Developmental Cognitive Neuroscience 16 (2015) 183–190

Contents lists available at ScienceDirect

Developmental Cognitive Neuroscience

journal homepage: http://www.elsevier.com/locate/dcn

Adolescent rats are resistant to forming ethanol seeking habits

Hannah Serlin, Mary M. Torregrossa*

Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA 15219, United States

ARTICLE INFO

Article history:
Received 11 August 2014
Received in revised form 3 December 2014
Accepted 16 December 2014
Available online 24 December 2014

Keywords:
Alcohol
Adolescent
Habit
Goal-directed
Contingency degradation
Self-administration

ABSTRACT

Early age of onset alcohol drinking is significantly more likely to lead to alcohol use disorders (AUDs) than alcohol drinking that begins after the age of 18. Unfortunately, the majority of people in the United States begin drinking in adolescence. Therefore, it is important to understand how early alcohol drinking leads to increased risk for AUDs so that better treatments and prevention strategies can be developed. Adolescents perceive greater rewarding properties of alcohol, and adolescents may be more likely to form alcohol-seeking habits that promote continued use throughout the lifetime. Therefore, we compared the development of alcohol seeking habits in adolescent and adult male, Sprague-Dawley rats. Rats were trained to lever press to receive 10% ethanol + 0.1% saccharin on a schedule that promotes habit formation. Rats were tested using a contingency degradation procedure at different points in training. Adult rats formed ethanol-seeking habits with only moderate training, while adolescents remained goal-directed even with extended training. Nevertheless, adolescents consumed more ethanol than adults throughout the experiment and continued to consume more ethanol than adults when they reached adulthood. Therefore, early onset alcohol use may promote AUD formation through establishment of high levels of drinking that becomes habitual in adulthood.

1. Introduction

Early age of onset of alcohol use is one of the best predictors of future development of an alcohol use disorder (AUD). Individuals who begin drinking before the age of 15 have significantly increased odds of developing an AUD than individuals who begin drinking after the age of 19 (Grant and Dawson, 1997; Grant et al., 2006; Bratek et al., 2013; Hingson and Zha, 2009). Adolescents consume more alcohol, and perceive the positive effects of alcohol as more positive and the negative effects as less negative than adults (Anderson et al., 2010; Spear, 2000, 2014; Silveri and Spear, 1998). However, this does not necessarily explain why early age of use is more likely to correlate with AUDs later in life. Indeed, alcohol use could simply decline once people reach adulthood. One possible explanation is that alcohol use initiated at a younger age is more likely to become a habitual behavior that is insensitive to changes in alcohol's rewarding or aversive properties. Alternatively, early alcohol use may result in changes to brain structure and function such that alcohol maintains greater rewarding properties in adulthood.

In order to begin to test these possibilities, we sought to determine whether there are differences in the propensity of adolescents and adults to form ethanol-seeking habits. A stimulus–response habit is defined as an action or behavior that is insensitive to changes in the value of the

* Corresponding author at: Department of Psychiatry, University of Pittsburgh, Bridgeside Point II, Room 228, 450 Technology Drive, Pittsburgh, PA 15219, United States. Tel.: +1 412 624 5723; fax: +1 412 624 5280.
E-mail address: torregrossam@upmc.edu (M.M. Torregrossa).

http://dx.doi.org/10.1016/j.dcn.2014.12.002

outcome produced by that action. Such actions also persist even when the contingency between the action and the outcome is changed (i.e., when the response no longer produce the outcome) (Yin and Knowlton, 2006; Balleine and O'Doherty, 2010). Current theories about the persistence of substance use disorders postulate that abnormal habit formation may explain why drug use persists even when the drug is no longer rewarding or its use results in adverse consequences (O'Tousa and Grahame, 2014; Belin et al., 2013; Everitt and Robbins, 2005). Adolescent substance use is known to be risk factor for the development substance use disorders; however, it is unclear if this increased risk is due to an increased propensity to form habits. Indeed, prior studies examining the vulnerability of adolescents to develop habitual or inflexible behaviors relative to adults have had mixed results. For example, in one study, adolescent rats demonstrated more habit-like behavior than adults when the contingency between the action and outcome was degraded, but did not appear habitual when tested using an outcome devaluation procedure (Naneix et al., 2012). In addition, Simon et al. (2013), found that adolescent rats exhibited more flexible behavior in a Pavlovian conditioning task relative to adults. Therefore, it is not clear if adolescents would be more or less likely than adults to form ethanol-seeking habits.

Furthermore, prior studies only examined habit formation for food reinforcers, but responding for ethanol becomes habitual more rapidly than responding for food (Dickinson et al., 2002; Corbit et al., 2012). Thus, adolescents may form ethanol-seeking habits at a different rate than food seeking habit. Indeed, previous research has demonstrated that food habits form more quickly in females than in males, but that the opposite is true for ethanol habit formation (Quinn et al., 2007; Barker et al., 2010). Therefore, in the present study we compared the rate of habit formation for an ethanol reinforcer in adolescent and adult rats using the contingency degradation paradigm. We also compared later adult ethanol self-administration in rats with adolescent vs. adult onset ethanol exposure.

2. Materials and methods

2.1. Subjects

Male Sprague-Dawley rats (Harlan, Frederick, MD) were delivered to the animal facility aged either 22 or 64 days. Rats were allowed to acclimate to the facility for 6 days before behavioral testing began on postnatal day (PND) 28, which is generally recognized as the beginning of early adolescence (Spear, 2000; Spear and Swartzwelder, 2014) or 70 (adulthood). Rats were housed two per cage for the entire experiment. Rats were maintained on a 12:12 h light–dark cycle in a temperature- and humidity-controlled environment. The rats were given ad libitum access to food and water except for periods of food restriction described below. All procedures conformed to the policies set forth by the University of Pittsburgh Institutional Animal Care and Use Committee and the National Institutes of Health Guidelines on the Care and Use of Laboratory Animals.

2.2. Behavioral testing

The experimental timeline and age of rats at each stage of testing are shown in Fig. 1A. Rats were food restricted beginning 3 days before behavioral testing. Rats were given sufficient daily food rations to maintain approximately 90% of their expected free-feeding body weight based on standard growth curves. Thus, adolescent rats were given sufficient food to grow and gain weight, but at a slower rate than rats fed ad libitum. The feeding procedure is based on published methods and produced no obvious detrimental effects to the animals and likely is similar to food intake that would be observed in the wild. Food restriction facilitates instrumental learning and ensured that all behavioral training and testing could occur within the age window of adolescence. In all phases of the experiment, rats were given or responded for a 10% v/v ethanol + 0.1% w/v saccharin solution made up in tap water. We used a slightly sweetened ethanol solution to best model initial ethanol drinking patterns in humans. Fig. 1 illustrates the timeline of behavioral testing and the details of each phase of testing are described below.

2.3. Ethanol habituation

On the day before operant training began rats were habituated to the ethanol solution to avoid neophobia (*i.e.*, fear of novelty) and facilitate acquisition of lever pressing behavior. Rats were placed in a novel cage containing one piece of standard chow for 15 min. The rats were allowed to become accustomed to the novel cage and eat the food pellet so that they would be more likely to be thirsty and sample the liquid solution. A bottle containing the ethanol solution was then placed on a wire top on each cage and the rats were allowed 30 min to sample the solution. The bottles were weighed before and after each session to verify that each rat consumed some of the solution.

2.4. Magazine training

All testing was conducted in standard operant chambers (MedAssociates, St. Albans, VT) and behavioral programs were controlled by MedPC software. All boxes contain a liquid dipper for delivery of liquid reinforcers. Two retractable levers were located on either side of a magazine (i.e., a receptacle) where reinforcers were delivered. The boxes were also equipped with a house light, stimulus lights above the levers, tone generators, and fans that allowed ventilation and produced background noise.

Rats underwent a single 30-min session of magazine training where the ethanol reinforcer was presented on a fixed-time 30-s schedule, which included the 10 s of access to the liquid dipper. The rats, therefore, had access to 60 reinforcers, and magazine entries were recorded to verify that rats learned to obtain reinforcers in the magazine.

2.5. Self-administration

Rats were trained to self-administer the oral ethanol solution in daily 30 min sessions. At the beginning of each session 2 levers were inserted into the chamber, one was