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ADOLESCENT INTERMITTENT ETHANOL EXPOSURE ENHANCES ETHANOL ACTIVATION OF THE NUCLEUS ACCUMBENS WHILE BLUNTING THE PREFRONTAL CORTEX RESPONSES IN ADULT RAT

W. LIU AND F. T. CREWS *

Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, CB 7178, Chapel Hill, NC 27599-7178, United States

Abstract—The brain continues to develop through adolescence when excessive alcohol consumption is prevalent in humans. We hypothesized that binge drinking doses of ethanol during adolescence will cause changes in brain ethanol responses that persist into adulthood. To test this hypothesis Wistar rats were treated with an adolescent intermittent ethanol (AIE; 5 g/kg, i.g. 2 days on–2 days off; P25–P54) model of underage drinking followed by 25 days of abstinence during maturation to young adulthood (P80). Using markers of neuronal activation c-Fos, EGR1, and phophorylated extracellar signal regulated kinase (pERK1/2), adult responses to a moderate and binge drinking ethanol challenge, e.g., 2 or 4 g/kg, were determined. Adult rats showed dose dependent increases in neuronal activation markers in multiple brain regions during ethanol challenge. Brain regional responses correlated are consistent with anatomical connections. AIE led to marked decreases in adult ethanol PFC (prefrontal cortex) and blunted responses in the amygdala. Binge drinking doses led to the nucleus accumbens (NAc) activation that correlated with the ventral tegmental area (VTA) activation. In contrast to other brain regions, AIE enhanced the adult NAc response to binge drinking doses. These studies suggest that adolescent alcohol exposure causes long-lasting changes in brain responses to alcohol that persist into adulthood.

Key words: adolescence, alcohol, networks, frontal cortex, development.

*Corresponding author. Tel: +1-919-966-5678.
E-mail addresses: wen_liu@med.unc.edu (W. Liu), ftcrews@med.unc.edu (F. T. Crews).

Abbreviations: AIE, adolescent intermittent ethanol; ANOVA, analysis of variance; aVTA, anterior ventral tegmental area; BECs, blood ethanol concentrations; BLA, basolateral nucleus of amygdala; CeA, central nucleus of amygdala; DA, dopamine; DAB, nickel-enhanced diaminobenzidine; dBNST, dorsal bed nucleus of stria terminalis; dPFC, dorsal prefrontal cortex; Ent, entorhinal cortex; EW, Edinger–Westphal nucleus; FC, frontal cortex; IL, infralimbic cortex; LaA, lateral nucleus of amygdala; mPFC, medial prefrontal cortex; NAc C, nucleus accumbens core; NAc Sh, nucleus accumbens shell; OFC, orbitalfrontal cortex; PBS, phosphate-buffered saline; pERK1/2, phophorylated extracellar signal regulated kinase; PFC, prefrontal cortex; PRh, perirhinal cortex; PrL, prelimbic cortex; PVA, paraventricular thalamic nucleus, anterior part; pVTA, posterior ventral tegmental area; vsNAc C, ventral striatal nucleus accumbens core; vsNAc Sh, ventral striatal nucleus accumbens shell.

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INTRODUCTION

Human studies find that adolescent brain maturation involves the transition of the immature child brain to the mature networks that characterize the adult brain (Giedd et al., 1999; Giedd, 2004). Unfortunately, adolescence is also a time when individuals initiate alcohol use and abuse (Spear, 2011). Studies find heavy episodic drinking in 5% of 8th grade, 14% of 10th grade, and 22% of 12th grade individuals (Johnston et al., 2013). This heavy drinking pattern increases in college with 44% of students reporting binge drinking every 2 weeks, and 19% reporting more than three binge drinking episodes per week (Wechsler et al., 1995; O'Malley et al., 1998). Routine binge drinking might lead to long-term changes in adult neurobiology due to the heightened neural plasticity and structural development that characterizes the adolescent brain (Crews et al., 2007). An earlier age of drinking onset (i.e., 11–14 years of age) increases the risk of developing an alcohol use disorder later in life (DeWit et al., 2000). Further, adolescent binge drinking is associated with diminished impulse inhibition (White et al., 2011), reduced attentional functioning (Koskinen et al., 2011), deficits in visuospatial ability (Giancola et al., 1998; Tapert et al., 2002), and impaired executive functioning (White et al., 2011). Although adolescents binge drink, the pattern is intermittent, and not daily. To investigate the impact of adolescent binge drinking on adult brain we treated rats with a model of intermittent binge drinking, i.e., adolescent intermittent ethanol (AIE) administration. Following AIE treatment, animals were abstinent during maturation to young adulthood. Alterations in the adult brain responses to alcohol challenge were used to test the hypothesis that AIE would induce long-term alterations in adult neuronal responses to ethanol.

Immediate early genes (IEGs) are genes that are transiently and rapidly induced in activated neurons. c-Fos protein, a product of an immediate early gene (c-Fos), is a transcription factor thought to play a role in neuronal adaptations and brain plasticity (Chaudhuri, 1997; Filipkowski et al., 2000) as a marker of neuronal activation (Brown et al., 1992; Liste et al., 1997). Transcription of c-Fos is triggered by intracellular calcium and cAMP (Cyclic adenosine monophosphate), which are increased through activation of NMDA (N-methyl-D-aspartic acid) receptors, L-type calcium channels, and/or other excitatory receptors, neuronal activity where action

potentials are coincident with synaptic activity (Bito et al., 1997). Another transcription factor that marks neuronal activation and long-term potentiation is EGR-1 (Cole et al., 1989) which along with phophorylated extracellar signal regulated kinase (pERK1/2), a kinase that marks neuronal activation (Gao and Ji, 2009), provide induction of neuronal activation that are implicated in long-term neuronal plasticity (Hiroi et al., 1999) and provide insights into neural signaling that may be altered by AIE. Ethanol- and stress-induced increases in brain c-Fos are independent of circulating levels of CORT (Helmreich et al., 1996; Ryabinin et al., 1999; Hansson et al., 2003) suggesting changes in neuronal activation markers provide direct insights into neuronal and neurocircuit changes in plasticity that may be altered by AIE.

Many studies have investigated ethanol induction of neuronal c-Fos and EGR1 and found that numerous and similar brain regions are activated by systemic ethanol exposure of rats and mice (Ryabinin et al., 1997, 1999; Knapp et al., 2001; Vilpoux et al., 2009). Ethanol activation of brain neurons likely occur through multiple mechanisms including directional pharmacological effects on neurons as well as through alterations in excitability through altered neuronal networks. Comparison of c-Fos responses to acute systemic ethanol with responses to intraventricular injections suggests direct ethanol effects on brain regions contributing to reward and feeding, with circuits regulating aversion and feeding behavior contributing to the broad neuronal activation during peripheral ethanol challenge in naïve rats (Crabbe et al., 1983; Crankshaw et al., 2003). Chronic ethanol administration leads to tolerance of c-Fos induction in many brain regions showing that loss of response is associated to a reduction in the aversive qualities of alcohol during chronic exposure (Hansson et al., 2008). We investigated the ethanol response of multiple brain regions involved in decision making, learning, reward, and negative affect using immediate early gene expression. We report here that AIE followed by abstinent maturation to adulthood markedly blunts the adult mPFC (medial prefrontal cortex) response to alcohol, but enhances the nucleus accumbens (NAc) response to alcohol.

EXPERIMENTAL PROCEDURES

Animals

Twelve timed-pregnant Wistar rats, young mothers at the same age, were ordered from Harlan Laboratories, Inc. (Indianapolis, IN, USA) under a protocol approved by the Institutional Animal Care and Use Committees at the University of North Carolina. Timed-pregnant dams were allowed to acclimate to our vivarium (University of North Carolina at Chapel Hill) at embryonic day 17 (E17), and pups were bred and reared to avoid pre-existing variation. Efforts were made to reduce environmental variation. All animals were maintained at 22 °C under 12:12-h light/dark cycles with free access to food and water. On the day following birth (postnatal day 1, P1), litters were culled to 10 pups (including male and female pups at this time). On weaning at P21, male offspring was pair-housed with a same-sex, same-age non-littermate and body weight match assigned to two experimental groups, control and ethanol (average 4–5 male rats used from each litter). This study was done in all males to avoid the confounds of sex differences in puberty and hormone cycles. Fig. 1A illustrates the intermittent treatment protocol (e.g., 2 days alcohol, 2 days off) with ethanol (5 g/kg, 25% ethanol w/v, i.g.) or water from early adolescence until well after puberty (P25–P54); the control group was administered the same volume of water. Body weight of animals, measured every four days was shown in Fig. 1B. Ethanol treatment stopped on P54. There was no treatment until postnatal day 80 (P80). At P80, the ethanol group was subdivided into three groups with body weight match (n = 7–8/each group); two groups of them were separately challenged with 2 or 4 g/kg i.g. ethanol (AIE-challenge 2 g/kg and AIE-challenge 4 g/kg groups), and the third one was administered with the same volume of water (AIE group). The water control group was subdivided into three adult-matched groups (n = 7–8/each group) identical to the adolescent ethanol-treated animals. Animals were handled at least 7 days before challenge day (P80), and sacrificed 2 h after either acute ethanol or water treatment.

Blood ethanol concentration

Tail blood samples were collected 1 h after ethanol treatment (5 g/kg i.g.) at P38 and P54, and 2 h after acute ethanol treatment (2 or 4 g/kg i.g.) at P80 (Fig. 1B). Blood ethanol concentrations (BEC) were measured using a GM7 Analyser (Analox, London, UK).

Tissue Collection and Preparation

Rats were deeply anesthetized with an overdose of sodium pentobarbital, and transcardially perfused with 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde (in 0.1 M phosphate buffer, pH 7.4). Brains were removed, and post-fixed for 24 h in 4% paraformaldehyde at 4 °C, and embedded with paraffin. Coronal sections (from the entire rostral to caudal of the whole brain) were obtained at a thickness of 10 μm in 1:20 series.

Histology procedure

All tissue sections embedded with paraffin were deparaffinized, rehydrated and retrieved with antigen retrieval buffer. Briefly, sections were incubated in 0.6% hydrogen peroxide (H_2O_2) for 30 min, and blocked in 3% goat serum (0.25% Triton X-100, Sigma, Saint Louis, MO) for 1 h at room temperature prior to 48 h for c-Fos (Ab-5)(4–17)(1:10,000, PC38; Calbiochem, EMD Chemicals, Inc., San Diego, CA, USA), and overnight for EGR1 (1:200, #4153; Cell Signaling Technology, Inc., Danvers, MA, USA) and phospho-p44/42 MAPK (ERK1/2) (1:200, #4370, Cell Signaling Technology, Inc., Danvers, MA, USA) at 4 °C. Then, sections were rinsed in PBS, and incubated with biotinylated secondary anti-rabbit antibody (Vector Laboratories Inc., Burlingame, CA, USA) for 1 h at room temperature. Subsequently, avidin–biotin complex (Vector ABC kit, Vector Laboratory Inc., Burlingame, CA, USA) was