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Ethanol-Metrecal Diets: II. Failure to Obtain Impaired Performance on a Series of Appetitively and Aversively Motivated Tasks[1]

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TOMBAUGH, T. N. *Ethanol-Metrecal diets: II. Failure to obtain impaired performance on a series of appetitively and aversively motivated tasks.* PHARMAC. BIOCHEM. BEHAV. 15(3) 463–469, 1981.—Rats were chronically maintained on ethanol-Metrecal diets where either 37%, 41%, 49% or 57% of the total kilocalories were derived from ethanol. In three experiments animals were tested on a series of problems (passive avoidance, shuttle avoidance, simultaneous and successive discrimination, and complex spatial maze learning) 6 to 8 weeks after the diet had been withdrawn. There was no evidence that chronic ethanol regimes impaired either learning or memory processes.

Ethanol Liquid diet Chronic ingestion Learning Conditioning Rats

CLINICAL assessment of alcoholics and reformed alcoholics frequently reveals severe learning and memory deficits which are attributable to previous drinking habits. These deficits include an impaired ability to learn or retain information even when subjects are no longer under the influence of alcohol. It is unclear, however, if these impairments are attributable to ethanol *per se* or if they more appropriately are related to the effects of malnutrition which typically accompanies alcoholism. The ability to directly control nutritional variables, as well as to maintain the quantity duration of alcohol exposure, has led many experimenters to employ animal models. Recently, a number of experimenters have assessed the behavioral effects of chronic ethanol consumption by combining ethanol with nutritionally adequate liquid diets. These studies were primarily concerned with the degree to which long term alcohol ingestion impaired the acquisition of learned behaviors when the experimental problems were introduced after alcohol was withdrawn and physiologically cleared from the system. This approach is contrasted with more traditional acute alcohol studies where interest centered on short term ethanol effects (e.g., tension reduction) that occur while subjects are under the direct influence of ethanol.

Many of the experiments concerned with the effects of prolonged continuous ethanol consumption have employed avoidance learning paradigms. Freund's [5] original report that chronic ingestion of an ethanol-Metrecal diet severely impaired the acquisition of shuttle avoidance performance in mice has been replicated and extended to rats [14]. Since behavioral testing occurred a minimum of two weeks after the ethanol diets were discontinued, the learning deficits were attributed to permanent changes caused by the ethanol rather than to temporary carry-over effects produced by either ethanol ingestion or withdrawal.

However, the data are much less clear when other types of tasks are employed. Bond and DiGuisto [1] reported that rats maintained on ethanol diets made more errors in a Hebb-Williams maze than did isocalorically pair-fed control groups. Two other studies have found that ethanol and control groups did not differ when tested for retrograde amnesia of shuttle avoidance [16] or after punishment of shuttle avoidance [5]. Finally, Walker and Freund [15] reported that while the ethanol-Metrecal diet did not disrupt retraining of a previously acquired bar-press response it did influence the acquisition and maintenance of responding under a DRL 20 sec schedule of reinforcement. Similar impaired DRL performance was reported by DeNoble and Begleiter [3] when ethanol served as the only source of fluids and inter-response

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intervals of 12, 18, and 24 sec were used. However, learning to respond under continuous reinforcement or DRL 6 sec was not altered.

No clear pattern of the conditions under which ethanol produced deficits in learning or performance emerges from these studies. Consequently, the present series of experiments were undertaken to assess whether chronic ethanol ingestion produces any systematic effects on learning or memory processes. In order to accomplish this, different paradigms, involving both appetitive and aversive stimuli as well as requiring response initiation and inhibition were selected—shuttle avoidance, passive avoidance, successive appetitive discrimination, simultaneous appetitive discrimination (including intra-dimensional shift and extinction) and complex spatial maze learning.

EXPERIMENT 1

The primary purpose of the first experiment was to replicate previously reported findings that prolonged ethanol maintenance impaired shuttle-avoidance performance [5, 6, 14]. In addition, Freund's [5] assertion that chronic ethanol ingestion does not alter learned inhibition was tested by using a step-down passive-avoidance task where initial "exploratory" behavior typically is suppressed by the single application of a brief electric shock. Thus, while ethanol is expected to impair acquisition of active avoidance it is not predicted to impair the acquisition of passive avoidance.

METHOD

Subjects

Thirty naive male (90 day old) rats of the Sprague-Dawley strain were purchased from the Holtzman Company. Upon receipt they were individually housed and maintained on ad lib food and water for two weeks. During the course of the experiment seven experimental animals died (two due to apparent seizures, two because of respiratory infections and three from nonspecified causes). Three subjects in the control group were sacrificed because of respiratory infections. An additional four control animals were randomly discarded at the beginning of testing to equate the number of subjects per group.

Apparatus

The shuttle avoidance apparatus was a rectangular plywood box (75.6×15.2×36.8 cm) with a Plexiglas top. The interior was painted flat black. The floor was made of 0.3 cm diameter stainless steel rods spaced 1.3 cm apart. A 7.5 W lamp was located 26.7 cm above the grid floor in the end wall of the chamber. A Mallory Sonalert was inserted in the Plexiglas top midway between the two chambers. The Sonalert was interfaced to the 24 V DC power supply through a 15 KΩ resister. A constant current shock source delivered a 0.6 mA shock through neon bulbs connected in series to the grid floor.

The passive avoidance apparatus consisted of a large wooden box (60.9×60.9×45.7 cm) with a smaller box (20.3×15.2×33.0 cm) positioned in one corner. The smaller box had a wooden floor and a 5 cm opening in the wall which faced the larger box. The larger box had a grid floor connected to a constant current stimulator and scrambler.

Liquid Diet

Half the animals were maintained on an ethanol-Metrecal diet for 170 days while the other half were pair-fed with a sucrose-Metrecal diet where sucrose was isocalorically substituted for ethanol. The ethanol-Metrecal diet combined 12% ethanol (v/v) with chocolate flavored Metrecal so that 49% of the total kilocalories were ethanol-derived kilocalories (EDK). Each ml of diet contained 1.28 kcal. The diet was fortified with vitamins and salts. Precise nutritional composition of metrecal and procedural details are described elsewhere [12]. After the last day of ethanol maintenance, all animals were returned to ad lib food and water for 60 days before introducing the shuttle-avoidance task. This intervening period insured that any behavioral effects would reflect permanent changes associated with chronic ingestion rather than temporary or transitional effects produced by the withdrawal of the ethanol-Metrecal diet.

Shuttle Avoidance

A compound CS (light and tone) was used with a delayed conditioning paradigm where the CS-UCS interval was 7.5 sec. The UCS was 0.6 mA shock. The CS remained on until the animal either performed a successful avoidance or escape response. All animals wers given 30 trials per day for five days. Half of the animals from each group were run during week one and the other half during week two.

Passive Avoidance

Training began one week following the end of shuttle-avoidance training. On day one each animals was placed in the holding chamber with its head facing away from the entrance to the test chamber. As soon as the animal oriented toward the entrance, the guillotine door was lifted and the step-out latency (SOL) clock started. The clock stopped and the guillotine door closed when the animal stepped onto the grid floor with all four feet. At this time a 1.0 mA shock was delivered for five seconds. The same procedure was followed on the succeeding day (retention test) except no shock was delivered. On both days, animals which had a step-out latency greater than 300 sec were removed from the holding chamber and returned to the home cage.

RESULTS AND DISCUSSION

Liquid Diet

Mean body weight (±SEM) at the beginning and end of the experiment were 368±5.50 and 432.7±10.8 grams for the control group and 360±4.2 and 399±19.4 for the ethanol group. An analysis of variance performed over the body weight data, averaged in three day blocks, showed that both groups significantly increased their body weight: $F(1,14)=24.78, p<0.01$. Degrees of freedom are those for the conservative Geiser-Greenhouse F-test [9]. However, the amount of increase was comparable for both groups: $F(1,14)<1$. These data are commensurate with other studies which have used diets containing 35%–40% EDK and isocaloric sucrose controls [14,16]. During the last three days of alcohol maintenance the rats consumed 11.6±0.25 SEM g of ethanol/kg of body weight and 54.0 ±2.1 SEM ml of liquid diets daily.

Shuttle Avoidance

The number of avoidance responses that occurred on

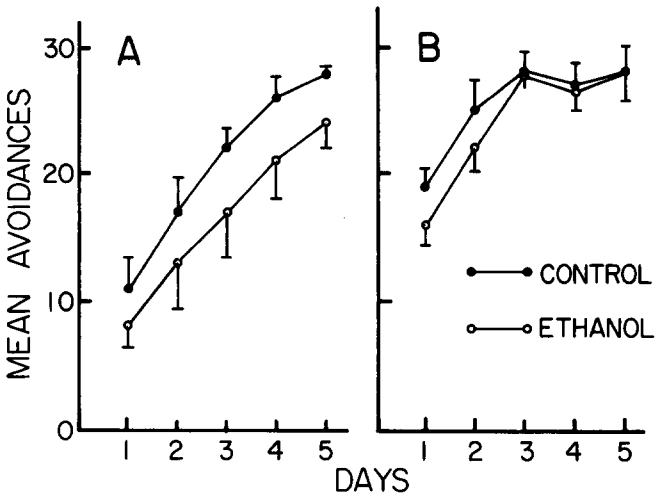

FIG. 1. Mean (±SEM) number of avoidance responses for Experiment 1 (Panel A) and Experiment 3 (Panel B). In Experiment 1 ethanol animals had been chronically maintained on 12% ethanol (49% EDK) for 170 days while in Experiment 3 a 9% ethanol diet (37% EDK) was administered for 215 days. Control animals in both experiments were given continuous access to food and water throughout the duration of the test phase. All animals were tested approximately 60 days after the ethanol diets had been withdrawn.

each of the five training days is shown in Figure 1A. An analysis of variance performed over these data revealed that there was a statistically significant increase in performance over days which occurred at the same rate for each group: ETOH, $F(1,14)=2.28, p>0.05$; Days $F(4,56)=51.77, p<0.01$; ETOH×Days, $F(4,56)<1$. Both groups had an average of 14 trials before making the first avoidance response and had a similar number of inter-trial interval crossings: $F(1,14)<1$. The alcohol group took more trials than did the control group to reach the criterion of nine avoidance responses in ten trials (75 vs 42). While these results are in the same direction as those previously reported [6,15] they failed to reach statistical significance: $F(1,14)=3.14$, $p>0.05$. All animals reached criterion by the end of day five. The failure to obtain differences between ethanol and control subjects supports unpublished data from our laboratory where rats maintained on the same level of diet for 255 days and tested 45 days after withdrawal also failed to produce an impairment of shuttle avoidance performance.

Passive Avoidance

The results from the passive avoidance task supports Freund's [5] contention that ethanol does not influence inhibitory control. That is, separate analyses performed over training and retention days showed that there were no differences on the mean SOL (±SEM) between the ethanol and control groups on either the original (22.7±5.7 and 17.9±5.5) or retention (300.0±0 and 188±54.4) day: $F(1,14)=4.20$, $p>0.05$, respectively.

EXPERIMENT 2

In Experiment 2 the emphasis was shifted from aversively to appetitively motivated behavior. A review of the literature showed that only a single study [1] has investigated chronic ethanol ingestion effects where learning was measured by number of errors in a choice situation—a measure generally accepted as reflecting learning impairments better than response rates or response speed measures where learning and motivational effects are frequently confounded. Bond and DiGuisto [1] reported that ethanol increased the number of errors in a Hebb-Williams maze. The present experiment extended this line of research by using a bar press response with a successive discrimination paradigm.

METHOD

Subjects

Eighteen female Sprague-Dawley rats purchased from BioBreeding Laboratories, Ottawa, Ontario were divided into the alcohol (10) and control (8) groups. Animals in the alcohol group were 66 days old and weighed between 220 and 230 g when the liquid diet was introduced.

Apparatus

Eight experimental chambers were used, each equipped with a 100 cfm Dayton blower for ventilation and white noise. Each chamber (61×71×74 cm) was constructed of 1.91 cm plywood and sound insulated with acoustic ceiling tile. A test cage (25×20×19 cm) was mounted in the center of each chamber and illumination was provided by a 24 V DC (6 W) lamp positioned behind an opaque face plate flush with the top of the cage. A retractable bar was positioned on the side of the cage. The bar was calibrated for a 30 G force requirement and had a 1 sec cycle time. A standard Lehigh Valley pellet dispenser delivered pellets to an aperture located on the left side of the bar. Located immediately above this opening was a 24 V DC magazine cue light (6 W) located behind an opaque glass.

Procedure

Liquid diet. During the first 50 days, ten animals were maintained on a 10% (41% EDK) ethanol-Metrecal diet. The ethanol percentage was then increased to 12% (49% EDK) for 35 days and 14% (57%EDK) for 40 days. All diets contained a constant 1.28 kcal/ml. At various times during the experiment blood samples were drawn from the tip of the tail into heparinized Natelson blood collecting pipets and subsequently enzymatically analyzed for blood alcohol levels (BALs): (Days: 10%–43,44,48; 12%—3,7,11,23,32; 14%—2,5,10,16,18,29,31). All samples were drawn between 10:00 a.m. and 12:30 p.m. After the last day of the 14% diet the eight remaining experimental subjects (two rats died during the course of the experiment) were placed on ad lib food and water for 46 days. Behavior was monitored for withdrawal symptoms during the five hours following removal of ethanol. Eight control rats of comparable age and weight were purchased from the same breeder at the beginning of the ad lib regime and as such constituted an *ad hoc* control group. The control animals were individually housed and were extensively handled prior to the beginning of discrimination training.

Successive discrimination. Ten days prior to the beginning of training all animals were placed on a daily restricted food regime of 12 g. Water was freely available. In order to familiarize the animals with the type of food which would be used as a reinforcer, a food cup containing six Noyes food pellets (45 mg) was placed in the home cage on five consecu-

tive days. During the next two days of magazine training a single 45 mg Noyes pellet was delivered at intervals of 30 sec. Thirty daily trials were employed. Onset of a 1.5 sec cue light and the concurrent offset of the house light accompanied the magazine cycle. A retractable bar procedure was used during the initial three days of bar press training to eliminate the need for hand shaping. At intervals of 30 sec the manipulandum was presented to the animals on 60 trials. For the first 30 trials each depression of the bar resulted in food delivery and retraction of the bar. If the bar was not depressed in 30 sec it was automatically retracted without food delivery. After 30 trials the reinforcement schedule was changed to a variable ratio 2 (VR2) for 30 trials. Three animals in each group failed to acquire the bar-press response and rather than introduce an additional training variable of hand-shaping, the animals were discarded from the study. The remaining animals were shifted to a VR-6 schedule for four sessions with 60 reinforcements per session. A 3000 Hz intermittent tone (1 sec on and 1 sec off) was presented throughout these sessions to minimize novelty effects that might occur when it was first introduced as a discriminative stimulus (S+) in the following phase. During the first 15 days of discrimination training the duration of S+ (tone) and S− (no tone) periods was 60 sec and a variable interval (VI) 15 sec schedule of reinforcement was used. Each session contained 100 trials, 50 S+ and 50 S−. On the following 18 days the VI schedule was increased to 60 sec and the duration of S+ and S− was extended to 120 sec. Each session consisted of 50 trials, 25 S+ and 25 S−. Throughout discrimination training, a 10 sec change-over delay was used—the onset of the S+ was withheld until no responding occurred during the last 10 sec of the S− period. The maximum session length was two hours. All animals were run six days a week.

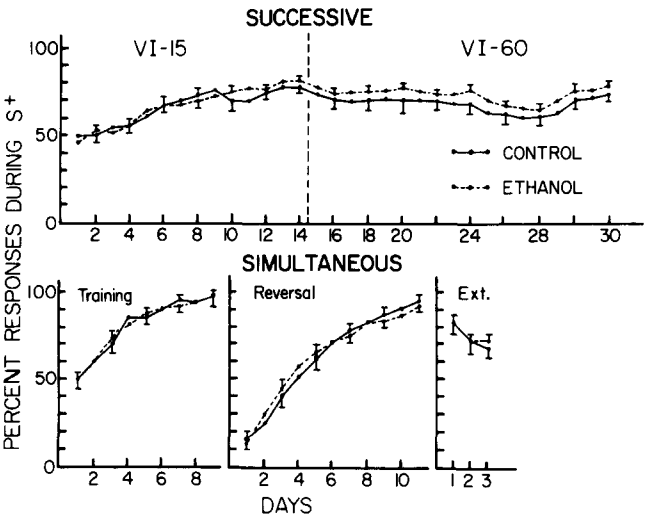

FIG. 2. Mean (±SEM) percent of the total number of responses occurring during reinforced (S+) periods in various phases of a successive discrimination (Experiment 2) and simultaneous discrimination (Experiment 3). In Experiment 2 ethanol rats were maintained on a 10% (41% EDK) ethanol-Metrecal diet for 50 days, a 12% (49% EDK) diet for 35 days and a 14% (57% EDK) diet for 40 days. In Experiment 3 a 9% (37% EDK) ethanol-Metrecal diet was administered for 215 days. Control animals in both experiments were maintained ad lib on food and water. All rats were tested 45–60 days after the withdrawal of the liquid diet.

RESULTS AND DISCUSSION

Liquid Diet

Detailed analyses of the effects which the various diets had on body weight, volume of liquid diet consumed, and grams of ethanol/kg of body weight are described elsewhere [12]. The mean daily amount of ethanol ingested over the last five days was 13.2±61 g of ethanol/kg of body weight, 34.9±2.2 ml of liquid diet was consumed, and body weight was 262±13.2 g. At the beginning of deprivation for discrimination training, mean body weight was 323±4.7 g. Mean BALs were as follows: 10%=123 mg/100 ml (±12.1); 12%=175 mg/100 ml (±10.1); 14%=202 mg/100 ml (±8.9). Pairwise comparisons showed that the blood alcohol levels were significantly different ($p<0.05$) between 10% and each of the other two levels. The difference between the 12% and 14% was not statistically reliable. Withdrawal symptoms, including tail stiffening, piloerections, and tail arching were observed. (For further detail concerning BALs and withdrawal symptoms see Tombaugh and Tombaugh [12]).

Successive Discrimination

Accuracy of responding was computed by dividing the number of responses that occurred during S+ periods by the total number of responses and multiplying this proportion by 100. Figure 2 (upper panel) shows the mean level of accuracy during discrimination training for both experimental and control groups. Inspection of this figure clearly shows that both groups had a similar rate of discriminated acquisition and terminal level of performance in both phases of the experiment. Analyses of variance performed over these data confirmed this observation: $F(1,14)<1$; $F(1,14)=1.60$, $p>0.05$. The two groups were also indistinguishable when the latency to make the first response in S+ and S− periods was compared over the last three days of training: $Fs(1,14)<1$. In order to determine if ethanol altered rates of responding an analysis of the responses per min during S+ and S− periods was computed over the last 3 days of VI 15 and VI 60 training. No significant differences were observed: $F(1,14)=1.73$, $p>0.05$; $F(1,14)=1.50$, $p>0.05$.

EXPERIMENT 3

The current failures, in comparison to previous findings, to show that chronic ethanol ingestion resulted in any type of learning deficit prompted one final attempt to replicate prior results. Since the first two studies employed a substantially higher level of ethanol derived kilocalories than previously employed, the level of ethanol used in Experiment 3 was adjusted accordingly to 9% (37% EDK). Several behavioral tasks other than shuttle-avoidance were employed. These included passive avoidance, simultaneous discrimination, intra-dimensional reversal and extinction of simultaneous discrimination, and complex radial-arm maze learning. The latter task was suggested by results showing that (1) chronic alcohol consumption produces morphological alteration in the hippocampus [11,13] and (2) hippocampal damage results in spatial memory impairments [17]. Thus, it was speculated that the present alcohol treatments might affect performance in a radial arm maze which Olton [10] has demonstrated to be sensitive for measuring spatial memory.

METHOD

Subjects

Fifty-six male rats of the Sprague-Dawley strain were purchased from the Holtzman Company. They were 120 days old and weighed 400–420 g at the beginning of the experiment. During the course of the experiment twelve animals were discarded from the ethanol group (three because of inner ear disease, four related to self-withdrawal from ethanol, two due to tumors, and three from undetermined causes). Six animals died in the control group (two because of inner ear disease, two due to tumors and two related to respiratory infections). During testing sixteen animals were randomly selected from each group in order to insure that the experimental conditions were precisely counterbalanced across multiple operant chambers.

Apparatus

The apparatus was the same as that described previously with the following exceptions. The shuttle-avoidance equipment was placed in a sound insulated chamber and automatically controlled by a micro processor system. Two-bar test cages were also used in the operant chamber which contained two fixed Gerbrands bars. Each bar was calibrated for a 30 G force. The bars were mounted to the side wall of the test cage 4 cm above the floor separated by 15 cm center-to-center. A 24 V DC lamp (no. 1819) with an opaque jewel was located 5.5 cm above each bar. A Gerbrand pellet dispenser delivered 45 mg Noyes pellets into a 4.5 cm square aperture 2 cm above the grid floor and centered between the two bars. An 8-arm radial maze elevated 77 cm from the floor was also utilized. Each arm (56 cm long, 9 cm wide) was constructed of 1 cm plywood. The center platform was 27 cm wide. Wooden restraining walls (7.5 cm high and 15 cm long) were joined to each arm at the center platform to inhibit subjects from crossing over the arms without going through the center platform. The rest of the arm contained 2.15 cm sides. Food cups (1.75 cm×4 cm) made of cardboard and wrapped with black electrical tape could be attached to the end of each arm.

Liquid Diet

Half of the animals were administered a 9% (37% EDK) ethanol-Metrecal diet for 215 days. After the termination of the ethanol diet the animals were placed on ad lib food and water for 63 days. Since previous data [14,16] have shown that isocaloric sucrose and free feeding procedures produce similar growth functions, the latter procedure was selected because of its relative ease of implementation. Consequently, control rats were maintained on ad lib Purina Laboratory Chow and water. The same subjects were used in all behavioral tests administered in the same order as described below.

Simultaneous Discrimination

Seven days prior to the beginning of the experiment all subjects were placed on a daily restricted feeding schedule of 15 g of Purina Laboratory Chow. Magazine training consisted of delivering a 45 mg Noyes pellet every 45 sec. Magazine cycles were accompanied by the onset of a 1.5 sec cue light and offset of the house light. Animals received 30 such trials on each of two days. Bar press training began on the following day. At intervals of 45 sec a retractable bar was presented for 45-sec periods on 30 successive occasions. Depression of the bar resulted in the delivery of the reinforcer and retraction of the bar. Failure to bar press resulted in bar retraction at the end of the 45-sec period without a food pellet being delivered. Subjects having fewer than 30 responses after two days of training were manually shaped to bar press. For the rest of the experiment the two bar chamber was employed. Two initial days of nondifferential training were given where a VI 15 sec reinforcement schedule was concurrently programmed on both bars. Session length was 45 minutes. The status of the cue lamps above the bars was varied in a random sequence. During discrimination training each trial consisted of the cue lamp positioned above one bar being illuminated (lamp-on) for 30 sec while the other cue lamp was not illuminated (lamp-off). The illumination of either the left or right lamp was varied randomly from trial to trial. For half the subjects "lamp-on" was associated with a VI 15 sec schedule of reinforcement (S+) while no reinforcement was delivered during "lamp-off" (S−). This relationship was reversed for the remaining half of the animals. Each session consisted of 100 S+ periods. After nine days of training, the S+ and S− conditions were reversed for all animals. Following 11 days of reversal training reinforcement was discontinued (extinction) for 3 sessions. During extinction the magazine dispenser did not cycle.

Passive Avoidance

The procedure previously described in Experiment 1 was employed. All animals were placed on ad lib food and water four days prior to beginning of testing.

Shuttle Avoidance

The procedure previously described in Experiment 1 was used.

Radial-Arm Maze

Nine experimental and nine control rats were randomly selected and maintained on ad lib food and water. On two separate days each rat was placed in the maze for two 15 min trials to establish a baseline of exploratory behavior (nondeprived exploratory phase). The animals were then placed on a 12 g daily feeding regime for six days. A third 15 min trial was used to determine if deprivation altered the pattern of responding (deprived exploratory phase). After eight more days of deprivation reinforced training began. Five daily placements were given with food cups placed at the end of each arm (food phase). Each cup contained a single 45 mg Noyes pellet. Subjects remained in the maze until all eight food pellets were consumed or 10 min transpired. In all phases the maze was washed at the start of each day and after each trial to eliminate any possible intra-maze cues (e.g., odor trails). Throughout the experiment the sequence of arms entered, duration of time spent in each arm and total number of entries were recorded.

RESULTS AND DISCUSSION

Liquid Diet

An analysis of variance performed over body weight averaged in five day blocks, showed both groups significantly increased their body weight: $F(1,32)=420.89$, $p<0.01$. A significant block by ethanol interaction: $F(1,32)=32.08$, $p<0.01$, indicated that the ethanol group gained weight at a

faster rate than did the control group. On the last trial block mean ethanol and control weight were 684±17.3 g and 625±8.0 g, respectively. This relationship between body weight is consistent with previously reported data [15,17]. During the last five days of ethanol maintenance, ethanol animals consumed 9.10±2.6 g of ethanol/kg of body weight and 96.5±1.52 ml of liquid diet.

Simultaneous Discrimination

Figure 2 (lower panel) shows percent correct for simultaneous discrimination, reversal and extinction. Inspection of the figure indicates that both groups performed in a similar manner in each phase. Subsequent analyses of variance showed that ethanol treatment did not produce statistically significant differences in any phase: Fs <1. All trial effects were reliable: acquisition, $F(8,240)=225$, $p<0.01$; reversal, $F(10,291)=421$, $p<0.01$; extinction, $F(2,58)=421$, $p<0.01$. However, the status of the light condition serving as the S+ did influence performance in reversal and extinction for both groups. In each phase performance was more persistent when the S+ was "light-on." This produced slower reversal learning and greater resistance to extinction for both ethanol and control conditions: $Fs(1,29)=9.22$ and 10.55, $p<0.01$, respectively. This relationship is consistent with the sign-tracking and feature-positive literature which shows that there is a greater tendency for animals to respond to a distinctive stimulus (e.g., "light-on") when it is associated with reward than when it is paired with non-reward [7]. The fact that both groups showed similar sign-tracking performance provides further evidence that ethanol maintenance did not disrupt the normal processes underlying learning.

Passive Avoidance

On day one the average SOL for the experimental and control groups was 59 sec and 44 sec. Subsequent analysis of variance showed this difference was not statistically significant: $F(1,30)$ <1. A similar analysis performed over the means for day two (243 and 246 sec) also was not significant: $F(1,29)$ <1. One animal which failed to leave the holding chamber within 300 sec on day one was not included in the retention day analysis.

Shuttle Avoidance

Figure 1B shows the number of avoidance responses which occurred during each day of training. An analysis of variance performed over these data revealed that the only significant effect was a gradual increase in performance for both groups: ETOH, $F(1,32) < 1$; Trials, $F(4,128)=52.53$, $p<0.01$; ETOH×Trials, $F(4,32)=1.37$, $p>0.05$. The mean number of trials to reach the first successful avoidance response was 8.7 and 11.8 for the control and experimental groups. This difference was not statistically significant: $F(1,32)=1.76$, $p>0.05$. The number of trials to reach the criterion of nine avoidance responses in ten trials was the same (28) for both groups. Except for a single subject in each group all rats reached the training criterion in two days. The relatively greater rate of acquisition observed in Experiment 3, in comparison to Experiment 1 is probably due to the increased degree of environmental control associated with the sound attenuated chamber used in Experiment 3.

Radial Arm Maze

The number of different arms selected during the first eight daily trials was calculated for the two groups during the three phases. Previous experiments have assumed this measure reflects short term spatial memory for either previously explored arms (exploratory phases) or the location of arms where food had previously been consumed (food phases). Analyses of variance performed over each phase failed to reveal any significant effects due to the ethanol treatment: $F(1,16)$ <1; $F(1,16)=1.25$, $p>0.05$; $F(1,16)=1.81$, $p>0.05$. There was, however, a significant increase in the number of novel arms entered on the second day of the nondeprived exploratory phase: $F(1,16)=6.04$, $p<0.01$. A similar days effect was not observed during the food phase: $F(4,64)=2.12$, $p>0.05$. The mean duration of time subjects remained in each arm during the first eight entries was computed to determine if different patterns of behavior existed between the two groups. No significant differences were observed during any phase of the experiment: $Fs(1,16)$ <1.

In order to determine if the ethanol treatments influenced exploratory activity the total number of arms entered during the two exploratory sessions was computed. No differences were observed between the groups for either phase: $Fs(1,16)$ <1. Finally individual response protocols were inspected to determine if different response sequences (i.e., exploration of adjacent arms) existed between the two groups. No differences were detected.

GENERAL DISCUSSION

The present series of experiments failed to provide any evidence suggesting that prolonged ethanol regimes impaired either learning or memory when testing was initiated several weeks after the ethanol-Metrecal diet had been discontinued. This conclusion is based on the results for both appetitive and aversive behavioral tasks (shuttle avoidance, passive avoidance, successive discrimination, simultaneous discrimination, reversal and extinction of simultaneous discrimination, and complex spatial maze learning) performed in a variety of different experimental apparatus by various experimenters over a three year period. The current failure to obtain performance impairments is surprising since several studies have reported that similar liquid diets produce learning and memory deficits in rats and mice (D. W. Walker, personal communication, 1980; see also [1, 5, 6, 13]). There are several sources of evidence which suggest that this lack of congruence does not reside in potential procedural differences associated with the administration of the diets. For example, Metrecal procedures used in the present series of studies produced comparable BALs and withdrawal symptoms to those previously observed [4,8]. Moreover, a high degree of correspondence also exists among these studies when body weight and ethanol consumption are compared. In the two studies reported by Walker and Freund [14] where ethanol retarded acquisition of shuttle-avoidance performance, the ethanol animals gained more weight than subjects maintained on lab chow and the mean amount of ethanol consumed over the last 30 days was 9.2 g/kg. These results are similar to those seen in Experiment 3 which employed a comparable ethanol-Metrecal diet. A detailed comparison of our shuttle-box avoidance data with those previously reported reveal striking performance differences existed between the control groups as well as between the experimental groups. Walker and Freund [14], using procedures corresponding to those employed in Experiment 3, reported that mean number of trials required to reach a 90% criterion was 246 for the control group while *none* of the

seven experimental animals reached criterion in 500 trials. In a second study, Walker and Hunter [16] pretrained animals on a shuttle avoidance task prior to introducing the liquid diet. Here 30% of the animals failed to acquire the task to a criterion of 90% avoidance in 450 trials. This is contrasted with the fact that in the present studies *all* experimental and control animals learned the task to 90% criterion in 60 trials in Experiment 3 and 150 trials in Experiment 1. Comparison of these results suggests that some aspect of the testing situation (e.g., intensity of the CS) may account for the lack of agreement. Dr. D. W. Walker indicated in personal correspondence that the intensity of the auditory CS used in their studies was initially dampened to reduce its magnitude and that intensity difference probably exists between our two series of experiments. It is possible, for example, that alcohol-induced impairment does exist, but is only measureable when the difficulty level of the shuttle-avoidance task is high. The fact that non-significant differences in the direction of previous results were obtained in Experiment 1 (where trials to criterion were intermediate to those reported by Walker and Freund [14]) and in Experiment 3 is consistent with this notion. A second possibility relates to the type of subjects employed. All of the studies previously reporting shuttle avoidance impairments used hooded rats (Long-Evans) while the present series of experiments used an albino strain (Sprague-Dawley). This contention is supported by unpublished results by Walker where avoidance deficits using Sprague-Dawley animals were not observed. A final possibility resides with the duration of time that transpired between the termination of the liquid diets and the beginning of behavioral testing. Walker and Freund [14] used a two week period while the current studies employed a minimum of eight weeks. Perhaps some type of functional recovery occurred during the longer time period which ameliorated previously reported impairments. However, other evidence [6] showing that avoidance deficits occur with mice when testing began four and one-half months after withdrawal of ethanol suggests that recovery of function probably did not occur in the present series of experiments. In any case, it is apparent that any conclusions concerning the effects which prolonged ethanol diets have on various learning processes will have to be held in abeyance until further experimentation has been able to isolate those variables which produced the differences between the current results and those which report that long term ethanol consumption produces learning deficits.

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