Alcohol xxx (2016) 1–10

Contents lists available at ScienceDirect

Alcohol

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

Mouse strain differences in punished ethanol self-administration

Lindsay R. Halladay*, Adrina Kocharian, Andrew Holmes

Laboratory of Behavioral and Genomic Neuroscience, National Institute on Alcoholism and Alcohol Abuse (NIAAA), NIH, Bethesda, MD, USA

ARTICLE INFO

Article history:
Received 9 November 2015
Received in revised form
5 February 2016
Accepted 23 May 2016

Keywords:
Alcohol
Mouse
Punishment
Addiction
Hippocampus
Amygdala

ABSTRACT

Determining the neural factors contributing to compulsive behaviors such as alcohol-use disorders (AUDs) has become a significant focus of current preclinical research. Comparison of phenotypic differences across genetically distinct mouse strains provides one approach to identify molecular and genetic factors contributing to compulsive-like behaviors. Here we examine a rodent assay for punished ethanol self-administration in four widely used inbred strains known to differ on ethanol-related behaviors: C57BL/6J (B6), DBA/2J (D2), 129S1/SvImJ (S1), and BALB/cJ (BALB). Mice were trained in an operant task (FR1) to reliably lever-press for 10% ethanol using a sucrose-fading procedure. Once trained, mice received a punishment session in which lever pressing resulted in alternating ethanol reward and footshock, followed by tests to probe the effects of punishment on ethanol self-administration. Results indicated significant strain differences in training performance and punished attenuation of ethanol self-administration. S1 and BALB showed robust attenuation of ethanol self-administration after punishment, whereas behavior in B6 was attenuated only when the punishment and probe tests were conducted in the same contexts. By contrast, D2 were insensitive to punishment regardless of context, despite receiving more shocks during punishment and exhibiting normal footshock reactivity. Additionally, B6, but not D2, reduced operant self-administration when ethanol was devalued with a bitter tastant. B6 and D2 showed devaluation of sucrose self-administration, and punished suppression of sucrose seeking was context dependent in both the strains. While previous studies have demonstrated avoidance of ethanol in D2, particularly when ethanol is orally available from a bottle, current findings suggest this strain may exhibit heightened compulsive-like self-administration of ethanol, although there are credible alternative explanations for the phenotype of this strain. In sum, these findings offer a foundation for future studies examining the neural and genetic factors underlying AUDs.

1. Introduction

Alcohol-use disorders (AUDs) are often characterized by persistent drinking despite negative consequences (American Psychiatric Association, 2013). Determining the neural factors contributing to such behaviors has become a significant focus of current preclinical research (Everitt et al., 2008; Koob & Volkow, 2010; Vengeliene, Celerier, Chaskiel, Penzo, & Spanagel, 2009). One potentially useful approach in this regard involves assaying ethanol self-administration after punishment (Hopf & Lesscher, 2014; Radke et al., 2015; Radwanska & Kaczmarek, 2012; Seif et al., 2013). For instance, comparing punished ethanol self-administration across genetically distinct mouse strains offers a means to identify brain regions, molecular pathways, and genetic factors contributing to ethanol self-administration in the face of aversive outcomes.

Four widely studied inbred mouse strains, C57BL/6J (B6), DBA/2J (D2), 129S1/SvImJ (S1), and BALB/cJ (BALB), have been shown to show disparities in neural function and anatomy (Andolina, Puglisi-Allegra, & Ventura, 2015) that may contribute to differences in learning (Holmes, Wrenn, Harris, Thayer, & Crawley, 2002; Lederle et al., 2011; Owen, Logue, Rasmussen, & Wehner, 1997; Paylor, Baskall, & Wehner, 1993), stress responsivity (Graybeal et al., 2014; Lattal & Maughan, 2012; Moy et al., 2007; Mozhui et al., 2010), and ethanol-related behaviors (Belknap, Crabbe, & Young, 1993; Boyce-Rustay, Janos, & Holmes, 2008; Chesler et al., 2012; Crabbe, 1983; Debrouse et al., 2013; Elmer, Meisch, & George, 1987a; Elmer, Meisch, & George, 1987b; Elmer, Meisch, Goldberg, & George, 1988; Fish et al., 2010; Ford, Steele, McCracken, Finn, & Grant, 2013; Palachick et al., 2008; Rhodes

* Corresponding author. 5625 Fishers Lane Room 2N09, Rockville, MD, 20852-9411, USA.
E-mail address: Lindsay.Halladay@nih.gov (L.R. Halladay).

http://dx.doi.org/10.1016/j.alcohol.2016.05.008
0741-8329/

et al., 2007; Rodgers & McClearn, 1962; Yoneyama, Crabbe, Ford, Murillo, & Finn, 2008). Of particular note, a now classic observation is that D2 exhibit reduced ethanol drinking and preference compared to B6 in two-bottle choice tests (Belknap et al., 1993; Boyce-Rustay et al., 2008; Crabbe, 1983; Rhodes et al., 2007; Rodgers & McClearn, 1962; Yoneyama et al., 2008), along with evidence that ethanol is a less effective reinforcer for D2 than B6 (Risinger, Brown, Doan, & Oakes, 1998). These findings have led to conceptualization of these two mouse strains as high- (B6) and low- (D2) ethanol preferring.

However, a number of recent observations have clouded the distinction between D2 and B6. D2 mice show stronger conditioned place preference (CPP) (Cunningham & Noble, 1992; Cunningham, Niehus, Malott, & Prather, 1992; Gremel, Gabriel, & Cunningham, 2006; Risinger, Malott, Riley, & Cunningham, 1992) and locomotor (Crabbe, 1983; Phillips, Dickinson, & Burkhart-Kasch, 1994; Rose, Calipari, Mathews, & Jones, 2013) responses to ethanol injections, and D2 < B6 differences in ethanol self-administration are attenuated when ethanol is delivered intragastrically or intravenously (Fidler et al., 2012, 2011; Grahame & Cunningham, 1997) or adulterated with certain tastants (e.g., monosodium glutamate) (McCool & Chappell, 2014). These findings suggest that taste aversion may at least partially account for lower two-bottle ethanol drinking in the D2 strain. They also raise interesting questions about how mouse strains, and D2 and B6 in particular, that have been characterized for their ethanol-related phenotypes in traditional behavioral assays would perform on measures posited to be more relevant to the addicted state, such as punished ethanol self-administration.

In the current study, we first compared the B6 and D2, along with S1 and BALB, strains on an operant measure of responding for ethanol after punishment, recently developed in our laboratory (Radke, Jury, et al., 2015; Radke, Nakazawa, & Holmes, 2015), based on prior studies of ethanol and cocaine self-administration in rats (Belin, Berson, Balado, Piazza, & Deroche-Gamonet, 2011; Marchant, Khuc, Pickens, Bonci, & Shaham, 2013; Pelloux, Murray, & Everitt, 2013, 2015). Additional experiments were then performed to further characterize differences in punished ethanol responding between B6 and D2 by testing for strain differences in sensitivity to ethanol devaluation and contextual cues. The results obtained offer novel insight into punished ethanol self-administration in mice and provide a foundation for exploiting these strains to delineate the neural and genetic basis of this behavior.

2. Materials and methods

2.1. Subjects

Male S1 (n=10), BALB (n=11), B6 (n=10), and D2 (n=12) mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). These strains were chosen based on their extensive use in neuroscience, their inclusion in the Mouse Phenome Project (www.jax.org/phenome) (Bogue & Grubb, 2004), and their use in our previous analyses of strain differences in behavioral phenotypes of active sensitivity and ethanol self-administration (Boyce-Rustay et al., 2008; Chen & Holmes, 2009; Lederle et al., 2011).

Mice were 9–10 weeks old at the start of the experiments. They were housed in pairs in a temperature (72 ± 5 °F) and humidity (45 ± 15%) controlled vivarium, under a 12-h light/dark cycle (lights on at 0630 h). Over approximately 1 week prior to behavioral training, mice were reduced to 85% of their free-feeding body weight, which was maintained through completion of behavioral testing. All experimental procedures carried out were approved by the NIAAA Animal Care and Use Committee and followed the NIH guidelines outlined in *Using Animals in Intramural Research*, as well as the local Animal Care and Use Committees. See Fig. S1 for a schematic depiction of the sequence of tests used.

2.2. Operant training

Behavioral training was conducted in 21.6 × 17.8 × 12.7 cm operant chambers housed within sound- and light-attenuating enclosures (Med Associates, St. Albans, VT, USA). Grid floors of the chambers were fully covered with Plexiglas® for all sessions except the punishment session, during which it was removed in order to administer footshock. Pellet and liquid dispensers delivered rewards into a receptacle at one end of the chamber, which was located in the middle of two ultra-sensitive response levers (5 cm from the receptacle). Speakers emitting a 3-kHz pure tone cue that signaled reward delivery were positioned above the levers. Med-PC software (Med Associates) controlled reward delivery and recorded lever presses.

Mice were initially trained to press one of the two levers (= 'active lever') to receive delivery of a 14-mg food pellet reward (40-min sessions on a fixed-ratio 1 [FR1]/continuous schedule of reinforcement). Presses on the second, inactive lever had no programmed consequences. Once responding was established (at least 35 active-lever presses in a 40-min session), mice were trained to respond for ethanol using a sucrose-fade procedure (Radke, Jury, et al., 2015), whereby the food pellet reward was replaced with a 10-μL liquid reward delivered over 0.3 s. The liquid solutions were 10% sucrose, 10% sucrose + 10% ethanol, 5% sucrose + 10% ethanol, and 10% ethanol, with training proceeding on a given solution until criterion was met (= consistent active-lever pressing with less than 20% inter-session variation on three consecutive sessions). The rate of active-lever pressing (per minute) for each reward type is reported as the mean average during the three sessions at criterion.

2.3. Punished responding for ethanol

Following training, there was a 40-min punishment session in which active-lever pressing alternated between being rewarded (10% ethanol) and being coincident with a 0.3-mA, 0.75-s footshock. Punishment sessions took place in the same room where the mice had received their training, using shock-equipped operant chambers that were identical to training chambers in all aspects other than having an exposed metal-rod floor due to removal of the Plexiglas® floor insert in order to deliver shock to the mouse. The number of shocks received during the punishment session was recorded. Post-punishment probe tests were conducted (using the procedure as pre-punishment training) on each of the 2 days following punishment, in the same operant chambers where training had occurred and with the Plexiglas® floor insert present. Three dependent variables were measured during the probe tests: 1) the per-minute rate of active-lever pressing, 2) the latency to first make an active-lever press, and 3) the vigor of active-lever pressing (= the maximum number of consecutive 1-min bins in which an active-lever press was made). These values were averaged over the two probe tests and compared with the average at pre-punishment criterion.

2.4. Ethanol devaluation

Beginning 24 h after probe testing, B6 and D2 were tested under the same procedures used for training until active-lever rates returned to pre-punishment levels (S1 and BALB were excluded from subsequent experiments because their performance did not differ from B6 during punished responding for ethanol). There were then an additional five daily sessions (testing procedures again equivalent to training) to ensure a reliable level of responding. Beginning on day 6, the ethanol solution was adulterated using the bitter compound, denatonium benzoate (DB) (Sigma Aldrich, Allentown, PA, USA) –

Please cite this article in press as: Halladay, L. R., et al., Mouse strain differences in punished ethanol self-administration, Alcohol (2016), http://dx.doi.org/10.1016/j.alcohol.2016.05.008