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Simple Method for Producing an Alcohol Withdrawal Syndrome in Rats[1]

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MUCHA, R. F., J. P. J. PINEL AND P. H. VAN OOT. *Simple method for producing an alcohol withdrawal syndrome in rats.* PHARMAC. BIOCHEM. BEHAV. 3(5) 765–769, 1975. – Rats received intragastric intubations of ethanol at 8 hr intervals for 1, 7, 15 or 30 days. The dosage for each animal was one which produced observable signs of intoxication 1 hr after the intubation. All of the rats in the experimental groups developed a tolerance to ethanol as indicated by the increasing dose required to induce intoxication, but the degree of tolerance was related to the duration of the ethanol administration. During the withdrawal period the incidence of hyperreactivity, convulsive symptoms, and the susceptibility to audiogenic seizures was determined for all 4 groups. Although every experimental animal displayed withdrawal symptoms, the incidence of these symptoms was found to be an increasing, negatively accelerated function of the duration of ethanol exposure. For situations where voluntary consumption of alcohol is not necessary this method is a simple, controlled, reliable, way of inducing ethanol tolerance and physical dependence in rats.

Ethanol Withdrawal syndrome Intragastric intubation Tolerance

A simple, reliable procedure for producing an alcohol withdrawal syndrome in rats would greatly facilitate the study of this phenomenon but periods of ethanol exposure as long as several months are usually required to produce an alcohol withdrawal reaction [2,3]. In those studies where ethanol dependence has been induced in rodents in shorter periods of time, the procedures have typically been complicated by the need for specialized equipment, the use of drugs to inhibit alcohol metabolism, or by the inadvertent development of extreme weight loss [1, 4, 7]. The purpose of the present paper is to report a simple technique for rapidly inducing alcohol tolerance and physical dependence in rats which circumvents these difficulties. In this report physical dependence and tolerance were measured following different durations of ethanol exposure.

METHOD

Animals and Surgery

Twenty-six naive, male black-hooded rats purchased from the Canadian Breeding Laboratories (La Prairie, Quebec) were used. Each rat was implanted under combined sodium pentobarbital (30 mg/kg) and chloral hydrate (125 mg/kg) anesthesia with 2, unipolar, epidural recording electrodes constructed of stainless steel jeweller's screws. One electrode was centered above each cerebral hemisphere and a similar reference electrode was placed rostal to the suture nasofrontalis. Following implantation, each rat was routinely injected with 0.2 cc of Metrazol and 0.2 cc of penicillin. Following at least 3 weeks of postoperative recovery, the animals were randomly divided into 4 groups which were intubated with alcohol at 8 hr intervals for 0 ($n = 5$), 7 ($n = 5$), 15 ($n = 8$), or 30 ($n = 8$) days. The beginning of alcohol administration was staggered for the different groups so that all the animals underwent withdrawal on the same day.

Procedure

All experimental animals were first intubated with a low, standard dose of 1,000 mg/kg of ethanol in a 20 percent volumetrically prepared solution and subsequent levels of ethanol administered to each individual animal were determined by its intoxicating effects. Since the initial dose was never sufficient to produce symptoms of intoxication, subsequent doses administered to each animal were increased for each intubation by 200 mg/kg until a dose was reached which produced symptoms of intoxication. A particular dose was employed until it failed to have an intoxicating effect, whereupon it was increased again by 200 mg/kg. For each animal the presence or absence of intoxication was assessed by a test administered 1 hr following intubation. An animal was considered intoxicated

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when it exhibited an impairment of 2 of the following 3 behaviors: general homecage activity, ambulatory behavior, and climbing ability. Specifically, signs of intoxication were the following: (1) comatose homecage behavior, (2) an irregular gait on a flat wooden surface and (3) failure to climb from the center of a vertical 25 x 60 cm wire mesh (1.5 cm squares) by falling off or by freezing for the 30 sec test period. For the first few days intoxication was assessed after each intubation, but following the third day, intoxication was assessed only once a day.

All animals were individually housed with continuous access to food and water. Their weights were monitored daily and the amount of alcohol administered was computed from these values. If a rat's weight fell below 90 percent of its weight measured before the beginning of alcohol exposure, the ethanol was administered as a 20 percent (v/v) solution in Metrecal (Mead Johnson Company). This dietary supplement was administered with each intubation until the animal's weight rose above the 90 percent criterion level.

The assessment of withdrawal effects commenced 9 hr after the last ethanol intubation. Each animal was observed individually every 2 hr over a 14 hr period and the presence or absence of hyperreactivity and convulsive symptoms was recorded. An animal was judged hyperreactive if it displayed marked resistance to handling upon removal from its homecage. During the alcohol exposure period there was seldom a negative reaction to handling, whereas during withdrawal, emotional behavior was commonly observed. Such behavior included crouching and shaking in response to the approaching hand of the experimenter and squealing, biting and twisting during actual handling. Convulsive activity was assessed by observing each rat for 10 min in a clear 15 x 20 x 40 cm Plexiglas box and recording the presence or absence of the following symptoms: headshakes, rhythmic facial twitches, generalized tremors, wet-dog shakes, forelimb clonus or body tonus. Moreover, on the second and seventh observation sessions electrographic activity was recorded from each hemisphere by measuring potential changes between each of the epidural electrodes and the reference electrode. Animals were placed in a grounded Faraday cage and cortical activity was monitored through shielded leads. The brain activity was amplified and displayed by a Model 78B Grass polygraph with a 1/2 low freq. amplitude filter fall-time constant setting of 30 msec.

The scoring of convulsive symptoms and hyperreactivity was discontinued after the eight observation session, 23 hr after the last inbubation. Then over the next 12 hr, 6 bihourly tests of audiogenic seizure susceptibility were administered. On each test an 86 ± 2 dB bell was sounded for 80 sec or until it elicited a running fit. A running response was scored as a seizure only if it outlasted the bell.

RESULTS

Physical dependence and tolerance were produced following as little as 1 week of ethanol exposure, but the severity of both increased in a negatively accelerated manner with increases in the duration of ethanol exposure. Changes in the dosage necessary to produce intoxication, as defined by our rating procedures, were used as a measure of tolerance. It was seen that during the alcohol exposure period every experimental animal developed some degree of tolerance, i.e., required an increase in the dosage to produce intoxication. Using the difference between the first and the last intoxicating dose the animals in both the 15 and 30 day groups displayed a significantly greater development of tolerance than the 7 day animals ($U = 0$, $N_1/N_2 = 5/8$; $p<0.001$ in both cases) but they did not differ significantly from each other ($U = 21$, $N_1/N_2 = 8/8$; $p>0.05$). The mean changes for the 7, 15, and 30 day groups were 360 ± 67.0, 1650 ± 91.9 and 1488 ± 113.1 mg/kg of alcohol respectively and the mean doses on the final intubation were 2840 ± 742, 3450 ± 140 and 3475 ± 112 mg/kg respectively (± SE).

The incidence of the withdrawal symptoms increased over the first 3 tests, 9, 11, and 13 hr after the last injection, but thereafter the manifestation of both the hyperreactivity and convulsive symptoms remained asymptotic up to and including the eighth and final test at 23 hr. Each animal was assigned a score between 0 and 8 for both hyperreactivity and convulsive symptoms which was the number of observation sessions in which any behavioral symptoms of these conditions were observed. The means of these scores for each group are presented in Fig. 1. In terms of these overall scores all 3 experimental groups were significantly different from controls for both measures (all Mann-Whitney comparisons were significant at $p<0.004$ or better). Among the experimental groups, the incidence of convulsive activity was found to be higher in the 30 day as opposed to the 7 day group ($U = 2.5$, $N_1/N_2 = 5/8$; $p<0.004$), but the differences between the 7 and 15 day groups, and between the 15 and 30 day groups were not significant ($p>0.05$). Hyperreactivity followed a similar pattern with only the difference between the 7 and 30 day groups being significant ($U = 4$, $N_1/N_2 = 5/8$; $p<0.009$).

With respect to other measures of withdrawal, electrographic recording indicated the presence of obvious cortical epileptiform spiking in 2 animals, one in each of the 15 and 30 day groups. For both animals this epileptic activity was in the form of 2 to 6 sec bursts of spike activity that ranged in amplitude from 200 to 500 μV and in frequency from 8 to 10 per sec. In addition, susceptibility to audiogenic seizures was also found to be related to the duration of alcohol exposure prior to withdrawal (Fig. 1). The number of animals showing susceptibility to audiogenic seizures in the 7 day group was not significantly different from the control value of zero, (Fisher Exact Probability, $p>0.05$) whereas the 15 and 30 day groups had a significant number of animals displaying audiogenic seizures (Fisher Exact Probability, $p<0.016$) but no significant difference was found between the incidence of audiogenic seizures in these two groups (Fisher Exact Probability, $p>0.05$).

The physical dependence and tolerance produced with the present technique was demonstrated without the drastic weight losses typical of many other procedures. Presented in Table 1 are the mean body weights of the rats prior to surgery, prior to the first intubation and at withdrawal. Statistical tests indicated no significant change in the weights of the 15 and 30 day animals between the first and last day of alcohol exposure (sign tests, $p>0.05$). The animals in the 7 day group, however, showed a slight but consistent decrease (sign test, $p>0.05$). A similar decrease in weight over the first week of alcohol exposure was also observed among animals in both the 15 day and 30 day groups (sign tests, $p<0.05$), with this significant decrease disappearing by the second week. Also presented in Table 1 is the number of animals in each group that received the Metrecal supplements. In each case the Metrecal was sufficient to restore the animal's weight so that at with-

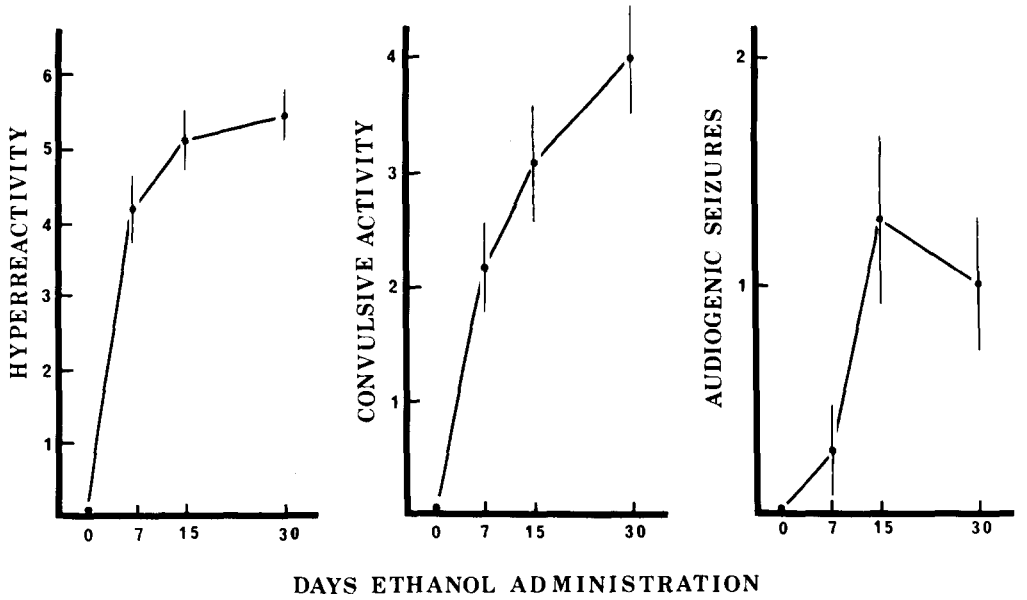

FIG. 1. The incidence of withdrawal symptoms as a function of the duration of alcohol exposure. Each point represents the mean ± SE number of tests during which a given symptom was observed for animals in that group. Eight tests of hyperreactivity and convulsive symptoms were administered while audiogenic seizure susceptibility was assessed a total of 6 times.

drawal all animals were well within the 10 percent weight loss criterion.

DISCUSSION

The method described in this report was found to be an effective technique for producing ethanol tolerance and physical dependence in rats with a relatively short period of ethanol exposure. Regardless of the particular procedure, most reports describing techniques for producing the withdrawal syndrome have typically described the effects of only a single alcohol exposure period. The problem with such reports is that they do not indicate whether the same syndrome could have been produced with shorter periods of exposure. However, we studied 4 periods of ethanol exposure and the negatively accelerated shape of all 3 of the curves in Fig. 1 implies that continuing the exposure beyond 30 days would not have led to an appreciable increase in the freqeuncy of the observed symptoms of withdrawal. Similarly, tolerance developed following only one week of alcohol intubation and approached a maximum after two weeks of exposure.

A number of other experimental procedures have been used to produce ethanol dependence in rats [12]. There are two methods for producing the alcohol withdrawal syndrome which have the advantage of allowing the rats to consume the alcohol themselves. The first procedure has been to limit fluid and/or food intake to an ethanol solution [1,4]. Branchey, *et al.* [1], for example, produced a withdrawal syndrome by maintaining rats at 66 percent of their normal body weight and restricting their diet to a Metrecal-ethanol solution for 3 weeks. The second procedure of this type involves the use of a schedule-induced polydipsia procedure to induce a withdrawal reaction. Falk

TABLE 1

MEAN BODY WEIGHTS AND NUMBER OF ANIMALS IN EACH GROUP RECEIVING METRECAL SUPPLEMENTS

Days of Alcohol Exposure	Mean Weight Prior to Surgery (± SE)	Mean Weight at First Intubation (± SE)	Mean Weight at Withdrawal (± SE)	Number of Animals Administered Metrecal
0 (n = 5)	249 ± 12.5	–	410 ± 15.5	–
7 (n = 5)	248 ± 10.7	386 ± 26.2	370 ± 25.6	0
15 (n = 8)	258 ± 6.3	397 ± 16.3	376 ± 13.9	1
30 (n = 8)	253 ± 6.9	352 ± 9.6	352 ± 9.1	4

et al. [3] maintained rats at their normal body weight and presented them with dry food pellets every 2 min during six 1 hr daily sessions. Following establishment of polydipsia with water, ethanol was added to the water in gradual increments until 6 percent solutions were reached. After 3 months on this regimen the alcohol was withdrawn and withdrawal symptoms ensued. Although both of these methods do seem to produce withdrawal symptoms similar to those reported here, they have two major shortcomings; both methods produce extreme weight loss in the animals and neither allows the experimenter to control the rate and pattern of ethanol consumption.

There are, however, techniques of producing the abstinence syndrome in rodents that do permit the strict experimental control of ethanol administration. Goldstein and Pal [8], for example, injected mice with pyrazole to inhibit alcohol metabolism and then exposed them to alcohol vapor. A withdrawal syndrome developed after only 3 days of exposure. This procedure, however, is complicated not only by the effects of weight loss (10–14 percent) but also by the toxic effects of pyrazole on liver function [12]. A number of variations of this technique have been employed which eliminate one or both of these problems, but generally speaking, when these problems are solved they are replaced by new ones. This is best illustrated by two recent experiments. First, Roach *et al.* [18] produced mild to severe withdrawal symptoms after 7 days without using pyrazole, but in this case the rats experienced an even more severe weight loss than that observed by Goldstein and Pal (average of 20.2 percent). Second, French and Morris [4] reported an inhalation technique employing low ethanol vapor concentrations which does not require pyrazole or produce weight loss, but unfortunately, no symptom of withdrawal more severe than an increase in reactivity to footshock was observed.

A number of investigators have reported procedures similar to our own but there are 3 independent aspects of the present technique which differ enough from the other intubation procedures to warrant emphasis. Any combination of these may have contributed to the reliability of the present results. First, if ethanol is to be present in the blood for a significant proportion of the exposure period, it is clear that alcohol must be administered in intoxicating doses more than once a day as employed by other investigators with the apparent exception of Majchrowicz [11] and Hunt [9]. Gibbins *et al.* [6], for example, administered daily increasing doses of alcohol to rats for 30 days. Following withdrawal there was a reduction in the startle threshold to electric shock but no spontaneous symptoms were observed other than hyperreactivity to handling. Majchrowicz [11] and Hunt [9], however, employed 3–5 daily doses of ethanol and produced severe withdrawal symptoms in their rats after as little as 5–6 days of exposure.

In the present experiment, 3 intoxicating doses of ethanol administered daily via the intragastric route proved to be a most practical regimen of ethanol administration. Intragastric administration was employed since it has been shown that ethanol administered by the intragastric route remains in the blood longer than ethanol administered intraperitoneally [14]. Since no obvious withdrawal signs were observed between intubations, it is probably safe to conclude that blood alcohol levels did not fall to zero during the intubation phase. We hesitate, however, to extend this conclusion to longer exposure periods, since there are indications that continued exposure to high doses of ethanol increases the rates of blood ethanol decline [15].

The second notable aspect of the present procedure was that severe weight loss, a confounding factor in many other procedures, was avoided. The importance of controlling this factor is apparent from the retarding influence that weight loss has on alcohol metabolism [5,14]. These decreases in the rate of metabolism may facilitate the production of dependence by allowing a more consistently high level of blood ethanol, but they also complicate the demonstration of a withdrawal syndrome because of the slower removal of blood ethanol after the last administration. In the present study this source of variance was specifically avoided by an ad lib food supply and the administration of dietary supplements when necessary.

A final important aspect of our technique was that every dose administered was intoxicating and individually determined for each rat. Other intubation procedures have generally administered prescribed doses of ethanol to all the rats. As a result, in the present situation every experimental animal developed tolerance and physical dependence. It is apparent from the literature, however, that similar results may be produced using the same series of doses for each animal provided that the fixed doses are high (11000–15000 mg/kg/day) [9,11]. This would suggest that our use of a method employing individually determined doses was unnecessarily complicated. However, we continued to employ it after noting in pilot experiments that a prescribed dose regimen resulted in serious health and weight losses in some animals that were difficult to offset. With the present method this was less of a problem since each animal received the minimum dose sufficient to produce intoxication.

Recently, studies on neurotransmitter involvement (cf. [16,17] and on the characteristics of subcortical and cortical electrical activity (cf. [10]) during ethanol withdrawal have been carried out. In studies such as these where self-administration of ethanol is not necessary, the procedure described in this report represents a highly controlled method of rapidly producing ethanol tolerance and physical dependence in rats. The simplicity and effectiveness of this technique should make it a valuable addition to those techniques already available for producing an alcohol withdrawal syndrome in experimental animals.

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