Neuropharmacology xxx (2015) 1–11

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Adolescent social isolation increases anxiety-like behavior and ethanol intake and impairs fear extinction in adulthood: Possible role of disrupted noradrenergic signaling

M.J. Skelly, A.E. Chappell, E. Carter, J.L. Weiner*

Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, USA

ARTICLE INFO

Article history:
Received 16 February 2015
Received in revised form
16 April 2015
Accepted 14 May 2015
Available online xxx

Keywords:
Duloxetine
Fear-potentiated startle
Prazosin
Propranolol
Social isolation

ABSTRACT

Alcohol use disorder, anxiety disorders, and post-traumatic stress disorder (PTSD) are highly comorbid, and exposure to chronic stress during adolescence may increase the incidence of these conditions in adulthood. Efforts to identify the common stress-related mechanisms driving these disorders have been hampered, in part, by a lack of reliable preclinical models that replicate their comorbid symptomatology. Prior work by us, and others, has shown that adolescent social isolation increases anxiety-like behaviors and voluntary ethanol consumption in adult male Long–Evans rats. Here we examined whether social isolation also produces deficiencies in extinction of conditioned fear, a hallmark symptom of PTSD. Additionally, as disrupted noradrenergic signaling may contribute to alcoholism, we examined the effect of anxiolytic medications that target noradrenergic signaling on ethanol intake following adolescent social isolation. Our results confirm and extend previous findings that adolescent social isolation increases anxiety-like behavior and enhances ethanol intake and preference in adulthood. Additionally, social isolation is associated with a significant deficit in the extinction of conditioned fear and a marked increase in the ability of noradrenergic therapeutics to decrease ethanol intake. These results suggest that adolescent social isolation not only leads to persistent increases in anxiety-like behaviors and ethanol consumption, but also disrupts fear extinction, and as such may be a useful preclinical model of stress-related psychopathology. Our data also suggest that disrupted noradrenergic signaling may contribute to escalated ethanol drinking following social isolation, thus further highlighting the potential utility of noradrenergic therapeutics in treating the deleterious behavioral sequelae associated with early life stress.

1. Introduction

Alcohol use disorder, anxiety disorders, and post-traumatic stress disorder (PTSD) frequently co-occur (Brady and Sinha, 2005; Kushner et al., 2000), and exposure to chronic stress in early life has been linked to the incidence of each of these conditions (Brady and Sinha, 2005; Enoch, 2011; Gillespie et al., 2009; Huot et al., 2001; Kaufman et al., 2007; Roman and Nylander, 2005). Individuals suffering from anxiety or trauma- and stressor-related disorders may drink to self-medicate, as acute alcohol relieves anxiety and transiently alleviates the symptoms of PTSD (Davis et al., 2013; Leeies et al., 2010; Robinson et al., 2011). In contrast, alcohol withdrawal enhances anxiety and exacerbates PTSD symptomatology, thereby promoting further intake (Becker, 2012; Driessen et al., 2001; Koob and Le Moal, 2005; Robinson et al., 2011). As alcohol dependence is characterized by cycles of intoxication and withdrawal, anxiety-related drinking has been implicated in advancing the progression of alcoholism (Breese et al., 2011; Koob, 2013), and individuals suffering from comorbid PTSD, alcohol use disorder, and anxiety disorders exhibit worsened clinical profiles and experience poorer treatment outcomes (Bruce et al., 2005; Jacobsen et al., 2001). As such, determining the shared mechanisms driving these commonly comorbid conditions may prove crucial to effectively preventing and reversing their co-occurrence.

Clinical research suggests that dysregulation of the noradrenergic system may contribute to the etiology of these disorders, as potentiated noradrenergic signaling has been observed in

* Corresponding author. Tel.: +1 336 716 8692; fax: +1 336 716 8501.
E-mail address: jweiner@wakehealth.edu (J.L. Weiner).

http://dx.doi.org/10.1016/j.neuropharm.2015.05.025
0028-3908/

individuals suffering from each condition (Brawman-Mintzer and Lydiard, 1997; Geracioti et al., 2001; Patkar et al., 2004) and putative noradrenergic inhibitors have been shown to decrease anxiety, ethanol intake, and PTSD symptomatology (Boehnlein and Kinzie, 2007; Famularo et al., 1988; Fox et al., 2012; Lader, 1988; Petrakis et al., 2012; Simpson et al., 2009). Noradrenergic signaling is an integral component of the stress response system, and sustained activation of locus coeruleus (LC) noradrenergic afferents in response to prolonged stress exposure has been linked to persistent increases in anxiety (Jedema et al., 2001; Ressler and Nemeroff, 2001). LC afferents project diffusely throughout the neuraxis, targeting many regions involved in generating and terminating appropriate behavioral and neuroendocrine responses to stressors (Berridge and Waterhouse, 2003); as such, noradrenergic pharmacotherapeutics likely exert their effects by acting on a number of LC targets. In support of this, chronic stress or ethanol exposure have been shown to disrupt noradrenergic signaling in many brain regions implicated in the maintenance of fear- and anxiety-related behaviors, including the hypothalamus, basolateral amygdala (BLA), and bed nucleus of the stria terminalis (BNST) (Aston-Jones and Harris, 2004; Morilak et al., 2005; Smith and Aston-Jones, 2008). Despite the potential importance of understanding how the noradrenergic system is disrupted by chronic stress or ethanol exposure and righted by treatment with drugs thought to decrease noradrenergic signaling, the specific neurobiological changes underlying these phenomena have yet to be identified. These efforts have been partially hampered by a lack of reliable animal models that engender enduring increases in anxiety-like behaviors, PTSD-like symptoms, and excessive ethanol intake.

Toward the goal of developing such a model, we have recently begun characterizing the effects of adolescent social isolation on subsequent ethanol consumption and anxiety measures in adult male Long–Evans rats. Preclinical models have demonstrated that exposure to chronic stress during development increases anxiety-like behaviors and ethanol intake in adulthood (Cruz et al., 2008; Huot et al., 2001; Roman and Nylander, 2005), and adolescence is arguably a particularly vulnerable developmental period during which the brain is especially sensitive to the effects of stress (Heim and Nemeroff, 2001; Jankord et al., 2011; Spear, 2009). In support of this, adolescent social isolation has been shown to increase anxiety-like behaviors (Chappell et al., 2013; Hall et al., 1998b; Hellemans et al., 2004; Yorgason et al., 2013) and ethanol self-administration (Chappell et al., 2013; Deehan et al., 2007; Ehlers et al., 2007; Hall et al., 1998a; McCool and Chappell, 2009) in adult rats. The experiments described herein extend previous studies by using the fear-potentiated startle paradigm, a highly translational and clinically relevant measure of fear learning (Grillon, 2008; Jovanovic et al., 2013), to examine whether adolescent isolation also produces disruptions in fear and extinction learning. Specifically, we hypothesized that adolescent social isolation would disrupt extinction memory acquisition following fear conditioning, as extinction learning is known to be disrupted among individuals suffering from PTSD (Grillon and Morgan, 1999; Jovanovic et al., 2010). Our results support this hypothesis, suggesting that exposure to chronic stress during adolescent development disrupts the ability to extinguish fear memories in later life.

Additionally, we examined the effect of three anxiolytic medications that target the noradrenergic system (the α1-adrenoreceptor (AR) antagonist prazosin, the β1/2-AR antagonist propranolol, and the serotonin–norepinephrine reuptake inhibitor (SNRI) duloxetine) on home-cage ethanol drinking in singly housed adult animals exposed to either social isolation or group housing during adolescence. Our findings confirm and extend our previous results, demonstrating that adolescent social isolation results in a robust and long-lasting increase in ethanol intake in adulthood, even relative to adolescent group housed subjects that were isolated during the adult drinking regimen. Furthermore, we report that all three modulators of noradrenergic signaling dose-dependently decrease ethanol intake in animals that were isolated throughout adolescence, while having little to no effect on intake among adolescent group housed conspecifics. Additionally, we found preliminary evidence that fear conditioning promotes greater ethanol intake among socially isolated animals, without effecting group-reared conspecifics.

2. Materials and methods

2.1. Subjects

A total of sixty-eight male Long Evans rats from four temporally distinct cohorts were used in these studies, all obtained from Harlan Laboratories (Indianapolis, IN). All animals were treated identically, except where indicated (Fig. 1). Animals arrived at postnatal day 21, and were group housed (4 animals/cage) in large, clear plexiglass cages (33.0 cm × 59.7 cm; Nalgene, Rochester, NY) for one week. Following this, socially isolated animals (SI n = 35) were moved to smaller clear cages (25.4 cm × 45.7 cm), while group housed animals (GH n = 33) remained in their original cages; animals stayed in these housing conditions with minimal handling (one cage change/week) for six weeks prior to behavioral testing. Rats had *ad libitum* access to food (Prolab RMH 3000, LabDiet; PMI Nutrition International, St. Louis, MO) and water throughout, and were maintained on a 12-h light/dark cycle in the same colony room. Animal care procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Wake Forest University Animal Care and Use Committee.

2.2. Anxiety-like behavior

Anxiety-like behavior was assessed in all animals during the seventh week of the adolescent housing manipulation using standard elevated plus-mazes (Med Associates, St. Albans, VT) raised 72.4 cm from floor level, with runways measuring 10.2 cm wide by 50.8 cm long. Open runways had 1.3 cm high lips and closed runways were enclosed in 40.6 cm high black polypropylene walls. Exits and entries from runways were detected via infrared sensors attached to the opening of each arm of the maze. Data were obtained and recorded via personal computer interfaced with control units and MED-PC programming (Med Associates). Animals were placed at the junction of the four arms at the beginning of the session, and activity was measured for five minutes. Anxiety-like behavior was assessed by measuring the total time spent on the open arms of the maze as well as the number of entries

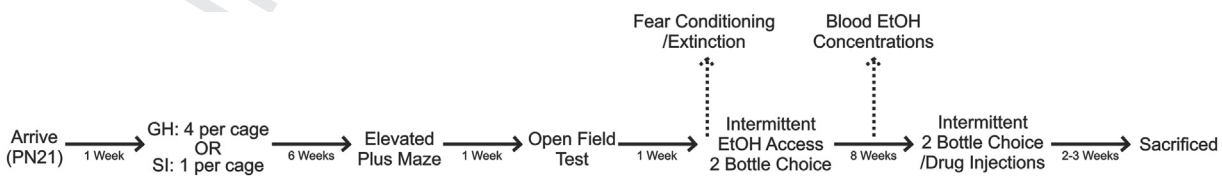

Fig. 1. Experimental Design. Animals arrived on postnatal day 21 and were housed in groups of four for one week. Following this, animals were randomly assigned to housing conditions, and either remained group housed (GH n = 33) or were socially isolated (SI n = 35) for the remainder of their adolescent development. At the end of six weeks, anxiety-like behavior and locomotor activity were assessed using the elevated plus-maze and open field tests. After this, 57 animals (GH n = 28; SI n = 29) were individually housed and began intermittent two-bottle choice ethanol self-administration in their home cages. A subset of animals (GH n = 13; SI n = 14) also underwent fear conditioning and extinction learning prior to ethanol access. All animals were allowed to self-administer ethanol on this schedule for eight weeks. A separate subset of animals (GH n = 15; SI n = 16) were used to determine blood ethanol concentrations three weeks into the drinking study. At the end of eight weeks, animals began receiving injections of either prazosin, propranolol, or duloxetine once per week to assess the dose-dependent effects of these drugs on drinking. Animals were sacrificed at the end of these studies.