Pharmacology Biochemistry and Behavior, Vol. 66, No. 1, pp. 143–151, 2000

0091-3057/00/$–see front matter

ELSEVIER

PII S0091-3057(00)00196-9

Withdrawal Symptoms in a Long-Term Model of Voluntary Alcohol Drinking in Wistar Rats

SABINE M. HÖLTER, ASTRID C. E. LINTHORST, JOHANNES M. H. M. REUL AND RAINER SPANAGEL

Max Planck Institute of Psychiatry, Kraepelinstr. 2, D-80804 Munich, Germany

Received 13 October 1999; Revised 4 January 2000; Accepted 4 January 2000

HÖLTER, S. M., A. C. E. LINTHORST, J. M. H. M. REUL AND R. SPANAGEL. *Withdrawal symptoms in a long-term model of voluntary alcohol drinking in Wistar rats.* PHARMACOL BIOCHEM BEHAV **66**(1) 143–151, 2000.— Long-term voluntary alcohol drinking with repeated alcohol deprivation episodes has been suggested as animal model for some aspects of alcoholism. Using a radiotelemetric system, the present study investigated the occurrence of withdrawal symptoms in long-term voluntarily alcohol drinking Wistar rats with (repeated alcohol deprivation group) and without (first alcohol deprivation group) prior alcohol deprivation experience. Six days after transmitter implantation, alcohol bottles were removed, and returned 4 days later. Alcohol deprivation induced hyperlocomotion in both groups. In the repeated alcohol deprivation group, hyperlocomotion was increased at the beginning of the alcohol deprivation phase and decreased during the following dark phase, suggesting that removal of the alcohol bottles might have become a conditioned withdrawal stimulus for this group. Both groups showed an enhanced alcohol intake after representation of alcohol bottles compared to preabstinence intakes (alcohol deprivation effect). However, alcohol intake of the repeated alcohol deprivation group was significantly increased compared to the first alcohol deprivation group at the end of the experiment. It is concluded that repeated alcohol deprivation experience might promote the development of alcohol addiction because of its latent stimulating effect on alcohol drinking that can be unveiled by (presumably mildly stressful) experimental situations. © 2000 Elsevier Science Inc.

Conditioning Ethanol Radiotelemetry Repeated alcohol deprivation Voluntary alcohol self-administration Withdrawal symptoms

WE recently established an animal model in unselected male Wistar rats with which certain aspects of the development of alcohol dependence can be modeled. After several months of voluntary alcohol consumption and repeated alcohol deprivation (withdrawal) experience the drug taking behavior following an alcohol deprivation phase is characterized by increased alcohol intake and preference and by changes in intake patterns resulting in an immediate increase in consumption of highly concentrated alcohol solutions, both under operant and nonoperant conditions (12,13,35). The phenomenon of a transient increase in alcohol intake and preference after a period of imposed abstinence has been termed the alcohol deprivation effect (ADE), and has been observed in several species including humans (5,32,33). Because the ADE in long-term alcohol drinking rats is characterized by an increased motivation to obtain alcohol, outlasts very long abstinence phases, and is hardly modified by external stimuli such as taste adulteration or social factors, it can be regarded as an animal model of relapse behavior and craving (14,35,37,43). The ADE has also been pharmacologically validated as a relapse model by drugs like acamprosate and naltrexone, which reduce relapse in weaned alcoholics, and also reduce the ADE (11,12,15,20,35).

It is a prevailing notion in the literature that voluntarily alcohol drinking rats do not become physically dependent, because their alcohol intake is too low to sustain elevated blood alcohol levels (6,7,25). However, 85% of alcohol-preferring P-rats exhibited mild physical withdrawal symptoms after 15–20 weeks of voluntary alcohol intake of 5.6–7.2 g/kg/day (42). The most frequent withdrawal symptom in these animals was hyperreactivity to external stimuli. We also found in our long-term alcohol-drinking rats that have an average alcohol preference of 50% and consume on average 3–4 g/kg alcohol per day withdrawal symptoms like hyperreactivity to stressors and increased anxiety-related behavior in the elevated plus-maze (13,37). Interestingly, anxiety-related behavior in the elevated plus-maze during withdrawal was higher in rats with repeated alcohol deprivation experience than in rats without

Requests for reprints should be addressed to Dr. S. M. Hölter, Max Planck Institute of Psychiatry, Kraepelinstr. 2, D-80804 Munich, Germany.

prior alcohol deprivation experience (13). It is assumed that blood alcohol levels attained, the length of chronic alcohol intake, and withdrawal experience play a role for the intensity of withdrawal symptoms (1,2,18).

The aim of the present study was to investigate further the occurrence of withdrawal symptoms in long-term voluntarily alcohol-drinking rats. A radiotelemetric system was used to this end with which locomotor activity and core body temperature could be continuously monitored in undisturbed rats. This system has been used successfully in rats and mice (22,23), and proved to be a very sensitive method to detect even mild alcohol-induced withdrawal symptoms in rats after only 1 week of forced alcohol drinking (36). Furthermore, we investigated the influence of repeated deprivation experience on the intensity of withdrawal symptoms. Therefore, rats with repeated alcohol deprivation experience (repeated alcohol deprivation group), age-matched alcohol drinking rats without prior alcohol deprivation experience (first alcohol deprivation group), and an age-matched water-drinking control group were compared in this study.

METHOD

Subjects

Twenty-two male Wistar rats (Max Planck Institute of Biochemistry, Martinsried, Germany) weighing 220–250 g upon arrival in our laboratory, were used in this study. All animals were housed individually in standard hanging rodent cages with food and tap water ad lib. Artificial light was provided daily from 0700 until 1900 h, and room temperature and humidity were kept constant (temperature: 23 ± 1°C; humidity: 60 ± 5%). The experiments were approved by the Committee on Animal Care and Use of the relevant local government body and carried out following the German Law on the Protection of Animals.

Long-Term Alcohol Self-Administration

After 1 week of habituation to the animal room, rats of the group "repeated alcohol deprivation" ($n = 8$) and of the group "first alcohol deprivation" ($n = 7$) were given access to tap water, 5, 10, and 20% (v/v) alcohol solutions in their home cages. Alcohol solutions were made up from 96% pure ethanol diluted with tap water to the different concentrations. The control group ($n = 6$) stayed alcohol naive. Spillage and evaporation were minimized by the use of bottle caps with ball bearings (Ehret, Emmendingen, Germany). With this procedure, the alcohol concentration in any of the solutions stayed constant for at least 1 week (13). All drinking solutions were renewed weekly and at that time the positions of the four bottles were changed to avoid location preferences. In the group "repeated alcohol deprivation" alcohol solutions were repeatedly withdrawn for 3 days (deprivation phase) every 4 weeks after the first 8 weeks of continuous access. The group "first alcohol deprivation" had continuous access to all three alcohol solutions in their home cages until their first alcohol deprivation experience during this experiment.

Measurement of Physical Signs of Alcohol Withdrawal

After 18 months of alcohol experience in the long-term paradigm described above, animals were transferred to the radiotelemetry room in their home cages. Alcohol drinking rats continued to have the choice between the three alcohol solutions and tap water. During the whole experiment the experimenter entered this room once daily between 1100 and 1200 h to weigh the bottles, the food, and the animals. Otherwise, the animals were left undisturbed in this sound-attenuated experimental room.

Core body temperature (°C) and locomotor activity (expressed in arbitrary units) were monitored with a radiotelemetric method using the Dataquest IV system (Data Sciences International, St. Paul, MN). After 1 week of habituation to the experimental room, on day 7, a battery-powered transmitter (TA-F40) was implanted into the intraperitoneal cavity of each animal under halothane anaesthesia. Collection of core body temperature and locomotor activity data started immediately after surgery. The frequency of the signal emitted by the transmitter was proportional to the animal's body temperature. This signal reached a receiver underneath the cage, and was transferred to and processed by an IBM PC. Body temperature was continually recorded at 3-min intervals. Locomotor activity of the animals was measured by continuously monitoring changes in the received signal strength from the transmitter that occurred upon movement of the animal. Changes in signal strength generate a digital pulse that was counted by the Dataquest IV system. The number of pulses was proportional to the distance the animal moved. Locomotor activity was continually recorded at 3-min intervals.

On day 13, the alcohol bottles were removed from the cages following the daily weighing routine, leaving all animals with food and tap water ad lib (alcohol deprivation). Four days later, on day 17, alcohol bottles were returned (alcohol representation), and data were collected for another 2 days. Data of the last 3 days before alcohol deprivation (days 10–12) served as measurements of baseline alcohol drinking conditions, and data of the last 2 days (days 17 and 18) represent measurements of the alcohol deprivation effect.

Data Analysis

Daily alcohol intake, food intake, weight changes, total fluid intake, total alcohol preference, and preferences for the three alcohol solutions were calculated from the daily measurements. Total alcohol preference was calculated as the percentage share of the sum of consumption from the three alcohol solutions in total fluid consumption, and the preference for a particular alcohol concentration was calculated as the percentage share of consumption from this alcohol solution in total fluid consumption. Body temperature and locomotor activity data were averaged over 3-h intervals, and these data were analyzed by three-way analysis of variance (ANOVA) with repeated measures (group × alcohol deprivation × days). The occurrence of an alcohol deprivation effect was tested by three-way ANOVA with repeated measures (group × alcohol deprivation × days) of alcohol intake and total alcohol preference data of days 11 and 12 and days 17 and 18 (2 days before and 2 days after alcohol deprivation). Concentration preference data was also analyzed by three-way ANOVA with repeated measures (concentration × alcohol deprivation × days). Because long-term alcohol drinking rats weighed less at the beginning of this study (group repeated alcohol deprivation: 530 g ± 3.9, group first alcohol deprivation: 534 g ± 4.0) than control rats (554 g ± 8.8), body weight was calculated as percentage of the body weight on day 12 (baseline of body weight before alcohol deprivation). Body weight was analyzed by two-way ANOVA with repeated measures (group × days), and food intake was analyzed by three-way ANOVA with repeated measures (group × alcohol deprivation × days). The chosen level of

significance was $p \leq .05$. Fisher's LSD (Protected-t) test was applied for post hoc comparisons when appropriate.

RESULTS

Locomotor Activity

Under baseline alcohol drinking conditions all animals had a normal circadian rhythm of locomotor activity. The daily weighing procedure between 1100 h and noon caused an increase in locomotor activity during this 3-h interval in all animals (Fig. 1). Alcohol deprivation caused a significant increase in locomotor activity during the 3-h interval of the daily weighing procedure (Fig. 1) compared to the activity during this 3-h interval at the 3 preceding days [factor alcohol deprivation: $F(1, 18) = 11.3$, $p < 0.01$; interaction group × alcohol deprivation × days: $F(4, 125) = 5.83$, $p = 0.001$]. Post hoc analyses revealed that this effect was only significant during the 3-h interval of removal of the alcohol bottles on day 13 (marked "AD" in Fig. 1), and that this effect was significantly stronger in the repeated alcohol deprivation group than in the first alcohol deprivation group. Interestingly, during this 3-h interval the locomotor activity of control rats, for which nothing changed, was also slightly increased.

In addition, alcohol deprivation induced an increase of locomotor activity during the dark phase compared to the level of activity during the dark phase of the 3 days under baseline drinking conditions (Fig. 1). This effect was strongest in the first alcohol deprivation group during the first dark phase after removal of the alcohol bottles (day 13) and declined over the following 2 nights (days 14 and 15). Increased locomotor activity during the dark phase during alcohol deprivation was significantly weaker in the repeated alcohol deprivation group compared to the first alcohol deprivation group. Interestingly, starting the second night after withdrawal of the alcohol bottles, control rats also showed a tendency towards an increase in locomotor activity (Fig. 1, days 14 and 15). Thus, it cannot be excluded that under these experimental conditions (common housing for several days in an experimental room in which external noise was minimized) behavioral changes of experimental animals affected control rats.

Representation of the alcohol bottles on day 17 caused a slight increase in locomotor activity in all animals during this 3-h interval, $F(1, 18) = 10.1$, $p < 0.01$. Only in the first alcohol deprivation group this effect was statistically significant compared to locomotor activity during this 3-h interval at the two preceding days (Fig. 2).

Body Temperature

Under baseline alcohol drinking conditions all animals had a normal circadian rhythm of body temperature. The daily weighing procedure between 1100 h and noon caused an increase in body temperature during this 3-h interval in all animals (Fig. 3). Alcohol deprivation caused a significant decrease in body temperature [factor alcohol deprivation: $F(1, 18) = 177.4$, $p < 0.0001$]. However, there was no significant interaction, indicating that all groups, including the control group, were similarly affected by alcohol deprivation.

Resumption of alcohol drinking after representation of the alcohol bottles on day 17 did not influence body temperature (Fig. 4).

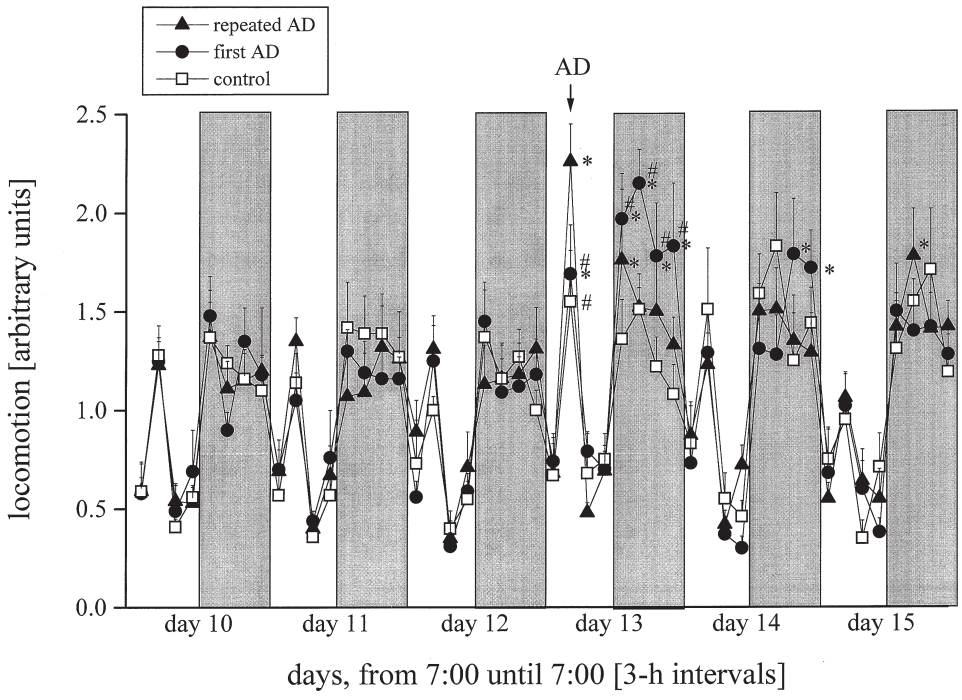

FIG. 1. Effects of alcohol deprivation on locomotor activity. Data are presented as means + SEM of 3-h intervals; $n = 6$–8. AD = alcohol deprivation. Shaded areas mark the dark phase (from 1900 h until 0700 h). *$p < 0.05$, significant difference vs. days 10 to 12 (before alcohol deprivation); #$p < 0.05$, significant difference vs. repeated alcohol deprivation.

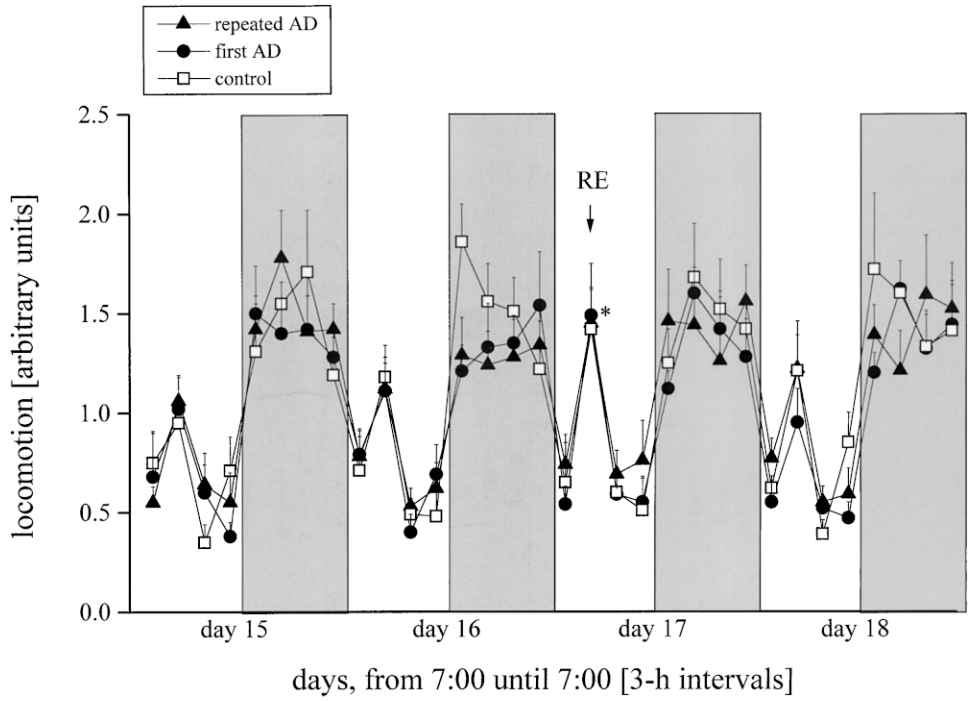

FIG. 2. Effects of representation of alcohol solutions on locomotor activity. Data are presented as means + SEM of 3-h intervals; $n = 6$–8. AD = alcohol deprivation, RE = Representation of alcohol solutions. Shaded areas mark the dark phase (from 1900 h until 0700 h). *$p < 0.05$, significant difference vs. days 15 and 16 (before alcohol representation).

Body Weight

Regarding body weight changes, there was a main effect on days [factor days: $F(5, 125) = 10.24$, $p < 0.0001$], but no significant group × days interaction (Fig. 5).

Alcohol Drinking Behavior

Alcohol intake and alcohol preference were slightly reduced in both groups following transfer to the experimental room compared to previous values in the animal housing

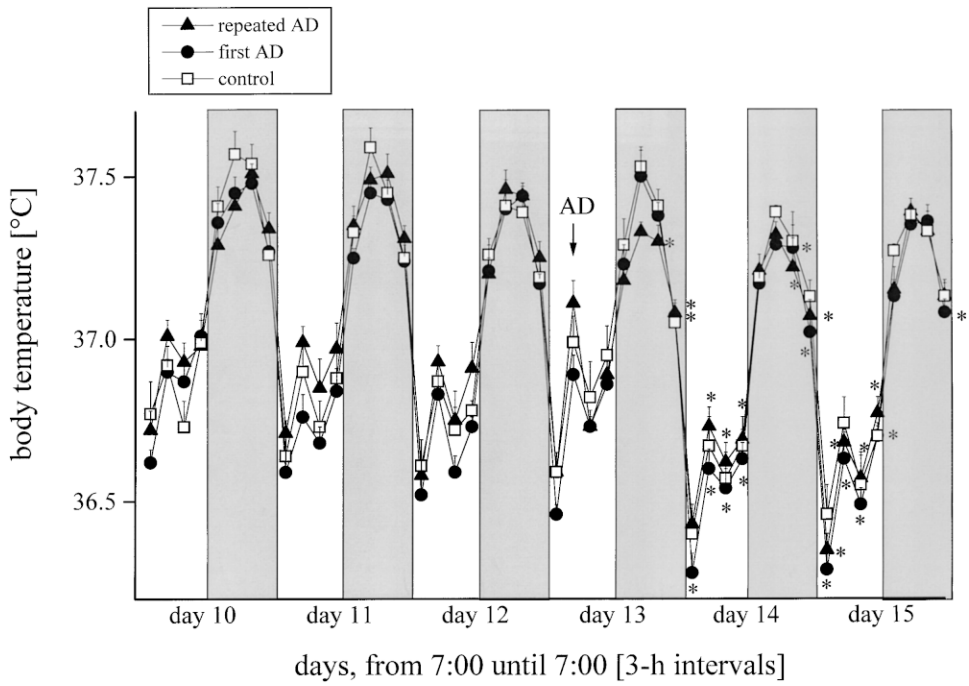

FIG. 3. Effects of alcohol deprivation on body temperature. Data are presented as means + SEM of 3–h intervals; $n = 6$–8. AD = alcohol deprivation. Shaded areas mark the dark phase (from 1900 h until 0700 h). *$p < 0.05$, significant difference vs. days 10 to 12 (before alcohol deprivation).

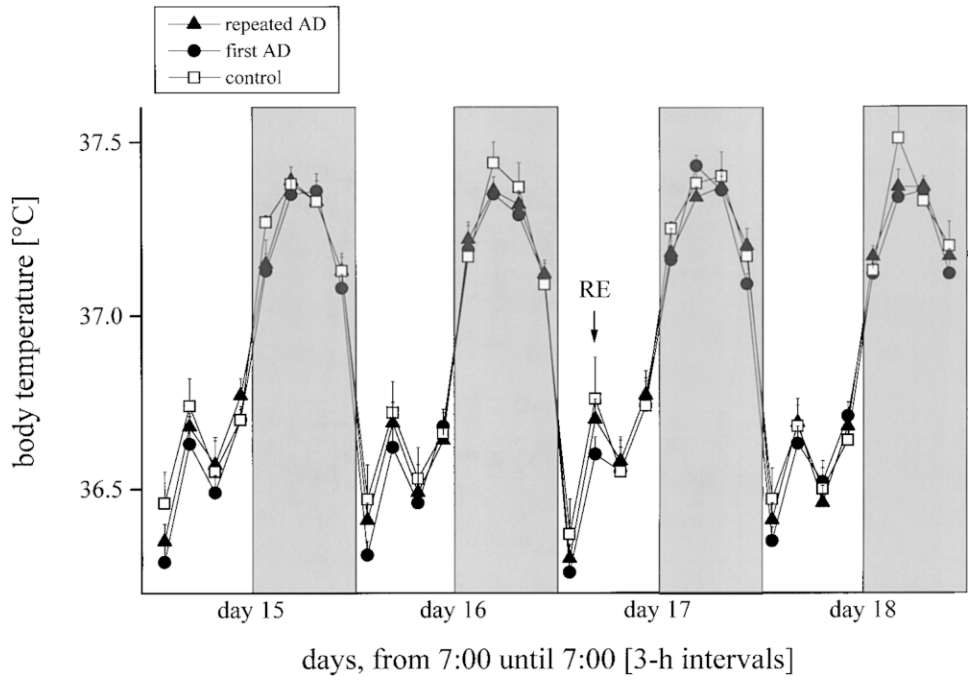

FIG. 4. Effects of representation of alcohol solutions on body temperature. Data are presented as means + SEM of 3-h intervals; n = 6–8. AD = alcohol deprivation, RE = Representation of alcohol solutions. Shaded areas mark the dark phase (from 1900 h until 0700 h).

room (3–4 g/kg/day for alcohol intake and about 50% for alcohol preference). Both parameters had returned to normal prior to surgery (Fig. 6). Surgery only shortly reduced alcohol intake without reducing alcohol preference, but following surgery there was a tendency towards an increase in alcohol intake, and particularly in alcohol preference, in both groups.

Both alcohol drinking groups showed an alcohol deprivation effect concerning alcohol intake, because alcohol intake

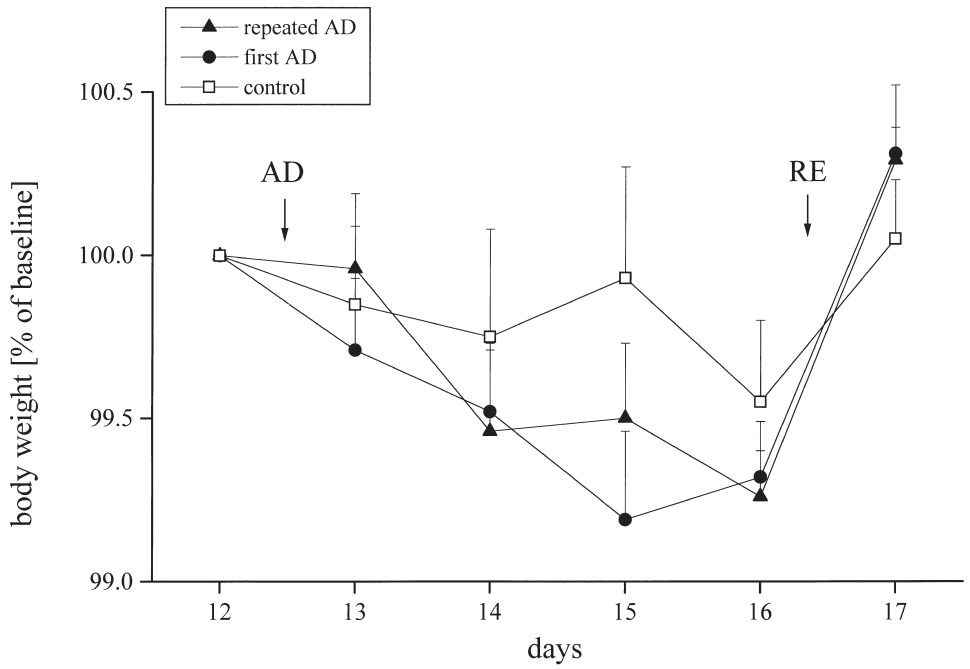

FIG. 5. Effects of alcohol deprivation and representation of alcohol solutions on body weight. Data are presented as means + SEM of 24 h measurements; n = 6–8. AD = alcohol deprivation, RE = Representation of alcohol solutions.

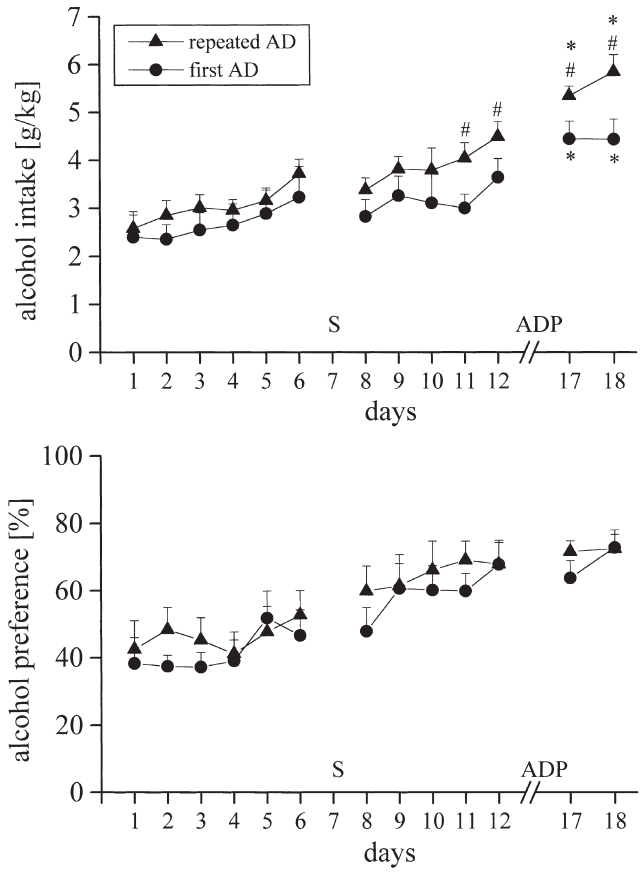

FIG. 6. Effects of repeated alcohol deprivation experience on alcohol intake (top) and alcohol preference (bottom). Data are presented as means + SEM of 24 h measurements; $n = 7$–8. AD = alcohol deprivation, S = Surgery, ADP = alcohol deprivation phase. *$p < 0.05$, significant difference vs. days 11 and 12; #$p < 0.05$, significant difference vs. first alcohol deprivation.

was increased after alcohol deprivation compared to intakes of the last 2 days before alcohol deprivation (Fig. 6, top) [factor alcohol deprivation: $F(1, 13) = 30.3$, $p < 0.001$], but not concerning total alcohol preference (Fig. 6, bottom). Thus, alcohol deprivation had the same effect on both groups. It should be noted that alcohol preference had risen to ⩾60% in both groups prior to alcohol deprivation. Interestingly, alcohol intake was significantly higher in the repeated alcohol deprivation group than in the first alcohol deprivation group during the last 2 days before alcohol deprivation as well as during the alcohol deprivation effect [factor group: $F(1, 14) = 8.9$, $p < 0.05$]. Both groups did not differ regarding alcohol preference (Fig. 6, bottom).

There was no difference between groups concerning preferences for the different alcohol concentrations offered, and no effect of alcohol deprivation on these preferences (data not shown).

Food Intake

Alcohol drinking rats consumed less food than control rats [factor group: $F(2, 18) = 10.2$, $p < 0.01$]. Food intake was reduced following surgery and had not returned to the levels before surgery before alcohol deprivation (Fig. 7). Despite the rising trend of food intake visible in control rats, alcohol deprivation significantly increased food intake in alcohol-deprived rats, and this effect was stronger in the repeated alcohol deprivation group than in the first alcohol deprivation group [factor group: $F(2, 18) = 8.18$, $p < 0.01$; factor alcohol deprivation: $F(1, 18) = 144.1$, $p < 0.0001$; interaction group × alcohol deprivation: $F(2, 18) = 8.95$, $p < 0.01$]. Resumption of alcohol intake after representation of alcohol bottles reduced food intake. This effect was stronger in the repeated alcohol deprivation group than in the first alcohol deprivation group [factor group: $F(2, 18) = 9.5$, $p < 0.01$; factor representation: $F(1, 18) = 11.07$, $p < 0.01$; interaction group × representation: $F(2, 18) = 10.75$, $p < 0.001$].

DISCUSSION

The results of this study show that: (a) baseline alcohol drinking does not influence the circadian rhythms of locomotor activity and core body temperature in long-term alcohol drinking rats, because these rats do not differ in these measures from control rats; (b) alcohol deprivation leads to mild physical withdrawal symptoms like hyperlocomotion within 9 to 48 h after withdrawal of alcohol; (c) repeated alcohol deprivation experience leads to an intensified reaction to the removal of alcohol bottles, but to a reduction of subsequent hyperlocomotion; (d) repeated alcohol deprivation experience does not necessarily increase baseline alcohol drinking in general, but can lead to enhanced alcohol intake in combination with experimental manipulations.

Neither baseline alcohol intake nor increased alcohol intake after alcohol deprivation affected locomotor activity or core body temperature. This is presumably due to the fact that in this voluntary drinking model the alcohol intake of up to 6 g/kg/day (after alcohol deprivation) is distributed over 24 h so that blood alcohol levels attained after individual drinking bouts are not sufficient to stimulate locomotor activity or induce hyperthermia, as was shown for acute oral and intraperitoneal bolus injections of 2–6 g/kg alcohol (10,16,34). However, it is also possible that this lack of effect might be due to the development of tolerance, as has been shown for the locomotor stimulating effect of voluntarily consumed alcohol in chronic voluntarily drinking rats (8) and for the hypothermia-inducing effect of acute alcohol administration (16,19,28). This conclusion is corroborated by the recent finding that long-term alcohol drinking rats are tolerant towards the locomotor stimulating effect of a low alcohol dose (0.5 g/kg IP), which is about the maximal dose consumed within an individual drinking bout (38).

Alcohol deprivation induced hyperlocomotion in animals both with and without prior alcohol deprivation experience. Hyperactivity, followed by hypoactivity during a strong withdrawal syndrome, is a frequently observed withdrawal symptom after chronic consumption of both high and low alcohol doses (4,21,25,36,42). In contradiction to our hypothesis, repeated alcohol deprivation experience did not increase withdrawal symptoms in general, as it intensified anxiety-related behavior (13). However, hyperlocomotion was increased at the beginning of the alcohol deprivation phase, and decreased during the following dark phase. This could represent conditioning of alcohol withdrawal. It is possible that animals with repeated alcohol deprivation experience learned to associate the removal of the alcohol bottles with the subsequent alcohol deprivation phase, which usually lasts 3 days, so that for

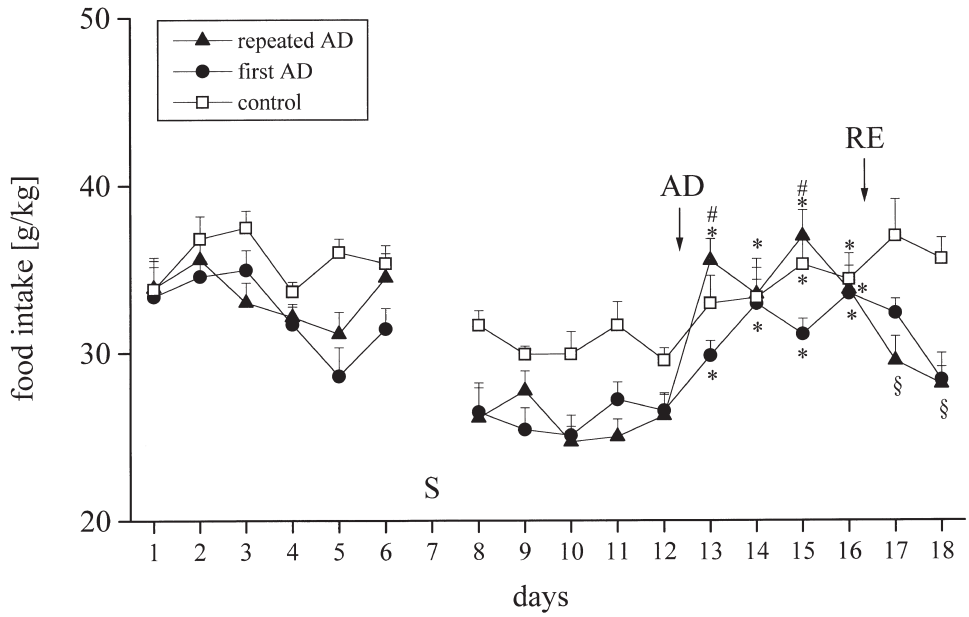

FIG. 7. Effects of alcohol deprivation and representation of alcohol solutions on food intake. Data are presented as means + SEM of 24 h measurements; $n = 6$–8. S = Surgery, AD = alcohol deprivation, RE = Representation of alcohol solutions. *$p < 0.05$, significant difference vs. days 9 to 12; #$p < 0.05$, significant difference vs. first alcohol deprivation; §$p < 0.05$, significant difference vs. days 15 and 16.

this group the removal of alcohol bottles became a conditioned stimulus signalling alcohol withdrawal. In contrast, animals without prior deprivation experience that could not learn that withdrawal followed the removal of the alcohol bottles reacted to that stimulus in the same way as control rats, but were hyperactive during the following dark phase, which is their first active phase after the removal of alcohol bottles. Thus, it is possible that this group only started "missing" the alcohol bottles when they became active in the following dark phase. This behavioral pattern also suggests that the hyperlocomotion observed during alcohol deprivation represents drug-seeking behavior.

There was no significant difference in body temperature between the control group and the alcohol drinking groups after alcohol deprivation, suggesting that the observed decrease in body temperature was not caused by alcohol deprivation but by an unknown environmental factor. Because room temperature did not change throughout the experiment, this factor can be excluded. It is unclear whether the putative influence of behavioral changes of experimental animals on the behavior of control rats observed in locomotor activity might play a role in this respect.

It is noteworthy that repeated alcohol deprivation experience led to increased alcohol intake at the end of the baseline measurement period just before alcohol deprivation and during the alcohol deprivation effect. In this model, repeated alcohol deprivation experience does not lead to sustained increases in alcohol consumption under undisturbed, home cage housing conditions, but might lead to increases in alcohol intake and preference under experimental conditions that are presumably stressful for the animals (13). In the present study, alcohol intakes were not significantly different between both alcohol drinking groups at the beginning of the experiment or immediately after surgery. However, alcohol preference slowly increased during the experiment in both groups, and alcohol intake significantly increased in the repeated alcohol deprivation group. Experimental conditions that involve changes, for example in housing conditions or handling, might stimulate increases in alcohol drinking in long-term alcohol experienced rats. It was shown that relatively mild, "psychological" stressors can induce increases in voluntary alcohol consumption (26,27,41). A change in alcohol drinking may not necessarily have to be an immediate reaction to a stimulus, but might also develop slowly with time. In the present study, the fact that the change in alcohol drinking behavior is more pronounced in the repeated alcohol deprivation group might be explained by the increased experience of this group of animals with higher alcohol doses due to the repeated alcohol deprivation effects these rats experienced after each alcohol deprivation phase. Several studies indicate that repeated withdrawal experience stimulates learning to use alcohol's pharmacological effects (17,31,39). The repeated possibility to associate alcohol consumption in a certain situation with its pharmacological effects may be important in this respect, because mere induction of physical dependence without the possibility to experiment with the pharmacological effects of alcohol by self-administering it does not necessarily induce an increase in voluntary alcohol intake [(3,24,30,40; but see also (9,29)]. The usage of a method of chronic alcohol intake that does not induce very strong physical withdrawal symptoms might also be important, because strong physical withdrawal symptoms may interfere with alcohol drinking behavior. In summary, it may be speculated that repeated alcohol deprivation experience could stimulate learning processes that can result in increased voluntary alcohol intake in experimental situations.

In conclusion, long-term voluntary alcohol consumption of only 3–4 g/kg/day in rats can induce mild withdrawal symptoms like hyperreactivity to a mild stressor (novel environ-

ment) (37), anxiety-related behavior (13), and hyperlocomotion (present study). These are well-known, relatively mild withdrawal symptoms in rats. Hyperlocomotion during alcohol deprivation presumably represents drug seeking behavior, and repeated alcohol deprivation experience might condition the animals to the removal of the alcohol bottles as withdrawal stimulus. Thus, repeated alcohol deprivation experience may promote the development of alcohol addiction, because it seems to have a latent stimulating effect on alcohol drinking that can be unveiled by (presumably mildly stressful) experimental situations.

ACKNOWLEDGEMENTS

This work was supported by the Bundesministerium für Bildung and Forschung (BMBF, FKZ 01EB 9706).

The authors would like to thank Christine Bartl and Thomas Pohl for their excellent technical assistance.

REFERENCES

1. Becker, H. C.; Hale, R. L.: Repeated episodes of ethanol withdrawal potentiate the severity of subsequent withdrawal seizures: An animal model of alcohol withdrawal "kindling." Alcohol. Clin. Exp. Res. 17:94–98; 1993.
2. Becker, H. C.: Positive relationship between the number of prior ethanol withdrawal episodes and the severity of subsequent withdrawal seizures. Psychopharmacology (Berlin) 116:26–32; 1994.
3. Begleiter, H.: Ethanol consumption subsequent to physical dependence. In: Gross, M. M., ed. Alcohol intoxication and withdrawal. New York: Plenum Press; 1975:373–378.
4. Branchey, M.; Rauscher, G.; Kissin, B.: Modifications in the response to alcohol following the establishment of physical dependence. Psychopharmacologia 22:314–322; 1971.
5. Burish, T. G.; Maisto, S. A.; Cooper, A. M.; Sobell, M. B.: Effects of voluntary short-term abstinence from alcohol on subsequent drinking patterns of college students. J. Stud. Alcohol 42:1013–1020; 1981.
6. Cicero, T. J.: A critique of animal analogues of alcoholism. In: Majchrowicz, E.; Noble, E. P, eds. Biochemistry and pharmacology of alcoholism. New York: Plenum Press; 1979:533–560.
7. Cicero, T. J.: Animal models of alcoholism? In: Eriksson, K.; Sinclair, J. D.; Kiianmaa, K., eds. Animal models in alcohol Research. New York: Plenum Press; 1980:99–117.
8. Coper, H.; Rommelspacher, H.; Wolffgramm, J.: The 'point of no return' as a target of experimental research on drug dependence. Drug Alcohol Depend. 25:129–134; 1990.
9. Deutsch, J. A.; Koopmans, H. S.: Preference enhancement for alcohol by passive exposure. Science 179:1242–1243; 1973.
10. Gallaher, E. J.; Egner; D. A.: Rebound hyperthermia follows ethanol-induced hypothermia in rats. Psychopharmacology (Berlin) 91:34–39; 1987.
11. Heyser, C. J.; Schulteis, G.; Durbin, P.; Koob, G. F.: Chronic acamprosate treatment eliminates the alcohol deprivation effect while having limited effects on baseline responding for ethanol in rats. Neuropsychopharmacology 18:125–133; 1998.
12. Hölter, S. M.; Landgraf, R.; Zieglgänsberger, W.; Spanagel, R.: Time course of acamprosate action on operant ethanol self-administration after ethanol deprivation. Alcohol. Clin. Exp. Res. 21:862–868; 1997.
13. Hölter, S. M.; Engelmann, M.; Kirschke, C.; Liebsch, G.; Landgraf, R.; Spanagel, R.: Long-term ethanol self-administration with repeated ethanol deprivation episodes changes ethanol drinking pattern and increases anxiety-related behaviour during ethanol deprivation in rats. Behav. Pharmacol. 9:41–48; 1998.
14. Hölter, S. M.; Spanagel, R.: Increased motivation to work for alcohol during the alcohol deprivation effect. Behav. Pharmacol. 10:S48; 1999.
15. Hölter, S. M.; Spanagel, R.: Effects of opiate antagonist treatment on the alcohol deprivation effect in long-term ethanol-experienced rats. Psychopharmacology (Berlin) 145:360–369; 1999.
16. Holloway, F. A.; Miller, J. M.; King, D. A.; Bedingfield, J. B.: Delayed ethanol effects on physiological and behavioral indices in the rat. Alcohol 10:511–519; 1993.
17. Hunter, B. E.; Walker, D. W.; Riley, J. N.: Dissociation between physical dependence and volitional ethanol consumption: Role of multiple withdrawal episodes. Pharmacol. Biochem. Behav. 2:523–529; 1974.
18. Hunter, B. E.; Riley, J. N.; Walker, D. W.: Ethanol dependence in the rat: A parametric analysis. Pharmacol. Biochem. Behav. 3:619–629; 1975.
19. Hutchison, W. D.; Gianoulakis, C.; Kalant, H.: Effects of ethanol withdrawal on beta-endorphin levels in rat brain and pituitary. Pharmacol. Biochem. Behav. 30:933–939; 1988.
20. Kornet, M.; Goosen, C.; Van Ree, J. M.: Effect of naltrexone on alcohol consumption during chronic alcohol drinking and after a period of imposed abstinence in free-choice drinking rhesus monkeys. Psychopharmacology (Berlin) 104:367–376; 1991.
21. Liljequist, S.; Ahlenius, S.; Engel, J.: The effect of chronic ethanol treatment on behaviour and central monoamines in the rat. Naunyn Schmiedebergs Arch. Pharmacol. 300:205–216; 1977.
22. Linthorst, A. C. E.; Flachskamm, C.; Holsboer, F.; Reul, J. M. H. M.: Intraperitoneal administration of bacterial endotoxin enhances noradrenergic neurotransmission in the rat preoptic area: Relationship with body temperature and hypothalamic–pituitary–adrenocortical axis activity. Eur. J. Neurosci. 7:2418–2430; 1995.
23. Linthorst, A. C. E.; Karanth, S.; Barden, N.; Holsboer, F.; Reul, J. M. H. M.: Impaired glucocorticoid receptor function evolves in aberrant physiological responses to bacterial endotoxin. Eur. J. Neurosci. 11:178–186; 1999.
24. Meisch, R. A.; Stewart, R. B.: Ethanol as a reinforcer: A review of laboratory studies of non-human primates. Behav. Pharmacol. 5:425–440; 1994.
25. Mello, N. K.: A review of methods to induce alcohol addiction in animals. Pharmacol. Biochem. Behav. 1:90–101; 1973.
26. Pohorecky, L. A.: The interaction of alcohol and stress. A review. Neurosci. Biobehav. Rev. 5:209–229; 1981.
27. Pohorecky, L. A.: Interaction of ethanol and stress: Research with experimental animals—An update. Alcohol Alcohol. 25:263–276; 1990.
28. Pucilowski, O.; Krzascik, P.; Trzaskowska, E.; Kostowski, W.: Different effect of diltiazem and nifedipine on some central actions of ethanol in the rat. Alcohol 6:165–168; 1989.
29. Samson, H. H.; Falk, J. L.: Alteration of fluid preference in ethanol-dependent animals. J. Pharmacol. Exp. Ther. 190:365–376; 1974.
30. Samson, H. H.; Harris, R. A.: Neurobiology of alcohol abuse. Trends Pharmacol. Sci. 13:206–211; 1992.
31. Schulteis, G.; Hyytiä, P.; Heinrichs, S. C.; Koob, G. F.: Effects of chronic ethanol exposure on oral self-administration of ethanol or saccharin by Wistar rats. Alcohol. Clin. Exp. Res. 20:164–171; 1996.
32. Sinclair, J. D.; Senter, R. J.: Increased preference for ethanol in rats following alcohol deprivation. Psychonom. Sci. 8:11–12; 1967.
33. Sinclair, J. D.: The alcohol-deprivation effect in monkeys. Psychonom. Sci. 25:21–22; 1971.
34. Sinclair, J. D.; Taira, T.: Hangover hyperthermia in rats: Relation to tolerance and external stimuli. Psychopharmacology (Berlin) 94:161–166; 1988.
35. Spanagel, R.; Hölter, S. M.; Allingham, K.; Landgraf, R.; Zieglgänsberger, W.: Acamprosate and alcohol: I. Effects on alcohol intake following alcohol deprivation in the rat. Eur. J. Pharmacol. 305:39–44; 1996.
36. Spanagel, R.; Putzke, J.; Stefferl, A.; Schöbitz, B.; Zieglgänsberger, W.: Acamprosate and alcohol: II. Effects on alcohol withdrawal in the rat. Eur. J. Pharmacol. 305:45–50; 1996.
37. Spanagel, R.; Hölter, S. M.: Long-term alcohol self-administra-

tion with repeated alcohol deprivation phases: An animal model of alcoholism? Alcohol Alcohol. 34:231–243; 1999.
38. Spanagel, R.; Hölter, S. M.: Pharmacological validation of a new animal model of alcoholism J. Neural Transm. (in press).
39. Trapold, M. A.; Sullivan, H. L.: A withdrawal-related reinforcing effect of alcohol. Bull. Psychonom. Soc. 13:327–329; 1979.
40. Veale, W. L.; Myers, R. D.: Increase of alcohol preference in rats following repeated exposures to alcohol. Psychopharmacologia 15:361–372; 1969.
41. Vogel, W. H.; Evans, B. D.: Stress and alcohol. In: Watson, R. R., ed. Drug and alcohol abuse reviews, vol. 6. Alcohol and hormones. Totowa, NJ: Humana Press Inc.; 1995:227–245.
42. Waller, M. B.; McBride, W. J.; Lumeng, L.; Li, T.-K.: Induction of dependence on ethanol by free-choice drinking in alcohol-preferring rats. Pharmacol. Biochem. Behav. 16:501–507; 1982.
43. Wolffgramm, J.; Heyne, A.: Social behavior, dominance, and social deprivation of rats determine drug choice. Pharmacol. Biochem. Behav. 38:389–399; 1991.