Systems genetics of intravenous cocaine self-administration in the BXD recombinant inbred mouse panel
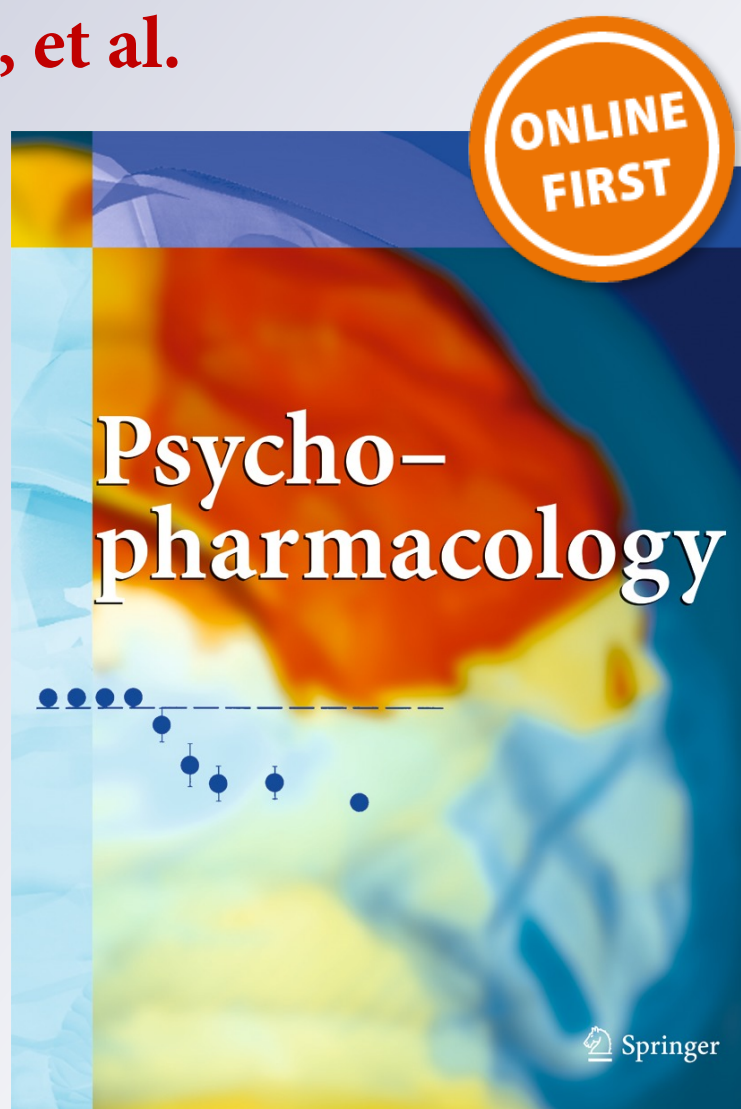
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ORIGINAL INVESTIGATION

Systems genetics of intravenous cocaine self-administration in the BXD recombinant inbred mouse panel

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Abstract

Rationale Cocaine addiction is a major public health problem with a substantial genetic basis for which the biological mechanisms remain largely unknown. Systems genetics is a powerful method for discovering novel mechanisms underlying complex traits, and intravenous drug self-administration (IVSA) is the gold standard for assessing volitional drug use in preclinical studies. We have integrated these approaches to identify novel genes and networks underlying cocaine use in mice.

Methods Mice from 39 BXD strains acquired cocaine IVSA (0.56 mg/kg/infusion). Mice from 29 BXD strains completed a full dose-response curve (0.032–1.8 mg/kg/infusion). We identified independent genetic correlations between cocaine IVSA and measures of environmental exploration and cocaine sensitization. We identified genome-wide significant quantitative trait loci (QTL) on chromosomes 7 and 11 associated with shifts in the dose-response curve and on chromosome 16 associated with sessions to acquire cocaine IVSA. Using publicly available gene expression data from the nucleus accumbens, midbrain, and prefrontal cortex of drug-naïve mice, we identified *Aplp1* and *Cyfip2* as positional candidates underlying the behavioral QTL on chromosomes 7 and 11, respectively. A genome-wide significant *trans*-eQTL linking *Fam53b* (a GWAS candidate for human cocaine dependence) on chromosome 7 to the cocaine IVSA behavioral QTL on chromosome 11 was identified in the midbrain; *Fam53b* and *Cyfip2* were co-expressed genome-wide significantly in the midbrain. This finding indicates that cocaine IVSA studies using mice can identify genes involved in human cocaine use.

Conclusions These data provide novel candidate genes underlying cocaine IVSA in mice and suggest mechanisms driving human cocaine use.

Keywords *Cyfip2* · *Fam53b* · *Aplp1* · Addiction · QTL · eQTL · Genetic correlation · Cocaine sensitization · Open field · Light dark box

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Introduction

After more than 40 years of sustained efforts to control the production, distribution, and consumption of cocaine (Global Commission on Drug Policy 2011), cocaine addiction persists as a widespread public health problem. Over 17 million people use cocaine worldwide (UNODC 2013). In the USA, there are over 1.6 million cocaine users, and 69 % of these individuals meet DSM-IV criteria for substance dependence or abuse (SAMHSA 2013). Cocaine has the highest abuse potential of all illicit drugs (Goldstein and Kalant 1990), and addiction to drugs including cocaine, alcohol, nicotine, and opiates is highly heritable (Goldman

et al. 2005). Twin studies reveal that heritability of heavy cocaine use and abuse is greater than 70 %, making cocaine addiction the most heritable of all addiction disorders (Goldman et al. 2005). The combination of high addiction liability and high heritability makes discovery of the genetic underpinnings of cocaine addiction a public health priority. Nevertheless, the genetic factors driving cocaine use and addiction remain largely unknown.

Although a variety of assays have been used to evaluate the reinforcing properties and behavioral effects of cocaine (Sanchis-Segura and Spanagel 2006), intravenous self-administration (IVSA) is widely considered to be the gold standard for assessing volitional drug use in preclinical studies (Mello and Negus 1996; Sanchis-Segura and Spanagel 2006; Thomsen and Caine 2007). The voluntary nature of drug use in IVSA mirrors that of human drug use and confers a high level of construct validity to the paradigm. To date, investigators utilizing the IVSA paradigm to probe the genetic mechanisms underlying cocaine use have primarily used a reverse genetics approach in which individual genes that are hypothesized to contribute to cocaine use are perturbed (e.g., knocked out), and the effect of these perturbations on the phenotype are assessed (Sora et al. 2010). While this approach provides rigorous genetic and environmental control, it relies on preexisting assumptions about genes underlying drug use (Hall et al. 2013), and this precludes discovery of novel and perhaps unexpected genes and mechanisms driving drug use. Furthermore, reverse genetics studies typically test the effect of gene perturbation on a single genetic background, hampering the identification of polygenic networks underlying behavioral traits. In contrast to the reverse genetics approach, systems genetics utilizes an experimental population with known trait variation to perform a global analysis of all biological molecules underlying that trait (Civelek and Lusis 2014). In this way, the genes and networks underlying a trait can be identified in the absence of a priori hypotheses.

In the present study, we used a systems genetics approach in the BXD recombinant inbred (RI) strains (Peirce et al. 2004) to discover novel genes and networks underlying cocaine IVSA. We assessed the acquisition of cocaine IVSA in mice from 39 BXD strains, obtained a complete dose-response curve in mice from 29 strains, and assessed heritability of these traits. We assessed genetic relationships between cocaine IVSA and commonly used assays indexing effects of experimenter-administered cocaine (sensitization, conditioned place preference) as well as phenotypes related to cocaine use in mice and rats (exploration in a novel open field and light/dark box). We identified genome-wide significant quantitative trait loci (QTL) associated with cocaine IVSA phenotypes. We used publicly available gene expression data from the nucleus accumbens (NAc), midbrain, and prefrontal cortex (PFC) of drug-naïve mice to prioritize candidate genes and identify genome-wide significant expression QTL (eQTL), genes, and networks associated with cocaine IVSA phenotypes.

Methods and materials

Subjects

Cocaine IVSA was assessed in male and female mice ($N=99$) from 44 BXD RI strains: BXD1/TyJ, BXD2/TyJ, BXD9/TyJ, BXD11/TyJ, BXD13/TyJ, BXD14/TyJ, BXD15/TyJ, BXD16/TyJ, BXD19/TyJ, BXD20/TyJ, BXD21/TyJ, BXD27/TyJ, BXD28/TyJ, BXD29/Ty, BXD31/TyJ, BXD32/TyJ, BXD33/TyJ, BXD34/TyJ, BXD36/TyJ, BXD39/TyJ, BXD40/TyJ, BXD43/RwwJ, BXD50/RwwJ, BXD55/RwwJ, BXD56/RwwJ, BXD61/RwwJ, BXD62/RwwJ, BXD63/RwwJ, BXD65/RwwJ, BXD65b/RwwJ (originally 92), BXD69/RwwJ, BXD70/RwwJ, BXD71/RwwJ, BXD73a/RwwJ (originally 80), BXD75/RwwJ, BXD77/RwwJ, BXD83/RwwJ, BXD84/RwwJ, BXD86/RwwJ, BXD87/RwwJ, BXD90/RwwJ, BXD98/RwwJ, BXD99/RwwJ, BXD100/RwwJ. BXD strains 1–40 were obtained from the Jackson Laboratory (Bar Harbor, ME), and BXD strains 43–100 were provided by Drs. Lu Lu and Robert W. Williams (University of Tennessee Health Science Center, Memphis, TN). Mice were bred in the Russell Vivarium at Oak Ridge National Laboratory (ORNL). At 3 weeks of age, litters were weaned and shipped from ORNL to the vivarium in the Department of Psychology at the University of Memphis for cocaine IVSA testing. Throughout the experiment, mice had free access to food and water and were maintained in a temperature-controlled environment (70 ± 2 °F) on a 14:10-light/dark cycle (lights on at 06:00).

Behavioral testing

The cocaine IVSA testing procedure and chambers have been described in detail previously (Dickson et al. 2014). Prior to catheter implantation, mice were housed in same-sex and same-strain groups of 3 to 5. At 12 weeks of age, an indwelling catheter was implanted into the right external jugular vein under oxygen/isoflurane anesthesia. Mice were individually housed following catheter implantation. Behavioral testing began following 3 days of post-surgical recovery. IVSA data were collected using Med Associates operant conditioning chambers. Manipulanda were two retractable response levers (active and inactive). Following 3 days of post-surgical recovery, mice began cocaine IVSA testing on a fixed-ratio 1 schedule at a dose of 0.56 mg/kg/infusion. Mice were tested in 2-h sessions at the same time daily 7 days per week throughout the experiment. Acquisition of cocaine IVSA was defined as two consecutive sessions during which ≥10 infusions occurred,

infusions did not vary by more than 20 %, and at least 80 % of lever presses were on the active lever. Following acquisition of cocaine IVSA, the self-administration response was allowed to stabilize at seven additional doses to establish a dose-response curve. Doses were presented in the following order: 0.56, 1.8, 1.0, 0.32, 0.18, 0.1, 0.056, 0.032 mg/kg/infusion. Stabilization was defined as two consecutive sessions during which infusions did not vary by more than 20 % and at least 70 % of lever presses were on the active lever.

To maintain patency, catheters were flushed before and after each daily testing session with 25 μl of a heparin lock solution (Hospira; Lake Forest, IL; 100 U/ml heparin/saline). To forestall bacterial infection, mice were infused (2 μl/g) with an enrofloxacin/saline solution (10 mg/kg) immediately before the heparin flush at the end of each session. Catheters were tested for patency with an infusion (2 μl/g) of a methohexital/saline solution (5 mg/kg) between each cocaine dose. Rapid loss of muscle tone was interpreted as an indication of patency. Cocaine hydrochloride was obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Cocaine doses were calculated as the salt. Methohexital (Brevital®) and enrofloxacin (Baytril®) were obtained from Henry Schein (Melville, NY). Methohexital and cocaine hydrochloride were dissolved in 0.9 % saline. All solutions were filtered through 0.22 μm syringe filters.

Genetic correlations

Using GeneNetwork (www.genenetwork.org), we assessed the genetic correlations between cocaine IVSA infusions and four other measures: cocaine conditioned place preference (CPP), cocaine sensitization, distance traveled in a novel open field, and time on the light side of a light/dark box. We have published these four measures previously (Philip et al. 2010) and they are publicly available on GeneNetwork (GeneNetwork IDs 12051, 12013, 12068, 11904). BXD mice tested on cocaine IVSA, cocaine CPP, cocaine sensitization, open field, and light/dark assays were generated at ORNL; testing on these five assays was performed at the University of Memphis. Consequently, all mice were exposed to the same environmental variables including light cycle, food, water, bedding, and animal handlers. Subjects tested on cocaine IVSA were not the same mice as those tested on other assays.

QTL mapping

We performed QTL mapping of cocaine IVSA data using GeneNetwork. Whole genome interval mapping was performed using 5000 permutations. When a genome-wide significant QTL ($p < 0.05$) was identified, we attempted to identify additional QTLs with composite interval mapping using the peak marker from the initial significant QTL as a control locus. One-way or factorial analysis of variance (ANOVA)

was used to assess effects of the C57BL/6J (B6) and DBA/2J (D2) alleles at the peak of identified QTL on IVSA phenotypes. Potential effects of sex were also examined. When repeated measures ANOVA was used, the assumption of homogeneity of variance across groups was assessed using Mauchly's test of sphericity. If this assumption was violated, the Huynh–Feldt correction was used.

When performing interval mapping using BXD RI strains, the allele at a locus (B6 or D2), not BXD strain, is the between-subjects factor. Consequently, only a single animal of each genotype (i.e., BXD strain) is required in a mapping study, and it is the total number of strains tested, not the number of replicates within a strain, that has the greatest effect on power to detect a QTL (Belknap 1998). Moreover, because allele at a locus is the between-subjects factor as opposed to strain, interactions of sex and allele can be assessed without phenotyping male and female mice from all strains.

Gene candidate identification and prioritization

Following the identification of significant QTL, we used QTL Miner (Alberts and Schughart 2010) on GeneNetwork to identify all genes located within the 2 logarithm of odds (LOD) confidence interval (CI) of a behavioral QTL. Genes from this set that exhibited genome-wide significant ($p < 0.05$) cis-acting eQTL (cis-eQTL) were considered positional candidates for behavioral QTL. To prioritize positional candidates, we examined co-variation of IVSA phenotypes and expression of candidate genes in the NAc, midbrain, and PFC using publicly available gene expression data sets (GeneNetwork study IDs: 44, 141, 36) (Wolen et al. 2012; Ye et al. 2014). These brain regions are critically involved in cocaine IVSA (Bocklisch et al. 2013; Ito et al. 2004; Marinelli et al. 2003; Pettit et al. 1984; Roberts and Koob 1982; Vassoler et al. 2013; Weissenborn et al. 1997; Wise 2009). For each QTL, candidate genes exhibiting expression levels that covaried significantly with the IVSA phenotype associated with the QTL were considered priority positional candidates. For covariation analysis, the family-wise error rate for each QTL was adjusted using the Bonferonni correction (.05/number of gene candidates).

Trans-acting eQTL associated with cocaine IVSA behavioral QTL

We reasoned that genes exhibiting expression levels that map to cocaine IVSA behavioral QTL in a brain region with known relevance to cocaine IVSA may be functionally related to the gene or genes underlying those same behavioral QTL. Therefore, we identified genes that exhibited genome-wide significant *trans*-acting eQTL (*trans*-eQTL) ($p < 0.05$) with a peak located within the 2 LOD confidence interval of a cocaine IVSA behavioral QTL.

Genome-wide covariation of cocaine IVSA phenotypes and gene expression

We reasoned that genes exhibiting expression levels that co-vary with a cocaine IVSA phenotype in a brain region with known relevance to cocaine IVSA may be part of a larger network involved in driving cocaine IVSA behaviors. Therefore, we identified all genes across the genome exhibiting expression levels that covaried genome-wide significantly with cocaine IVSA phenotypes associated with a behavioral QTL. To calculate the genome-wide error rate for each brain region, we used the Bonferroni correction (.05/number of probe sets). Probe sets not associated with a gene were excluded. Bonferroni-adjusted genome-wide significance levels for the NAc, midbrain, and PFC were 1.1×10^{-6}, 1.5×10^{-6}, and 1.1×10^{-6}, respectively.

Results

Acquisition of cocaine IVSA

Mice from 39 strains acquired cocaine IVSA (Table S1). The number of days to meet acquisition criteria ranged from 2 to 11 ($M=4.62$, $SD=2.12$) across the 39 BXD strains. Heritability of this measure was 0.28 (Table S2). To assess acquisition of cocaine IVSA, we performed a repeated measures ANOVA using number of lever presses as the dependent measure, session (1–7) as a within-subjects factor, and lever (active or inactive) as a within-subjects factor. For mice that reached criterion prior to day 7, the mean of active and inactive lever presses on the final two acquisition sessions was carried forward. As a group, BXD strains rapidly learned to lever press for cocaine (Fig. 1a) as indicated by a significant session × lever interaction [$F_{(6, 228)}=9.43$, $p=3.70 \times 10^{-5}$]. Main effects of lever [$F_{(1, 38)}=44.79$, $p=6.42 \times 10^{-8}$] and session [$F_{(6, 228)}=8.51$, $p=8.30 \times 10^{-5}$] were also significant. Post hoc tests indicated that the number of active presses was significantly greater than the number of inactive presses on all session ($p<0.01$ for all comparisons). The number of active presses increased significantly ($p=1.14 \times 10^{-4}$) from session 1 ($M=23.79$; $SD=33.00$) to session 7 ($M=114.48$; $SD=137.75$). In contrast, inactive presses did not change significantly from session 1 ($M=5.16$; $SD=8.25$) to session 7 ($M=3.10$; $SD=3.27$).

Cocaine IVSA dose-response curve

Mice from 29 strains completed a dose-response curve (Table S1). Heritability of infusions at each of the eight doses ranged from 0.46 to 0.60 (Table S2). The dose-response curve (Fig. 1b) exhibited a characteristic ascending limb and descending limb, with a peak at 0.1 mg/kg/infusion. The peak of the dose-response curve varied across individual BXD strains and ranged from 0.032 to 0.18 mg/kg/infusion (Fig. 1c, S1). To assess performance across the dose-response curve, we conducted a repeated measures ANOVA using number of infusions as the dependent measure and dose as a within-subjects factor. Number of infusions varied significantly between doses as indicated by a significant main effect of dose [$F_{(7, 196)}=77.74$, $p=5.09 \times 10^{-23}$]. Post hoc comparisons indicated that (1) the number of infusions at all doses on the ascending limb, including the peak dose, differed significantly, and (2) the number of infusions at all doses on the descending limb, including the peak dose, differed significantly ($p<0.01$ for all comparisons). Analysis of lever press data (Fig. 1d) revealed the same pattern. Cocaine intake across doses is provided in Fig. 1e.

Genetic correlations

Using GeneNetwork, we assessed genetic correlations of cocaine IVSA and two commonly used assays relevant to the behavioral effects of cocaine: cocaine CPP and cocaine sensitization. Cocaine CPP (3.2 mg/kg i.p.) was not genetically correlated with cocaine IVSA at any dose. Cocaine sensitization (10 mg/kg i.p.) was positively genetically correlated with cocaine IVSA infusions at the two lowest cocaine doses (Fig. 2a, b). We also assessed genetic correlations among cocaine IVSA and (1) distance traveled in a novel open field and (2) time spent in the light side of a light/dark box. Distance traveled (cm) during 60 min in a novel open field was positively genetically correlated with cocaine IVSA at the lowest cocaine dose (Fig. 2c); this relationship approached significance at the 0.1 mg/kg/infusion dose ($r=0.34$, $p<0.06$) and 0.18 mg/kg/infusion dose ($r=0.307$, $p<0.07$). Percentage of time spent in the light side of a light/dark box during a 10-min testing session was positively genetically correlated with cocaine IVSA at the three lowest cocaine doses (Fig. 2d–f).

Notably, the three traits which were genetically correlated with cocaine IVSA infusions (cocaine sensitization, distance traveled in a novel open field, and time on the light side of a light/dark box) were not genetically intercorrelated. This suggests that dissociable underlying mechanisms drive the genetic relationship between cocaine IVSA and each of these behaviors in the BXD RI panel. To assess the hypothesis that these three traits explained unique genetic variance on cocaine IVSA, we performed a simultaneous multiple regression using cocaine IVSA infusions at the 0.032 mg/kg/infusion dose as the dependent measure and cocaine sensitization, distance traveled in a novel open field, and time on the light side of a light/dark box as predictors. The overall regression was statistically significant [$F_{(3, 21)}=12.26$, $p=7.50 \times 10^{-5}$], and the three predictor variables collectively accounted for 63.7 % of the genetic variance around IVSA infusions at the lowest cocaine dose ($R=0.798$). After controlling for the effects of the other two predictors, each
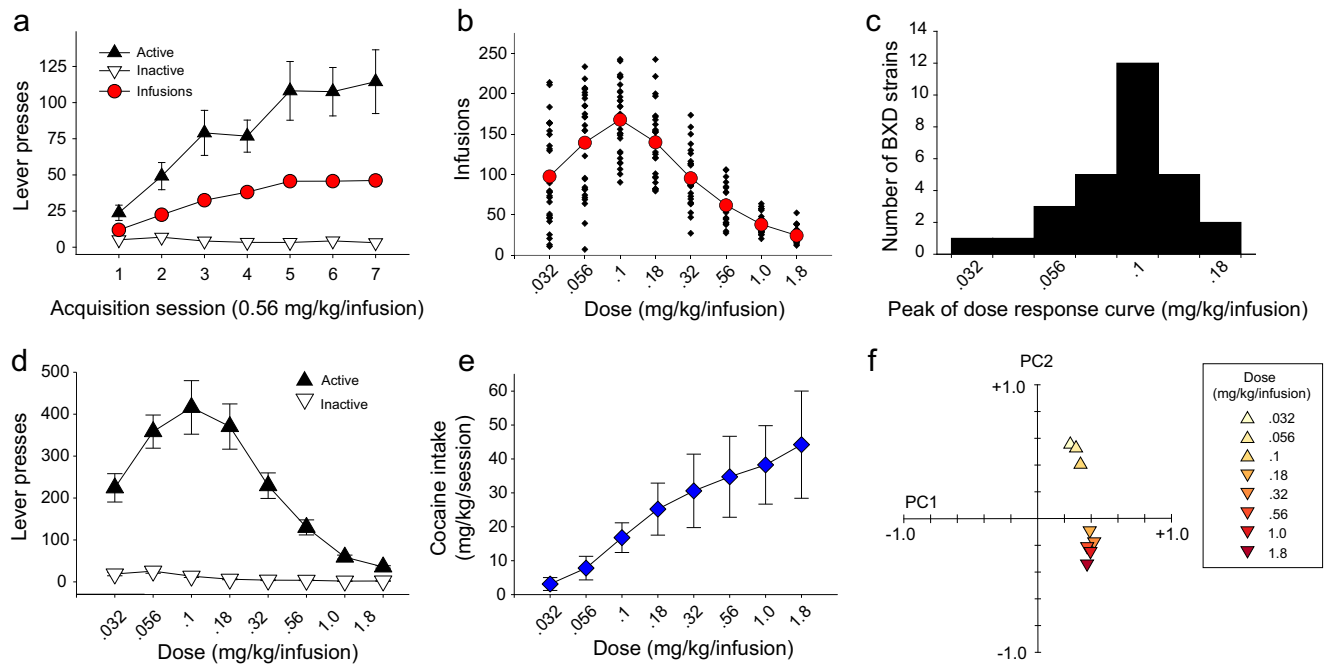
Psychopharmacology

Fig. 1 Intravenous cocaine self-administration in the BXD recombinant inbred panel. **a** Active presses, inactive presses, and cocaine infusions (mean±SE) during the first seven 120-min sessions of cocaine IVSA testing in 39 BXD strains. Number of active presses was significantly greater than number of inactive presses on all sessions ($p<0.01$). **b** Number of cocaine infusions at each dose (mean of final two 120 min sessions) along the dose-response curve in 29 BXD strains. *Red circles* represent the mean of all BXD strains at each dose and *black diamonds* represent individual strain means. **c** Frequency distribution of the peak of the dose-response curve for individual BXD strains. **d**, **e** Active and inactive lever presses (mean±SE) and cocaine intake (mean±SD) across the dose-response curve. **f** Principal components associated with cocaine IVSA infusions at all doses along the dose-response curve
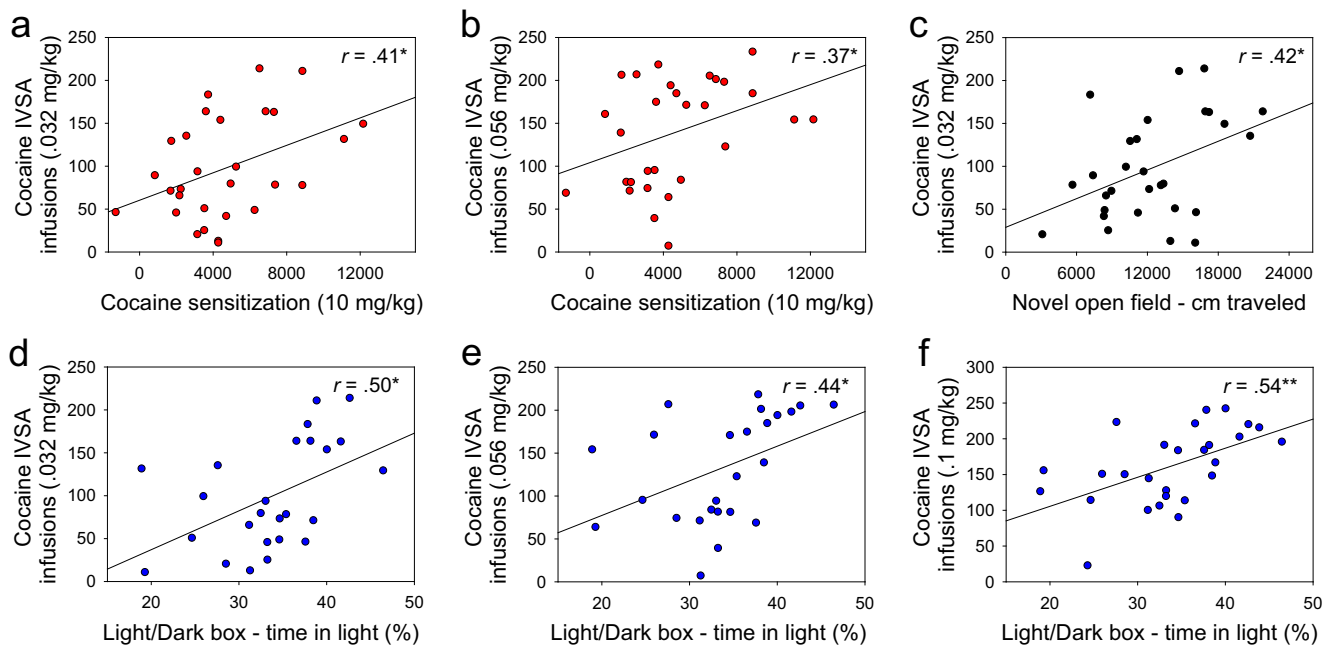

Fig. 2 Genetic correlations. **a**, **b** Cocaine sensitization was positively genetically correlated with cocaine IVSA infusions at the two lowest cocaine doses. **c** Distance traveled over 60 min in a novel open field was positively genetically correlated with cocaine IVSA infusions at the lowest dose. **d–f** Percentage of time in the light side of a light/dark box during a single 10-min session was positively genetically correlated with cocaine infusions at the three lowest cocaine doses. After adjusting for the effects of the other two phenotypes, each of these measures explained unique genetic variance on cocaine IVSA infusions at the lowest cocaine dose: cocaine sensitization (26.2 %, $p<0.01$), light/dark box (29.8 %, $p<0.001$), and open field (11.7 %, $p<0.05$). Data points in all panels represent BXD strain means

predictor explained statistically significant unique variance on cocaine IVSA infusions at the lowest cocaine dose: cocaine sensitization (26.2 %, $p<0.01$), time on the light side of a light/dark box (29.8 %, $p<0.001$), and distance traveled in a novel open field (11.7 %, $p<0.05$).

QTL mapping of cocaine IVSA phenotypes

Indexing dose-response curve characteristics To facilitate QTL mapping of traits affecting the shape of the cocaine IVSA dose-response curve, we used the Correlation Matrix and PCA tool in GeneNetwork to generate principal components associated with the number of infusions at each of the eight cocaine doses. This procedure generated two principal components (Fig. 1f): all cocaine doses loaded on the first principal component, and the highest five doses loaded strongly. The three cocaine doses comprising the ascending limb and peak of the dose-response curve loaded strongly on the second principal component. From this point, we refer to these principal components as (1) infusions on the descending limb of the dose-response curve (IDL) and (2) infusions on the ascending limb of the dose-response curve (IAL), respectively.

At low cocaine doses, it can be challenging to determine if lever pressing reflects titration of cocaine intake or responding in extinction. To dissociate these two types of responding, we created an index termed consistency of cocaine intake across the ascending limb of the dose-response curve (CAL) by calculating the relative standard deviation of mean cocaine intake of the three lowest doses. Mice with low CAL values maintained relatively consistent cocaine intake across these doses, whereas mice with high CAL values exhibited variable intake across doses. Thus, low CAL values indicate relatively precise titration of cocaine intake across the three lowest doses and suggest that lever presses on the ascending limb of the dose-response curve reflect volitional cocaine intake. In contrast, high CAL values reflect a failure to titrate cocaine intake and may indicate that mice were in extinction.

Genome-wide significant QTL associated with sessions to acquire cocaine IVSA Three BXD strains were identified by GeneNetwork as outliers (13, 27, 33) and were excluded from the QTL analysis. We identified a significant QTL (Fig. 3a, b) on chromosome (Chr) 16 with a peak locus of 86.393 Mb (LOD=4.28, $p=1.80\times10^{-2}$). The 2 LOD CI was 83.407–88.783 Mb and the closest listed marker to the QTL peak was rs4213255 at 86.458 Mb. To assess the effect of genotype at this locus on sessions to acquire cocaine IVSA, we grouped the 36 BXD strains by the B6 ($n=21$) or D2 ($n=15$) genotype at the rs4213255 locus (B6- and D2-allele groups, respectively). The D2-allele group required significantly more sessions to reach acquisition criteria ($M=5.32$, SD=1.32) relative to the B6-allele group ($M=3.40$, SD=1.18) [$F(1, 34)=20.97$, $p=6.00\times10^{-5}$], and this effect accounted for 38.19 % of the variance on the sessions to acquire cocaine IVSA measure (Fig. 3c). Including sex as a between-subjects factor in the analysis (i.e., calculating strain means in males and females separately) resulted in the same main effect of genotype [$F(1, 43)=14.76$, $p=3.97\times10^{-4}$] but no main effect of sex or interaction of genotype and sex.

Genome-wide significant QTL associated with infusions on the ascending limb (IAL) of the dose-response curve We identified a significant QTL (Fig. 4a, b) on Chr 11 with a peak locus of 47.436 Mb (LOD=5.87, $p=7.00\times10^{-3}$). The 2 LOD CI was 46.00–50.39 Mb and the closest listed marker to the QTL peak was rs13481014 at 47.931 Mb. To assess the effect of genotype at this locus on the IAL factor, we grouped the 29 BXD strains by the B6 ($n=17$) or D2 ($n=12$) genotype at the rs13481014 locus. IAL factor scores of the B6-allele group ($M=-.76$, SD=1.02) differed significantly from those of the D2-allele group ($M=1.08$, SD=0.60) [$F(1, 27)=31.08$, $p=7.00\times10^{-6}$], and this effect accounted for 53.52 % of the variance on the IAL factor (Fig. 4c).

Genome-wide significant QTL associated with consistency of cocaine intake across the ascending limb of the dose-response curve (CAL) We identified a significant QTL (Fig. 5a, b) on Chr 7 with a peak locus of 31.221 Mb (LOD=5.73, $p=2.00\times10^{-3}$). The 2 LOD CI was 31.206–31.467 Mb, and the marker at the QTL peak was rs8261944. To assess the effect of genotype at this locus on CAL, we grouped the 29 BXD strains by the B6 ($n=19$) or D2 ($n=10$) genotype at the rs8261944 locus. CAL scores of the B6-allele group ($M=0.68$, SD=0.11) were significantly lower than those of the D2-allele group ($M=1.10$, SD=0.25) [$F(1, 27)=40.04$, $p=8.96\times10^{-7}$], and this effect accounted for 59.73 % of the variance on the CAL index (Fig. 5c).

Effect of genotype at the IAL and CAL QTL on the cocaine IVSA dose-response curve To assess the effect of genotype at the IAL and CAL QTL on infusions across the dose-response curve, we performed a repeated measures ANOVA using number of cocaine infusions as the dependent measure and dose as the within-subjects factor (.032–1.8 mg/kg/infusion). Between-subjects factors were sex, genotype at the IAL QTL peak (B6 or D2 allele), and genotype at the CAL QTL peak (B6 or D2 allele). Infusions varied as a function of cocaine dose [$F(7, 161)=84.36$, $p=5.06\times10^{-42}$], and this effect was moderated by genotype at the IAL QTL [dose × IAL genotype: $F(7, 161)=5.19$, $p=1.01\times10^{-4}$] (Fig. 4d, e) and genotype at the CAL QTL [dose × CAL genotype: $F(7, 161)=5.84$, $p=2.60\times10^{-5}$] (Fig. 5d, e). Genotypes at the IAL and CAL QTL did not interact. There was no main effect of sex and sex did not interact with other factors.

Fig. 3 QTL associated with the number of sessions to meet cocaine IVSA acquisition criteria. **a**, **b** Genome-wide significant QTL on chromosome 16 associated with sessions to reach cocaine IVSA acquisition criteria (2 LOD CI, 83.407–88.783 Mb). **c** BXD strains carrying the D2 allele at the QTL peak required significantly more sessions to reach acquisition criteria (M= 5.32, SD=1.32) relative to BXD strains carrying the B6 allele (M= 3.40, SD=1.18). This effect accounted for 38 % of the variance on the sessions to acquire cocaine IVSA measure
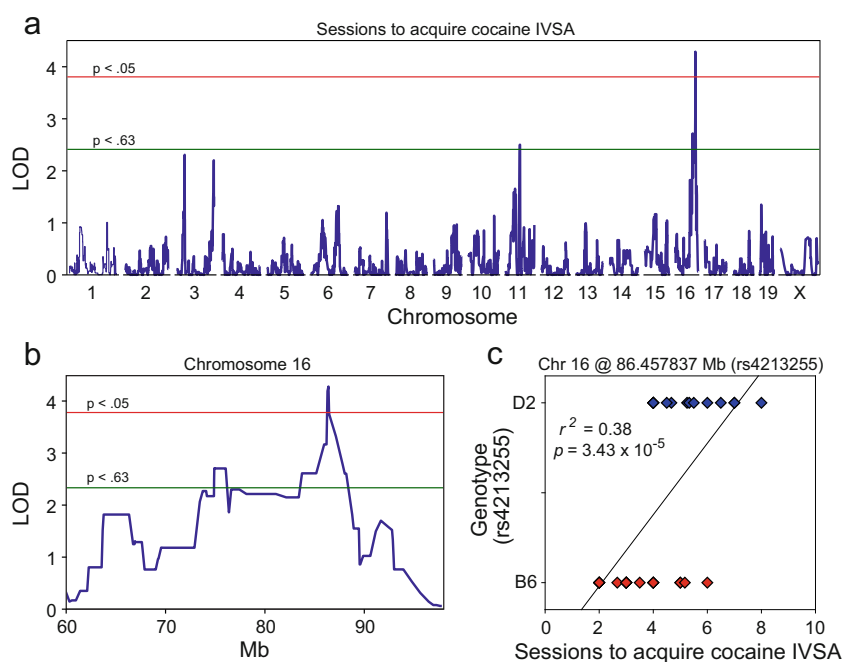
BXD strains in the B6-allele group at the QTL associated with IAL (i.e., B6 allele at rs13481014) infused significantly more cocaine on the ascending limb of the dose-response curve relative to BXD strains in the IAL D2-allele group (Fig. 4d, e). Conversely, the IAL B6-allele group infused significantly less cocaine on the descending limb of the dose-response
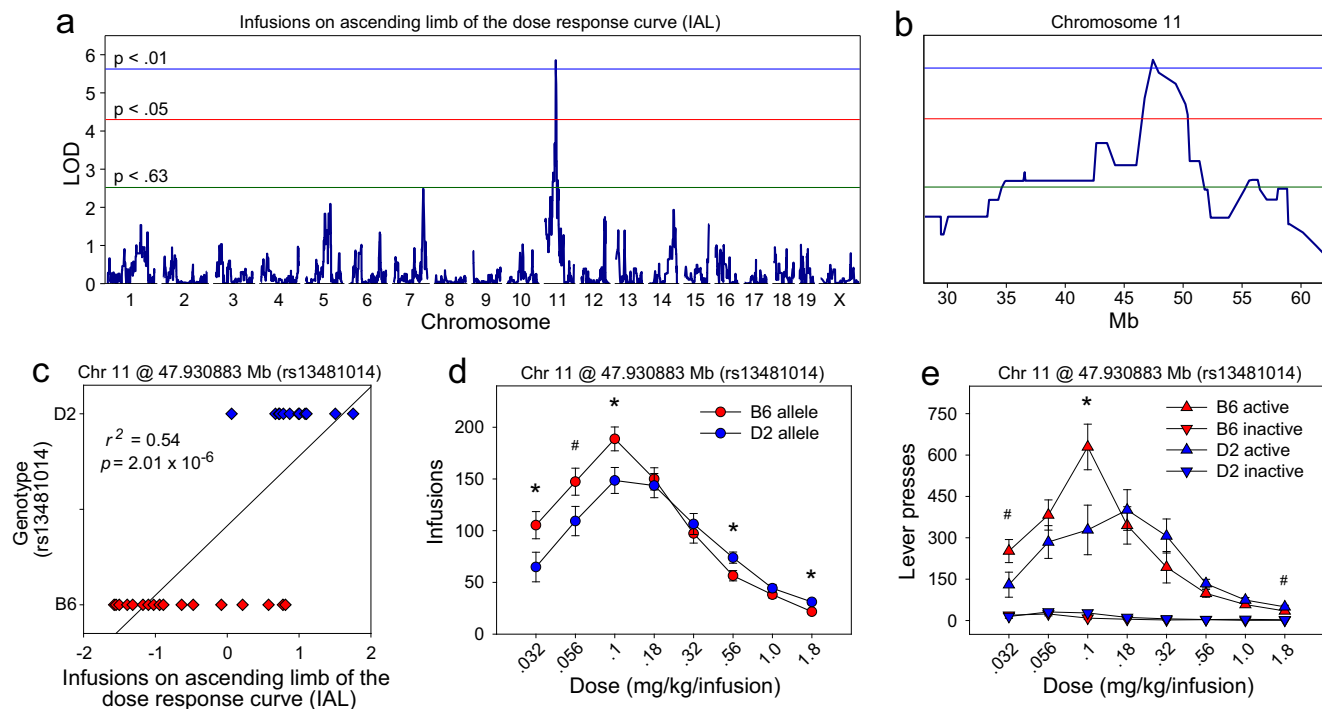
Fig. 4 QTL associated with infusions on the ascending limb of the dose-response curve. **a**, **b** Genome-wide significant QTL on chromosome 11 associated with infusions on the ascending limb of the dose-response curve (IAL) (2 LOD CI, 46.00–50.39 Mb). **c** IAL factor scores of BXD mice carrying the B6 allele at the QTL peak differed significantly from scores of BXD mice carrying the D2 allele at the QTL peak, and this effect accounted for 54 % of the variance on the IAL factor. **d**, **e** BXD strains carrying the B6 allele at the QTL peak infused significantly more cocaine on the ascending limb of the dose-response curve relative to strains carrying the D2 allele at the QTL peak. This effect was significantly reversed on the descending limb, indicating a leftward shift of the dose-response curve in BXD strains carrying the B6 allele at the QTL peak. Data points represent group means±SE. *p<.05, #p<.10
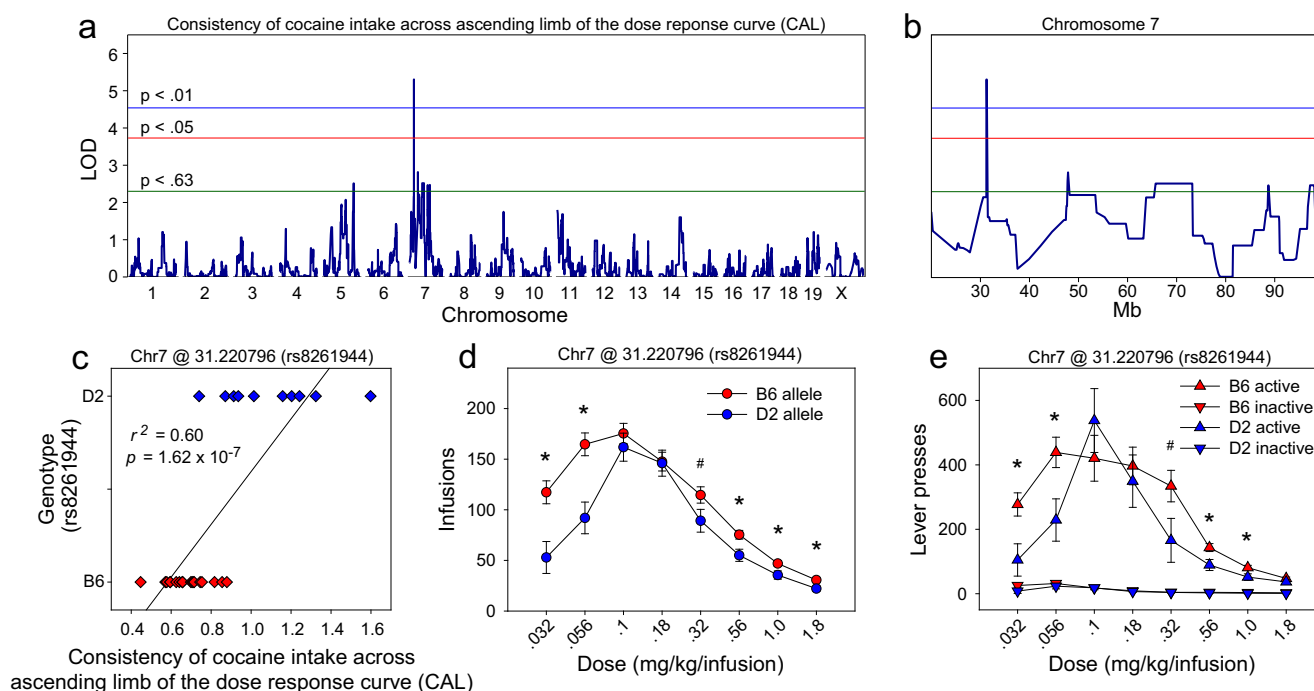
Fig. 5 QTL associated with consistency of cocaine intake across the ascending limb of the dose-response curve. **a, b** Genome-wide significant QTL on chromosome 7 associated with consistency of cocaine intake across the ascending limb of the dose-response curve (CAL) (2 LOD CI, 31.206–31.467 Mb). **c** CAL index scores of BXD strains carrying the B6 allele at the QTL peak differed significantly from scores of BXD strains carrying the D2 allele at the QTL peak, and this effect accounted for 60 % of the variance on the CAL index. **d, e** BXD strains carrying the B6 allele at the QTL peak infused significantly more cocaine on the ascending and descending limbs of the dose-response curve relative to strains carrying the D2 allele at the QTL peak. Collectively, these observations indicate an upward shift of the dose-response curve in BXD strains carrying the B6 allele at the QTL peak. Data points represent group means±SE. *p<.05, #p<.10

curve relative to the IAL D2-allele group. Collectively, these data reveal a leftward shift of the dose-response curve in BXD strains carrying the B6 allele at Chr 11 at 47.93 Mb.

BXD strains in the B6-allele group at the QTL associated with CAL (i.e., B6 allele at rs8261944) infused significantly more cocaine on both the ascending and descending limbs of the dose-response curve relative to BXD strains in the CAL D2-allele group (Fig. 5d, e). These data reveal an upward shift of the dose-response curve in BXD strains carrying the B6 allele at Chr 7 at 31.22 Mb.

BXD strains carrying the B6 allele at both Chr 11 at 47.93 Mb (IAL QTL peak) and Chr 7 at 31.22 Mb (CAL QTL peak) infused significantly more cocaine and made significantly more active lever presses on the ascending limb of the dose-response curve relative to BXD strains carrying the D2 allele at both QTL (Fig. 6a, b). The combined allelic effects were significantly greater than the independent effects on the two lowest cocaine doses ($p<0.05$). Dose-response curves of individual BXD strains grouped by allele at the IAL and CAL QTL are provided in Fig. S1.

QTL positional candidates

Positional candidates were those genes within the 2 LOD confidence interval of a behavioral QTL that exhibited significant cis-eQTL in the NAc, midbrain, or PFC. Candidates for the QTL on Chr 11 at 47.43 Mb associated with the IAL phenotype were *Cyfip2* and *Itk*. Candidates for the QTL on Chr 7 at 31.221 Mb associated with the CAL phenotype were *Aplp1*, *Nfkbid*, *Hspb6*, *Kirrel2*, *Lin37*, *Nphs1*, *Psenen*, and *Tmem149*. Candidates for the QTL on Chr 16 at 86.393 Mb associated with sessions to acquire cocaine IVSA were *Adamts1*, *App*, *Bach1*, *Cyyr1*, *Grik1*, and *Mrpl39*. *App* exhibited a suggestive cis-eQTL in the PFC and was included as a candidate because of its functional relevance to *Aplp1* (Aydin et al. 2012; Cousins et al. 2015; Korte et al. 2012). With the exception of *Mrpl39*, all candidates contained non-synonymous SNPs. With the exception of *Psenen*, all candidates contained indels.

QTL positional candidates exhibiting expression levels that covaried significantly with their cocaine IVSA phenotype in one of the three brain regions were elevated to priority candidates (Table 1). All priority candidates exhibited genome-wide significant cis-eQTL in the same brain region that covariation of phenotype and expression was observed. *Cyfip2* and *Aplp1* also exhibited genome-wide significant trans-eQTL in those regions. Interval mapping of *Cyfip2* expression in the midbrain revealed a genome-wide significant trans-eQTL on Chr 3 with a peak locus of 28.53 Mb (LOD=4.09, $p=4.40\times10^{-2}$) and
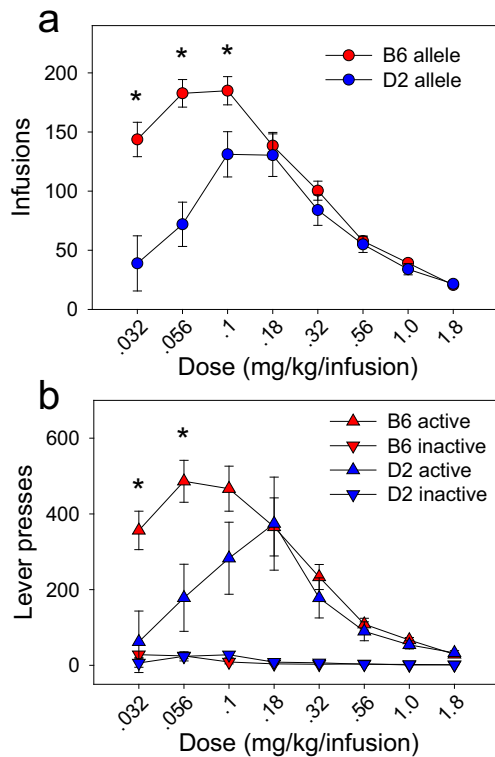
Fig. 6 Combined allelic effects on the cocaine IVSA dose-response curve of the behavioral QTL on chromosome 7 and 11. BXD strains carrying the B6 allele at both Chr 11 at 47.93 Mb (IAL QTL peak) and Chr 7 at 31.22 Mb (CAL QTL peak) (**a**) infused significantly more cocaine and (**b**) made significantly more active lever presses on the ascending limb of the dose-response curve relative to BXD strains carrying the D2 allele at both QTL. The combined allelic effects were significantly greater than the independent effects on the two lowest cocaine doses ($p < 0.05$). Data points represent group means±SE. *p<.05

a 2 LOD CI of 24.78–36.34. Notably, the fragile X-related gene *Fxr1* (Chr 3 at 33.96 Mb) resides within this CI and is functionally related to *Cyfip2* (Schenck et al. 2001).

Interval mapping of *Aplp1* expression in the midbrain revealed a genome-wide significant *trans*-eQTL on Chr 11 with a peak locus of 57.65 Mb (LOD=3.94, $p=3.90 \times 10^{-2}$) and a 2

LOD CI of 50.19–61.28. The location of this *trans*-eQTL is notable because the genes exhibiting expression levels that covaried most strongly with the IAL phenotype were located within this CI. These relationships reached genome-wide significance for *Hist3h2a* and *Skp1a* (Table 3). These associations suggest a functional relationship between a priority gene candidate for the CAL phenotype (*Aplp1*) and genes strongly associated with the IAL phenotype.

Trans-eQTL associated with cocaine IVSA behavioral QTL

We identified genes exhibiting genome-wide significant *trans*-eQTL that mapped to a cocaine IVSA behavioral QTL. We performed this analysis using the midbrain gene expression data set because four of the five priority positional candidates covaried significantly with their QTL phenotype in the midbrain (Table 1). We identified six genes, *Fam53b*, *Mapk12*, *Lppr5*, *Chchd7*, *Serpini1*, and *Rps6ka6*, that exhibited genome-wide significant *trans*-eQTL located within the CI of the IAL behavioral QTL on Chr 11 at 47.43 Mb (Table 2). Notably, the only genome-wide association study of cocaine dependence in humans to date identified *FAM53B* as the sole genome-wide significant gene candidate (Gelernter et al. 2014). Figure 7a shows the genome-wide significant *trans*-eQTL associated with expression of *Fam53b* (Chr 7 at 139.95 Mb) superimposed on the behavioral QTL associated with the IAL phenotype. *Fam53b* and the highest priority gene candidate associated with the IAL QTL (*Cyfip2*) were co-expressed genome-wide significantly in the midbrain (Fig. 7b). There were no genome-wide significant *trans*-eQTL within the CI of the behavioral QTL on Chr 7 at 31.221.

Covariation of cocaine IVSA phenotypes and gene expression across the genome

We identified genes that covaried genome-wide significantly in the NAc, midbrain, or PFC with one of the three cocaine

Table 1 Priority positional candidates for behavioral QTL associated with cocaine IVSA phenotypes

		Gene					*cis*-eQTL				Covariation[a]		References
Phenotype	Region	Symbol	Chr	Mb	*n*	Probe set	Chr	Mb	LRS	p[b]	*r*	p[c]	
IAL	Midbrain	*Cyfip2*	11	46.007	15	A_55_P1962771	11	42.844	62.7	<1.00E−03	0.79	2.16E−04	Kumar et al. (2013)
	Midbrain	*Itk*	11	46.138	15	A_55_P1953788	11	42.631	19.4	2.80E−02	0.77	4.48E−04	
CAL	PFC	*Lin37*	7	31.341	12	1426577_a_at	7	31.220	34.9	1.00E−03	0.74	4.01E−03	
	Midbrain	*Kirrel2*	7	31.233	15	A_51_P512306	7	31.220	35.6	<1.00E−03	0.74	9.36E−04	
	Midbrain	*Aplp1*	7	31.220	15	A_55_P1991605	7	31.220	21.2	9.00E−03	0.66	5.56E−03	

[a] Covariation of phenotype and gene expression in the listed brain region

[b] p values <.05 indicate genome-wide significant *cis*-eQTL

[c] All p values are statistically significant (family-wise error rate=0.05) following Bonferroni adjustment for the total number of positional candidates within the QTL confidence interval

Table 2 Genes exhibiting midbrain expression levels that map genome-wide significantly to the behavioral QTL associated with infusions on the ascending limb of the dose-response curve (IAL phenotype)

Gene					trans-eQTL				
Symbol	Chr	Mb	n	Probe set	Chr	Mb	LRS	p^{a}	References
Fam53b	7	139.951	37	A_55_P2178496	11	49.367	23.51	2.00E−03	Gelernter et al. (2014)
Mapk12	15	88.961	37	A_52_P252258	11	47.930	23.58	2.00E−03	
Lppr5	3	117.392	37	A_55_P1987291	11	50.386	20.50	8.00E−03	
Chchd7	4	3.866	37	A_51_P510939	11	50.386	17.77	1.30E−02	
Serpini1	3	75.421	37	A_66_P100165	11	50.197	20.18	1.80E−02	
Rps6ka6	X	108.502	37	A_55_P2020911	11	50.386	19.92	2.40E−02	

[a] p values <.05 indicate genome-wide significant trans-eQTL

IVSA phenotypes associated with a behavioral QTL (Table 3). Covariation of phenotype and expression of all genes listed in Table 3 reached statistical significance in the listed brain region after Bonferroni adjustment (.05/number of probe sets). Three of these genes (Hist3h2a, Skp1a, Fin14) exhibited genome-wide significant eQTL.

Discussion

Systems genetics is a powerful method for identifying the biological mechanisms underlying complex traits, and cocaine IVSA is the gold standard for assessing volitional drug use in animals. In the present study, we integrated these two approaches to identify novel genes and networks underlying cocaine IVSA in the BXD recombinant inbred mouse panel. To our knowledge, this is the first report of a systems genetics analysis of cocaine IVSA phenotypes. Mice from 39 BXD strains acquired cocaine IVSA, and mice from 29 BXD strains

completed a full dose-response curve (Fig. 1). We identified independent genetic associations between cocaine IVSA and measures of environmental exploration and cocaine sensitization (Fig. 2). We identified a genome-wide significant QTL for sessions to acquire cocaine IVSA (Fig. 3) and two genome-wide significant QTL associated with cocaine IVSA on the ascending limb of the dose-response curve (Figs. 4, 5, and 6). Using publicly available gene expression data from the NAc, midbrain, and PFC, we prioritized gene candidates within behavioral QTL (Table 1) and identified genome-wide significant trans-eQTL (Table 2, Fig. 7) and genes (Table 3) associated with cocaine IVSA.

Genetic correlations

We identified positive genetic correlations between cocaine IVSA and cocaine sensitization, distance traveled in a novel open field, and time in the light side of a light/dark box (Fig. 2). Importantly, each of these measures explained unique

Table 3 Genome-wide significant covariation of IVSA phenotypes and gene expression in the nucleus accumbens (NAc), midbrain, and prefrontal cortex (PFC)

Phenotype	Region	Gene						eQTL				Covariation[a]	
		Symbol	Chr	Mb	n	Probe set	Chr	Mb	LRS	p^{b}	r	p^{c}
Sessions to	NAc	Nudt8	19	4.001	17	1450111_a_at	–	–	–	–	0.873	4.99e−07
acquisition	Midbrain	Gm5797	14	8.156	19	A_55_P2036083	–	–	–	–	0.872	8.22e−08
	PFC	Rpl35	2	38.857	13	1436840_x_at	–	–	–	–	0.934	9.13e−08
	PFC	Rpl35	2	38.857	13	1455950_x_at	–	–	–	–	0.933	1.07e−07
IAL	NAc	Hist3h2a	11	58.769	16	1455712_at	11	56.515	35.5	<1.00E−03	0.922	7.75e−09
	NAc	Skp1a	11	52.057	16	1423148_at	11	50.196	72.0	<1.00E−03	0.881	6.63e−07
	PFC	Rpl31	1	39.427	12	1460008_x_at	–	–	–	–	0.928	7.85e−07
CAL	Midbrain	Pate4	9	35.415	15	A_51_P194293	–	–	–	–	0.886	1.15e−06
	PFC	Fin14	X	12.869	12	1423043_s_at	7	31.212	18.9	3.40E−02	0.930	6.25e−07

[a] Covariation of phenotype and gene expression in the listed brain region

[b] p values <.05 indicate genome-wide significant eQTL

[c] All p values are statistically significant (family-wise error rate=0.05) following Bonferroni adjustment for number of probe sets in the brain region
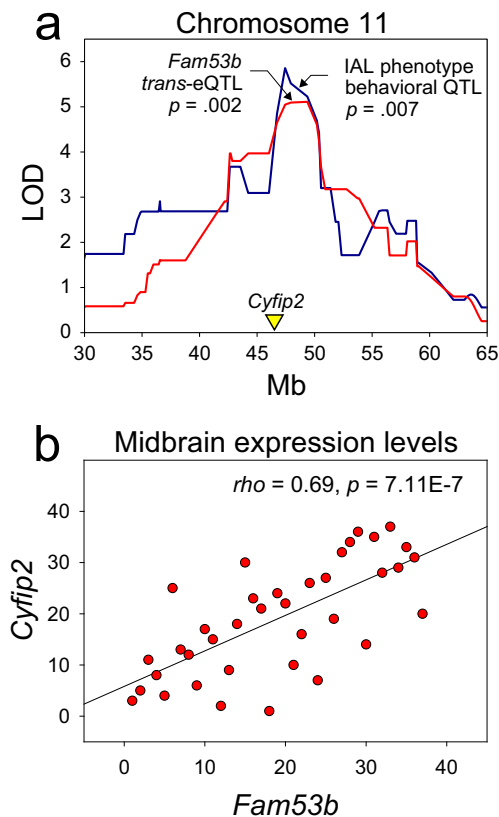
Fig. 7 *Fam53b* maps to a cocaine IVSA behavioral QTL on chromosome 11. **a** *FAM53B* is a human GWAS candidate for cocaine dependence (Gelernter et al. 2014). Midbrain expression of *Fam53b* (Chr 7 at 139.95 Mb) maps genome-wide significantly to the IAL behavioral QTL located on Chr 11. **b** *Fam53b* and the top gene candidate for the IAL behavioral QTL (*Cyfip2*) were co-expressed genome-wide significantly in the midbrain. Collectively, these data suggest a functional relationship between *Cyfip2* and *Fam53b* in the context of cocaine use

genetic variance on low-dose cocaine IVSA. Time in the light side of a light/dark box explained the most genetic variance of the three phenotypes (29.8 %). Elevated time in the dark compartment of a light/dark box may reflect an anxious phenotype, whereas elevated time in the light compartment may reflect a risk taking or impulsive phenotype (Arrant et al. 2013). Regarding this interpretation of light/dark box behavior, the genetic associations reported here are consistent with previous findings of positive associations between psychostimulant IVSA and operant measures of impulsive action and impulsive choice (Dalley et al. 2007; Marusich and Bardo 2009). In humans, trait-impulsivity is reported to be a risk factor for the development of psychostimulant dependence (Ersche et al. 2010, 2012, 2013).

Distance traveled in a novel open field explained 11.7 % of the genetic variance on low-dose cocaine IVSA. High levels of exploration in a novel open field may be viewed as reflecting a sensation seeking phenotype, and sensation seeking may be a risk factor for drug use or abuse

(Blanchard et al. 2009). Previous studies in outbred rats have shown a phenotypic relationship between exploration in a novel open field and psychostimulant IVSA (Cain et al. 2005; Gancarz et al. 2011; Klebaur et al. 2001; Piazza et al. 1990), but the genetic component of this relationship has been unclear. The present study illustrates that shared genetic mechanisms drive open field exploration and low-dose cocaine IVSA in the BXD population.

Cocaine sensitization explained 26.2 % of the genetic variance on low-dose cocaine IVSA, whereas cocaine CPP did not explain statistically significant genetic variance on cocaine IVSA. Collectively, these data suggest that the genetic mechanisms driving cocaine IVSA are largely, though not completely, dissociable from those driving the most commonly used indexes of the behavioral effects of experimenter-administered cocaine. Moreover, the pattern of genetic associations among all examined phenotypes indicates that the observed genetic relationship between cocaine and the open field and light/dark exploratory traits is captured by the cocaine IVSA paradigm, but not by the cocaine CPP or sensitization paradigms. It should be noted, however, that expanded genetic diversity in mouse populations such as the Collaborative Cross RI lines or Diversity Outbred mice may allow identification of relationships not detectable in the BXD lines (Chesler 2014). The use of IVSA methods such as a progressive ratio schedule (Richardson and Roberts 1996), a second order schedule (Everitt and Robbins 2000), or extended-access sessions (Mantsch et al. 2004) may enable identification of relationships not observed in this study.

Genome-wide significant QTL on chromosomes 7 and 11 associated with shifts in the cocaine IVSA dose-response curve

We detected a QTL on Chr 11 at 47.43 Mb associated with infusions on the ascending limb of the dose-response curve (IAL QTL) and a QTL on Chr 7 at 31.22 Mb associated with consistency of cocaine intake across the ascending limb of the dose-response curve (CAL QTL). Presence of the B6 allele at the peak of the IAL QTL (Fig. 4d, e) and CAL QTL (Fig. 5d, e) was strongly associated with elevated cocaine intake on the ascending limb of the dose-response curve, and the effect of carrying the B6 allele at both loci was striking (Fig. 6a, b).

Some lever pressing at low doses was likely due to extinction responding, and we attempted to quantify this phenomenon with the CAL phenotype. Specifically, mice with a high CAL score exhibited relatively variable cocaine intake across the lowest cocaine doses, suggesting that these mice did not adjust active lever pressing across doses to titrate cocaine intake. The effect of the D2 allele at the CAL QTL was a precipitous drop in responding on the lowest two doses relative to the peak of the dose-response curve (Fig. 5d). In contrast, the effect of the D2 allele at the peak of the IAL QTL was an

ascending limb that was shifted down equivalently at the three lowest doses (Fig. 4d). These differential effects suggest that the genes underlying the IAL and CAL QTL drive dissociable aspects of cocaine IVSA. In support of this hypothesis, the alleles at the peak of the IAL and CAL QTLs differentially affected the overall dose-response curve. Specifically, we observed a statistically significant leftward shift in the dose-response curve in BXD strains carrying the B6 allele at the IAL QTL, whereas a statistically significant upward shift was observed in BXD strains carrying the B6 allele at the CAL QTL. Studies in knockout mice will be required to confirm the effect of gene candidates proposed to underlie these QTL (Table 1) and to dissociate the effects of these genes on dose-response curve shifts, responding at low doses, and responding during extinction.

QTL positional candidates

Cyfip2 and *Itk* were the sole positional candidates for the IAL QTL on Chr 11. Expression of both genes was significantly correlated with the IAL phenotype in the midbrain (Table 1). Two recent studies suggest that *Cyfip2* may be the driver of this QTL. First, *Cyfip2* plays a critical role in the behavioral sensitization to cocaine (Kumar et al. 2013), and the observation of a genetic correlation between cocaine sensitization and low-dose cocaine IVSA (Fig. 2a, b) indicates shared genetic underpinnings of these phenotypes. Second, expression of *Fam53b* in the midbrain maps genome-wide significantly to the IAL QTL (Fig. 7a), and *Fam53b* and *Cyfip2* (but not *Itk*) are co-expressed genome-wide significantly in the midbrain (Fig. 7b). This association is relevant because *FAM53B* was the sole genome-wide significant candidate in the only human genome-wide association study of cocaine dependence to date (Gelernter et al. 2014). Notably, all relationships involving the IAL phenotype, *Cyfip2* expression, and *Fam53b* expression were observed in the midbrain; this suggests that the putative effect of *Cyfip2* and *Fam53b* on cocaine use may occur in this region.

The priority candidates for the CAL QTL on Chr 7 were *Aplp1*, *Lin37*, and *Kirrel2* (Table 1). Two observations suggest that *Aplp1* may be the driver of this QTL. First, expression of *App*, which is closely related to *Aplp1* (Aydin et al. 2012; Cousins et al. 2015; Korte et al. 2012), is significantly associated with chronic cocaine and heroin abuse in humans (Albertson et al. 2004, 2006). Notably, *App* is a gene candidate for the QTL on Chr 16 associated with sessions to IVSA acquisition. Second, gene expression data suggest a functional relationship between *Aplp1* and the genetic mechanisms underlying the IAL phenotype. Specifically, *Aplp1* exhibits a genome-wide significant *trans*-eQTL on Chr 11 at 57.65 Mb, and two of the three genes exhibiting genome-wide significant covariation with the IAL phenotype reside within the CI of this *trans*-eQTL (Table 3). Moreover,

Wolen et al. (2012) observed an association between these regions on Chr 7 and 11 in the context of ethanol response and proposed involvement of *Aplp1* and *Gria1* (Chr 11 at 57.14). Considering this putative association and that the CAL phenotype may index extinction responding, it is notable that *Gria1* knockouts over-respond during extinction in a cocaine IVSA paradigm (Mead et al. 2007).

The positional candidates for the QTL on Chr 16 associated with sessions to acquire cocaine IVSA were *Adamts1*, *App*, *Bach1*, *Cyyr1*, *Grik1*, and *Mrpl39*. We did not identify a priority candidate because covariation of expression and phenotype did not reach statistical significance for any gene candidate. However, other sources of evidence suggest *App* and *Grik1* as genes of interest. *App* is significantly associated with chronic cocaine and heroin abuse in humans (Albertson et al. 2004, 2006) and is a member of the same gene family as *Aplp1*, a positional candidate for the CAL QTL on Chr 7. *Grik1* encodes the GluR5 kainate receptor subunit (Contractor et al. 2011), and deletion of this subunit affects cocaine sensitization in B6 mice (Gregus et al. 2010).

The positional candidates described here putatively underlie their associated cocaine IVSA phenotypes. Future studies using knockout mice will be necessary to confirm involvement of these gene candidates in cocaine IVSA and uncover the mechanisms driving observed involvement.

Relevance to human cocaine addiction

Addiction is a complex phenomenon, the core of which is persistent drug use in the face of harmful consequences (American Psychiatric Association 2000). Although addiction-like qualities have been demonstrated in rats (Deroche-Gamonet et al. 2004), the addiction construct is difficult to measure in a laboratory setting. In the present study, we tested mice under time-limited access sessions to identify candidate genes underlying characteristics of the cocaine IVSA dose-response curve and latency to acquire cocaine IVSA. Although not direct indexes of addiction, these measures may serve as addiction endophenotypes and, as such, enable identification of genes and mechanisms underlying human addiction. A critical observation from the present study supports this hypothesis: The only genome-wide association study of cocaine dependence in humans to date identified *FAM53B* as the sole genome-wide significant gene candidate (Gelernter et al. 2014). Remarkably, in the present study, we found that midbrain expression of *Fam53b*, which is located on chromosome 7, mapped genome-wide significantly to a behavioral QTL on chromosome 11 (Fig. 7a). This behavioral QTL was associated with a lateral shift in the cocaine IVSA dose-response curve (Fig. 4). Moreover, expression of *Fam53b* and the top gene candidate for this behavioral QTL (*Cyfip2*) were co-expressed genome-wide significantly in the midbrain (Fig. 7b). These data suggest that the gene underlying the

behavioral QTL on chromosome 11, putatively *Cyfip2*, mediates expression of *Fam53b* on chromosome 7. Collectively, these data illustrate that variation in classical pharmacological measures such as dose-response curves may serve as addiction endophenotypes which can reveal putative genes and mechanisms associated with human addiction.

Conclusions

We conducted a systems genetics study using the BXD RI mouse strains to identify novel genes and networks underlying cocaine IVSA. To our knowledge, this is the first report of a systems genetics analysis of cocaine IVSA. We identified novel genome-wide significant behavioral QTL, expression QTL, and genes associated with cocaine IVSA phenotypes. A genome-wide significant *trans*-eQTL linking *Fam53b* (a GWAS candidate for human cocaine dependence) on chromosome 7 to a cocaine IVSA behavioral QTL on chromosome 11 suggests the ability of mouse preclinical studies using the cocaine IVSA paradigm to identify genes and networks involved in human cocaine dependence. Collectively, these data provide novel candidate genes driving cocaine IVSA in mice and suggest novel mechanisms underlying cocaine use in human populations.

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