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Research report

# Nest building is a novel method for indexing severity of alcohol withdrawal in mice

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## HIGHLIGHTS

- Chronic ethanol vapor inhalation dose-dependently suppressed nest building for up to 32 h after withdrawal.
- Withdrawal from an acute, high dose (4 g/kg) of ethanol reduced nest building for up to 32 h.
- Body temperature predicted nest building in naïve HS/Npt mice but not in the same mice following ethanol vapor chamber exposure.

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## ABSTRACT

Withdrawal after chronic ethanol (EtOH) affects body temperature, goal-directed behavior and motor function in mice and increases general central nervous system excitability. Nest-building tests have been used to assay these states but to this point have not been employed as measures of EtOH withdrawal severity. We first refined nest-scoring methods using a genetically heterogeneous stock of mice (HS/Npt). Mice were then made physically dependent following three days of chronic EtOH vapor inhalation to produce average blood EtOH concentrations (BECs) of 1.89 mg/mL. EtOH withdrawal affected the progression of nest building over time when mice were tested 2–4 days after removal from three days of chronic exposure to EtOH. In a separate group of mice, chronic EtOH vapor inhalation (BECs 1.84 mg/mL) suppressed nest building over days 1–2 but not days 2–3 of withdrawal. In a following experiment, EtOH withdrawal dose-dependently slowed recovery of nest building for up to 32 h. Finally, we determined that long-lasting nest-building deficits extend to mice undergoing withdrawal from a high dose (4 g/kg) of acute EtOH. Sex differences for nest building were absent following EtOH exposure. In mice naïve to EtOH treatments, male mice had lower pre-test body temperatures and increased nest scores across a two-day testing period compared to females. These results suggest that nest building can be used to assess chronic and acute EtOH withdrawal severity in mice.

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## 1. Introduction

Although alcohol withdrawal is a complex syndrome, many studies using rodent models have focused on a single physical response; the enhanced sensitivity to and/or severity of seizures. When quantified as a convulsion score in mice, this response can be used as a measure that is sensitive to the duration of exposure and dose of alcohol [1]. In mice, handling-induced convulsions (HICs) have been used for four decades to assess the severity of withdrawal from chronic EtOH vapor exposure, and they are also observed during withdrawal from a single, high-dose injection of EtOH [1,2]. Although seizures following chronic alcohol administration occur in a number of species [3,4], HICs in particular have only been described in mice. Additionally, convulsions represent only one effect that appears during a limited time window of withdrawal. In humans, the subjective effects of alcohol withdrawal may be present for up to weeks or months after abstinence [5]. Therefore, behavioral tests that can identify alcohol withdrawal states

*Abbreviations:* EtOH, ethanol; HIC, handling-induced convulsion; BEC, blood ethanol concentration; i.p., intraperitoneally; h, hours; PDT, Pacific Daylight Time.

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for extended time periods and can be potentially applied to many model organisms would be valuable.

Nest building is a behavior performed by mice and many other species. A mouse provided with nesting material will move it to a centralized location and build up walls. Nests in mice have been quantified based on the quantity of material used [6] or the quality of the nest, with high-quality nests having built-up walls that produce cupped or dome shapes [7,8]. Nest measures have been used to assess thermoregulatory behavior [9], positive motivational states [10,11] and sensorimotor function in mice [12]. These states are disrupted in mice over various time periods during withdrawal from chronic EtOH. During the initial 24 h of withdrawal from three days of chronic EtOH vapor inhalation, dysregulated body temperature [13] and motor abnormalities (e.g., increased tremor) [14] were observed in mice from a genetically heterogeneous stock and in mice selectively bred for severe EtOH withdrawal HICs, respectively. Longer-lasting motor impairments on the balance beam and accelerating rotarod were dose-dependently induced by chronic EtOH vapor inhalation and observed up to 3 days after withdrawal in mice from a genetically segregating background [15]. Effects of alcohol withdrawal on affective states may also be longer-lasting [5], and C57BL/6J mice display increased depression-like behavior (i.e., increased immobility during a forced swim test) for up to 14 days after withdrawal from two-bottle choice voluntary alcohol drinking [16].

Here, we refined nest-scoring methods to test for effects of EtOH withdrawal in a genetically heterogeneous stock of mice (HS/Npt). Nest building allows for a reduction of heat loss and has been studied extensively for thermoregulatory purposes in singly-housed mice. Mice selectively bred for greater weight of cotton used for nesting at room temperature had higher body temperatures than the low-nesting line (positive correlated response) [17]. In our initial experiments, we assessed body temperature and nest building before and after withdrawal from chronic EtOH vapor inhalation. In separate groups of mice, we tested for time-and dose-dependent effects of chronic EtOH. Finally, we tested whether effects of EtOH withdrawal on nest building extended to an acute, high-dose EtOH injection. With these experiments, we present nest building as a novel marker of EtOH withdrawal severity that can be detected for extended periods of time during withdrawal, and potentially be applied to many species.

## 2. Materials and methods

### 2.1. Animals and husbandry

Naïve male and female mice from a genetically' heterogeneous stock (HS/Npt) were bred in the VA Portland Health Care System's Veterinary Medical Unit and were 64–82 days old at the start of experiments. With the exception of one singly-housed animal, mice were housed 2–5 animals per plastic cage (28 × 17 × 11.5 cm) lined with Bed-O-Cob bedding (The Andersons, Maumee, OH, USA) and stainless steel wire bar tops with rodent chow 5001 (PMI Nutrition International, Brentwood, MO, USA) and tap water available *ad libitum*. These mice were derived from an 8-way cross of inbred mouse strains for which details have been previously described [18]. The colony room was held on a 12 h:12 h light:dark cycle [lights on at 2130 Pacific Daylight Time (PDT)] at a temperature of 21 ±1 °C, and procedural rooms were kept within the same temperature range. Mice were not exposed to nesting material prior to being included in experiments. Animal numbers for each experiment are detailed in Table 1. All procedures were in accordance with NIH Guidelines for the Care and Use of Laboratory Animals and approved by the Portland VA Health Care System's Institutional Animal Care and Use Committee.

**Table 1**
Sample sizes and sample losses across experiments.

| Experiment no. | Initial *N* | Final *N* | No. of trt groups | Final *N*/group |
|---|---|---|---|---|
| 1 | 8 | 8 | N/A | N/A |
| 2 | 40 | 38 | 2 | 16–22 |
| 3 | 71 | 59 | 2 | 28–31 |
| 4 | 80 | 76 | 4 | 16–23 |
| 5 | 43 | 43 | 2 | 21–22 |

### 2.2. Behavior

#### 2.2.1. Nest-building test

Mice were singly housed and 8 g of Enviro-Dri® (FiberCore, Cleveland, OH, USA) shredded paper nesting material was flattened on the half of the cage opposite the water bottle spout and a picture was taken from an overhead, high angle. The start time of the nest test depended on the design for experiments described below, and nests were scored at various time points during the light phase. Images were captured at each time point for scoring confirmation and comparison with the initial placement of nesting material. The nest-scoring rubric was adapted from methods previously described in mice [7,8]. If the nesting material was untouched, a nest was scored a 0. If the majority of nesting material was moved but scattered throughout the cage, the nest was scored a 1. Scores 2–5 were based on a centralized site built towards a maximum score of 5. We determined from experiment 1 (Fig. S1) that complete dome heights (measured from the floor of the cage to the apex of the dome) ranged from 7.60 to 10.10 cm (mean 9.38 ± 0.60 cm). Therefore, once a centralized site was present, the nest was conceptually split into quadrants, and the height of the nest wall for each quadrant determined the score. A score of 5 was given when the wall height was greater than half the height of a dome (>5 cm), a score of 4 was given when a wall was approximately half the height of a dome (4–5 cm), a score of 3 was given when the nest wall was less than half the height of a dome, but still cupped (<4 cm), and a score of 2 was given when the wall was flat against the cage bedding. The experimenter scored all four quadrants of the nest, took measurements as needed, and a picture was taken from an overhead, high angle. The four scores were averaged for a total nest score. Mice had *ad libitum* access to food and water throughout the experiments.

#### 2.2.2. Handling-induced convulsions

To confirm withdrawal severity during experiment 2, we tested mice for HICs following exit from the EtOH vapor chambers. Mice were first observed for HICs after being picked up gently by the tail. If mice did not display a convulsion, they were spun 180° and scored using a 0–7 rubric that has previously been described [19].

#### 2.2.3. Body temperature

Body temperatures were assessed at baseline and during withdrawal in experiment 2. Mice were first singly housed without nesting material for at least 60 min prior to temperature assessments. Mice were then weighed and placed briefly in restrainers before taking rectal temperatures with a glycerol-lubricated probe for 5 s (TH-8; Sensortek, Clifton, NJ). We tested for EtOH withdrawal induced changes in body temperature by subtracting the baseline body temperature from the body temperature recorded during withdrawal.

### 2.3. Drugs and administration

#### 2.3.1. EtOH injections

Mice were injected intraperitoneally (i.p.) with EtOH (Decon Laboratories, Inc., King of Prussia, PA) diluted in 0.9% saline (20%