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Chronic Intermittent Ethyl Alcohol Inhalation and Avoidance Learning[1]

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SOTZING, J. H. AND T. S. BROWN. *Chronic intermittent ethyl alcohol inhalation and avoidance learning.* PHARMAC. BIOCHEM. BEHAV. 5(4) 417–421, 1976. – Weanling male and female rats were chronically exposed to alcohol for 50 days using an intermittent inhalation technique which does not cause alcohol dependency. After 17 days of no exposure to alcohol, animals began two-way active avoidance testing. Results indicated that males were impaired on this task and females were not while at the same time males had reduced body weights. The male weight reduction was not responsible for the avoidance impairment. It was concluded that impaired avoidance learning following chronic esposure to alcohol is not specific to dependency models of animal alcoholism.

Alcohol Shock Avoidance Alcohol Inhalation Chronic Intermittent Alcohol Exposure Nondependency

PREVIOUS papers have reported a two-way active avoidance impairment in female mice and male rats following chronic alcohol exposure (3–9 months) and two weeks of no exposure to alcohol prior to testing [7,20]. In these studies, alcohol was administered in the form of a liquid diet, a procedure which has been found to lead to withdrawal reactions upon the termination of alcohol exposure [6]. Using an alcohol inhalation technique it has been reported that developing physical dependence requires continuously elevated blood alcohol levels; short periods of sobriety are sufficient to decay a developing addiction [9]. The same conclusion has been reached using a number of other alcohol administration techniques (see [18]). The alcohol liquid diet procedure reportedly meets the requirement of blood alcohol maintenance over time [6,12]. Withdrawal reactions were observed in female mice following chronic liquid diet treatment and prior to the obtained impairment in two-way active avoidance [7]. When male rats were used no overt signs of withdrawal were reported although it was not completely clear whether dependency did or did not develop [20].

The present experiment was an attempt to extend the above findings. Firstly, an attempt was made to determine if the avoidance learning impairment is specific to a dependency model of alcoholism by using a non-dependency technique for alcohol administration. Secondly, both sexes were included in the present study to determine if the impairment is sex specific. Because there is evidence that female rats metabolize alcohol more rapidly than males [4] it was thought possible that a sex difference would occur. Thirdly, while previously adult male rats were chronically exposed to alcohol for 6 or 9 months in order to obtain an avoidance impairment [20], the present study sought to determine if a shorter period of chronic alcohol exposure during development would lead to a similar impairment.

METHOD

Animals

Sixteen male and 16 female 21 day old weanling albino rats, equally divided by sex into two groups, were used. One group of 8 males and 8 females was chronically exposed to alcohol while the other group of identical numbers serves as controls. Another 14 male and 14 female weanling rats were used to obtain blood samples by cardiac puncture. All animals were housed in individual cages throughout the experiment with ad libitum availability of food (Teklad rat chow) and water.

Apparatus

An alcohol inhalation chamber consisted of an ordinary glasswalled aquarium (24 × 12½ × 11½ in.) which was covered by a sheet of clear Plexiglas and divided into two equal parts by a screen barrier. A vacuum pump pushed air (9.5 liters/min) through rubber tubing (inside dia. ¼ in.) into a one liter glass bottle containing 520 ml of pure ethyl alcohol. Air saturated with alcohol vapor exited the bottle through the second of two openings in its cap and traveled through tubing into the chamber. A piece of tubing 9 in. long extending into the chamber through the plastic covering at one corner distributed the vapor into the chamber. Vapor pressure in the sealed chamber was relieved by a series of small holes drilled in the covering at the opposite end of the chamber from the vapor distribution

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tube. A second chamber much the same as the first except without the vacuum pump and vapor system was used as a control enviroment.

A two-way shuttle box (21½ × 7 × 12in.) was used for avoidance testing. It was constructed with clear Plexiglass sides and a hardboard ceiling. The two compartment floors each consisted of 20 grids and were separated by a 2 in. wooden barrier on which 4 grids were situated to prevent straddling. A 0.2 mA constant current was delivered on each trial to the grids in the appropriate compartment while grids situated on the barrier were activated on all trials. Grid polarity was scrambled to eliminate the possibility of no shock. A buzzer was built into the hardboard lid of the box and masking noise was generated by a pair of exhaust fans located directly in front of the apparatus. The shuttle box was in one room and the experimenter and controls for presenting the CS and the UCS were in an adjoining room separated by a one-way vision mirror. The apparatus was illuminated by a 7½ W light bulb placed outside the box and adjacent to the dividing hurdle.

Blood alcohol determination was made using the method and reagents purchased from the Calbiochem Company (Ethyl Alcohol Stat-Pak). This method involves the enzymatic oxidation of ethyl alcohol and the subsequent change in photometric absorbancy at 340 nm.

Procedure

Weanling rats were either chronically exposed to alcohol vapor or to the control chamber environment for 50 days. Animals were removed from their home cages and placed as a group either in the alcohol chamber or the control chamber. Within both chambers males were separated from females by means of the screen barriers. Daily vapor treatment lasted for 2 hr during which time vapor saturated air was continuously circulated through the experimental chamber. After 2 hr animals were returned to their home cages. Males and females were alternated daily between the two compartments of the chambers. The one liter bottle was refilled each day before treatment. Record was kept of body weights throughout the 50 day chronic treatment period and until 180 days after treatment initiation. In order to determine the success of the inhalation technique for producing intoxication, blood alcohol assays were carried out on 2 males and 2 females once a week for 7 weeks during the alcohol treatment period. Blood sampling animals were placed in the vapor chambers along with experimental animals on appropriate days. Blood was collected by cardiac puncture following the 2 hr exposure period and was immediately put on ice until centrifugation. Assays were performed on the same day as collection. A total of 28 blood alcohol determinations from 28 different male and female rats were made. Although a number of methods for chronic repeated blood sampling are available and perhaps would have been advisable in this experiment, the terminal cardiac puncture approach was used for two reasons. Firstly, samples were taken from developing rats from which it would have been difficult to obtain adequate blood sample sizes using a more peripheral sampling route. Secondly, the purpose of the blood assays was merely to indicate that alcohol was successfully entering the blood stream.

Following alcohol treatment there were 17 days of no experimental manipulations before avoidance testing. This period was intended to insure the complete removal of alcohol from body tissues and any associated effects of acute alcohol intoxication. Animals were 88 days of age when avoidance testing began. The animals received 10 trials per day. Each rat was removed from its home cage and placed in one side of the shuttle box. Fans generating masking noise remained on throughout the course of testing. A buzzer (CS) preceded a 0.2 mA shock (UCS) to the occupied side of the box by 5 sec and remained paired with it until the animal jumped the barrier into the safe compartment thereby closing a photoelectric relay. When the animal avoided the UCS prior to its onset the CS was immediately terminated. The intertrial interval was randomly set at 20, 25, 30, 35, or 40 sec. The number of successful avoidance trials and the shock escape latency on the initial avoidance trial were recorded. Animals were discontinued from testing when a 90% avoidance criterion was reached, i.e. any series of 18 out of 20 successful trials.

RESULTS

The alcohol inhalation technique was found to successfully elevate blood alcohol concentrations. The mean blood alcohol level from 28 blood sampling animals over seven weeks following weaning was equal to 113.571 mg/100 ml (range: 60–190 mg/100 ml). Males and females had exactly the same mean levels. These results do not reflect the effects of chronic exposure to alcohol since blood sampling animals received only a single exposure to alcohol treatment prior to terminal cardiac puncture blood collection.There was a tendency for younger blood sampling animals to develop higher blood alcohol levels than older animals. A Students t comparing blood concentrations obtained from animals 22–50 days of age (mean = 133.13 mg/100 ml, range: 60–190 mg/100 ml) with animals 51–70 days of age (mean = 90.00 mg/100 ml, range: 60–170 mg/100 ml) statistically supported this tendency, $t(25) = 2.84$, $p<0.01$.

Seventeen days intervened between chronic alcohol treatment and avoidance testing. In this period animals were handled and observed daily. None of the typical behavioral symptoms of withdrawal [12] or any other types of unusual behavior were seen.Results from the first 6 days of avoidance testing are presented in Fig. 1. In the first 3 days of testing there was an apparent sex difference in the effects of alcohol treatment, i.e. a sex by treatment interaction effect. Alcohol exposed males successfully avoided the UCS fewer times than did alcohol exposed females or control males and females. A three-way ANOVA (sex × treatment × days) indicated that this interaction was reliable, $F(1,28) = 4.9$, $p<0.05$, Fig. 1 indicates that this effect was eliminated by the second three days of avoidance testing. An ANOVA indicated no significant effects beyond the third day of testing. A trials to criterion measure was also analyzed. The number of trials necessary until a criterion of 20 total successful avoidance trials was determined for each rat. A two-way ANOVA (treatment × sex) yielded a significant interaction, $F(1,28) = 5.60$, $p<0.05$; alcohol exposed males required more trials to reach criterion (43.75) than alcohol exposed females (32.50) or control males (35.75) and females (30.75). These results indicate that while males were detrimentally effected on avoidance learning as a result of chronic exposure to alcohol, females were not.

Chronic exposure to alcohol not only differentially effected the sexes on avoidance behavior, but body weights were also differentially effected. Mean body weight data are

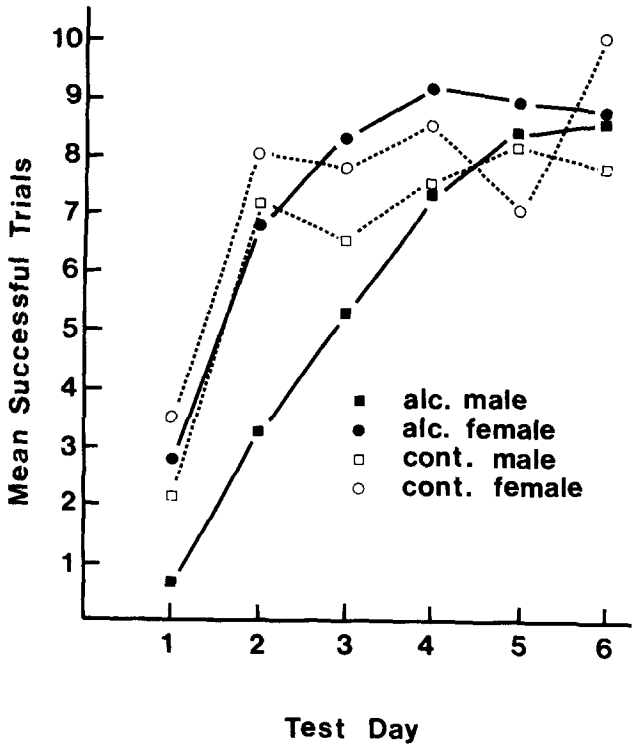

FIG. 1. Mean number of successful avoidance trials during the first 6 days of testing.

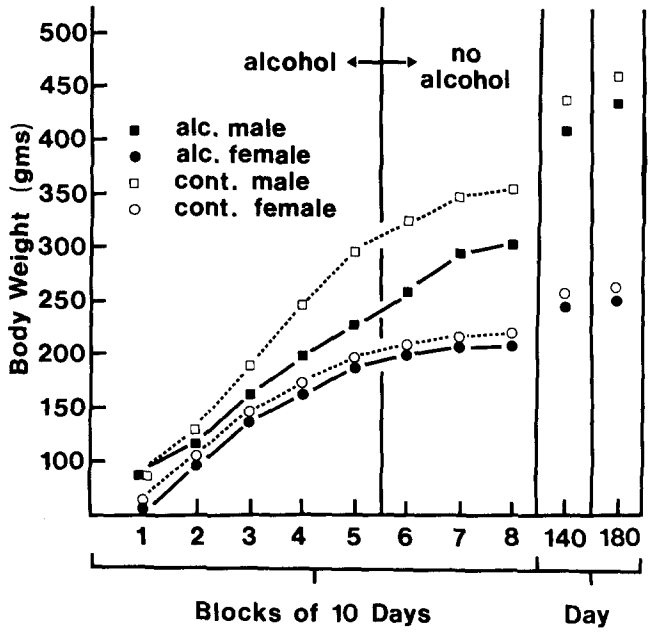

FIG. 2. Mean body weights during the 50 days of alcohol exposure and up to 180 days after the beginning of alcohol treatment.

presented in Fig. 2. As can be seen, by the end of the chronic alcohol treatment period alcohol exposed males were reduced in body weight while alcohol exposed females were relatively uneffected. An ANOVA verified this by a significant sex by treatment interaction in the fourth and fifth blocks of 10 days, $F(1,28) = 13.60$, $p<0.01$. By Day 180, however, no significant effects were obtained indicating that the male body weight reduction had reversed itself.

Although improbable, the possibility existed that the treatment by sex interaction on avoidance behavior could be predicated upon a similar interaction in body weight. An Analysis of Covariance indicated that the trials to criterion avoidance interaction remained even when body weight variance was taken into account, $F(1,27) = 4.53$, $p<0.05$. The avoidance interaction could not, therefore, be considered dependent upon the body weight covariate.

Another possibility is that the results can be attributed to differential shock sensitivity. An ANOVA was performed on shock escape latencies on the initial trial of avoidance testing. No significant differences in escape latencies were found.

DISCUSSION

The results indicate that the inhalation technique did not cause dependency but was successful in raising blood alcohol to effective levels. Following chronic intermittent alcohol exposure males were impaired on avoidance learning but females were not. While males were reduced in body weight following alcohol treatment, the avoidance impairment was not predicated upon the weight loss. These results confirm and extend previous findings in which a two-way avoidance impairment was found in female mice [7] and male rats [20] following chronic exposure to alcohol. While those studies used a liquid diet method of alcohol administration known to cause physical dependence, the present study used an intermittent inhalation technique which does not meet the critical requirement for alcohol dependency, i.e. continuously elevated blood alcohol levels (see [18]). Despite this important procedural difference, the present results indicate a similar avoidance learning impairment in male rats. No impairment was found in alcohol exposed females. Because the male avoidance performance returned to control levels on the fourth day of testing, animals were apparently able to compensate for an initial rate deficit. The avoidance learning impairment reported earlier [7,20] was nonreversible and in this regard was probably the result of the relatively greater severity of the accompanying alcohol administration technique.

Peak daily blood alcohol levels obtained using the inhalation procedure fell within the range of levels reported for many of the other techniques that result in alcohol dependency [16]. Blood alcohol levels were elevated for 2 hr per day; during the remaining 22 hr levels presumably dropped to zero. Assuming an alcohol metabolism rate of 270 mg/kg hr [2], 22 hours is more than a sufficient time period for the complete removal of alcohol from the blood stream. Also, there is recent evidence indicating that chronic daily unimodal increases in blood alcohol are ineffective in causing alcohol dependency [18]. The intermittent inhalation technique was therefore successful in raising blood alcohol to levels which if continuously maintained would lead to addiction. In the time between daily bouts, however, any possibility of dependency was eliminated concomitant with the metabolized removal of the alcohol.

Previously rats were exposed to alcohol for 6 or 9 months beginning on Day 75 [20]. Anatomical [3,5], electrophysiological [11,13], and behavioral [14] evidence suggests that central nervous system maturity occurs at

30–50 days of age. Because in the present study alcohol exposure began on Day 21, the results might therefore be attributed to a detrimental effect of alcohol interacting with CNS maturation, a possibility that earlier studies were able to eliminate [7,10]. Whether the avoidance impairment can be attributed with certainty, however, to alcohol obstruction of CNS development or to alcohol destruction of intact nerve tissue cannot be determined from this study.

It is possible that the male avoidance impairment (and presumed neural damage) was only indirectly caused by alcohol and was more directly caused by alcohol-induced malnutrition. This is unlikely, however, in view of the finding that early malnutrition facilitates rather than inhibits avoidance learning [17]. That the avoidance impairment was due to inadequate nutrition also does not correspond to the fact that males and females did not differ in availability of food or water. There is no ready explanation for the male and not female reduction in body weight.

The obtained sex difference in avoidance learning can perhaps be attributed to differential endocrinological susceptibilities to the effects of alcohol. Female rats have larger anterior pituitary and adrenal glands than males [19] and higher blood corticosterone levels [1,15] suggesting higher circulating ACTH levels. There is convincing evidence that ACTH levels are positively related to avoidance performance [21]. Since it is possible that the intermittent inhalation technique was chronically stress-inducing, endogenous male and female differences in circulating ACTH levels may have been effected by chronic alcohol exposure in such a way as to lead to differences in ACTH mobilization. As a result, alcohol exposed females may have had greater elevations in ACTH levels than males thus resulting in more efficient avoidance performance. If this were true, however, then alcohol exposed males would have done better than control males on the avoidance task. Since this was not the case, it is reasonable to assume that the male impairment cannot be accounted for on the basis of altered ACTH levels.

There is evidence suggesting that there is a sex difference in alcohol metabolism rates: males exhibit longer sleep times than females to a challenge dose (3.5 g/kg) of alcohol [4]. Chronic intermittent vapor exposure may therefore have effectively maintained male blood alcohol levels for a longer period than females on each day of treatment. It is possible that a slower metabolism rate in males is responsible for a sex difference in the CNS toxicity of alcohol.

As was indicated in the results, the 17 days intervening between treatment and testing make it unlikely that residual symptoms of acute alcohol exposure could have been responsible for the obtained avoidance deficit. Also, shock sensitivities were probably not effected by alcohol treatment. The previously reported avoidance deficits have not been caused by differential activity rates [7,20]. Although an intertrial activity measure was not recorded in the present study, no obvious differences in intertrial activity were observed.

In sum, the above considerations make it improbable that the male learning deficit can be attributed to body weight, endocrine functioning, acute pharmacological effects, shock sensitivity, or activity. Rather, the more reasonable explanation is that some sort of CNS damage accrued from long-term exposure to alcohol. Whether this was due to an indirect developmental disturbance or to immediate neural damage is not clear from this study.

It should be mentioned that the intermittent inhalation technique for alcohol administration used in this study is the first of its kind in the modern literature. Goldstein [8] has used a continuous inhalation technique with mice resulting in physical dependence. This technique uses low levels of vapor concentration and necessitates the use of pyrazole injections to block alcohol metabolism. With continuous maintenance of blood alcohol at 200 mg/100 ml, physical dependence develops in as little time as three days of vapor exposure [9]. The advantages and disadvantages of this model have been expounded on [10]. The critical difference between the continuous and the intermittent inhalation techniques is that the former results in dependency while the latter does not. At the same time, the intermittent technique retains many of the advantages of the continuous method such as immediate dosage control, control of exposure time, unobtrusive route of intake, and no nutritional manipulation. It is also important to note that the apparatus for intermittent inhalation is relatively easy to construct. Further research on the long-term effects of chronic but nonaddictive exposure to alcohol should find the intermittent inhalation technique very useful.

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