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## Chapter 7

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### Enhanced alcohol self-administration and relapse in a highly impulsive inbred mouse strain

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*In preparation*



## **Abstract**

Impulsivity has frequently been associated with alcohol abuse disorder. However, it is unknown to what extent pre-existing levels of impulsive behavior predict the motivation to take alcohol or the vulnerability to relapse to alcohol seeking. BXD16, a recombinant inbred strain derived from a cross between high alcohol-drinking C57BL/6J and alcohol-avoiding DBA/2J mice, was identified in the 5-CSRTT as the strain with the highest level of impulsive action. We compared BXD16 with C57BL/6J mice in a simple choice reaction time task (SCRTT) and confirmed the impulsive and inattentive phenotype of BXD16. In a sucrose/alcohol fading self-administration procedure C57BL/6J and BXD16 did not differ in fixed ratio (FR1) responding for 10% sucrose. However, BXD16 showed increased motivation to earn 10% alcohol solution, both under FR1 and progressive ratio (PR2) schedules of reinforcement. Responding readily decreased in both strains during extinction training. Upon re-exposure to alcohol-associated cues after extinction, alcohol seeking was reinstated to a larger extent in BXD16 than C57BL/6J mice. Although additional studies are required to dissect the genetic relation between impulsivity and addiction-related behavior, this is the first study in mice suggesting that impulsivity predicts the motivation to consume alcohol as well as relapse vulnerability.

## **Introduction**

The multifaceted construct of impulsivity has frequently been associated with substance use disorders, including alcohol abuse (Verdejo-Garcia *et al.*, 2008). Accumulating evidence from human prospective studies suggests that pathological levels of impulsivity may not only be a consequence of alcohol use, but could pre-date the development of alcohol abuse and dependence (Caspi *et al.*, 1996; Dawes *et al.*, 1997). In line with these observations in humans, increased levels of impulsive behavior have been observed in alcohol-naïve individuals of rat (Steinmetz *et al.*, 2000; Wilhelm & Mitchell, 2008), as well as mouse lines (Gubner *et al.*, 2010; Logue *et al.*, 1998; Oberlin & Grahame, 2009) that are genetically predisposed to show a high preference/consumption of alcohol. This suggests that genetic factors that contribute to impulsive behavior may facilitate the preference or consumption of alcohol, and thereby the initiation and maintenance of alcohol use. However, whether impulsivity predicts other stages of alcohol abuse, such as the motivation and persistence to take and seek alcohol has not been investigated.

Using a 5-CSRTT, an operant task of impulsive action (Winstanley *et al.*, 2006a), we previously identified a highly impulsive mouse inbred strain among the panel of BXD recombinant inbred strains of mice (BXD16; see **chapter 6**; Peirce *et al.*, 2004). This strain was substantially more impulsive than either of its founders, the DBA/2J and C57BL/6J strains of mice (**chapter 5 and 6**). In the current study, we investigated whether we could reproduce and extent the

impulsive phenotype of BXD16 compared with C57BL/6J mice in another task of impulsive action, a simple choice reaction time task (SCRTT) that requires the animals to wait an unpredictable number of responses rather than a predictable amount of time. In addition to this, we investigated to what extent the SCRTT could detect differences in attentional performance, as detected previously in the 5-CSRTT for these strains (**chapter 6**). Subsequently, we compared BXD16 and C57BL/6J mice in an operant sucrose-fading alcohol self-administration protocol to address the question whether differences measured in impulsivity between strains are predictive of the motivation and persistence to take and seek alcohol. Specifically, we investigated the motivation to self-administer alcohol under a schedule of progressive ratio responding, as well as relapse of alcohol seeking behavior after extinction in a cue-induced reinstatement protocol (De Vries *et al.*, 2001).

## Materials and Methods

### Animals

Male seven-week-old mice were bred in the facility of the Neuro-Bsik consortium from the VU University Amsterdam, after receiving breeding pairs from Jackson Laboratory. Mice were singly housed on sawdust in standard Makrolon type II cages (26.5 cm long, 20.5 cm wide and 14.5 cm high) enriched with cardboard nesting material and habituated for minimally 1 week before behavioral testing commenced. In the week prior to operant training, body weights were recorded and mice were food-restricted to gradually decrease their body weight to 90 - 95% of their initial body weight before daily training in operant cages commenced (5 days each week, 30 min. per session).

### 5-CSRTT and SCRTT

Mice (C57BL/6J  $n = 25$ ; BXD16  $n = 12$ ) were trained to perform the 5-CSRTT on an individually-paced schedule, as described previously (Loos *et al.*, 2010b; Loos *et al.*, 2009). In a 5-CSRTT, mice were required to respond to a 1 s stimulus in one of 5 stimulus holes to earn a food pellet dispensed in the magazine at the opposite side of the cage. Impulsivity (impulsive action) was defined in terms of the percentage premature responses:  $[100 \times (\text{number of premature responses}) / (\text{number of omissions} + \text{correct} + \text{incorrect responses})]$ . The two measures of attentional performance in the 5-CSRTT were response accuracy, defined as  $[100 \times (\text{number of correct responses}) / (\text{number of correct and incorrect responses})]$  and response variability, defined by the standard deviation of the correct response latencies. These measures, as well as the mean and mode of correct latencies (using the half range method, Hedges & Shah, 2003) were calculated from the 6th until the 10th session at stimulus duration of 1 s, and the average of these sessions was used as standard 5-CSRTT performance.

An independent group of mice (C57BL/6J  $n = 10$ ; BXD16  $n = 9$ ) that acquired the 1 s stimulus duration limit in 5-CSRTT training were trained to perform the SCRTT. In this paradigm, the cue light in the middle of the five response holes (response hole 3) was designated as start-stimulus, and the cue light in the response hole immediately to the left or right (response hole 2 or 4, counterbalanced across strains) was designated go-stimulus. During the first 4 training sessions, a trial started with the illumination of the start-stimulus. A response into the start-stimulus hole extinguished the start-stimulus and switched on the go-stimulus. A Go-response into the go-stimulus hole switched off the stimulus and was immediately followed by distribution of a reward into the magazine. After an interval of 5 s the next trial commenced. Premature start and Go-responses in non-illuminated response holes, as well as perseverative start-responses after presentation of the go-stimulus were not rewarded but followed by a 5 s time out period during which house light and stimulus light were switched off.

In subsequent sessions, responding at a variable 3 ratio (VR3) schedule into the illuminated start-stimulus hole was required to ignite the go-stimulus. The go-stimulus was only switched on for the duration of an individually-titrated limited hold (LH) period, which was set to 5 s during the first session. A Go-response during the LH period resulted in the delivery of a reward, whereas an omission of a Go-response was followed by a 5 s time out period. The percentage of omissions of Go-responses in each session was defined as follows:  $100 * [\text{omissions of Go-response} / (\text{omissions of Go-response} + \text{correct Go-responses} + \text{perseverative start-responses})]$ . To titrate the percentage of omissions to 30% for each subject, in subsequent sessions LH periods were decreased 0.7 fold if the percentage of omissions during the previous session was less than 25%, and increased by 1.25 fold if the percentage of omissions during the previous session was larger than 35%. Impulsivity in the SCRT was defined as the percentage of premature Go-responses, calculated as follows:  $100 * [\text{number of trials with premature Go-response} / \text{number of started trials}]$ . Furthermore, we recorded the latencies between the onset of the go-stimulus and a Go-response into the go-stimulus hole (GoRT). Prior experiments indicated that GoRTs larger than 1.7 s are observed when mice travel to the magazine in between start and Go-responses. Therefore, GoRTs larger than 1.7 s were excluded in the calculation of mean GoRT, the mode of the GoRTs (using the half range method, Hedges & Shah, 2003) and the variability of the GoRTs (standard deviation of the GoRTs). Performance was considered stable when the mean GoRT and percentage of premature responses did not change significantly for either strain for three consequent sessions.

### **Operant alcohol-self administration**

A third group of BXD16 ( $n = 10$ ) and C57BL/6J ( $n = 9$ ) mice was trained in operant conditioning cages in sound attenuating chambers (TSE Systems, Bad

Homburg, Germany) 5 days per week. Food and water were available *ad libitum* throughout the experiment, except during the testing session, which lasted 1 h. During the entire session a red house light provided dim illumination of the chamber. Two levers, of which one was active, were located at opposite walls of the cage. A predefined number of responses (see below) onto the active lever resulted in the delivery of 10  $\mu$ l liquid reward into the receptacle located to the left of the left lever, and switched on a white stimulus light located above the receptacle for 2 s. After a time out period of 15 s, during which lever presses were without consequence but which were recorded, a red cue light above the receptacle switched on indicating the availability of the next reward. In addition to the number of rewards and active lever responses, the preference of responding onto the active lever was used to quantify behavior, calculated as follows: [number active lever responses / (number active lever responses + number inactive lever responses)].

*Sucrose fading, FR1 and PR2 responding for alcohol.* Mice were trained to respond for a 10% alcohol solution using a sucrose fading protocol at a fixed ratio 1 (FR1) schedule of reinforcement, in which reward consisted of the following solutions (wt/vol in tap water): 10% sucrose (S1-S9), 10% sucrose and 2% alcohol (S10-S13), 10% sucrose and 4% alcohol (S14-S15), 10% sucrose and 6% alcohol (S16-S17), 10% sucrose and 8% alcohol (S18-S20), 10% sucrose and 10% alcohol (S21-S25), 5% sucrose and 10% alcohol (S27-S32) and finally 10% alcohol (S33-S38). In the subsequent 5 sessions, mice were subjected to a progressive ratio (PR2) schedule. During these sessions the response requirement is increased by 2 after each reward delivery. The breakpoint was determined in the 5th en last session, and defined as the last completed ratio.

*Extinction and reinstatement of alcohol seeking behavior.* After PR2 sessions, mice received 6 sessions of FR1 training for 10% alcohol. During the subsequent 20 sessions of extinction training responding on the previously active lever was without programmed consequences. During the last training session reinstatement of alcohol seeking behavior was determined in response to presentation of alcohol conditioned cues: cue lights in response to active lever responses and a droplet of 10% alcohol placed in the receptacle at the start of the session.

### **Data analysis**

For the evaluation of strain differences, analysis of variance (ANOVA) was used with 'strain' as between-subjects factor. The effects of task manipulations across different sessions were analyzed using ANOVA with repeated measures with 'session' as within-subjects factor. When Mauchly's test for sphericity of data was significant, more conservative Huynh-Feldt corrected degrees of freedom and related probability values were reported. Where appropriate, post hoc tests were performed with Student's t-tests for 'strain' effects and paired Student's t-tests for 'session' effects. All data are depicted as means  $\pm$  standard error of the

mean (SEM), and the level of significance was set at  $P < 0.05$ . All analyses were performed using the Statistical Package for the Social Sciences for Windows version 15.0 (SPSS, Chicago, IL, USA).

## Results

### 5-CSRTT

After reaching the baseline stimulus duration of 1 s in our standard 5-CSRTT training protocol, BXD16 mice were found to exhibit a higher impulsivity in terms of the percentage of premature responses (**Fig. 1a**; strain:  $F(1,35) = 29.87$ ,  $P < 0.001$ ) compared with C57BL/6J mice. Attentional performance of C57BL/6J mice was superior compared to BXD16 mice, with a significantly higher response accuracy (strain:  $F(1,35) = 13.17$ ,  $P < 0.001$ ; data not shown) and lower response variability (**Fig. 1c**; strain:  $F(1,35) = 7.30$ ,  $P < 0.05$ ). Analysis of the distribution of correct response latencies (**Fig. 1b**) indicated that BXD16 mice were slower in terms of the mean (strain:  $F(1,35) = 12.20$ ,  $P < 0.01$ ) and mode (strain:  $F(1,35) = 12.50$ ,  $P < 0.01$ ) of the correct response latencies (**Fig. 1c**).

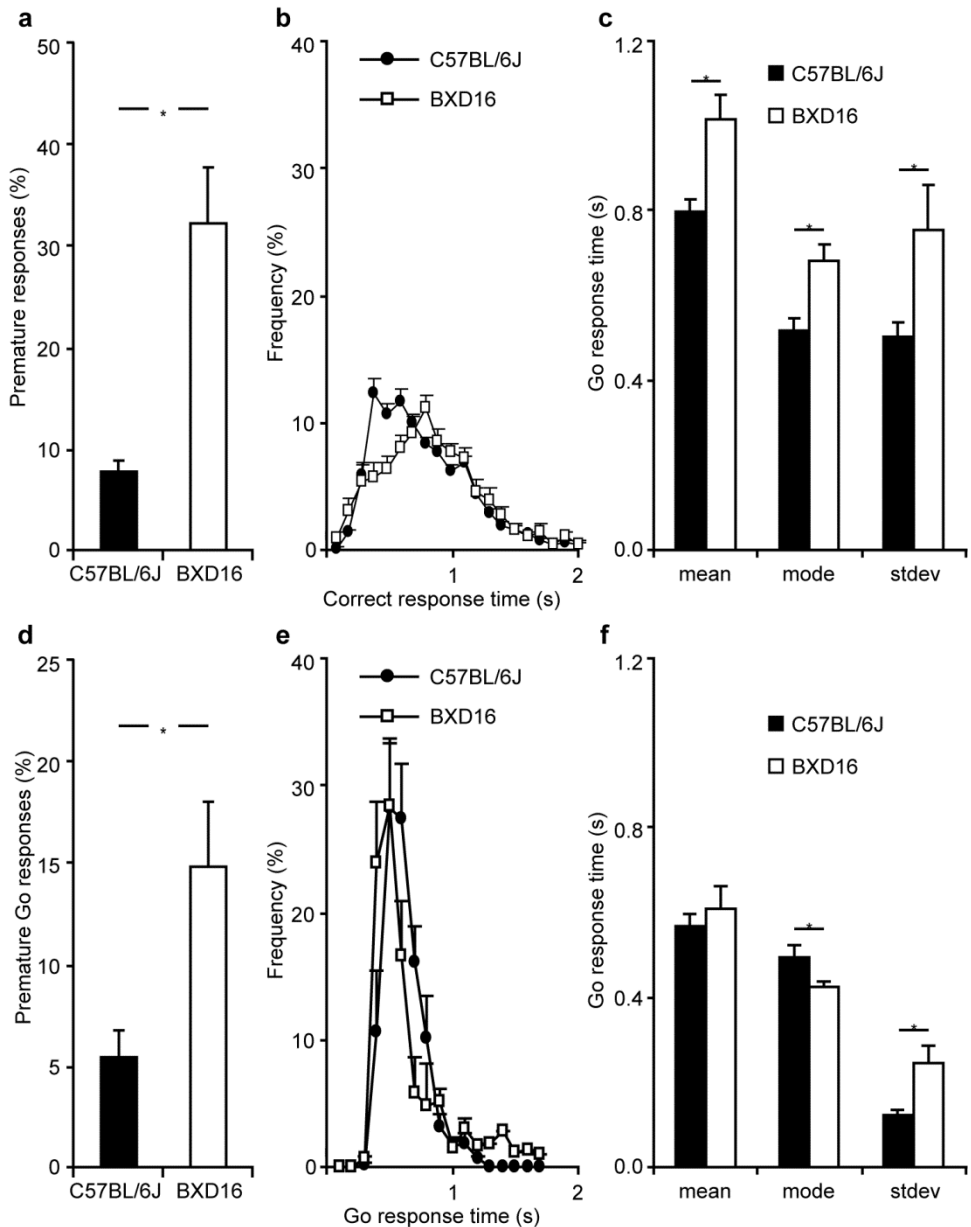
### SCRTT

An independent group of C57BL/6J and BXD16 mice was trained to perform the SCRTT. Performance stabilized after 10 training sessions, and data of the subsequent sessions was taken for average SCRT performance. BXD16 mice were more impulsive in terms of percentage of premature Go-responses during presentation of the start stimulus compared with C57BL/6J mice (**Fig. 1d**; strain:  $F(1,17) = 6.87$ ,  $P < 0.05$ ). Although the required limited hold time was significantly longer for BXD16 mice (strain:  $F(1,17) = 8.85$ ,  $P < 0.01$ ), their mean GoRTs were not different from C57BL/6J mice (**Fig. 1e**; strain:  $F(1,16) = 0.48$ , n.s.). This was explained by the observation that BXD16 mice had a faster mode of GoRTs (**Fig. 1f**; strain:  $F(1,16) = 4.72$ ,  $P < 0.05$ ) but also a larger GoRT variability (strain:  $F(1,16) = 8.73$ ,  $P < 0.01$ ). No significant differences were found in the number of initiated trials (strain:  $F(1,17) = 3.40$ , n.s.) and the percentage of omission of Go-responses (strain:  $F(1,17) = 3.87$ , n.s.).

### Alcohol self administration

*Sucrose fading, FR1 and PR2 responding for alcohol.* There was a significant effect of strain on the number of earned rewards across the first 37 sessions (session:  $F(36,612) = 21.72$ ,  $P < 0.001$ ; strain  $\times$  session:  $F(36,612) = 5.4$ ,  $P < 0.001$ ; strain:  $F(1,17) = 7.53$ ,  $P < 0.05$ ). Post hoc testing revealed a significant difference during the first session of sucrose acquisition. Analysis of the first 9 sucrose sessions revealed only a trend for session  $\times$  strain interaction ( $P = 0.08$ ), and development of strain differences starting from the fading step of 10% sucrose with 8% alcohol and onwards (**Fig. 2a**). There was a significant



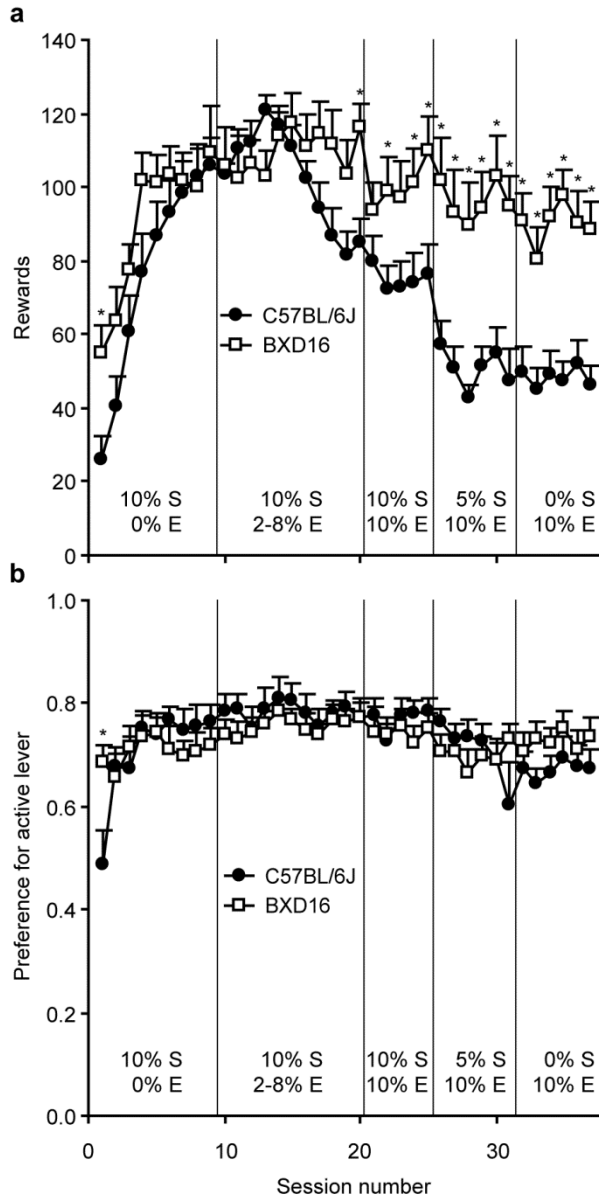


**Figure 1** | Consistent differences in impulsivity and attention between BXD16 and C57BL/6J mice in two operant tasks. (a, d) BXD16 mice were more impulsive in terms of the number of premature responses in the 5-CSRTT (A) and SCRRT (d). (b, e) Analysis of the distribution of responses in the 5-CSRTT (b) and SCRRT (e) indicated that (c, f) C57BL/6J mice showed lower response variability than BXD16, indicative of fewer lapses in attention in both tasks.  $P < 0.05$ .

difference in the development of the preference of the active over the inactive lever between strains (session:  $F(36,612) = 4.87$ ,  $P < 0.001$ ; strain  $\times$  session:  $F(36,612) = 2.18$ ,  $P < 0.001$ ; strain:  $F(1,17) = 0.01$ , n.s.). Post hoc testing revealed an increased preference in BXD16 mice only during the first session of sucrose acquisition (**Fig. 2b**), suggesting a faster development of the dissociation between active and inactive lever in these mice. Similar effects were found on the number of active lever responses, which include non-rewarding active lever responses during the time out (not shown; session:  $F(36,612) = 11.02$ ,  $P < 0.001$ ; session  $\times$  strain:  $F(36,612) = 4.06$ ,  $P < 0.001$ ; strain:  $F(1,17) = 7.07$ ,  $P < 0.05$ ). Again, post hoc testing revealed a significant difference during the first session, and the development of strain differences starting from the fading step of 10% sucrose with 8% alcohol and onwards (not shown). There was a significant session  $\times$  strain effect with respect to the number of inactive responses (session:  $F(36,612) = 1.68$ ,  $P < 0.01$ ; session  $\times$  strain  $F(36,612) = 2.03$ ,  $P < 0.001$ ). However, post hoc testing indicated that BXD16 mice only showed increased inactive lever responses during two fading steps (5% sucrose with 10% alcohol, 5% sucrose with 10% alcohol), but not during the FR1 sessions with 10% alcohol.

To test the motivation to receive a 10% alcohol reward, mice were tested in a PR2 schedule. During the 5th session of PR2 training, BXD16 mice reached a higher breakpoint (i.e., last completed ratio) than C57BL/6J mice (**Fig. 3a**; strain:  $F(1,17) = 20.01$ ,  $P < 0.001$ ), in line with a significantly elevated number of active lever responses ( $F(1,17) = 16.85$ ,  $P < 0.01$ ). No difference was detected in the preference of the active versus inactive lever (**Fig. 3b**; strain:  $F(1,17) = 0.71$ , n.s.) and in inactive lever responding ( $F(1,17) = 2.84$ , ns; not shown). During the six FR1 sessions following PR2 training, BXD16 mice again displayed higher alcohol self-administration behavior in terms of earned rewards compared with C57BL/6J mice (strain:  $F(1,17) = 9.18$ ,  $P < 0.01$ ).

*Extinction and reinstatement of alcohol seeking behavior.* Over the course of the last FR1 session and 20 subsequent extinction sessions, alcohol-seeking behavior in terms of the number of responses on the previously active lever decreased (**Fig. 4a**; session:  $F(20,320) = 8.57$ ,  $P < 0.001$ ). No difference in the rate of extinction or preference of the active lever between groups was detected (session  $\times$  strain:  $F(20,320) = 1.54$ , n.s.). The absolute number of previously active lever responses remained significantly higher in BXD16 (strain:  $F(1,16) = 17.3$ ,  $P < 0.001$ ). Similar to extinction of the active lever responses, there was a significant decrease in the preference of the active over the inactive lever in both strains (**Fig. 4b**; session:  $F(20,320) = 3.36$ ,  $P < 0.001$ ; session  $\times$  strain:  $F(20,320) = 1.06$ , n.s.). No difference between strains was detected in the preference for the previously active lever (strain:  $F(1,16) = 1.64$ , n.s.). The number of responses on the previously inactive lever showed an interaction effect (session:  $F(20,320) = 2.31$ ,  $P < 0.01$ ; session  $\times$  strain:  $F(20,320) = 3.10$ ,  $P < 0.001$ ). Post hoc testing



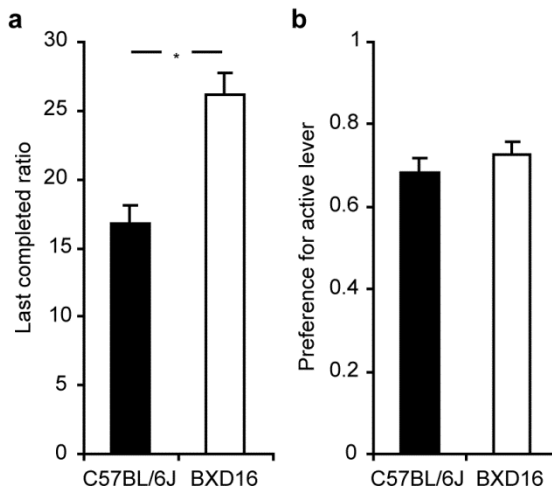
**Figure 2** | Enhanced alcohol seeking in BXD16 mice at an FR1 schedule. During the first sessions an active lever response was rewarded with 10  $\mu$ l sucrose solution (10%). This reward was faded towards an alcohol solution (10%) after 32 sessions. (a) A strain difference in obtained rewards developed during fading in of alcohol that was persistent with a 10% alcohol solution. (b) Except for the first session, strains did not differ in the preference for the active lever over the inactive lever, indicating a similar capacity to dissociate the active from the inactive lever. \*  $P < 0.05$ .

indicated that, whereas the number of these responses did not differ during the last FR1 session, inactive lever responding increased in BXD16 from the second extinction session onwards (not shown). Next, sensitivity to alcohol-conditioned cues was tested in a reinstatement session. In comparison to the last extinction session, the number of responses on the previously active lever increased significantly (session:  $F(1,16) = 25.18$ ,  $P < 0.001$ ) upon presentation of alcohol-conditioned cues during the reinstatement session (C57BL/6J: from  $52.9 \pm 9.5$  to  $85.8 \pm 13.4$  responses; BXD16: from  $106.7 \pm 17.0$  to  $207.2 \pm 37.1$  responses). This effect was strain dependent (session  $\times$  strain:  $F(1,16) = 6.47$ ,  $P < 0.05$ ), indicating that the relative increase in number of responses on the previously active lever in BXD16 (one-way Student's *t*-test  $P < 0.01$ ) was larger than in C57BL/6J (one-way Student's *t*-test  $P < 0.05$ ; **Fig. 4c**). During the reinstatement session, the preference of the previously active lever over the inactive lever increased in both strains (**Fig. 4d**; session:  $F(1,16) = 4.64$ ,  $P < 0.05$ ; session  $\times$  strain:  $F(1,16) = 0.47$ , n.s.). The number of previously inactive lever responses also increased (session:  $F(1,16) = 9.30$ ,  $P < 0.01$ ), but in contrast to active lever responses, no significant session  $\times$  strain interaction was detected (session  $\times$  strain:  $F(1,16) = 0.34$ , n.s.; data not shown).

## Discussion

### Consistent strain differences in impulsivity and attention

Our previous studies (**chapters 5 and 6**) indicated that BXD16 is the most impulsive and least attentive strain among the panel of BXD recombinant inbred strain as assessed in a 5-CSRTT. In the current study, we compared the performance of C57BL/6J and BXD16 mice in a novel SCRRT. In this SCRRT, the onset of a go-stimulus was rendered unpredictable by randomly varying the



**Figure 3** | Enhanced motivation of alcohol self-administration in BXD16 mice at a PR2 schedule. (a) BXD16 mice reached a higher last completed ratio of responding to obtain 10  $\mu$ l alcohol solution (10%), indicative of enhanced motivation to self-administer alcohol. (b) After adaptation to the procedure, strains did not differ in the preference for the active lever during the 5<sup>th</sup> session at PR2 schedule of reinforcement, indicating a similar capacity to dissociate the active from the inactive lever, as observed during the sucrose-fading paradigm. \* $P < 0.05$ .

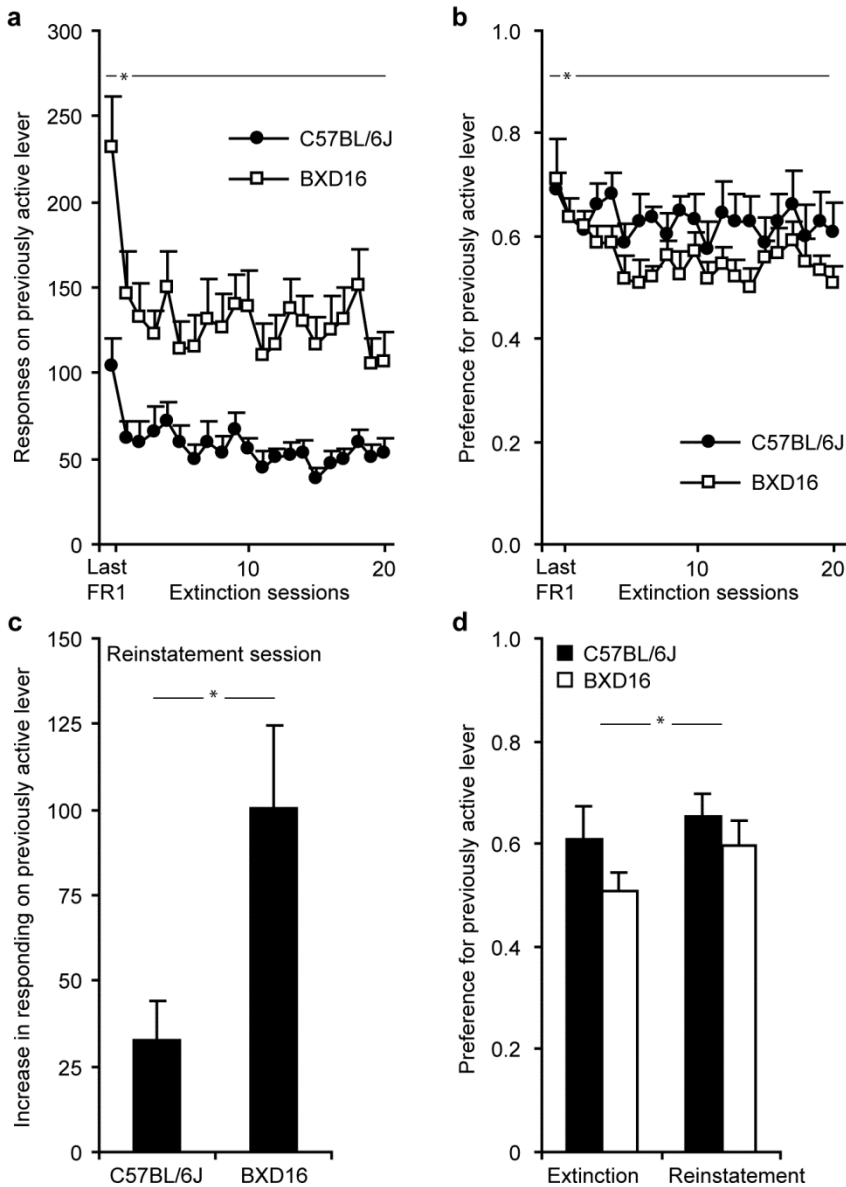
number of start-responses required to initiate a go-stimulus. This required mice to inhibit prepotent Go-responses for an unpredictable amount of time. Compared with C57BL/6J, BXD16 mice made more premature Go-responses. This extends the observation that BXD16 mice make more premature responses in the 5-CSRTT, in which mice need to inhibit responding during fixed inter-trial interval time of 5 s. As such, we can conclude that BXD16 mice have a reduced ability to inhibit responding in situations, in which waiting is required, whether or not the duration of this waiting period is predictable.

In comparison to C57BL/6J mice, BXD16 mice showed increased intra-individual variability in Go-response latencies. This can be explained by an increased frequency or duration of brief lapses in attention during which the onset of a go-stimulus is not detected by BXD16 mice. This observation is in line with previous observations of low attentional performance in the 5-CSRTT, in terms of low choice accuracy and large intra-individual variability (**chapter 5**). Together, these data clearly indicate reduced attentional performance of BXD16 mice compared with C57BL/6J mice.

We detected a faster mode of response latencies in BXD16 mice in the SCRT in the current study. It has been suggested that the mode of response latencies can be taken as measure of sensorimotor processing speed (Leth-Steensen *et al.*, 2000; Sabol *et al.*, 2003), suggesting that BXD16 mice have faster sensorimotor processing speed. The contrasting finding that C57BL/6J mice have a faster mode of response latencies in the 5-CSRTT (**chapter 5**) may be explained by two procedural differences between 5-CSRTT and SCRTT that have an impact on movement time and timing ability. In the 5-CSRTT, mice have to travel to a stimulus in one of the 5 holes spaced along one side of the operant cage, while in the SCRTT only a small movement from the middle hole to the adjacent hole is required. This is reflected by the markedly lower mode of the response latencies in the SCRTT in the current study compared to the 5-CSRTT. Hence, strain difference in movement speed would affect performance in the 5-CSRTT to a larger extent, which could explain inconsistent strain difference in the mode of response latencies between 5-CSRTT and SCRTT. Secondly, only in the 5-CSRTT mice can use a timing strategy to anticipate the onset of the stimulus after the 5 s ITI. It is possible that C57BL/6J mice have a superior timing ability that would contribute to a shorter mode of responding in the 5-CSRTT, but not in the SCRTT. Taken together, the comparison of the mode of response latencies in the SCRT and 5-CSRTTs suggests that C57BL/6J mice may have faster movement times or better timing ability, while BXD16 mice have faster processing speed.

### **Strain differences in alcohol-seeking behavior**

During the first self-administration session, when reward consisted of a 10% sucrose solution, BXD16 mice made more responses on the active lever, earned more rewards and had a higher preference for the active lever than C57BL/6J



**Figure 4** | Stronger reinstatement of alcohol-seeking in BXD16 mice. Compared to the last FR1 session, responding at the previously active lever (a) and the preference for the previously active lever (b) decreased during extinction training in both strains (paired t-tests for comparison between last FR1 and each subsequent extinction session). Upon exposure to alcohol-conditioned cues (c), a larger increase in alcohol-seeking behavior in BXD16 than in C57BL/6J mice in terms of the increase in the number of responses on the previously active lever compared with the last extinction session. (d) Exposure to alcohol-conditioned cues after extinction increased the preference of responding on the previously active lever indicative of alcohol-seeking. \*  $P < 0.05$ , \*\*  $P < 0.01$ .

mice. This suggests that BXD16 mice were slightly faster in acquiring instrumental responding. No strain difference in the number of rewards was detected in each of the following 8 sessions with 10% sucrose reward, suggesting that BXD16 and C57BL/6J did not differ in motivation to consume sucrose. Also, no differences in number of inactive lever responses were detected, indicating that there was no evidence for a higher general activity of BXD16 mice.

During the sucrose fading procedure, a significant strain difference in number of active lever responses and earned rewards developed, starting when reward consisted of 10% sucrose mixed with 8% alcohol. The absence of a concomitant change in the preference for the active lever, and the absence of differences in number of inactive lever responses during the sessions with 10% alcohol solution indicate that the higher number of active lever responses of BXD16 was not merely a higher general activity.

The FR1 data suggested that BXD16 mice were more motivated to self-administer alcohol. The latter was confirmed when animals were subjected to the progressive ratio (PR2) procedure for 10% alcohol reward. PR schedules provide a measure of motivational strength of the drug (Mendrek *et al.*, 1998). Compared with C57BL/6J mice, the BXD16 strain reached higher breakpoints in the absence of a difference in the preference for the active lever, indicative of a stronger motivation to self-administer 10% alcohol reward.

In the subsequent 20 extinction sessions, the strains did not differ in rate at which responding on the previously active lever decreased, suggesting a similar extinction rate of alcohol-seeking behavior. Similarly, the preference for the previously active lever decreased at the same rate in both strains. However, the number of active lever responses remained significantly higher in BXD16 during extinction sessions and the number of inactive lever responses reached significantly higher levels than C57BL/6J mice from the second extinction session onwards. Such a general activity difference between C57BL/6J and BXD16 was not observed earlier in the operant protocol, and has not been observed before in tests of locomotion in novel environments (Yang *et al.*, 2008). Therefore it is possible that this general activity difference between strains only appears after prolonged periods of habituation, i.e., 50 sessions of training in the operant chambers in the current experiment.

Both strains showed reinstatement of alcohol-seeking behavior upon exposure to alcohol-associated stimuli, reflected in responding on, and preference for, the previously active lever. Importantly, statistical analysis indicated that this increase was strain-specific, and a more robust increase was detected in BXD16 than in C57BL/6J mice.

C57BL/6J mice are known to show voluntary alcohol intake and alcohol metabolism similar to, or even higher than BXD16 (Crabbe *et al.*, 1983; Grisel *et al.*, 2002; Phillips *et al.*, 1994; Rodriguez *et al.*, 1994). When placed in the context of the concept that different factors predict different stages in the

development of addiction (Kreek *et al.*, 2005), these observations do not contradict the observation of enhanced alcohol-seeking in BXD16 mice in the current study. A sucrose fading protocol may overcome an initial aversive effect of alcohol in low-alcohol drinking rodents, and enhance subsequent voluntary alcohol intake in the home cage (Tolliver *et al.*, 1988). Therefore, a fading protocol may facilitate the intake of alcohol to levels that provide enough reinforcement to initiate and maintain alcohol-seeking behavior. Thus, in comparison to C57BL/6J, high impulsive BXD16 mice have an equal or lower propensity to initiate alcohol self-administration, but once alcohol self-administration is established, they show enhanced alcohol-seeking behavior, even after a prolonged period of extinction training.

The oral consumption of alcohol is accompanied by chemosensory perception of its flavor, which plays an important role in its acceptance and rejection. Like humans, rodents depend on the possibility to detect the sweet (sucrose-like) and bitter (quinine-like) taste of alcohol. BXD16 mice showed the least voluntary consumption of a bitter quinine solution of a panel of BXD strain including the parental lines (Phillips *et al.*, 1991), indicating a good perception of bitterness, as well as a low-moderate voluntary consumption of saccharine (Lush, 1989). This indicates that enhanced alcohol self-administration cannot be explained by a reduced perception of bitterness or increased perception of sweetness of alcohol in the BXD16 strain.

In conclusion, this is the first study in rodents linking impulsive action to the enhanced motivation to self-administer alcohol, as well as to the stronger reinstatement of alcohol-seeking upon exposure to alcohol-associated stimuli after extinction. Our results extend previous studies in rodents indicating that impulsive action measured in Go-NoGo tasks (Gubner *et al.*, 2010; Logue *et al.*, 1998) and impulsive choice in a delayed reward paradigm (Oberlin & Grahame, 2009; Poulos *et al.*, 1995; Wilhelm & Mitchell, 2008) coincide with the consumption, preference and sensitizing effects of alcohol. In general, these data are in line with the association between impulsivity and drug self-administration behavior in rats and substance abuse in humans (Belin *et al.*, 2008; Diergaarde *et al.*, 2008; Verdejo-Garcia *et al.*, 2008; Winstanley *et al.*, 2010). Although the current data point towards a common mechanism underlying impulsivity and addiction-related behavior in mice, we underscore that the association of impulsivity and addiction-related behavior may depend on separate genetic loci that are both present among BXD16 and C57BL/6J mice. Studies that identify genetic loci underlying impulsivity and determine their effect on addiction-related behavior are currently underway.

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