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Adolescent binge ethanol treatment alters adult brain regional volumes, cortical extracellular matrix protein and behavioral flexibility

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ABSTRACT

Adolescents binge drink more than any other age group, increasing risk of disrupting the development of the frontal cortex. We hypothesized that adolescent binge drinking would lead to persistent alterations in adulthood. In this study, we modeled adolescent weekend underage binge-drinking, using adolescent mice (post-natal days [P] 28–37). The adolescent intermittent binge ethanol (AIE) treatment includes 6 binge intragastric doses of ethanol in an intermittent pattern across adolescence. Assessments were conducted in adulthood following extended abstinence to determine if there were persistent changes in adults. Reversal learning, open field and other behavioral assessments as well as brain structure using magnetic imaging and immunohistochemistry were determined. We found that AIE did not impact adult Barnes Maze learning. However, AIE did cause reversal learning deficits in adults. AIE also caused structural changes in the adult brain. AIE was associated with adulthood volume enlargements in specific brain regions without changes in total brain volume. Enlarged regions included the orbitofrontal cortex (OFC, 4%), cerebellum (4.5%), thalamus (2%), internal capsule (10%) and genu of the corpus callosum (7%). The enlarged OFC volume in adults after AIE is consistent with previous imaging studies in human adolescents. AIE treatment was associated with significant increases in the expression of several extracellular matrix (ECM) proteins in the adult OFC including WFA (55%), Brevican (32%), Neurocan (105%), Tenacin-C (25%), and HABP (5%). These findings are consistent with AIE causing persistent changes in brain structure that could contribute to a lack of behavioral flexibility.

1. Introduction

Adolescent human drinkers participate in heavy and binge drinking (>5 drinks/episode) at substantially higher rates than adults (Ehlers et al., 2011; Lucantonio et al., 2012; SAMSA, 2007), with the rate of binge-drinking peaking during adolescence (Ehlers et al., 2011). Alcohol consumption during adolescence is highly prevalent as 8% of 8th grade, 16% of 10th grade, and 25% of 12th grade adolescent individuals reported heavy episodic drinking (i.e., <5 consecutive alcohol drinks per episode) over the past 2 weeks (Johnston et al., 2009). This heavy drinking pattern persists into college as 44% of students report binge drinking every 2 weeks with 19% reporting more than 3 binge drinking episodes per week (O'Malley et al., 1998; Wechsler et al., 1995). Early onset of alcohol use (<13 years of age) is associated with increased drinking frequency and physical violence (Gruber et al., 1996). Further, large population studies find that across the teenage years the earlier the age of drinking onset the greater the risk of lifetime alcohol use disorder (Zhu et al., 2010) and lifetime alcohol related violence and injuries (Hingson and Winter, 2003; A.M. White et al., 2011; H.R. White et al., 2011). Adolescents with alcohol use disorders have deficits in executive functioning (Brown et al., 2000; Hanson et al., 2011; Tapert and Brown, 2000) consistent with frontal cortical dysfunction. Although studies have found that heavy drinking among adolescent males increases impulsivity the following year in those individuals predisposed to adolescent-typical impulsivity (A.M. White et al., 2011; H.R. White et al., 2011), human studies are all confounded by the difficulty in distinguishing a pre-existing condition that leads to alcohol abuse from an alcohol induced dysfunction that alters the individual beyond the period of intoxication.

Adolescence is a developmental period associated with maturation of cognitive ability, personality and frontal cortical executive functions (Crews et al., 2007; Gong et al., 2012; Spear, 2000). The frontal cortex continues to develop throughout adolescence and is uniquely sensitive to ethanol neurotoxicity (Crews et al., 2000). Adolescent rat ethanol

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treatment results in greater frontal cortical damage than adults as well as other alterations in adult brain neurotransmission (Basak and Bandyopadhyay, 2013; Coleman et al., 2011; Sharma and Bhatia, 2004; Torchilin, 2001), electrophysiological activity and sleep wave patterns (Escobar-Chavez et al., 2006), reduced forebrain cholinergic-choline acetyltransferase + neurons (Coleman et al., 2011; Ehlers et al., 2011) and reduced hippocampal neurogenesis (Ehlers et al., 2011). Further, adolescent rat ethanol treatment increases alcohol intake (Fabio et al., 2013; Pascual et al., 2007) and risk-taking preference (Qin et al., 2013), as well as impairing conditioned discrimination, object recognition (Pascual et al., 2007), and reversal learning (Coleman et al., 2011). These studies support the hypothesis that adolescent binge-drinking impacts the brain and behavior in a manner that persists into adulthood.

Human adolescents drink mostly in a unique binge drinking 'on–off–on' intermittent temporal pattern, drinking at the end of the week and on weekends and abstaining during the first 3–4 days of the week (Zou and Crews, 2012). Daily and intermittent ethanol treatments can have different effects on brain function (Breese et al., 2005). In this study we used an adolescent intermittent ethanol (AIE) treatment (5 g/kg, i.g., 2 days on–2 days off) designed to model the weekend binge drinking pattern of human adolescents. To assess the persistence of effects following AIE treatment animals remained abstinent during 2 months of maturation into adulthood. We report here that AIE treatment of adolescent rats disrupts adult Barnes Maze reversal learning, but not initial learning acquisition (i.e., the learning acquisition curve). Also, AIE increased specific brain regional volumes determined by magnetic resonance imaging (MRI) and increases expression of extracellular matrix proteins in the orbital frontal cortex (OFC).

2. Material and methods

2.1. Animal treatment

Male C57BL/6 mice were ordered from Charles River Labs (Raleigh, NC), and were allowed to acclimate to the animal facilities for seven days in our animal facility prior to treatment. Adolescent mice were requested with the stipulation that all mice were the same weight, in order to reduce potential variability in brain size. Mice were given either water (N = 7) or ethanol (5 g/kg, i.g. 25% ethanol w/v, N = 8) once a day during adolescence on post natal day (P)28–37 in an intermittent fashion (Fig. 1).

Either water or ethanol was administered by intragastric (i.g.) administration on days P28, P29, P32, P33, P36, and P37. In our lab this concentration of ethanol given to adolescent mice results in average blood alcohol levels of 310 mg/dL ± 93.7 (mean ± SEM) measured 1 h after ethanol treatment. This is a very high binge drinking blood level. There were no differences in body weight between the two groups during the ethanol treatment (Supplemental Fig. 1). All protocols were approved by the University of North Carolina Institutional Animal Care and Use Committee and were in accordance with the Congressional Animal Welfare Act.

2.2. Adult behavior

Behavioral testing began in young adulthood on P60. The following tests were performed: open field locomotion with center time assessment (P60), learning acquisition using the Barnes Maze at P68 (30 days after the last dose of ethanol), reversal learning using the Barnes Maze (P81–91), and forced swim test P104. Mice were sacrificed by perfusion on P110 for MRI. All behavioral testing was performed in the UNC Neurodevelopmental Disorders Research Center Mouse Behavioral Phenotyping Core using previously published methods (Al-Saffar et al., 2013).

2.2.1. Locomotor activity

Mice spent 2 h in the open field chamber (40 cm × 40 cm × 30 cm, VersaMax system, AccuScan Instruments) (Al-Saffar et al., 2013). Total locomotor activity and time spent in the center of the open field were measured.

2.3. Barnes Maze spatial learning with reversal

The Barnes Maze is a large, brightly-lit, circular platform (diameter = 122 cm), elevated 96.5 cm from the floor and positioned like a table, with 40 holes (diameter = 5 cm) drilled along the perimeter (Fig. 2C). An escape box was located under one of the holes, and mice learned which hole allowed for escape from the maze surface. Each mouse was given his own individual escape location, which remained constant across the learning days. Mice were given one trial per day,

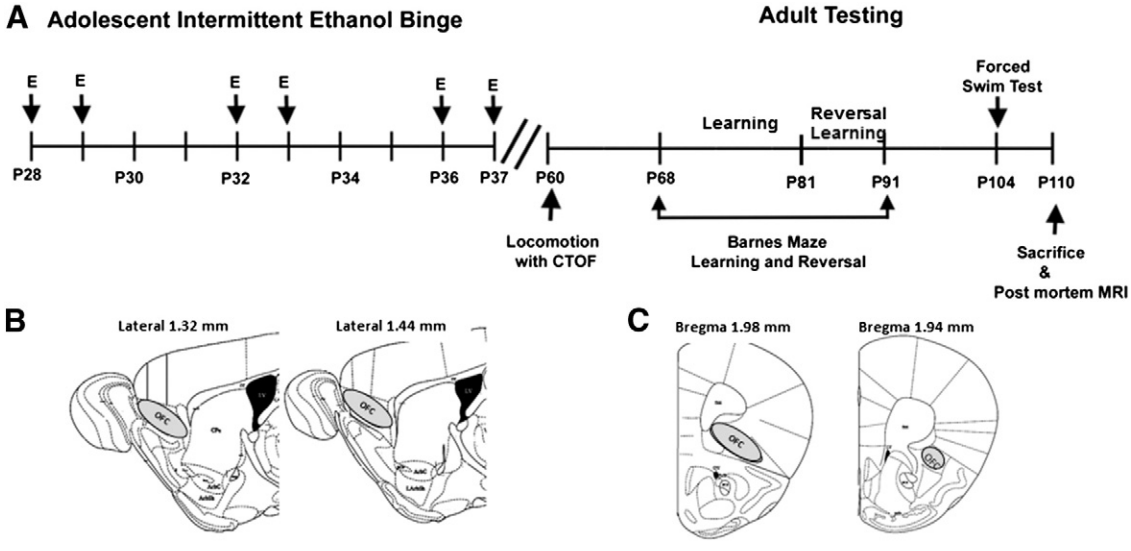

Fig. 1. Adolescent intermittent ethanol (AIE) binge and adult behavioral testing schedules. Mice were given either water or ethanol (5 g/kg, i.g. 25% ethanol w/v) once a day during adolescence (P28–37) in an intermittent fashion. Water or ethanol was administered on days P28, P29, P32, P33, P36, and P37. Behavioral testing began in young adulthood on P60. The following tests were performed: open field locomotion with center time (P60) and studies using the Barnes Maze to assess learning started at P68, 30 days after the last dose of ethanol. Reversal learning studies were P81–91 and forced swim test P104. Mice were sacrificed by perfusion on P110 for MRI. Following MRI scans using the intact head, the brain was removed and sectioned for immunohistochemistry either sagittal (B) or coronal (C). Coronal sections were used for brevican as shown above. To better localize OFC we shifted to sagittal sections for WFA, neurocan, HABP, Phosphocan and Tenacin-C.