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Behavioral Effects of Ethanol Inhalation in Rats

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GHOSH, T. K., R. L. COPELAND, JR., P. K. ALEX AND S. N. PRADHAN. *Behavioral effects of ethanol inhalation in rats.* PHARMACOL BIOCHEM BEHAV **38**(4) 699–704, 1991.—Behavioral effects of ethanol inhalation were studied on two fixed-ratio (FR) liquid-reinforced schedules and a continuous reinforcement (CRF) schedule intracranial self-stimulation (SS) in rats using the inhalational behavioral chamber designed in our laboratory. In the FR-24 schedule ethanol caused a decrease of reinforcement rate at 161 ppm and higher concentrations. In the FR-50 schedule decreases of the rate were observed at 102 ppm and 203 ppm. In the SS behavior ethanol produced a decrease in the rate of reinforcement at 603 ppm and higher concentrations. In rats of this schedule, blood ethanol concentrations were measured to be 393 μg/ml and 545 μg/ml after exposure to 600 ppm and 1200 ppm of ethanol respectively. Acute tolerance to ethanol was observed in these experiments, particularly in the FR-24 schedule. Thus ethanol inhalation could produce adequate blood concentrations so as to produce behavioral effects.

Ethanol Inhalation Fixed-ratio (FR) liquid-reinforced behavior Self-stimulation Tolerance Blood ethanol concentration Rats

NEUROBEHAVIORAL effects and physical dependence induced by ethanol, one of the most abused chemicals as well as an industrial solvent, have been studied following its administration by various techniques such as gastric intubation (12,15), self-administration (1), and feeding with liquid diet (17). Administered by these and other techniques, ethanol has been shown to produce biphasic effects in fixed-ratio (FR), fixed-interval (FI), differential reinforcement at low rates (DRL) and self-stimulation schedules in pigeons and rats (11, 14, 15). In a multiple (FI-FR) schedule in pigeons a biphasic effect was also seen in the FR component, but the response rate was only decreased in the FI component (12).

Although ethanol is almost always abused by oral consumption, self-administration by inhalation of its vapor has also been reported to achieve euphoria (2). Administration of ethanol by inhalation over periods of 1 to 13 days (along with the use of pyrazole, an inhibitor of alcohol dehydrogenase to maintain continuously elevated blood ethanol levels) has been shown to produce physical dependence and functional tolerance in mice (7–10). Behavioral effects of ethanol have also been studied during its administration by inhalation. Concentration-dependent decreases of response rate were shown on fixed-ratio (FR) schedule performance in mice at 14,600 to 37,400 ppm (19). Fixed-interval (FI) responding was also decreased by ethanol, but at very high concentrations (36,000 and 48,000 ppm by inhalation), or 4 g/kg oral (20). In the latter experiment, the behavioral performance was conducted following 30-min inhalation to ethanol to show recovery from the effect of the solvent exposure. In both of these experiments the concentrations of ethanol used appear to be very high. Ethanol inhalation studies (6) in rats from our laboratory show that at 100 and 400 ppm, it caused an increase in the duration of waking and a decrease in duration of rapid eye movement sleep, in the sleep-wake cycle; higher concentrations (up to 1600 ppm) did not have any further effect on the sleep-wake cycle or EEG power spectrum. The present report describes effects of ethanol inhalation in rats trained in three schedules of behavior differing in types of reinforcers as well as contingencies [e.g., FR-24 or FR-50 liquid-reinforced schedules and a continuous reinforcement intracranial self-stimulation (SS) behavior] during exposure to much lower concentrations.

METHOD

ANIMALS

Male rats (F344, Charles River Breeding Lab) having initial body weight of 180–210 g were used. They were housed individually in stainless steel cages. Animals were kept in artificial light-dark cycle of 12/12 h (light 7 a.m. to 7 p.m.) and the mean ± S.E. of the temperature and relative humidity of the animal room were 24 ± 1°C and 55 ± 5%, respectively.

EXPOSURE TO ETHANOL

Rats were exposed to ethanol (100%, anhydrous; U.S. Industrials Co.) in a dynamic inhalational behavioral chamber devised

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by Pradhan and Copeland (27). The chamber consisted of an inverted cylindrical glass chromatography jar which covered a circular grid floor with a vertical metal plate accommodating a lever and a liquid dipper. The chamber was infused with a flow of air derived from house air supply. The house air was filtered, mixed with ethanol vapor generated in an evaporating flask and passed through a water-cooled condenser to lower the temperature of the mixture before entering into the exposure chamber.

The concentration of ethanol in this chamber was monitored by collecting gas samples in a sampling bulb from inside the chamber at 15-min intervals, and then injecting 1 ml of the sample into a Shimadzu dual-column gas chromatograph (GC) equipped with flame ionization detectors (Model GC Mini 2). The concentration was measured with the help of a digital integrator connected to the GC. The average concentration of ethanol in samples collected during a daily session was expressed as the grand mean ± S.E.

Behavioral Schedules and Experimental Design

To study the behavioral effects of ethanol inhalation, rats were trained in two operant schedules, FR liquid-reinforced schedules and SS behavior. After training for 3–4 weeks under various protocols, when the performance of rats was more or less stabilized, they were placed within the inhalational behavioral chamber for acclimatization and further training. After a stable performance for 3–4 consecutive days (when the daily response rate did not vary more than 10% of the average), exposure to ethanol inhalation was started. Only house air was pumped on nontreatment days.

FR Liquid-Reinforced Behavior

Rats were maintained at 80% of their original starting weights by controlled postsession feeding. They were trained to press a lever 24 or 50 times for delivery of the reinforcement (0.2 ml of 5% sucrose solution), controlled by a minicomputer system. Behavioral sessions started with a noncontingent delivery of sucrose solution which was given by manipulation of an external switch connected to the minicomputer system. In all experiments recordings were made for both responses and reinforcements which were approximately proportional. For calculation and evaluation purposes, reinforcement rate has been taken into consideration in these experiments.

FR-24 liquid-reinforced schedule.

Two-hour ethanol exposure at 4 concentrations. The schedule consisted of a 15-min period of behavioral performance alternating with a 15-min period of time-out (TO), during a daily session of 2 h 15 min. Reinforcement was available on five episodes during a session. All rats were randomly exposed to 4 graded concentrations of ethanol (140.2 ± 0.4, 160.8 ± 0.5, 202.4 ± 0.9 and 397.7 ± 1.4 ppm) for 2 h on different days. To prevent the development of tolerance the same rat was not exposed to the next concentration for at least 2 weeks. Exposure to ethanol was started following the first 15-min behavioral performance (preexposure control). Behavioral performance on the previous day of exposure was considered as the previous day control.

Five-hour ethanol exposure to the lowest level concentration. Rats were trained to a schedule of 15-min behavioral performance followed by 45 min of TO, which continued for a daily session of 5 h 15 min. Therefore, six episodes of behavioral performance were available in a daily session. After control studies in the inhalational behavioral chamber, rats were exposed to 140.9 ± 0.5 ppm of ethanol for 5 h. Fifteen min of behavioral performance was recorded as preexposure control before ethanol exposure started. Behavioral performance on the previous day of exposure was considered as the previous day control.

Two-hour daily exposure to a medium ethanol concentration for 5 consecutive days. The daily session of this experiment consisted of a behavioral schedule as described in the earlier 2-h exposure schedule. When the reinforcement rate was stabilized in the inhalational behavioral chamber, control data were recorded for 3–4 days. After that rats were exposed to an intermediate ethanol concentration (206.3 ± 0.3 ppm) for 2 h daily for 5 consecutive days. A 15-min preexposure control was recorded every day before exposure had started. The reinforcement rate on the previous day before the first day of exposure was considered as an unexposed control.

FR-50 liquid-reinforced schedule. Four rats were trained to press the lever 50 times (FR-50) to receive 0.2 ml of sucrose solution, and were allowed to perform continuously without any interruption. The reinforcement rate was counted at 20-min intervals. After recording a consistent control reinforcement rate for 3–4 days, rats were exposed to 102 ppm of ethanol for 2 h. Exposure started following a 20-min control period.

Self-Stimulation Behavior

Rats were trained to press a lever to receive an electrical stimulation through a set of implanted electrodes in the posterior hypothalamus. The methods of electrode implantation, training and other procedures were essentially the same as previously described (4,26), with slight modifications.

In brief, a set of bipolar stainless steel electrodes (0.01 inch in diameter and insulated except at the tip from Plastic Products Inc., Roanoke, VA) was implanted stereotaxically in the posterior hypothalamus. Coordinates for the area were: 3.5 mm posterior to bregma, 0.5 mm lateral to midline, 8.4 mm ventral to the top of the skull held flat according to the atlas of Paxinos and Watson (22). Electrode implantation was done under sodium pentobarbital anesthesia (40 mg/kg, IP).

One week after surgery rats were trained to press the lever in a behavioral box to be reinforced with electrical stimulation. Each lever press provided an electrical stimulus that was a sine wave consisting of a train of pulses of 60 Hz and 0.3 s duration. The current intensity (as rms) remained constant at slightly above the threshold level and varied from rat to rat between 30–110 μA; The threshold level was arbitrarily defined as the level that will induce 2 or 3 SS responses during a 5-min session. After a period of three to six weeks of training, rats in the initial behavioral box as well as in the inhalational behavioral chamber, when their SS behavior was stabilized (response rate not varying more than 10% of the average), ethanol exposure was started.

Two-hour ethanol exposure. Rats were allowed to press the lever in 2-h 20-min sessions. Lever-pressing responses were recorded every 20-min period. When the control data from two successive daily sessions were within 10% of their average, rats were exposed to four graded concentrations of ethanol (129.5 ± 10.6, 372.9 ± 10.7, 603.4 ± 14.4, 1287.2 ± 73.0 ppm) at random for 2 h on separate days. The same rat was not exposed to the next concentrations for at least 2 weeks. Exposure to ethanol started after a 20-min control period.

MEASUREMENT OF BLOOD ETHANOL CONCENTRATION

Blood was collected from the tail vein of the rat after 2-h ethanol exposure under the SS behavioral schedule and ethanol concentration in the blood sample was determined by alcohol dehydrogenase (ADH) assay (25). Briefly, protein-free supernatant from 25 μl of whole blood was prepared by adding 100 μl 0.38 N trichloroacetic acid into a microtube on ice. This mixture

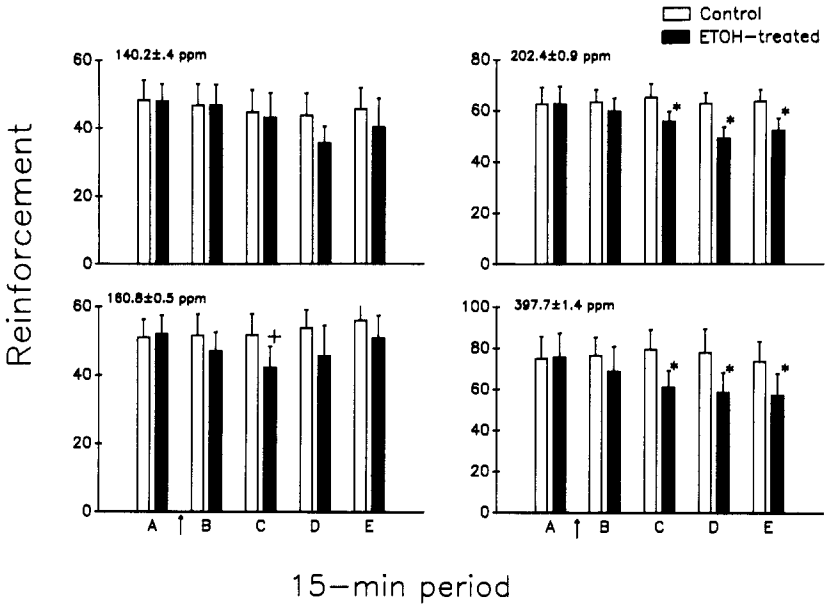

FIG. 1. Changes in the number of reinforcements for rats responding under a fixed-ratio (FR-24) liquid-reinforced schedule during a 2-h exposure to ethanol at 4 different concentrations. Reinforcement was given for 15-min periods at 15-min intervals: A, control −15–0 min; B, 16–30 min; C, 46–60 min; D, 76–90 min; E, 106–120 min. Ethanol-treatment day data (ETOH-treated) was compared with corresponding previous day control data (control). Exposure to ethanol was started at arrow after A (control period). Mean ± S.E. of data for 6 rats. *$p<0.05$. (F value, sig.; +, sig. with LSD; *, sig. with LSD, DT and NKT.)

was then centrifuged at 3000 rpm for 5 min. The supernatant was added to another microtube containing 600 μl of ADH-NAD/glycine assay enzyme mixture and allowed to stand at room temperature for 20 min. Ethanol standards were treated in the same manner as the whole blood. Absorbance was read at 340 nm with a spectrophotometer. All specimens were analyzed in duplicate. The concentration of ethanol in blood specimens was determined from the calibration curve for the difference in background absorbance between plasma and aqueous standards. The blood from nontreated rats provided the control level.

STATISTICAL ANALYSIS

FR Liquid-Reinforced Behavior

Differences of reinforcement rates between preexposure control and each exposure period were determined for each animal on both the previous day control and experimental day. These differences from corresponding periods of the previous day control and experimental day were analyzed by ANOVA using the SAS general linear model procedure, modeling with within-period exposure-control comparison directly to determine the significant effect at an overall level $p<0.05$. A similar procedure was followed to compare the raw data of unexposed control (day 0) and different experimental days involving 2-h daily exposure for 5 days.

SS Behavior

For calculation and evaluation, responses for the consecutive 20-min periods during the behavioral session were taken into consideration. Differences of rates of response (responses/20-min period) between preexposure control and each exposure period, were determined for each animal on both the control on the previous day and experimental day, were analyzed by ANOVA using general linear model procedure, modeling the within period exposure control comparison directly to determine the significant effect at an overall level, $p<0.05$.

If the F value was significant in the ANOVA test, then significance of difference between the previous day control and the corresponding experimental day data was tested by using three procedures reflecting the grades of their stringency in increasing order: Least Significance Difference (LSD), Duncan's test (DT) and Newman-Keuls test (NKT). The level of significance and the test(s) used are indicated in the legends to the respective figures.

RESULTS

FR-24 LIQUID-REINFORCED BEHAVIOR

Two-Hour Ethanol Exposure

Rats were exposed to four graded concentrations of ethanol in increasing sequence at an interval of about a week. During these intervals, the baseline rates of responses and the reinforcements gradually increased with time, as reflected in Fig. 1. Furthermore, significant decreases in the reinforcement rate were noted in the FR-24 schedule during exposure to ethanol at 160.8 ± 0.5, 202.4 ± 0.9 and 397.7 ± 1.4 ppm, but not at 140.2 ± 0.4 ppm. At 160.8 ppm, significant decreases were noted during the 46–60-min period; the rate returned to normal during the 76–90-min period, but the decrease persisted during the rest of the exposure period at 202.4 and 379.7 ppm (Fig. 1).

Five-Hour Exposure

In order to investigate the possible cumulative effect, of ethanol, rats were exposed to a noneffective concentration as indi-

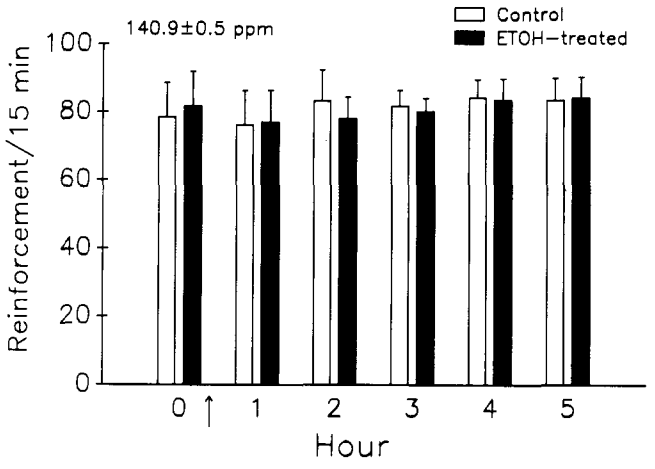

FIG. 2. Fixed-ratio (FR-24) liquid-reinforced behavior of rats during 5-h exposure to 140.9 ± 0.5 ppm of ethanol. Reinforcement was given for last 15 min at each hour during the session. Exposure to ethanol was started at arrow after 15 min of control recording (0). Mean ± S.E. for data from 4 rats.

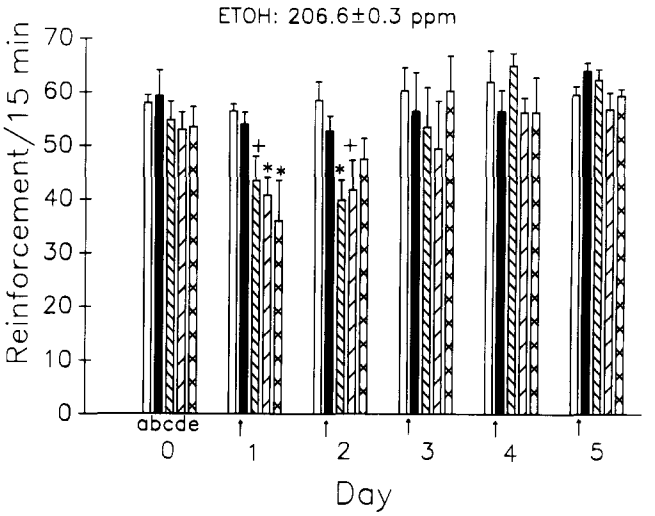

FIG. 3. Effect of 2-h daily exposure to 206.6 ± 0.3 ppm of ethanol for 5 consecutive days on fixed-ratio (FR-24) liquid-reinforced behavior. Reinforcement was given for 15 min at 15-min intervals: a, control (−15–0 min); b, 16–30 min; c, 46–60 min; d, 76–90 min; e, 106–120 min. Ethanol exposure was started at arrow after 15 min of control recording each day. Mean ± S.E. for data from 4 rats. *$p<0.05$. (Two-way ANOVA, F value sig.; +, sig. with LSD; *, sig. with DT.)

cated in the previous experiment (e.g., 140.9 ppm) over a prolonged (5-h) session. In this experiment no significant change in the reinforcement rate was observed at any time compared to the previous control day (Fig. 2).

Two-Hour Daily Exposure for 5 Days

In order to investigate possible development of tolerance, on repeated administration of ethanol, a moderately effective concentration (e.g., 206.6 ppm) of ethanol was selected from the previous experiment for repeated daily exposure for 5 days. Comparison of the preexposure control data from day 1 to day 5 with corresponding data from day 0 (control day) did not show any significant difference. However, during 2-h exposure on day 1, the reinforcement rate was significantly decreased during 46–60-min, 76–90-min and 106–120-min periods. On day 2, the decrease of the reinforcement rate occurred during the 46–60-min and 76–90-min periods, but not during the 106–120-min period. On day 3, day 4 and day 5 the reinforcement rate did not show any significant change during the exposure period, thus showing development of tolerance to ethanol (Fig. 3).

FR-50 LIQUID-REINFORCED SCHEDULE

In this schedule, there was no change in the reinforcement rate during 80 min of exposure to 102.1 or 202.5 ppm of ethanol except for a trend in decrease during the initial 20–40-min exposure to 202.5 ppm and a trend in increase during the postexposure 20 min for 102.1 ppm. However, these changes were not significant (Fig. 4).

Self-Stimulation Behavior

Two-hour ethanol exposure. SS behavior, as indicated by the reinforcement rate, showed a trend in increase during exposure to 129.5 ppm of ethanol at the 1–20-min period, but not altered during the rest of the exposure period. Trends in decreases of the reinforcement rate were noted at the 81–100-min period during exposure to 603.4 ppm, and at the 81–100-min, 101–120-min and 121–140-min periods during 1287.2 ppm exposure. These changes were not significant (Fig. 5).

Blood Ethanol Concentration

Figure 6 shows the data for blood ethanol concentrations reached after exposure to different ethanol concentrations during

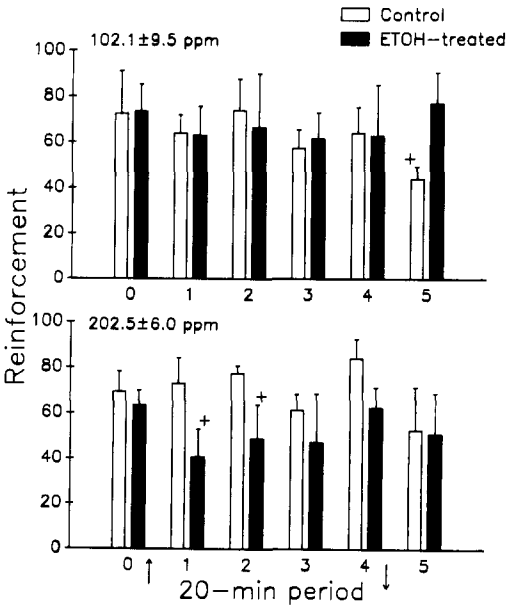

FIG. 4. Changes in the reinforcement rate of rats responding under fixed-ratio (FR-50) liquid-reinforced schedule during 2-h continuous exposure to 102.1 ± 9.5 ppm of ethanol. Responses were recorded for consecutive 20-min periods. Exposure to ethanol was started at arrow after the first 20-min control recording (0). Ethanol-treatment day data (ETOH-treated) was compared to corresponding previous day control data (control). Mean ± S.E. of data for 3–4 rats. (F value, insig.). +, sig. with LSD.

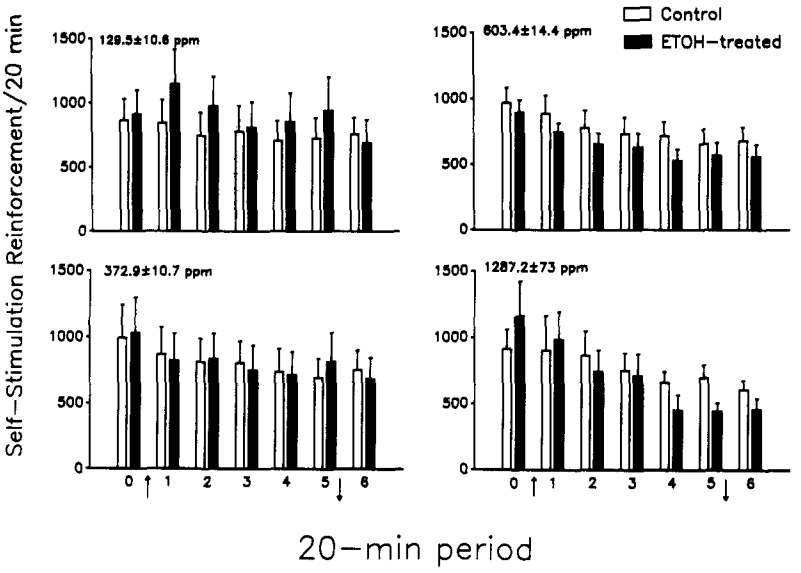

FIG. 5. Changes in self-stimulation behavior during 2-h exposure to ethanol at 4 different concentrations. Other details are same as in Fig. 4. (F value, insig.)

performance of self-stimulation behavior. Thus the blood ethanol concentration was observed to be approximately 393 μg/ml after exposure to 600 ppm and 545 μg/ml after exposure to 1200 ppm of ethanol.

DISCUSSION

Our study mainly demonstrated a behavioral deficit following ethanol inhalation. Under the liquid-reinforced FR-24 schedule the behavioral depression was transient at 160.8 ppm, but became more marked at 202.4 and 397.7 ppm (Fig. 1). On the other hand, in the SS study, there was a trend in depression (although insignificant), which was transient at 603 ppm and more persistent at 1287 ppm (Fig. 5). Thus, for ethanol, the FR schedule was found to be more sensitive than the SS schedule; a similar trend was also observed for xylene on these schedules (5,28). This could be due to difference in the reinforcers (SS being a stronger reinforcer) as well as in schedule contingencies.

In many physiological, behavioral and biochemical studies, biphasic effects of ethanol have been reported, with stimulatory effects being observed at low doses [see (23) for references]. In SS behavior, facilitation of responding at low doses and its decreases at high dose have been reported (13). Such biphasic effects have not been observed in our behavioral studies, except for SS behavior, in which a trend in initial response increase (although insignificant) was observed.

The effective concentrations (for depression) of ethanol in our behavioral schedules (FR and SS) are much lower than those used by Moser and Balster (20) on their F1 schedule. This discrepancy may be partly due to the difference in the experimental animal (mouse vs. rat), behavioral chamber (static vs. dynamic), procedure of exposure (before vs. during the behavioral performance), and behavioral schedule used.

Our threshold for behavioral depression due to ethanol exposure also appears to be lower than that set up by OSHA (Occupational Safety and Health Administration). Permissible Exposure Limit (PEL) as well as Time Weighted Average (i.e., the employee's average airborne exposure in any 8-h work shift of 40-h work week) for ethanol as set by OSHA is 1000 ppm or 1900 mg/m^3 (21).

In ethanol inhalation studies from other laboratories, blood concentrations of ethanol have been monitored and correlated with their pharmacological effects. Thus mice showed ataxia, tremor and sleep with blood levels above 1.5 mg/ml, and coma above 3.5 mg/ml level (8). Physical dependence could be produced by maintaining the blood ethanol level at 2 or even 1 mg/ml with the peak of withdrawal reaction progressively increasing between 6 to 9 days (9). In the present behavioral studies, blood ethanol concentrations have been found to be as low as 120 μg/ml to produce decrement in a fixed-ratio liquid-reinforced schedule. This could be due to difference in experimental subjects (mice vs. rats), method of ethanol exposure (mice were in groups of 24 in two cages, whereas a rat was in a cage) and behavior studied (liquid-reinforced FR 24 schedule is very sensitive to drug effect, as already stated).

Rapid tolerance to ethanol inhalation has been observed in our FR-24 schedule both during a single exposure (Fig. 1, 160.8 ppm)

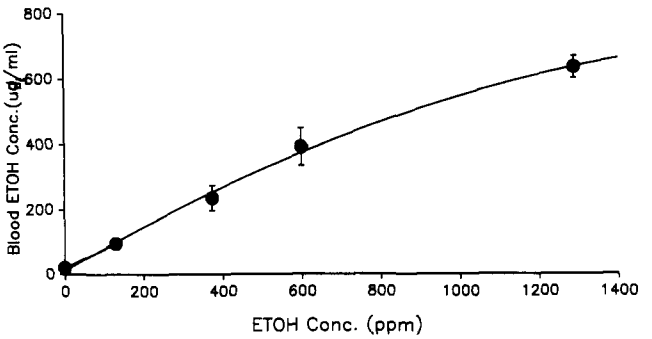

FIG. 6. Blood concentration of ethanol at the end of 2-h exposure to various ethanol concentrations during self-stimulation behavior sessions. Mean ± S.E. of data from 3 to 6 rats. Blood from nontreated rats provided the controls.

as well as repeated daily exposure (Fig. 3, day 3 and later). Acute tolerance to ethanol first observed by Mellanby (18) has been confirmed with a wide range of physiological and behavioral measures of ethanol effects (3, 10, 16, 24). The precise mechanism for acute ethanol tolerance which has been thought to be a part of a general adaptive response of the organism is not clearly known; however, it has been shown to involve alteration in the activity of central monoamines and peptide hormones as well as in the central neuronal membrane [see (24), for review]. Although our study did not involve ethanol dependence, it may be mentioned that dependence to ethanol has been produced by an inhalation procedure in mice by Goldstein (7,8). Thus, like other procedures for ethanol administration, its exposure by inhalation is also effective for producing behavioral effects, tolerance and dependence.

ACKNOWLEDGEMENTS

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