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Association of novelty-related behaviors and intravenous cocaine self-administration in Diversity Outbred mice

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Abstract

Rationale—Preference for and reaction to novelty are strongly associated with addiction to cocaine and other drugs. However, the genetic variants and molecular mechanisms underlying these phenomena remain largely unknown. Although the relationship between novelty- and addiction-related traits has been observed in rats, studies in mice have failed to demonstrate this association. New, genetically diverse, high-precision mouse populations including Diversity Outbred (DO) mice provide an opportunity to assess an expanded range of behavioral variation enabling detection of associations of novelty- and addiction-related traits in mice.

Methods—To examine the relationship between novelty- and addiction-related traits, male and female DO mice were tested on open field exploration, hole board exploration, and novelty preference followed by intravenous cocaine self-administration (IVSA; ten 2-hour sessions of fixed-ratio 1 and one 6-hour session of progressive ratio).

Results—We observed high variation of cocaine IVSA in DO mice with 43% reaching and 57% not reaching conventional acquisition criteria. As a group, mice that did not reach these criteria still demonstrated significant lever discrimination. Mice experiencing catheter occlusion or other technical issues ($n = 17$) were excluded from analysis. Novelty-related behaviors were positively associated with cocaine IVSA. Multivariate analysis of associations among novelty- and addiction-related traits revealed a large degree of shared variance (45%).

Conclusions—Covariation among cocaine IVSA and novelty-related phenotypes in DO mice indicates that this relationship is amenable to genetic dissection. The high genetic precision and phenotypic diversity in the DO may facilitate discovery of previously undetectable mechanisms underlying predisposition to develop addiction disorders.

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INTRODUCTION

Personality traits such as impulsivity, reaction to novelty, and preference for novelty predispose individuals to initiate or progress from controlled to compulsive psychostimulant use. Although exposure to psychostimulants may itself affect novelty related traits in humans (Ersche et al. 2010), rats exhibiting elevated impulsivity (Belin et al. 2008; Molander et al. 2011), elevated preference for novelty (Belin et al. 2011), elevated reactivity to a novel environment (Piazza et al. 1990), or reduced anxiety (Bush and Vaccarino 2007; cf. Dilleen et al. 2012), are more likely to initiate or develop compulsive patterns of psychostimulant use (reviewed in Bardo et al. 2013; Belin and Deroche-Gamonet 2012; Dalley et al. 2011; Flagel et al. 2014).

The relationship between predisposing and addiction-related traits in experimental animals suggests the existence of common underlying mechanisms. In support of this hypothesis, Cervantes et al. (2013) have recently provided evidence which suggests a genetic relationship between impulsivity and cocaine self-administration in BXD recombinant inbred mice. However, the genetic underpinnings of the relationship between novelty- and addiction-related traits remain largely unexplored. Despite this, these traits are heritable in humans (Goldman et al. 2005) and mice (Kliethermes and Crabbe 2006; Ruiz-Durantez et al. 2006) making them amenable to genetic dissection. This is particularly true for cocaine addiction as it has the highest heritability of all abused drugs ($h = 0.72$; Goldman et al. 2005).

Past studies examining interrelationships between novelty- and addiction-related traits have been conducted using mouse and rat populations with limited genetic diversity and precision. This limits interpretability of these studies for two reasons. First, the low allelic diversity in these populations contributes to limited behavioral variation, a characteristic that has been suggested as the reason for prior failures to observe relationships between novelty- and addiction-related traits in mice (Kliethermes et al. 2007). Second, due to widespread linkage of loci across the genome in these populations (Payseur and Place 2007; Petkov et al. 2005), it is possible that some previously observed relationships between novelty- and addiction-related traits in rats are not the result of pleiotropic actions of the same polymorphic loci, but instead reflect parallel effects due to genetic linkage.

Recently developed mouse populations including the Collaborative Cross inbred strains (CC) (Aylor et al. 2011; Chesler et al. 2008; Churchill et al. 2004; Collaborative Cross Consortium 2012; Philip et al. 2011) and the Diversity Outbred mouse population (DO) (Churchill et al. 2012; Logan et al. 2013; Svenson et al. 2012) were designed to overcome these limitations through high allelic diversity and recombination precision. These populations were derived from an intercross of eight mouse strains consisting of five commonly used strains derived from the earliest laboratory strains (A/J, C57BL/6J, 129S1/

SvImJ, NOD/LtJ, NZO/HILtJ) and three wild derived strains (CAST/EiJ, PWK/PhJ, and WSB/EiJ) (Chesler et al. 2008). Both the DO and CC offer advantages over commonly used experimental populations such as (1) substantially increased genetic diversity compared to classical laboratory mouse strains (Yang et al. 2011), (2) high behavioral diversity (Philip et al. 2011, Logan et al. 2013), (3) high precision quantitative trait locus (QTL) mapping of behaviors (Philip et al. 2011, Logan et al. 2013), and (4) reduced linkage disequilibrium enabling dissociation of relationships caused by true pleiotropic effects from those secondary to genetic linkage. Moreover, DO mice are outbred, providing a tremendous source of novel allelic combinations, the potential for high sample size mapping studies, and restoration of the broad and continuous range of behavioral phenotypes which were constrained in the derivation of common mouse resources (for review, Chesler 2014). This expanded range of variation enables detection of variation and covariation not typically observed in laboratory mice.

In the present study, we assessed the relationship between intravenous cocaine self-administration (IVSA) and several novelty-related behaviors (activity and center time in a novel open field, exploration of a hole board, and novelty preference) in male ($n = 51$) and female ($n = 47$) DO mice. We chose to examine multiple novelty-related behaviors because they likely represent phenotypically distinct and genetically independent constructs (Kliethermes and Crabbe 2006). The added diversity in the DO, combined with the high construct validity of the cocaine IVSA paradigm, provides an opportunity to discover interrelationships between novelty- and addiction-related traits which have been undetected in previous mouse genetic studies.

MATERIALS AND METHODS

Subjects

The DO population was derived from 144 CC lines obtained from Oak Ridge National Laboratory (Chesler 2008) at generations $F_4 - F_{12}$ of inbreeding (Churchill et al. 2012). DO mice (J:DO, JAX stock number 009376) from $G_{10} - G_{12}$ were obtained from the mouse production facility at The Jackson Laboratory (JAX) at 4 weeks of age and transferred to the housing and phenotyping facility via standard JAX shipping container. Mice were housed in same sex groups of 3 – 5 in duplex polycarbonate cages on ventilated racks which provided 99.997% HEPA filtered air to each cage. The lid of each cage was fitted with a filtered top which reduced cross-cage odor exposure when mice were removed from the ventilated racks for testing.

Mice were maintained in a climate-controlled room under a standard 12:12 light-dark cycle (lights on at 0600 h). Bedding was changed weekly and mice were provided free access to food (NIH31 5K52 chow, LabDiet/PMI Nutrition, St. Louis, MO) and acidified water. A Nestlet and Shepherd Shack were provided in each cage for enrichment. All procedures and protocols were approved by The Jackson Laboratory Animal Care and Use Committee and were conducted in compliance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Behavioral Testing

DO mice (N = 98) were assessed for exploration in an open field, novelty preference in a divided open field, and exploration in a hole board apparatus (Kliethermes and Crabbe 2006). Increased time spent in the center of an open field is commonly considered to reflect reduced levels of anxiety in mice, but the motivation to explore a novel environment is dependent on neophobia of that environment (Bailey and Crawley 2009; Crawley 1985). Therefore, time spent in the center of the open field during novel open field testing was used as an additional novelty-related measure.

Following assessment of novelty-related behaviors, mice were implanted with a jugular catheter and tested on a cocaine IVSA task (1 mg/kg/infusion) using a fixed-ratio 1 (FR1) schedule. Following 10 sessions of testing using these criteria, mice were tested for a single session on a progressive ratio (PR) schedule to assess motivation to take cocaine. Following behavioral testing, interrelationships between IVSA and novelty-related measures were assessed.

Novelty-related behavioral testing

Open field testing occurred on day 1, novelty preference testing occurred on day 2, and hole board exploration testing occurred on day 3. Mice were between 12 – 16 weeks of age on the first day of testing and were habituated to the testing room for at least 48 hours prior to testing. All pieces of apparatus were cleaned using 70% ethanol between each mouse. Novelty testing was conducted between 10 AM and 2 PM.

Apparatus—All behavioral tests were performed using eight VersaMax Animal Activity Monitors (AccuScan Instruments, Inc; Columbus, OH). Each square arena (39 × 39 × 39 cm) was constructed of clear acrylic. Infrared detectors enabling automated data collection were positioned around the bottom edges of the arena walls. Arenas were housed in a rectangular room (3 × 4.5 meters) and illumination in each apparatus was maintained at 150 – 200 lux. Open field activity was collected in an empty arena. An insert which separated each arena into two equally-sized rectangular-halves (one with clear walls, the other with black walls) was used for novelty preference testing. The two sides were connected by a small opening in the central partition. A separate insert consisting of 16 holes positioned in a 4 × 4 grid was used for hole board exploration testing. The open field apparatus was in the same location when assessing open field activity, hole board exploration, and novelty preference.

Open field activity—Mice were placed into the center of the empty arena and allowed to explore for 20 minutes. Total distance traveled, time in center, and additional measures were recorded. We chose to use a relatively short 20 minute session because the environment becomes less novel over time and our goal was to assess novelty reactivity.

Novelty preference—A partition was placed into the arena to create two equally spaced halves. Mice were placed into one side of the two-sided arena (the familiar side) and allowed to explore for 10 minutes. The wall color of the familiar side (black or clear) was randomized for each mouse. During this initial phase, access to the other side of the chamber

(the novel side) was blocked. Immediately following this initial 10-minute familiarization phase, mice were allowed access to both the familiar and novel sides of the arena for 10 minutes. The time spent in each side of the arena during this 10-minute testing phase was recorded.

Hole board exploration—The hole board insert was placed into the arena. Mice were placed into the center of the arena and allowed to explore for 10 minutes. The total number of exploratory hole pokes was recorded.

Intravenous cocaine self-administration

Catheterization surgery—Following testing on novelty-related paradigms, an indwelling catheter was implanted into the right external jugular vein under oxygen/isoflurane anesthesia using procedures described in detail by Thomsen and Caine (2007) and Kmietek et al. (2012). Briefly, the catheter was inserted 12 mm into the jugular vein and anchored with sutures. The catheter traveled subcutaneously to an incision at the mid scapular region and attached to an external vascular access harness (Instech Laboratories, Inc., Plymouth Meeting, PA).

Apparatus—IVSA data were collected using 16 Med Associates operant conditioning chambers (307W) enclosed in sound attenuating cubicles (ENV-022MD). Two retractable response levers (ENV-310W) were mounted on the front wall. A stimulus light (ENV-321W) was mounted above each lever. A house light (ENV-315W) was centrally mounted on the rear wall. A 25 gauge single-channel stainless steel swivel was mounted to a counterbalanced lever arm attached to the outside of the chamber. Tubing was used to connect a syringe mounted on the infusion pump to the swivel, and to connect the swivel to a vascular access harness. Operant conditioning chambers were controlled by a Med Associates control unit using MED-PC IV software.

Acquisition of intravenous cocaine self-administration—Following three days of post-surgical recovery, mice were tested for 5 consecutive days on a cocaine IVSA paradigm using an FR1 schedule at a dose of 1.0 mg/kg/infusion. Infusion rate was 11.76 μ l per second and infusion duration was 2 seconds. Each day mice were tested for two 2-hour sessions separated by a 2-hour timeout resulting in a total of 10 testing sessions. IVSA testing began at 9 AM each day. During the entire IVSA study, mice were continuously housed in the operant conditioning chambers with free access to food and water. To verify and maintain patency, mice were briefly removed from the chamber once daily at which point blood was aspirated and catheters were flushed with 25 μ l of a heparin lock solution (100 U/ml heparin/saline). Mice were dropped from the study if catheters became non-patent.

Each 2-hour session began with the extension of the two response levers. A left (active) lever press resulted in a cocaine infusion and the illumination of both stimulus lights for two seconds with house light off. This was followed by a twenty-second time-out during which the house light was off and lever presses were recorded but had no consequences. The following dependent variables were collected during each 120 min cocaine IVSA session:

(1) number of infusions, (2) number of responses on the active lever, and (3) number of responses on the inactive lever. All mice were tested for 10 sessions.

Progressive ratio—Following the acquisition stage of cocaine IVSA testing, all mice were tested on a PR schedule for a single session to assess motivation to take cocaine. The schedule was identical to the FR1 schedule used during the acquisition stage with the exception that, following an earned cocaine infusion, the number of active lever presses required to receive a subsequent cocaine infusion was increased within the session as follows: 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, etc. The number of responses required for each infusion was determined using the following equation: $[5e^{(\text{infusion number} \times 0.2)}] - 5$ (Richardson and Roberts 1996). Breakpoint was reached when 60 min had elapsed without earning a cocaine infusion and corresponded to the last completed step (i.e., the total number of infusions earned during the session). If breakpoint was not reached within 6 hours the session was terminated.

Drugs

Cocaine hydrochloride was obtained from the NIDA Drug Supply Program. Non-sterile cocaine solution was filtered through 0.22 μm syringe filters. Heparinized saline was purchased from Fisher Scientific (Pittsburgh, PA).

Mortality and attrition of experimental subjects

Although 98 mice were tested on the three novelty-related behavioural tasks, hole board exploration data were unavailable for 6 mice and novel place-preference data were unavailable for 3 mice because of equipment failure. Open field data were unavailable for 1 mouse and novel place-preference data were unavailable for 1 mouse because these mice escaped from the apparatus during testing.

Following testing on novelty-related paradigms, 87 mice were successfully catheterized. Out of these mice, 17 mice did not complete cocaine IVSA testing for the following reasons: non-patent catheter ($n = 7$), systemic infection ($n = 5$), local infection at the catheter port ($n = 3$), cocaine overdose ($n = 1$), air embolism ($n = 1$). These mice were not included in analyses involving cocaine IVSA measures. Mice from cohort 1 were tested on a different version of the PR schedule than those from subsequent cohorts, and were therefore excluded from the PR analysis. Performance distributions of mice that completed novelty testing but did not complete cocaine IVSA testing are provided in Supplementary Figure 1. Performance of these two groups did not differ significantly on any of the novelty-related assays ($p > .21$ for all tests).

Statistical analysis

Prior to performing inferential statistics, normality and linearity of all measures were assessed by inspecting normal probability plots and scatterplots. Z-scores and Mahalanobis distance scores were examined to identify univariate and multivariate outliers, respectively. Z-scores in excess of the absolute value of 3.29 (outlier at $p < .001$, two-tailed test) and extreme Mahalanobis distance scores (outlier at $p < .001$, χ^2 value) were considered significant outliers. Data which violated statistical assumptions were, depending on the

severity of the violation, square root or log base 10 transformed using the methods recommended by Tabachnick and Fidell (2007). One-way and factorial mixed-design analysis of variance (ANOVA) was used to assess group differences on novelty-related and cocaine IVSA measures. Pearson product-moment correlations and canonical correlation (Sherry and Henson 2005; Tabachnick and Fidell 2007) were used to assess interrelationships between novelty-related and cocaine IVSA measures. When repeated-measures ANOVA was used, the assumption of homogeneity of variance across groups was assessed using Mauchly's test of sphericity. When this assumption was violated, the Huynh-Feldt correction was used.

RESULTS

Outlier detection

Univariate outliers were identified for several measures including active lever presses ($z = 3.67$), inactive lever presses ($z = 5.23$), time in the center of the open field ($z = 3.42$), and exploratory hole pokes ($z = 4.27$). Notably, the elevated z-scores in these four mice reflect statistically significant abnormal behaviour on these measures as opposed to equipment failure or some other artifact related to testing. In isogenic populations, these aberrant values typically reflect environmentally driven events or other experimental noise. In a high diversity population such as the DO, outliers are to be expected due to the presence of rare combinations of alleles. With high sample sizes, the genetic basis of systematic outlier behaviour can be uncovered, highlighting a key advantage of utilizing an outbred population with high genetic and phenotypic diversity such as the DO.

Novelty preference

Prior to examining the relationships between novelty- and addiction-related behaviors, we examined performance on the novelty preference task to determine if mice exhibited a preference for the novel side during the choice stage and if this preference was affected by side color. We performed a $2 \times 2 \times 2$ mixed-design ANOVA with percentage of time on a side as the dependent variable, color of the novel side (black or clear) and sex as between-subjects factors, and side-novelty (novel or familiar) as a within-subjects factors. Repeated measures ANOVA revealed a statistically significant two-way interaction of side-novelty and color of the novel side [$F(1, 91) = 16.80, p = 9.00 \times 10^{-5}$] as well as a significant main effect of color of the novel side [$F(1, 91) = 22.75, p = 6.98 \times 10^{-6}$]. Post hoc tests indicated that mice spent significantly more time on the novel side when walls were black ($M = 58.87\%$, $SD = 15.03$) than when walls were clear ($M = 44.89$, $SD = 17.50$). When collapsing across color of the novel side, mice as a group demonstrated a small but non-significant preference for the novel side ($M = 51.96$, $SD = 17.67$) over the familiar side ($M = 48.04$, $SD = 17.67$) [$F(1, 91) = 1.25, p = .26$].

Interrelationships between novelty-related measures

To assess relationships among the four novelty-related measures, we performed Pearson product-moment correlations. As shown in Table 1, all dependent measures were positively correlated. With the exception of novelty preference, all of these relationships reached statistical significance. Additionally, total distance traveled in the three novelty-related tasks

was positively associated. Specifically, total distance traveled in the open field was positively correlated with total distance traveled in the novelty preference task ($r = .45$, $p < .001$) and the hole board exploration task ($r = .53$, $p < .001$). In order to statistically correct for the significant effect of novel side color on novelty preference scores, the novelty preference measure was regressed on color of the novel side to create a residual. This residual was used as the novelty preference measure in this and all subsequent analyses.

Sex differences on novelty-related measures

To assess the effect of sex on performance during novelty-related tasks, we performed one-way ANOVAs with sex as the independent factor and each of the novelty-related measures as dependent variables. As shown in Table 2, mean values for all novelty-related measures were higher in female mice. These differences reached statistical significance on the hole pokes measure [$F(1, 90) = 11.94$, $p = 8.37 \times 10^{-4}$] and approached statistical significance on the total distance traveled in the open field measure [$F(1, 95) = 3.42$, $p = 6.71 \times 10^{-2}$].

Intravenous cocaine self-administration in DO mice

Acquisition—Out of the 70 DO mice that completed the 1 cocaine IVSA testing sessions, 18 males and 12 females met and 20 males and 20 females did not meet conventional acquisition criteria (i.e., ≥ 10 infusions and $\geq 75\%$ on the active lever for 3 consecutive sessions). As would be expected in a phenotypically diverse population such as the DO, we observed substantial variability in both active and inactive lever pressing across all mice. All 70 mice earned cocaine infusions during the course of testing. The range of infusions summed across the 10 sessions was 4 to 236, respectively ($M = 113.21$, $SD = 63.96$).

To assess performance in mice meeting acquisition criteria, we performed a $10 \times 2 \times 2$ mixed-design ANOVA with number of lever presses as the dependent variable, session (1 – 10) and side (active or inactive) as within-subjects factors, and sex as a between-subjects factor. ANOVA revealed significant main effects of session [$F(9, 252) = 3.65$, $p = 1.35 \times 10^{-3}$] and lever [$F(1, 28) = 160.75$, $p = 4.02 \times 10^{-13}$] as well as a significant session $\times$ lever interaction [$F(9, 252) = 2.50$, $p = 2.79 \times 10^{-2}$]. Sex differences did not reach statistical significance. Post hoc tests were examined to determine the nature of the significant session $\times$ lever interaction. As shown in Figure 2A, mice that met acquisition criteria began to discriminate between the active and inactive levers on the first session ($p = 1.45 \times 10^{-4}$) and continued to do so for all subsequent sessions. The percentage of responses on the active lever increased across the first five sessions and remained stable at 80 – 90% for all subsequent sessions.

Although it is common to categorize mice as having acquired or not acquired cocaine IVSA based on a set of criteria including number of infusions and percentage of responses on the active relative to the inactive lever (Thomsen and Caine 2007), these acquisition criteria are somewhat arbitrary and may obscure the detection of genetic effects on variation in acquisition related traits. It is likely that some, though not all mice which fail to reach these criteria are responding for cocaine, albeit to a somewhat lesser degree. To examine this possibility, we assessed IVSA performance in the group of 40 DO mice that did not to reach acquisition criteria. Repeated measures ANOVA revealed significant main effects of session

[$F(9, 342) = 5.63, p = 1.98 \times 10^{-5}$] and lever [$F(1, 38) = 33.89, p = 1.00 \times 10^{-6}$]. As shown in Figure 2B, post hoc tests indicated that, as a group, mice that did not reach formal acquisition criteria individually nevertheless showed a statistically significant preference for the active lever ($p < .05$) on all sessions with the exception of session 8.

Progressive ratio—Following 10 sessions of testing on the FR1 schedule, all mice were tested for a single session on the PR schedule. Breakpoints ranged between 0 and 19 in male mice ($M = 5.28, SD = 4.93$) and 0 and 16 in female mice ($M = 5.68, SD = 5.27$). There was no significant effect of sex on breakpoint. Mice that met acquisition criteria reached a higher breakpoint ($M = 6.85, SD = 5.43$) than mice that did not meet acquisition criteria ($M = 5.51, SD = 5.16$), although this difference did not reach statistical significance. As shown in Figure 3D and 3H, the range of breakpoints was the same for both groups. As shown in Table 1, breakpoint was significantly positively correlated with active lever presses on the FR1 schedule. However, the strength of this relationship was modest ($r = .26$), supporting the hypothesis that progressive and fixed ratio schedules measure distinct constructs (Richardson and Roberts 1996).

Relationships between novelty- and addiction-related behaviors

Bivariate relationships—Although we tested mice in two separate sessions each day to maximize training during the five day acquisition period, we used the mean of the last two days of cocaine IVSA testing when assessing the relationship between novelty-related measures and IVSA FR1 measures. We specifically considered the second 2-hour session on each of these days in order to minimize inclusion of (1) variability due to the initial loading phase which occurs during cocaine IVSA (Ahmed and Koob 2005; Lynch and Carroll 2001; Panlilio et al. 2003; Tornatzky and Miczek 2000), and (2) responding for non-drug stimuli such as stimulus lights which occurs most robustly early during exposure (Olsen and Winder 2009; compare first hour responses of 6-hour group presented in Fig 4B with total session responses presented in Fig 4A of this paper). In the present study, these effects are evidenced by significantly higher active lever responding on the first relative to the second testing session on all testing days with the exception of the first, $F(1, 69) = 22.12, p = 1.30 \times 10^{-5}$.

To estimate trait covariation in a highly diverse population, it is important to avoid range restriction. Therefore, all mice that completed cocaine IVSA testing were included in correlational analyses, regardless of whether they met conventional acquisition criteria. This also served to maintain sufficient power to detect correlations. Distributions of mean performance on four measures obtained during the last session of the final two days of IVSA are shown in Figure 3, separated by attainment of acquisition criteria. Overall, there were significantly fewer self-infusions [$F(1, 68) = 26.03, p = 3.00 \times 10^{-6}$] and active lever presses [$F(1, 68) = 17.17, p = 9.60 \times 10^{-5}$] performed by mice that did not reach acquisition criteria, as expected by definition. The number of inactive lever presses did not differ significantly between the two groups, although six mice that did not reach acquisition criteria responded on the inactive lever more than ten times, while only one mouse that reached acquisition criteria exhibited this behavior.

To assess the bivariate relationships between novelty-related behaviors and cocaine IVSA, we performed Pearson product-moment correlations using each of the novelty- and addiction-related measures. As shown in Fig 4A, the number of exploratory hole pokes was significantly positively correlated with the number of cocaine infusions. Also, as shown in Figure 4B, time in the center of the open field was significantly positively correlated with the number of active lever presses. Bivariate relationships of all novelty- and addiction-related measures are shown in Table 1.

Multivariate relationships—A canonical correlation analysis (Sherry and Henson 2005; Tabachnick and Fidell 2007) was performed to assess the multivariate shared relationship between the two novelty- and addiction-related variable sets. The set of novelty-related variables consisted of distance traveled in the open field, time in the center of the open field, novelty preference, and hole board exploration. The set of addiction-related variables consisted of infusions on the FR1 schedule, breakpoint, and IVSA acquisition (i.e., met or did not meet IVSA acquisition criteria). Both breakpoint and FR1 infusions were included in the analysis because these measures index independent facets of drug use (Richardson and Roberts 1996), evidenced by the modest strength of their association in the present study ($r = .23$). To avoid high multicollinearity, active lever presses on the FR1 schedule was excluded from the analysis because it was highly correlated with FR1 infusions ($r = .925$, $p = 3.80 \times 10^{-23}$). All other within-set Pearson product-moment correlation coefficients were $.54$. Only mice with scores on all 6 dependent measures were included in the analysis, resulting in an overall sample size of $N = 53$. To meet assumptions of normality, a square root transformation was applied to center time in the open field. Following this transformation, no univariate or multivariate outliers were identified.

The analysis yielded four canonical correlations which had magnitudes of $.61$ (37% overlapping variance), $.32$ (11% overlapping variance), $.14$ (2% overlapping variance), and $< .01$ ($< 1\%$ overlapping variance). When all four canonical functions were included, the full model was statistically significant [$F(16, 138.11) = 1.86$, $p = 2.80 \times 10^{-2}$, Wilks $\lambda = .5495$] and explained 45% of the shared variance between the variable sets. Canonical functions 2 to 4, 3 to 4, and 4 alone did not explain a statistically significant amount of shared variance between the variable sets ($p > .70$ on all tests). Therefore, as shown in Figure 5, the first set of canonical variates was sufficient to account for the significant relationships between the novelty- and addiction-related variable sets.

The standardized canonical function coefficients, structure coefficients, and squared structure coefficients for the first canonical function are presented in Table 1. Using a cutoff correlation of $.3$, the novelty-related measures that were correlated with the first canonical variate were distance traveled in the open field, exploratory hole pokes in the hole board, and time in the center of the open field. Novelty preference was not correlated with the first canonical variate. The addiction related measures that were correlated with the first canonical variate were infusions on the FR1 schedule and breakpoint. Thus, the first pair of canonical variates indicates that a high propensity to explore novel environments, as assessed using multiple measures, is associated with a high propensity to self-administer cocaine. Notably, the novelty-related measures explained a relatively large degree of variance (45%) in the addiction-related measures.

DISCUSSION

In the present study, we have demonstrated in a high-precision and genetically diverse mouse population that multiple novelty-related measures are predictive of cocaine IVSA. DO mice were assessed on a group of four novelty-related measures consisting of open field exploration, time in the center of the open field, hole board exploration, and novelty preference. Following assessment on the novelty-related tasks, jugular catheters were implanted and mice were tested on a cocaine IVSA task. Multivariate analysis revealed that open field exploration, time in the center of the open field, and hole board exploration were significantly positively related to cocaine IVSA (Table 3 and Figure 5). Collectively, novelty-related measures accounted for a large degree of variance (45%) in the addiction-related measures.

Although phenotypic relationships between novelty-related behaviors and cocaine IVSA have previously been reported in rats, we are unaware of any previous studies showing that novelty-related behaviors are predictive of intravenous cocaine IVSA in mice. Thus, results from the present study indicate that these relationships can be modeled in the mouse species and offer further support for the hypothesis that similar biological mechanisms underlie novelty- and addiction-related traits. Most importantly, the present study indicates that the genetically and phenotypically diverse DO population can be used to discover the underlying genes and mechanisms driving these relationships. Indeed, as discussed below, results from prior studies suggest that the additional phenotypic diversity in the DO may be required to detect relationships between novelty- and addiction-related traits in mice.

Novelty-related behaviors in Diversity Outbred mice

With the exception of novelty preference, all novelty related variables were significantly, though modestly, positively intercorrelated (Table 1). One reason for the modest strength of these relationships may be the genetic independence of these novelty-related traits (Kliethermes and Crabbe 2006). In the case of novelty preference, which was least related to other tests, it is possible that mice experienced less anxiety during this task relative to other tasks due to a habituation phase. Specifically, in order to create a situation in which one side of the apparatus was relatively less familiar (i.e., more novel), mice were introduced to the apparatus and allowed to explore one side prior to the testing phase. In the other two tasks, testing began immediately when mice were placed into the apparatus. Previous studies using different testing methods and apparatus have observed a more robust preference for novelty in mice (Adriani et al. 1998; Palanza et al. 2001). Thus, it is possible that refinements to the task, such as the addition of tactile cues or extending habituation to the familiar side, may result in a more robust side preference.

Intravenous cocaine self-administration in Diversity Outbred mice

Mice that met acquisition criteria (43%) exhibited variation in active lever preference, with some mice responding almost exclusively on the active lever following acquisition, while others continued to sample the inactive lever. DO mice that did not meet acquisition criteria individually (57%) nevertheless showed significant lever discrimination as a group. What is most striking about these mice is the range of response patterns. For example, some mice

sampled the active lever but never consistently responded, others consistently responded on both levers but did not discriminate between them, and others appeared to acquire but then did not maintain that pattern of responding across sessions. That some mice did not meet conventional acquisition criteria may be viewed as a limitation. However, several factors suggest that high variability in performance observed in the present study was due to the expanded genetic diversity in the DO population, not experimental artifacts. First, mice experiencing catheter failure, systemic infection, or other technical issues ($n = 17$) were excluded from analysis, indicating that these factors do not explain response patterns in mice that did not reach conventional acquisition criteria. Second, all mice included in the analysis received cocaine infusions during the study (range 4 – 236). Thus, all mice experienced pairings of active lever pressing and cocaine infusions. Third, in contrast to an inbred strain such as C57BL/6J, mice from the genetically diverse DO population would be expected to express high phenotypic diversity on all traits which control responding in the cocaine IVSA paradigm. Therefore, it seems likely that mice which did not meet conventional acquisition criteria did so as a result of the combined effects of broad phenotypic diversity on the spectrum of processes affecting cocaine acquisition and use in IVSA paradigms such as reaction to novelty (present study), impulsivity (Cervantes et al. 2013), rewarding and aversive effects of cocaine (Rademacher et al. 2000), regulation of drug intake (Lynch and Carroll 2001), rewarding effects of non-drug stimuli (Olsen and Winder 2009), and instrumental and Pavlovian learning and memory processes (Beckmann et al. 2011; Schindler et al. 2002). Furthermore, this suggests that the common practice of excluding from statistical analysis mice that do not reach predetermined acquisition criteria may be ill-advised when using genetically diverse populations as this technique serves to reduce the phenotypic diversity of the sample and, as a consequence, the power to detect relationships between behavior and underlying biological processes.

Association of novelty-related behaviors and intravenous cocaine self-administration in Diversity Outbred mice

Statistically significant bivariate relationships were observed (Figure 4A and 4B) between cocaine IVSA and two of the four novelty-related traits (hole board exploration and center time in the open field). These findings in mice are consistent with previous findings in humans indicating that high novelty seeking and low anxiety are positively related to cocaine and other drug use (Gunnarsdottir et al. 2000; Wills et al. 1994). However, these bivariate relationships were fairly modest with respect to overall variance explained. In contrast, when we considered the multivariate relationship between the four novelty-related traits and the addiction related traits in the present study, it was evident that a much larger degree of variance was shared between these two variable sets than was suggested by the bivariate relationships alone (Figure 5 and Table 3). This observation is consistent with that from a previous study in which Cain et al. (2005) observed that the use of multiple novelty-related traits (open field activity, novel object exploration, and novelty preference), in comparison to a single trait, significantly improved prediction of amphetamine IVSA in rats using a regression model. One reason for this may be that bivariate analysis does not adequately account for the complex relationships within and between the varied psychological constructs which underlie novelty-related and addiction-related behaviors. (Arnett 1994; Belin et al. 2011; Bush and Vaccarino 2007; Castellani and Rugle 1995;

Deroche-Gamonet et al. 2004; Dilleen et al. 2012; Molander et al. 2011; Patkar et al. 2002; Patkar et al. 2004; Piazza et al. 1990).

Although there were no main effects of sex on addiction related measures, effects on novelty-related measures were observed; some of these have been previously reported by others (Adriani and Laviola 2000; Beatty 1979; Goodrich and Lange 1986; Gray 1971; Hughes et al. 2004; Ray and Hansen 2004; Siuciak et al. 2007). The high correlation of sex to the first canonical covariate in the multidimensional analysis reflects that variation in novelty-related traits are in part influenced by sex of the mouse, and this must be accounted for when assessing the correlation among novelty- and addiction-related measures. We included this measure on the addiction side of the canonical correlation because it is a predictor of novelty related traits. It is independent of IVSA measures, but enables detection of the canonical correlation of novelty and addiction related behaviors.

In contrast to a previous study in which Kliethermes et al. (2007) found that hole board exploration and oral methamphetamine self-administration were unrelated in mice selectively bred for high and low exploration of a hole board apparatus, we were able to detect such a relationship in the DO. High and low responders in Kliethermes et al. were derived from a B6D2 cross. The increased genetic diversity in DO mice (Chesler 2014) may have enabled detection of relationships which were driven by polymorphisms not present in the B6 and D2 inbred strains from which selected lines were derived in Kliethermes et al. Indeed, several behavioral traits exhibit low between-strain variation in the B6 and D2 strains (Philip et al. 2010), and Kliethermes et al. suggested that the low levels of methamphetamine intake observed in their study were likely due to limited genetic diversity in these strains. A second consideration is that the route of administration differed between the two studies, with Kliethermes et al. using an oral self-administration paradigm while an intravenous self-administration paradigm was employed in the present study. This may be relevant because Kliethermes et al. observed marked aversion to the methamphetamine solution which resulted in low levels of intake across groups, likely creating a floor effect which limited the ability to detect relationships between methamphetamine oral self-administration and hole board exploration. Finally, it should be noted that Kliethermes et al. examined genetic correlations between methamphetamine preference and hole board exploration using selected lines, while in the present study we examined phenotypic correlations between novelty-related traits and cocaine IVSA in individual DO mice.

Although we assessed relatively short-term cocaine use in the present study, some studies in rats have revealed that certain addiction-related traits only emerge following longer-term cocaine use (Belin et al. 2011; Deroche-Gamonet et al. 2004). In the present study, mice were tested daily in two independent two-hour sessions separated by a two-hour timeout and were housed in the testing chambers 24 hours a day during the six day IVSA testing period. In similar studies, mice have been tested in a single daily session and, with the exception of those testing sessions, were housed in the home cage (e.g., Cervantes et al. 2013; Dickson et al. 2014; Griffin and Middaugh 2003; Ruiz-Durantez et al. 2006). Housing in the testing chamber could reduce anxiety during the testing session or reduce the drive to explore the relatively less-novel environment. Prior to IVSA testing, mice were group housed and provided with enrichment materials consisting of a Nestlet and Shepard Shack.

Environmental enrichment (Chauvet et al. 2009; Gipson et al. 2011; Green et al. 2010) and group housing (Smith 2012; van der Veen et al. 2007) have been shown to affect cocaine self-administration. In the case of group housing, gene by environment interactions have been observed (van der Veen et al. 2007). In the present study, assessment of IVSA and novelty behaviors occurred during the light phase of the light:dark cycle. Strain-dependent effects of light-phase on some high-throughput behavioral tasks such as the elevated plus maze and open field have been observed (Hossain et al. 2004; Post et al. 2011). Many (Caine et al. 2012; Soria et al. 2008; Thomsen and Caine 2006; 2011; Zhang et al. 2009) though not all (Fuchs et al. 2003; Trigo et al. 2009; Ward et al. 2009) mouse IVSA studies have been conducted during the light phase.

The Diversity Outbred population as a resource for the discovery of genes and mechanisms underlying addiction and predisposing traits

The ability to use mice to examine the genetic underpinnings of the novelty-addiction relationship carries distinct advantages, and the mouse remains the most deeply characterized and cost effective resource for genetic analysis. Several mouse genetic resources have been employed for the study of addiction including the BXD recombinant inbred strains (Belknap and Crabbe 1992; Belknap et al. 1993; Crabbe et al. 1994; Gallaher et al. 1996; Grisel et al. 1997; Kest et al. 2004; Philip et al. 2010; Phillips et al. 1998; Roberts et al. 1995; Tarantino et al. 1998), F₂ hybrid crosses (Bergeson et al. 2001; Doyle et al. 2008; Ferraro et al. 2005; Kest et al. 2004), the Hybrid Mouse Diversity Panel (Bryant et al. 2012a; Ghazalpour et al. 2012; Park et al. 2011; Parker et al. 2012a), and advanced intercross lines (Bryant et al. 2012b; Parker et al. 2012a; Parker et al. 2012b; Samocha et al. 2010). While these resources have enabled substantial progress in the understanding of mechanisms underlying the effects of addictive drugs, each has some limitations in power, precision, genetic diversity, and genetic architecture. The Collaborative Cross inbred strains (CC), DO mouse population, and related populations (Ghazalpour et al. 2012; Iancu et al. 2010) complement these resources with a wealth of allelic variation, high mapping precision and a greater range of behavioral diversity (reviewed in Chesler 2014). Allelic variants can be identified using a QTL mapping approach in combination with functional genomic data resources (Recla et al. 2014). We have successfully mapped several additive behavioral QTLs in the DO using approximately 300 mice, though more power will enable reliable detection of a larger number of loci and more complex genetic effects (Logan et al. 2013). Because DO mice are generated from the same eight founder strains as the CC and these founders have been sequenced (Keane et al. 2011), CC lines can be intercrossed to assess allelic effects at the QTLs identified using the DO. Once identified, positional candidate genes can be validated with knockout mice from the knockout mouse project (Austin et al. 2004) or derived using CRISPR/Cas systems (Wang et al. 2013; Yang et al. 2014).

Conclusions

Using mice from the genetically and phenotypically diverse DO population, we have shown that performance on multiple novelty-related tasks is predictive of cocaine IVSA. Novelty-related traits as a group explained 45% of the variance in addiction related traits, indicating the high explanatory power of the observed relationships in the DO population. A broad range of behavioral variation in acquisition and discrimination patterns was observed. The

high genetic diversity in the DO combined with the high construct validity of the cocaine IVSA paradigm enabled the detection of relationships between novelty- and addiction-related behaviors which have been undetected in past studies. These results indicate for the first time that the relationship between novelty-related traits and traits related to cocaine addiction can be modeled in mice, and that the enhanced genetic diversity in the DO population can be utilized to discover the underlying genetic mechanisms which drive the development of addiction through predisposing personality traits.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Performance distributions of all mice tested on novelty and intravenous cocaine self-administration assays.

Figure 2.

Intravenous cocaine self-administration in Diversity Outbred mice. (A) As a group, male ($n = 18$) and female ($n = 12$) mice that reached conventional acquisition criteria began to discriminate between the active and inactive levers on the first session ($p = 1.45 \times 10^{-4}$) and continued to do so for all subsequent sessions. (B) As a group, male ($n = 20$) and female ($n = 20$) mice that did not reach conventional acquisition criteria also showed significant lever discrimination on most days.

Figure 3.

Performance distributions of mice that reached (A – D) and did not reach (E – F) conventional intravenous cocaine self-administration acquisition criteria. Relative frequency (percentage) is plotted against mean performance on the last session of the final two days of testing for each of the measures.

Figure 4.

Bivariate relationships between novelty-related variables and cocaine IVSA. (A) Number of exploratory hole pokes and (B) center time in the open field were significantly positively correlated ($p < .05$) with number of cocaine infusions and active lever presses, respectively. The highest novelty and IVSA correlations are shown. Cocaine infusions and active lever presses were highly positively correlated (Table 1). $*p < .05$

Figure 5.

The multivariate shared relationship between the sets of novelty- and addiction-related variables. Novelty-related variables comprise distance traveled in the open field (cm), novelty preference, hole board exploration, and time in the center of the open field (s). Addiction-related variables comprise infusions on the FR1 schedule, breakpoint, IVSA acquisition, and sex. $*p < .05$

Pearson product-moment correlations of novelty-related and addiction-related dependent measures

Table 1

Dependent measures	Novelty-related				Addiction-related			
	Distance traveled OF (cm)	Hole pokes	Novelty preference (%)	Time in center OF (s)	Infusions FR1	Active presses FR1	Inactive presses FR1	Breakpoint PR
Novelty								
Distance traveled OF (cm)	-	-	-	-	-	-	-	-
Hole pokes	.273**	-	-	-	-	-	-	-
Novelty preference (%)	.156	.200	-	-	-	-	-	-
Time in center OF (s)	.547**	.316**	.157	-	-	-	-	-
Addiction								
Infusions FR1	.037	.257*	-.227	.192	-	-	-	-
Active presses FR1	.090	.220	-.225	.298*	.916**	-	-	-
Inactive presses FR1	.144	.181	-.103	.146	.260*	.394**	-	-
Breakpoint PR	.117	.203	-.002	.190	.231	.262*	.086	-

Notes. Degrees of freedom for correlations between two novelty-related variables were 88 – 95. Degrees of freedom for correlations between novelty- and addiction-related variables were 54 – 67. Degrees of freedom for correlations between two addiction-related variables were 68 if both variables were from the FR1 schedule and 59 if the correlation contained PR breakpoint.

**
p < .01

*
p < .05

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Table 2
Means and standard deviations of novelty-related behaviors in male and female Diversity Outbred mice.

Dependent Measures	Males	Females
Distance traveled OF (cm)	2125.18 (1037.97)	2516.28 (1041.53)
Hole pokes ^{**}	26.13 (11.17)	36.91 (18.20)
Novelty preference (%)	48.95 (17.50)	55.16 (17.47)
Time in center OF (s)	78.02 (71.82)	92.01 (73.08)

^{**}
p < .01

Table 3

Canonical correlation analysis of the relationship between novelty-related behaviors and addiction-related behaviors

Dependent Measures	Coef	r_s	r_s^2 (%)
Novelty			
Distance traveled OF (cm)	0.2905	<u>0.6381</u>	40.71
Hole pokes	0.8282	<u>0.9435</u>	89.01
Novelty preference (%)	-0.1445	-0.0541	0.29
Time in center OF (s)	0.0482	<u>0.5290</u>	27.98
Addiction			
Infusions FRI	0.7249	<u>0.6092</u>	37.11
Breakpoint PR	0.2550	<u>0.4181</u>	17.48
Acquired IVSA	-0.2250	0.2183	4.77
Sex of mouse	0.7237	<u>0.6922</u>	47.91

Notes. Structure coefficients greater than |.30| are underlined. Coef = standardized canonical function coefficient, r_s = structure coefficient, r_s^2 = squared structure coefficient;