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BRIEF COMMUNICATION

A Rapid Technique for Producing Ethanol Dependence in the Rat[1]

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CANNON, D. S., T. B. BAKER, R. F. BERMAN AND C. A. ATKINSON. *A rapid technique for producing ethanol dependence in the rat.* PHARMAC. BIOCHEM. BEHAV. 2(6) 831–834, 1974. – Ethanol dependence in the rat is demonstrated following 2–4 days of intragastric intubation with 8–12 g/kg/day. Withdrawal symptoms include tremulousness, hyperactivity, and seizures. Nutritional deficiency is shown to be an insufficient explanation of the withdrawal symptoms produced.

Ethanol Rat Dependence Seizures

IN the 1950's the alcohol withdrawal syndrome was first demonstrated to be the result of the cessation of excessive alcohol consumption rather than nutritional deficiency or intercurrent illness [13,19]. Since that time investigators have sought techniques for producing dependence in laboratory animals to permit well-controlled investigations of alcohol dependence and the alcohol withdrawal syndrome.

With the exception of a schedule-induced polydipsia technique [8], no animal studies in which alcohol consumption is voluntary have demonstrated dependence. However, dependence has been demonstrated through forced ingestion in the rhesus monkey [5,6], dog [7], mouse [9, 10, 11, 12, 18] and rat [1, 3, 16]. Mello [17] has recently provided a thorough review of methods used to induce alcohol addiction in animals.

The rat is a commonly used laboratory animal in other areas of research, and its use in the investigation of alcohol dependence permits direct use of the techniques and results of a large body of research. Reported withdrawal symptoms in the rat include tremulousness, spasticity, tonic-clonic seizures, fatal convulsions, hyperactivity, and an exaggerated startle response [17]. One study with rats as subjects reported alcohol withdrawal symptoms after more than four months of alcohol consumption maintained by scheduled-induced polydipsia [8]. In that study, evidence of withdrawal included fatal convulsions. Another study using rats [1] employed a modification of Freund's [9] ethanol-Metrecal diet for three weeks. Two of fourteen experimental animals in that study had unelicited seizures during withdrawal. Cicero *et al.* [3], restricted the fluid intake of rats to an ethanol-water solution for four months following weaning. The only withdrawal symptom reported was hyperactivity, but this study did demonstrate behavioral tolerance.

Majchrowicz [16] has reported the use of an intubation technique, similar to one employed with the monkey [5,6], to produce alcohol dependence in the rat. He reported that rats were administered ethanol at doses ranging from 12–15 g/kg/day over 1–12 days. He concluded that elevated blood ethanol concentrations for 3–5 days were required for a major withdrawal syndrome. The present paper reports the use of an intubation technique that produces physical dependence to alcohol in 2–3 days after the administration of a mean of 9.7 to 8.2 g/kg/day of alcohol.

METHOD

Animals

Twenty-four naive male Long-Evans rats with an initial

[1] Portions of this paper were presented at the 54th annual convention of the Western Psychological Association, San Francisco, California, April, 1974.

[2] The authors are grateful to David Osborne for determining the blood alcohol levels reported in this paper.

mean weight of 340 g were individually housed in wire mesh cages. Neither food nor water was available during the study. All animals were weighed every eight hours.

Procedure

Pre-withdrawal phase. Rats were assigned at random to 4 groups of 6 each. Group 1 received an initial 6 g/kg dosage of 45% (^{V}/v) ethanol-water solution. Subsequent dosages of a 22.5% (^{V}/v) ethanol-water solution varied from 0–3 g/kg, depending on behavioral intoxication criteria. Ambulatory animals received 3 g/kg. Non-ambulatory, conscious animals received 1.5 g/kg. No dose was given unconscious animals. Doses were scheduled at 8 hr intervals for 2 days (i.e., 6 doses, including the initial 6 g/kg dose). Because of dehydration of throat and mouth mucosa of experimental animals, intubation tubes were coated with a surgical lubricant. Group 2 received the same procedure but for 3 days for a total of nine doses. Group 3 was intubated once every 8 hr with a 315.5 g/l sucrose solution isocaloric to the 22.5% ethanol-water solution. Group 3 rats were given this solution for 2 days (6 doses). Group 4 was given the sugar solution for 3 days (9 doses).

Blood samples were drawn from 2 animals in Group 1 and 2 animals in Group 2 during the pre-withdrawal phase of the study. Samples were drawn from the 4 animals 1, 4 and 7 hours following the first and fourth scheduled doses, and from the two Group 2 animals 1, 4, and 7 hours following the seventh scheduled dose. Blood samples were drawn from the tail vein in 50 μl heparinized capillary tubes. BAC was determined by gas chromatography [4].

Withdrawal phase. An attempt to elicit seizures was made in all animals with auditory stimuli (key jangling and the striking of a tin pan with a spoon) and manipulation (picking rats up by their tails or turning them over repeatedly). These seizure tests were conducted at 2 hr intervals beginning 8 hr and ending 24 hr after the last scheduled intubation.

RESULTS

One animal in Group 1 and another in Group 2 died during the pre-withdrawal phase of the study, apparently of ethanol overdose.

The mean daily dosage for the remaining 10 rats in Groups 1 and 2 combined was 12 g/kg/day on the first intubation day and 7.5 g/kg/day the second day. The five Group 2 animals received a mean of 5.1 g/kg on the third day. Thus the animals in Group 1 received a mean of 9.7 g/kg/day across 2 days while those in Group 2 received a mean of 8.2 g/kg/day across three days. The mean individual dosage across groups was 2.93 g/kg.

The BAC of each of the 4 rats sampled during the pre-withdrawal intubation phase is shown in Fig. 1. Mean BAC rose from 150 mg/100 ml on the first day to 718 mg/100 ml the second. The mean BAC for the two Group 2 rats was 779 mg/100 ml on the third day even though neither animal had received the dose scheduled prior to this series of samples.

All Group 1 and 2 rats evidenced the withdrawal symptoms of tremulousness and heightened excitability during the withdrawal phase of the study, while none of the Group 3 and 4 rats showed these symptoms. The median length of time from the last dose until these symptoms developed was 14 hr. Three Group 1 and four Group 2

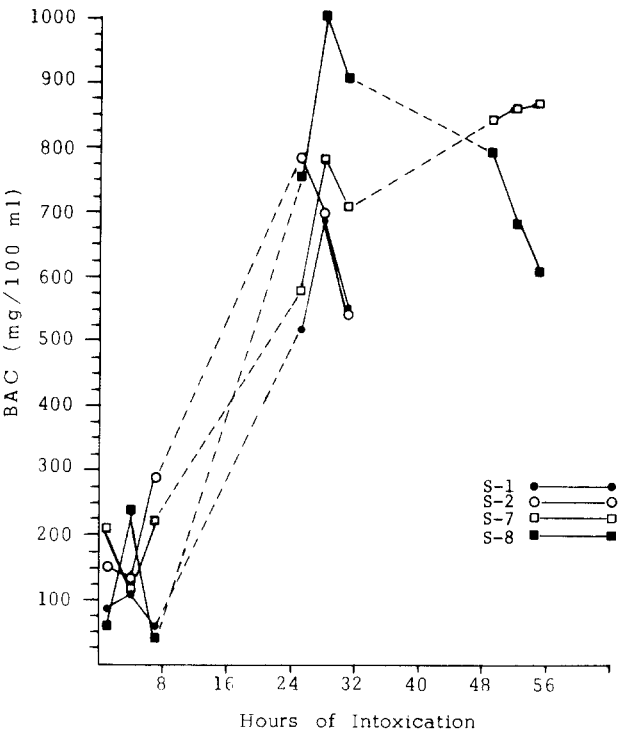

FIG. 1. Blood alcohol concentrations (mg/100 ml) as a function of hours of intoxication during the pre-withdrawal phase for animals S-1, S-2, S-7 and S-8. All animals were given a 6g/kg dosage at 0 hours. A 3g/kg dosage was scheduled at 8-hour intervals. S-7 was not given a dose at 48 and 56 hours. S-8 was not given a dose at 32 and 48 hours.

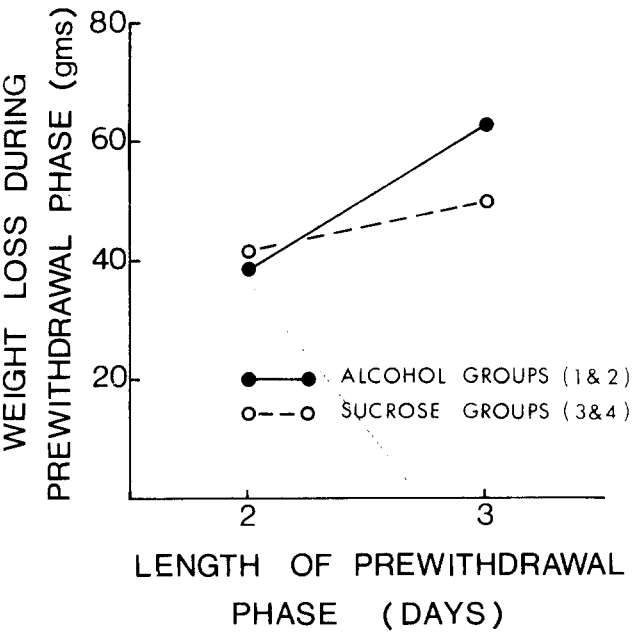

FIG. 2. Mean weight loss per group during the pre-withdrawal phase of the study.

animals had seizures during the withdrawal period, while none of the twelve control animals did. Seizures for 3 of the 7 animals in Groups 1 and 2 were of the tonic-clonic convulsive type, while the 4 remaining animals exhibited opisthotonos seizure activity including tetanic back arch, tail lift and head elevation with fasciculation of facial and forelimb muscles. The difference in seizure incidence between experimental and control groups was significant (Fisher Exact Test, $p<0.01$). The difference between Groups 1 and 2 was not significant. The median length of time from the last dose until an experimental animal had its first seizure was 16 hr. No tremulousness was observed 36 hr following the last dose.

There was not a significant difference in the initial mean weights of the four groups. Mean weight loss per group during the pre-withdrawal phase was analyzed by means of a 2 X 2 analysis of variance for unequal cell frequencies and is presented graphically in Fig. 2. Due to a significant interaction between treatment, i.e., ethanol or sucrose, and length of treatment, i.e., 2 vs. 3 days, $F(1,18) = 5.7$, $p<0.05$, tests of simple main effects were made. These tests indicate no difference between treatments in weight loss after 2 days, and no difference between 2 and 3 days for animals given sucrose. Group 2, given ethanol for 3 days, lost more weight during the pre-withdrawal phase than either the group given ethanol for two days, $F(1,18) = 25.9$, $p<0.01$, or the group given sucrose three days, $F(1,18) = 7.26$, $p<0.05$. Mean weight loss during the withdrawal phase (i.e., the period from 8 to 32 hr following the last ethanol or sucrose dose) was similarly analyzed and is shown in Fig. 3. Again, a significant interaction, $F(1,18) = 7.7$, $p<0.05$, necessitated analysis of simple main effects. Group 1, which received ethanol for two days, lost more weight during withdrawal than Group 2, which had been administered ethanol for three days, $F(1,18) = 19.9$, $p<0.01$. Thus, Group 2 lost more weight than Group 1 during its longer pre-withdrawal phase, but Group 1 lost more than Group 2 during the withdrawal phase. There was no difference in the weight loss of the sucrose groups during withdrawal. During withdrawal the two-day ethanol group lost more weight than the two-day sucrose group, $F(1,18) = 42.6$, $p<0.01$, and the three-day ethanol group lost more weight than the three-day sucrose group, $F(1,18) = 6.7$, $p<0.05$. An analysis of total weight loss from the first dose to 32 hours following the last dose indicates the ethanol animals lost more weight than sucrose animals, $F(1,18) = 25.4$, $p<0.01$, and animals with a three-day pre-withdrawal period lost more weight than those with only a two-day pre-withdrawal period, $F(1,18) = 9.4$, $p<0.01$. There was no interaction between treatment and the length of the pre-withdrawal phase on total weight loss. Mean total weight loss, expressed as a percentage of initial weight, was 23% for the two-day ethanol group, 27% for the three-day ethanol group, 19% for the two-day sucrose group, and 21% for the three-day sucrose group.

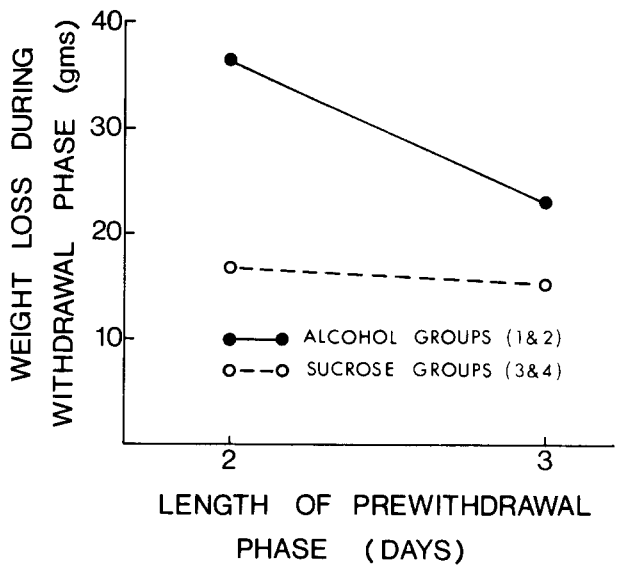

FIG. 3. Mean weight loss per group during the withdrawal phase of the study.

Several pilot studies using a four hour ethanol intubation schedule were conducted prior to the main study, and the results are summarized in Table 1. The four hour and eight hour schedules were similarly effective in producing alcohol dependency.

DISCUSSION

Intragastric intubation of ethanol in total daily amounts of 8–12 g/kg produces ethanol dependence in the rat in

TABLE 1

RESULTS OF PILOT STUDIES IN WHICH ANIMALS WERE GIVEN ETHANOL ONCE EVERY FOUR HOURS UNLESS UNCONSCIOUS

Hours on EtOH Regimen	Maximum Dose (g/kg)	Mean Dose (g/kg)	Mean Dose Day (g/kg/day)	Seizure Incidence
44	3	1.71	10.25	2/2
68	3	2.00	12.00	2/2
96	3	2.18	13.08	5/6
60	2	1.56	9.4	6/10

2–4 days. The withdrawal symptoms of tremulousness and hyperexcitability were observed in all rats following termination of ethanol intubation. Seizures were elicited in a total of 22 of the 30 experimental animals in the studies reported in this paper.

Nutritional deficiencies have been associated with the severity of withdrawal symptoms in previous studies [9,18]. While the studies reported in this paper do not indicate whether such deficiencies are necessary for the development of withdrawal symptoms, they suggest nutritional deficiencies are not sufficient. Control animals maintained on an isocaloric sucrose diet were equally deprived of vitamins and other essential nutrients. No control animals, however, evidenced any withdrawal symptoms. The greater weight loss by ethanol animals during the withdrawal phase may, in part, be due to their hyperactivity during that period.

The data do not demonstrate the superiority of maintaining rats on alcohol for three rather than two days prior to withdrawal.

Figure 1 indicates that the BAC's of two experimental animals (S-2 and S-7) were higher seven hours following the initial 6 g/kg dosage of 45% ethanol than they were at one and four hours following the dose. This finding is consistent with studies reviewed by Kalant [14] which indicate that ethanol in concentrations of 30–40% or more can produce pylorospasm, thereby retarding absorption. Figure 1 also indicates no decrease in BAC of S-7 from the 49th to the 55th hour of intoxication even though the dose scheduled for the 49th hour was not given. A possible explanation for the failure of the BAC to decrease during this six hour period is suggested by the report of Carré and Trémolières [2] that ascitic fluid may contain a higher ethanol concentration than the blood, but that the two concentrations are equalized as ethanol is metabolized. The BAC's of S-7 and S-8 from the 49th to the 55th hour of intoxication (i.e., 9–15 hours following their previous dose) remained high even though the dose scheduled for Hour 48 was not given. This demonstrates that when doses are not given due to the behavioral criteria used in this study, BAC's remain high over a 16 hour interval. Failure to use behavioral criteria to individualize dosages was fatal in several cases.

The amount of ethanol administered per day decreased across days. Group 2 animals received a mean of 12 g/kg/day the first day of the pre-withdrawal period and a mean of 5.1 g/kg/day the third day. Part of this decrease was undoubtedly due to the fact that the rate at which ethanol is metabolized is reduced by 50% in fasted animals [15].

This technique is much quicker than others reported thus far for producing ethanol dependence in the rat. Mello [17] points out that a forced administration technique such as this is of little value to investigators interested in studying the determinants of voluntary acquisition of ethanol dependence. However, to those interested in dependence per se and its sequalae, the technique described in this paper should be very useful because of the minimal amount of equipment and time required to produce dependence.

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