Psychoneuroendocrinology 76 (2017) 154–161

Contents lists available at ScienceDirect

Psychoneuroendocrinology

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

Adolescent binge alcohol exposure increases risk assessment behaviors in male Wistar rats after exposure to an acute psychological stressor in adulthood

Audrey Torcaso, AnnaDorothea Asimes, Margaret Meagher, Toni R. Pak*

Loyola University Chicago, Stritch School of Medicine, Department of Cell and Molecular Physiology, Maywood, IL 60153, United States

ARTICLE INFO

Article history:
Received 5 July 2016
Received in revised form 21 October 2016
Accepted 28 November 2016

Keywords:
Alcohol
Puberty
Stress
Behavior

ABSTRACT

Teenage binge drinking is a common practice that has been shown to increase the risk for developing mood disorders in adulthood. The hypothalamo-pituitary-adrenal (HPA) axis is often dysfunctional in mood disorder patients, and animal models of adolescent binge alcohol exposure similarly show disordered HPA axis function, even after long periods of alcohol abstinence. Here, we sought to investigate the anxiety-like behavioral consequences of binge alcohol exposure in a Wistar rat model. Male rats were administered alcohol in a binge pattern during *peri*-puberty, and one month later, anxiety-like behaviors were measured using the elevated plus maze. A subset of the rats then underwent 30 min of restraint stress, and the anxiety-like behaviors were measured again. We observed an increase in risk assessment behaviors due to both adolescent binge alcohol exposure and restraint stress, but no differences in canonical anxiety-like behaviors. We also repeated the observation that adolescent binge alcohol induces long-term changes in HPA axis sensitivity. Therefore, we concluded that a history of *peri*-pubertal binge alcohol exposure subtly alters the behavioral response to subsequent acute psychological stress during adulthood, which may over time contribute to the development of mood disorders. This relatively pragmatic animal model represents a more clinically relevant tool in understanding the molecular mechanisms underlying the long-term effects of adolescent binge drinking.

1. Introduction

Teenage binge drinking is an increasing public health concern in the United States and many other developed countries worldwide. In the United States, the costs associated with underage drinking totaled $24.3 billion in 2010 (Sacks et al., 2015), and in 2015, 46.7% of high school seniors reported having been drunk at some point in their lives (Johnston et al., 2016). Because adolescence is a critical period of brain development, teenage binge alcohol consumption has the potential to produce long-lasting effects on health and behavior beyond the immediate effects of being acutely intoxicated. Teenagers who drink alcohol have region-specific cortical thinning and white matter disorganization (Luciana et al., 2013; Wilson et al., 2015). In addition to these anatomical changes, adolescent binge drinking increases the risk for developing alcohol dependence and other mental health disorders during adulthood (McCambridge et al., 2011; Rose et al., 2014; Viner and Taylor, 2007).

The hypothalamo-pituitary-adrenal (HPA) axis comprises the major neuroendocrine stress response system. Following exposure to an acute psychological or physical stressor, parvocellular neurons in the hypothalamic paraventricular nucleus (PVN) are activated, releasing corticotropin releasing factor (CRF) and arginine vasopressin (AVP) into the portal system of the anterior pituitary gland. This stimulates the release of adrenocorticotropic hormone (ACTH) into the systemic circulation, which acts on the adrenal cortex to release glucocorticoids into the blood. Glucocorticoids have a myriad of effects throughout the body, including the regulation of glucose homeostasis and immune function, as well as exerting negative feedback on the hypothalamus and pituitary gland to decrease further release of CRF, AVP, and ACTH (Smith and Vale, 2006).

The HPA axis undergoes important changes during puberty and the function of this axis can be permanently altered by exposure to a stressor during this developmental timeframe (Romeo et al., 2006; Sisk and Zehr, 2005). Heavy-drinking youth show greater volume decreases in the ventral diencephalon (the brain region which

* Corresponding author.
E-mail address: tpak@luc.edu (T.R. Pak).

http://dx.doi.org/10.1016/j.psyneuen.2016.11.032
0306-4530/

contains the hypothalamus) than age-matched controls (Squeglia et al., 2015, 2014). HPA axis dysfunction has also been reported among female college students who engage in problematic drinking (Wemm et al., 2013). Importantly, dysfunction of the HPA axis is associated with many psychiatric disorders, including anxiety (Naughton et al., 2014), raising the possibility that adolescent binge drinking might increase the risk of mental health disorders by altering the function of the HPA axis.

To examine the causal effects of teenage binge drinking on HPA axis dysfunction, our lab and others have established animal models of adolescent binge alcohol exposure (Allen et al., 2011; Przybycien-Szymanska et al., 2010). We have previously shown that our animal model of adolescent binge alcohol exposure (which utilizes male Wistar rats exposed to alcohol via oral gavage from post-natal day 37–44) results in long-term biochemical alterations of the HPA axis (Przybycien-Szymanska et al., 2011b). However, it was unknown if these biochemical changes translated to an altered behavioral phenotype. Here we tested the hypothesis that male Wistar rats exposed to our adolescent binge-pattern alcohol paradigm would exhibit increased anxiety-like behaviors in the elevated plus-maze after subsequent exposure to an acute psychological stressor during adulthood. Our data demonstrated that adolescent binge alcohol exposure increased risk assessment behaviors in adulthood when the animals were exposed to an acute mild psychological stressor; however there were no differences in baseline anxiety-like behavior in the absence of a stressor. Together, these data suggest that adolescent binge alcohol exposure could sensitize individuals to subsequent mild stressors and increase their risk of developing anxiety disorders as adults.

2. Methods

2.1. Ethics statement

All animal protocols were approved by the Loyola University Medical Center Institutional Animal Care and Use Committee (IACUC) permit #2013034. All measures were taken to minimize animal numbers and suffering.

2.2. Animals

Male Wistar rats were purchased from Charles River Laboratories (Wilmington, MA) at weaning (post-natal day [PND] 25) and allowed to acclimate for 5 days after arrival. Animals were pair-housed on a 12:12 light/dark cycle with lights on at 7:00 h. Food and water were available *ad libitum*.

2.3. Experimental paradigm

2.3.1. Repeated binge alcohol exposure

After acclimation to the housing environment, beginning on PND 30, animals were handled for 5 min once per day for 7 days by the same individual, between 09:30 and 11:00 h. Pubertal binge ethanol (EtOH) treatments commenced on PND 37, which is defined as *peri*-puberty in this species (Ketelslegers et al., 1978; Södersten et al., 1977). Animals were randomly assigned to either (1) binge EtOH treated (n = 20), or (2) water treated control (n = 20) groups. The binge EtOH treated animals received 3 g/kg ethanol (20% v/v in water) intragastrically (i.g.) *via* oral gavage once per day at 10:00 h for 3 consecutive days, then an equivalent volume of water i.g. for 2 days, then an additional 3 days with EtOH. This once/day (total of 8-days) binge paradigm has been used previously to mimic the pattern of binge alcohol consumption in adolescents (Lauing et al., 2008; Przybycien-Szymanska et al., 2010). Our previous studies, and others, have demonstrated that this dose and method of EtOH delivery resulted in blood alcohol concentrations (BAC) between 150 and 180 mg/dL one hour after the last dose, and does not interfere with normal growth rates or feeding behavior (Prins et al., 2014; Przybycien-Szymanska et al., 2010; Walker and Ehlers, 2009). The water treated control group received 8 days of an equivalent volume of water i.g. *via* oral gavage.

2.3.2. Acute stress paradigm

After pubertal binge alcohol treatments, both groups of animals were left undisturbed for 3 weeks (see Fig. 1). At the end of this 3-week period, the animals were again handled 5 min once per day for 7 days, as described above. At 10:00 h on PND 73, prior to further manipulation, animals were given a trial test in the elevated plus-maze (EPM) to establish a baseline level of anxiety-like behavior. Then, animals within each group were randomly assigned to either (a) a restraint stress group (n = 10 per group), or (b) an unstressed control group (n = 10 per group). Next, on PND 74 at 09:30 h, animals in the restraint stress group were placed in a plastic rodent restraint tube (Stoelting Co. #51335) inside a fresh cage for 30 min, then 5 min after being removed from the restraint tube, the rats were tested in the EPM again, and 5 min after ending the EPM test, the animals were euthanized. It has been previously demonstrated that plasma corticosterone (CORT) levels reach a peak after 30 min inside a plastic restraint tube (Cole et al., 2000). Animals in the unstressed control group were placed singly in a fresh cage for 35 min, then tested in the EPM again, and 5 min after ending the EPM test, the animals were euthanized.

2.4. Elevated plus-maze testing

Elevated Plus-Maze testing was conducted with the Rat Elevated Plus-Maze apparatus (Stoelting Co. #60240) and recorded using a video camera and ANY-maze software (Stoelting Co.). Testing was conducted in a dimly lit room (~5 lux) with white noise generated by a HoMedics Sound Spa Relaxation machine (~70 dB, equivalent to the white noise generated by the HVAC system in the animal housing room). Rats were placed singly in the center of the maze facing an open arm by a female experimenter, marking the beginning of the test period. The rat was then allowed 5 min to explore the maze freely, after which the recording stopped automatically, and then the rat was returned to its cage. Later, both spatiotemporal and ethological analyses were conducted using the recorded videos and ANY-maze.

2.5. Elevated plus-maze analysis

All parameters were analyzed from the video recordings by an investigator blinded to the animal treatment paradigms. For the spatiotemporal analysis, the maze was divided into 3 zones, specified using the ANY-maze software: the open arms, the closed arms, and the intersection. In order to be considered in the open or closed arms, at least 80% of the rat's body surface area had to be inside that zone (consistent with the "four-paw rule"). The rat was considered to be in the intersection if it was not considered to be in either the open or closed arms. The amount of time spent in any given zone was divided by the total test duration to calculate the percentage of time spent in the zone. The total distance travelled and average speed were also measured to consider differences in overall locomotor activity. For the ethological behavioral analyses, scoring of head dips, stretched attend postures, and rearing behaviors were manually recorded by a blinded trained observer. A head dip was defined as the rat extending its head over the edge of the maze and down toward the floor. A stretched attend posture was defined as the rat extending forward with its front paws, then retracting back to its original position. A rearing was defined as the rat sitting back on its hind paws and elevating its front paws, moving vertically.