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Absence of Impairments in Spatial and Temporal Discrimination Learning in Lewis Rats After Chronic Ethanol Consumption

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BLOKLAND, A., J. PRICKAERTS AND W. RAAIJMAKERS. *Absence of impairments in spatial and temporal discrimination learning in Lewis rats after chronic ethanol consumption.* PHARMACOL BIOCHEM BEHAV **46**(1) 27–34, 1993.—Many studies reported that chronic ethanol consumption leads to cognitive dysfunction in rodents. It has been suggested that the effects of chronic ethanol consumption resemble those of aging because of the behavioral and neurochemical similarities between the two processes. The present study examined the effects of a chronic ethanol treatment (20% aqueous solution) in Lewis rats on performance in three different tasks: the Morris spatial navigation task, a cone-field task, and a temporal discrimination task. Although an age-related deficit was found in water escape learning, chronic ethanol consumption did not affect the performance of adult and old rats. The results of this experiment were, however, not conclusive. No differences between old control and ethanol-treated rats were found for spatial (cone-field task) and temporal discrimination learning. However, old ethanol-treated rats showed a transient tendency to perseverate in the temporal discrimination task. The present results are at variance with the generally found cognitive impairments after chronic ethanol consumption using aqueous solutions. It is suggested that the effects of ethanol could be related to strain of rat, task complexity, method of ethanol administering, and housing conditions and may explain the discrepancy between results.

Chronic ethanol treatment Aging Spatial learning Temporal discrimination learning Lewis rat

IT has been reported that chronic ethanol consumption results in a cognitive dysfunction in rats and mice that is independent of a nutritional, that is thiamine, deficiency (12). Learning and memory impairments after chronic ethanol treatment have been found in shock avoidance tasks (13,31), temporal discrimination learning (26,32), and maze learning (3,7). More recently, impairments in spatial learning and memory have been reported to be related to deficiencies in the cholinergic system after prolonged ethanol consumption (1,15). These behavioral and neurochemical findings after chronic ethanol consumption in young subjects are comparable to those associated with normal aging and support the "premature aging" hypothesis of chronic ethanol consumption (25). This premature aging hypothesis, however, has recently been challenged by Beracochea et al. (4), who found evidence for different forms of memory impairments in aged vs. chronic ethanol-treated mice. Further, chronic ethanol treatment impairs shuttlebox avoidance learning in middle-aged and old rats in a similar fashion (17). This indicates that chronic ethanol treatment does affect the process of aging. In humans, it has been reported that the mechanisms involved in normal aging and alcoholism are different (8). However, support for the premature aging hypothesis has been found in a study with aged, alcoholic, and alcoholic Korsakoff individuals (20). Irrespective of the difference between experimental subjects, these results indicate that the relation between chronic ethanol consumption and aging has still to be clarified.

It has been reported that chronic ethanol consumption is more detrimental to old than to young subjects, both at a biochemical [e.g., adaptive response of density of muscarinic receptors (22)] and behavioral level [e.g., sleeping time, locomotor activity (35); hypnosis (36)]. However, it should be mentioned that there is a complex interaction between ethanol consumption and behavioral effects with aging (21). Based upon these findings, it was assumed that learning and memory deficits would also be more pronounced in old subjects. However, only a few studies evaluated the effects of chronic ethanol consumption on learning and memory impairments in old rats (17).

We carried out a series of experiments to evaluate the effects of chronic ethanol treatment on learning and memory performance in different tasks in old rats. First, we tested

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adult and old control and ethanol-treated rats in a spatial discrimination paradigm. If chronic ethanol consumption affects the aging process (see above), learning and memory performance of old rats should be more impaired by a chronic ethanol diet than the performance of adult rats. Because we found no effects of chronic ethanol treatment in the first behavioral study, we tested only old rats in a more complex spatial discrimination task and in a temporal discrimination task. This was done to evaluate whether it was possible to detect an ethanol-induced learning and memory deficit in old rats, which were assumed to be more susceptible to chronic ethanol treatment.

EXPERIMENT 1: BLOOD ETHANOL CONCENTRATIONS IN YOUNG RATS FROM FOUR DIFFERENT STRAINS AND OLD LEWIS RATS

It is well documented that strains of rats differ in many aspects of behavior and biochemistry. Therefore, before starting behavioral testing we determined blood ethanol concentrations (BECs) in four different strains of rats. This was done to evaluate whether there were strain differences in BECs and determine what strain of rats would be best for the subsequent behavioral tests. Apart from basal BEC measurement, we also determined BECs per amount of consumed ethanol. In addition, BEC was determined in old Lewis rats.

METHOD

Animals

Male young rats of four different strains were used [n = 10 for each strain: Wistar (WIS), Brown-Norway (BN), Sprague-Dawley (SD), and Lewis (LEW)]. Also, eight 25-month-old male Lewis (n = 8) rats were used. At the age of 4 weeks, the young rats were housed individually in standard Makrolon cages on sawdust bedding in an air-conditioned room (20°C). Old rats were housed in the same room but housed in groups of four animals in Makrolon cages. They had free access to standard dry food (RMH-TM, Hope Farms) and tapwater and were kept under a reverse light-dark cycle (light on between 2100 and 0900 h).

Ethanol Treatment

For young rats, ethanol treatment started when rats were 5 weeks old. In the first week of treatment, rats were given a 5% (v/v) ethanol solution as the only source of liquid. The ethanol solutions were prepared weekly in 250-ml water bottles with standard drinking spouts. In weeks 6–8, the ethanol concentration was increased weekly by steps of 5% to reach a concentration of 20% in week 8. If rats did not drink the solution, they were given some tapwater in another cage, after which they were returned to their home cage. After 2 weeks, all animals drank the ethanol solution without complications. The concentration of 20% was chosen according to other studies on the effects of chronic ethanol consumption (1,15). For old rats, the concentration of ethanol was also increased gradually and started when rats were 25 months old. Dry food was freely available.

Blood Ethanol Concentration

After young rats had been on the 20% ethanol diet for 8 weeks, BEC was determined. BEC of old rats was determined after rats had been on a 20% ethanol diet for 4 weeks. Blood samples of young and old rats were taken 1 h after the onset of light.

Because of the great variance in BECs [see also (15)], we also determined BECs per amount of consumed ethanol in young rats. To control for duration of ethanol consumption, the bottles with the ethanol solution were removed from the cages for 5 h at the end of the dark period (active period). One hour before blood samples were taken, the bottles were replaced. The amount of ethanol consumed in that hour was measured.

Blood samples were taken by orbita puncture with a heparin-coated capillary tube; 0.5 ml blood was collected in vials containing 10 μl heparin solution. The blood samples were frozen and stored at −20°C. BEC was determined in duplicate by high-resolution gas chromatography.

RESULTS AND CONCLUSIONS

There was no difference between basal BECs (mg/ml) of young rats of different strains [mean (SEM); WIS, 0.27 (0.06); BN, 0.50 (0.12); SD, 0.39 (0.15); LEW, 0.44 (0.10); $F(3, 30) = 0.81$, n.s.]. Basal BECs of old rats was similar to that of young rats of the four strains [old LEW, 0.48 (0.18); $F(4, 37) = 0.55$, n.s.].

In the hour before blood samples were taken for measurement of BECs per amount of ethanol consumed, young rats of the four strains consumed the same amount of ethanol, $F(3, 39) < 1.0$, n.s., which was approximately 2 g/kg. An analysis of covariance (ANCOVA) with ethanol consumption as covariate showed that there was a strain effect for BECs per amount of ethanol consumed, $F(3, 39) = 4.06$, $p < 0.05$. A Duncan–Waller posthoc analysis revealed that LEW rats had a higher BEC per amount of ethanol consumed than SD or WIS rats. The BEC per amount of ethanol consumed in BN rats was higher than in WIS rats but was the same as for LEW and SD rats. These results indicated that the mean BEC per amount of ethanol consumed was highest in LEW rats.

EXPERIMENT 2: PERFORMANCE OF YOUNG AND OLD RATS IN A MORRIS SPATIAL NAVIGATION TASK AFTER CHRONIC ETHANOL CONSUMPTION

The Morris spatial navigation task has been widely used to evaluate age-related impairments in spatial discrimination learning (23). Therefore, with respect to the hypothesis that chronic ethanol consumption affects the process of aging, adult and old ethanol-treated rats should show a learning deficit when compared with adult and old controls, respectively.

METHOD

Animals

Three- and 18-month-old male Lewis rats were used. Animals were housed in groups of four to five animals in standard Makrolon cages on sawdust bedding in an air-conditioned room (20°C). They had free access to food and tapwater and were kept under a reverse light-dark cycle (light on between 2100 and 0900 h).

Ethanol Treatment

Young and old rats were randomly assigned to either a control group (young control, n = 11; old control, n = 10) or an ethanol-treated group (young ethanol, n = 12; old ethanol, n = 10). The control groups had free access to both tapwater and food. The ethanol-treated group was given a 20%

(v/v) ethanol solution as the only source of liquid. In the first week, a few animals in the ethanol groups did not drink the solution. These rats were given some tapwater in another cage, after which they were returned to their home cage. After 2 weeks, all animals drank the ethanol solution without complications.

Treatment was ended in two steps [1 week a 10% (v/v) ethanol solution] after 6 months. After the end of treatment, rats were housed individually in standard Makrolon cages and had access to food and tapwater ad lib. During the treatment, two old control and three old ethanol-treated rats died for unknown reasons. Behavioral experiments were started 6 weeks after cessation of the ethanol diet.

There was an ethanol-induced reduction in body weight (g) in both young and old rats [mean (SEM); young control, 538 (25.6); young ethanol, 420 (17.2); old control, 663 (19.1); old ethanol, 462 (6.4); ethanol effect, $F(1, 34) = 60.64$, $p < 0.01$].

Behavioral Procedures

Rats were tested on the standard Morris task (19) in a black water tank with a diameter of 1.22 m. Briefly, rats were started from four different, randomly chosen, start positions and trained to find an invisible platform (diameter 11 cm) that was at a fixed position in the water tank, 1 cm below the surface of the water (temperature of water: 22–23°C). A trial lasted until a rat had found the platform or 60 s had elapsed. If a rat did not find the platform within 60 s, it was placed on the platform for a few seconds and removed from the water tank. Rats were trained with massed trials (day 1, four trials; days 2–4, eight trials) to a total of 28 trials.

Statistical Analysis

The escape latencies of a block of four successive trials were averaged. Data for the first three trial blocks, corresponding with the first phase of acquisition, were analyzed with a two-factorial (age and treatment) analysis of variance (ANOVA). The learning curves were analyzed with a three-factorial (age, treatment, and trial blocks) ANOVA with repeated measures over trial blocks. In addition, differences in the shapes of the learning curves were analyzed by a two-factorial design (age and treatment) ANOVA on trend coefficients calculated over the first three trial blocks (33).

To evaluate the difference in asymptotic performance, a two-factorial design (age and treatment) was performed on the last two trial blocks separately.

RESULTS AND CONCLUSION

Adult rats had shorter escape latencies than old rats (see Fig. 1) on the first three trial blocks [age effect, $F(1, 34) > 7.89$, $p < 0.01$]. Ethanol treatment, however, did not affect the performance of adult or old rats [treatment effect and age × treatment interaction effect, $F(1, 34) < 1.0$, n.s.]. Adult and old rats improved their performance over the first three trial blocks [block effect, $F(2, 68) = 47.24$, $p < 0.01$], an improvement characterized by a linear trend that explained 96% of the variation in the decrease in escape latencies. Again, ethanol treatment did not affect the improvement in water escape learning in adult and old rats [block × treatment interaction effect and block × age × treatment interaction effect, $F(1, 34) < 1.0$, n.s.]. Older rats were slower to learn than adult rats [age effect on linear trend, $F(1, 34) = 10.14$, $p < 0.01$], but the rate of learning was not affected by the ethanol treatment, $F(1, 34) < 1.0$, n.s. At the end of training (last two trial blocks), old rats still showed a poor performance in locating the position of the platform, $F(1, 34) > 43.03$, $p < 0.01$. All old control and ethanol-treated rats sometimes did reach the platform within 5 s, irrespective of the start position. This finding argues against the explanation that the differences in escape latency is caused by an age-related difference in swimming speed. Ethanol treatment did not affect the performance of adult and old rats at the end of training, that is, asymptotic level [treatment effect and age × treatment interaction effect, $F(1, 34) < 1.0$, n.s.].

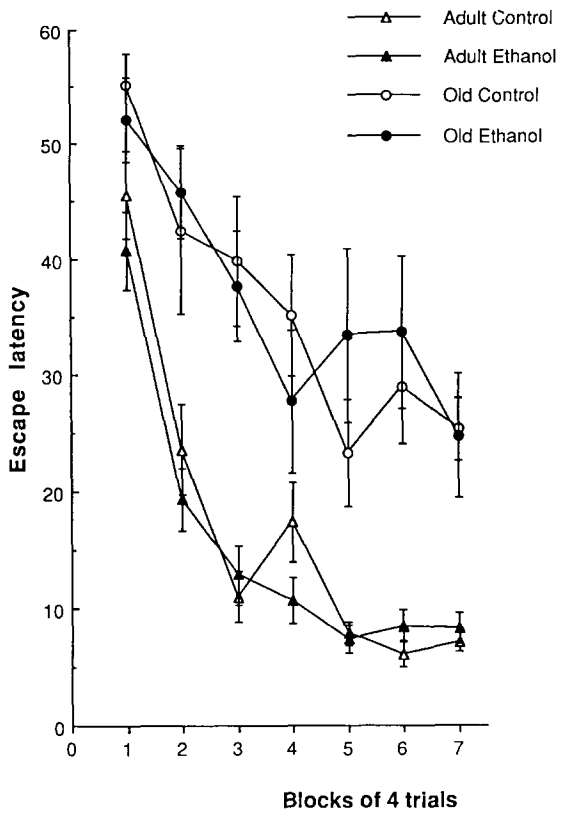

FIG. 1. Escape latencies (in seconds ± SEM) of adult and old control and ethanol-treated Lewis rats during acquisition of the Morris water escape task.

These results clearly show that old rats had an impaired spatial discrimination performance but that chronic ethanol treatment did not affect the learning performance of Lewis rats in the Morris spatial navigation task. Also, at the end of training, when rats' performance had reached asymptotic levels, ethanol treatment did not affect the performance of young and old rats. Although we expected that old rats would be more susceptible to chronic ethanol treatment than young rats, chronic ethanol treatment had no effect on the performance of old rats in the Morris task. One possible explanation for the lack of effect of ethanol treatment in old rats is that there were floor effects because of the low level of performance of old control rats (about 30 s). Another possible explanation for these results could be related to the significant weight loss of ethanol-treated rats in this experiment. Therefore, the results of this study are not conclusive with respect to the effects of chronic ethanol consumption and cognitive impairments.

EXPERIMENT 3: PERFORMANCE OF OLD RATS IN SPATIAL DISCRIMINATION LEARNING IN A CONE-FIELD TASK AFTER CHRONIC ETHANOL CONSUMPTION

The cone-field task has been developed to evaluate age-related differences in rats and allows the simultaneous assessment of working memory (WM) and reference memory (RM) (30). WM holds information that is relevant only within a specific trial (e.g., a list of visits to places within a trial) whereas RM holds trial-independent information [e.g., the location(s) of food]. This task is more complex than the Morris spatial navigation task. Acquisition of the cone-field task takes about 50–60 trials, which is not errorless (7 visits to collect the 4 food rewards), whereas acquisition of the Morris spatial navigation task takes about 16–20 trials in Lewis rats. Further, in the cone-field a rat has to obtain a food reward at four different locations, which increases the chance of making errors. Old Lewis rats do reach fairly high levels of performance for RM and WM (6,29). We therefore reasoned that the cone-field task would detect spatial discrimination learning impairments in old Lewis rats after chronic ethanol consumption if such ethanol effects were present.

METHOD

Animals

Twenty-one 18-month-old Lewis rats (from the same cohort as rats used in Experiment 2) were used. Housing conditions were identical to those of the previous experiment.

Ethanol Treatment

The two groups were randomly divided into a control ($n = 11$) and an ethanol group ($n = 10$). The ethanol treatment was identical to that of the previous experiment and lasted 6 months. During the period of ethanol treatment, two control and three ethanol-treated rats died for unknown reasons. Behavioral experiments were started 3 weeks after the end of the ethanol diet.

At the start of training, the body weight (g) of ethanol-treated rats tended to be lower than that of control rats [mean (SEM); control, 643 (14.1); ethanol, 588 (24.7); $t(14) = 2.02$, $0.10 > p > 0.05$].

Behavioral Procedures

Rats were deprived of food and body weights were gradually reduced to 77.5% of the free-feeding weight.

Apparatus. The cone-field task has been described in detail elsewhere (29,30). In short, the cone-field is an open field with 16 cones and four starting boxes connected to it. Food can be obtained from the tops of the cones. A visit to a cone is operationalized as a learning response against the top of the cone. Visits to cones were scored automatically and data were collected on an MS-DOS-compatible microcomputer.

Behavioral testing. Three weeks after the treatment ended, rats were familiarized with the cone-field in four adaptation sessions (10 min/day). In adaptation sessions, all cones were baited with one 45-mg pellet (Bioserve). After the four adaptation sessions, acquisition of the cone-field task started (rats were also subjected to Skinner box training on the same day; see Experiment 4). During acquisition, a fixed subset of four cones was baited. Rats were trained with massed trials (days 1–5, two trials; days 6 and 7, four trials; days 8–14, six trials) to a total of 60 trials. The starting position within a series of daily trials was determined by random permutations of the numbers 1–4. A trial was started by placing the rat in the startbox. The sliding door was then opened. As soon as the rat had entered the cone-field, the sliding door was closed. A trial was terminated when the rat had found and consumed all four food pellets or when 10 min had elapsed, whichever occurred first. The animal was put back into its home cage between trials. When the cone-field had been cleaned with a damp sponge and the four cones had been rebaited, the next trial was started.

Statistical Analyses

Two measures of the training sessions were analyzed: WM and RM [see (30)]. WM represents the percentage of all visits to the baited set of cones that had been rewarded and was defined as the ratio (number of rewarded visits)/(number of visits to the baited set of cones). RM represents the number of visits to the baited set of cones as a percentage of the total number of visits to all cones and was defined as the ratio (number of visits to the baited set of cones)/(number of visits to all cones).

Means of blocks of 10 trials each were calculated. WM and RM performance were analyzed in a two-factorial (treatment and trial block) ANOVA with repeated measures over trial blocks. In addition, differences in the shapes of the learning curves were analyzed with a one-factorial ANOVA on orthogonal trend components calculated over the successive trial blocks (33).

RESULTS AND CONCLUSIONS

Working Memory

There was no difference in overall WM performance (see Fig. 2A) between control and ethanol-treated rats [general mean, $F(1, 14) < 1.0$, n.s.]. Also the rate of learning, which was for 88% characterized by a linear trend, was not different for control and ethanol-treated rats, $F(5, 70) < 1.0$, n.s.

Reference Memory

There was also no difference in RM performance between control and ethanol-treated rats (see Fig. 2B) when averaged over all trial blocks [general mean, $F(1, 14) < 1.0$, n.s. The rate of learning, for 90% characterized by a linear trend, was the same for control and ethanol-treated rats, $F(5, 70) < 1.0$, n.s.

Although the cone-field task is more complex than the Morris spatial navigation task, both ethanol-treated and control rats reached a fairly high level of WM and RM performance. Thus, chronic ethanol treatment did not affect the two measures of spatial memory in the cone-field task. More detailed analyses of behavior, as measured by interchoice interval, trial duration, and choice correspondence of reinforced visits [see (29,30)], also did not reveal any effects of the ethanol treatment.

EXPERIMENT 4: PERFORMANCE OF OLD RATS ON A DISCRETE TRIAL FIXED-INTERVAL 60-S SCHEDULE AFTER CHRONIC ETHANOL CONSUMPTION

The previous experiments showed that chronic ethanol treatment did not affect spatial discrimination learning in Lewis rats. Apparently, chronic ethanol treatment in Lewis

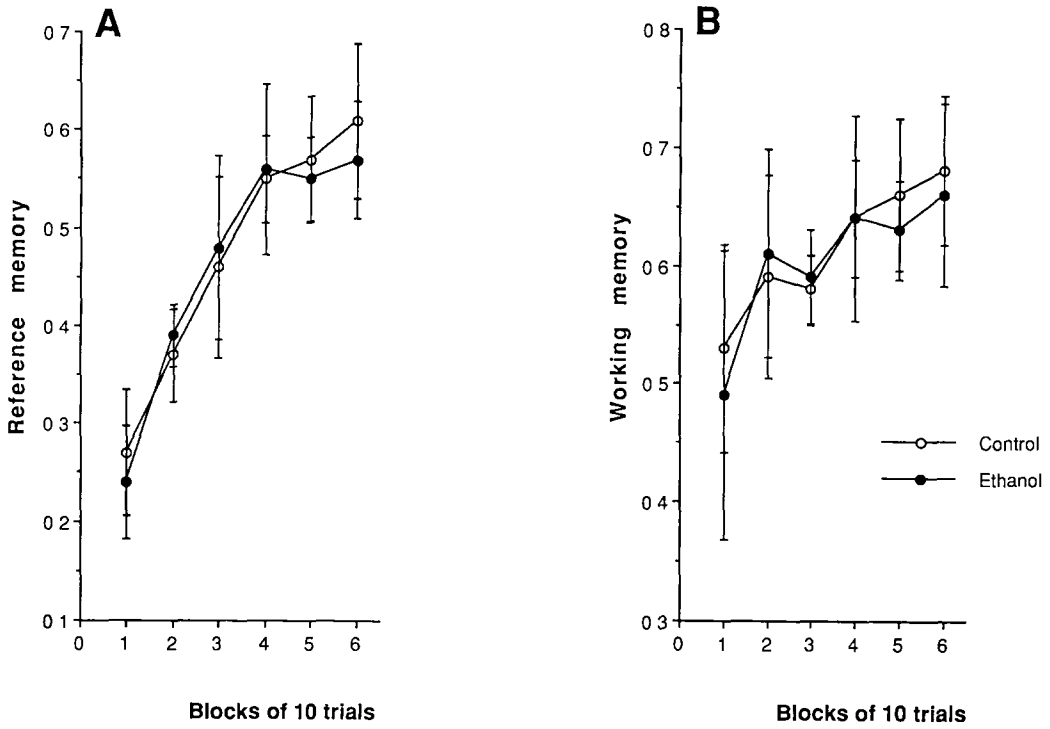

FIG. 2. Acquisition of the spatial cone-field task by old control and ethanol-treated Lewis rats. (A). Working memory performance (number of rewarded visits)/(number of visits to the baited set of cones) (±SEM). (B). Reference memory performance (number of visits to the baited set of cones)/(number of visits to all cones) (±SEM).

rats does not affect spatial learning and memory processes. However, chronic ethanol consumption could affect other learning and memory processes. Because it has been reported that temporal discrimination learning is impaired after prolonged ethanol consumption (11,32), it could be argued that chronic ethanol consumption affects temporal discrimination learning processes in Lewis rats. Therefore, we subjected old Lewis rats to a discrete trial fixed interval of 60 s (DFI 60) schedule [see (24)] to evaluate whether temporal discrimination performance is affected after chronic ethanol consumption. In a DFI 60 schedule, an interval is started by a stimulus or a response (in this experiment, the rat had to press the lever to start the interval) and a reinforcement is contingent upon the first lever press after 60 s. Thus, when a rat presses the lever once and presses the lever once again after 60 s its performance is optimal.

METHOD

Animals and Ethanol Treatment

The same animals were used as in Experiment 3. Magazine training in the Skinner box started after the first week of cone-field training, that is, 4 weeks after treatment ended. Thus, on one day rats were trained simultaneously in the cone-field task and the DFI 60 schedule. Intertesting time was about 2 h (the cone-field task first and then the DFI 60 schedule).

Behavioral Procedures

Apparatus. Animals were trained in four identical Skinner boxes (40 × 30 × 33 cm). Holding cages, in which rats were placed, were made of transparent poly(vinyl chloride). The left- and right-side walls of the holding cage were sliding doors. This cage could be inserted into the conditioning chamber. The ceiling of the Skinner boxes contained a light that illuminated the conditioning chamber during the experiments. The left- and right-side walls served as control panels and included manipulanda and discriminanda. A recess (5 × 5 cm) was build into the left-side panel 2.5 cm above the grid floor and contained a food tray, into which a pellet dispenser delivered 45-mg food pellets (Bioserve). Two retractable stainless steel levers (4 cm wide) projected 2 cm into the Skinner box. The levers were located 6 cm from both sides of the recess, 12 cm above the grid floor. The conditioning chambers were enclosed in sound-attenuating housing. An exhaust fan produced background noise. An MS-DOS microcomputer controlled the experimental equipment and collected the data.

At the start of a session, a rat was placed in a holding cage that was inserted into the conditioning chamber. After the sliding doors had been removed, the rat had free access to the manipulanda and a session was started. At the end of the training/test sessions, the sliding doors were again inserted and the rat was withdrawn from the apparatus while remaining in the holding cage.

Rats received two 30-min magazine training sessions before they were trained on a continuous-reinforcement schedule to press the left lever on the left wall.

Food motivation. After rats had acquired the lever press response, they were subjected to a progressive-ratio schedule (PR 5, where 5 refers to the incremental steps of lever presses to obtain a food reward) to evaluate whether there were differences in food motivation between control and ethanol-treated

rats. A PR 5 session was ended when the time between two lever presses exceeded 10 min.

Discrete trial fixed-interval 60 s. Temporal discrimination training started 1 day after the PR 5 session. Rats were subjected to 18 daily 1-h DFI 60 sessions.

Statistical Analysis

Performance in the PR 5 session was analyzed by comparing the total number of lever presses of control and ethanol-treated rats by using *t*-statistics.

The interval of 60 s of the DFI 60 schedule was divided into six classes of 10 s. The mean number of lever presses in class 6 of blocks of two sessions was analyzed by using *t*-statistics. Further, the percentage of lever presses per interval of 10 s was analyzed, with the number of lever presses in class 6 being taken as 100%. Means of blocks of two sessions were calculated and group effects analyzed in a three-factorial (treatment, class, and block) ANOVA with repeated measures over the factors class and block. In addition, treatment effects were evaluated by analyzing the percentage of lever presses in the second interval class in a two-factorial (treatment and block) ANOVA with repeated measures over blocks. Differences in the percentage of lever presses in class 2 in the course of training were analyzed with a one-factorial ANOVA on orthogonal trend components calculated over the successive trial blocks (33).

RESULTS AND CONCLUSIONS

Food Motivation

Control and ethanol-treated rats had comparable food motivation, as indicated by an equal number of lever presses during the PR 5 session [$t(13) = 0.57$, n.s.; data not shown].

Discrete Trial Fixed-interval 60 s

Control and ethanol-treated rats did not differ in the number of lever presses in the sixth class during the nine blocks of two training sessions ($t < 0.86$, n.s.). Although rats learned the temporal demands of the task (block and class effect, and block × class interaction effect, $F > 2.27$, $p < 0.05$), ANOVA revealed that performance during DFI 60 training was not affected by ethanol treatment (treatment × class, treatment × block, and treatment × class × block interaction effects, $F < 1.0$, n.s.; see Figs. 3A and 3B). Apparently, chronic ethanol consumption did not affect timing behavior in old Lewis rats. Analysis of the second interval class showed that the percentage of lever presses in class 2 was increased in ethanol-treated rats [general mean, $F(1, 13) = 11.31$, $p < 0.01$; see Fig. 3C]. Analysis of orthogonal trend components revealed that ethanol-treated rats showed a transient impairment in inhibiting their responses after they had pressed the lever to start the interval [quadratic trend component × treatment effect, $F(1, 13) = 6.02$, $p < 0.05$]. This was interpreted as a transient tendency of ethanol-treated rats to perseverate.

GENERAL DISCUSSION

The major finding of the present experiments was that chronic ethanol consumption did not impair spatial discrimination performance in the cone-field and timing behavior in old LEW rats. The only effect was a transient tendency in old rats to perseverate in the DFI 60 task. These findings are at variance with the generally reported learning and memory impairments after chronic ethanol consumption [(1,7,12, 15,26,31,32); but, see (27)]. We therefore tried to explain our results in relation to studies that evaluated the effects of chronic ethanol consumption on learning and memory performance.

In the first experiment, it was found that there was no difference in BECs in four different rat strains and that BECs of old LEW rats were similar to those of young rats. In addition, we evaluated whether there was a difference in BECs per amount of ethanol consumed because there was a great variance in BECs [see also (15)]. It is likely that this variance in BECs is due to different amounts of ethanol consumed just before blood samples are taken. It was found that young LEW rats had the highest BECs per amount of ethanol consumed.

It could be argued that old LEW rats have a different ethanol uptake than young LEW rats as a result of age-related changes in drug metabolism (28). However, it was found that BEC was similar in young and old LEW rats. In addition, old rats are generally found to be more sensitive to acute, short-term, and chronic ethanol treatment (21,22,35,36). Therefore, it is unlikely that the findings of our study can be explained by differences in metabolism in young adult (used in most studies) and old LEW rats.

The lack of any learning and memory impairments after chronic ethanol consumption in old LEW rats is open to other explanations. It could be argued that chronic ethanol treatment does not cause neuronal damage in LEW rats or does not affect structures that are critically involved in learning and memory processes (e.g., the hippocampus). Chronic ethanol treatment in LEW rats, for example, could have an effect on structures in the frontal cortex, which could explain the transient tendency of old ethanol-treated rats to perseverate in the DFI 60 task [see (34)]. Besides, there are studies in humans showing a neuronal loss in the frontal cortex in alcoholic patients (14). However, although a frontal lobe dysfunction is not found in alcoholics such a dysfunction can be found in Korsakoff patients (16), which might explain the tendency to perseverate in Korsakoff patients (18).

Another possibility could be that the ethanol-induced neuronal damage could have been reversed within the period between the end of treatment and the start of behavioral testing (i.e., 3 weeks in Experiments 3 and 4). However, it has been reported that the effects of chronic ethanol consumption can still be found 4.5 months (31) or even 6 months (2) after the cessation of ethanol administration, which makes this explanation less plausible.

The method of ethanol administration (liquid diet vs. ethanol in tapwater) has been reported to lead to discrepancies in the effects on β-endorphin regulation [see (9)]. In most studies that report learning and memory impairments after ethanol treatment, liquid diets were given to control for nutritional and caloric factors between the experimental groups. Arendt and coworkers (1) administered ethanol in tapwater and were able to demonstrate learning and memory impairments after chronic ethanol treatment. This indicates that both diets can lead to learning and memory impairments. However, there have been no studies that systematically evaluate the possible effects of different methods of ethanol administration on learning and memory performance.

It could be argued that the use of a 20% ethanol solution does not result in a BEC that affects learning performance. However, we performed an experiment in which we used a 15% ethanol solution and found an ethanol-induced performance deficit in WIS rats in a two-way active avoidance task (17). In the present study, it was found that the BEC per

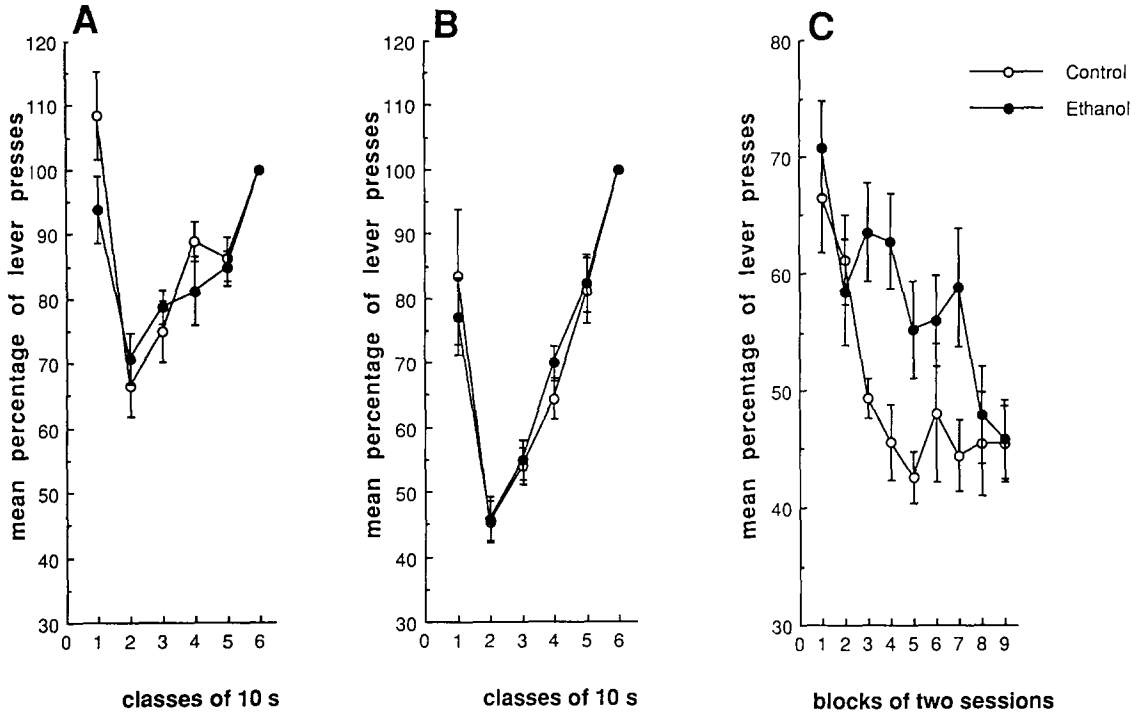

FIG. 3. Performance of old control and ethanol-treated Lewis rats on a discrete trial fixed interval of 60-s schedule in a Skinner box. (A). Mean percentage of lever presses (± SEM) in the six interval classes of 10 s during the first block of two sessions of training. (B). Mean percentage of lever presses (± SEM) in the six interval classes of 10 s during the last block of two sessions of training. (C). Mean percentage of lever presses (± SEM) in the second class of 10 s during training.

amount of ethanol consumed of WIS rats was lower than that of LEW rats. Another study reported ethanol-induced impairments in the acquisition of a DRL schedule of reinforcement in hooded rats given a 10% ethanol solution for 163 days (11). Further, Hodges et al. (15) reported learning impairments in a group of rats (low-alcohol group) of which the BECs ranged between 23.5 and 100 mg%. This range in BECs is comparable to the range in BECs found in our study. Low BECs can thus lead to learning and memory impairments in rats. On the other hand, no learning and memory impairments have been reported after treatment with a metrecal diet (27), which leads to a higher ethanol intake than a treatment with an ethanol solution. Apparently, there is a dissociation between the amount of ethanol consumed and learning and memory impairments observed after prolonged ethanol consumption: Low levels of ethanol intake can lead to learning impairments and high levels of ethanol intake do not necessarily lead to learning impairments.

Finally, in the studies that report a decline in learning and memory performance after chronic ethanol consumption (see above) animals were housed individually when they were given the ethanol diet. In the present study, however, rats were housed in groups of five to six animals when they were on the ethanol diet. It has been reported that the behavior of isolated rats differs from group-housed rats with respect to activity, fear response, and learning ability (10). It is therefore possible that the housing conditions could have interfered with the effects of chronic ethanol treatment. However, to what extent rearing conditions interact with the effects of chronic ethanol consumption has, to our knowledge, not been studied.

The present results, however, are in line with the findings of one other study that reported a lack of learning and memory impairments after chronic ethanol consumption (27). It was suggested that the lack of learning and memory impairments in ethanol-treated rats could be due to the strain of rats used (Sprague-Dawley) and/or a low level of complexity of the tasks used. Arendt and coworkers (1) used Sprague-Dawley rats in their studies and were able to reveal learning and memory deficits in radial maze performance after chronic ethanol consumption. These different findings could be attributed to the different diets used in these studies (see above). An ethanol-metrecal diet did not affect learning and memory performance in Sprague-Dawley rats (27) whereas a 20% ethanol solution as the only source of liquid did (1). In the present study, a 20% ethanol solution was given for 6 months but did not cause learning and memory deficits, even in old rats.

As mentioned above, the level of task complexity could be another factor that could explain the different outcomes of the chronic ethanol treatment studies. It is assumed that ethanol-induced learning and memory impairments can only be found in complex tasks. Although old Lewis rats perform at a fairly high level in the cone-field task (29), the performance is not errorless for WM and RM during the end of training. It is, therefore, not likely that the lack of learning and memory impairments in the present study could be attributed to a low level of task complexity. Nor could the absence of learning impairments be attributed to floor effects because the performance of the control rats was fairly high.

Apparently, chronic ethanol consumption does not necessarily lead to learning and memory impairments in the rat. Differences in results may be attributed to several variables, that is, strain of rats, task complexity, method of ethanol administration, and housing conditions, for which little attention has been given in ethanol research thus far. Careful stud-

ies should evaluate the possible contributions of the variables mentioned above to the effects of chronic ethanol treatment on cognitive functions. In addition, such studies should also include neuroanatomic/biochemical verification of structures that are potential targets of ethanol (14). Finally, we found that the level of anxiety is reduced after chronic ethanol treatment in adult LEW rats (5). This indicates that attention should also be given to the effects of chronic ethanol consumption on nonmnemonic processes that could affect learning and memory performance.

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