Pharmacology Biochemistry & Behavior, Vol 16, pp 501–507, 1982 Printed in the U S A

Induction of Dependence on Ethanol by Free-Choice Drinking in Alcohol-Preferring Rats[1]

M B WALLER, W J McBRIDE,[2] L LUMENG AND T -K LI

Departments of Psychiatry, Medicine and Biochemistry
Indiana University School of Medicine, V A Medical Center
and the Institute of Psychiatric Research, Indianapolis, IN 46223

Received 31 July 1981

WALLER, M B , W J McBRIDE, L LUMENG AND T -K LI *Induction of dependence on ethanol by free-choice drinking in alcohol-preferring rats* PHARMAC BIOCHEM BEHAV **16**(3) 501–507, 1982 —Studies were performed to examine whether chronic, voluntary consumption of ethanol by the selectively-bred, alcohol-preferring P-rats produces physical dependence Body weight reduction, food restriction and flavoring the 10% ethanol solution increased ethanol consumption from 7 to 14 g ethanol/kg body weight/day when water was freely available Under similar conditions, consumption by selectively-bred, alcohol-nonpreferring NP-rats increased from 1 to 12 g/kg/day Removal of ethanol after eight weeks induced physical signs of withdrawal in both lines of animals In two subsequent studies, P-rats were given food, water and unflavored 10% ethanol ad lib for 15 and 20 weeks, ethanol consumption was 7 2 and 5 6 g/kg/day, respectively Upon removal of ethanol, manifestations of withdrawal, scored blind in one experiment, developed in 85% of the animals and persisted for 72 hours Importantly, none in the control groups of P and NP rats given water only exhibited these signs The ethanol withdrawn groups were hyperactive in both the open-field and the head-poke apparatus These results indicate that sufficient ethanol was voluntarily consumed by the selectively-bred alcohol-preferring P-rats under free-feeding conditions to produce physical dependence

Ethanol Alcohol-preferring rats Oral ethanol consumption Physical dependence Withdrawal syndrome

TWO lines of rats, one with a natural preference for ethanol, the P line, and the other with an aversion to drinking ethanol solutions, the NP line, have been raised in our laboratories by selective breeding [12,14] The P line of animals consume 25% or more of their daily calories as ethanol when both food and water are available ad lib This consumption is not contingent on caloric restriction and the amount approaches their apparent maximum capacity for ethanol elimination [12] The amount consumed remains constant when the concentration of the ethanol solution is varied from 10 to 30% or when both water and the 10% ethanol solution are flavored with sucrose [14] On the other hand, caloric restriction in combination with the flavoring of ethanol enhances ethanol consumption in the P line of rats [12,15] Importantly, after training to bar-press for reward, the P-rats will work in order to obtain the ethanol, even with concurrent access to food and water [18]

This communication reports that the chronic free-choice drinking of 10% (v/v) ethanol can lead to physical dependence in the P line of rats A study was first performed in weight-reduced P and NP animals given water and a 10% ethanol solution, flavored with saccharin and NaCl, in order to establish the manifestations of the withdrawal syndrome in these selectively bred animal lines are similar to those previously observed in unselected rats [9,16] In subsequent experiments, the P rats were given food ad lib and free-choice of water and ethanol, without flavor additives The assessment of withdrawal was performed in both unblinded and blinded fashion In addition, the pattern of alcohol drinking by P rats in relation to the light-dark cycle was monitored during free access to food, water and ethanol

METHOD

Animals

Adult, male, alcohol-preferring (P) and -nonpreferring (NP) rats from generations S12-18 were housed individually in a temperature- and humidity-controlled environment with a 12 hr day-night cycle (8 a m –8 p m , light and 8 p m –8 a m , dark) Each animal was first tested for alcohol preference by a procedure reported previously [14] For these studies, the criteria of selection for P animals were >5 0 g ethanol/kg body weight/day and an alcohol (10%, v/v) water drinking ratio of >2 1 (v/v) For the NP animals the criteria were <1 5 g ethanol/kg/d and an alcohol water drinking ratio of <0 2 1 (v/v) Rats meeting these criteria were randomly divided into ethanol-exposed (experimental) and water-only (control) groups for each experiment

[1]Supported by PHS AA-03243
[2]Recipient of Research Scientist Development Award MH-00203

The availability of food (Wayne Lab-Blox, Allied Mills, Inc , Chicago, IL), water and 10% (v/v) ethanol is described below for the individual experiments

Withdrawal Testing

After discontinuation of ethanol, the spontaneous behavior of each animal was assessed in an open-field arena and in a head-dip apparatus similar to those previously described [4,19] In the open-field, grids crossed, rearing, grooming, rotation within a square and the number of fecal pellets excreted were recorded for 3 minutes A total activity score was calculated for each animal by summing the number of grids crossed, rearings, rotations and grooming episodes Head-dips or head-pokes and rearing by the animals in the head-dip apparatus were counted for a 10 minute period

Physical signs of dependence were evaluated according to the criteria described by Hunter *et al* [9] and Majchrowicz [16] Briefly, the stages were I, tail stiffening, II, tail arching, broad-based gait, III, hypoactivity, mild tremor, hyperreactivity, IV, wet-dog shakes, teeth chatter, V, audiogenic seizure (induced by sound from a bell for a maximum of 60 seconds) In addition, bizarre behaviors (e g , stereotyped body movements, aimless locomotion) similar to those reported by Majchrowicz [16] were recorded

Each animal was observed individually for spontaneous activity and physical signs of withdrawal, at 20 hours before ethanol was removed, at 2, 4, 6 and 8 hours after the removal of ethanol on the first day of withdrawal and at 24, 48 and 72 hours thereafter Susceptibility to audiogenic seizure was assessed only at 3 and at 7 hours In Experiment 3, the animals were retested at 168 hours On the day of withdrawal, the animals were observed between 10 a m and 4 p m Retesting at 24–72 hours and at 168 hours postwithdrawal was done between 8 a m and 9 a m The assessment of withdrawal was always conducted in a room other than that in which the animals were housed

Unless otherwise indicated, the results are expressed as mean values±S E M Student's t-test was used to determine the statistical significance ($p<0$ 05) of the differences between the means

Experiment 1

Eighteen rats of the P-line (S12-13 generations) and 16 of the NP-line (generations S12-13) were randomly subdivided into P-experimental, P-control, NP-experimental and NP-control groups of similar size The experimental groups were given the free-choice of 10% ethanol and water whereas the control groups received water only After baseline values for food, water and 10% ethanol intakes were established, the animals were reduced to 80% of their free-feeding weight, and maintained at that level by adjusting the amount of food provided daily Water and 10% ethanol remained freely available to the experimental groups After three weeks, saccharin (0 125 g %) and sodium chloride (1 0 g %) were added to the ethanol solution and offered along with unflavored water Food, water and ethanol intakes and body weights were monitored daily throughout the experiment After a total of 8 weeks of free-choice drinking by the experimental groups, alcohol was discontinued at the end of a dark period and all animals were evaluated by an unblinded observer for physical signs of withdrawal

Experiment 2

In this experiment, 13 P rats of the S13–14 generations were given food ad lib and the free-choice drinking of unflavored 10% ethanol and water A control group (generations S13–14) of equal size was given free access to food and water only Water and ethanol intakes were monitored daily and the animals weighed each week After 15 weeks, the alcohol was discontinued at the end of a dark period and both ethanol-exposed and water-only animals were evaluated by an unblinded observer for both spontaneous activity and physical signs of withdrawal In a separate experiment, NP animals were similarly studied the experimental group (N=6) was given food ad lib and free-choice drinking of unflavored 10% ethanol and water, while the control group (N=6) was given food ad lib but only water to drink The experimental group consumed less than 1 5 g ethanol/kg/day The experimental and control groups were indistinguishable behaviorally when the ethanol was removed from the experimental group after 15 weeks

Experiment 3

Thiry-eight adult male rats of the P-line (S14–18 generations) were randomly divided into ethanol-exposed (experimental) and water-only (control) groups of equal size Food, water and 10% ethanol were freely available to the experimental group and food and water only to the control group throughout the experiment Water and alcohol consumption was monitored daily and the animals weighed weekly In addition, drinkometers (Columbus Instruments International Corp , Columbus, OH) were used to monitor alcohol drinking patterns in 8 of the experimental animals After 20 weeks of free-choice drinking, alcohol was removed at the end of a dark cycle Spontaneous activity and physical signs of withdrawal in both experimental and control animals were then scored by a blinded observer, in order to eliminate observer bias

RESULTS

Alcohol Consumption and Pattern of Drinking

In Experiment 1, with water freely available, food restriction and the addition of saccharin and sodium chloride to the 10% ethanol solution increased mean alcohol consumption from 7 0 to about 14 0 g of ethanol/kg body weight/day in the P rats and from 1 0 to 12 0 g/kg/day in the NP animals We have shown previously that, under similar conditions, weight-restricted P rats exhibit blood alcohol concentrations in the range of 80–250 mg % [15] In Experiment 2 and 3, with food, unflavored 10% ethanol and water available ad lib, ethanol intakes by the P rats averaged 7 2 g/kg/day and 5 6 g/kg/day, respectively These amounts are typical for a P rat, the variation being largely attributable to differences in body weight

Figure 1 shows the mean ethanol consumption and mean body weight of P rats during the 20 weeks of free-choice drinking in Experiment 3 The mean amount of ethanol consumed per day remained constant at 2 7 g However, mean body weight increased from 435 g to 520 g during the same period Accordingly, the mean ethanol intake of 6 7 g/kg/day at the start of the experiment decreased to 4 5 g/kg/day just prior to withdrawal Importantly, initial body weight and the subsequent rate of weight gain during the 20 weeks did not differ significantly from those of the control group given free access to food and water only

TABLE 1

PHYSICAL MANIFESTATIONS OF WITHDRAWAL IN P AND NP RATS AFTER CHRONIC FREE-CHOICE DRINKING OF 10% ETHANOL*

		Experiment 1 Weight-Reduced		2 Free-Fed	3† Free-Fed
Maximum Stage Attained on Day 1		P	NP	P	P
0		1	0	2	1
I	(Tail Stiffening)	0	0	0	0
II	(Straub Tail, Broad-Based Gait)	0	1	1	2
III	(Muscle Fasciculation, Hyper-Reactivity, Etc)	3	3	2	5
IV	(Wet-Dog Shakes, Teeth Chatter, Etc)	2	3	8	11
V	(Convulsions)	3	1	0	0
	Total	9	8	13	19

*None of the water-only control P animals developed physical signs of withdrawal
†Assessed by blind observer

While generally recognized that rats drink more during the dark cycle [21,22], the pattern of alcohol intake by P rats in relation to the light-dark cycle has not been defined As measured with drinkometers in 8 rats over 20 weeks, the percentage of licks for ethanol made by P rats during the light cycle remained rather constant at about 25% of the 24 hr total In the first two weeks, daylight licking was highly variable, ranging from 19–43% of total licks However, with continued exposure to ethanol, variability decreased to 20–33% of the total licks for alcohol We have shown previously that voluntary ethanol consumption by free-fed P rats produces blood ethanol concentrations in the range of 10–80 mg % during the dark cycle [12,15]

The voluntary ethanol consumption of P rats occurred in bursts, distributed rather evenly over the 24 hour period A typical pattern for a single animal on consecutive days is shown in Fig 2 Although the pattern varied from day-to-day, the maximum difference in the number of daily licks for alcohol during the light period was only 170 and, for a complete day-night cycle, 750 licks (Fig 2, day 1 vs day 3)

Physical Signs of Withdrawal

Table 1 summarizes the physical manifestations of withdrawal, shown as the maximum stage of withdrawal attained on day 1 in the 3 experiments In Experiment 1, the daily consumption of 14 and 12 g of ethanol/kg body weight by the weight-restricted P and NP animals, respectively, for 8

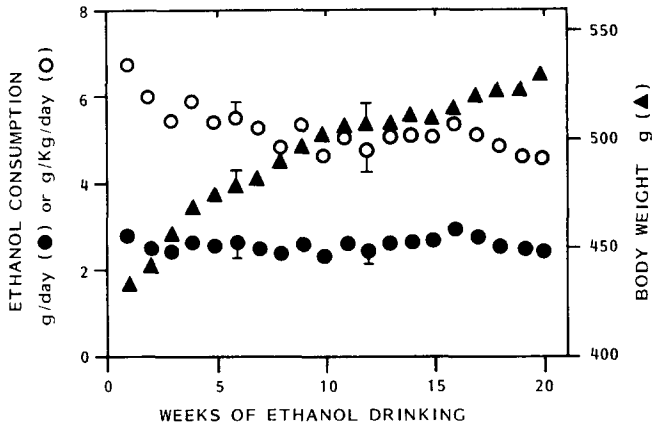

FIG 1 Ethanol (10% v/v) intake and body weight of P rats during 20 weeks of free-choice drinking The amount of ethanol consumed is expressed on the left ordinate as g/day (●) and g/kg/day (○) Body weight expressed in grams (▲) is on the right ordinate Results shown are the mean values±S E M for 19 animals

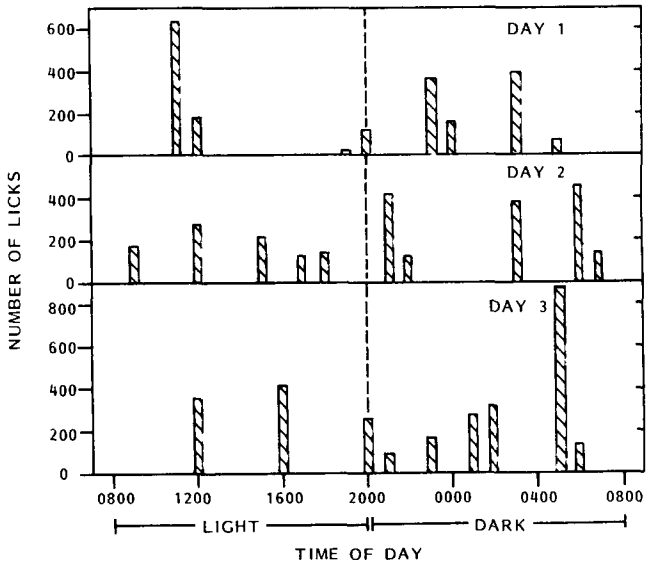

FIG 2 Illustrative pattern of voluntary ethanol (10% v/v) consumption on consecutive days by a single P rat The results shown are the number of licks per hour

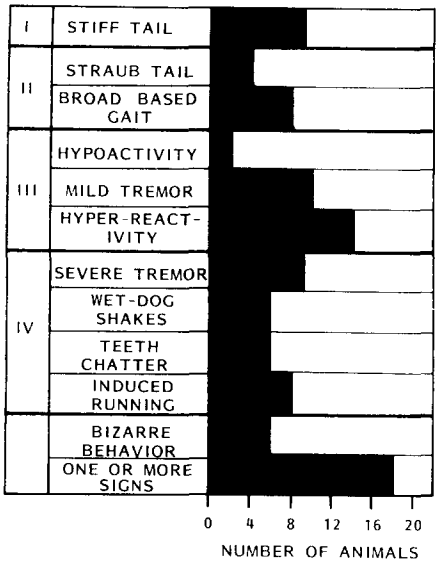

FIG 3 Physical signs of withdrawal, scored by blind assessment, in 19 P rats after 20 weeks of voluntary ethanol (10% v/v) consumption Bars indicate the number of animals exhibiting each sign Roman numbers refer to Stages after Hunter *et al* [9] and Majchrowicz [16]

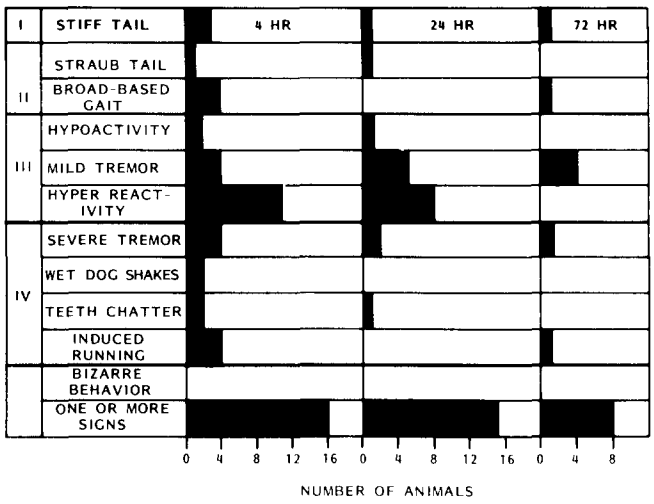

FIG 4 Physical signs of withdrawal scored by blind assessment at 4 24 and 72 hours postwithdrawal, in P rats after 20 weeks of voluntary ethanol (10% v/v) consumption See Fig 3 for details

weeks resulted in physical signs typical of the abstinence syndrome [9,16] in 8 of 9 ethanol-exposed P rats (Stage III–V) and in all 8 ethanol-exposed NP rats (Stage II–V) Sound from the bell induced convulsions (Stage V) in 3 of the P animals and in 1 of the NP animals None of the control P and NP animals exhibited any signs of withdrawal

In Experiment 2, the free-choice, daily consumption of 7 2 g of unflavored 10% ethanol by P rats for 15 weeks produced physical signs of withdrawal in 11 of 13 animals All animals had food and water available ad lib While 8 of the 11 animals attained Stage IV of withdrawal, no audiogenic seizures were observed Again, none of the P animals in the control group exhibited signs of withdrawal

Experiment 3 replicates the conditions of Experiment 2 with the exception that the rating of withdrawal signs was performed by blind assessment The duration of ethanol exposure was extended to 20 weeks because the mean daily ethanol consumption was somewhat lower, 5 6 g/kg/day Physical signs of withdrawal were observed in 18 of 19 ethanol-exposed P rats, 11 of the 18 exhibited one or more signs indicative of Stage IV of withdrawal on the first day No audiogenic seizures were observed (Table 1) No signs of withdrawal were observed in the water-only control groups

Figure 3 shows the distribution of the physical signs observed on the first day in the 18 experimental animals in Experiment 3 manifesting one or more signs of withdrawal The most frequently observed sign was hyper-reactivity to sound and touch seen in 14 of the animals Other commonly occurring signs included mild and severe tremor (10 and 9 animals, respectively) and tail stiffening (9 animals) Bizarre behaviors, predominantly stereotyped head and body movements, were seen in 6 of the ethanol-withdrawn rats

The physical manifestations of withdrawal diminished over time (Fig 4) At the 4 hours assessment period, 16 of the 18 animals exhibited one or more signs with hyper-reactivity occurring in 11 of the 16 P rats The occurrence of other signs was evenly distrubuted At 24 hours, 15 animals continued to display one or more signs of withdrawal and the number exhibiting Stage IV signs was greatly diminshed Most physical signs disappeared by 72 hours post-withdrawal

In all experiments, the experimental and control groups were indistinguishable from each other and no signs of withdrawal were observed, when they were examined 20 hours before the removal of ethanol

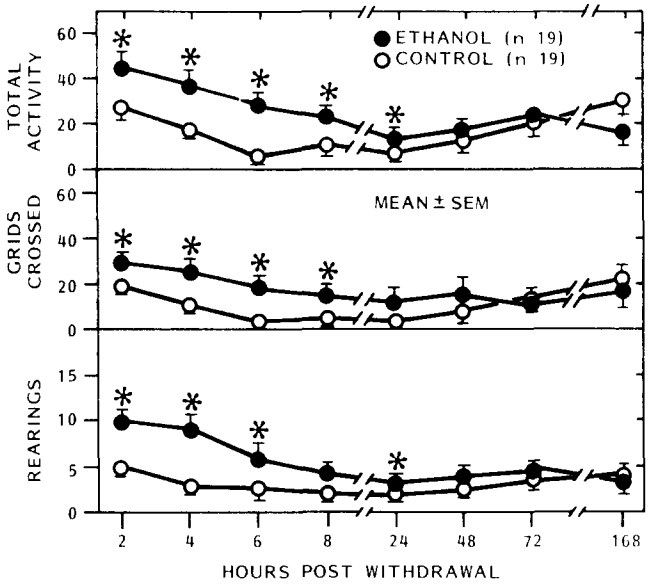

FIG 5 Effect of ethanol withdrawal on open field activity of ethanol-exposed and water-only P-rats, scored by blind assessment Shown is the mean±S E M for 19 animals per group Statistical significance ($p<0$ 05) is indicated by an asterisk

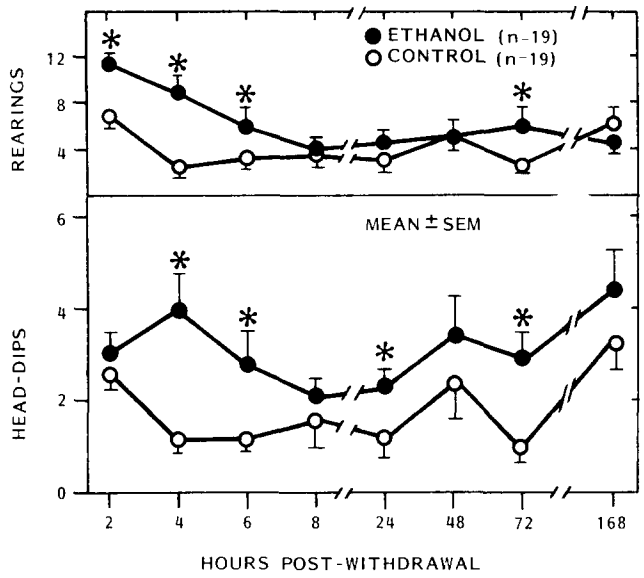

FIG 6 Effect of ethanol withdrawal on head-poke activity of ethanol-exposed and water-only P rats, measured by blind assessment Asterisks indicate statistical significance ($p<0$ 05)

Behavioral Disruptions During Withdrawal

In Experiments 2 and 3, spontaneous activity testing was performed in the open-field arena and head-poke apparatus by unblind and blind assessment, respectively Similar results were obtained and those from Experiment 3 are presented in Figs 5 and 6

The experimental animals were more active than the control animals in the open field test (Fig 5) On the first day of withdrawal, the scores of the ethanol-exposed animals differed significantly from those of the control group at 3 or more time points for rearing, grids crossed and total activity The hyperactivity of the withdrawn group persisted through the 24-hour testing period for rearings and total activity Later retesting revealed no significant differences

Similar results were observed in the head-dip apparatus (Fig 6) In general, withdrawn animals were more active than the control group Significant differences in the number of rearings were seen at the 2, 4 and 6 hour testing periods and in the number of head-dips at 4 and 6 hours These differences persisted up to 72 hours on both measures When the animals were retested after 1 week, there was no differences between the groups

In all experiments, there were no significant differences in spontaneous activity between experimental and control animals when they were tested 20 hours before the removal of ethanol

DISCUSSION

It is now well established that the chronic administration of ethanol produces physical dependence in experimental animals [8,20] The dose-response relationships and duration of ethanol exposure necessary to produce dependence have been characterized in rodents and other animal species using experimental designs intended to maintain intoxicating levels of blood alcohol almost continuously These methods employ forcible means (e g , inhalation, intubation), operant procedures or the incorporation of ethanol into a sole source of fluid or diet and, in the rat, amounts of ethanol averaging 10–20 g/kg/day are administered Severe manifestations of the withdrawal syndrome, including seizures and death, were elicited The advantages and disadvantages of such procedures have been reviewed [20]

There is a prevailing notion that the voluntary oral consumption of ethanol by experimental animals is insufficient to produce physical dependence [2], because most animals dislike solutions containing high concentrations of ethanol [17] However, the mean, voluntary, oral consumption of aqueous solutions of ethanol by both the selectively bred P- and AA (Alko, Alcohol-preferring)-lines of rats is 5–7 g/kg/day [6,12] This daily dose has been reported to produce physical dependence when administered by other means [1, 4, 13], even though this amount of ethanol cannot be expected to produce continuously elevated blood alcohol levels The present study confirms these earlier observations and additionally demonstrates that, in the rat lines selectively bred for ethanol preference, this can be accomplished by free-choice drinking of 10% ethanol with water and food available ad lib (Fig 1) Importantly, the ethanol consuming animals exhibited identical weight gains as the free-feeding control animals given water as the sole source of fluid Although the ethanol constitutes 20–25% of the caloric intake, commercially available rat chow is well-known to contain an excess of the daily requirement of nutrients for rats Thus, nutritional impairment does not appear to be a factor

In both the P- and AA-lines of rats, free-choice drinking of ethanol consistently produces elevated blood alcohol levels as high as 100 mg % during the dark cycle [5, 11, 12, 15] During the light cycle, however, ethanol consumption is about 25% of the daily total (Fig 2) Accordingly, blood ethanol concentrations during the light cycle should be substantially lower Presumedly, this amount is sufficient to prevent overt signs of withdrawal, since none were observed during the light cycles when ethanol was freely available In these studies, ethanol was removed at the end of a dark cycle in order to initiate withdrawal

We have shown previously that reduction of the body weight of P-rats to 80% of the free-feeding weight and the flavoring of the 10% ethanol solution increases the consumption of ethanol to 10 g/kg/day or more [15] These observations are confirmed in the present study weight-restriction and the addition of saccharin and NaCl to the ethanol solution increased ethanol consumption to about 14 g/kg/day, an amount comparable to that obtained with liquid diet regimens [20] However, weight-restricted NP animals, similarly provided with water ad lib, also consumed large amounts of ethanol, 12 g/kg/day These results indicate that the enhanced consumption is, to a large extent, dictated by the caloric value of ethanol, since flavoring alone increases ethanol consumption of NP animals only to about 3 g/kg/day [15] Predictably, the weight-restricted, ethanol-consuming, P and NP animals exhibited characteristic manifestations of withdrawal after 8 weeks Four out of the 17 had audiogenic seizures and 5 of them showed signs characterized as Stage IV of withdrawal (Table 1) Therefore, very little difference in the severity of the withdrawal syndrome was seen between the P and NP group This is not unexpected, since the P and NP lines were selectively bred for divergent ethanol drinking behaviors and not for differences in susceptibility to physical dependence

Because free-fed P animals drinking unflavored 10%

ethanol consumed only about 1/2 of the amount ingested by the weight-restricted animals, they were exposed to longer periods of chronic free-choice drinking, 15 weeks in Experiment 2 and 20 weeks in Experiment 3 (Fig 1) In Experiment 3, physical signs of withdrawal were scored by blind assessment in order to minimize observer bias Although none of the 32 ethanol-consuming animals in these experiments had audiogenic seizures, only 3 did not show signs of withdrawal and 19 met the criteria for Stage IV of withdrawal (Table 1) The abnormalities receded over a period of 72 hours in the ethanol-consuming animals Importantly, none of the control animals exhibited signs of withdrawal The blind assessment of the occurrence of physical signs of withdrawal and the time course of their disappearance provide particularly convincing evidence that the chronic free-choice consumption of ethanol by free-fed rats of the P-line produces physical dependence (Figs 3 and 4)

The occurrence of physical dependence in the free-fed P rats voluntarily drinking ethanol was corroborated by the testing of spontaneous activity in Experiments 2 and 3 (Figs 5 and 6), although these tests appeared to be less sensitive than the rating of physical signs In both the open-field arena and the head-poke apparatus, the ethanol-consuming animals exhibited increased activity in comparison to the control animals during the first 24 hours after the removal of ethanol The duration of these behavioral changes is shorter than that reported by Cicero *et al* [4] and Liljequist *et al* [13] who found persistence and even increased hyperactivity up to 72 hours of withdrawal In contrast to these observations, Pohorecky [19] has reported hypoactivity during withdrawal The different temporal relationships and the disparate finding of hypo- and hyperactivity in the different studies are difficult to reconcile unless methodologic variables, such as dose, duration of exposure, the reference point chosen to mark the start of withdrawal, ethanol administration technique and test schedules, influence outcome, as they may Further studies to elucidate such issues are needed It should be noted that, in the present study, the animals were not primed or standardized by ethanol injection or intubation to a high blood ethanol level prior to testing [16,19], because such a procedure would have disrupted the voluntary oral consumption study design The reference point for onset of withdrawal was taken as the end of the last dark period of ethanol availability Accordingly, the actual time that each of the animals last consumed ethanol may have been different This variability might have lessened the activity differences between the experimental and control groups seen with testing

A secondary objective of the present studies was to evaluate the potential of the P-line of rats as an animal model of alcoholism Such a model should ideally simulate the human condition in all aspects other than those psychosocial variables uniquely encountered in humans [3, 7, 10] Voluntary oral self-administration of ethanol is considered by most investigators to be a key and essential requirement, and has been a crucial stumbling block in this regard, because most experimental animals do not voluntarily drink aqueous solutions containing high concentrations of ethanol [17] The development of alcohol-preferring lines of rats through selective breeding has overcome this obstacle We have reported previously that the drinking behavior of the P-rats also satisfies several other perceived requirements of an animal model [12] The present studies demonstrate that the voluntary oral consumption of ethanol in such animals under free-feeding conditions can produce physical dependence, thus satisfying another key criterion The ability of food-restriction and flavoring to increase ethanol consumption to levels that result in signs of severe withdrawal is particularly interesting, suggesting that other environmental variables may be effective in enhancing drinking Accordingly, it may be possible to induce episodic bouts of overt intoxication, a feature not exhibited by the P-rats when food, water and 10% ethanol are continuously available in the environment

ACKNOWLEDGEMENTS

We thank M Clark and P Hall for their technical assistance

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