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CHRONIC INTERMITTENT ETHANOL EXPOSURE LEADS TO ALTERATIONS IN BRAIN-DERIVED NEUROTROPHIC FACTOR WITHIN THE FRONTAL CORTEX AND IMPAIRED BEHAVIORAL FLEXIBILITY IN BOTH ADOLESCENT AND ADULT RATS ☆

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Abstract—Chronic intermittent exposure to ethanol (EtOH; CIE) that produces binge-like levels of intoxication has been associated with age-dependent deficits in cognitive functioning. Male Sprague–Dawley rats were exposed to CIE (5 g/kg, 25% EtOH, 13 intragastric gavages) beginning at three ages: early adolescence (postnatal day [PD] 28), mid-adolescence (PD35) and adulthood (PD72). In experiment 1, rats were behaviorally tested following CIE. Spatial memory was not affected by CIE, but adult CIE rats were impaired at acquiring a non-spatial discrimination task and subsequent reversal tasks. Rats exposed to CIE during early or mid-adolescence were impaired on the first reversal, demonstrating transient impairment in behavioral flexibility. Blood EtOH concentrations negatively correlated with performance on reversal tasks. Experiment 2 examined changes in brain-derived neurotrophic factor (BDNF) levels within the frontal cortex (FC) and hippocampus (HPC) at four time points: during intoxication, 24 h after the final EtOH exposure (acute abstinence), 3 weeks following abstinence (recovery) and after behavioral testing. HPC BDNF levels were not affected by CIE at any time point. During intoxication, BDNF was suppressed in the FC, regardless of the age of exposure. However, during acute abstinence, reduced FC BDNF levels persisted in early adolescent CIE rats, whereas adult CIE rats displayed an increase in BDNF levels. Following recovery, neurotrophin levels in all CIE rats recovered. Our results indicate that intermittent binge-like EtOH exposure leads to acute disruptions in FC BDNF levels and long-lasting behavioral deficits. However, the type of cognitive impairment and its duration differ depending on the age of exposure. © 2017 Published by Elsevier Ltd on behalf of IBRO.

Key words: chronic intermittent ethanol exposure, discrimination learning, reversal learning, adult, early adolescence, mid-adolescence.

INTRODUCTION

Adolescent and early adult alcohol drinking has been linked to development of alcohol use disorders, which can lead cognitive deficits and behavioral problems (Crews et al., 2007; Spear and Swartzwelder, 2014; Risher et al., 2015). Early adolescent ethanol (EtOH) exposure appears to solidify an adolescent-like behavioral phenotype in adulthood, which includes impulsivity, impaired behavioral flexibility, and increased anxiety (Semenova, 2012; Vetreno and Crews, 2012; Risher et al., 2013; Coleman et al., 2014; Gass et al., 2014; Mejia-Toiber et al., 2014). However, mid-adolescent and adult chronic intermittent exposure to ethanol (CIE) has also been associated with deficits in attention, reversal learning and extinction learning (Slawecki, 2006; Kuzmin et al., 2012; Broadwater et al., 2014; Badanich et al., 2016). Thus, further examination of the long-term effects of EtOH exposure across early adolescence into early adulthood is critical for understanding the unique age-specific effects of CIE on cognition and neural adaption.

Binge-like EtOH exposure, particularly during adolescence, leads to reductions in neurogenesis in the hippocampus (HPC), decreased gliogenesis in the frontal cortex (FC), as well as a loss of forebrain cholinergic neurons (Crews and Nixon, 2009; Koss et al., 2012; Broadwater et al., 2014; Vetreno and Crews, 2015). Such pathology is believed to be caused by EtOH-mediated induction of neuroimmune genes within the FC and HPC that persist into adulthood (Vetreno and Crews, 2012; Crews et al., 2015). Ethanol-induced activation of proinflammatory signaling in the brain can lead to neurodegeneration through exacerbated oxidative stress and excitotoxicity. As such, damage to both the FC, such as decreases in myelination, and neural degeneration in the HPC, visualized using an amino-culpric silver technique, have been observed following adolescent CIE exposure (Crews et al., 2000; Vargas et al., 2014; Vetreno et al., 2014).

Neurotrophins are key modulators of neurodegeneration associated with aging and disease. It has been shown that prenatal and adult chronic EtOH exposure alters levels of neurotrophin, such as brain-

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Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CIE, Chronic intermittent exposure to ethanol; ELISA, enzyme-linked immunosorbent assays; EtOH, ethanol; FC, frontal cortex; HPC, hippocampus.

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derived neurotrophic factor (BDNF), in the FC and HPC (Miller et al., 2002; Davis, 2008; Nixon and McClain, 2010; Mooney and Miller, 2011; Vedder et al., 2015). However, few studies have assessed neurotrophin expression after adolescent CIE and the results are variable (Briones and Woods, 2013; McClain et al., 2014; Sakharkar et al., 2016). One key factor in alcohol-associated neurotrophin dysfunction is the timing or stage of the disease process during which neurotrophin measures are assessed (see Davis, 2008).

Our goal was to determine an ontogenetic profile across early adolescence into early adulthood regarding the effect of binge-like EtOH exposure on hippocampal and frontal cortical neurotrophin adaption. We employed a CIE model in early adolescent, mid-adolescent and young adult rats. In experiment 1, following a 3-week EtOH-free recovery period, which matured both early and mid-adolescent rats to adulthood, spontaneous alternation and a non-spatial discrimination task with reversals were conducted to determine deficits in hippocampal-dependent spatial memory and frontocortical-dependent cognitive flexibility. Since BDNF has been shown to modulate neuroadaption, we examined the effects of CIE on mature BDNF levels in the FC and HPC in experiment 2. BDNF levels were measured at differing time points during CIE: During the final EtOH exposure (intoxication), 24 h after the final EtOH exposure (acute abstinence), 3 weeks following final EtOH exposure (recovery) and post-behavioral testing.

EXPERIMENTAL PROCEDURES

Subjects

Early Adolescent (PD28), mid-adolescent (PD35), and adult (PD65-78) male Sprague–Dawley rats were obtained from litters bred at Binghamton University. No more than one rat from each litter was randomly assigned within each treatment condition.

Rats were pair housed in a temperature- (20 °C) and humidity-controlled colony under a 12-h light/dark cycle (onset at 7:00 am). Rats were provided with *ad libitum* access to lab chow and water. During CIE treatment, rats were weighed on each treatment dosing date. After CIE, rats were weighed on a weekly basis to ensure normal weight gain and health. Experimental procedures were in compliance with the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee (IACUC) at the State University of New York at Binghamton.

Experiment 1: Behavioral testing and BDNF measurement

Rats at each age range were randomly divided into CIE (Early Adolescent: $n = 10$; Mid-adolescent: $n = 9$; Adult: $n = 9$) and water-treated control groups (Early Adolescent: $n = 10$; Early Adolescent: $n = 11$; Adult: $n = 10$). Three weeks following CIE cessation, rats were behaviorally tested. This cohort also served as the time-point 4 (behaviorally tested) cohort in Experiment 2. Fig. 1 demonstrates a schematic of the exposure and treatment timeline.

Chronic intermittent ethanol treatment

For both experiments 1 and 2, adolescent and adult rats were subject to 13 intragastric gavages of either 25% EtOH (*v/v*) or water, administered at a dose of 5 g/kg. The dosing schedule followed a modified 2-day on/off cycle, where animals were dosed once per day for 2 days, followed by a 2-day recovery period until the 12th gavage. The final gavage (#13) was administered 2 days following gavage #12. Blood samples were collected via a small incision in the lateral tail vein 30 min to an hour following the first, fifth, and final gavage. Blood collection occurred during the time course when BEC levels would be increasing, but not at peak intoxication (Livy et al., 2003; Quertemont et al., 2003). Plasma was separated using a centrifuge and stored at −20 °C until blood ethanol content (BEC) levels were measured using an AM1 Alcohol Analyzer (Analox Instruments, MA, USA). Throughout treatment, all animals gained weight, and there was no significant effect of CIE treatment on animal weights.

Following the cessation of CIE, rats in experiment 1 had a 3-week recovery period, during which they were weighed and handled once per week. Prior to the start of behavioral testing, rats were food restricted to 90% of their free feed weight over the course of 5 days to induce searching and digging motivation. Spontaneous alternation testing occurred first, followed by training in a multiple phase, non-spatial discrimination task. Fig. 1 illustrates the exposure protocol.

Spontaneous alternation

Details for our spontaneous alternation protocol can be found in Fernandez et al., 2016. In brief, rats were tested once for spontaneous alternation behavior in a plus maze (105.5 cm × 14.4 cm × 15 cm) with clear, plastic walls and black, wooden floors. The animal was habituated to the testing room for 20 min, after which it was placed on the center of the maze. Each rat explored the maze for 18 min. Arm entries were recorded during testing, and percent alternation scores were analyzed. An alternation was defined as entry into four different arms in a successive sequence. Spontaneous alternation scores were corrected to account for significant differences in activity between groups: arm entries were only recorded up to 27 possible arm entries, which was the average number of arm entries made by the lowest activity group (adults). The normalization of percent alternation scores is adapted from Savage (2012) and Fernandez et al. (2016).

Non-spatial discrimination learning and reversal task

Details regarding the non-spatial discrimination and reversal task can be found in Fernandez et al. (2016). In brief, the day after spontaneous alternation testing, rats began dig training in their home cage. Ceramic bowls were filled with wood shavings and baited with Cheerios.

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