Alcohol 51 (2016) 89–100

Contents lists available at ScienceDirect

Alcohol

journal homepage: http://www.alcoholjournal.org/

Age-related effects of chronic restraint stress on ethanol drinking, ethanol-induced sedation, and on basal and stress-induced anxiety response

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ARTICLE INFO

Article history:
Received 27 June 2015
Received in revised form
16 October 2015
Accepted 16 November 2015

Keywords:
Restraint stress
Rat
Ethanol
Adolescent
Adult

ABSTRACT

Adolescents are sensitive to the anxiolytic effect of ethanol, and evidence suggests that they may be more sensitive to stress than adults. Relatively little is known, however, about age-related differences in stress modulation of ethanol drinking or stress modulation of ethanol-induced sedation and hypnosis. We observed that chronic restraint stress transiently exacerbated free-choice ethanol drinking in adolescent, but not in adult, rats. Restraint stress altered exploration patterns of a light–dark box apparatus in adolescents and adults. Stressed animals spent significantly more time in the white area of the maze and made significantly more transfers between compartments than their non-stressed peers. Behavioral response to acute stress, on the other hand, was modulated by prior restraint stress only in adults. Adolescents, unlike adults, exhibited ethanol-induced motor stimulation in an open field. Stress increased the duration of loss of the righting reflex after a high ethanol dose, yet this effect was similar at both ages. Ethanol-induced sleep time was much higher in adult than in adolescent rats, yet stress diminished ethanol-induced sleep time only in adults. The study indicates age-related differences that may increase the risk for initiation and escalation in alcohol drinking.

Preclinical studies suggest that adolescents are uniquely sensitive to ethanol's pharmacological effects and that this pattern of response may put them at risk for alcohol initiation and escalation (Spear & Swartzwelder, 2014). Adolescent rats exhibit, when compared to adults, greater sensitivity to the appetitive (Pautassi, Myers, Spear, Molina, & Spear, 2008) and social facilitating effects of ethanol (Varlinskaya & Spear, 2002). This pattern, however, is somehow different in adolescent mice, which require lengthier training or higher ethanol doses to exhibit the same magnitude of conditioned place preference (CPP) by ethanol found in their adult counterparts (Dickinson, Kashawny, Thiebes, & Charles, 2009). The adolescents from both species, however, are less sensitive than adults to the aversive motivational effects of the drug (Holstein, Spanos, & Hodge, 2011; Vetter-O'Hagen, Varlinskaya, & Spear, 2009). Moreover, compared to adults, adolescents usually consume more ethanol on a gram by kilogram (g/kg) basis (Doremus, Brunell, Rajendran, & Spear, 2005) and are much less sensitive to the hypnotic effect induced by relatively high (≥3.5 g/kg) doses of ethanol (Silveri & Spear, 1998).

Evidence suggests that adolescent rats may be more sensitive to stress than adults (Stone & Quartermain, 1997). An intriguing study (Song et al., 2007) observed CPP by ethanol in adult, but not in adolescent, mice. Stress exposure facilitated the expression of ethanol-induced CPP in the adolescents but did not modify its expression in the adults. Relatively little is known, however, about age-related differences in stress modulation of ethanol drinking or

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http://dx.doi.org/10.1016/j.alcohol.2015.11.009
0741-8329/

stress modulation of ethanol-induced sedation and hypnosis. Exposure to social isolation enhances ethanol intake in adolescent, but not in adult, mice (Lopez, Doremus-Fitzwater, & Becker, 2011; Schenk, Gorman, & Amit, 1990). More in detail, the study by Lopez et al. (2011) revealed that social isolation between weaning and adulthood, but not during adulthood, increased subsequent ethanol intake, when compared to control, group-housed, C57BL/6J mice. The stress-induced by social isolation can also attenuate the duration of loss of the righting reflex, as shown in mice selectively bred for exhibiting differential sensitivity to the sedative and hypnotic effects of ethanol (i.e., long sleep [LS] and short-sleep [SS] mice) (Parker, Ponicsan, Spencer, Holmes, & Johnson, 2008). Siegmund, Vengeliene, Singer, and Spanagel (2005) reported greater alcohol intake after footshock in adult rats that had begun drinking during adolescence, but not in counterparts who experienced alcohol initiation as adults. Brunell and Spear (2005) examined modulation of ethanol intake by chronic footshock in adult and adolescent rats housed in isolation, and found a lack of stress-induced differences at any age.

The present study analyzed whether the effects of restraint stress (RS) upon ethanol intake and upon sensitivity to ethanol-induced sedation and hypnosis are similar in adolescent and adult rats. Increased ethanol intake has been found after protracted RS in Wistar rats (Lynch, Kushner, Rawleigh, Fiszdon, & Carroll, 1999; Ploj, Roman, & Nylander, 2003; Roman, Ploj, & Nylander, 2004) and sometimes in mice, although predominantly in males exposed to repeated cycles of RS and ethanol access (Chester, de Paula Barrenha, DeMaria, & Finegan, 2006). Yet rat and mice studies in which RS significantly decreased (rat: Chester, Blose, Zweifel, & Froehlich, 2004; Ng Cheong Ton, Brown, Michalakeas, & Amit, 1983) or did not affect ethanol intake (rat: Bertholomey, Henderson, Badia-Elder, & Stewart, 2011; Rockman, Hall, Hong, & Glavin, 1987; mice: Tambour, Brown, & Crabbe, 2008) have also been reported. Acute RS increased anxiety in adolescent and adult rats, and this anxiogenic effect was reversed by ethanol only in adolescents (Varlinskaya & Spear, 2012). Daily restraint sessions, for 5 days, exacerbated the social facilitating effects of ethanol in adolescents and reversed the inhibitory effects of ethanol commonly observed in non-stressed adults (Varlinskaya, Doremus-Fitzwater, & Spear, 2010). Findings concerning the effects of chronic RS upon ethanol-induced sedation revealed that it decreased and increased the duration of loss of the righting reflex, in LS and SS mice, respectively (dose: 4.1 g/kg; Jones, Connell, & Erwin, 1990).

It is still unknown if the effects of RS upon ethanol intake or upon ethanol-induced sedation and hypnosis are similar in adolescent and adult rats. Yet, the age-dependent interactions between ethanol and acute and chronic RS suggest that chronic RS may enhance ethanol drinking in adolescent, but not in adult, rats. This hypothesis was analyzed in Experiment 1. Adolescent and adult rats were given RS (five daily sessions, duration: 120 min) and then assessed for ethanol-induced behavioral stimulation and ethanol drinking in two-bottle choice tests. The studies conducted in LS and SS mice, in turn, suggest that the consequences of chronic RS on ethanol-induced sedation and hypnosis may be different in adolescents and adults (Jones et al., 1990). As indicated, adolescents are much less sensitive than adults to the hypnosis induced by relatively high ($\geq$3.5 g/kg) doses of ethanol (Silveri & Spear, 1998). Experiment 3 assessed stress modulation of this difference, which has important implications. The sedative and narcotic effects of ethanol serve as natural deterrents to alcohol drinking, and lower basal or stress-related sensitivity to these consequences constitutes a vulnerability factor for problematic alcohol use (Spear & Varlinskaya, 2010). Additionally, Experiment 2 explored the effects of chronic RS on basal and stress-induced corticosterone levels and anxiety response.

Materials and methods

Experimental designs and overview of procedures and aims

This section provides a description of the experimental designs and a brief overview of each experiment's aims and procedures, which are then described at length in the next section.

Experiments 1a and 1b

Experiment 1a analyzed acute motor-stimulating effects of ethanol and ethanol intake and employed a 2 (age: adolescent or adult) $\times$ 2 (stress condition: 120 min of restraint a day, for 5 days; or non-stressed) $\times$ 2 (ethanol dose before open-field measurement: 0.0 or 2.5 g/kg) factorial design, with 11–13 animals per group. Animals were exposed to RS on postnatal days (PD) 30–34 (adolescents) or 70–74 (adults) and were given ethanol (0.0 or 2.5 g/kg, intragastrically [i.g.]) 2 h after termination of the last stress exposure on PD34 or PD74. Five minutes after this intubation, animals were placed in the central area of an open field and tested for 10 min. Ethanol intake assessments were conducted on PD37 to PD40 (adolescent group) or PD77 to PD80 (adult group). Experiment 1b assessed metabolic processing of alcohol in naïve adolescent and adult animals ($n = 6$ per group).

Experiment 2

A 2 (age: adolescent or adult) $\times$ 2 (stress condition: 120 min of restraint a day, for 5 days; or non-stressed) factorial design was employed. Adolescents and adults were exposed to RS and then, on PD38 (adolescent group) or PD78 (adult group), assessed for anxiety response in a light–dark box (LDB) test (5 min), and then exposed for 5 min to inescapable stress (confinement in the white section of the light–dark box with illumination of 1200 lux). Blood samples, collected 90 min before the LDB test and immediately after termination of the inescapable stress, were used to measure corticosterone levels. Each group had 12 animals.

Experiment 3

A 2 (age: adolescent or adult) $\times$ 2 (stress condition: 120 min of restraint a day, for 5 days; or non-stressed) $\times$ 2 (ethanol dose: 4.0 or 4.5 g/kg) factorial design was employed. Each of the 8 groups included 10–12 animals. Adolescent and adult rats were either exposed or not to RS, and on PD35 or PD75 (adolescent and adult group, respectively), challenged with ethanol (4.0 or 4.5 g/kg, intraperitoneally [i.p.]) and assessed for loss of righting reflex and sleep time. Trunk blood samples were taken after recuperation from ethanol-induced sleep and processed for blood ethanol concentration (BEC) and CORT levels.

Subjects

Two-hundred forty-six male rats were used. The animals in Experiments 1a, 1b, and 2 (44, 6, and 29 adolescents; and 47, 6, and 30 adults, respectively) were Wistar rats born and reared in the animal facility of the Instituto Ferreyra (INIMEC-CONICET-UNC, Córdoba, Argentina). Rats in Experiment 3 (39 adolescents and 45 adults) were Sprague–Dawley rats (SD) born and reared in an animal facility at the Psychology Department of Binghamton University (Binghamton, NY), within an AAALAC-accredited facility. Dams were checked for births every day, and the day of delivery was considered PD0. Weaning was conducted on PD21, and unless specified, animals were housed in groups of four and given continuous *ad libitum* access to water and food. Both colonies were kept at an ambient temperature of 22 $\pm$ 1 °C with lights turned on and off at 8:00 AM and 8:00 PM, respectively. The rationale for using Wistar rats in Experiments 1 and 2 but SD rats in Experiment