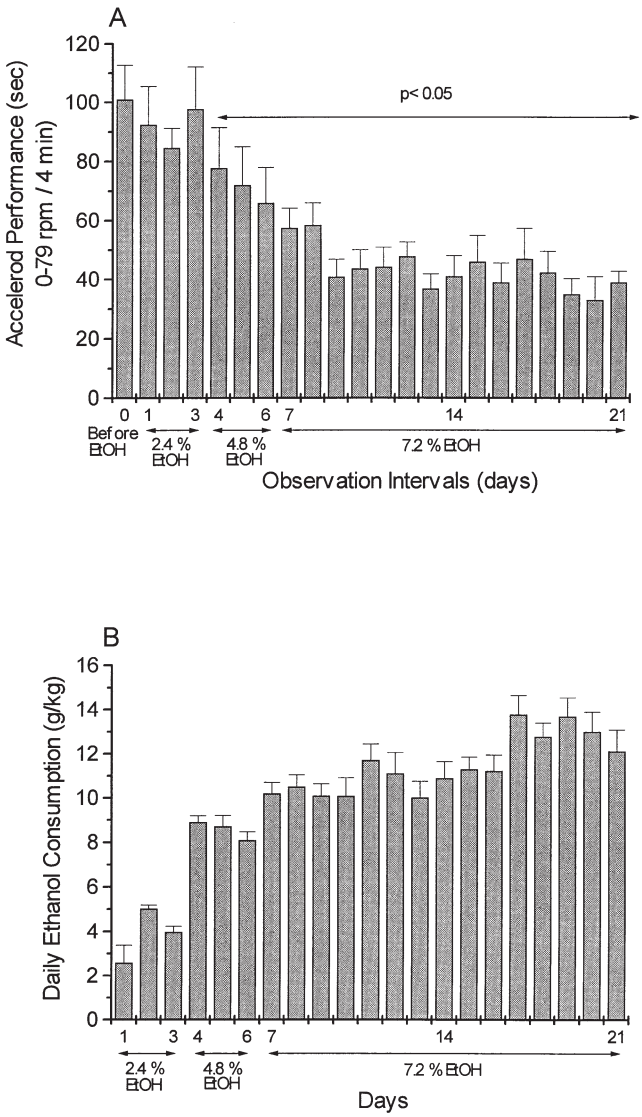


FIG. 3. Effects of ethanol on accelerod performance in the test from 0 to 79 rpm within 4 min (A) and daily ethanol consumption of the rats (B) ($n = 8$, $p < 0.05$ significantly different from preethanol baseline day).

Statistical Analysis

Values were given as mean ± SEM. Changes in accelerod performance were statistically evaluated using analysis of variance (ANOVA) for repeated measures (one-way MANOVA) with post hoc comparisons made by Dunnett's tests. The level of significance was set at $p < 0.05$.

RESULTS

Accelerod performances of the rats before and during ethanol administration are shown in Figures 1–4, together with daily ethanol consumption. Ethanol caused some significant inhibitory effects on accelerod performances of the rats tested at all rotational velocities, independently [one-way MANOVA; Fs(21, 147) > 6.569, ps < 0.001]. Lower concentrations (2.4 and 4.8%, v/v) of the ethanol liquid diet administration did not significantly change accelerod performance of the rats at rotational velocities increasing from 0 to 79 rpm within 20 or 10 min compared to day 0 (Dunnett's test, $p > 0.05$; Figs. 1 and 2A). Significant decreases in the accelerod performance of the rats were observed at 4.8% ethanol in the groups tested at a rotational velocity of 0–79 rpm/4 min and 0–79 rpm/2 min (Dunnett's test, $p < 0.05$; Figs. 3 and 4A). The 2.4% concentration of ethanol produced significant decreases only at the highest acceleration of rotational velocity tested (0–79 rpm/2 min) (Dunnett's test, $p < 0.05$; Fig. 4A). The highest concentration of ethanol (7.2%, v/v) produced a significant decrease on accelerod performance of the rats throughout the observation period (15 days) in all velocities (Dunnett's test, $p < 0.05$; Figs. 1–4A). Mean daily ethanol consumption of the rats ranged from 2.58 to 5.38, from 7.25 to 9.0, and from 10.3 to 14.4 g/kg for concentrations of 2.4, 4.8, and 7.2% (v/v) of ethanol, respectively (Figs. 1–4B).

Mean blood ethanol levels were found to be 266.34 ± 13.11 and 285.20 ± 9.77 mg/dl the 7th and 15th days of ethanol (7.2%) consumption. Daily ethanol intakes of the rats in these groups ranged from 11.98 ± 0.55 to 13.77 ± 0.95 mg/dl. Daily average ethanol consumptions were measured as 12.65 ± 0.48 and 13.11 ± 1.18 mg/dl at the 7th and 15th days of exposure to ethanol, respectively.

DISCUSSION

In the present study, ethanol caused a prominent concentration-dependent impairment of accelerod performance in rats. This observation is consistent with the previous literature in that ethanol administered by gastric intubation, intraperitoneal injection, or liquid diet has been shown to result in impaired motor coordination (ataxia) (3,17,22,24).

The motor impairment by ethanol was greatest at the highest concentration used in the study and at all rates of acceleration. An interaction was also observed between the extent of motor impairment produced by ethanol and the rate of acceleration of rotational velocity of the rod. This result indicates that the motor impairment effects of ethanol also depend on

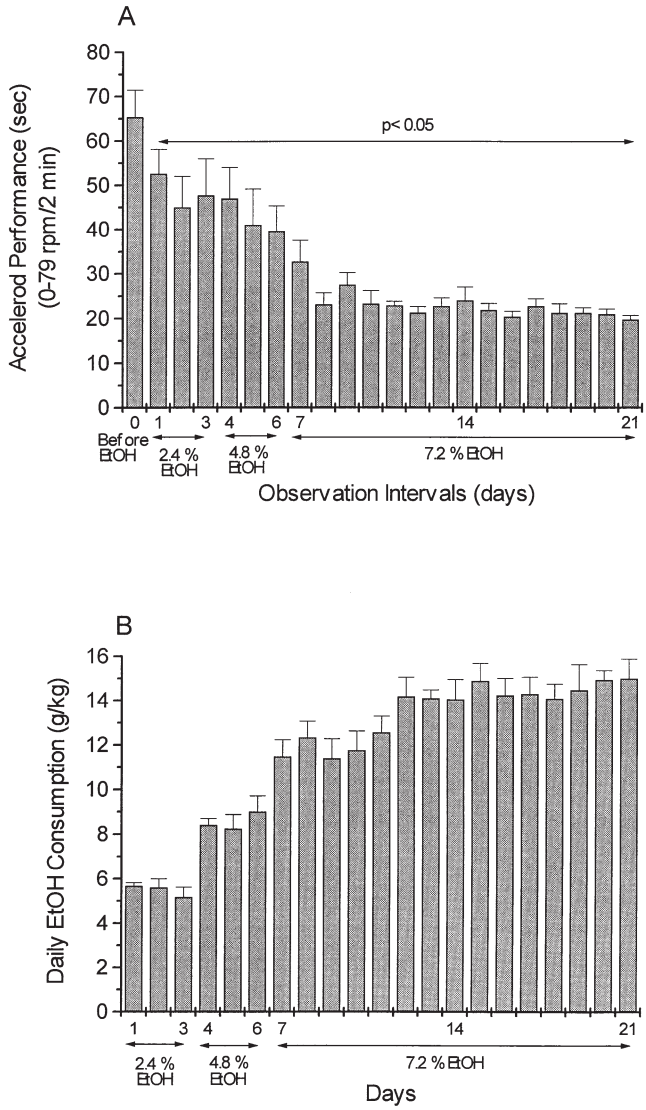


FIG. 4. Effects of ethanol on accelerod performance in the test from 0 to 79 rpm within 2 min (A) and daily ethanol consumption of the rats (B) ($n = 8, p < 0.05$ significantly different from preethanol baseline day).

the acceleration of the rod or difficulty of the test in addition to ethanol concentration.

Significant impairment effects of ethanol (7.2%) on accelerod performance were observed throughout the testing period (15 days). During this period, the mean daily ethanol consumption of the rats was above 9.5 mg/dl and high blood ethanol concentrations (above 266 mg/dl) measured on the 7th and 15th days of ethanol (7.2%) intake. These findings indicate that tolerance to the ethanol-induced impairment on accelerod performance was not detected for up to 15 days in animals receiving ethanol in liquid diet ad lib. The data also confirm our previous results indicating a lack of tolerance to the impairment effect of ethanol on accelerod performances in Wistar rats (22,24).

Ahmad and Nicholls (1) observed that dose-dependent tolerance developed to the effects of aminoglutethimide, a barbiturate-like hypnosedative, on the accelerating rotarod in mice. Van der Laan et al. (25) have also reported the development of tolerance to the effects of lorazepam, a benzodiazepine, on accelerod performance in rats. Although these classes of drugs are known to show extensive crosstolerance to ethanol, the present data are not in line with their results. The discrepancy may be related to the different doses, routes of administration, and/or treatment period by drugs used in these studies. In addition, they used a much slower acceleration than those used in the present study.

The rotarod test has been used with rodents to evaluate drug effects on motor coordination and capacitation despite its methodological limitations. In our previous study, we observed tolerance development to the motor impairment effect of ethanol on rotarod performance in rats fed ethanol in a liquid diet ad lib (22). Because the accelerod test is much more difficult to perform than the constant-speed rotarod test, it is much more subject to impairment by ethanol. However, we did not test whether tolerance developed to motor impairment effects of ethanol on accelerod performance in much slower rotational velocities of the rod because the existing apparatus did not provide a rate lower than 0–79 rpm/20 min. This apparatus or rotational velocities used in the present study may not be appropriate to detect tolerance development to motor impairment effects of ethanol.

Studies on the characteristics of ethanol tolerance have shown that it is a complex phenomenon (9,10), with the rate and extent of tolerance development being influenced not only by the amount of ethanol exposure, but also by various behavioral processes. For example, it has been shown that the development and expression of ethanol tolerance can be greatly affected by the contextual cues that accompany ethanol exposure (7,15,21). In addition, the opportunity to practice while under ethanol intoxication may produce behavioral tolerance (6,16). Thus, it could be stated that tolerance to ethanol and other drugs is influenced by behavioral factors such as practice under the influence of the drug, variation of the test system, and conditional influences of environmental cues (12). These findings suggest that tolerance is unlikely to be accounted for by a single mechanism. In the present study, the motor impairment of accelerod performance was maintained for 3 days even at low concentrations of ethanol. Significant increases in ethanol consumption of the rats within a give ethanol concentration resulted in more prominent motor impairment.

In conclusion, ethanol given by a liquid diet exhibited concentration- and rate of acceleration-dependent impairment effects on accelerod performance of rats. Our results support the hypothesis that accelerod performance testing in rotational velocities and/or apparatus used in the present study may not be appropriate for detecting tolerance development to the motor impairment effects of ethanol given by a liquid diet in rats.

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