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Prolonged Alcohol Consumption in the Rat: Absence of Retrograde Amnesia for an Avoidance Response[1]

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WALKER, D. W., AND B. E. HUNTER. *Prolonged alcohol consumption in the rat: absence of retrograde amnesia for an avoidance response.* PHARMAC. BIOCHEM. BEHAV. 2(1) 63–66, 1974. – Prolonged alcohol consumption (18 weeks) did not result in the disruption of retention of a previously learned avoidance response. Rats were pretrained to a criterion of 80% correct responses on a shuttlebox avoidance task and subsequently divided into three groups matched for performance and weight. One group received ethanol incorporated into a liquid diet which served as the source of calories and fluid. One control group was individually pair-fed the identical liquid diet except sucrose was isocalorically substituted for ethanol. A second control group was maintained on pelleted laboratory food and water. After 18 weeks of maintenance on the respective experimental diets, all rats were given laboratory food and water ad lib. Three months after the ethanol-containing and sucrose-containing diets were replaced with laboratory food and water, the rats were tested for retention of the avoidance response. It was found that the groups were statistically indistinguishable on the measures of retention used. The results were interpreted as being in agreement with clinical descriptions of alcoholic Korsakoff patients.

Alcohol Ethanol Shock avoidance Korsakoff's psychosis Chronic alcohol consumption Retrograde amnesia

CHRONIC ethanol consumption has been found to result in central nervous system (CNS) pathology including morphological deterioration in a variety of brain regions and associated behavioral symptomatology; the most prominent being impairment in learning and recent memory [10]. Traditionally, the pathology has been attributed to malnutrition rather than toxicity of ethanol, based primarily upon the frequent associations in the clinical literature between Korsakoff's amnestic syndrome and symptoms of malnutrition; especially thiamine deficiency [11]. Victor, Adams, and Collins [12] have suggested that the amnesia, characteristic of alcoholic Korsakoff's patients, is actually a "psychic manifestation of Wernicke's disease". Presumably, intake of a substantial amount of empty calories in the form of ethanol results in a reduction in more nutritive calories inducing a state of relative malnutrition, although total caloric intake may remain normal.

Although evidence for morphological and behavioral pathology resulting from thiamine deficiency appears unequivocal, it is unlikely that all the symptomatology found in Korsakoff's psychosis can be attributed exclusively to malnutrition. Institutionalized alcoholics who show evidence of associated malnutrition often are of the skid row variety representing only about 5% of the problem drinkers in our society [1]. Since brain damage and associated mental deterioration have been reported in alcoholic patients with no clinical history of malnutrition [5,9], it is conceivable that ethanol may exert toxic effects on CNS function and learning despite adequate nutrition.

Recent investigations using laboratory animals have supported this conclusion. By incorporating ethanol into liquid diets, it has been shown that mice [2,15] or rats [6] will consume substantial quantities of ethanol and subsequently develop withdrawal symptoms. The use of liquid diets as the sole source of calories and fluid provides for precise nutritional control. The empty calories provided by ethanol can be replaced in control diets by isocaloric quantities of sucrose. Further supplemental nutrients can be added to insure proper intake of essential vitamins and minerals. Using this technique, it had been shown that mice [3,4] or rats [13] consuming alcohol liquid diets for 3–7 months show impairment in shock avoidance learning when tested

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2–18 weeks after ethanol was omitted from the diet. However, the possibility remained that the alcohol-induced impairment was specific to the shock avoidance situation. To test the generality of this result, rats were tested for the acquisition of a differential reinforcement of low rate response (DRL) following a period of prolonged consumption. When compared to shock avoidance, the DRL is food motivated rather than shock motivated, requires response suppression rather than rapid response initiation and acquisition is not contingent upon sensory processing of external stimuli. It was found that prolonged alcohol consumption resulted in severe impairment of the acquisition of the DRL task [14]. Taken together, these results strongly supported the conclusion that prolonged alcohol consumption, despite adequate nutrition, results in impairment of the associative processes of learning. The present experiment represents the first in a series of experiments which will attempt to characterize the nature of the alcohol-induced impairment. More specifically, we attempted to determine if prolonged alcohol consumption disrupted the retention of a previously learned response.

METHOD

Animals

The animals were 30 male Long Evans hooded rats, approximately 90 days old, weighing 250–300 g, purchased from Blue Spruce Farms, Altamont, New York.

Liquid Diets

The composition and nutritional adequacy of the liquid diets has been reported in detail previously [13]. Briefly, the experimental diets contained 35% ethanol-derived calories and were prepared from a 63.3% V/V stock solution of ethanol mixed with Metrecal Shape (Mead Johnson).

The final concentration of ethanol was 8.1% (V/V), providing 1.3 KCal/ml. Control diets were prepared in the same manner except sucrose was isocalorically substituted for ethanol. The diets were additionally fortified with Vitamin Diet Fortification Mixture, 0.3g/100 ml, and Salt Mixture XIV, 0.5g/100 ml (Nutritional Biochemical Corporation).

Apparatus

The apparatus consisted of a Lehigh Valley Electronics model #146-04 toggle floor shuttlebox enclosed in a Lehigh Valley sound-attenuating cubicle. The two compartments of the shuttlebox were separated by a metal barrier 3 1/4 in. in diameter. Shock was delivered by a Lehigh Valley Electronics model #113-04 constant current shock generator and scrambled by a Lehigh Valley model #113-14 dual shock scanner and scrambler, and programmed by Grason-Stadler Series 1200 solid state modules.

Procedure

The rats were trained in the shuttlebox avoidance task until a criterion of 12/15 avoidances had been attained during a single session. Each session (one session/day) consisted of 15 CS-UCS presentations separated by an intertrial interval of 30 sec. The CS was a compound stimulus of light and tone presented simultaneously. The tone was generated by a Mallory Sonalert (standard on Lehigh Valley shuttlebox). The light stimulus was the onset of a cue light in the compartment occupied by the animal at the beginning of each trial. The CS-UCS interval was 7 sec. UCS was a shock of 0.6 ma. Both CS and UCS remained on until the rat either avoided or escaped and were then terminated. The number of avoidances, the mean latency to respond following CS presentation, and the number of ITI responses were recorded in blocks of 15 trials.

After 30 sessions, 9 animals had not reached the 80% criterion and were eliminated from the experiment. The remaining 21 rats were divided into 3 groups of seven, matched for number of sessions to criterion.

For the following 4 1/2 months, one group was maintained on the alcohol-containing liquid diet, one control group was individually pair-fed the sucrose diet, and the remaining group received lab chow and water ad lib. Following this experimental diet period, all rats were placed on lab chow and water ad lib. for a period of 3 months before retention testing was begun. This time period was chosen to prevent any possible transient effects of alcohol withdrawal on performance and to evaluate the permanency of the retention deficit, if one occurred. We had previously found that the alcohol-induced deficit in acquisition of shuttlebox avoidance was present for at least 18 weeks following discontinuation of alcohol in the diet, the longest interval tested [4].

Retention of the avoidance response was tested by the presentation of the CS alone for five trials/day for two days. The conditions during this retention test were identical to those during acquisition, except no shock was delivered. The number of avoidances (responses occurring in less than 7.0 sec) and latency to respond follwoing CS presentation were recorded for the 10 retention trials. As an additional measure of retention, reacquisition of the avoidance response was initiated on the day following the last retention test. Ten reacquisition sessions of 15 trials each were given. The experimental conditions and CS and UCS parameters were identical to those during original acquisition. The number of sessions to reacquisition of the criterion of 12/15 avoidances (80%) were recorded for each rat.

RESULTS AND DISCUSSION

On the day of alcohol withdrawal each rat was given lab chow and water ad lib and was carefully observed for behavioral symptoms of withdrawal. No overt behavioral signs other than hyperactivity were observed. It should be noted, however, that the procedures used in the present experiment, as in those discussed previously [3, 4, 13, 14] differ considerably from those used to induce physical dependence in mice [2,15] or rats [6], since no weight reduction was used prior to the introduction of the experimental diets and the concentration of ethanol was not gradually increased during the treatment period. Furthermore, as has been discussed previously [13], it is likely that some adaptation to alcohol takes place during the extended treatment period.

Table 1 shows the mean body weights for alcohol, sucrose and lab chow groups during the 18 week treatment period. It is apparent that the rats consuming the experimental diets, either alcohol or sucrose, maintain their weight at levels equal to or slightly above rats allowed free access to lab chow and water. Mean daily ethanol consumption during this period was 8.0 g/kg/rat/day (SD: ± 0.8).

The relative changes in body weights of the three groups together with the level of ethanol consumption are compa-

TABLE 1

MEAN AND RANGE OF WEIGHT IN GRAMS FOR EACH GROUP

WEEK	ALCOHOL		SUCROSE		LAB CHOW	
	Mean	Range	Mean	Range	Mean	Range
0*	433	336–512	422	276–519	424	342–514
4	453	346–566	442	297–522	434	355–514
8	493	354–657	485	310–582	471	381–559
12	519	361–706	503	306–612	478	375–574
16	539	373–739	527	332–644	504	367–599
18†	573	393–772	558	357–668	501	378–594

*Weights taken on day experimental diets were begun.
†All rats given pelleted laboratory food and water ad lib.

TABLE 2

RETENTION DATA

	ALCOHOL					SUCROSE					LAB CHOW			
Rat	SC*	NA†	SR‡	% SAV.§	Rat	SC	NA	SR	% SAV.	Rat	SC	NA	SR	% SAV.
2	8	8	2	75	1	3	2	3	0	9	9	10	2	78
24	9	9	1	89	3	10	9	2	80	27	9	10	1	89
29	12	10	1	92	7☆	13	2	–	–	20	11	10	1	91
19	14	5	2	86	10	15	3	3	80	12	14	8	4	71
30	18	7	1	94	25	19	7	8	58	28	14	3	2	86
8☆	20	2	–	–	11	22	10	1	95	22	20	6	4	80
32	23	7	3	87	21	22	7	1	95	26	26	10	2	92
Mean	14.8	6.8	1.7	87	Mean	14.8	5.7	3.0	68	Mean	14.7	8.1	2.3	84
SD	5.7	2.7	0.8	6.7	SD	6.9	3.3	2.6	35.9	SD	6.2	2.7	1.2	7.7

*The number of sessions to criterion of 80% avoidances (12/15) on original acquisition.
†The number of avoidances during the 10 retention trials in which the CS alone was presented.
‡The number of sessions for reacquisition to 80% criterion.
§The per cent savings score calculated by subtracting SR from SC and dividing by SC.
☆Since the body weights of these rats were significantly above those of the other rats, their SR and % savings data are not included.

rable to previous reports in which rats were found to be severely impaired on the acquisition of different behavioral tasks [13,14] following a period of prolonged alcohol consumption. However, the results of the present investigation indicate that prolonged ethanol consumption does not impair the retention of a previously learned shock avoidance response.

Three measures of retention were considered: 1) the number of avoidances during the 10 presentations of CS alone (NA), 2) sessions to reacquisition (SR), and 3) the per cent savings. The results for each rat together with the group means and standard deviations are presented in Table 2. These results were subjected to nonparametric analysis of variance (Kruskal-Wallis). The animals in the three groups were initially matched on the pretraining sessions to criterion (SC), and these scores are shown in the second column of Table 2. Statistical analysis revealed that the groups were not significantly different on this baseline measure of acqui-

sition (H=0.696, $p>0.05$). Three months after ethanol had been discontinued from the diet (total of 7 1/2 months following original acquisition), retention testing was begun. The number of avoidances during the 10 presentations of CS alone was extremely variable within each group. Statistical analysis revealed that the retention scores of the three groups were not significantly different (H=0.289, $p>0.05$).

The sessions to reacquisition were not as variable as the previous measure, perhaps as a result of the prior experience with the training apparatus and procedures [7]. Kruskal-Wallis nonparametric analysis of variance revealed no significant differences between groups (H=0.861, $p>0.05$). The data for one rat in the alcohol group (#8) and one rat in the sucrose group (#7) were not included in the caluclations of the SR and per cent savings. Both of these animals failed to reach the 80% criterion level by the tenth session (when reacquisition training was terminated). The body weights of these rats were significantly above the other animals in the experiment and their poor reacquisition was attributed to a deficit in performance rather than retention.

The foregoing results indicate that prolonged alcohol consumption does not lead to an impairment in the retention of a previously learned response. This conclusion, however, must be qualified by two important factors. First, the 80% criterion used in pretraining may have resulted in overlearning, making this response less sensitive to the possible disruptive effects of chronic ethanol consumption. It is conceivable that responses learned to a less stringent criterion might be more sensitive to the potentially disruptive effects of ethanol. Secondly, the 7 1/2 month interval between training and tests for retention must be considered an extended time period. We suggest that in this situation the shock avoidance response could be considered a remote memory, which is consistent with the clinical descriptions of Korsakoff's patients since memories of childhood and adolescence often remain intact [8,12].

Although the results of the present experiment suggest that previously stored information is unaffected by chronic ethanol consumption, we believe that the retention of the shock avoidance response should be considered a remote memory; consistent with the clinical descriptions of Korsakoff's patients. It appears that the question of whether retrograde amnesia in an integral component of the symptomatology found in Korsakoff's patients or whether it is only an apparent retrograde amnesia resulting instead from a progressive deterioration in the ability to acquire new information, remains unclear [8, 10, 12]. Since it has been shown that 3 months, but not 6 weeks, of ethanol consumption in mice, results in the impairment of the acquisition of a shock avoidance response [4], experiments in which pretraining and tests for retention are more closely locked to this time course may provide some clarification of this question. Although we must be cautious, when generalizing from animal experimentation to the human condition, it is clear that the proper controls necessary in investigations of this sort can be attained in animal studies. The recent finding that prolonged alcohol consumption, concomitant with adequate nutrition, leads to an impairment in the associative processes of learning in mice [3,4] or rats [13,14] together with the results of the present experiment, indicate that this model may be useful in determining the mechanisms involved in the amnesia found in Korsakoff's psychosis.

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