2004

Enhanced operant self-administration of alcohol in Wistar rats receiving intermittent versus continuous alcohol vapor exposure

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0145-6008/04/2811-1676$03.00/0
ALCOHOLISM: CLINICAL AND EXPERIMENTAL RESEARCH

Vol. 28, No. 11
November 2004

Enhanced Alcohol Self-Administration after Intermittent Versus Continuous Alcohol Vapor Exposure

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Background: Ethanol self-administering rats exhibit enhanced responding during withdrawal from continuous exposure to ethanol vapor. This study compared self-administration of ethanol during withdrawal from continuous versus intermittent ethanol vapor.

Methods: Experiment 1 examined self-administration of ethanol in rats trained to self-administer ethanol after continuous, intermittent (14 hr on and 10 hr off), or no (i.e., controls) ethanol vapor exposure. Exposure time was equalized such that the intermittent group received 4 weeks of exposure and the continuous group received 2 weeks of exposure. Four self-administration tests were conducted 2 hr after removal from vapor, and each test was separated by 3 to 4 days of ethanol vapor. Experiment 2 examined self-administration of ethanol after 2 weeks of intermittent vapor either 2 or 8 hr after removal from vapor. Experiment 3 addressed the specificity of the increased responding for ethanol by examining saccharin self-administration after 2 weeks of intermittent vapor.

Results: Four weeks of intermittent exposure produced an increase in ethanol self-administration during the first withdrawal relative to controls and relative to animals receiving 2 weeks of continuous exposure. The continuous group was indistinguishable from controls on the first test and gradually increased their responding across tests. Two weeks of intermittent exposure also increased ethanol self-administration, and there was no difference in this effect 2 or 8 hr after removal from vapor. There was no difference in saccharin self-administration in control rats and those given 2 weeks of intermittent exposure.

Conclusions: The finding that intermittent exposure produces more rapid increases in self-administration of ethanol relative to continuous exposure suggests that intermittent exposure may be associated with a more rapid escalation of the allostatic processes responsible for excessive ethanol self-administration. The mechanisms that drive the increases in drinking during withdrawal are similar after 2 and 8 hr of withdrawal and seem to be specific to ethanol.

Key Words: Alcohol, Intermittent, Continuous, Vapor, Self-Administration.

MUCH RESEARCH HAS focused on producing animal models of ethanol dependence in humans. Early work using a technique of feeding ethanol as part of a totally liquid diet found that the physical dependence and withdrawal observed in alcoholics were reproduced in animal models [for a review, see Lieber and DeCarli (1982)]. Work in our laboratory has shown that animals trained to self-administer ethanol exhibit increases in operant self-administration of ethanol after chronic exposure to ethanol vapor sufficient to produce dependence, as measured by physical and motivational signs upon withdrawal (Roberts et al. 1999; Schulteis et al. 1995). For example, animals exhibit reliable increases in self-administration of ethanol during withdrawal from ethanol vapor exposure (the intake approximately doubles), and the animals maintain blood alcohol levels (BALs) from 100 to 150 mg/100 ml for up to 12 hr of withdrawal (Roberts et al., 1996). Additionally, animals exposed to chronic ethanol vapor exhibit a time-dependent decrease in brain reward function with the intracranial self-stimulation procedure that persists up to 72 hr after removal from ethanol vapor (Schulteis et al., 1995). The decrease in brain reward function observed in intracranial self-stimulation studies has been well established as a tool for assessing the negative aspects of withdrawal from various drugs of abuse (Markou et al., 1998).

Studies using the chronic ethanol vapor exposure model have supported its predictive validity as a model of alcohol dependence in humans. For example, increased operant self-administration of ethanol after chronic vapor exposure is blocked by acamprosate and naltrexone (Heyser et al., 2003; Morse and Koob, 2002), compounds that display

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Received for publication May 3, 2004; accepted August 9, 2004.

Supported by Grants AA06420, AA08459, and AA12602.

This is publication 16389-NP from The Scripps Research Institute.

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DOI: 10.1097/01.ALC.0000145781.11923.4E

clinical efficacy in treating alcoholism (Littleton and Zieglgansberger, 2003). Moreover, dependent rats exhibiting increased operant self-administration of ethanol after chronic ethanol vapor exhibit alterations in drinking behavior after manipulations that contribute to excessive drinking in humans, such as those involving the stress axis. Specifically, competitive corticotropin-releasing factor antagonists have no effect on ethanol self-administration in nondependent rats but effectively eliminate excessive drinking in dependent rats (Valdez et al., 2002).

Studies in our laboratory have used a regimen of continuous ethanol vapor exposure with repeated withdrawals after an extended period of continuous vapor. The repeated withdrawals refer to the short 2-hr withdrawal periods on test days that are inherent in our model of continuous ethanol exposure, and they do not constitute enough time to consider our procedure an intermittent treatment regimen. However, continuous ethanol vapor exposure may not optimize the neuroadaptive changes that are hypothesized to contribute to the development of dependence, because continuous vapor exposure does not mimic the commonly observed on/off nature of binge ethanol intake patterns in humans. Thus, it is reasonable to hypothesize that an intermittent ethanol vapor exposure procedure may produce a more valid animal model of the progression of alcoholism. For example, studies comparing the effects of alcohol after continuous or intermittent exposure have shown that withdrawal-induced seizures seem to be more prevalent after intermittent ethanol treatment regimens relative to continuous ones (Becker, 2000; Matsumoto et al., 2001; Mhatre et al., 2001). Comparison studies have also observed more profound alterations in γ-aminobutyric acid systems after intermittent treatment regimens, and this is relevant in light of the role of this system in mediating ethanol withdrawal–induced seizures (Mhatre et al., 2001; Petrie et al., 2001). Also, rats trained to self-administer ethanol exhibit enhanced self-administration with repeated periods of abstinence from an ethanol liquid diet (Schulteis et al., 1996). Enhanced ethanol intake has also been observed in a two-bottle free-choice situation after 7 weeks, but not after 2 or 4 weeks, of intermittent ethanol vapor exposure relative to controls that did not receive vapor exposure (Rimondini et al. 2002, 2003).

The goal of this study was to compare operant self-administration of ethanol in animals trained to self-administer ethanol and exposed to ethanol vapor either continuously (24 hr on) or intermittently (14 hr on/10 off) during each day of vapor exposure. Control rats were trained but not exposed to ethanol vapor. Operant self-administration of ethanol was also compared at two time points (2 and 8 hr) of withdrawal from intermittent vapor exposure. A final experiment compared self-administration of an alternative reinforcer (i.e., saccharin) in control rats and those exposed to intermittent ethanol vapor.

METHODS

Animals

Male Wistar rats weighing 180 to 200 g at the start of the experiment were obtained from Charles River Laboratory (Kingston, NY). Rats were housed two to three per cage with food and water available ad libitum, except for 3 days of water restriction during the initiation of operant testing. Separate groups of rats were used for each of the three experiments. Lights were on a 12-hr light/dark cycle, with lights on at 6:00 AM. All procedures met the guidelines of the NIH *Guide for the Care and Use of Laboratory Animals*.

Operant Ethanol Self-Administration

Ethanol (10% w/v) was prepared with 95% ethyl alcohol and water. Saccharin (Sigma Chemical Co., St. Louis, MO) was added to water or the ethanol solutions to achieve a concentration of 0.2% w/v. Ethanol self-administration was established in standard operant chambers (Coulbourn Instruments, Allentown, PA) that were housed in sound-attenuated ventilated cubicles. A continuous reinforcement (fixed ratio-1) schedule was used such that each response resulted in delivery of 0.1 ml of fluid delivered into either of two stainless-steel drinking cups mounted 4 cm above the grid floor in the middle of one side panel. Two retractable levers were located 4.5 cm to either side of the drinking cups. Fluid delivery and recording of operant responding were controlled by a microcomputer. During the 0.5 sec in which the pumps were activated, lever presses were not recorded.

Rats were trained to self-administer ethanol by using a sweet saccharin solution fading procedure. This paradigm has been shown to produce pharmacologically relevant BALs with limited access to ethanol over approximately 6 weeks (Roberts et al., 1996, 1999). Briefly, rats were restricted to 3 hr of water for 3 days only and were allowed access to the operant boxes where responding on the one extended lever resulted in delivery of a saccharin solution. Thereafter, water restriction was discontinued. Ethanol concentrations increased from 5 to 8% to a final concentration of 10% over the following 20 days. Each concentration was first mixed with saccharin and then presented alone. During this saccharin fading procedure, both levers were extended with one lever producing ethanol/saccharin and the other lever producing water. Initially, the levers associated with each solution were alternated between left and right on consecutive days, and then ultimately one of the levers produced ethanol and the other lever produced water. Before exposure to ethanol vapor, rats were allowed to respond for 10% ethanol versus water for 4 to 6 weeks until responding across three consecutive days varied less than 25% and preference for ethanol over water was at least 60%. In each experiment, control and ethanol vapor–treated groups were matched for similar levels of ethanol self-administration according to the last 5 days of operant responding before the vapor-exposure period. All operant sessions were 30 min long and were conducted 5 days per week between 9:00 AM and 12:00 PM (lights on from 6:00 AM to 6:00 PM).

Alcohol Vapor Chamber Procedure

Two standard rat cages were housed in separate sealed clear plastic chambers into which ethanol vapor was introduced. Ethanol vapor was created by dripping 95% ethanol into 2000-ml Erlenmeyer vacuum flasks kept at 50°C on a warming tray. Air was blown over the bottom of the flask at 11 liters/min to vaporize the ethanol. Concentrations of ethanol vapor were adjusted by varying the rate at which ethanol was pumped into the flask and ranged from 22 to 27 mg/liter. Chambers administering intermittent vapor were connected to a timer that would turn the ethanol vapor on and off every day so that the animals received vapor for 14 hr, and then it was off for 10 hr, during which time the rats did not receive ethanol vapor.

Previous work in our laboratory has demonstrated that ethanol vapor exposure is a reliable technique for inducing ethanol dependence in that BALs can be maintained at a certain level, and animals are free-moving

and gain weight normally (Roberts et al., 2000). Moreover, this paradigm has been shown to produce physical dependence, as evidenced by the appearance of observable withdrawal symptoms upon removal from the chambers (Roberts et al., 1996). Blood was sampled for BAL determination every 3 days during vapor exposure and upon removal from the chambers. Tail blood (0.5 ml) was collected into heparinized Eppendorf tubes. After centrifugation, the plasma was extracted with trichloroacetic acid and assayed for ethanol content by using the nicotinamide adenine dinucleotide-alcohol dehydrogenase (NAD-ADH) enzyme spectrophotometric method (Sigma). Target BALs were 150 to 200 mg/100 ml across both the intermittent and continuous exposure procedures.

Experiment 1: Ethanol Self-Administration After Intermittent or Continuous Vapor Exposure

This experiment examined whether intermittent or continuous ethanol vapor exposure produces different levels of operant self-administration of ethanol after immediate withdrawal (i.e., 2 hr) from ethanol vapor relative to rats that were not exposed to the ethanol vapor. The animals were first trained to respond for 10% ethanol by using the saccharin fading procedure. Rats were then divided into three groups that received intermittent (14 hr on/10 hr off; $n = 16$), continuous ($n = 12$), or no ethanol vapor exposure (i.e., controls; $n = 16$). To equalize the total number of hours spent in ethanol vapor, rats exposed to continuous ethanol vapor were in the chambers for 2 weeks, whereas rats exposed to intermittent vapor were in the chambers for 4 weeks. Control rats were left alone during the vapor exposure period. The 4-week time point was selected according to previous work from our laboratory which demonstrated that once a stable BAL of 150 to 200 mg/100 ml is reached, enhanced drinking is observed after 4 weeks of exposure to continuous ethanol vapor (Roberts et al., 2000). On test days, rats receiving intermittent and continuous exposure were removed from the ethanol vapor chambers after termination of the intermittent vapor. All vapor-exposed rats were then immediately sampled for BALs by cutting the tip of the tail and stroking the tail to draw a small volume of blood (i.e., at least 0.05 ml). Control rats were handled in the same manner except that the tip of the tail was pinched to mimic the bleeding procedure in the vapor-treated rats. The control rats were kept in similar housing conditions (i.e., light cycle, food access, and handling). Housing these animals outside of the vapor chambers ensured that the control group did not receive any alcohol exposure. With respect to the tail-bleeding procedure, because ethanol is an analgesic, the vapor-treated rats experienced the tail bleed quite differently (i.e., a milder stress) relative to the control rats. Therefore, tail-pinching the controls is likely a good control for this procedure. Control and vapor-treated animals received the same handling during the tail-pinching and -bleeding procedure, and their behavioral responses were identical. Two hours later, rats were tested for ethanol self-administration in 30-min self-administration tests. Five tests were run with two to three intervening days, when the animals were re-exposed to the ethanol vapor on their respective schedules. Rats given intermittent vapor started 2 weeks early so that testing was performed for all groups on the same day. This experiment was replicated in two separate cohorts representing each group of animals.

Experiment 2: Ethanol Self-Administration After 2 Weeks of Intermittent Vapor Exposure

This experiment examined whether rats exposed to intermittent ethanol vapor for only 2 weeks would exhibit enhanced operant self-administration of ethanol. Additionally, we wanted to examine whether subsequent operant self-administration of ethanol would be higher in rats tested 8 hr into withdrawal versus the immediate 2-hr time point that was used in experiment 1. Rats were trained and tested in a procedure similar to that described in experiment 1. Two groups of rats were exposed to intermittent vapor for 2 weeks, and on the test days, one group of rats ($n = 6$) was tested 2 hr after removal from vapor, whereas the other group ($n = 6$) was tested 8 hr after removal from vapor. Testing was performed as in experiment 1, and control rats were tested at an intermediate time point between the withdrawal groups. Control rats were left alone during the vapor-exposure period, and subsequent tests revealed that there was no difference in operant self-administration of ethanol in control rats that were tested at these different time points during the day (1:00 PM versus 5:00 PM).

Experiment 3: Saccharin Intake After 2 Weeks of Intermittent Vapor Exposure

This experiment compared saccharin self-administration in control rats exposed to intermittent ethanol vapor for 2 weeks. The purpose of this experiment was to determine whether dependent rats would exhibit enhanced self-administration of an alternate reinforcer other than alcohol and to determine whether the excessive drinking effect was specific to ethanol. This study also served as a replication of the finding that excessive drinking is observed after 2 weeks of intermittent vapor exposure. Rats were trained and tested in a procedure similar to that described for experiments 2 and 3. Rats then received 2 weeks of intermittent vapor exposure ($n = 6$), and control rats ($n = 5$) were left alone during the vapor-exposure period. Five self-administration tests were conducted 2 hr after removal from intermittent vapor exposure. A final test was conducted in a similar manner, but the animals were given access to 0.004% saccharin or water in the operant session. Previous work in our laboratory has shown that this dose of saccharin produces the same level of operant responding as 10% ethanol (Roberts et al., 1999; Schulteis et al., 1996).

Statistical Analysis

Baseline measures of ethanol self-administration were compared between treatment groups by using a one-way ANOVA. Alterations in ethanol self-administration were analyzed by using repeated-measures ANOVA with vapor treatment as a between-subjects factor and test session as a repeated measure. Analyses were performed with values from test sessions 1 to 5 only. Significant main effects were further analyzed with post hoc analyses to compare between treatment groups with Fisher's protected least significant difference (PLSD) test ($p < 0.05$). For experiment 3, a separate one-way ANOVA was performed to compare saccharin intake in dependent and nondependent rats.

RESULTS

The results from experiment 1 are displayed in Fig. 1. Baseline values reflect mean operant responses for ethanol in the 5 days before vapor exposure. There were no significant differences in mean baseline ethanol deliveries across the treatment conditions [$F(2,36) = 0.28$, $p < 0.75$; mean responses ± SEM: controls, 30.3 ± 1.70; intermittent, 33.2 ± 3.6; continuous, 34.1 ± 5.6]. There were no significant interaction effects across vapor treatment groups during test sessions 1 through 5 [$F(8,144) = 0.95$; $p = 0.47$]. However, the analyses of ethanol self-administration during the test sessions revealed a main effect of treatment, with rats receiving intermittent vapor exposure displaying significantly more ethanol deliveries relative to the other treatment groups [$F(2,36) = 8.5$; $p < 0.001$]. This analysis is reflected in the area under the curve inset in Fig. 1 (Fisher's PLSD test; $p < 0.05$).

The results from experiment 2 are displayed in Fig. 2. There were no significant differences in mean baseline ethanol deliveries across treatment conditions [$F(2,14) = 0.8$, $p < 0.46$; mean responses ± SEM: controls, 37.9 ± 4.2; intermittent 2-hr withdrawal, 44.2 ± 3.2; intermittent 8-hr withdrawal, 43.7 ± 3.8]. There were no significant interaction effects across vapor treatment groups during test ses-

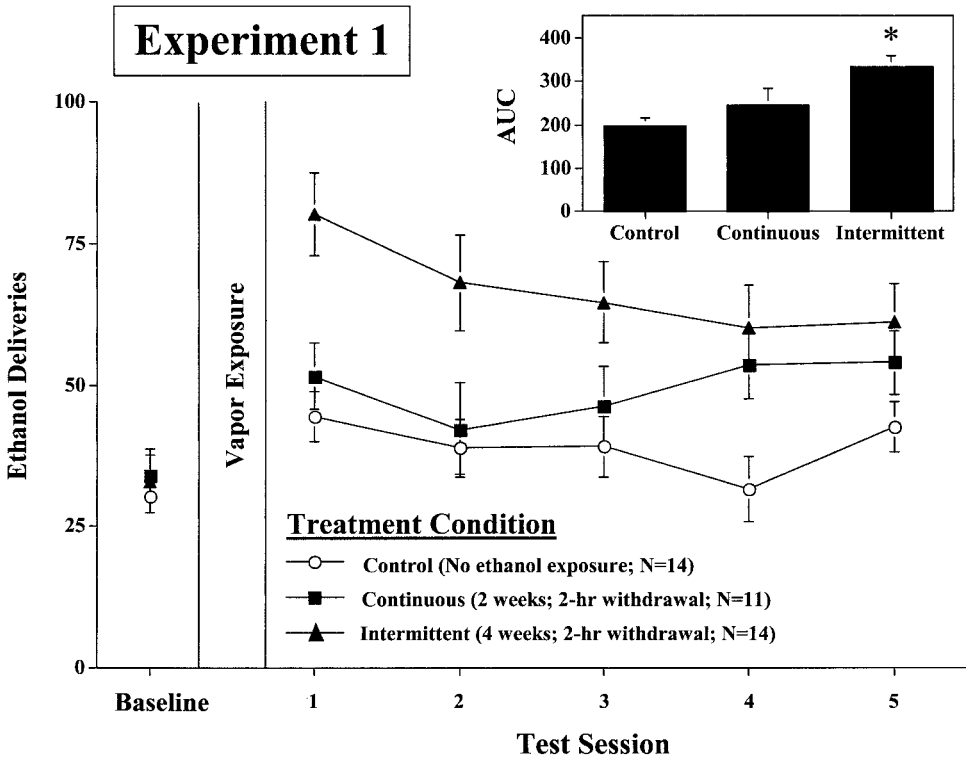

Fig. 1. Ethanol deliveries (mean ± SEM) in rats trained to respond for 10% ethanol and then either not exposed to ethanol vapor (i.e., controls; $n = 14$) or exposed to intermittent (14 hr on/10 hr off; $n = 14$) or continuous ($n = 11$) ethanol vapor. Rats exposed to continuous ethanol vapor were in the chambers for 2 weeks, whereas rats exposed to intermittent vapor were in the chambers for 4 weeks. All rats were tested 2 hr after removal from the vapor chambers. The area under the curve (AUC) reflects the mean total ethanol deliveries across the five test sessions. *Significant increase in operant self-administration of ethanol in rats receiving intermittent vapor exposure relative to controls or rats receiving continuous vapor exposure ($p < 0.05$).

sions 1 through 5 [$F(8,56) = 1.7$; $p = 0.12$]. However, the analyses of ethanol self-administration during the test sessions revealed a main effect of treatment, with rats receiving intermittent vapor exposure displaying significantly more ethanol deliveries relative to the other treatment groups [$F(2,14) = 30.2$; $p < 0.0001$]. There was no difference between rats that were tested 2 or 8 hr after withdrawal from intermittent ethanol vapor. This analysis is reflected in the area under the curve inset in Fig. 2 (Fisher's PLSD test; $p < 0.05$).

The results from experiment 3 are displayed in Fig. 3. There were no significant differences in mean baseline

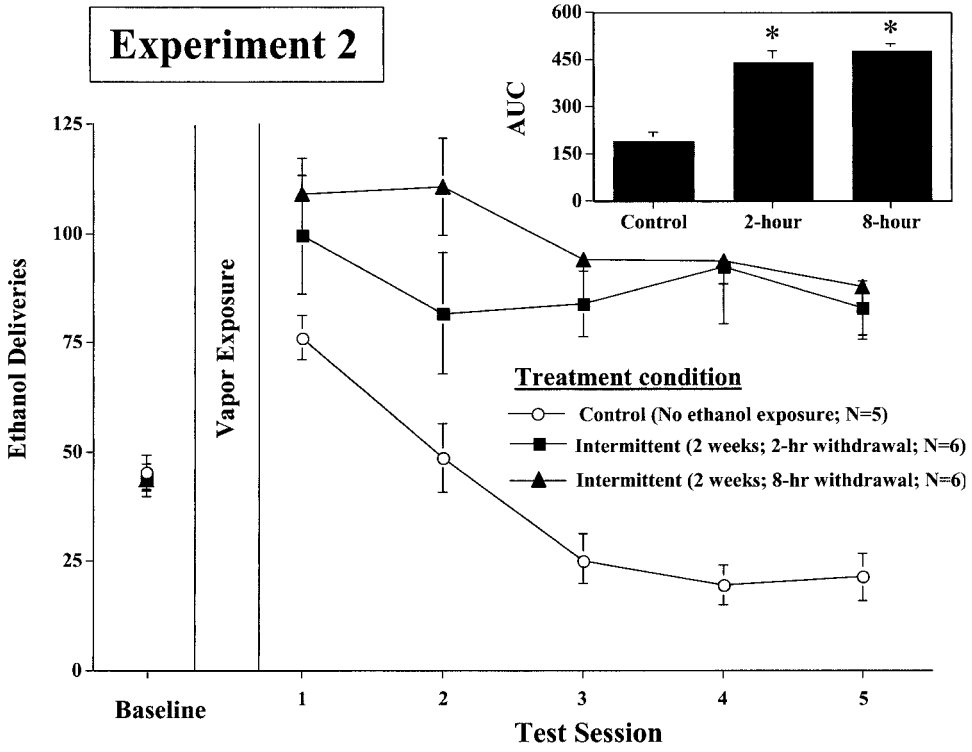

Fig. 2. Ethanol deliveries (mean ± SEM) in rats trained to respond for 10% ethanol and then either not exposed to ethanol vapor (i.e., controls; $n = 5$) or exposed to intermittent vapor (14 hr on/10 hr off) for 2 weeks and then tested either 2 hr ($n = 6$) or 8 hr ($n = 6$) after removal from ethanol vapor. The area under the curve (AUC) reflects the mean total ethanol deliveries in the five test sessions. *Significant increase in operant self-administration of ethanol in rats receiving intermittent vapor exposure relative to control rats ($p < 0.05$). There was no difference between rats exposed to intermittent vapor and tested either 2 or 8 hr after withdrawal from ethanol.

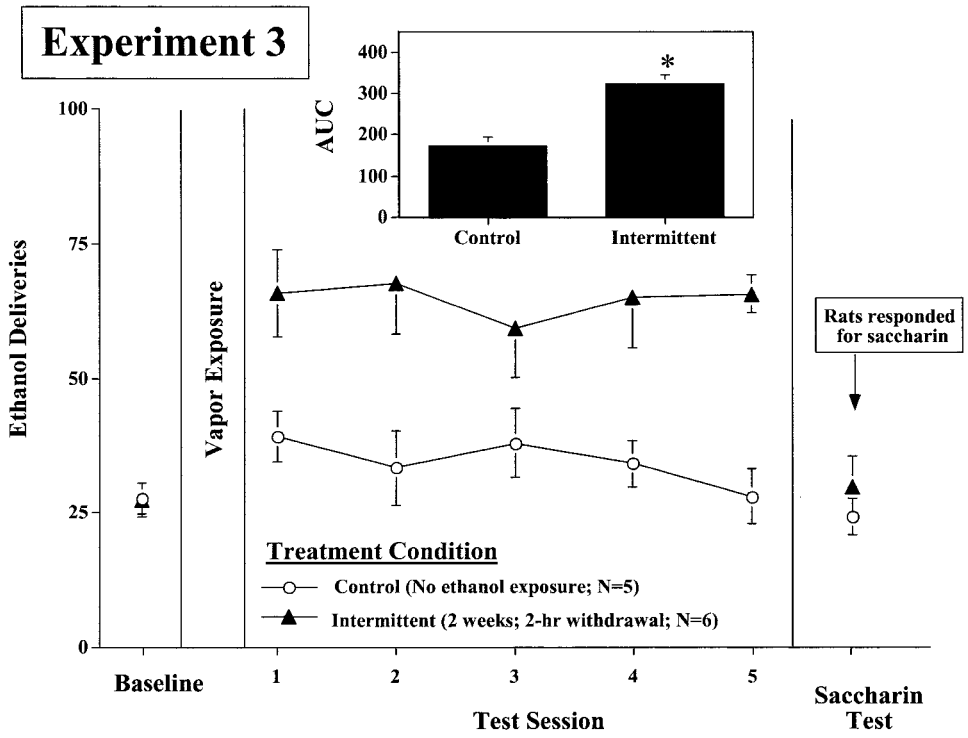

Fig. 3. Ethanol deliveries (mean ± SEM) in rats trained to respond for 10% ethanol and then either not exposed to ethanol vapor ($n = 5$) or exposed to intermittent ethanol vapor (14 hr on/10 hr off) for 2 weeks ($n = 6$) and tested 2 hr after removal from ethanol vapor. An additional test was run in which both groups of rats responded for 0.004% saccharin 2 hr after removal from ethanol vapor. The area under the curve (AUC) reflects the mean total of ethanol deliveries in the five test sessions. *Significant increase in operant self-administration of ethanol in rats receiving intermittent vapor exposure relative to control rats ($p < 0.05$). There was no significant difference in saccharin self-administration in control rats and rats receiving intermittent vapor exposure.

ethanol deliveries across treatment conditions [$F(1,9) = 0.44$, $p < 0.52$; mean responses ± SEM: controls, 28.8 ± 4.4; intermittent group, 32.5 ± 3.5]. There were no significant interaction effects across vapor treatment groups during test sessions 1 through 5 [$F(4,36) = 0.45$; $p = 0.76$]. However, the analyses of ethanol self-administration during the test sessions revealed a main effect of treatment, with rats receiving intermittent vapor exposure displaying significantly more ethanol deliveries relative to the control group [$F(1,9) = 25.3$; $p < 0.001$]. This analysis is reflected in the area under the curve inset in Fig. 3 (Fisher's PLSD test;
$p < 0.05$). On average, the rats displayed a significant preference (88%) for saccharin relative to water, and preference was defined as [(saccharin deliveries/saccharin deliveries + water deliveries) × 100]. There were no significant differences in saccharin deliveries across control rats and those exposed to intermittent vapor [$F(1,9) = 1.8, p < 0.21$; mean responses ± SEM: controls, 24 ± 4.1; intermittent group, 33.3 ± 5.2]. This concentration of saccharin produced levels of operant responding similar to those with 10% ethanol (i.e., approximately 25–30 responses).

DISCUSSION

Experiment 1 demonstrated that rats trained to self-administer alcohol displayed significantly more operant self-administration of ethanol after intermittent ethanol vapor exposure for 4 weeks relative to rats that received 2 weeks of continuous ethanol vapor exposure. In addition, experiment 2 illustrated that operant self-administration of ethanol was similar after 2 and 8 hr of withdrawal from intermittent ethanol vapor, thus suggesting that the mechanisms driving excessive ethanol self-administration in this model are similar during these early withdrawal time points. Further, the finding from experiment 3 that there was no difference in self-administration of an alternative reinforcer, such as saccharin, in rats that received intermittent vapor relative to controls suggests that the mechanisms driving excessive ethanol self-administration are likely specific to ethanol.

Previous work from our laboratory has demonstrated that at least 4 to 6 weeks of continuous ethanol vapor are necessary to observe significant increases in operant self-administration of ethanol during withdrawal (Roberts et al., 1999; Schulteis et al., 1995). The first experiment found that rats exposed to 2 weeks of continuous ethanol vapor showed increases in ethanol self-administration only at later tests, when the rats had been exposed to continuous vapor for 2 weeks plus the additional 3 weeks of re-exposure to vapor between test days. Given approximately the same number of hours of ethanol vapor exposure, rats exposed to intermittent vapor for 4 weeks displayed significantly more ethanol self-administration relative to rats exposed to continuous vapor for 2 weeks. Thus it seems that the repeated daily withdrawals experienced by the intermittent group produced a greater facilitation of ethanol self-administration relative to rats that received continuous exposure. Another possible explanation is that intermittent ethanol vapor was experienced for more days relative to continuous vapor. However, an increase in operant self-administration of ethanol was also observed only after 2 weeks of intermittent vapor relative to control rats

(experiment 2), an effect that has not been observed within such a short time after continuous vapor exposure.

This study compared alterations in ethanol self-administration 2 hr after removal from intermittent or continuous ethanol vapor. The 2-hr withdrawal period was chosen on the basis of large motivational effects that we have observed in the elevated plus maze and increased drinking behavior at this time point (Valdez et al., 2002). In addition, at this time point, BALs are significantly decreased, but not to such an extent that major physical signs of withdrawal are present that may interfere with responding. These findings cannot be explained on the basis of different BALs across experimental groups, because the average BALs across the five test sessions were similar for both intermittent (140.2 mg/100 ml) and continuous (141.8 mg/100 ml) administration groups. The BALs were also not different for any respective test session.

The findings from experiment 2 illustrate similar increases in operant self-administration of ethanol after 2 and 8 hr of withdrawal from intermittent vapor exposure. It was expected that more physical signs of withdrawal and negligible BALs would be observed 8 hr after removal from ethanol vapor (Roberts et al., 1996). Therefore, the similar increases in drinking observed at these time points suggest that motivational effects for ethanol surpass any potential competition by physical signs. The BALs in these experiments are associated with mild to moderate dependence and very mild signs of physical withdrawal. Therefore, under these conditions, increased ethanol self-administration is likely to be evident across the first 8 hr of withdrawal from ethanol.

Slight differences in baseline ethanol self-administration were observed between experiments 1, 2, and 3 (mean responses of 32.3, 42.2, and 30.8, respectively). These group differences are commonly observed between animal shipments and might be expected from this outbred strain of rats. This level of variability is also within the range of different studies in our laboratory that have used this model [i.e., 25–30 responses in Valdez et al. (2002), 33–36 responses in Roberts et al. (1996), and 40 responses in Schulteis et al. (1996)]. In each experiment, control animals exhibited an increase in ethanol self-administration from baseline to test 1 values, a phenomenon referred to as the alcohol deprivation effect (ADE). Slight differences in the ADE were also observed between experiments 1, 2, and 3 (mean increase of 32, 50, and 28%, respectively). This variability might also be related to differences in shipments of animals. Alternatively, the larger ADE in experiment 2 may be related to longer maintenance at 10% ethanol in these animals, a notion supported by studies demonstrating that longer training periods facilitate the ADE [for review, see Spanagel and Holter (1999)]. It is important to note that higher baseline and ADE values in experiment 2 do not confound the major conclusions of this report. In each experiment, animals were matched for baseline intake before vapor-exposure manipulations, and the conclusions were made from independent studies.

Work from other laboratories is consistent with the observation that the behavioral effects of ethanol are enhanced after intermittent ethanol exposure. Spanagel and Holter (1999) have also demonstrated that rats given long-term access to various concentrations of ethanol in a four-bottle-choice procedure exhibit enhanced intake after repeated periods of abstinence (i.e., ADE) and that these rats display physical signs of withdrawal. Increases in voluntary ethanol consumption and ethanol preference have also been observed after intermittent exposure for 7 weeks, but not after 2 to 4 weeks of intermittent ethanol vapor (Rimondini et al., 2003). Increases in alcohol intake have also been observed in a free-access situation in alcohol-preferring rats given repeated alcohol deprivations (Rodd-Henricks et al., 2001).

Studies comparing continuous and intermittent ethanol exposure have found that more changes in brain morphology are observed after an intermittent treatment regimen. For example, intermittent exposure to ethanol drinking produced a decrease in the number and density of sympathetic neurons in the superior cervical ganglion, whereas no changes were observed after continuous ethanol access (Riikonen et al., 1999). Similarly, a significant reduction in hippocampal synapses was observed after intermittent, but not continuous, ethanol access (Lundqvist et al., 1994). Despite more profound neural changes after intermittent exposure, the latter study also observed lower levels of total ethanol intake in rats given intermittent versus continuous access to ethanol. This suggests that the pattern of ethanol exposure is more important than the amount of ethanol experienced in the initiation of neural changes. Comparison studies have also found that repeated exposures to multiple cycles of ethanol withdrawal increase the sensitivity to seizures produced by a γ-aminobutyric acid inverse agonist relative to continuous ethanol exposure (Mhatre et al., 2001). The concentration of synaptosomal phospholipids is increased after intermittent, but not continuous, ethanol access (Alling et al., 1991; Gustavsson and Alling, 1989). Collectively, these studies suggest that an intermittent pattern of ethanol exposure enhances neural changes relative to continuous ethanol exposure.

The finding that intermittent ethanol exposure produces a larger increase in operant self-administration of ethanol relative to continuous vapor suggests that intermittent ethanol exposure may be associated with a more rapid escalation of allostatic processes that are responsible for excessive ethanol self-administration. We have argued that chronic alcohol exposure elicits allostatic changes within the brain's reward mechanisms as a means of maintaining stability in the face of chronic demands (Koob, 2003). Allostasis allows for the continuous re-evaluation and readjustment of all physiological parameters toward environmental demands, as well as for the anticipation of such demands. In an animal experiencing repeated ethanol with-

drawal, the set point for functioning is even further altered because of heightened demands on the mechanisms that regulate systems that are constantly undergoing withdrawal from ethanol. Therefore, repeated withdrawal episodes may be hypothesized to produce a more rapid alteration in the reward set point that drives excessive ethanol self-administration and dependence.

The neural mechanisms that drive excessive drinking after intermittent versus continuous vapor exposure are presently unclear. Although previous studies indicate that these mechanisms likely involve changes in neuroadaptive processes, these changes may occur at the system, cellular, or molecular level. For example, decreased dopamine functioning and increased corticotropin-releasing factor activity have been observed in the basal forebrain of rats made dependent on alcohol (Merlo Pich et al., 1995; Weiss et al., 1996). Future studies will explore how various molecular and cellular events drive these neuroadaptive processes and how they contribute to excessive drinking after intermittent ethanol exposure.

The results from this study represent a significant advancement in our animal model of ethanol dependence in rats. Intermittent ethanol vapor exposure produces a larger increase in excessive drinking in a shorter period of time relative to continuous vapor exposure. More importantly, intermittent exposure more closely mimics the binge intake pattern in human alcoholics and, thus, provides a better model of the development of dependence and compulsive use and relapse after periods of abstinence in human alcoholics.

ACKNOWLEDGMENTS

We thank Drs. Annika Thorsell and Brendan Walker for their insightful comments on this manuscript, and Mike Arends for his excellent editorial assistance.

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