Neuroscience 339 (2016) 64–71

EXPOSURE TO ALCOHOL DURING ADOLESCENCE EXERTS LONG-TERM EFFECTS ON STRESS RESPONSE AND THE ADULT BRAIN STRESS CIRCUITS

CAMRYN D. ALLEN,[a†] JAN-SEBASTIAN GRIGOLEIT,[b‡] JOONHO HONG,[a‡] SEJIN BAE,[a] JOAN VAUGHAN[a] AND SOON LEE[a*]

[a] *The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037, USA*

[b] *Laboratory of Neuronal Structure and Function, The Salk Institute, La Jolla, CA 92037, USA*

Abstract—The hypothalamic–pituitary–adrenal (HPA) axis undergoes critical developments during adolescence. Therefore, stressors experienced during this period potentially have long-term effects on adult HPA axis function. We hypothesized that adolescent intermittent ethanol (AIE) exposure would affect adult HPA axis function, resulting in altered responses to an alcohol challenge in young adults or adults. To test these hypotheses, male rats were exposed to alcohol vapor for 6 h per day from post-natal day (PND) 28–42, then acutely challenged with alcohol intragastrically (3.2–4.5 g/kg) in young adults (PND 70) or adults (PND 90). Overall, we observed blunted HPA axis responses to an alcohol challenge due to AIE exposure. Specifically, AIE tended to inhibit the alcohol challenge-induced increase in plasma corticosterone (CORT) concentrations in young adult and adult rats. As well, AIE significantly blunted the alcohol challenge-induced arginine vasopressin (*Avp*) *mR*NA expression in the paraventricular nucleus (PVN) of the hypothalamus of adult rats. Results of the present study are similar to what we have previously shown, that these changes in PVN responsiveness may result from AIE-induced alterations in adrenergic neurons in brain stem regions C1–C3 known to project to the PVN. AIE elevated the number of colocalized c-fos/phenylethanolamine N-methyltransferase (PNMT)-positive cell bodies in the C1 region of adult rats. Together, these data suggest that AIE exposure produces alterations in male HPA axis responsiveness to administration of an acute alcohol challenge that may be long-lasting.

Key words: alcohol, corticosterone, *Crf*, *Avp*, PNMT.

INTRODUCTION

Alcohol drinking is generally initiated during adolescence when the brain is still developing. In comparison to adults, adolescents are less sensitive to the aversive properties of alcohol, while they are more sensitive to the positive rewarding effects of alcohol (Spear and Varlinskaya, 2010). Genetic vulnerability, stress exposure or previous alcohol use may exacerbate these sensitivities to the aversive and rewarding aspects of alcohol (Spear and Varlinskaya, 2010). The effects of exposure to alcohol during adolescence are varied and could contribute to increased alcohol consumption in adulthood; these consequential effects may involve changes in the mesolimbic dopaminergic and glutamatergic systems (Pascual et al., 2009), impairment of neurogenesis (Morris et al., 2010), and differences in brain damage than is found in adults (Crews et al., 2000). Interestingly, adolescents also seem to be more susceptible to the effects of stress on alcohol drinking (Siegmund et al., 2005; Füllgrabe et al., 2007). Our laboratory is interested in alcohol's effects on the central circuits known to regulate stress response – the hypothalamic–pituitary–adrenal (HPA) axis, which includes the paraventricular nucleus (PVN) of the hypothalamus and adrenergic brain stem regions that provide catecholamine inputs to the hypothalamus. It is widely known that alcohol exposure alters corticotropin releasing factor (*Crf*) activity in the PVN (Rivier et al., 1984, 1990) with reported increases in PVN neuronal activity (Lee et al., 2000b) as well as corticosterone (CORT) release from the adrenal glands in response to alcohol consumption (Richardson et al., 2008). Our lab has also shown that alcohol causes changes in catecholaminergic cell numbers and activation following alcohol exposure (Logrip et al., 2013). However, it is important to distinguish between the response of a brain structure to alcohol itself, and the consequence of previous exposure to alcohol on the HPA axis' ability to respond to an additional stressor such as an alcohol challenge in young adulthood and adulthood. We have examined the effects

*Corresponding author. Address: The Salk Institute for Biological Studies; PBL, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA. Fax: +1-858-552-1546.
E-mail address: slee@salk.edu (S. Lee).
† Present address: Marine Mammal and Turtle Division, Southwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, La Jolla, CA 92037, USA.
‡ Equal second authors.

Abbreviations: AIE, adolescent intermittent ethanol; ANOVA, analysis of variance; *Avp*, arginine vasopressin; BAL, blood alcohol level; CON, control group; CORT, corticosterone; *Crf*, corticotropin releasing factor; EDTA, Ethylenediaminetetraacetic acid; HPA, hypothalamic–pituitary–adrenal; Ig, intragastric; IHC, immunohistochemistry; PFA, paraformaldehyde; PND, post-natal day; PNMT, phenylethanolamine N-methyltransferase; PVN, paraventricular nucleus.

http://dx.doi.org/10.1016/j.neuroscience.2016.09.031
0306-4522/

of adolescence binge drinking (self-administration) and adolescent intermittent ethanol (AIE) exposure (vapor chambers) on the brain stress response in young adulthood. In adolescent self-administering binge-drinking animals, we found a decrease in the number of *Crf* cells in the young adult amygdala (Allen et al., 2011a), a brain region that is important in conveying the emotional component of the stress response (Pich et al., 1995). Additionally, we have shown that AIE exerts long-term effects on the ability of the PVN to respond to an alcohol challenge in young adulthood, possibly mediated by catecholaminergic input from the brain stem to the PVN as seen by changes in activation (measured via c-fos immunoreactivity) of phenylethanolamine N-methyltransferase (PNMT) neurons (Allen et al., 2011b; Logrip et al., 2013). These results prompted us to investigate whether AIE exposure similarly or differentially affects the response to a distinct acute stressor, an alcohol challenge in young adult and adult male rats.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (n = 45) were obtained from Harlan Laboratory (San Diego, CA, USA). Animals were housed three or four per cage with food and water *ad libitum* in a humidity- and temperature-controlled vivarium under a 12-h light/12-h dark cycle with lights off at 18:00. All experiments were carried out in the morning and met the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by The Salk Institute Animal Care and Use Committee.

Intermittent alcohol vapor exposure

Adolescent rats [post-natal day (PND) 28–42] were exposed to alcohol vapor in an airtight chamber system provided by La Jolla Alcohol Research, Inc. (La Jolla, CA, USA, http://www.ljari.com), as described in detail previously (Lee et al., 2000a, 2000b). Rats were exposed to alcohol as vapor daily for 6 h (07:00–13:00; AIE) or air [Control (CON)] for the duration of the 15-day adolescent period (PND 28–42). After each daily exposure, the rats were returned to clean housing cages. Blood alcohol levels (BALs) were obtained and monitored on alternate days to maintain BALs at approximately 200 mg/dL. This exposure paradigm was used to generate controlled daily cycles of alcohol intoxication and withdrawal during the adolescent period as described previously (Logrip et al., 2013).

Blood alcohol levels

Blood samples for BAL measurements were obtained from the tails of all animals, including the air-exposed rats to control for any procedural stress effects. Blood plasma (5 μL) was used to determine BALs via an Analox AM 1 analyzer (Analox Instruments Ltd., Lunenburg, MA, USA, (Lee et al., 2000a, 2000b)). The precision of this assay is 1–2%, the sensitivity is 0.1 mg/dL and the curve is linear up to 400 mg/dL.

Corticosterone assay

Plasma CORT levels were measured in unextracted plasma from iv blood according to manufacturer's instructions using a widely applied commercial double antibody RIA kit for rat and mouse samples (MPBiomedicals, Diagnostic Division, Orangeburg, NY, USA, catalog # 07120103). Specifically, blood was collected into chilled plastic tubes containing EDTA (0.6 mg/500 mL whole blood) and centrifuged. The resulting plasma was aspirated and a 5-μL aliquot immediately diluted into 995 L CORT assay buffer, 1/200 final dilution per manufacturers' instructions, and stored frozen until thawing once for CORT analysis. All samples from a single experiment were analyzed in the same CORT RIA and all samples displayed B/B_0 of 90–20%. The reliable range of the assay is 10–1000 ng/mL with an intra-assay coefficient of variance of $<10\%$. This assay is highly specific for CORT; the cross-react with desoxycorticosterone is 0.34%, testosterone is 0.1% and cortisol is 0.05%.

Animal surgery and alcohol injection

AIE and control male rats were implanted with ig or iv catheters upon reaching PND 62–63 or PND 68–69, respectively, under isoflurane anesthesia (Butler Animal Health Supply, Dublin, OH, USA) [see Ogilvie et al., 1997 for methods], and were allowed to recover from surgery in individual cages for 7–8 days (intragastric, ig) or 1–2 days (intravenous, iv) before experimentation. On the day of the experiment (PND 70 or 90), the animals were placed in individual buckets with wood chip bedding in a quiet room with extension cannulae connected to a syringe containing heparinized saline such that the animals could be injected without being handled, to prevent any procedural stress. They were free-moving and left undisturbed for 2–3 h in an effort to acclimatize rats, then administered 3.2–4.5 g/kg alcohol ($<20\%$ v/v in water) via the ig cannula. The alcohol challenge dose corresponds to that previously used in our laboratory (Lee et al., 2001; Lee and Rivier, 2003; Seo and Rivier, 2003). Injections were slowly infused over a 2-min period because of the large volume needed to be administered.

Acute stressor

Plasma CORT concentration in young adult (PND 70) rats was determined to confirm a hormonal stress response to alcohol – a previously published stressor known to elicit changes in HPA-related brain circuitry [i.e., alcohol challenge; (Logrip et al., 2013): n = 6–7/group]. We also determined CORT concentration in adult animals (PND 70 or 90) to identify a hormonal stress response to an alcohol challenge; blood was collected from control (air: n = 4–7) and treatment (AIE: n = 4–7) male rats at 0, 60, 120, 180 and 240 min (PND 70) and at 0, 60, 120 and 210 min (PND 90) after an alcohol challenge (3.2–3.6 g/kg, ig). Finally, the effects of AIE exposure on the brain stress response to an alcohol challenge in adult animals were examined. Following an alcohol challenge in adulthood, the expression of *Crf* or arginine