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Assessment of affective and somatic signs of ethanol withdrawal in C57BL/6J mice using a short-term ethanol treatment

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ABSTRACT

Alcohol is one of the most prevalent addictive substances in the world. Withdrawal symptoms result from abrupt cessation of alcohol consumption in habitual drinkers. The emergence of both affective and physical symptoms produces a state that promotes relapse. Mice provide a preclinical model that could be used to study alcohol dependence and withdrawal while controlling for both genetic and environmental variables. The use of a liquid ethanol diet offers a reliable method for the induction of alcohol dependence in mice, but this approach is impractical when conducting high-throughput pharmacological screens or when comparing multiple strains of genetically engineered mice. The goal of this study was to compare withdrawal-associated behaviors in mice chronically treated with a liquid ethanol diet vs. mice treated with a short-term ethanol treatment that consisted of daily ethanol injections containing the alcohol dehydrogenase inhibitor, 4-methylpyrazole. Twenty-four hours after ethanol treatment, mice were tested in the open field arena, the elevated plus maze, the marble burying test, or for changes in somatic signs during spontaneous ethanol withdrawal. Anxiety-like and compulsive-like behaviors, as well as physical signs, were all significantly elevated in mice undergoing withdrawal, regardless of the route of ethanol administration. Therefore, a short-term ethanol treatment can be utilized as a screening tool for testing genetic and pharmacological agents before investing in a more time-consuming ethanol treatment.

Introduction

Alcohol abuse and dependence are a significant concern within the United States and many other countries (Carlson et al., 2012). In the United States, the rate of alcohol dependence is double that of other drugs of abuse, with more than 80 million individuals meeting the criteria for dependence (Carlson et al., 2012). Withdrawal symptoms promote drug relapse and represent a significant obstacle to successful alcohol cessation. They include increased anxiety, agitation, hypervigilance, insomnia, irritability, tremors, and, in the most severe cases, seizures occurring within 8–24 h after cessation of ethanol ingestion (Hall & Zador, 1997).

Current preclinical models used to induce alcohol dependence in both rats and mice include involuntary/forced exposure to ethanol through vapor, intragastric intubation, or liquid diet (Braconi et al., 2010; Fidler, Clews, & Cunningham, 2006; Fidler et al., 2012; Gilpin, Misra, & Koob, 2008; Gilpin et al., 2009; Kurokawa, Mizuno, & Ohkuma, 2013; Macey, Schulteis, Heinrichs, & Koob, 1996; O'Dell, Roberts, Smith, & Koob, 2004). Each procedure has advantages and disadvantages. Ethanol vapor produces very stable blood ethanol concentrations for specific durations of time and temporal patterns (O'Dell et al., 2004), but the equipment is expensive and requires a suitable amount of space. Intragastric intubation is a short-duration procedure but can produce excessively elevated blood ethanol concentrations, resulting in increased mortality rates (Majchrowicz, 1975). Ethanol liquid diets delivered as the only nutritional source to the animal produce high volume intakes and withdrawal symptoms upon ethanol cessation (Lieber & DeCarli, 1982). The liquid diet paradigms are more time consuming and require dedicated space because each experimental subject is single-housed. In addition, the diet is consumed

Abbreviations: i.p., intraperitoneal; 4MP, 4-methylpyrazole; OFA, open field arena; EPM, elevated plus maze; MBT, marble burying test.

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throughout the day and this leads to increased variability in blood ethanol concentrations and behavior within groups. All this requires larger animal numbers for accurate comparisons. Given the increasing number of genetically modified mouse models available for the identification of potential therapeutic approaches to ethanol dependence, it would be advantageous to have an inexpensive, short-duration preclinical model that can be used for drug screens before investing in a costly and time-consuming ethanol treatment.

The goal of this study was to survey an array of alcohol withdrawal symptoms in a short-term ethanol treatment paradigm that included an alcohol dehydrogenase inhibitor to prolong the half-life of ethanol in the plasma. Such a paradigm has been successfully employed in mice to induce handling-induced seizures and anxiety-like behavior during withdrawal (Farook et al., 2008; Farook, Morrell, Lewis, Littleton, & Barron, 2007). Our results indicate that ethanol cessation after a short course of intraperitoneal (i.p.) injections can produce withdrawal symptoms comparable to those observed after a 6 week ethanol diet. The study also expands the battery of behavioral tests that can be used to detect ethanol withdrawal symptoms in mice to include compulsive-like and physical symptoms.

Materials and methods

Animals

C57BL/6J mice of both sexes were used throughout the experiments. C57BL/6J is an inbred strain commonly used for behavioral studies because mice exhibit average behavior in most behavioral tasks during baseline conditions (Brooks, Pask, Jones, & Dunnett, 2005; Crawley et al., 1997). In addition, C57BL/6J is a relatively good breeder, easily available from the Jackson Laboratory, and is widely used as a background strain for many genetically engineered mouse models. Mice were no older than 3 months of age at the beginning of the ethanol treatments. All procedures complied with the directives of the Institutional Animal Care and Use Committee (IACUC) and the Center for Comparative Medicine. Weaning was performed 21 days after birth, and same-sex littermates were housed in cages containing 1 cm of corncob bedding with a maximum of five animals. The animals had *ad libitum* access to water and food pellets (Labdiet 5001, PMI®, Brentwood, MO), and were maintained on a 12 h light/dark cycle (lights on 7:00 AM and off at 7:00 PM). Ambient light level in the housing rooms was 350 lux, while the average light on the housing shelves, where cages were kept, was 80 lux.

Chronic alcohol treatment

Chronic injections

We adopted the chronic ethanol treatment described by Farook et al. (2007). Mice were injected daily with either 2 g/kg (20% w/v) ethanol or saline for a minimum of 9 days. Both ethanol and control solutions contained 9 mg/kg 4-methylpyrazole (4MP), an alcohol dehydrogenase inhibitor that helps to achieve elevated blood ethanol levels for longer durations (Farook et al., 2007). All behavioral paradigms were performed during spontaneous ethanol withdrawal (24 h after the last injection), during the lights on phase of the light/dark cycle (12:00 PM–5:30 PM). All treatments were performed in the housing room, under a hood (73 lux). During experiments, ambient light level was kept at 11 lux unless otherwise stated. All animals were acclimated to the behavior room for a minimum of 45 min before testing. Multiple testing was planned so that mice received their respective treatment immediately after each behavioral test and during the following day before being tested in another behavioral paradigm. Behavioral tasks were performed in the order of increasing stress: open field arena, elevated plus maze, marble burying, and somatic signs. Mice were weighed daily throughout the treatment and withdrawal testing, and no significant differences were observed between treatment groups (data not shown).

Blood ethanol concentrations (BECs) were measured using a separate group of mice. Mice were treated with saline + 4MP or ethanol (2 g/kg) + 4MP for 9 days. On the last day of treatment, blood was collected 1, 4, and 6 h after ethanol injections. Blood plasma was analyzed using the colorimetric ethanol assay kit from Sigma–Aldrich™ (St. Louis, MO). One hour after ethanol injection, BEC was 194.88 mg/dL (STE ± 11.13, $n = 6$ per time point) and was reduced by 40% and 79%, 4 h and 6 h later, respectively.

Liquid ethanol diet

A separate group of mice was exposed to an ethanol liquid diet for 6 weeks. Liquid diets have extensively been used in rodents to produce ethanol dependence and physiological changes associated with chronic ethanol use (Gilpin et al., 2008; Macey et al., 1996; Nan et al., 2013; Umathe, Bhutada, Dixit, & Shende, 2008; Verleye, Heulard, & Gillardin, 2009; Zhang et al., 2013). Mice were single-housed with a nestlet (a cotton square that provides rodents with enrichment and sterile material to make a nest) and were provided for 1 week with a basic diet consisting of the chocolate-flavored high protein nutritional drink, Boost® (Nestlé Health Science, Switzerland), supplemented with 3 g/L vitamin mixture (MP Biomedicals, LLC, Solon, OH) and 5 g/L mineral mix (ICN Biomedicals, Inc., Aurora, OH) (Gilpin et al., 2008; Verleye et al., 2009). After a habituation period to the liquid diet, mice were divided into two groups based on age, sex, and weight. The first group received the basic diet containing 4% v/v ethanol for 6 weeks (Nan et al., 2013; Verleye et al., 2009; Zhang et al., 2013). The second, yoked-control group continued to receive the basic diet supplemented with an isocaloric amount of sucrose in order to account for the increased calories associated with ethanol consumption. Ethanol-treated mice received an unlimited amount of liquid diet throughout the day, while control mice received a specific amount of control diet based on the average intake of the ethanol group on the previous day. All diets were made and replaced daily 2 h after lights out (under red lights not to disrupt the light cycle). All mice had unlimited access to drinking water. Animal weights and amount of diet consumed were recorded daily. Mice consumed on average 17.4 g/kg (±0.55 g/kg) ethanol during the last 2 weeks of the 6 week treatment. It should be noted that female mice on average consumed significantly more ethanol than male mice ($F_{sex}[1,14] = 6.51$, $p = 0.023$), 18.2 g/kg (±0.5 g/kg) and 16.6 g/kg (±0.39 g/kg), respectively. Similar sex differences in ethanol consumption have been observed in rodents exposed to liquid ethanol diet, continuous two-bottle choice, intermittent two-bottle choice, and drinking in the dark paradigms (Hwa et al., 2011; McCall et al., 2013; Piano, Carrigan, & Schwertz, 2005; Trujillo, Do, Grahame, Roberts, & Gorman, 2011).

Mice were tested during spontaneous ethanol withdrawal, 24 h after removal of the ethanol diet. To that end, on the last day of liquid diet treatment, mice received fresh diet for a minimum of 3 h before the ethanol diet was replaced with control diet. All behavioral testing was performed during the dark phase of the cycle (12:00 AM–5:30 AM), using the same ambient light levels (11 lux) used for behavioral testing in ethanol-injected mice. Mice were acclimated to the behavior room for a minimum of 45 min before testing. Immediately after testing, ethanol diet was reinstated for a minimum of 3 days before subsequent behavioral testing. Behavioral testing was performed in the same order as the chronic ethanol injection treatment paradigm: open field arena, elevated plus maze, marble burying test, and somatic signs.