Impairment of Acquisition of a DRL Schedule Following Prolonged Ethanol Consumption[1]

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DENOBLE, V. J. AND H. BEGLEITER. *Impairment of acquisition of a DRL schedule following prolonged ethanol consumption.* PHARMAC. BIOCHEM. BEHAV. 10(3) 393–396, 1979.—Two groups of male hooded rats (N=9) were given either ethanol or sucrose solutions as their only source of fluid for six months. Thirty days after the ethanol treatment, the rats were reduced to 85% of their free-feeding weights and allowed to obtain 45 mg food pellets on an FR 1 schedule for five consecutive days. Subsequently, the rats were tested on four DRL schedules (6, 12, 18, 24 sec). There was no difference between the two groups on FR 1 or DRL 6 sec schedules; however, when the DRL interval was lengthened to 12, 18, and 24 sec, the ethanol group required more sessions than the sucrose group to reach criterion performance. After increases in the DRL interval, the modal interresponse time of the ethanol group shifted more slowly than that of the sucrose group.

Ethanol   DRL   Aging   Rats

THE POTENTIALLY deleterious effects of prolonged ethanol consumption on behavior have been recognized for centuries. Brain damage and/or learning impairments have been demonstrated in numerous alcoholic patients [2, 13, 15, 16]. Until recently it has been generally accepted that this brain damage and resulting abnormalities in learning and memory were a direct result of malnutrition, especially thiamine deficiency [17]. The role of direct ethanol toxicity in the production of human brain damage is difficult to determine because conditions collateral to alcoholism may themselves contribute to central nervous system damage. Liver malfunction, head trauma, as well as dietary insufficiencies are among those conditions which frequently accompany alcoholism and can lead to brain and behavioral dysfunctions. For these reasons it is desirable to use controlled animal populations to study the potential ethanol-induced behavioral dysfunctions.

In a number of studies the effects of chronic ethanol consumption on learning have been investigated in laboratory animals which were maintained on a diet adequate in all essential nutrients [1, 5, 18, 19, 20]. Generally, the results indicate that prolonged ethanol consumption concomitant with adequate nutrition leads to a deterioration of behaviors involved in the acquisition of new material. An attenuation of learning following prolonged ethanol intake (6–12 months) has been shown to occur with mice and rats on shock avoidance tasks, on acquisition of behavior reinforced according to a differential reinforcement of low rates (DRL) schedule, and maze performance [1, 5, 8, 19]. Reinforcement according to a DRL schedule is contingent upon two responses being separated by some minimum specified time period. The first response after the time period has elapsed produces a food pellet; responses prior to the completion of the time period are not reinforced and start the interval over.

Walker and Freund [19] showed that prolonged ethanol consumption impairs acquisition of DRL performance. However, the initial difference between the groups in the number of responses per session gradually diminished with repeated training sessions. During the last three sessions of the experiment, there were no significant differences between the groups in the number of responses in each session, and the mean number of reinforcers obtained per session for the ethanol group showed a positive accelerating slope [19]. These data suggest that with extended training the DRL performance of animals previously maintained on ethanol would be similar to that of control animals. The present study was conducted to test this notion and to further examine the DRL performance of rats with a history of chronic ethanol intake.

METHOD

Animals

Eighteen male hooded rats weighing between 210–261 g were used. The animals were weighed daily for two weeks and then divided into two equal groups matched for weight. All animals were housed individually in a temperature controlled room (23–25°C) with a 12 hr light-dark cycle.

Apparatus

Two identical Lehigh Valley operant conditioning cham-

bers were used. The chambers were enclosed in sound attenuated boxes and programmed with electromechanical and solid state logic modules. Each chamber contained a white stimulus light positioned above a response lever. To the left of the response lever a food receptacle was mounted 2.0 cm above the grid floor.

Procedure

Drug history. Following group assignments, nine rats were given as their only source of liquid an increasing progression of ethanol solutions as follows: 3.0% (v/v) solution for the first five days, 5.0% (v/v) for next five days, 8.0% (v/v) for the next seven days, and 10.0% (v/v) for the remaining 163 days. The solutions were mixed from 95% ethanol (5.25 calories/ml), and were available to the animals in two 100 ml bottles with stainless steel drinking spouts attached by means of rubber stoppers. The bottles were cleaned and refilled every other day. Food (Purina Rat Chow) was freely available throughout the 180 days, and the animals were weighed three times each week. Following 120 days of continuous ethanol intake, six withdrawal probes, one every ten days, were conducted to determine if the rats were physically dependent. Probes consisted of enforced ethanol abstinence for 12 consecutive hours, during which time the animals were observed for signs of withdrawal (activity, muscle tone, muscle rigidity, muscle fasciculations, tremors, and hyperactivity). The control group received as their only source of liquid an isocaloric solution of sucrose (87% w/v=3.5 calories/ml) according to the same regimen as the ethanol group. All animals were given 0.5 ml of poly-vi-sol multivitamin supplement two times each week throughout the 180 days. The vitamins were administered orally with a 1.0 ml syringe.

Following 180 days of ethanol or sucrose consumption, tap water was substituted in the drinking bottles for thirty days. During this time, the animals were weighed daily. Subsequently, they were reduced to 85% of their free-feeding weights calculated from the mean of the last five days of the 30 day period. Behavioral testing began on the 45th day after ethanol discontinuation.

Behavioral testing. After weight reduction, all animals were shaped to lever press and were allowed to receive 100 45 mg Noyes food pellets on a Fixed-ratio 1 schedule (FR 1) on each of five days. The schedule was then changed to a DRL 6 sec schedule. The DRL interval was lengthened by 6 sec when the rat reached an efficiency ratio of at least 0.50 for three out of five days or remained in the existing DRL contingency for 30 days. The efficiency ratio was calculated by dividing the number of food pellets obtained in each session by the total number of responses emitted in that session. Daily sessions lasted 3 hr and the behavioral testing continued until all rats reached criterion on an interval of 24 sec.

Upon completion of the experiment, all rats were sacrificed and wet liver weights were measured.

RESULTS

Ethanol consumption and weight gain. During the first month, ethanol intake was variable and low; however, the mean g/kg intake for the remaining five months was 6.2 g/kg/day. Casual observation of the rats showed that all rats in the Ethanol group appeared intoxicated on at least one occasion with the major sign being motor incoordination and a lethargic appearance. During the six withdrawal probes,

mild signs of withdrawal (general hyperactivity and occasional tail tremors) were observed in all ethanol consuming rats, whereas sucrose removal did not produce an observable effect. After 180 days of treatment, the general appearance of the ethanol group was indistinguishable from that of the sucrose group. The mean weights ($\pm$SEM) of the sucrose and ethanol groups before treatment were 238.1 ($\pm$6.1) and 236.0 ($\pm$5.2), and after treatment, they were 429.3 ($\pm$3.9) and 382.5 ($\pm$4.2) respectively. The sucrose group had a mean liver weight of 3.01 g/100 g body weight, while the ethanol group had a mean liver weight of 2.86 g/100 g body weight. A Student's t test between liver weights of the two groups showed no significant difference ($t=1.50$; $p>0.05$).

Fixed-ratio and DRL performance. At FR 1, the mean responses per second for the sucrose group was 0.25 while the mean for the ethanol group was 0.28, suggesting that the two groups were performing equally well. Figure 1 presents the mean number of days to criterion at the four DRL intervals for both experimental and control rats. At DRL 6-sec, the difference between the sucrose and ethanol consuming groups in the number of days to criterion was small (Mean=7.8 for sucrose and 8.9 for the ethanol group). However, when the interval was switched from 6 to 12 to 18 to 24 sec, the ethanol animals required significantly more time to reach criterion at each stage than did sucrose rats. None of the sucrose rats required 30 days of training to reach criterion and only three rats in the ethanol group reached the 30 day limit, all at DRL 12 sec. A two-way analysis of variance with repeated measures comparing sucrose versus ethanol animals for days to criterion at the four DRL intervals showed a significant difference between the groups, $F(3,48)=15.5$, $p<0.01$. The interaction between the group and DRL interval was not significant, $F(3,48)=2.1$, $p>0.05$.

The first two columns in Fig. 2 present the frequency distributions of interresponse times averaged for all animals within each group for the last five days prior to increasing the DRL interval. When the DRL interval was increased, the second mode of these bi-modal distributions occurred at the required delay interval for both groups at the four DRL time intervals. When performance was stable, there was found to be no major difference in the frequency distributions of the control and ethanol animals. In contrast to stable performance, a difference is found between ethanol and sucrose rats if a comparison is made after both groups have had equal training. The third column in Fig. 2 shows the frequency distributions for the ethanol rats that were obtained after the same number of sessions that the sucrose group had at each DRL interval. A comparison of sucrose group performance (column 1) with ethanol group performance which occurred after the same number of sessions as the sucrose group (column 3) shows that the ethanol group emitted a larger proportion of responses in the intervals immediately prior to the required interval. A Student's t test between the number of premature responses of the control and ethanol group (column 1 and 3, Fig. 2) showed significant differences at DRL 12, 18, and 24 ($t=4.21$, $p<0.01$, $t=3.71$, $p<0.01$, $t=4.93$, $p<0.01$). This pattern of emitting too many responses in the previously reinforced interval may be thought of as response perseveration, for the rats had a history of being reinforced at shorter IRT intervals when the DRL length was one value lower. The numbers to the left of each frequency distribution show the percent of responses occurring prior to the reinforcement interval. Inspection of the distributions (column 3) indicates that the ethanol group emitted a larger proportion of responses prior to the required DRL interval.
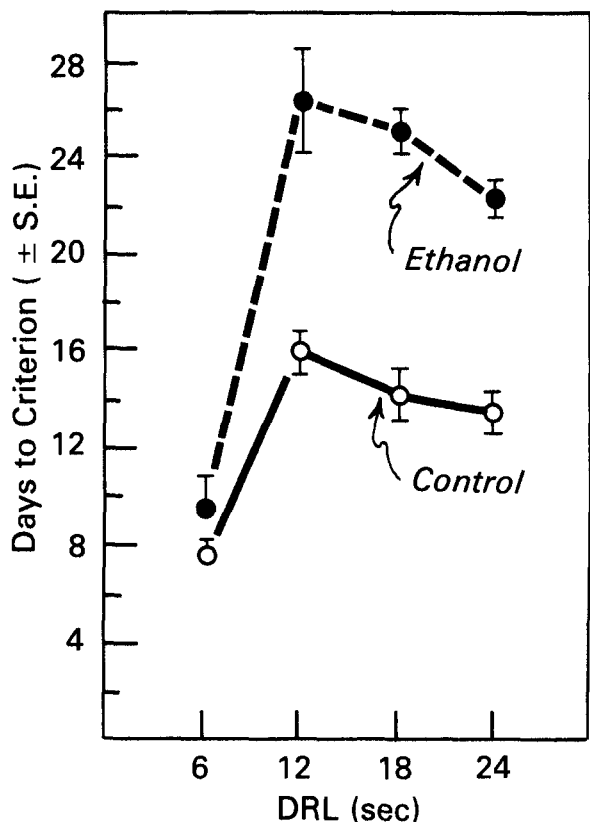
FIG. 1. Days to criterion as a function of the DRL intervals. Each point represents the mean number of sessions to criterion for nine rats. Brackets show the standard error of the mean.
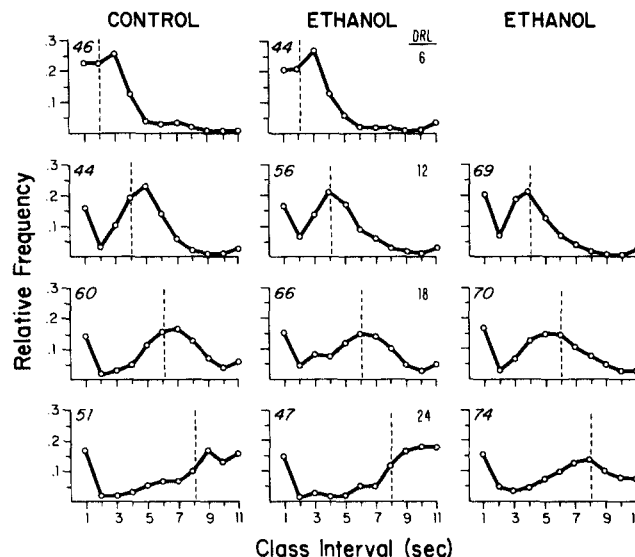

FIG. 2. The relative frequency of interresponse times as a function of the class interval (each class interval equals 3.0 sec). Each point represents the mean (N=45) for the nine rats in each group for the last five sessions prior to increasing the DRL interval. Vertical lines indicate the reinforcement interval and the numbers to the left of each graph show the mean percent of responses that occurred prior to the reinforcement interval. Column one presents the stable performance for sucrose rats for sessions 3–7 (DRL 6), 12–16 (DRL 12), 10–14 (DRL 18), and 9–13 (DRL 24). Column two presents the stable performance for the ethanol rats for sessions 5–9 (DRL 6), 23–27 (DRL 12), 21–25 (DRL 18), and 18–22 (DRL 24). The third column presents the interresponse times of the ethanol rats measured after the same number of sessions as the sucrose rats (3–7 DRL 6, 12–16 DRL 12, 10–14 DRL 18, and 9–13 DRL 24).

DISCUSSION

The results show that there were no differences between the ethanol and sucrose groups in either FR 1 training or performance at the first DRL interval (6 sec). However, when the DRL interval was lengthened, differences in transition behavior occurred. The rats subjected to prolonged ethanol consumption required more time to reach criterion at specific DRL intervals [12, 18, 24] even though the final performance at these intervals was similar to that of control rats. Our findings corroborate previous reports showing that prolonged ethanol consumption results in learning and performance deficits in rodents [5, 8, 11, 19].

Despite the 180 day ethanol treatment and the mild signs of withdrawal observed in the ethanol group, there were no differences in the body weight, liver weight and general physical appearance of the two groups. Stable performance of the ethanol consuming rats was just as efficient as the sucrose group. Thus, the ethanol-induced performance deficits which occur on DRL schedules can be ameliorated with extended training. Further, since the only difference in performance between the ethanol and sucrose groups was in the acquisition of lengthened DRL performance, the behavioral deficit appears to be specific as opposed to a general toxic effect that would produce an impaired final level of performance.

It should be noted that a pair-fed control group was not included, and differences in the nutritional status of the rats cannot be ruled out. In view of the similarity of body and liver weights and since the operant task began 45 days following the end of drug treatment, it is unlikely that nutritional states or differences in growth rate were responsible for the difference in DRL acquisition.

Another possible explanation for the differences in performance is that the ethanol group had six withdrawal probes. Freund [7] has shown that repeated withdrawal from ethanol results in impairment of acquisition of shuttle box avoidance, whereas continuous ethanol consumption for the same length of time (5 weeks) did not impair learning ability. Comparisons between the two studies are difficult due to the extended period of alcohol exposure in the present study. However, since the DRL performance was obtained more than 45 days after the last withdrawal probe, it is unlikely that these probes affected behavior 45 days later, unless these withdrawal probes produced brain damage.

The rate of acquisition of lengthened DRL performance in ethanol consuming rats following discontinuation of an aqueous ethanol solution was slower than that of sucrose consuming rats. Similar response patterns have been shown when the learning performance of mature-young (8 months old) and aged (27 months old) rats were compared [9,10]. In these studies the aged rats exhibited behavioral rigidity (the tendency to perseverate in making the same errors) when

compared to young rats. While in the present study there was no age differences between the groups, the behavioral pattern of the ethanol consuming rats appears similar to that of aged rats. A hypothesis that ethanol is conducive to premature aging has been previously suggested [6], and recently this hypothesis was supported in a study of the pathophysiology of alcoholic cerebral atrophy [4]. However, it should be noted that the deleterious effects of alcoholism appear more reversible than those seen in aged individuals [3]. The findings of the present study combined with findings in other studies suggest that chronic high ethanol intake induces a behavioral deficit that is similar to a deficit seen in aged rats. Perhaps, future studies will reveal a common mechanism for the production of such deficits.

ACKNOWLEDGEMENT

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