Published in final edited form as:
Alcohol Clin Exp Res. 2011 February ; 35(2): 326–337. doi:10.1111/j.1530-0277.2010.01348.x.

The influence of selection for ethanol withdrawal severity on traits associated with ethanol self-administration and reinforcement

Matthew M. Ford, Ph.D.[1,*], **Andrea M. Fretwell, M.S.**[1,2], **Allison M.J. Anacker, B.S.**[1], **John C. Crabbe, Ph.D.**[1,2], **Gregory P. Mark, Ph.D.**[1], and **Deborah A. Finn, Ph.D.**[1,2]

[1]Department of Behavioral Neuroscience, Oregon Health & Science University Portland, OR 97239

[2]Portland Alcohol Research Center, Veterans Affairs Medical Center, Portland, OR 97239

Abstract

Background—Several meta-analyses indicate that there is an inverse genetic correlation between ethanol preference drinking and ethanol withdrawal severity, but limited work has characterized ethanol consumption in one genetic animal model, the Withdrawal Seizure–Prone (WSP) and –Resistant (WSR) mouse lines selected for severe or mild ethanol withdrawal, respectively.

Methods—We determined whether line differences existed in: 1) operant self-administration of ethanol during sucrose fading and under different schedules of reinforcement, followed by extinction and reinstatement of responding with conditioned cues; and 2) home cage drinking of sweetened ethanol and the development of an alcohol deprivation effect (ADE).

Results—WSP-1 mice consumed more ethanol than WSR-1 mice under a fixed ratio (FR)-4 schedule as ethanol was faded into the sucrose solution, but this line difference dissipated as the sucrose was faded out to yield an unadulterated 10% v/v ethanol solution. In contrast, WSR-1 mice consumed more ethanol than WSP-1 mice when a schedule was imposed that procedurally separated appetitive and consummatory behaviors. After both lines achieved the extinction criterion, reinstatement was serially evaluated following oral ethanol priming, light cue presentation, and a combination of the two cues. The light cue produced maximal reinstatement of responding in WSP-1 mice, whereas the combined cue was required to produce maximal reinstatement of responding in WSR-1 mice. There was no line difference in the home cage consumption of a sweetened ethanol solution over a period of one month. Following a two week period of abstinence, neither line developed an ADE.

Conclusions—Although some line differences in ethanol self-administration and reinstatement were identified between WSP-1 and WSR-1 mice, the absence of consistent divergence suggests that the genes underlying these behaviors do not reliably overlap with those which govern withdrawal severity.

Keywords

alcohol; Withdrawal Seizure-Prone mice; Withdrawal Seizure-Resistant mice; extinction; reinstatement; conditioned cue; genetics; selected lines

[*]Corresponding Author: Matthew M. Ford, Ph.D., Department of Behavioral Neuroscience (L-470), Oregon Health & Science University, 3181 SW Sam Jackson Park Road, Portland, OR 97239-3098; Phone: 503-346-0050; Fax: 503-494-6877; fordma@ohsu.edu..

Introduction

Several studies suggest common genetic influences on high ethanol drinking and low withdrawal severity, and vice versa. Strong evidence comes from studies conducted in inbred mouse strains, as the correlation between inbred strain mean values on two phenotypes (e.g., withdrawal severity and ethanol intake) provides an index of the potential contribution of a common set of genes to the two traits (Crabbe et al., 1990). A meta-analysis previously tested the hypothesis that genetic overlap existed between ethanol consumption and ethanol withdrawal across several genetic populations of mice (Metten et al., 1998). This study reported a significant negative correlation between the g ethanol/kg body weight (g/kg) consumption of a 10% v/v ethanol (10E) solution under continuous access, two-bottle choice conditions and acute or chronic ethanol withdrawal severity as assessed by hourly handling-induced convulsion (HIC) scores. Significant negative correlations were reported in combined data sets from populations derived from inbred C57BL/6 and DBA/2 strains ($r = -0.39$; $P = 0.00003$) and from standard inbred strains including C57BL/6 and DBA/2 ($r = -0.42$; $P = 0.05$). Because each inbred strain is comprised of animals that are genetically identical, data can be accumulated over time and across laboratories. Subsequent inbred mouse strain comparisons reported a similar inverse relationship between consumption of 10E and chronic ethanol withdrawal severity ($r = -0.40$, 13 strains in common, Metten & Crabbe, 2005; $r = -0.65$, 6 strains in common, Yoneyama et al., 2008). Support for the inverse genetic relationship between ethanol consumption and withdrawal severity is more mixed with regard to selected lines derived from genetically heterogeneous stocks (Metten et al., 1998; Hitzemann et al., 2009). Heterogeneous stocks are developed initially by intercrossing either 8 or 4 inbred strains, which leads to the presence of as many as 8 possible alleles at each gene locus (as distinguished from a single allele at each gene in each inbred strain). Lines selectively bred from a heterogeneous stock differ markedly in their genetic composition at genes affecting the selected trait, and studies in selected lines are useful for determining correlated responses to selection. When a pair of lines selected to differ on one trait is found to differ significantly on a trait other than the one for which they were selected, this significant genetic correlation between the two traits implies the action of a common set of genes on the two phenotypes (discussed in Crabbe et al., 1990).

Early work in Withdrawal Seizure-Prone (WSP) and Withdrawal Seizure-Resistant (WSR) lines, which were selectively bred for severe (WSP) or mild (WSR) chronic ethanol withdrawal-induced HICs following 72 hrs of continuous ethanol vapor exposure (Crabbe et al., 1985), measured ethanol consumption in femals of both replicate pairs of WSP (WSP-1, WSP-2) and WSR (WSR-1, WSR-2) mice (Kosobud et al., 1988). In a standard two-bottle preference test (2.2% v/v ethanol (2E), 4.6E, and 10.0E versus water), WSR mice of both replicates drank more 2.2E and 4.6E than the respective WSP mice (Kosobud et al., 1988). At the highest concentration, only the WSR-2 line expressed an elevated level of consumption relative to the other lines. When ethanol concentrations (1–14.5%) were adjusted up or down every two days depending on the individual animal's intake, the selected lines exhibited very different patterns of intake. Only WSR-2 animals consumed consistently greater amounts of ethanol than WSP-2 mice. Thus, the selected line difference (WSR>WSP) was more consistent across conditions in replicate 2.

Additionally, a short term selection for chronic withdrawal severity (derived from the same genetically heterogeneous mice as the WSP/WSR lines) observed a negative association with 10E consumption ($r = -0.20$) as well as a trend towards a significant difference ($p = 0.09$) in intake between lines (Metten et al., 1998). More recent short term selection studies using a different heterogeneous stock (but one that also included C57BL/6 and DBA/2 mice

among the 4 inbred strains used to create it) determined that selection for 10E consumption produced lines with the predicted reciprocal relationship for acute ethanol withdrawal. However, a separate selection for acute withdrawal severity did not produce lines of mice that differed in 10E consumption (Hitzemann et al., 2009).

Collectively, the negative genetic relationship between preference drinking for 10E and withdrawal severity was less consistent in more complex genetic populations than in standard inbred strains. With this in mind, the purpose of the present studies was to examine multiple measures of ethanol self-administration and reinforcement in the WSP and WSR selected lines. The WSP and WSR selected lines exhibit greater than a 10-fold difference in chronic ethanol withdrawal severity (i.e., WSP >> WSR; Crabbe et al., 1985). While the withdrawal profiles and correlated responses have been reviewed (Kosobud & Crabbe, 1995; Metten & Crabbe, 1996), limited work has investigated whether the lines differed in measures of ethanol reward and reinforcement. In the single drinking study described above (Kosobud et al., 1988), the authors concluded that line differences in consumption might reflect differences in the relative sensitivity to the rewarding or aversive effects of ethanol. Related to this point, only WSP mice of both genetic replicates exhibited a conditioned place preference for the ethanol associated floor (Crabbe et al., 1992). Also, male WSP mice of both replicates exhibited a smaller magnitude of conditioned taste aversion relative to the WSR line on the first trial, but both lines developed taste aversions to a similar degree thereafter (Chester et al., 1998). An earlier operant self-administration study that used a post-prandial induction procedure was unable to establish an 8% v/v ethanol solution as a reliable reinforcer in either replicate of WSP or WSR mice (Barbera et al., 1994). This pattern of results across multiple procedures that are all presumed to assess ethanol's motivational or aversive properties suggests that separate genes contribute to differences in ethanol drinking, operant ethanol self-administration, ethanol-induced conditioned place preference, and ethanol-induced conditioned taste aversion in mice selectively bred for differences in chronic ethanol withdrawal severity (discussed in Chester et al., 1998).

A review of behavioral phenotypes in inbred mouse strains surmised that the genetic contributions to a given response to ethanol should not be extrapolated beyond the specific task conducted (Crawley et al., 1997). With regard to the WSP and WSR selected lines, the current work sought to extend the spectrum of observations regarding potential line differences in ethanol reinforcement and relapse by assessing a panel of related traits. We predicted that if some or all of the genetic determinants underlying ethanol withdrawal severity and reinforcement co-segregated during the selection process for the WSP and WSR lines (in a manner that was consistent with the negative genetic relationship between withdrawal severity and consumption of 10E), then WSR mice would demonstrate greater ethanol self-administration and reinforcement, more rapidly meet extinction criteria, and exhibit a more pronounced reinstatement of responding or relapse-induced drinking when compared to their WSP counterparts. Alternatively, WSP and WSR mice could differ in some, but not all of the reinforcement-related tests, indicating that the negative genetic correlation is more specifically related to withdrawal severity versus preference drinking. To test these predictions, parallel investigations were performed in separate groups of male WSP-1 and WSR-1 mice: 1) an operant self-administration procedure to identify potential line differences in initiation of ethanol self-administration via sucrose fading, maintenance of responding with various schedules of reinforcement, extinction, and reinstatement of responding with conditioned cues (stimulus light, oral ethanol prime, or both in combination); and 2) a home cage drinking procedure to measure consumption of a sweetened ethanol solution prior to, and following a two week period of abstinence, to determine whether line differences exist for consumption maintenance or in the expression of an alcohol deprivation effect (ADE). Since continuous access home cage drinking procedures often produce only marginal blood ethanol concentrations (BECs), it was

reasoned that implementation of a sucrose fading procedure in concert with operant ethanol self-administration, as initially pioneered by Samson (1986), would produces consistent responding for unadulterated ethanol and result in self-administered quantities of ethanol within a 30 min session that are believed to be pharmacologically active (e.g., Elmer et al., 1987; Roberts et al., 1999, 2000; Czachowski et al., 2002; Bäckström & Hyytiä, 2005; Tsiang & Janak, 2006; Ford et al., 2007a, 2007b, 2009).

Materials and Methods

Animals

The WSP and WSR lines were selected (in replicate) from a genetically heterogeneous population of HS/Ibg mice for their susceptibility to HICs during withdrawal from chronic ethanol vapor exposure, as previously described in detail (Crabbe et al., 1985). Both replicates of these selected lines were generated and bred in the Veterinary Medical Unit at the Veterans Affairs Medical Center (Portland, OR, USA). Naïve male mice from the replicate-1 line (i.e., WSP-1 and WSR-1) were used in the present studies, as sufficient numbers of female mice and replicate-2 line mice were unavailable at experiment onset. The animals were from selection generation 26 and filial generation 110, with ages ranging from 62 to 83 days old at experiment onset and no significant difference in age range between the selected lines. Mice were group housed and acclimated to a 12hr/12hr light/dark cycle (lights on at 0600 hrs), and were given *ad libitum* access to food and water in the home cage (except when noted below). The local Institutional Animal Care and Use Committee approved all procedures in accordance with the guidelines outlined in the Guide for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council of the National Academies, 2003).

Operant Conditioning Chambers and Equipment

Eight modular test chambers with stainless steel grid floors (interior dimensions 21.6 × 17.8 × 12.7 cm; Med-Associates Inc, St. Albans, VT) were used for these studies. Each chamber contained the following accessories: house light, two stimulus lights, two ultra-sensitive retractable levers (protruding 1 cm into chamber; positioned 2.2 cm above grid floor), and a retractable sipper apparatus. A custom-built 10-ml graduated pipette with double ball bearing metal drinking tube was mounted on each retractable sipper, and permitted volume of consumption to be measured to the nearest 0.05 ml. Further, to monitor the lick patterns, contact lickometers were interfaced between the retractable sippers, the steel grid floors (via quick disconnect harnesses), and an IBM-compatible computer running the MED-PC IV software package (Med Associates Inc.). Cumulative records of ongoing licks and lever presses were recorded during the daily sessions. On one wall of the chamber the house light was mounted near the chamber ceiling. The opposing wall contained the levers positioned 11 cm apart, the stimulus lights mounted above each lever, and an access port for the retractable sipper located between the levers. Each conditioning chamber was positioned within a sound-attenuating cubicle (61 × 38 × 33 cm; Fisher Custom Woodworking, Portland, OR). An exhaust fan provided ventilation to each chamber.

Lever Acquisition Training and Sucrose Fading

At the start of the operant study, WSP-1 (n=16) and WSR-1 (n=19) mice were housed two per cage, and body weights (25.7 ± 0.6 grams) did not differ between the lines. Mice were trained to respond on an active lever under a fixed ratio 1 (FR1) schedule for periodic access to a retractable sipper tube containing a 10% w/v sucrose solution (10S). Although the active lever remained fixed throughout the experiment, its location was counterbalanced between the left and right sides across all subjects to account for the possibility of a native side preference. Upon successful completion of each FR the house light was extinguished as the

stimulus light located above the active lever was activated for 5-sec and the retractable sipper was extended into the chamber for 30-sec. Following the 5-sec light cue, the house light was re-illuminated. Additional responses on the active lever during the light cue and sipper presentation sequence were recorded, but had no scheduled consequences. Reponses on the inactive lever were similarly recorded, and were always without experimental consequence. Mice were water restricted for 16 hrs prior to each of the 8 training sessions to enhance motivational drive, but thereafter were given continuous water access in the home cage. The overall goal of training was for the mice to exhibit ≥ ten 30-sec sipper presentations within a 30-min session. This behavioral criterion was achieved by the majority of WSP-1 and WSR-1 mice within 6 sessions.

After lever training, the reinforcement schedule was incrementally increased from FR1 to FR4 over two weeks. Once mice were stably responding on the FR4 schedule, a modified sucrose fading procedure was implemented, as previously described in detail (Finn et al., 2008; Ford et al., 2009). In brief, ethanol was gradually added to the 10S solution up to 10% v/v (10E), and then sucrose was faded out to yield 10E alone. Three to five sessions were run with each solution in the following sequence: 10S/2E, 10S/5E, 10S/10E, 5S/10E, 2S/10E, and 10E. Ethanol (ethyl alcohol; 200 proof; Pharmco Products, Inc., Brookfield, CT) and sucrose (Sigma-Aldrich Company, St. Louis, MO) solutions were constituted in tap water.

Operant Schedule Manipulation

After a week of responding on a FR4 schedule for 10E, reinforcement schedule manipulations were undertaken in the selected lines. In brief, the appetitive and consummatory phases of operant self-administration were procedurally separated. In the appetitive phase, mice completed a single response requirement of 8 presses on the active lever (termed RR8 schedule) to obtain 30-min of uninterrupted access to 10E (i.e., the consummatory phase). This procedure was previously described in detail as the `sipper' procedure in the rat (Samson et al., 1998, 2000), and was replicated in a mouse model in recent work from our laboratory (Finn et al., 2008; Ford et al., 2007a, 2007b, 2009). It offers the distinct advantage of a drinking contingency in which the animal can regulate its own rate of consumption (and onset of ethanol's pharmacological effects) throughout the consummatory phase of the session without the interference of intermittent access periods as experienced under a FR schedule. It also explicitly prevents ethanol's pharmacological effects from influencing performance of the operant response during the appetitive phase. Although the response requirement was subsequently increased to 12 (i.e., RR12) in the current work, approximately half the mice from each selected line were unable to fulfill this response demand on a consistent basis. Consequently, all mice were subsequently maintained on a FR4 schedule for an additional 6 weeks prior to extinction onset.

Extinction and Reinstatement Tests

Due to inconsistent responding on the FR4 schedule in some mice of each selected line after the schedule manipulation, only a subset of WSP (n = 11) and WSR (n = 10) mice were examined for extinction and subsequent reinstatement. Specifically, mice that were unable to consistently earn ≥5 reinforcers per session and consume detectable amounts (≥ 0.05 ml) of the 10E solution were excluded from further study. Under extinction conditions, responding on either the previously active lever or the inactive lever had no scheduled consequences throughout 30-min sessions. The overall goal was to reduce responding on the active lever to ≤ 30% of the baseline response levels, consistent with previously published extinction criterion in mice (Sanchis-Segura et al., 2006). This was accomplished in both selected lines of mice within a 3-week period. Once extinction was stably expressed (three consecutive sessions with ≤ 20% variability in active lever responses), the mice were exposed to

conditioned cue tests intended to elicit reinstatement of responding on the previously assigned active lever. Based on earlier work (Bäckström & Hyytiä, 2004; Samson & Chappell, 2002), an oral prime was provided in which a sipper containing 10E was presented after a single press on the previously active lever until 10 licks (i.e., contacts) were generated. Upon completion of 10 licks, the sipper was retracted for the remainder of the 30-min session. Under a second reinstatement test condition, mice were presented with a stimulus light, as reported earlier in detail (Bäckström & Hyytiä, 2004; Finn et al., 2008). In brief, a stimulus light positioned above the previously active lever was illuminated while the house light was concurrently extinguished for 5-sec on a FR1 schedule. Active lever responses that occurred during stimulus light activation periods were recorded, but had no additive effect on the length of cue presentation. Lastly, a combination of the oral prime and the stimulus light were tested for their combined influence on reinstatement. Stable baseline extinction levels of responding were re-established with a minimum of 7 sessions interspersed between reinstatement tests.

Home Cage Two-Bottle Preference Drinking of Sweetened Ethanol

A separate cohort of male WSP-1 (n=20) and WSR-1 (n=18) mice was tested, and body weights at the start of the study (23.6 ± 0.2 grams) were not different between the lines. Animals were individually housed with water and food always freely available. The procedure was a modification of that described by Yoneyama et al. (2008). Mice were provided continuous access (i.e., 24 hr) to two inverted 25 ml glass graduated cylinders with metal sippers placed on the stainless steel cage top. Food was distributed near both cylinders to avoid food-associated side preferences. Initially, mice were acclimated with both cylinders containing tap water. A modified sucrose fading procedure was then used to slowly introduce ethanol to a 5% w/v sucrose (5S) solution constituted in tap water. First, one water cylinder was replaced with one containing 5S, followed by the addition of increasing concentrations of ethanol (2E, 5E, then 10E) to the 5S solution. Access to 5S, 5S/2E, and 5S/5E solutions were provided for 4 days each, followed by access to the 5S/10E solution for an additional 30 days. Throughout the experiment, cylinder positions were alternated every 2nd day. At each concentration change, fresh fluids were provided and body weights were measured. During 5S/10E access, body weights were measured every 4 days, and fresh fluids were provided every 7 days. Fluid intake (to the nearest 0.2 ml) was recorded by measuring the level of the meniscus on the graduated cylinder each day. Ethanol and water cylinders placed on two empty cages allowed for the measurement of leakage and evaporation. The average volume depleted from these “control” cylinders was subtracted each day from the raw values to yield “corrected” drinking volumes.

Alcohol Deprivation Effect (ADE)

Following 30 days of 5S/10E consumption, WSP and WSR mice underwent a 2 week period of abstinence, during which time the 5S/10E solution was replaced with a cylinder containing tap water. After 2 weeks, access to the 5S/10E solution was restored for 4 days. Volume intakes were recorded as described above.

Data Analysis

For the operant studies, cumulative records of responding and licking were monitored using MED-PC IV software (Med Associates). The dependent variables analyzed included active lever responses, inactive lever responses, number of reinforcers (i.e., sipper presentations), volume consumed (ml), ethanol dose consumed (g/kg), and sipper contacts (i.e., licks). Twoway repeated measures ANOVAs were conducted to identify significant main effects of selected line (WSP-1 vs. WSR-1) and the repeated factor of sucrose fading solution, reinforcement schedule, extinction session, or reinstatement cue condition. When appropriate, pair-wise differences were evaluated with the Tukey's multiple comparisons

procedure. For the home cage drinking studies, the dependent variables were ethanol/sucrose dose consumed, volume of ethanol/sucrose or water consumed, total volume, body weight and ethanol/sucrose preference ratio (calculated as the volume of ethanol/sucrose consumed divided by the total volume consumed). ANOVA assessed line (WSP-1 vs. WSR-1) and concentration/day (repeated measure) effects on the dependent variables. Significant interactions were pursued with one-way ANOVAs at each concentration and post-hoc tests. In all cases, data are presented as the mean ± SEM. The level of significance was set at $P \leq 0.05$. Statistical analyses were conducted with the computer program SYSTAT (version 11, SYSTAT Software, Inc., Richmond, CA).

Results

Sucrose Fading and Initiation of Operant Ethanol Self-Administration

Appetitive and consummatory measures were monitored throughout the fading procedure to assess selected line differences with the various reinforcer solutions offered. Although a 2-way repeated measures ANOVA revealed no effect of either selected line or solution for active lever responding (Figure 1A), a notable trend towards a line x solution interaction was observed [$F(6,198) = 2.08$; $P = 0.06$]. A parallel analysis of reinforcers earned (on a FR schedule) discovered a significant main effect of solution [$F(6,198) = 4.92$; $P < 0.001$] and a significant line x solution interaction [$F(6,198) = 3.03$; $P < 0.01$]. However, there were no significant pair-wise differences in the number of reinforcers obtained for any solution offered (data not shown). In contrast, a 2-way repeated measures ANOVA of inactive lever responses throughout sucrose fading found main effects of selected line [$F(1,33) = 11.08$; $P < 0.01$] and solution [$F(6,198) = 10.81$; $P < 0.001$], as well as a line x solution interaction [$F(6,198) = 2.74$; $P < 0.05$]. WSR mice demonstrated consistently higher levels of inactive lever responding when compared to WSP mice (Figure 1B), especially during the presentation of sucrose-containing solutions ($P < 0.01$ for 10S and 10S/10E; $P < 0.05$ for all others).

In contrast to the dependent variables of appetitive responding, there were pronounced selected line differences in consummatory measures throughout sucrose fading. A 2-way repeated measures ANOVA detected significant main effects of line [$F(1,33) = 6.60$; $P < 0.05$], solution [$F(6,198) = 45.68$; $P < 0.001$], and a line x solution interaction [$F(6,198) = 8.01$; $P < 0.001$] for volume consumed. Step-wise increases in ethanol concentration to the sucrose training solution (i.e., 10S) resulted in a steady decline of volume intake in WSR mice, whereas this same process initially led to enhanced volume intake in WSP mice (Figure 2A). WSP mice consumed significantly more volume than WSR mice when the 10S/2E ($P < 0.05$), 10S/5E ($P < 0.001$), 10S/10E ($P < 0.001$), and 5S/10E ($P < 0.05$) solutions were presented. Consistent with intake volume profiles during sucrose fading, a separate 2-way repeated measures ANOVA determined a significant main effect of line [$F(1,33) = 11.84$; $P < 0.01$] and solution [$F(5,165) = 50.53$; $P < 0.001$], as well as a significant line x solution interaction [$F(6,165) = 13.30$; $P < 0.001$] for the ethanol dose (g/kg) consumed. Specifically, WSP mice self-administered significantly higher ethanol doses than WSR mice during access to the 10S/2E ($P < 0.01$), 10S/5E ($P < 0.001$), 10S/10E ($P < 0.001$), and 5S/10E ($P < 0.01$) solutions (Figure 2B). Most notable was the 2.2-fold greater ethanol dose consumed in WSP versus WSR mice with the 10S/10E solution.

Reinforcement Schedule and Ethanol Self-Administration

Once stable self-administration of 10E was established on a FR4 schedule, mice were exposed to a reinforcement schedule manipulation. A 2-way repeated measures ANOVA determined a significant effect of schedule [$F(1,33) = 14.81$; $P < 0.01$] and a significant line x schedule interaction [$F(1,33) = 6.57$; $P < 0.05$] for ethanol intake. Subsequent analyses

revealed that transition from a FR4 to a RR8 schedule led to a significant increase in the ethanol dose consumed by WSR mice ($P < 0.001$), but the schedule transition exhibited no effect in WSP mice (Figure 3). Further, WSR mice self-administering ethanol on a RR8 also consumed 76% more ethanol than WSP mice on this schedule ($P < 0.05$).

Extinction of Ethanol-Reinforced Responding

Responding on the active and inactive levers under the FR4 schedule prior to extinction onset was 52 ± 8 and 6 ± 1 in WSP mice and 58 ± 10 and 10 ± 2 in WSR mice, respectively. Concomitant ethanol intake at this time was 0.53 ± 0.16 g/kg in WSP and 0.44 ± 0.11 g/kg in WSR mice. A 2-way repeated measures ANOVA of the extinction time course (i.e., extinction sessions 1–21) revealed that there was neither an effect of selected line nor a line x session interaction on active lever responding (Figure 4A). However, there was a significant main effect of extinction session [$F(21,397) = 20.07$; $P < 0.001$] for this measure. Specifically, active lever responding on extinction session 2 ($P < 0.01$) and sessions 3–21 (all $Ps < 0.001$) were significantly reduced when compared to baseline responding. Consistent with the absence of a line effect on active lever responding (and despite the appearance of greater responding by WSR than by WSP mice on Days 4–12, see Figure 4A), an area under the curve analysis of active lever responses during extinction sessions 1–21 also revealed no significant line difference (data not shown). By extinction session 21, responding on the previously active lever in WSP and WSR mice decreased to 21 ± 8% and 26 ± 6% of their pre-extinction baseline, respectively.

With regard to inactive lever responding, a 2-way repeated measures ANOVA found no effect of selected line, but a significant main effect of extinction session [$F(21,397) = 3.48$; $P < 0.001$] and a line x session interaction [$F(21,397) = 2.53$; $P < 0.001$]. WSR mice exhibited significantly greater inactive lever responses than WSP mice (session 5, $P < 0.05$; sessions 6–7 and 11, $P < 0.01$; Figure 4B). A significant increase in inactive lever responses was also determined for WSR mice on session 11, when compared to its within-group baseline prior to extinction onset.

Reinstatement of Ethanol-Reinforced Responding

Due to selected line differences in the baseline level of responding at extinction (9 ± 1 versus 16 ± 4 responses on the previously active lever in WSP and WSR mice, respectively; Figure 5A) and our *a priori* prediction that the reinstatement tests would differ between the lines, separate 1-way repeated measures ANOVAs were conducted for the analysis of responding during reinstatement tests. Significant main effects of cue manipulations on active lever responding in WSP [$F(3,30) = 9.54$; $P < 0.001$] and WSR [$F(3,27) = 6.48$; $P < 0.01$] mice were determined. In WSP mice, non-reinforced active lever responding was significantly elevated following tests with the light cue ($P < 0.01$) and the combined oral prime + light cue ($P < 0.001$), when compared to baseline extinction responding (Figure 5A). Furthermore, the combined cue test in WSP mice also significantly increased active lever responding over levels observed subsequent to the oral prime alone ($P < 0.01$). In WSR mice only the combined cue generated significantly greater levels of previously active lever responding versus within-group baseline extinction values ($P < 0.01$; Figure 5A). However, the combined cue test in WSR mice was also significantly greater than within group levels of responding subsequent to challenge with the oral prime ($P < 0.01$) and tended to be higher than that produced by the light cue ($P < 0.10$). Reinstatement tests were without effect on inactive lever presses in either WSP or WSR mice (Figure 5B).

Home Cage Preference Drinking and ADE

Daily 5S consumption (mls) was significantly higher in WSR versus WSP mice [$F(1,32) = 4.13$, $P = 0.05$] and increased significantly across days [$F(3,96) = 6.28$, $P = 0.001$] (data not

shown). The interaction between line and time was not significant, consistent with the daily higher sucrose intake in the WSR line. Average 5S consumption across the 4 days was 5.35 ± 0.37 mls for WSP-1 mice and was 6.33 ± 0.30 mls for WSR-1 mice. Consistent with the amount of 5S consumed, preference for the 5S solution was significantly higher in WSR versus WSP mice [$F(1,33) = 5.96$, $P < 0.05$] (data not shown). Average preference for the 5S solution was 0.94 ± 0.02 for WSR and 0.83 ± 0.04 for WSP mice. There was no line difference in total fluid intake (data not shown).

The addition of ethanol to the sucrose solution eliminated the line difference in intake, as there was no significant main effect of line for the ethanol dose consumed from the 5S/2E, 5S/5E or 5S/10E solutions (Figure 6). Fluctuations across time were observed for intake of the 5S/2E [$F(3,108) = 40.72$, $P < 0.001$] and 5S/10E [$F(29,1044) = 10.37$, $P < 0.001$] solutions, and the interaction between line and time was significant only for consumption of the 5S/10E solution [$F(29,1044) = 1.57$, $P < 0.05$]. This interaction appeared to be due to a transient line difference in ethanol intake. Specifically, the ethanol dose consumed from the 5S/10E solution was significantly higher in WSP versus WSR mice on days 15 and 24 ($P < 0.05$) and tended to be higher on days 13 and 26 ($P < 0.09$). Similar results were observed in the analysis of preference ratio for the 3 solutions (data not shown). There was neither a line difference nor a time x line interaction in total fluid intake for any solution offered (data not shown).

To analyze ADE, the last 4 days of ethanol intake prior to the 2 week period of abstinence were averaged as a pre-ADE baseline (days 39 – 42) and compared with the 4 days of intake following abstinence (Figure 6, inset). While WSP and WSR mice did not differ in the ethanol dose consumed, there was a main effect of time [$F(4,144) = 7.04$, $P < 0.001$] and a line × time interaction [$F(4,144) = 2.94$, $P < 0.05$]. The significant interaction appeared to be due to the fact that ethanol intake was persistently suppressed in WSR mice across all 4 days of measurement ($P < 0.01$ on day 68; $P < 0.001$ on days 69 – 71), but it was only suppressed in WSP mice on the final 2 days of measurement ($P < 0.05$ on day 70; $P < 0.01$ on day 71) when compared to respective selected line baselines. Thus, neither line exhibited an ADE after a 2 week period of abstinence.

Analysis of preference ratio yielded similar results to that for ethanol dose (data not shown). The pre-ADE preference ratio was 0.22 and 0.20 for WSR and WSP mice, respectively. Following the 2 week period of abstinence, preference on days 68 – 71 ranged from 0.13 – 0.18 for WSR mice. Preference was decreased in WSP mice only on days 70 and 71 (0.16 – 0.17). Total fluid intake was not significantly altered (data not shown).

Discussion

The purpose of the present series of experiments was to examine in greater detail several different measures of ethanol reinforcement and relapse in WSP and WSR mice. Hence, multiple phenotypes were investigated in an attempt to determine whether the segregation of alleles related to ethanol withdrawal severity conferred line differences in a subset of behaviors related to ethanol reinforcement, more generally across all measures, or whether withdrawal severity and reinforcement appeared to be genetically independent. In general, the present results indicate that there were subtle line differences in several aspects of ethanol self-administration, which depended on the reinforcer solution presented and the reinforcement schedule imposed. The results are summarized in Table 1.

The first set of studies examined operant ethanol self-administration under different reinforcement schedules. Importantly, we were able to successfully train all WSR mice (19/19) and most WSP mice (13/16) to respond for access to ethanol on a FR schedule of

reinforcement. When responding on a FR4 schedule of reinforcement, WSR mice exhibited a higher overall level of responding on both levers throughout the sucrose fading procedure than the WSP mice (Figure 1). This result is consistent with early work demonstrating that operant responding maintained by a sucrose-milk solution was significantly higher in male WSR-2 than in WSP-2 mice (Balster et al., 1993).

While the responding maintained by sucrose/ethanol combinations was unaltered in WSR-1 mice as the ethanol was introduced and subsequently increased in a stepwise fashion (Figure 1A), the introduction of ethanol into the sucrose solution did alter responding in the WSP-1 mice. The near significant line × solution interaction on active lever responding ($P=0.06$) and the significant line × solution interaction for the number of reinforcers was most likely attributable to the changes in reinforcers earned by WSP-1 mice as the ethanol content of the solution was altered. It should be noted that the current procedure implemented a procedure in which the quantity of the acquired reinforcer was not fixed (i.e., as is the case with a dipper cup or a set infusion volume). Instead, mice were presented with 30-sec sipper access periods during which the rate and quantity of consumption was entirely self-regulated by the animal. Therefore, while one interpretation of the small changes in responding upon introduction of ethanol could be that ethanol exhibited little reinforcing efficacy, an equally plausible explanation could be that mice simply consumed more or less ethanol per reinforcer earned. The waxing and waning of ethanol intake in WSP-1 mice across the sucrose/ethanol solutions offered (Figure 2) would favor the latter interpretation, and would suggest that WSP-1 mice were more attentive to changes in the sucrose and ethanol content of the solutions presented during fading. However, the lines did not differ in their self-administration (volume or dose) of unadulterated 10E.

There are several possible explanations for the line differences in the initiation phase of operant 10E self-administration. First, WSP mice may find sweetened ethanol solutions more rewarding when responding in an operant setting, as they self-administered doses of ethanol that are presumed to be pharmacologically active (e.g., 1.2 – 2.0 g/kg with intake of the 10S/5E, 10S/10E, and 5S/10E solutions). Consistent with this idea, WSP (but not WSR) mice of both replicates exhibited a conditioned place preference to ethanol (Crabbe et al., 1992), which suggests that they are more sensitive to the rewarding effects of ethanol in the place conditioning procedure. Second, the higher intake in WSP versus WSR mice could reflect a line difference in overall fluid intake. While an earlier home-cage drinking study reported that overall fluid intake was consistently higher in WSP than in WSR mice (Kosobud et al., 1988) there was no line difference in total fluid intake during any phase of our home cage drinking study. Third, WSR mice exhibited a gradual decline in ethanol intake throughout the sucrose fading procedure (i.e., beginning with the initial introduction of 2E). It is possible that WSR-1 mice are more sensitive than WSP-1 mice to ethanol's chemosensory properties, which were revealed in a limited access setting. This suggestion also would be consistent with the transient development of a greater conditioned taste aversion to ethanol in both replicates of WSR versus WSP mice (Chester et al., 1998), and would suggest that WSR -1 may be slightly more sensitive than WSP-1 mice to ethanol's aversive effects. Another possibility is that WSR mice simply did not consume ethanol from the sipper upon each presentation when responding on the FR4 schedule. We recently observed that C57BL/6 mice, which exhibit high ethanol preference and self-administration, only approached the sipper approximately 50% of the time when they were responding on a FR4 schedule (Ford et al., 2007a). A preliminary examination of sipper contacts during the initial sipper presentation of 10E in the current study suggested that both WSP and WSR mice were not consistently sampling during the 30 sec of sipper access. Thus, inconsistent consumption from the sipper on a FR4 schedule may partially contribute to the decreased consumption throughout sucrose fading in WSR mice.

The current understanding of conditioned responding for ethanol under a non-food restricted condition suggests that ethanol is a weak behavioral reinforcer in mice. As described by Ford et al. (2007a), oral self-administration of ethanol supports a much lower response rate than that observed for intravenous infusions of psychostimulants such as cocaine (e.g., Little, 2000). The difficulty in assessing the reinforcing properties of orally administered ethanol in animal models is due in part to the delayed onset of pharmacological effects (Meisch, 2001) and the quantity of fluid volume necessary to support a behaviorally-relevant dose. Both of these procedural challenges are problematic for operant conditioning procedures that incorporate a FR schedule of responding. This potential difficulty may be overcome with recent modifications in operant conditioning procedures in the rat, where a "sipper" model procedurally separated the appetitive and consummatory phases of ethanol self-administration (Samson et al. 1998, 2000). By providing continuous access to ethanol (typically 30 min) following the completion of a single response requirement (RR), this experimental procedure permits the animal to regulate its own consumption (and hence the onset of ethanol pharmacology) rather than having intake dictated by intermittent access following repeated response demands that are associated with a FR schedule of reinforcement. It also removes the possibility that intoxication will gradually interfere with FR performance during later parts of the session. With this in mind, we determined whether the two schedules of reinforcement would detect line differences in the maintenance of ethanol self-administration.

An examination of the maintenance phase of ethanol self-administration revealed that there was no line difference in 10E intake when mice were responding on a FR4 schedule. However, when a schedule was imposed (RR8) that allowed mice to regulate their own rate of 10E consumption for 30 uninterrupted minutes, WSR mice significantly increased their 10E intake by 76% over the intake in WSP mice (Figure 3). Notably, the enhancement of drinking in WSR mice following a schedule manipulation from FR4 to RR8 is similar to our recent results in male C57BL/6 mice, where transition from a FR4 to a RR4 schedule of reinforcement was associated with a doubling of the ethanol dose consumed during a 30 min session (Ford et al., 2007a). Although BECs were not measured, WSR mice were consuming a dose of ethanol (0.8 g/kg) within 30 minutes that has previously been shown to produce BECs ≥ 50 mg/dl (or 10.9 mM; e.g., Elmer et al., 1987;Czachowski et al., 2002). As reviewed by Spanagel (2009), the subjective effects of ethanol can be detected by social drinkers experiencing BECs of 30 mg/dl and the function of ion channels and receptors can be inhibited by concentrations of ethanol in the range of 10 – 20 mM. These findings suggest that WSR mice consumed a pharmacologically active dose of ethanol and that they did find ethanol to be reinforcing when responding on the RR schedule. Additionally, comparison of the results on the FR4 versus RR8 schedule would suggest that there are line differences in the manner by which WSP and WSR self-regulate their 10E intake.

The present studies also utilized the reinstatement procedure, where non-contingent exposure to drug, non-drug stimuli or stress after extinction can cause an animal to resume a previous drug-reinforced behavior, to determine whether there were line differences in this model of drug craving (see reviews by Lê & Shaham, 2002; Shaham et al., 2003; Stewart, 2003; Epstein et al., 2006). When responding under non-reinforced conditions, both lines achieved the extinction criterion of ≤ 30% of baseline responding prior to the reinstatement tests (even though overall responding was higher in WSR than WSP mice). Importantly, both selected lines exhibited significant levels of reinstatement following either the presentation of the light cue (WSP only) or the combination of light cue and oral ethanol priming (WSP and WSR) when compared to their respective baselines (Figure 5). These findings are consistent with recent work, which demonstrated that conditioned stimuli (CS) and contexts could reinstate ethanol seeking behavior in C57BL/6 mice (Tsiang & Janak, 2006; Finn et al., 2008).

In earlier work with rats (Bäckström & Hyytiä, 2004), an oral ethanol prime paired with a CS (i.e., discriminating odor plus light cue), robustly reinstated ethanol-seeking behavior to levels greater than that seen with the CS alone (approximately 60% versus 40% of pre-extinction baseline levels of responding, respectively). We chose to use an oral ethanol prime, as we reasoned that it would be a more potent stimulus (i.e., it incorporated smell, taste and pharmacological onset just as when the mice were self-administering during reinforced sessions) than an ethanol injection. However, the oral ethanol prime did not promote reinstatement in either WSP or WSR mice, and it only tended to increase non-reinforced responding when paired with the light CS in WSR mice. These results are not entirely surprising, given the difficulty in promoting ethanol seeking with an ethanol priming injection (e.g., Lê & Shaham, 2002; Nie & Janak, 2003; Finn et al., 2008) and the potential aversive chemosensory properties of ethanol in WSP and WSR mice. Nonetheless, pairing the light CS with an oral ethanol prime produced a non-significant increase in non-reinforced responding on the active lever over that following light cue exposure only in WSR mice, an effect that was comparable in magnitude to that previously reported in rats (65% versus 43% of pre-extinction response levels; Bäckström & Hyytiä, 2004). The absence of a similar effect in WSP mice may be explained by the near-maximal response of this selected line to the light CS alone (65% of pre-extinction responding). In general, the present results suggest that non-drug stimuli are highly effective at promoting ethanol-seeking in WSP and WSR mice, but that there may be line differences in the relative salience of the CS components.

Home cage drinking of a sweetened ethanol solution (5S/10E) did not differ in male WSP-1 and WSR-1 mice. This result is similar to the early report by Kosobud et al. (1988), where female WSP-1 and WSR-1 mice did not differ in their consumption of 10E at the end of a preference test, although the WSR-1 mice drank more at lower concentrations. It was anticipated that intake of a sweetened ethanol solution would be higher than for unsweetened ethanol, but a comparison of the present work with that of Kosobud et al. (1988) suggests that this may not be the case (for both studies: ethanol dose ~ 3.5 g/kg; ethanol preference ratio ≤ 0.2). A direct, contemporaneous comparison of intakes of sweetened versus unsweetened ethanol, each versus water, would be needed to confirm the effect of sweetening. However, across 22 inbred mouse strains, there was no strain for which addition of sweetener did not significantly increase ethanol intake (Yoneyama et al, 2008). The lines did, however, differ in their intake of, and preference for, the 5S solution. Specifically, sucrose intake and preference was significantly higher in WSR versus WSP mice. Thus, the lack of line difference in intake of the sweetened ethanol solutions suggests that chemosensory factors such as the taste and smell of alcohol might be more important determinants of sweetened ethanol intake than an innate line difference in preference for sucrose.

A comparison of the operant and home cage drinking results revealed that the significant line difference in operant self-administration of most of the sweetened ethanol solutions did not correspond with the home cage drinking results where no overall line differences in consumption of 5S/10E were detected. The different results for intake of the 5S/10E solution in operant versus home-cage drinking procedures could be due to line differences in patterns of consumption that are only revealed under limited access conditions. This suggestion is consistent with the recent examination of 24 hr versus 2 hr limited access home cage drinking in knockout (KO) versus wild type (WT) mice with a null mutation in the 5α-reductase type 1 gene (*Srd5a1*). Specifically, 24 hr 10E intake was significantly decreased in KO versus WT male mice, but 2 hr 10E intake was significantly increased in KO versus WT male mice (Nickel et al., 2006).

We also measured the “alcohol deprivation effect” or ADE, which refers to the transient increase in ethanol consumption that occurs after abstinence, in WSP and WSR mice following over 30 days of consumption of sweetened ethanol. As described by Sanchis-Segura and Spanagel (2006), the ADE is considered a model of relapse, since the procedure allows the animal to self-administer alcohol after a period of protracted abstinence. However, it is unclear whether the ethanol itself is acting as a cue (i.e., taste and smell), as a priming stimulus, or both with regard to facilitating the transient increase in ethanol intake following deprivation. The present results indicate that neither WSP-1 nor WSR-1 mice developed an ADE, when the sweetened alcohol solution was presented after a 2 week period of abstinence. Ethanol intake was decreased in both lines, but the suppression was more persistent in WSR (4 days) than in WSP (2 days) mice. This result was not entirely surprising, since the increase in ethanol consumption is typically transient and requires repeated deprivations to extend beyond a single day of increased drinking (e.g., Rodd-Hendricks et al., 2000; Bell et al., 2004; discussed in more detail in Sanchis-Segura & Spanagel, 2006). Another consideration is that the emergence of an ADE appears to be more robust and consistent in rat models, but it appears to be more sensitive to procedural variables and inconsistent in mouse genotypes. Specifically, a single 2-week deprivation (similar to that used in the present study) decreased daily ethanol intake in C57BL/6J mice (Melendez et al., 2006), consistent with the present findings. A 6-day deprivation period with a single alcohol re-exposure per week was required to generate an ADE in C57BL/6J mice, but the emergence of this effect (week 1 vs. week 6) was quite variable between studies (Melendez et al., 2006). Additionally, a 4-day deprivation produced varying effects in C57 substrains (ADE in C57BL/6NCr1, no effect in C57BL/6J), with evidence that repeated deprivations produced decreases in post-deprivation ethanol intake (Khisti et al., 2006). Future studies will need to examine hourly intake in an attempt to capture the transient rise in drinking following abstinence and also employ multiple deprivations and procedural variations to determine whether an ADE would emerge differentially in WSP and WSR mice.

Two caveats regarding the current studies should be mentioned. Although the WSP/WSR replicate lines were derived from the same genetically heterogeneous stock, each pair of lines (WSP-1/WSR-1 versus WSP-2/WSR-2) was developed from a different set of 9 original mating pairs. Thus, there are many alleles that were excluded from these relatively small starting populations at the outset (this is called the “founder effect”). In consequence, the genes selected for in WSP-1 mice, for example, may be somewhat different from those selected for in the WSP-2 mice, even though the selection trait was identical. Thus, the strongest evidence for or against a correlated response to selection would be to find that the trait differed across both replicate pairs of WSP versus WSR selected lines. The second replicate lines were unavailable for the current studies. Further studies in the second replicate pair of lines could strengthen, or alter, the results we report here (for discussion, see Crabbe et al.,1990). The second point relates to comparisons between these findings and reports from Kosobud and colleagues (1988). Mice had been selected for 17 or 19 generations when the earlier data were collected, but selection continued for another several generations (until S25). Continued selection undoubtedly produced genetic changes in all 4 selected lines. Thereafter, mice were randomly mated for an additional 85 generations (within each selected lines), and additional genetic change occurred during those years. The changes in gene frequency since S25 are due to the necessarily small population sizes, and are termed genetic drift. Thus, it is not unexpected to find differences between contemporary and archival data. That said, we did not test here two-bottle preference for ethanol versus water with unlimited access, so strict comparisons between these data and Kosobud and colleagues (1988) are not possible.

In summary, the present series of experiments indicate that selected line differences in ethanol self-administration behavior depended on the qualities of the ethanol solution offered and the reinforcement schedule imposed. The strongest evidence supporting the negative genetic relationship between ethanol intake and withdrawal severity was provided by operant self-administration under an RR schedule of reinforcement. When animals were able to self-regulate their 10E consumption during 30 min of continuous ethanol access, ethanol intake was significantly higher in WSR-1 versus WSP-1 mice. However, the overall pattern of behavioral phenotypes examined reveal the absence of a consistent line difference in responses that reflect the rewarding or aversive effects of ethanol in WSP-1 and WSR-1 mice. Thus, the genes underlying ethanol self-administration and reinstatement behaviors do not consistently overlap with those which govern withdrawal severity in the WSP-1 and WSR-1 selected lines.

Acknowledgments

Supported by Veterans Affairs Merit Awards (to DAF and JCC), a Research Career Scientist Award (to JCC), and NIH grants AA16849 (to MMF), AA12439 (to DAF), and AA10760 and AA13519 (to JCC).

References

Bäckström P, Hyytiä P. Ionotropic glutamate receptor antagonists modulate cue-induced reinstatement of ethanol-seeking behavior. Alcohol Clin Exp Res. 2004; 28:558–565. [PubMed: 15100606]

Bäckström P, Hyytiä P. Suppression of alcohol self-administration and cue-induced reinstatement of alcohol seeking by the mGlu2/3 receptor agonist LY379268 and the mGlu8 receptor agonist (S)-3,4-DCPG. Eur J Pharmacol. 2005; 528:110–118. [PubMed: 16324694]

Balster RL, Wiley JL, Tokarz ME, Tabakoff B. Effects of ethanol and NMDA antagonists on operant behavior in ethanol Withdrawal Seizure–Prone and –Resistant mice. Behav Pharmacol. 1993; 4:107–113. [PubMed: 11224177]

Barbera TJ, Baca K, George FR. Regulation of operant ethanol-reinforced behavior is genetically independent of regulation of withdrawal severity in WSP and WSR mice. Alcohol. 1994; 11:371–377. [PubMed: 7818794]

Bell RL, Rodd ZA, Boutwell CL, Hsu CC, Lumeng L, Murphy JM, Li T-K, McBride WJ. Effects of long-term episodic access to ethanol on the expression of an alcohol deprivation effect in low alcohol-consuming rats. Alcohol Clin Exp Res. 2004; 28:1867–1874. [PubMed: 15608603]

Chester JA, Risinger FO, Cunningham CL. Ethanol reward and aversion in mice bred for sensitivity to ethanol withdrawal. Alcohol Clin Exp Res. 1998; 22:468–473. [PubMed: 9581655]

Crabbe JC, Kosobud A, Young ER, Tam BR, McSwigan JD. Bidirectional selection for susceptibility to ethanol withdrawal seizures in *Mus musculus*. Behav Genet. 1985; 15:521–536. [PubMed: 4096679]

Crabbe JC, Phillips TJ, Cunningham CL, Belknap JK. Genetic determinants of ethanol reinforcement. Ann NY Acad Sci. 1992; 654:302–310. [PubMed: 1632589]

Crabbe JC, Phillips TJ, Kosobud A, Belknap JK. Estimation of genetic correlation: Interpretation of experiments using selectively bred and inbred animals. Alcohol Clin Exp Res. 1990; 14:141–151. [PubMed: 2190477]

Crawley JN, Belknap JK, Collins A, Crabbe JC, Frankel W, Henderson N, Hitzemann RJ, Maxson SC, Miner LL, Silva AJ, Wehner JM, Wynshaw-Boris A, Paylor R. Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. Psychopharmacology. 1997; 132:107–124. [PubMed: 9266608]

Czachowski CL, Santini LA, Legg BH, Samson HH. Separate measures of ethanol seeking and drinking in the rat: Effects of remoxipride. Alcohol. 2002; 28:39–46. [PubMed: 12377359]

Elmer GI, Meisch RA, George FR. Differential concentration-response curves for oral ethanol self-administration in C57BL/6J and BALB/cJ mice. Alcohol. 1987; 4:63–68. [PubMed: 3828066]

Epstein DH, Preston KL, Stewart J, Shaham Y. Toward a model of drug relapse: An assessment of the validity of the reinstatement procedure. Psychopharmacology. 2006; 189:1–16. [PubMed: 17019567]

Finn DA, Mark GP, Fretwell AM, Gililland KR, Strong MN, Ford MM. Reinstatement of ethanol and sucrose seeking by the neurosteroid allopregnanolone in C57BL/6 mice. Psychopharmacology. 2008; 201:423–433. [PubMed: 18758755]

Ford MM, Fretwell AM, Mark GP, Finn DA. Influence of reinforcement schedule on ethanol consumption patterns in non-food restricted male C57BL/6J mice. Alcohol. 2007a; 41:21–29. [PubMed: 17452296]

Ford MM, Fretwell AM, Nickel JD, Mark GP, Strong MN, Yoneyama N, Finn DA. The influence of mecamylamine on ethanol and sucrose self-administration. Neuropharmacology. 2009; 57:250–258. [PubMed: 19501109]

Ford MM, Mark GP, Nickel JD, Phillips TJ, Finn DA. Allopregnanolone influences the consummatory processes that govern ethanol drinking in C57BL/6J mice. Behav Brain Res. 2007b; 179:265–272. [PubMed: 17376546]

Hitzemann R, Edmunds S, Wu W, Malmanger B, Walter N, Belknap J, Darakjian P, McWeeney S. Detection of reciprocal quantitative trait loci for acute ethanol withdrawal and ethanol consumption in heterogeneous stock mice. Psychopharmacology. 2009; 203:713–722. [PubMed: 19052728]

Khisti RT, Wolstenholme J, Shelton KL, Miles MF. Characterization of the ethanol-deprivation effect in substrains of C57BL/6 mice. Alcohol. 2006; 40:119–126. [PubMed: 17307648]

Kosobud, AE.; Crabbe, JC. Genetic influences on the development of physical dependence and withdrawal in animals. In: Begleiter, H.; Kissin, B., editors. The Genetics of Alcoholism. Oxford University Press; Oxford: 1995. p. 221-256.

Kosobud A, Bodor AS, Crabbe JC. Voluntary consumption of ethanol in WSP, WSC and WSR selectively bred mouse lines. Pharmacol Biochem Behav. 1988; 29:601–607. [PubMed: 3362955]

Lê AD, Shaham Y. Neurobiology of relapse to alcohol in rats. Pharmacol Ther. 2002; 94:137–156. [PubMed: 12191599]

Little HJ. Behavioral mechanisms underlying the link between smoking and drinking. Alcohol Res Health. 2000; 24:215–224. [PubMed: 15986716]

Meisch RA. Oral drug self-administration: An overview of laboratory animal studies. Alcohol. 2001; 24:117–128. [PubMed: 11522433]

Melendez RI, Middaugh LD, Kalivas PW. Development of an alcohol deprivation and escalation effect in C57BL/6J mice. Alcohol Clin Exp Res. 2006; 30:2017–2025. [PubMed: 17117967]

Metten P, Crabbe JC. Alcohol withdrawal severity in inbred mouse (*Mus musculus*) strains. Behav Neurosci. 2005; 119:911–925. [PubMed: 16187819]

Metten, P.; Crabbe, JC. Dependence and withdrawal. In: Deitrich, RA.; Erwin, VG., editors. Pharmacological Effects of Ethanol on the Nervous System. CRC Press; New York: 1996. p. 269-290.

Metten P, Phillips TJ, Crabbe JC, Tarantino LM, McClearn G, Plomin R, Erwin VG, Belknap JK. High genetic susceptibility to ethanol withdrawal predicts low ethanol consumption. Mamm Genome. 1998; 9:983–990. [PubMed: 9880664]

National Research Council of the National Academies. Guide for the Care and Use of Mammals in Neuroscience and Behavioral Research. National Academies Press; Washington, DC: 2003.

Nickel JD, Ford MM, Yoneyama N, Murillo AR, Finn DA. Modulation of ethanol intake patterns in male and female *Srd5a1* knockout mice. Alcohol Clin Exp Res. 2006; 30:65A.

Nie H, Janak PH. Comparison of reinstatement of ethanol- and sucrose-seeking by conditioned stimuli and priming injections of allopregnanolone after extinction in rats. Psychopharmacology. 2003; 168:222–228. [PubMed: 12719962]

Roberts AJ, Heyser CJ, Koob GF. Operant self-administration of sweetened versus unsweetened ethanol: Effects on blood alcohol levels. Alcohol Clin Exp Res. 1999; 23:1151–1157. [PubMed: 10443980]

Roberts AJ, McDonald JS, Heyser CJ, Kieffer BL, Matthes HW, Koob GF, Gold LH. Muopioid receptor knockout mice do not self-administer alcohol. J Pharmacol Exp Ther. 2000; 293:1002–1008. [PubMed: 10869404]

Rodd-Hendricks ZA, McKinzie DL, Shaikh SR, Murphy JM, McBride WJ, Lumeng L, Li T-K. Alcohol deprivation effect is prolonged in the alcohol-preferring (P) rat after repeated deprivations. Alcohol Clin Exp Res. 2000; 24:8–16. [PubMed: 10656186]

Samson HH. Initiation of ethanol reinforcement using a sucrose-substitution procedure in food- and water-sated rats. Alcohol Clin Exp Res. 1986; 10:436–442. [PubMed: 3530023]

Samson HH, Chappell A. Reinstatement of ethanol seeking responding after ethanol self-administration. Alcohol. 2002; 26:95–101. [PubMed: 12007584]

Samson HH, Czachowski CL, Slawecki CJ. A new assessment of the ability of oral ethanol to function as a reinforcing stimulus. Alcohol Clin Exp Res. 2000; 24:766–773. [PubMed: 10888063]

Samson HH, Slawecki CJ, Sharpe AL, Chappell A. Appetitive and consummatory behaviors in the control of ethanol consumption: a measure of ethanol seeking behavior. Alcohol Clin Exp Res. 1998; 22:1783–1787. [PubMed: 9835295]

Sanchis-Segura C, Borchardt T, Vengeliene V, Zghoul T, Bachteler D, Gass P, Sprengel R, Spanagel R. Involvement of the AMPA receptor GluR-C subunit in alcohol-seeking behavior and relapse. J Neurosci. 2006; 26:1231–1238. [PubMed: 16436610]

Sanchis-Segura C, Spanagel R. Behavioural assessment of drug reinforcement and addictive features in rodents: An overview. Addiction Biol. 2006; 11:2–38.

Shaham Y, Shalev U, Lu L, de Wit H, Stewart J. The reinstatement model of drug relapse: History, methodology and major findings. Psychopharmacology. 2003; 168:3–20. [PubMed: 12402102]

Spanagel R. Alcoholism: A systems approach from molecular physiology to addictive behavior. Physiol Rev. 2009; 89:649–705. [PubMed: 19342616]

Stewart J. Stress and relapse to drug seeking: Studies in laboratory animals shed light on mechanisms and sources of long-term vulnerability. Am J Addiction. 2003; 12:1–17.

Tsiang MT, Janak PH. Alcohol seeking in C57BL/6 mice induced by conditioned cues and contexts in the extinction-reinstatement model. Alcohol. 2006; 38:81–88. [PubMed: 16839854]

Yoneyama N, Crabbe JC, Ford MM, Murillo A, Finn DA. Voluntary ethanol consumption in 22 inbred mouse strains. Alcohol. 2008; 42:149–160. [PubMed: 18358676]

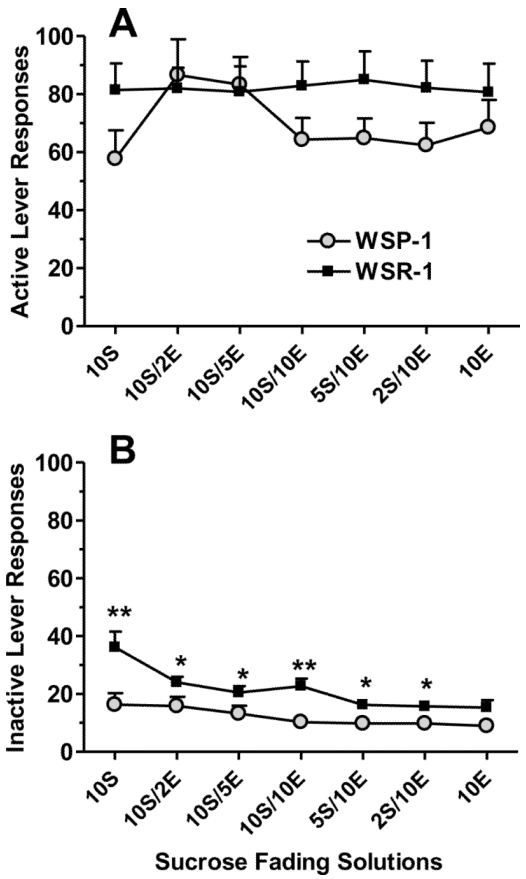

Figure 1. Appetitive measures during sucrose fading

Responding on the active (**panel A**) and inactive (**panel B**) levers throughout the sucrose fading procedure are depicted. Each point represents the mean ± SEM of responding for WSP-1 (n = 16) or WSR-1 (n = 19) mice across 3–5 sessions at each sucrose fading solution. **P* < 0.05 and ***P* < 0.01 between selected lines; Tukey's post-hoc test.

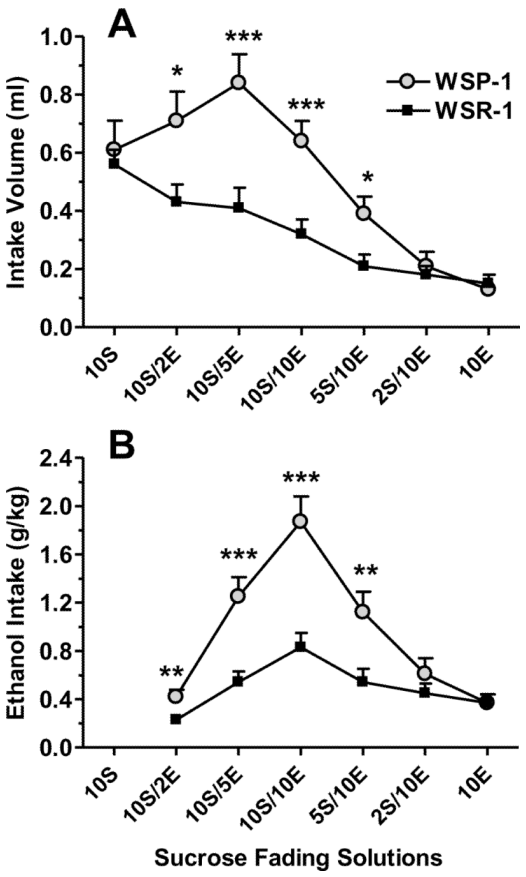

Figure 2. Line difference in consummatory measures during sucrose fading

The volume (**panel A**) and ethanol dose (**panel B**) consumed throughout the sucrose fading procedure are shown. Each point represents the mean ± SEM for WSP-1 (n = 16) or WSR-1 (n = 19) mice across 3–5 sessions at each sucrose fading solution. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 between selected lines; Tukey's post-hoc test.

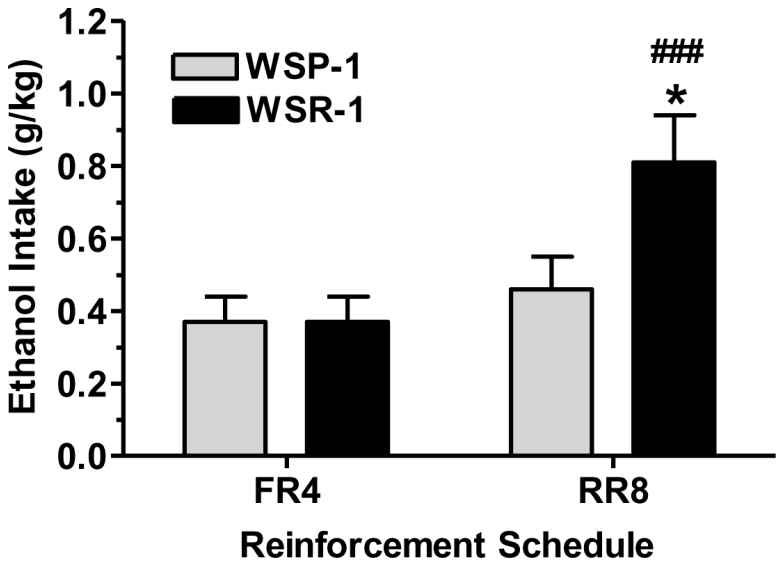

Figure 3. Reinforcement schedule alters line difference in ethanol self-administration maintenance

Comparisons of ethanol intakes during fixed ratio (FR)-4 and response requirement (RR)-8 schedules are illustrated. Each bar represents the mean ± SEM of WSP-1 (n = 16) or WSR-1 (n = 19) mice during 4 sessions on the FR4 schedule and a subsequent 2 sessions on the RR8 schedule. *$P < 0.05$ between selected lines for RR8 schedule; $^{###}P < 0.001$ within WSR line versus FR4 schedule; Tukey's post-hoc test.

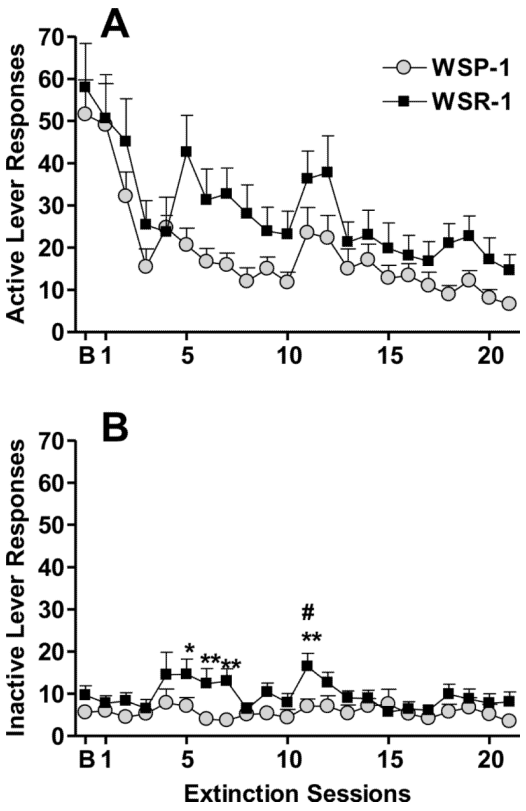

Figure 4. Responding throughout the extinction time course

Responding on the previously active (**panel A**) and inactive (**panel B**) levers are shown. There were no scheduled consequences to responding during extinction. Each point represents the mean ± SEM of responding for WSP-1 (n = 11) or WSR-1 (n = 10) mice during single extinction sessions (except baseline which represents a 3-session average on FR4 schedule). **P* < 0.05 and ***P* < 0.01 between selected lines; #*P* < 0.05 within WSR line versus baseline; Tukey's post-hoc test.

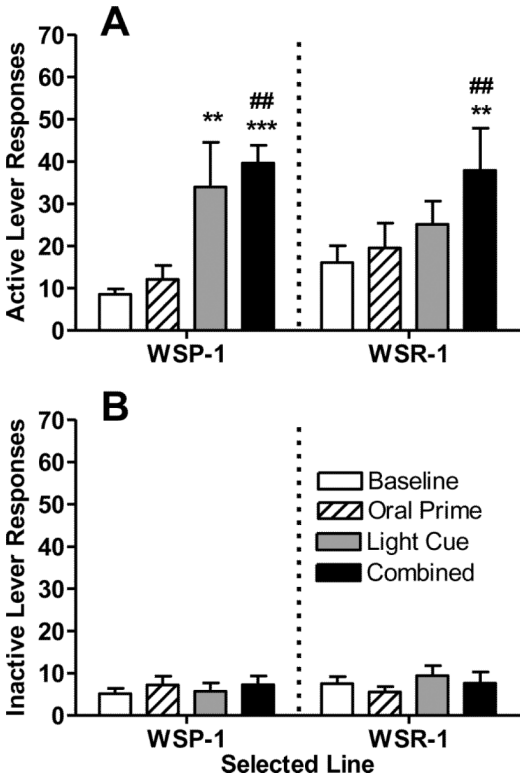

Figure 5. Subtle line differences in reinstatement of responding for ethanol

Responding on the previously active (**panel A**) and inactive (**panel B**) levers are depicted. Each bar represents the mean ± SEM of responding for WSP-1 (n = 11) or WSR-1 (n = 10) mice. A collapsed baseline composed of the sessions immediately preceding the reinstatement tests is shown (the three baseline sessions did not significantly differ from one another). $^{**}P < 0.01$ and $^{***}P < 0.001$ versus respective within line baseline value; $^{\#\#}P < 0.01$ versus within line oral prime value; Tukey's post-hoc test.

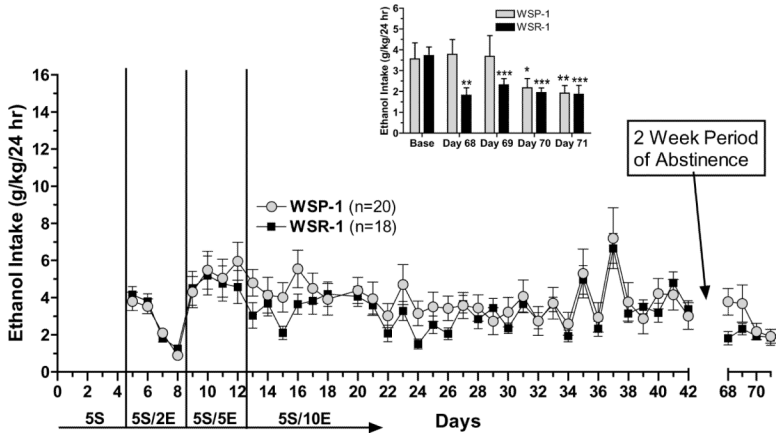

Figure 6. No line difference in home cage drinking of sweetened ethanol or in the development of an ADE

Daily intake of each sweetened ethanol solution is depicted for WSP-1 (n=20) and WSR-1 (n=18) mice. Mice consumed the 5S/10E solution for 30 days prior to the 2 week period of abstinence and then the 4 days of re-access to the ethanol solution to determine whether animals would exhibit an ADE. Inset to the figure depicts the ADE data, with baseline intake reflecting the average of the last 4 days of intake prior to the 2 week period of abstinence. Shown are mean ± SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 versus respective baseline.

Table 1

Summary of findings in WSP-1 and WSR-1 mice.

Phenotype	Outcome
Sucrose fading (acquisition)	
Appetitive measures	
Active lever responses	WSR-1 ≥ WSP-1
Inactive lever responses	WSR-1 > WSP-1
Consummatory measures	
10S volume (ml)	WSR-1 = WSP-1
Sucrose/ethanol volume (ml)	WSR-1 < WSP-1
10E volume (ml)	WSR-1 = WSP-1
Sucrose/ethanol intake (g/kg ethanol)	WSR-1 < WSP-1
10E intake (g/kg ethanol)	WSR-1 = WSP-1
Reinforcement schedule	
FR4: 10E intake (g/kg)	WSR-1 = WSP-1
RR8: 10E intake (g/kg)	WSR-1 > WSP-1
Extinction time course	
Rate	WSR-1 = WSP-1
Active lever responses	WSR-1 = WSP-1
Inactive lever responses	WSR-1 > WSP-1
Reinstatement (active lever)	
Baseline	WSR-1 > WSP-1
Oral prime	No reinstatement
Light cue	WSP-1 only
Combined cues	WSR-1 = WSP-1
Inactive lever responses	No effects
Home cage preference drinking	
5S intake (g/kg sucrose)	WSR-1 > WSP-1
5S/10E intake (g/kg ethanol)	WSR-1 = WSP-1
Alcohol deprivation effect (5S/10E intake; g/kg ethanol)	No ADE; WSR-1 < WSP-1 for intake during first 2 days postdeprivation