ELSEVIER

Physiology & Behavior 85 (2005) 479 – 488

PHYSIOLOGY & BEHAVIOR

Home cage activity and ingestive behaviors in mice following chronic ethanol vapor inhalation

Christopher L. Kliethermes*, Kim Cronise, John C. Crabbe

Department of Behavioral Neuroscience, Oregon Health & Science University and the Portland Alcohol Research Center, c/o Portland VA Medical Center, R&D 12, 3710 SW US Veterans Hospital Road, Portland, OR 97239, United States

Received 8 November 2004; received in revised form 16 March 2005; accepted 25 May 2005

Abstract

Although drug withdrawal may induce an anxiety-like state, decreased locomotion in tests of anxiety-like behavior is an almost universal finding in rodent studies of ethanol withdrawal. Decreased locomotion in many behavioral apparatus, either as a result of a withdrawal-induced lethargy, malaise, or reduced motivation to explore confounds interpreting the effects of withdrawal as specifically increasing an anxiety-like state. To address this issue, we measured home cage activity levels as well as food and water intake for 3 days prior to and up to 5 days after chronic ethanol vapor exposure in genetically heterogeneous mice. In the first experiment, ethanol-withdrawing WSC-2 mice drank less water than controls, but did not differ from controls on any other behavioral measure during the withdrawal assessments. When the dose of ethanol was elevated in a subsequent experiment in WSC-2 mice, a similar temporary decrease in food and water intake, but not in locomotion, was observed during withdrawal. These results differed from those of mice placed into activity monitors during peak withdrawal, which exhibited profoundly reduced activity levels compared to controls. Finally, home cage activity levels during withdrawal were only transiently decreased in a mouse line that has been selectively bred to display high ethanol withdrawal handling-induced convulsion severity (WSP mice). The reduction in food and water consumption seen in most experiments suggests that withdrawal may induce a temporary malaise-like state, but this state is not reflected in altered home cage activity levels. Further, even in a relatively severe mouse model of alcohol withdrawal, any decreases in general home cage activity are short-lived.
Published by Elsevier Inc.

Keywords: Alcohol withdrawal; Mice; Anxiety-like behaviors; Home cage

1. Introduction

Anxiety is a characteristic of ethanol withdrawal [25–27], and has been frequently studied in rodents in order to evaluate the efficacies of potential therapeutic agents and to understand the neurobiology of ethanol withdrawal (e.g. [16,21]). A common finding in studies of ethanol withdrawal-induced anxiety-like behavior in rodents is decreased locomotor activity (e.g. [10,15,37–39], see [22] for review).

Most rodent studies of anxiety-like behavior employ a task dependent on voluntary exploration of the apparatus, such as the elevated plus (or zero) maze, the light–dark box, or an open arena. The elevated plus maze, currently the most frequently utilized of these tests, consists of two open and two closed arms radiating from a central square. Drugs with known anxiolytic effects in humans increase the amount of time that rodents spend on the normally aversive open arms of the maze, while anxiogenic drugs decrease this time. Interpreting the effects of a drug as specifically increasing or decreasing an anxiety-like state, however, is complicated if the drug also alters locomotor activity [7,24]. In the elevated plus maze, activity is indicated by the numbers of closed or total arm entrances during the test.

In studies utilizing the elevated plus maze during ethanol withdrawal, decreased time spent on the open arms has been observed in rats [9,10,12,29,30,39] and mice [13,14,37–39], but in almost all cases, concurrent decreases in closed or total arm entrances have also been observed (see [22] for

* Corresponding author. Tel.: +1 503 220 8262x56675(lab), +1 503 220 8262x54392(office); fax: +1 503 721 1029.
E-mail address: kliether@ohsu.edu (C.L. Kliethermes).

0031-9384/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.physbeh.2005.05.009

review). Similarly, general decreases in activity during ethanol withdrawal have been observed in the social interaction test [28], the light–dark box [37] and other tests that possibly reflect an anxiety-like state [3,20]. These findings of decreased locomotor activity make it difficult to attribute the reduced open arm time or entries to increased anxiety during ethanol withdrawal and not simply to the overall decreased locomotion.

In at least three studies, withdrawal-induced anxiety-like behavior has been investigated using the elevated plus maze in mice during withdrawal from chronic ethanol vapor inhalation [13,14,31]. These studies differed in the duration of ethanol vapor exposure (3–25 days), ethanol dose administered, and genotype of mouse used (Withdrawal Seizure-Prone (WSP) vs. Withdrawal Seizure-Resistant (WSR); C57BL/6J vs. DBA/2J; and NIH/S, respectively). Even though none of these studies reported increased anxiety-like behavior in withdrawing as compared to control mice (such as selective avoidance of the open arms by withdrawing mice), all three observed decreased locomotion during withdrawal as measured by the number of total arm entrances. In contrast, we recently demonstrated that withdrawal from 3 days of chronic vapor inhalation produced an anxiety-like response on the elevated zero maze in genetically heterogenenous WSC-2 mice, but that this response was only detectable after relatively extensive prior exposure to the maze [23]. However, this presumably increased anxiety-like behavior during withdrawal was also accompanied by decreased locomotion.

We considered the possibility that withdrawal from alcohol may induce a general “malaise” in rodents that manifests as reduced activity, among other signs, and may give the appearance of an anxiety-like state when behavior is measured in an exploration-based test of anxiety-like behavior. If ethanol withdrawal induces malaise, it seems probable this would be evidenced by changes in several behaviors relevant to normal functioning, including locomotor activity and food and water consumption in the home cage. These behaviors are not necessarily equivalent to those that define classic physiological sickness, as in the case of an immune challenge with antigens, which is characterized by multiple behavioral, hormonal, and immunological alterations [18]. However, reduced activity and feeding and altered corticosterone levels would perhaps be expected in either condition. Similar to procedures used in previous research with rats [19,33], the current experiments evaluated these basic behaviors in mice following ethanol vapor inhalation for evidence of a withdrawal-induced malaise.

2. Materials and methods

2.1. Subjects

Male mice of a locally maintained genetically outbred stock (WSC-2), aged 50–70 days at the beginning of the experiment, were used for Experiments 1 and 2. Male 70- to 90-day-old Withdrawal Seizure-Prone (WSP-1 and WSP-2) mice were used for Experiment 3. These lines of mice were selectively bred from the same foundation stock as WSC-2 for severe handling induced convulsions during withdrawal following chronic ethanol vapor exposure, and each of the two replicate lines has been maintained as an independent population [4]. Prior to the experiments, naïve mice were housed 2–4 per cage on Bed-o-cob bedding on a 12-h light/dark cycle (lights on at 0600 h). Food (Purina 5001) and water were available ad libitum. All polysulfone cages measured 17×28 cm.

2.2. General procedure

2.2.1. Home Cage behaviors (all experiments)

At 0700 h, two mice were individually housed in a home cage placed in 1 of 12 activity monitors (Accuscan Instruments, Columbus OH) for 3 days prior to exposure to the inhalation chambers. Each monitor was placed in a sound attenuating plastic enclosure equipped with an individual fan and light source. For home cage behavioral measures, two cages were placed into each activity monitor in a manner that allowed independent recordings from each home cage. Activity counts (beam interruptions) were cumulated in 1-h bins throughout each of 3 days of acclimation and the subsequent assessments during withdrawal. Water and food intake and body weights were recorded daily between 0600 and 0700 h, during which time the activity monitors were not recording locomotion. Water intake was measured using standard 25-mL tubes with no ball bearing, and food intake was recorded as the difference between daily measurements in the weight of the cage top which contained the food. When large pieces of food were found in the bedding, these were collected and returned to the cage top. For Experiments 1 and 2 with WSC mice, these variables were recorded for 3 days before and 5 days after 72 h of ethanol vapor inhalation, but not during the inhalation itself. Experiments 1 and 2 used the same procedure for home cage behavior recordings, except that mice in Experiment 2 began withdrawal at a higher blood ethanol concentration (BEC) than those in Experiment 1. In Experiment 3 with WSP mice, behaviors during the baseline period were measured for 3 days, but behaviors during withdrawal were measured for 2 days only. Each activity monitor housed the same two mice between baseline and withdrawal assessments, but a new home cage was used for each mouse after removal from the inhalation chambers. The home cage bedding was not changed during either the baseline or the withdrawal assessments. Sample sizes for Experiments 1 and 2 were 14 ethanol- and 10 air-exposed WSC-2 mice each, while for Experiment 3, 13 ethanol- and 11 air-treated mice were used, with 5–7 WSP-1 and WSP-2 mice each in the ethanol- and air-exposed groups.