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Behavioral phenotypes revealed during reversal learning are linked with novel genetic loci in diversity outbred mice

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ABSTRACT

Impulsive behavior and impulsivity are heritable phenotypes that are strongly associated with risk for substance use disorders. Identifying the neurogenetic mechanisms that influence impulsivity may also reveal novel biological insights into addiction vulnerability. Our past studies using the BXD and Collaborative Cross (CC) recombinant inbred mouse panels have revealed that behavioral indicators of impulsivity measured in a reversal-learning task are heritable and are genetically correlated with aspects of intravenous cocaine self-administration. Genome-wide linkage studies in the BXD panel revealed a quantitative trait locus (QTL) on chromosome 10, but we expect to identify additional QTL by testing in a population with more genetic diversity. To this end, we turned to Diversity Outbred (DO) mice; 392 DO mice (156 males, 236 females) were phenotyped using the same reversal learning test utilized previously. Our primary indicator of impulsive responding, a measure that isolates the relative difficulty mice have with reaching performance criteria under reversal conditions, revealed a genome-wide significant QTL on chromosome 7 (max LOD score = 8.73, genome-wide corrected $p<0.05$). A measure of premature responding akin to that implemented in the 5-choice serial reaction time task yielded a suggestive QTL on chromosome 17 (max LOD score = 9.14, genome-wide corrected <0.1). Candidate genes were prioritized (*2900076A07Rik, Wdr73 and Zscan2)* based upon expression QTL data we collected in DO and CC mice and analyses using publicly available gene expression and phenotype databases. These findings may advance understanding of the genetics that drive impulsive behavior and enhance risk for substance use disorders.

1. Introduction

Many people initiate experience with potentially addictive substances, yet only a fraction of those develop a clinically impairing substance use disorder [1]. Stimulant drugs, including cocaine, are no exception; a majority of people who initiate cocaine use in their lifetime will not become addicted to it [1]. The transition from subclinical, recreational use to a SUD is influenced by both genetic and environmental factors, as well as interactions among them [2–4]; at least 50% of the risk for developing a cocaine use disorder is attributable to genetic variation [4]. Moreover, genetic risk for cocaine addiction is, to a substantial degree, shared with other illicit drugs of abuse [2,4,5], meaning that identifying genetic loci regulating cocaine-related behaviors indirectly informs us about the genetics that influence clinically-impairing use of other substances. To date, the specific genes and gene networks that influence the vulnerability to transition to compulsive drug-seeking and -taking remain mostly unknown. This knowledge gap represents a barrier that limits the ability to design and develop effective prevention and treatment options.

Impulsivity, which can be described as either difficulty with inhibiting impulsive reward pursuit or consumption (impulsive action) and/or as impulsive reasoning about reward-related behaviors (impulsive choice) [6–8], has been repeatedly linked with the initiation of drug and alcohol use and progression into a SUD [7–10]. Although impulsive action and choice phenotypes may be distinct in terms of underlying biological mechanisms [7,11–14], both predict aspects of the response to cocaine in animal models and humans. For example, inter-individual differences in impulsivity predict the propensity to experience altered subjective effects of potentially addictive substances [15] and to relapse after periods of abstinence in human subjects [16]. Research with ani-

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mals has further demonstrated that impulsivity predicts the propensity to initiate cocaine intravenous self-administration (IVSA) [9,10,17–20], transition to habitual/inflexible use [11,18], and relapse after periods of withdrawal or abstinence [11,20]. Our work has revealed that the predictive relationship between impulsive action and cocaine IVSA is attributable to a genetic correlation, also known as co-heritability [9].

Impaired impulsive action may result from deficient inhibitory control over behavior and ultimately manifest as a proclivity to persist in drug use despite negative outcomes. Laboratory tasks that measure inhibitory control provide opportunities to investigate the biology of behavioral flexibility, including indirectly uncovering the neurogenetic mechanisms of addiction vulnerability. One procedure, called reversal learning, measures a subject's ability to suppress the response to a previously reinforced behavior when response-outcome contingencies unexpectedly change [21]. Reversal learning deficits are associated with drug use and SUDs, both in laboratory animals and human subjects, and therefore may be informative of biological factors that drive impulsivity and subsequent risk for SUDs [9,21–26].

Reversal learning is influenced by genetic variation in rodent populations that can be utilized to map associated genetic loci [27,28]. Laboratory rodent populations offer some distinct advantages in forward genetic approaches. Genetically diverse populations can be tested in prospective, highly controlled experimental designs that can reveal quantitative trait loci (QTL) associated with impulsive traits and addiction liability. Concurrent study of genome-wide transcript expression can support discovery of candidate genes and gene networks that affect behavioral flexibility.

The Diversity Outbred (DO) mouse population and Collaborative Cross (CC) inbred strain panel were developed by interbreeding a highly genetically diverse set of founder strains [29–33]. High genetic diversity can expand phenotypic distributions and provide unique opportunities for discovery of variants that drive extreme phenotypes [29]. Reversal learning is heritable in CC strains and their founders [27], indicating these populations may be suitable for genetic dissection of this trait. The DO mice may thus be utilized for relatively high-resolution QTL mapping studies. The CC strains support discovery of genetic correlations among gene expression and behavioral traits, in a fully replicable population that allows for cumulative research and inter-study analyses.

Here, we describe QTL mapping for reversal learning using DO mice. We also advance trait regulatory candidate discovery using reversal learning data from the CC strains along with complementary whole-transcriptome gene expression measures generated from bulk RNA sequencing of striatal tissue (previously described [27,34] to advance positional candidate discovery. The striatum is a key brain region of interest in reversal learning performance and SUDs [35–38]. Collectively, these experiments may reveal genes that moderate reversal learning and enhance understanding of SUD neurogenetics.

2. Methods

2.1. Subjects

Diversity outbred (DO) mice (n = 230 male, 295 female) and CC strains (n = 33 strains, 272 mice, approximately equal numbers of of males and females within each strain) [27] were born at the Jackson Laboratory, Bar Harbor, ME. Additional details of testing and further analyses of data procured from the CC strains are included in [27,34]. The mice were maintained in dedicated mouse colony rooms on a 12:12 h light:dark cycle and at an average temperature of 69–70˚F. Food (Lab Diet 5001, ScottPharma Solutions) and water was available *ad libitum* prior to initiation of food restriction and behavioral testing (described below). A nestlet and a disposable dome-shaped shack were provided in the home cage (Shepherd Specialty Papers, Inc., Watertown, TN, USA). Mice were group housed post-weaning, transitioned to single housing at 6 weeks of age, and maintained under single housing for the duration of testing. All DO/CC mice were tested at JAX by the Behavioral Phenotyping Core, a component of the Systems Neurogenetics of Addiction. Animal studies were performed according to the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 2011) in the AAALAC accredited programs at JAX. All studies were reviewed and approved by the Institutional Animal Care and Use Committee.

2.2. Novelty-related behavioral testing

The DO mice utilized for reversal learning were initially tested (7-8 weeks of age) for locomotor and novelty related behaviors beginning at 8 weeks of age, as previously described [34]. These tests included the open field, light-dark box, hole board and a measure of novel place preference. All mice experienced all forms of testing under equivalent protocols and conditions. The data from these studies are not reported here.

2.3. Food restriction

Food restriction was initiated prior to operant testing. Mice were weighed daily during food restriction and percent of free-feeding body weight was calculated by dividing the current weight by the pre-restriction weight. Mice were fed once daily, with a titrated food amount to maintain a body weight that was 85 +/- 5% of their initial (pre-restriction) weights. Once mice reached their target weight, operant testing began. If a mouse dropped below 80% body weight at any point, increased food was provided. If recovery to greater than 80% was not achieved by the following day, the mouse was returned to *ad libitum* food access until its body weight increased to the target range; the mouse then returned to food restriction and continued testing.

2.4. Reversal learning

Reversal learning testing began at 9-13 weeks of age. Testing took place in 8.5″ L × 7″ W × 5″ H (21.6 × 17.8 × 12.7 cm) operant conditioning modular chambers (Model ENV-307W, Med Associates Inc.) that were fitted with stainless-steel grid floors (Model ENV-307W-GFW, Med Associates Inc.) and located in sound attenuating cubicles. The operant box contained a horizontal array of five nose poke apertures on one side of the box, and a central food magazine on the opposite wall. A house light and white noise maker were positioned within the cubicle above the operant box.

Immediately prior to testing, mice were removed from their home cage by grasping the tail with large, padded forceps and placed inside the operant box. Each mouse was sequentially tested in a series of programs; mice transitioned from program to program individually, as they met criterion performance (see below).

Stage 1: *Box habituation*. Mice were placed in the box for 1 h. The house light and white noise were active, but no other programmed events occurred.

Stage 2: *Magazine training*. Twenty microliter aliquots of Strawberry-flavored Boost (Nestlé HealthCare Nutrition, Inc., Florham Park, NJ) were dispensed every 30s into the food magazine. Reward retrieval was recorded by infrared beam break, and the session terminated when the mouse retrieved 50 rewards or 1 h elapsed, whichever came first. Stage 2 was complete when the mouse retrieved a minimum of 30 rewards in a session.

Stage 3: *Initial operant* (*nose-poke*) *conditioning*. All sessions in stages 3-7 were initiated by activation of the house light and white noise, followed by activation of the center aperture light 10 s later. In stage 3, mice were trained to nose-poke the center aperture (aperture 3 of 5) to obtain a Boost reinforcer. Activation of the aperture by beam break or continuous beam break for a specified minimum nose-poke duration (no minimum imposed, 100 ms, or 200 ms; varied randomly trial to trial) resulted in delivery of 20 μl of Boost in the food magazine and extinguished the aperture light. Upon retrieval of the reinforcer, the next trial

was initiated 1.5 s later (signaled by illumination of the aperture light). If the nose-poke duration was not met, the aperture light and house light were extinguished for a 2 s time-out period. White noise was active for the duration of the session. Sessions ended after 1 h or when 50 reinforcers were earned, whichever came first. Stage 3 was complete when the mouse earned 50 reinforcers in a session.

Stage 4: The same testing procedure as stage 3 was utilized with a nose-poke duration of 100 or 200 ms (varied randomly trial to trial). Failure to pass stage 4 in 10 sessions resulted in regression to stage 3. Mice were only allowed 1 regression across all stages; the second stage failure resulted in exclusion from the study.

Stage 5: The same testing procedure as stage 3/4 was utilized with a nose-poke duration of 100, 200 or 300 ms (varied randomly trial to trial). Failure to pass stage 5 in 10 sessions resulted in regression to stage 4.

Stage 6: *Discrimination learning*. Mice were required to initiate a trial by a nose poke in the center aperture with a nose-poke duration of 100 or 200 ms (varied randomly trial to trial). Following trial initiation, the flanking apertures (2 and 4) were both illuminated. One of the two apertures (left or right) was pseudo-randomly assigned (55% of the subjects were assigned left in the final dataset) as the reinforced aperture; a response in the correct aperture resulted in delivery of a reinforcer and was counted as a correct response. A response in the opposite aperture (incorrect response) or failure to respond at all in 30 s (omission) resulted in a time-out (house light extinguished). The side assignments remained the same for the duration of stage 6. Responses in either flanking aperture prior to initiating a trial (by a center nose-poke) were counted as a correct premature response (premature response in the reinforced aperture) or incorrect premature response (premature response in the opposite aperture). Responses in either flanking aperture after initiating a trial and responding in the reinforced aperture but prior to retrieving the reward were not counted. Stage 6 was complete when the mouse achieved 80% accuracy (trials with a successful response in reinforced aperture/total trials) in a sliding 20 trial window within the session. Sessions lasted for 1 h or until 80% accuracy was achieved. Failure to complete 10 trials over 3 consecutive sessions resulted in regression to stage 5.

Stage 7: *Reversal learning stage*. Testing conditions were identical to stage 6 except that the aperture reinforcement contingencies were reversed and remained reversed for the duration of stage 7. If a mouse failed to complete stage 7 by 8 weeks of testing, it was excluded from the experiment.

Key dependent variables included total trials to criteria (TTC); the number of trials the mouse initiated in stage 6 and 7, total correct premature responses in stage 6 divided by TTC in stage 6, and total incorrect premature response in stage 7 divided by the TTC in stage 7. Phenotype data has been made public in the Mouse Phenome Database (https://phenome.jax.org/projects/CSNA03). Genetic data and code utilized for analyses have been made public in the CSNA GitHub (https://github.com/TheJacksonLaboratory/CSNA).

2.5. Genotyping

Tails were removed from each animal at euthanasia, placed into 1.5 mL Eppendorf tubes, and stored in saline at -80°C until DNA extraction. Tail samples were shipped to GeneSeek (Neogen Inc., Lincoln, NE, USA) for DNA extraction and genotyping on the GigaMUGA (N = 500) Illumina array platforms. The GigaMUGA assays 143,259 genetic markers spanning the 19 autosomes and X chromosome of the mouse, with a mean spacing of 18 Kb (GRCm38 - mm10) [39]. Markers were optimized for information content in DO mice. Genotypes were imputed to a 69K grid to allow for equal representation across the genome. We performed quality control tests that are described in detail in Broman et. al. 2019 [40]. These procedures included ensuring a minimum of missing genotypes and errors, no sample duplicates, and verified congruence of sex chromosomes to labeled sex. No samples were excluded for QC failure. CC strains were bred under JAX quality control standards to ensure genetic stability and no further genotyping was necessary in this panel.

2.6. Heritability

Heritability was estimated in DO mice using the heritability function in R/QTL2, which makes use of a linear mixed model to estimate heritability using the est_herit() function. To compute confidence intervals for estimated heritability we employed a bootstrap-based approach. Specifically, for each trait, we performed 1000 bootstraps by simulating the trait using the rmvnorm() function (R/mvtnorm) with covariance matrix specified using the kinship matrix and original trait heritability. Using these 1000 bootstraps, we computed the 90% confidence intervals.

2.7. Quantitative trait locus mapping

The TTC difference score and premature responding in the reinforced (during acquisition) and incorrect (during reversal) aperture were the *a priori* impulsivity-related traits utilized for QTL mapping. As the TTC in acquisition and reversal were utilized to calculate the difference, we secondarily mapped these measures independently, as a common QTL between TTC (in either stage) and the difference score may clarify the role of that QTL. DO genome reconstruction, sample and marker quality control and QTL mapping were carried out using R/qtl2 software (v 0.28) as described previously [41–45]. Briefly, R/qtl2 software constitutes a set of functions designed for QTL mapping in multi-parent populations derived from more than two founder strains. R/qtl2 allows users to perform genome scans using a linear mixed model to account for population structure and permit the imputation of SNPs based on founder strain genomes. Sex and generation (ranged from 30 to 36) were included as additive covariates for association and linkage mapping. Sex was additionally assessed as an interactive covariate to test for possible QTL by sex interactions. Data points greater than 5 standard deviations from the mean, within each trait, were identified as outliers and excluded from all mapping analysis and heritability calculations. This resulted in the exclusion of 2 mice from premature correct responses in stage 6 and 2 additional mice from premature incorrect responses in stage 7.

2.8. Linkage mapping

For linkage mapping, we used an additive haplotype model with kinship correction to estimate founder effects for each QTL. We accounted for genetic relatedness between mice by using a kinship matrix based on the leave-one-chromosome-out (LOCO) method [46]. The LOCO method was chosen because kinship calculations that include the causative marker are known to produce overly conservative mapping results [47,48]. The genome-wide significance thresholds corresponding to p-values < 0.01, 0.05, 0.10 and 0.63, for each trait, were calculated using 1000 permutations to create a null distribution of LOD scores. A QTL was deemed significant if the genome-wide p-value was less than 0.10, otherwise it was deemed suggestive. When a QTL peak was identified above any of the above thresholds, a 1.5 LOD drop off from the peak marker was used to determine the corresponding QTL region [42,44]. Power analyses indicated 800 mice as sufficient to map a relatively small effect QTL (~5% variance explained) at 80% power, with 400 mice providing 80% power to map a medium/large effect QTL (~10% variance explained) [44].

2.9. Local association mapping

For each significant and/or suggestive QTL region, we imputed all high-quality SNPs from the Sanger Mouse Genome Project (build REL 1505; [49] onto DO genomes and fit an additive genetic model at each SNP. This approach is widely used in human GWAS and increases power and precision by measuring the effects at individual variants by mapping at the two-state SNP level [44].

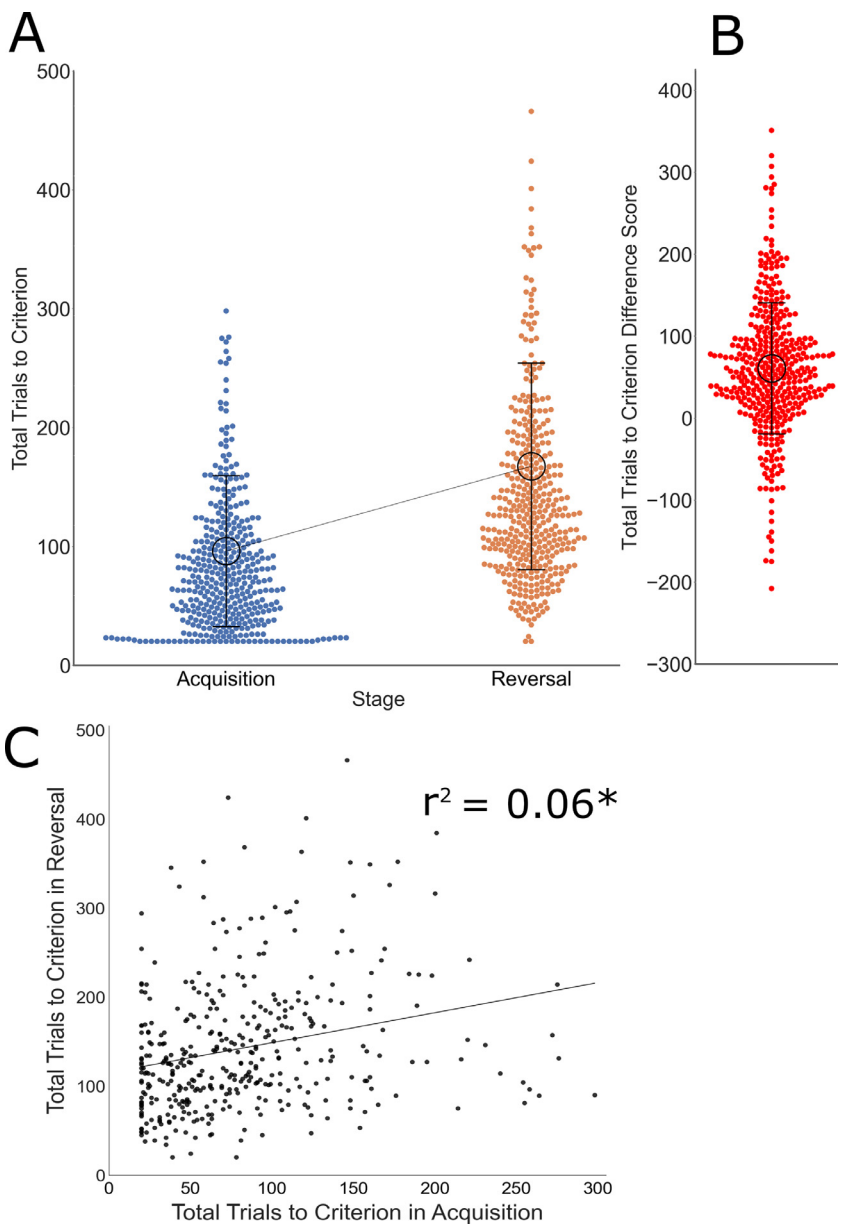

Fig. 1. Reversal learning in DO mice. (A) As expected, the average number of trials required to reach preset performance criteria was larger in the reversal, as compared to the acquisition, stage. DO mice required a wide range of total trials in both the acquisition and reversal learning stages. (B) A difference score captures relative difficulty in reaching criterion in the reversal stage. Again, DO mice displayed a broad range of performance and this measure was found to be heritable. (C) A significant correlation was detected between acquisition and reversal stages; however, only 6% of variance is shared between these measures.

2.10. Gene expression

RNA sequencing was performed on striatal tissue collected from 33 CC strains and 369 DO mice (drug naïve), as previously described [34]. Each strain was tested under a sensitization protocol following exposure to either cocaine or saline control (two groups of mice per strain) as described in Schoenrock et al, 2020. Tissue was collected 24 to 48 h after the final injection.

2.11. Expression QTL mapping

Briefly, gene expression counts were obtained by summing expected counts over all transcripts for a given gene. eQTL mapping was performed on regression residuals of 17,248 genes using the R/qtl2 package with the founder haplotype regression method. Kinship matrices to correct for population structure were computed with the LOCO method for kinship correction [44] (http://k.org/qtl2). We randomly selected 100 genes and permuted each gene 1000 times to obtain genome wide significance thresholds, from a null distribution derived from 100,000 permutations, corresponding to p-values < 0.01, 0.05 and 0.10 . Sex and generation were included as additive covariates. We then used the interactive, web-based analysis tool QTL viewer (http://34.74.187.222/) to visualize the expression data with profile, correlation, LOD, effect, mediation and SNP association plots. Detailed information about the structure of the QTL viewer objects are available at: https://github.com/churchill-lab/qtl-viewer/blob/master/docs/QTLViewerDataStructures.md.

2.12. Positional candidate gene prioritization

Gene expression and reversal learning data obtained from CC strains [27,34] was utilized to prioritize positional candidate genes for the behavioral QTL detected in DO mice. Pearson's correlations were calculated for strain-level gene expression, in cocaine and saline exposed mice, to reversal learning in the same strains. The reversal difference score and total trials to acquisition and reversal were assessed. Genes with correlations of FDR < 0.25 were considered prioritized candidates.

These candidates were further assessed for genetic association to other traits of potential interest by use of the ePHeWAS tool available on systems-genetics.org, which calculates correlations of strain-level gene

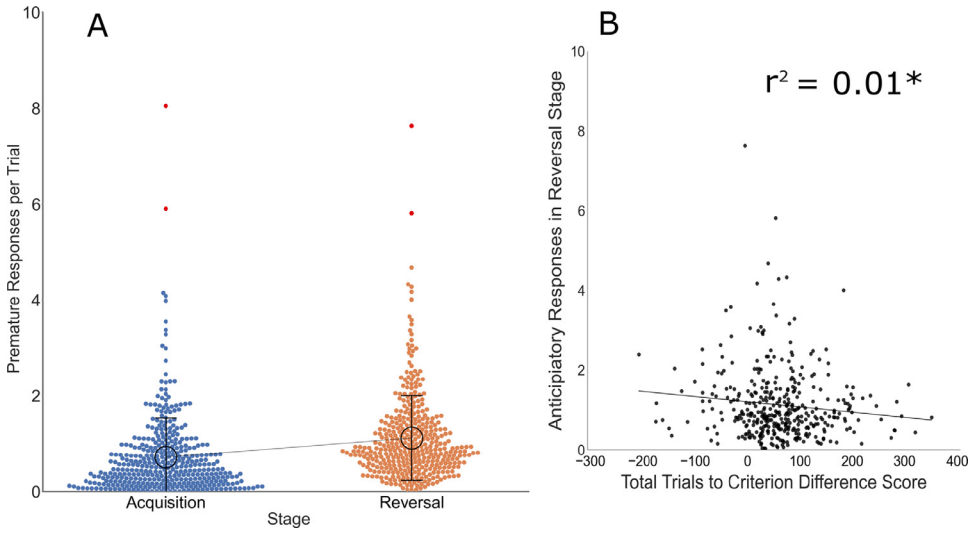

Fig. 2. Premature responding and its relationship to total trials to criterion. (A) Premature responding in acquisition (correct aperture) and reversal (incorrect aperture) are expressed as a fraction of the total trials initiated. DO mice displayed a broad range of responding in these measures. Scatter points colored red were identified as > 5 standard deviation outliers and excluded from mapping analysis. (B) A significant correlation was detected between premature responding in reversal and the reversal learning difference score; however, only 1% of variance is shared, indicating these two measures may capture largely distinct traits. A similar, strain-level r^2 value (r^2=0.2, p=0.06) was found for CC strains [27], indicating a similarly small genetic correlation between these traits.

Table 1
Reversal learning statistics for all DO mice.

Phenotype	Acquisition					Reversal				
	Min	Max	Mean	St. Dev.	Heritability (90% CI)	Min	Max	Mean	St. Dev.	Heritability (90% CI)
Days To Criteria	1	20	3	3	0.44 (0.24-0.62)	1	38	5	5	0.08 (0.00-0.28)
Total Trials	20.0	298.0	81.6	53.9	0.37 (0.16-0.56)	20.0	466.0	142.3	73.9	0.18 (0.00-0.37)
Percent Correct	35.3	95.0	62.1	12.1	0.31 (0.08-0.49)	20.3	80.0	47.7	10.1	0.15 (0.00-0.34)
Difference Score	n/a	n/a	n/a	n/a	n/a	-208.0	351.0	60.6	80.1	0.10 (0.00-0.29)
Premature Correct Responses (per trial)	0.0	8.0	0.7	0.8	0.32 (0.10-0.51)	0.0	6.6	0.9	0.8	0.11 (0.00-0.30)
Premature Incorrect Responses (per trial)	0.0	5.2	0.5	0.5	0.09 (0.0-0.28)	0.03	7.6	1.1	0.9	0.19 (0.23-0.44)
Total Time (min)	6.6	1195.6	153.0	181.7	0.44 (0.22-0.61)	14.4	2264.6	290.0	326.1	0.10 (0.00-0.31)
Trial Initiation Latency (sec., per trial)	2.0	257.8	34.7	30.8	0.36 (0.13-0.53)	3.3	314.6	51.9	51.2	0.09 (0.00-0.27)
Reward Retrieval Time (sec., per correct trial)	7.3	58.4	19.4	6.6	0.41 (0.22-0.58)	8.8	54.7	21.0	7.4	0.35 (0.15-0.52)

expression from publicly available databases to all traits in the phenome database on genenetwork.org [50]. The striatum and frontal cortex (FC) were selected as regions of interest for this analysis [35–38,51–53]. Multiple comparisons were corrected by Bonferroni adjustment.

3. Results

3.1. Reversal learning

DO mice displayed a wide range of performance in reversal learning. During acquisition, total trials to criterion ranged from 20 to 298, with a mean of 81.6 and a standard deviation of 53.9. During the reversal stage, totals trials to criterion ranged from 20 to 466, with a mean of 142.3 and a standard deviation of 73.9. Average number of trials completed per testing session was calculated per mouse and demonstrated a range of 6.5 – 99, mean 34.8 +/- 17.8 (standard deviation) for acquisition and 4.9-123, 40.5 +/- 21.7 for reversal. A mixed ANOVA for trials to criterion, with stage as a repeated measure and sex as a between-subjects factor revealed main effects of stage [$F(1,390)=227.3$, $p<0.001$, $\eta_p^2=0.37$] (Fig. 1A) and sex [$F(1,390)=8.0$, $p=0.005$ $\eta_p^2=0.02$], with males requiring a larger number of trials to reach the preset performance criterion at both stages (male mean ± SEM = 120.8 ± 4.5; female mean ± SEM = 106.0 ± 3.0). A Pearson's correlation analysis performance on acquisition and reversal data from individual mice revealed a modest correlation ($r=0.25$, $r^2=0.06$, $p<0.001$) (Fig. 1C).

The difference score (total trials in reversal minus total trials in acquisition) ranged from -208 to 351, with a mean of 60.6, a standard deviation of 80.1 and heritability of 0.10 (Fig. 1B). The DO mean was higher than that of CC and founder mice obtained in an earlier study [27]; however, variance is similar between the populations (-271 to 383, mean = 37.2, SD = 85.1).

DO mice displayed a wide range of premature responding (response prior to initiating a trial by a center aperture nosepoke) phenotypes in the correct aperture during the acquisition stage (0 to 8.0 premature responses/trial, mean=0.7, SD=0.8) or in the incorrect aperture during the reversal stage (0.03 to 7.6 premature responses/trial, mean = 1.1, SD = 0.9). The range, mean and variance were greater relative to CC/Founder mice in acquisition (0 to 5.7, mean 0.7, SD = 0.7) and reversal (0 to 5.3, mean = 1.0, SD = 0.80) [27] (Fig. 2A). See Table 1 for descriptive statistics of additional variables collected during testing.

A Pearson's correlation was calculated between the reversal learning difference score and premature responding on the incorrect aperture during the reversal stage. A modest correlation was detected ($r = -0.12$, $p = 0.02$, $r^2 = 0.01$) (Fig. 2B), indicating a large proportion of unshared variance and suggesting these measures may capture distinct phenotypes.

Of the mice that initiated testing, 25% failed to successfully complete reversal learning due either to testing criteria failure (17.2%), health problems (5.1%), technical error (2.1%) or other reasons (0.6%). 55.6% of mice that failed were male, suggesting a potential sex-bias in attrition (44.0% of total mice tested were male). Additionally, one mouse was not genotyped due to a technical error and could not be included in mapping/heritability analysis.

3.2. QTL mapping

The reversal learning difference score was subject to QTL mapping. A significant QTL on chromosome 7 (position is in GRCm38, Mbp): Chr07, Peak = 80.80581, LOD = 8.725234, Confidence Interval = 80.26511-81.51397, MAF = 0.46, 12% variance explained) was detected, suggesting a variant(s) at this locus associated with reversal learning performance (Fig. 3A). The additive effects of haplotypes indicated the

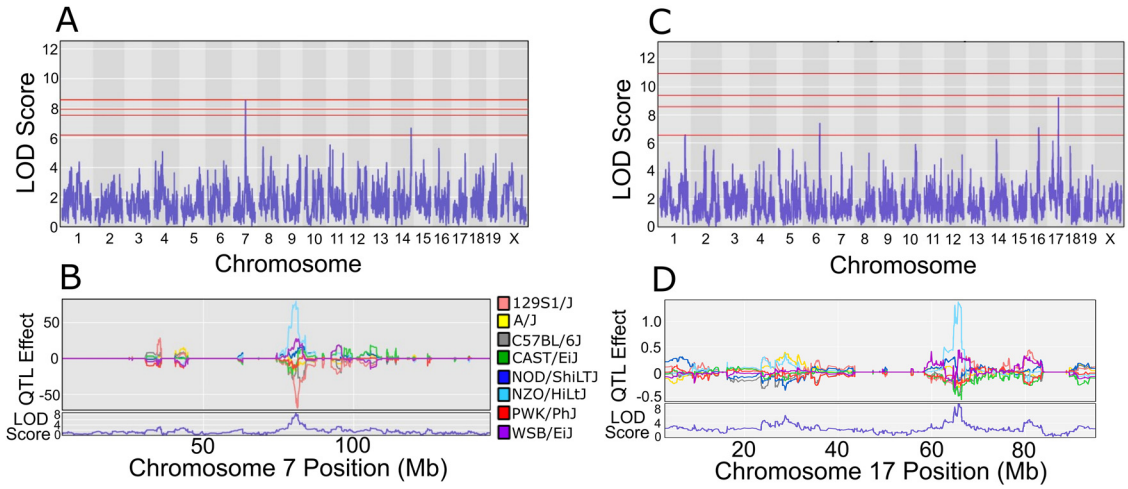

Fig. 3. A significant QTL was detected for the reversal learning difference score and a suggestive QTL was detected for premature responses. (A) A significant QTL was mapped on chromosome 7 (80.26511-81.51397 Mb) for the reversal learning difference score, indicating one or more variants at this locus associated with reversal learning. (B) Haplotype analysis indicated the NZO/HlLtJ haplotype associated with larger difference scores and the 129/SvlmJ haplotype associated with smaller scores. (C) A suggestive QTL was mapped on chr 17 (64.84549 - 66.34104 Mb) for premature responding. (D) Haplotype analysis indicated the NZO/HlLtJ associated with greater premature responding.

Table 2
Correlation (r, p-value) between gene expression in 33 cocaine or saline exposed CC strains and reversal learning in independent groups of the same strains. These genes are positioned within the reversal learning difference score QTL confidence interval and have cis-eQTL. Text in bold indicates a significant p-value.

Gene	cis-eQTL LOD	Saline			Cocaine			Residual Variation		
		Acquisition	Reversal	Difference Score	Acquisition	Reversal	Difference Score	Acquisition	Reversal	Difference Score
2900076A07Rik	9.61	-0.17, 0.33	0.22, 0.23	0.41, 0.02	-0.05, 0.78	0.36, 0.04	**0.46, 0.01**	0.05, 0.78	0.29, 0.1	0.28, 0.11
Ap3b2	16.46	-0.17, 0.34	-0.06, 0.72	0.09, 0.62	-0.06, 0.75	0.05, 0.79	0.11, 0.54	0.11, 0.56	0.14, 0.42	0.06, 0.73
Cpeb1os1	14.98	-0.06, 0.73	-0.09, 0.61	-0.04, 0.81	-0.04, 0.81	-0.09, 0.61	-0.06, 0.72	-0.01, 0.98	-0.05, 0.8	-0.05, 0.8
Crtc3	40.29	0.16, 0.36	0.04, 0.85	-0.12, 0.52	0.06, 0.75	0.14, 0.45	0.1, 0.59	-0.13, 0.49	0.18, 0.31	0.33, 0.06
Furin	9.06	0.03, 0.86	-0.07, 0.71	-0.11, 0.56	0.09, 0.62	0.13, 0.48	0.06, 0.74	0.08, 0.64	0.18, 0.33	0.12, 0.51
Gm15880	31.22	-0.01, 0.98	0.02, 0.91	0.03, 0.88	-0.07, 0.69	0.1, 0.57	0.18, 0.3	-0.1, 0.59	0.13, 0.48	0.24, 0.18
Gm18310	41.24	0.03, 0.87	0.17, 0.36	0.16, 0.38	0.04, 0.81	0.2, 0.27	0.18, 0.31	0.05, 0.78	0.14, 0.45	0.1, 0.57
Gm45718	13.56	-0.07, 0.69	0.01, 0.96	0.08, 0.66	0, 0.98	0.25, 0.16	0.28, 0.11	0.08, 0.65	0.35, 0.04	0.32, 0.07
Hddc3	48.14	0.05, 0.78	0.04, 0.81	0, 1	0.05, 0.79	0, 0.99	-0.05, 0.79	0, 0.99	-0.13, 0.46	-0.15, 0.41
Iqgap1	34.73	-0.02, 0.93	-0.03, 0.87	-0.02, 0.92	-0.23, 0.2	0.05, 0.77	0.28, 0.12	-0.24, 0.17	0.07, 0.7	0.31, 0.08
Man2a2	23.24	0.04, 0.84	0.26, 0.14	0.26, 0.14	0.01, 0.97	0.25, 0.16	0.28, 0.12	-0.05, 0.8	0.05, 0.79	0.1, 0.59
Ngrn	22.18	-0.07, 0.72	-0.06, 0.74	-0.01, 0.97	0.18, 0.31	0.26, 0.15	0.12, 0.52	0.3, 0.09	**0.39, 0.02**	0.16, 0.38
Pde8a	10.36	0.25, 0.16	0.07, 0.72	-0.17, 0.35	-0.09, 0.61	-0.01, 0.98	0.08, 0.66	-0.2, 0.27	-0.03, 0.86	0.15, 0.4
Prc1	39.45	0.11, 0.56	-0.01, 0.95	-0.11, 0.53	-0.02, 0.93	-0.03, 0.86	-0.02, 0.91	-0.22, 0.22	-0.04, 0.81	0.16, 0.38
Rccd1	55.25	0.05, 0.76	0.13, 0.48	0.09, 0.61	-0.03, 0.85	0.17, 0.35	0.22, 0.22	-0.19, 0.29	0.12, 0.52	0.31, 0.08
Rps17	31.75	0.12, 0.52	-0.09, 0.63	-0.21, 0.25	0.15, 0.4	0.02, 0.91	-0.12, 0.51	0.1, 0.6	0.15, 0.41	0.08, 0.68
Unc45a	12.63	0.26, 0.15	0.12, 0.5	-0.11, 0.55	0.21, 0.25	0.16, 0.38	-0.02, 0.93	-0.01, 0.97	0.1, 0.59	0.12, 0.52
Vps33b	20.69	0.27, 0.12	0.15, 0.41	-0.09, 0.6	0.05, 0.78	0.3, 0.09	0.3, 0.1	-0.1, 0.6	0.26, 0.14	0.39, 0.03
Wdr73	17.54	-0.13, 0.47	0.2, 0.26	0.35, 0.05	-0.07, 0.69	0.31, 0.08	**0.42, 0.02**	0.09, 0.63	0.28, 0.12	0.23, 0.2
Zscan2	8.35	-0.03, 0.87	0.05, 0.78	0.09, 0.63	0.02, 0.92	0.41, 0.02	**0.45, 0.01**	0.03, 0.85	**0.43, 0.01**	**0.46, 0.01**

NZO/HILtJ haplotype associated with positive difference scores (relatively poor reversal learning) and the 129/SvlmJ haplotype associated with negative differences scores (relatively good reversal learning) (Fig. 3B). Analysis with sex as an interactive covariate did not provide evidence that this QTL interacts with sex ($p > 0.1$).

The QTL interval contained 58 genes. 20 of these genes were associated with *cis*-eQTL (Table 2). When these genes were assessed for strain-level correlation to reversal learning outcomes in 33 CC strains [27], three were found to positively correlate with the reversal learning difference score (*2900076A07Rik, Wdr73 and Zscan2)*.

Prioritized candidate genes were assessed by ePheWAS (systems-genetics.org) [54] for correlation between BXD strain-level expression levels in the striatum or FC and all traits in the genenetwork.org phenome database. The candidate gene, *Wdr73,* demonstrated genetic correlations to dopamine receptor traits including: D1/D2 ratio (genenetwork ID 15554, -log10(p)=10.76), D1 expression (genenetwork ID 15185, -log10(p)=7.29), D2 expression (genenetwork ID 15186, -log10(p)=5.98) and expression signature of D1 medium spiny neurons (genenetwork ID15552, -log10(p)=5.87).

A suggestive QTL on chromosome 17 (position is in GRCm38 Mbp): Chr 17, Peak = 65.68404, LOD = 9.136811, Confidence Interval = 64.84549 - 66.34104, MAF = 0.12, 9% variance explained) was detected for premature responses on the incorrect aperture in the reversal stage (Fig. 3C). The additive effects of haplotypes indicated the NZO/HILtJ haplotype associated with greater premature responding (Fig. 3D). Analysis with sex as an interactive covariate did not provide evidence that this QTL interacts with sex ($p > 0.1$). The QTL interval contains 17 genes and 8 of these genes demonstrated *cis*-eQTL (Table 3). However, no genes demonstrated a correlation between gene expression and premature responses. Genes with *cis*-eQTL were also assessed for correlation to the reversal learning difference score. Expression of *Ralbp1* in the cocaine group demonstrated a positive correlation to the reversal learning difference score. Analysis by ePheWAS revealed that this gene is associated with acquisition of a visual discrimination

Table 3
Correlation (r, p-value) between gene expression in 33 cocaine or saline exposed CC strains and premature responding during reversal learning in independent groups of the same strains. These genes are positioned within the premature responding QTL confidence interval and have cis-eQTL.

Gene	cis-eQTL LOD	Saline	Cocaine	Residual Variation
Ankrd12	26.12	(0, 0.99)	(0.02, 0.93)	(0.02, 0.93)
Ddx11	19.63	(-0.13, 0.47)	(-0.1, 0.6)	(0.01, 0.95)
Ppp4r1	14.68	(0.1, 0.59)	(0.03, 0.87)	(-0.04, 0.82)
Rab31	9.41	(0.31, 0.08)	(0.17, 0.36)	(0.05, 0.77)
Ralbp1	29.44	(0.21, 0.25)	(0.17, 0.33)	(0.02, 0.9)
Twsg1	46.59	(0.02, 0.92)	(0.13, 0.48)	(0.21, 0.23)
Vapa	28.49	(0.06, 0.76)	(0.07, 0.7)	(0.05, 0.8)
Washc1	15.22	(0.07, 0.7)	(0.07, 0.69)	(0.03, 0.86)

operant response (genenetwork ID 16202, -log10(p)=5.10) and aggregate protein formation on a Huntington's disease model crossed to the BXD panel (genenetwork ID 16190, -log10(p)=5.40). Furthermore, the *Ralbp1* gene harbors a non-synonymous variant (Table 4). Considering independent evidence that indicates *Ralbp1* may influence a similar operant task to that tested here, this gene may be considered an interesting candidate for further examination.

4. Discussion

Impulsive action is a heritable trait that associates with risk for SUDs [6,9,21–27,55–57], and to some degree, this association may be due to a genetic correlation (coheritability). As a consequence, identifying the genetic regulators of impulsive behaviors may indirectly illuminate SUD genetics and neurobiology. We have previously found that the Collaborative Cross (CC) inbred strains and their founders demonstrate heritable variation in impulsive action, as measured by the reversal learning task [27]. In the present study, we utilized the Diversity Outbred (DO) mice, derived from the same founders as the CC strains, to characterize reversal learning and perform genome-wide QTL mapping to discover loci that may influence reversal learning traits. As expected, DO mice demonstrated a broad range of reversal learning performance. Our analyses of these data revealed a significant QTL that influenced reversal learning performance and a suggestive QTL that influenced premature responding.

The difference score for reversal learning captures the relative difficulty subjects have in adapting to the unexpected switch in response-outcome contingencies that happens at reversal. On average, trials to criterion were greater in the reversal stage however, the range of performance in the DO mice is broad. Some mice took ~200 fewer trials in reversal while mice at the other extreme required >300 additional trials to complete the reversal stage relative to acquisition. This variation is, in part, due to genetic differences in the DO mouse and is thus amenable to genome-wide QTL studies. QTL mapping revealed a significant QTL on chromosome 7 for this trait. The broadly defined confidence interval contained 58 genes. Gene expression data from the DO mice and 33 CC strains was utilized to determine positional candidate genes on the basis of striatum *cis*-eQTL and heritable expression patterns that are correlated with reversal learning difference scores in the same CC strains. This analysis indicated three genes as top candidates (*2900076A07Rik, Wdr73, Zscan2)*. Notably, we did not replicate the chromosome 10 QTL discovered by Laughlin et. al. 2011 for reversal learning in the BXD mouse population. This QTL may be dependent on the DBA/2J founder strain allele; this founder strain is not included in the CC/DO populations and therefore this QTL may not be detectable in DO mice. Furthermore,

Table 4
Positional candidate genes (within the 1.5 lod interval) with genetic variants.

Gene	Chr	Gene Type	Gene ID (MGI)	3 prime UTR	Splice region	Down-stream	Inter-genic	Intron	Intron, non coding	Non coding exon	Up-stream gene	Synon-ymous	Mis-sense	Structural
1700023D08Rik	7	unclassified	1921473	0	0	0	0	0	0	0	0	0	0	DEL
Alpk3	7	protein coding	2151224	0	0	4	0	10	0	0	5	0	0	
Ap3b2	7	protein coding	1100869	0	0	0	0	0	1	0	1	0	0	
Cpeb1	7	protein coding	108442	0	0	18	0	126	11	0	1	0	0	
Crtc3	7	protein coding	1917711	0	0	1	0	0	0	0	0	0	0	
Gm15544	7	pseudo	3782993	0	0	0	0	0	0	0	1	0	0	
Gm18392	7	pseudo	5010577	0	0	0	0	0	0	0	0	0	0	DEL
Gm18922	7	pseudo	5011107	0	0	0	0	0	0	0	0	0	0	DEL
Gm32112	7	lncRNA	5591271	0	0	0	0	0	0	0	0	0	0	DEL
Gm32178	7	lncRNA	5591337	0	0	0	0	0	0	0	0	0	0	DEL
Gm42398	7	lncRNA	5625283	0	0	0	0	0	0	0	0	0	0	DEL
Gm45991	7	lncRNA	5825628	0	0	0	0	0	0	0	0	0	0	DEL
Iqgap1	7	protein coding	1352757	0	0	0	0	0	0	0	0	1	0	
Nmb	7	protein coding	1915289	0	0	0	0	0	0	0	7	0	0	
Pde8a	7	protein coding	1277116	0	0	5	0	74	23	0	10	0	0	INS
Platr32	7	lncRNA	3801726	0	0	1	0	0	10	0	8	0	0	INS
Rps17	7	protein coding	1309526	0	0	6	0	0	0	0	0	0	0	
Sec11a	7	protein coding	1929464	0	0	4	0	0	21	3	6	0	0	INV
Slc28a1	7	protein coding	3605073	1	1	4	0	9	0	0	0	0	0	INS
Wdr73	7	protein coding	1919218	0	0	6	0	0	10	0	12	0	0	INV
Zfp592	7	protein coding	2443541	0	0	2	0	2	0	0	1	0	0	
Zscan2	7	protein coding	99176	0	0	38	0	14	0	0	8	0	0	INS
Ankrd12	17	protein coding	1914357	0	0	1	0	0	0	0	1	0	0	
Ddx11	17	protein coding	2443590	0	0	0	0	4	0	0	0	0	0	
Gm23264	17	snRNA	5453041	0	0	0	0	0	0	0	1	0	0	
Mtcl1	17	protein coding	1915867	0	0	14	0	0	0	0	0	0	0	INS
Ppp4r1	17	protein coding	1917601	0	0	0	0	0	3	0	0	0	0	
Rab31	17	protein coding	1914603	1	0	1	0	4	0	0	1	0	0	
Ralbp1	17	protein coding	108466	0	0	0	0	0	0	0	0	0	1	
Tmem232	17	protein coding	2685786	0	0	0	0	1	0	0	0	0	0	
Twsg1	17	protein coding	2137520	1	0	0	0	0	0	0	0	0	0	
Txndc2	17	protein coding	2389312	0	0	0	0	1	0	0	2	0	0	
Vapa	17	protein coding	1353561	0	0	0	0	1	0	0	0	0	0	
Washc1	17	protein coding	1916017	0	0	0	0	0	0	0	0	1	0	

false negatives are expected under the sample sizes tested and, assuming this QTL is detectable in DO mice, it may have gone undetected in this study.

Further analysis of these prioritized genes by ePheWAS of publicly available gene expression and phenome datasets in the BXD recombinant inbred mouse panels revealed that *Wdr73* associated with heritable variation in striatal dopamine receptor transcript expression. Given the importance of striatal dopamine in reversal learning and risk for SUDs [35–38], *Wdr73* may impact reversal learning by affecting dopamine system function in this brain region. Furthermore, mutations in *Wdr73* are associated with Galloway-Mallowat syndrome, a developmental/neurological disorder [58] and this gene was recently highlighted as a positional candidate in a multivariate GWAS of mood disorders and psychosis in human subjects [59]. Given the collection of evidence to suggest *Wdr73* may influence comorbid psychiatric conditions and striatal dopamine, this gene is considered a top candidate.

Premature responding during reversal learning is a measure of impulsive action analogous to measures in five choice serial reaction time [60]. Given that this trait demonstrated a very modest correlation to the reversal learning differences score, it may provide unique and valuable genetic information. DO mice demonstrated a broad range of premature responding (near 0 to ~ 6 premature responses per trial). We discovered a suggestive QTL for premature responding on chromosome 17. The confidence interval contained 17 positional candidate genes. Eight of these genes have striatal cis-eQTL; however, none demonstrated genetic correlation to premature responding, suggesting that the causal variant may not act through striatal gene expression regulation. These genes were also tested for genetic correlation to reversal learning difference scores. The gene *Ralbp1* positively correlated to differences scores, and ePheWAS analysis of his gene revealed that it is genetically correlated to phenotypes gathered in a similar operant discrimination task in the BXD mouse panel (genenetwork ID 16202). Additionally, this gene also correlated to aggregate protein formation in a Huntington's disease model that was tested across BXD strains (genenetwork ID 16190). This gene also harbors a non-synonymous variant. Collectively, this evidence may indicate *Ralbp1* a candidate gene for further consideration.

The DO and CC mouse populations are genetically diverse mouse resources that have proven valuable for the study of impulsive action and addiction genetics. We have utilized the DO mice to follow up previous research in the CC strains that indicated reversal learning is heritable in these populations and amenable to forward genetic approaches. This approach has revealed a novel QTL for reversal learning difference scores and a suggestive QTL for premature responding during reversal learning. Additional work is underway to characterize cocaine self-administration and other traits related cocaine use disorder in the DO/CC populations [34,61,62]. Future analysis will integrate data presented here with these additional studies to facilitate further discovery of the genetics that simultaneously influence impulsivity and SUD-related traits.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data is available on mouse phenome database. Analysis code is available on the CSNA github. Both of these resources have been linked in the manuscript.

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References

[1] F.A. Wagner, J.C. Anthony, From first drug use to drug dependence: developmental periods of risk for dependence upon marijuana, cocaine, and alcohol, Neuropsychopharmacology 26 (2002) 479–488, doi:10.1016/S0893-133X(01)00367-0.
[2] E.C. Prom-Wormley, J. Ebejer, D.M. Dick, M.S. Bowers, The genetic epidemiology of substance use disorder: a review, Drug Alcohol Depend. 180 (2017) 241–259, doi:10.1016/j.drugalcdep.2017.06.040.
[3] R.H.C. Palmer, S.E. Young, C.J. Hopfer, R.P. Corley, M.C. Stallings, T.J. Crowley, et al., Developmental epidemiology of drug use and abuse in adolescence and young adulthood: Evidence of generalized risk, Drug Alcohol Depend. 102 (2009) 78–87, doi:10.1016/j.drugalcdep.2009.01.012.
[4] D. Goldman, G. Oroszi, F. Ducci, The genetics of addictions: uncovering the genes, Nat. Rev. Genet. 6 (2005) 521–532, doi:10.1038/nrg1635.
[5] D.M. Dick, The genetics of addiction: where do we go from here? J. Stud. Alcohol Drugs 77 (2016) 673–675, doi:10.15288/jsad.2016.77.673.
[6] J.W. Dalley, B.J. Everitt, T.W. Robbins, Impulsivity, compulsivity, and top-down cognitive control, Neuron 69 (2011) 680–694, doi:10.1016/j.neuron.2011.01.020.
[7] J.D. Jentsch, J.R. Ashenhurst, M.C. Cervantes, S.M. Groman, A.S. James, Z.T. Pennington, Dissecting impulsivity and its relationships to drug addictions, Ann. NY Acad. Sci. 1327 (2014) 1–26, doi:10.1111/nyas.12388.
[8] C.A. Winstanley, P. Olausson, J.R. Taylor, J.D. Jentsch, Insight into the relationship between impulsivity and substance abuse from studies using animal models, Alcohol. Clin. Exp. Res. 34 (2010) 1306–1318, doi:10.1111/j.1530-0277.2010.01215.x.
[9] M.C. Cervantes, R.E. Laughlin, J.D. Jentsch, Cocaine self-administration behavior in inbred mouse lines segregating different capacities for inhibitory control, Psychopharmacology 229 (2013) 515–525, doi:10.1007/s00213-013-3135-4.
[10] J.W. Dalley, T.D. Fryer, L. Brichard, E.S.J. Robinson, D.E.H. Theobald, K. Laane, et al., Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement, Science 315 (2007) 1267–1270, doi:10.1126/science.1137073.
[11] N. Broos, L. Diergaarde, A.N. Schoffelmeer, T. Pattij, T.J. De Vries, Trait impulsive choice predicts resistance to extinction and propensity to relapse to cocaine seeking: a bidirectional investigation, Neuropsychopharmacol 37 (2012) 1377–1386, doi:10.1038/npp.2011.323.
[12] J.W. Dalley, A.C. Mar, D. Economidou, T.W. Robbins, Neurobehavioral mechanisms of impulsivity: fronto-striatal systems and functional neurochemistry, Pharmacol. Biochem. Behav. 90 (2008) 250–260, doi:10.1016/j.pbb.2007.12.021.
[13] J.W. Dalley, T.W. Robbins, Fractionating impulsivity: neuropsychiatric implications, Nat. Rev. Neurosci. 18 (2017) 158–171, doi:10.1038/nrn.2017.8.
[14] J. MacKillop, J. Weafer, J. Gray, A. Oshri, A. Palmer, H. de Wit, The latent structure of impulsivity: impulsive choice, impulsive action, and impulsive personality traits, Psychopharmacology 233 (2016) 3361–3370 (Berl), doi:10.1007/s00213-016-4372-0.
[15] J. Weafer, H. De Wit, Inattention, impulsive action, and subjective response to d-amphetamine, Drug Alcohol Depend. 133 (2013) 127–133, doi:10.1016/j.drugalcdep.2013.05.021.
[16] B. Adinoff, T.J. Carmody, R. Walker, D.M. Donovan, G.S. Brigham, T.M. Winhusen, Decision-making processes as predictors of relapse and subsequent use in stimulant-dependent patients, Am. J. Drug Alcohol Abuse 42 (2016) 88–97, doi:10.3109/00952990.2015.1106550.
[17] J.J. Anker, J.L. Perry, L.A. Gliddon, M.E. Carroll, Impulsivity predicts the escalation of cocaine self-administration in rats, Pharmacol. Biochem. Behav. 93 (2009) 343–348, doi:10.1016/j.pbb.2009.05.013.
[18] D. Belin, A.C. Mar, J.W. Dalley, T.W. Robbins, B.J. Everitt, High impulsivity predicts the switch to compulsive cocaine-taking, Science 320 (2008) 1352–1355 (New York, NY), doi:10.1126/science.1158136.
[19] J.L. Perry, E.B. Larson, J.P. German, G.J. Madden, M.E. Carroll, Impulsivity (delay discounting) as a predictor of acquisition of IV cocaine self-administration in female rats, Psychopharmacology 178 (2005) 193–201, doi:10.1007/s00213-004-1994-4.
[20] J.L. Perry, S.E. Nelson, M.E. Carroll, Impulsive choice as a predictor of acquisition of IV cocaine self- administration and reinstatement of cocaine-seeking behavior in male and female rats, Exp. Clin. Psychopharmacol. 16 (2008) 165–177, doi:10.1037/1064-1297.16.2.165.
[21] A. Izquierdo, J.D. Jentsch, Reversal learning as a measure of impulsive and compulsive behavior in addictions, Psychopharmacology 219 (2012) 607–620, doi:10.1007/s00213-011-2579-7.
[22] D.J. Calu, T.A. Stalnaker, T.M. Franz, T. Singh, Y. Shaham, G. Schoenbaum, Withdrawal from cocaine self-administration produces long-lasting deficits in orbitofrontal-dependent reversal learning in rats, Learn. Mem. 14 (2007) 325–328, doi:10.1101/lm.534807.
[23] J. Camchong, A.W. MacDonald, B. Nelson, C. Bell, B.A. Mueller, S. Specker, et al., Frontal hyperconnectivity related to discounting and reversal learning in cocaine subjects, Biol. Psychiatry 69 (2011) 1117–1123, doi:10.1016/j.biopsych.2011.01.008.
[24] M.J. Gullo, C.J. Jackson, S. Dawe, Impulsivity and reversal learning in hazardous alcohol use, Personal. Individ. Differ. 48 (2010) 123–127, doi:10.1016/j.paid.2009.09.006.
[25] J. Jentsch, Impairments of reversal learning and response perseveration after repeated, intermittent cocaine administrations to monkeys, Neuropsychopharmacology 26 (2002) 183–190, doi:10.1016/S0893-133X(01)00355-4.

[26] P. Smith, N. Benzina, F. Vorspan, L. Mallet, K. N'Diaye, Compulsivity and probabilistic reversal learning in OCD and cocaine addiction, Eur. Psychiatry 30 (2015) S110–S111, doi:10.1016/j.eurpsy.2015.09.210.
[27] L.S. Bailey, J.R. Bagley, R. Dodd, A. Olson, M. Bolduc, V.M. Philip, et al., Heritable variation in locomotion, reward sensitivity and impulsive behaviors in a genetically diverse inbred mouse panel, Genes Brain Behav. (2021), doi:10.1111/gbb.12773.
[28] R.E. Laughlin, T.L. Grant, R.W. Williams, J.D. Jentsch, Genetic dissection of behavioral flexibility: reversal learning in mice, Biol. Psychiatry 69 (2011) 1109–1116, doi:10.1016/j.biopsych.2011.01.014.
[29] E.J. Chesler, Out of the bottleneck: the diversity outcross and collaborative cross mouse populations in behavioral genetics research, Mamm. Genome 25 (2014) 3–11, doi:10.1007/s00335-013-9492-9.
[30] G.A. Churchill, D.C. Airey, H. Allayee, J.M. Angel, A.D. Attie, J. Beatty, et al., The collaborative cross, a community resource for the genetic analysis of complex traits, Nat. Genet. 36 (2004) 1133–1137, doi:10.1038/ng1104-1133.
[31] G.A. Churchill, D.M. Gatti, S.C. Munger, K.L. Svenson, The diversity outbred mouse population, Mamm. Genome 23 (2012) 713–718, doi:10.1007/s00335-012-9414-2.
[32] V.M. Philip, G. Sokoloff, C.L. Ackert-Bicknell, M. Striz, L. Branstetter, M.A. Beckmann, et al., Genetic analysis in the collaborative cross breeding population, Genome Res. 21 (2011) 1223–1238, doi:10.1101/gr.113886.110.
[33] D.W. Threadgill, G.A. Churchill, Ten years of the collaborative cross, Genetics 190 (2012) 291–294, doi:10.1534/genetics.111.138032.
[34] Saul M.C., Bagley J.R., Bailey L.S., Datta U., Dickson P.E., Dodd R., et al. Consideration of genetic and sex effects in mice enhances consilience with human addiction studies. BioRxiv 2020:2020.02.(14).949784. 10.1101/2020.02.14.949784.
[35] H.C. Bergstrom, A.G. Lieberman, C. Graybeal, A.M. Lipkin, A. Holmes, Dorsolateral striatum engagement during reversal learning, Learn. Mem. 27 (2020) 418–422, doi:10.1101/lm.051714.120.
[36] H.F. Clarke, T.W. Robbins, A.C. Roberts, Lesions of the medial striatum in monkeys produce perseverative impairments during reversal learning similar to those produced by lesions of the orbitofrontal cortex, J. Neurosci. 28 (2008) 10972–10982, doi:10.1523/JNEUROSCI.1521-08.2008.
[37] R. Cools, M.J. Frank, S.E. Gibbs, A. Miyakawa, W. Jagust, M. D'Esposito, Striatal dopamine predicts outcome-specific reversal learning and its sensitivity to dopaminergic drug administration, J. Neurosci. 29 (2009) 1538–1543, doi:10.1523/JNEUROSCI.4467-08.2009.
[38] B.J. Everitt, T.W. Robbins, From the ventral to the dorsal striatum: devolving views of their roles in drug addiction, Neurosci. Biobehav. Rev. 37 (2013) 1946–1954, doi:10.1016/j.neubiorev.2013.02.010.
[39] A.P. Morgan, C.P. Fu, C.Y. Kao, C.E. Welsh, J.P. Didion, L. Yadgary, et al., The mouse universal genotyping array: from substrains to subspecies, G3 6 (2015) 263–279 (Bethesda, Md), doi:10.1534/g3.115.022087.
[40] K.W. Broman, D.M. Gatti, K.L. Svenson, Ś. Sen, G.A. Churchill, Cleaning genotype data from diversity outbred mice, G3 Genes Genomes Genet. 9 (2019) 1571, doi:10.1534/g3.119.400165.
[41] K.W. Broman, Fourteen years of R/qtl: just barely sustainable, J. Open Res. Softw. 2 (2014), doi:10.5334/jors.at.
[42] K.W. Broman, D.M. Gatti, P. Simecek, N.A. Furlotte, P. Prins, Ś. Sen, et al., R/qtl2: software for mapping quantitative trait loci with high-dimensional data and multiparent populations, Genetics 211 (2019) 495–502, doi:10.1534/genetics.118.301595.
[43] R.J. Church, D.M. Gatti, T.J. Urban, N. Long, X. Yang, Q. Shi, et al., Sensitivity to hepatotoxicity due to epigallocatechin gallate is affected by genetic background in diversity outbred mice, Food Chem. Toxicol. 76 (2015) 19–26, doi:10.1016/j.fct.2014.11.008.
[44] D.M. Gatti, K.L. Svenson, A. Shabalin, L.Y. Wu, W Valdar, P Simecek, et al., Quantitative trait locus mapping methods for diversity outbred mice, G3 4 (2014) 1623–1633 (Bethesda, Md), doi:10.1534/g3.114.013748.
[45] K.L. Svenson, D.M. Gatti, W. Valdar, C.E. Welsh, R. Cheng, E.J. Chesler, et al., High-resolution genetic mapping using the mouse diversity outbred population, Genetics 190 (2012) 437–447, doi:10.1534/genetics.111.132597.
[46] R. Cheng, A.A. Palmer, A simulation study of permutation, bootstrap, and gene dropping for assessing statistical significance in the case of unequal relatedness, Genetics 193 (2013) 1015–1018, doi:10.1534/genetics.112.146332.
[47] E.G. King, A.D. Long, The beavis effect in next-generation mapping panels in drosophila melanogaster, G3 7 (2017) 1643–1652 (Bethesda), doi:10.1534/g3.117.041426.
[48] J. Yang, N.A. Zaitlen, M.E. Goddard, P.M. Visscher, A.L. Price, Advantages and pitfalls in the application of mixed-model association methods, Nat. Genet. 46 (2014) 100–106, doi:10.1038/ng.2876.
[49] T.M. Keane, L. Goodstadt, P. Danecek, M.A. White, K. Wong, B. Yalcin, et al., Mouse genomic variation and its effect on phenotypes and gene regulation, Nature 477 (2011) 289–294, doi:10.1038/nature10413.
[50] M.K. Mulligan, K. Mozhui, P. Prins, R.W. Williams, GeneNetwork: a toolbox for systems genetics, in: K. Schughart, R.W. Williams (Eds.), Systems Genetics: Methods and Protocols, Springer, New York, NY, 2017, pp. 75–120, doi:10.1007/978-1-4939-6427-7_4.
[51] R.Z. Goldstein, N.D. Volkow, Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex, AJP 159 (2002) 1642–1652, doi:10.1176/appi.ajp.159.10.1642.
[52] J. Hornak, J. O'Doherty, J. Bramham, E.T. Rolls, R.G. Morris, P.R. Bullock, et al., Reward-related reversal learning after surgical excisions in orbito-frontal or dorsolateral prefrontal cortex in humans, J. Cogn.e Neurosci. 16 (2004) 463–478, doi:10.1162/089892904322926791.
[53] R.A. Wise, M.A. Robble, Dopamine and addiction, Ann. Rev. Psychol. 71 (2020) 79–106, doi:10.1146/annurev-psych-010418-103337.
[54] H. Li, X. Wang, D. Rukina, Q. Huang, T. Lin, V. Sorrentino, et al., An integrated systems genetics and omics toolkit to probe gene function, Cell Syst. 6 (2018) 90–102 e4, doi:10.1016/j.cels.2017.10.016.
[55] J.A. Brewer, M.N. Potenza, The neurobiology and genetics of impulse control disorders: relationships to drug addictions, Biochem. Pharmacol. 75 (2008) 63–75, doi:10.1016/j.bcp.2007.06.043.
[56] H. de Wit, Impulsivity as a determinant and consequence of drug use: a review of underlying processes, Addict. Biol. 14 (2009) 22–31, doi:10.1111/j.1369-1600.2008.00129.x.
[57] J.L. Perry, M.E. Carroll, The role of impulsive behavior in drug abuse, Psychopharmacology 200 (2008) 1–26, doi:10.1007/s00213-008-1173-0.
[58] R.O. Rosti, E. Dikoglu, M.S. Zaki, G. Abdel-Salam, N. Makhseed, J.C. Sese, et al., Extending the mutation spectrum for Galloway–Mowat syndrome to include homozygous missense mutations in the WDR73 gene, Am. J. Med. Genet. Part A 170 (2016) 992–998, doi:10.1002/ajmg.a.37533.
[59] T.T. Mallard, R.K. Linnér, A.D. Grotzinger, S. Sanchez-Roige, J. Seidlitz, A. Okbay, et al., Multivariate GWAS of psychiatric disorders and their cardinal symptoms reveal two dimensions of cross-cutting genetic liabilities, Genetics (2019), doi:10.1101/603134.
[60] A. Bari, J.W. Dalley, T.W. Robbins, The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats, Nat. Protoc. 3 (2008) 759–767, doi:10.1038/nprot.2008.41.
[61] S.M. Kim, C.A. Vadnie, V.M. Philip, L.H. Gagnon, K.V. Chowdari, E.J. Chesler, et al., High-throughput measurement of fibroblast rhythms reveals genetic heritability of circadian phenotypes in diversity outbred mice and their founder strains, Sci. Rep. 11 (2021) 2573, doi:10.1038/s41598-021-82069-8.
[62] S.A. Schoenrock, P. Kumar, A.A. Gómez, P.E. Dickson, S.M. Kim, L. Bailey, et al., Characterization of genetically complex Collaborative Cross mouse strains that model divergent locomotor activating and reinforcing properties of cocaine, Psychopharmacology 237 (2020) 979–996, doi:10.1007/s00213-019-05429-3.