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Research report

Impulsive choice and anxiety-like behavior in adult rats exposed to chronic intermittent ethanol during adolescence and adulthood

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HIGHLIGHTS

- CIE exposure and age had no effect on baseline impulsive choice.
- Ethanol increased impulsivity in younger adult rats regardless of CIE exposure.
- CIE withdrawal-induced decreases in anxiety and arousal were not age-specific.
- Subsequent ethanol withdrawal produced age-dependent increases in anxiety.

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ABSTRACT

Binge drinking during adolescence and adulthood may have differential long-term effects on the brain. We investigated the long-term effects of chronic intermittent ethanol (CIE) exposure during adolescence and adulthood on impulsivity and anxiety-like behavior. Adolescent (adolescent-exposed) and adult (adult-exposed) rats were exposed to CIE/water on postnatal days (PND) 28–53 and PND146–171, respectively, and a 4-day ethanol/water binge on PND181–184 and PND271–274, respectively. During withdrawal from CIE and 4-day binge exposures, anxiety-like behavior and arousal were measured in the light-potentiated startle (LPS) and acoustic startle (ASR) procedures, respectively. Impulsive choice was evaluated in the delay discounting task (DDT) at baseline and after ethanol challenges. Independent of age, ASR and LPS were decreased during withdrawal from CIE exposure. In contrast, LPS was increased in adult-exposed, but not adolescent-exposed, rats during withdrawal from the 4-day ethanol binge. CIE exposure had no effect on preference for the large delayed reward at baseline, independent of age. During DDT acquisition, CIE-exposed, compared with water-exposed rats, omitted more responses, independent of age, suggesting the CIE-induced disruption of cognitive processes. Ethanol challenges decreased preference for the large reward in younger adolescent-exposed rats but had no effect in older adult-exposed rats, independent of previous CIE/water exposure. Taken together, the present studies demonstrate that CIE withdrawal-induced decreases in anxiety and arousal were not age-specific. CIE exposure had no long-term effects on baseline impulsive choice. Subsequent ethanol exposure produced age-dependent effects on impulsivity (increased impulsivity in younger adolescent-exposed rats) and anxiety-like behavior (increased anxiety-like behavior in older adult-exposed rats).

1. Introduction

The high level of alcohol binge drinking early in life (17% among adolescents aged 12–20 years and 40% among young adults aged 18–25 years) remains an important public health concern [1]. Younger drinkers (<25 years), categorized as type 2 alcoholics based on age of drinking onset and personality type [2], are characterized by high levels of impulsivity and novelty seeking and low levels of harm avoidance. In contrast, type 1 alcoholics (>25 years) start heavy drinking later in life and show low levels of novelty seeking and high levels of harm avoidance. Anxiety-like behavior is another behavioral trait that may be affected by drinking (for review, see [3]). Differences in personality traits, including impulsivity and anxiety, between type 1 and type 2 alcoholics may precede drinking or may result from heavy alcohol use during either adolescence or adulthood. However, studies investigating the long-term consequences of drinking during adolescence or adulthood on impulsivity and anxiety-related behaviors are very limited.

Abbreviations: CIE, chronic intermittent ethanol; PND, postnatal day; LPS, light-potentiated startle; BEC, blood ethanol concentration; DDT, delay discounting task.

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High self-reported impulsivity has been documented in alcohol-dependent adults [4], adolescents [5], and college students [6]. A small number of studies have investigated the impact of drinking on multiple aspects of impulsive behavior in laboratory settings. The delay discounting task (DDT) is commonly used in both human and animal studies to measure impulsive choice [7]. In this task, impulsivity is defined and measured as the preference for a smaller immediate reward over a larger delayed reward [8,9]. Increased impulsive choice has been reported in adult abstinent alcoholics and heavy drinkers compared with light drinkers and control subjects [10–12]. Acute alcohol intoxication decreased impulsive choice in healthy undergraduate students [13] but increased impulsive choice in healthy adults [14] and non-dependent alcohol drinkers in a laboratory [15,16] or bar setting [17] suggesting age-dependent effects of acute ethanol on impulsive choice. In experimental animals without a history of ethanol exposure, an acute ethanol challenge increased impulsive choice in non-selected adult rats [18–21] and in rats and mice bred for high but not low ethanol drinking [22,23]; but see [24]. However, the long-term effects of binge ethanol exposure during adolescence or adulthood on impulsive choice during adulthood have been largely unexplored.

Increased anxiety and an enhanced startle response are often associated with ethanol withdrawal [25–28]. In contrast to the majority of physical ethanol withdrawal symptoms that usually disappear within a few days, increased anxiety may last for months and even years, resulting in relapse to drinking [26,29]. In both humans and animals, anxiety-like behavior can be assessed in the light-potentiated startle (LPS) procedure, in which startle responses are measured in successive sessions, during which the startle chambers are either dark or brightly lit. The startle response is potentiated by the aversive bright light in rodents. The degree to which light enhances startle reactivity is used as an operational measure of anxiety, and this response is selectively reduced by anxiolytic compounds [30,31]. Work in our laboratory has shown that LPS was increased during spontaneous nicotine withdrawal in rats [32]. To the best of our knowledge, no studies have assessed LPS during ethanol withdrawal in rats with or without a history of previous ethanol exposure.

The aim of the present work was to investigate impulsive choice and anxiety-like behavior in rats exposed to chronic intermittent ethanol (CIE) during either adolescence or adulthood. To ensure ethanol exposure during the developmentally sensitive adolescent period, male Wistar rats were exposed to CIE throughout adolescence (PND28–53), broadly defined from postnatal day (PND) 28 to PND42 or PND60 in males [33]. Adult rats were exposed to an identical CIE regimen during adulthood (PND146–171) to determine whether the effects of CIE exposure on impulsivity and anxiety are specific to ethanol exposure during adolescence. CIE exposure consisted of ethanol binges for two consecutive days at 48-h intervals of abstinence for 25 days. Similar intermittent ethanol administration regimens produced inflammatory brain damage and long-term alterations in cognitive and motor function [34] and resulted in tolerance to the hypnotic effects of an ethanol challenge in rats during adulthood [35]. Thus, we hypothesized that CIE exposure during adolescence or adulthood would have long-lasting effects on impulsivity and anxiety. The effects of CIE exposure on impulsivity were assessed using the DDT. We assessed impulsive choice under baseline conditions and after acute ethanol challenges in adult rats exposed to CIE during adolescence or adulthood. Anxiety-like behavior was assessed in the LPS procedure. This procedure also provides measures of the acoustic startle response (ASR). The ASR has been used to characterize arousal during ethanol withdrawal in both humans [25,36] and rodents [37,38]. LPS and the ASR were assessed in both adult and adolescent rats during withdrawal from CIE exposure as well as during withdrawal from a 4-day ethanol binge in adulthood. Both CIE and 4-day ethanol binge exposures produced an average blood ethanol concentration (BEC) of 300 mg/dl, mimicking heavy alcohol use in humans [39].

2. Materials and methods

2.1. Animals

Two cohorts of 12 pregnant female Wistar rats (Charles River, Raleigh, NC, USA) arrived in the laboratory on gestational day 13. Male rats were weaned from each litter at 21 days of age (PND21; average body weight, 83.7 ± 1.8 g), assigned to the adolescent experimental groups (water and CIE), and tested in two cohorts (n = 50 total). Another 26 adult male Wistar rats (Charles River, Raleigh, NC, USA) arrived in the laboratory on PND 134 (average body weight, 361.4 ± 4.2 g) and were assigned to the adult experimental groups (water and CIE). The experimental design is described below and presented in Fig. 1. All of the rats were pair-housed and maintained in a humidity- and temperature-controlled vivarium under a reverse 12 h/12 h light/dark cycle (light off at 8:00 AM). Food and water were available *ad libitum* except during training and testing in the DDT. During behavioral training and testing in the DDT, the rats were food-deprived and received from 16 to 20 g/rat/day of food chow, including food pellets obtained during behavioral testing. The rats were fed 1 h after the experimental session. Training and testing occurred during the dark phase of the light/dark cycle. All of the experiments were in accordance with the guidelines of the American Association for the Accreditation of Laboratory Animal Care and National Research Council's Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

2.2. Chronic intermittent ethanol, ethanol challenge, and 4-day ethanol binge exposures

Adolescent (PND28–53) and adult (PND146–171) rats were exposed to CIE or water administered intragastrically (IG) using stainless steel gavage needles (Roboz Surgical Instruments, Gaithersburg, MD, USA). The rats were administered 1–5 g/kg of a 25% (v/v) ethanol solution three times per day according to a 2-day on/2-day off regimen, for a total of seven 2-day binges. During CIE exposure, each subsequent ethanol dose was adjusted based on the behavioral intoxication score (see Supplementary Materials). Adolescent and adult control rats were administered sterile water IG according to the same regimen as the ethanol-treated rats.

Acute saline or ethanol challenge injections (0.5, 1, and 2 g/kg intraperitoneally [IP] in a volume of 1 ml/kg, 15 min before the session) were administered to the rats during adulthood (PND144–163 and PND251–270 in the adolescent and adult groups, respectively) once per week according to a within-subjects Latin-square experimental design.

After completion of the experiment with acute ethanol challenges, the rats were exposed to a single 4-day ethanol or water binge during adulthood (PND181–184 and PND271–274 in the adolescent and adult groups, respectively, 90–103 days after the termination of CIE exposure) counterbalanced with previous CIE/water exposure. The rats were administered 1–4 g/kg IG of a 25% (v/v) ethanol solution in sterile water via gavage twice per day with a 6-h interval between injections for 4 days. During the ethanol binge, each subsequent ethanol dose was adjusted based on the behavioral intoxication score (see Supplementary Materials). Control rats were administered sterile water IG according to the same regimen as the ethanol-treated rats.

Blood samples (200 μl) were collected from the tip of the tail 60–90 min after the last ethanol dose on the 2nd, 4th, and 6th