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Differing genetics of saline and cocaine self-administration in the hybrid mouse diversity panel

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ABSTRACT

To identify genes that regulate the behavioral and brain transcriptomic responses to the potentially addictive drug cocaine, we performed genome-wide association studies (GWASs) and RNA-Seq using a panel of inbred and recombinant inbred mice undergoing intravenous self-administration of saline as a control for cocaine. A linear mixed model increased statistical power for the longitudinal behavioral data, which was acquired over 10 days, and identified 145 loci for responding when saline was delivered compared to 17 for the corresponding cocaine GWAS. Only one locus overlapped. Transcriptome-wide association studies (TWASs) using RNA-Seq data from the nucleus accumbens and medial frontal cortex identified *5031434O11Rik* and *Zfp60* as significant for saline self-administration. Two other genes, *Myh4* and *Npc1*, were nominated based on proximity to loci for multiple endpoints or a *cis* locus regulating expression. All four genes have previously been implicated in locomotor activity, despite a lack of significant relationship between saline IVSA and distance traveled in the open field. Our results indicate a distinct genetic basis for saline and cocaine self-administration, and suggest some common genes for saline self-administration and locomotor activity.

INTRODUCTION

In the US, over 2 million individuals use cocaine more frequently than once a month [1–5]. Deaths due to overdose in 2018 were 4.5 per 100,000 standard population. Cocaine misuse in humans has broad and narrow sense heritabilities of ~0.32–0.79 and ~0.27-0.30, respectively. However, identifying genes for cocaine misuse using genome-wide association studies (GWASs) in humans has been difficult because of the obstacles in recruiting properly ascertained subjects.

To identify genes involved in intravenous self-administration (IVSA) of cocaine, we used an array of inbred and recombinant inbred mice called the hybrid mouse diversity panel (HMDP) [6,7]. As a control, and to enable studies of differential gene expression, a parallel experiment was performed in which saline was employed instead of cocaine, with all other factors (handling, surgery, behavioral testing) being identical. The HMDP was evaluated over 10 days using four dependent variables for cocaine and saline IVSA.

Though we found evidence that cocaine served as a more effective behavioral reinforcer than saline, individual strains engaged in levels of saline self-administration that varied considerably relative to cocaine [6,7]. Three additional sets of observations supported differing genetic causes of cocaine and saline IVSA in the HMDP. First, the behavioral endpoints were much more highly correlated within than between infusates. Second, both

narrow and broad sense heritabilities were significantly higher for saline IVSA (~0.31 and ~0.44, respectively) than cocaine (~0.20 and ~0.32, respectively). Third, the genome scans for cocaine and saline using individual days segregated nearly completely when subjected to unsupervised clustering, although neither infusate had significant loci.

To increase the statistical power of the cocaine GWASs, we took advantage of the longitudinal data using a linear mixed model that employed fixed and random effects of testing day as a continuous variable, while correcting for population structure using a genetic relatedness matrix. A total of 15 unique significant cocaine loci were identified. To further increase statistical power, we used transcriptome-wide association studies (TWASs) to combine the longitudinal genome scans with RNA-Seq data from medial frontal cortex (mFC) and nucleus accumbens (NAc) of the cocaine cohort. Both the TWASs and GWASs highlighted the ionotropic cannabinoid receptor *Trpv2* as a key locus for cocaine self-administration, partly explaining the shared genetic risk factors for cocaine addiction and cannabis use [8].

In this report, we use both longitudinal GWASs and TWASs to identify genes for saline-taking and to better understand the genetic basis for the differences in cocaine and saline IVSA.

METHODS

Saline and cocaine intravenous self-administration

A total of 479 and 477 mice from 84 strains of the HMDP were used for cocaine and saline IVSA respectively, as described [6,7]. The average number of mice per strain was 5.7 ± 0.1, 11.3 ± 0.07 weeks of age, and 50.4 ± 0.7% males, averaged over cocaine and saline. Mice were individually housed and evaluated in the light phase of a 12 h/12 h cycle. Animals were tested over 10 consecutive daily sessions.

Testing chambers had two response levers, one of which, when actuated, produced an infusion of cocaine or saline depending on cohort, the other of which was inactive. To encourage conditioning, infusion of the agent was signaled by a visual cue (flashing of the house light) for 20 s. Pressing the inactive lever had no programmed effect. After an infusion, active lever presses were recorded but no infusion given for 20 s. Testing was for 2 h or until 65 infusions were given, whichever came first.

Mapping loci for IVSA

Quantitative trait loci (QTLs) for saline IVSA were mapped using `GMMAT` to evaluate the normalized endpoints as longitudinal traits over the 10 testing days, as described [7,9]. This linear mixed model included fixed and random effects of testing day as well as correcting for population structure via a kinship matrix. Covariates were sex, active lever (left or right), testing chamber, cohort and age. `GMMAT` reports Z statistics. Genome-wide significance thresholds of 5% obtained from permutation were employed. Genotypes used single nucleotide polymorphism (SNPs) [10], and after removing SNPs with minor allele frequency < 5% or missing genotype frequency > 10%, 340,097 remained. Mouse genome build GRCm38/mm10 was employed [11].

Open field

Comparison of saline IVSA endpoints and open field locomotor activity used data downloaded from the Mouse Phenome Database [12]. The open field measures were normalized distance traveled in the first 10 min of a 55 min testing session for 62 inbred strains [13] and a 20 min testing session of 55 BXD recombinant inbred strains [14].

RNA-Seq

RNA-Seq was performed on NAc (core and shell) and mFC of 41 strains exposed to either cocaine or saline IVSA [6,7]. A total of 73 M reads of 75 bp paired-ends were obtained per region per strain. GWASs of transcript, spliceform and RNA editing abundance were performed as described using FaST-LMM to correct for population structure [6,7,15,16]. Covariates were sex and sequencing batch. *Cis* expression quantitative trait loci (eQTLs), splicing (percent spliced in, or ψ) QTLs (ψQTLs) and editing QTLs (ϕQTLs) were defined as residing within 2 Mb of the regulated gene. Behavioral or molecular QTLs, were deemed coincident if located < 2 Mb apart [15]. The ascertainment rate for RNA editing was defined as the proportion of informative sequence reads for a given site. All editing sites were A to I.

Transcriptome-wide association studies

`FUSION` and `FOCUS` software were used to perform transcriptome-wide association studies (TWASs) [17,18]. `FUSION` reports Z statistics. Significance thresholds used $P < 0.05$, Bonferroni corrected for the number of genes tested. `FOCUS` significance thresholds employed a posterior inclusion probability (pip) > 0.8.

eCAVIAR

eCAVIAR was used to find single nucleotide polymorphisms (SNPs) that co-regulated *cis* eQTLs and behavioral loci with the highest colocalization posterior probability (CLPP) [19]. Markers within 200 SNPs of the *cis* eQTL were evaluated. The support threshold for a co-regulating SNP was CLPP > 0.01.

Statistical analyses

Relevant statistical tests are provided in descriptions of the various analyses.

RESULTS

Saline self-administration

We evaluated 84 strains of the HMDP for IVSA of saline (477 mice) or cocaine (479 mice) over a 10 day testing period, as described previously [6]. Pressing one lever in the testing chamber caused delivery of the infusate (saline or cocaine, depending on experiment), while the other lever was inactive. Infusate delivery was accompanied by flashing of the house light. Four behavioral endpoints were evaluated: number of infusions, active lever presses, percent active lever presses and inactive lever presses.

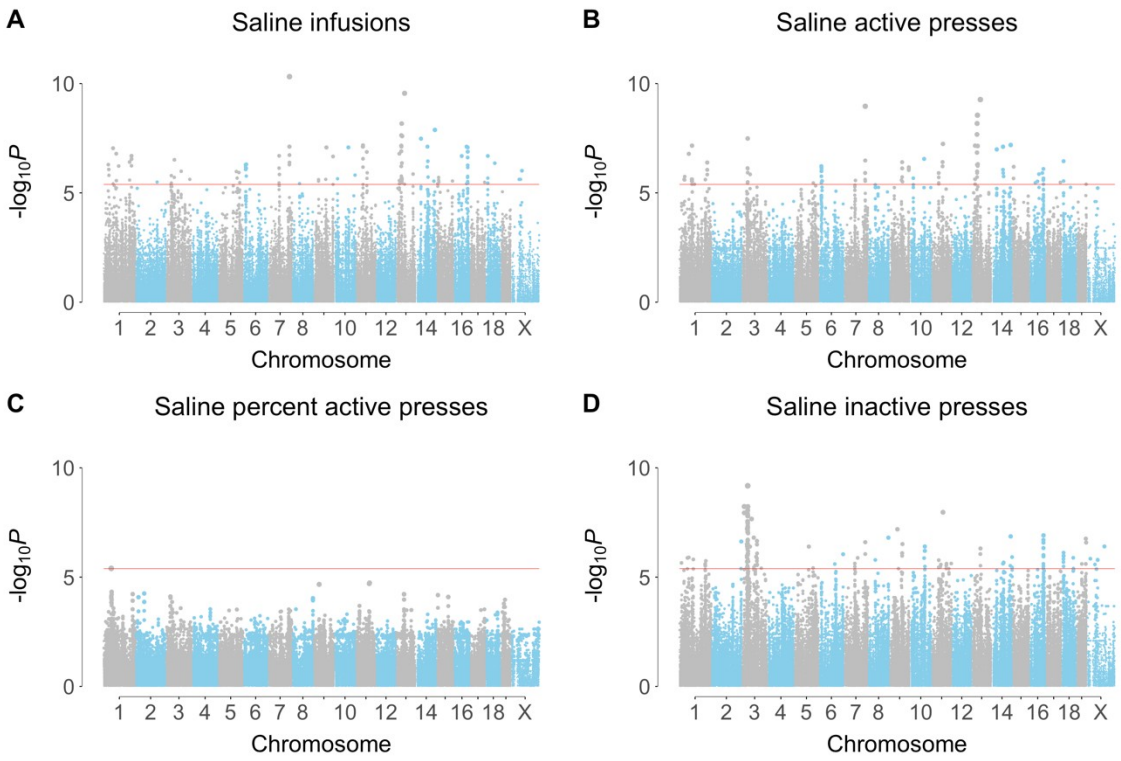

FIGURE 1 Longitudinal genome scans for saline IVSA. (A) Infusions. (B) Active lever presses. (C) Percent active lever presses, (D) Inactive lever presses. Red horizontal line, family-wise error rate = 5%.

Genome-wide association studies (GWASs) for saline IVSA using the four endpoints on each of the ten days produced no genome-wide significant loci [6,7]. To increase statistical power, we used `GMMAT` software to incorporate the longitudinal phenotypes via a linear mixed model [9]. The day of assay was used as both a fixed and random effect together with random effects of SNPs to correct for genetic relatedness, as described [6,7].

A total of 145 significant loci were identified for saline IVSA using the four behavioral endpoints, of which 85 were unique (Figure 1, Table S1, Figure S1). Of the 145 loci for saline IVSA, 56 were for infusions, 35 were for active lever presses, 1 for percent active lever presses and 53 for inactive lever presses. In contrast, 17 significant loci were obtained for cocaine using `GMMAT`, of which 15 were unique [7]. The greater number of loci for saline IVSA is consistent with its higher broad and narrow sense heritabilities.

The only locus significant for both cocaine and saline IVSA was for inactive lever presses on Chromosome 3 [7]. The peak SNP for saline was rs30114031 (37,799,968 bp, $P = 6.6 \times 10^{-10}$), which was more significant than the peak SNP for cocaine, rs30059671 (38,178,200 bp, $P = 3.2 \times 10^{-7}$). *Spry1* is centromeric to both SNPs, but closer to the saline SNP (157,696 bp distant) than the cocaine SNP (535,928 bp).

A triplet of loci for infusions, active lever presses, and inactive lever presses spanned from 66,206,986 bp to 68,567,223 bp (2,360,237 bp) on Chromosome 11 (Figure 2A). *Myh4* was located at the approximate center of this region (67,249,238 bp) and encodes a myosin heavy chain gene. Selection of mice for high voluntary wheel running resulted in mini-muscle mice, which have decreased muscle mass as a result of an intronic SNP in *Myh4* [20].

RNA-Seq

To identify pathways for saline IVSA, RNA-Seq was performed on NAc and mFC from 41 of the cocaine- and saline-exposed strains from the HMDP. NAc and mFC were chosen because of their role in operant self-administration of cocaine [2,5]. A total of 72.8 ± 0.8 M paired-end reads were obtained for each brain region per strain [6]. Transcripts, spliceforms and RNA editing sites regulated by infusate, brain region and sex were previously discussed [7].

Expression QTLs

Cis and *trans* QTLs were mapped for transcript (expression QTLs, or eQTLs), splicing (sQTLs or ψQTLs) and RNA editing (edit QTLs or ϕQTLs) levels in the saline samples using `FaST-LMM` [16]. The number of *cis* eQTLs averaged over the two brain regions for saline was 4,878 ± 253. The distance between the *cis* eQTLs and their corresponding gene was 0.62 Mb ± 0.009 Mb, averaged across brain regions, and is consistent with the linkage disequilibrium of the HMDP [21–23].

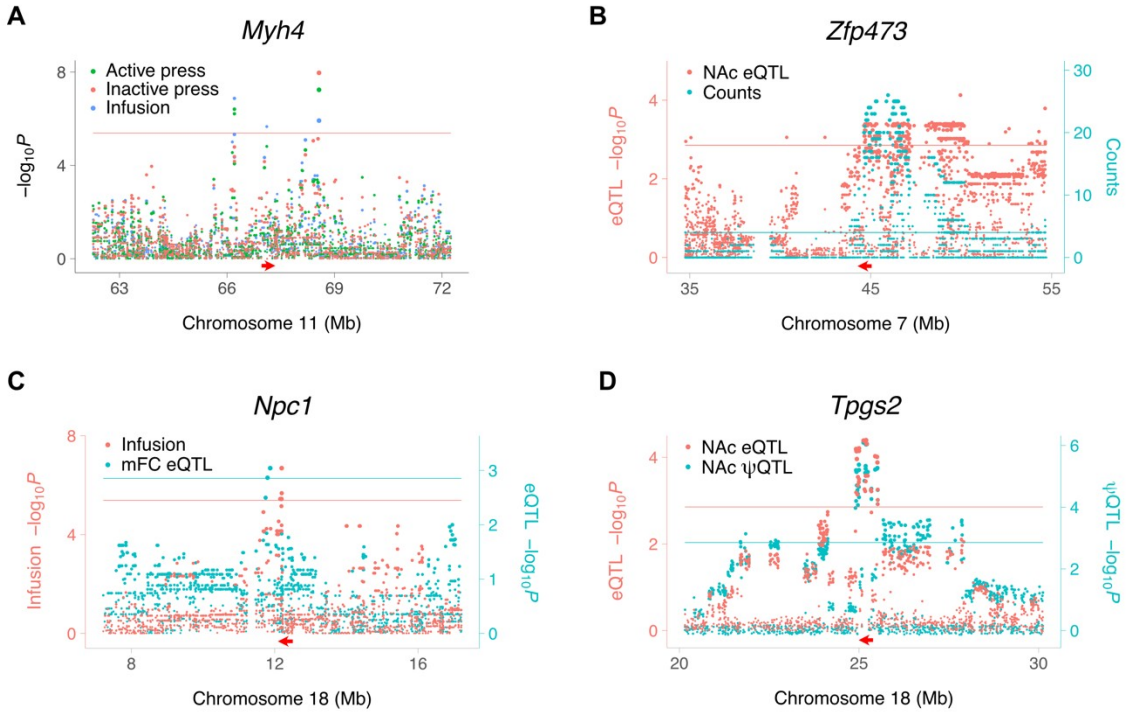

FIGURE 2 Regulation of gene expression in NAc and mFC of saline-exposed mice. (A) Myh4 is close to loci for three different saline IVSA endpoints. (B) Co-aligned NAc eQTL hotspot and Zfp473 cis eQTL. Red arrow, location of Zfp473. Blue horizontal line, eQTL hotspot significance threshold, FDR < 0.05. Red horizontal line, cis eQTL significance threshold. (C) Coincident loci for saline infusions and Npc1 cis eQTL in mFC.

(D) Coincident NAc cis ψQTL for exon 6 and eQTL for Tpgs2. Peak marker rs31436205 for both QTLs. Blue and red horizontal lines, respective significance thresholds.

Hotspots, in which a locus regulates many genes, were identified for transcript abundance [15,24]. A total of 12 hotspots regulating ≥ 20 genes were present in NAc saline samples (FDR < 2.2×10^{-16}), and 22 hotspots in mFC saline samples (FDR < 2.2×10^{-16}). We sought candidate genes for hotspots by looking for co-aligned *cis* eQTLs. One NAc saline hotspot was coincident with a *cis* eQTL for the transcription factor *Zfp473* (Figure 2B).

Npc1 showed a *cis* eQTL co-aligned with a QTL for infusions, suggesting a possible link between *Npc1* and this behavior (Figure 2C). This gene was significant in a human GWAS study for walking pace ($P = 3 \times 10^{-11}$) [25,26].

Splicing QTLs

A total of 1,418 ± 46 *cis* splicing QTLs (ψQTLs) were detected for saline exposed mice, averaged over the two brain regions. Transcript abundance can be affected by genetic variants that alter spliceform preference and hence mRNA stability [27,28]. To evaluate the prevalence of this phenomenon, we examined whether there was a statistically significant enrichment in coincident *cis* eQTLs and ψQTLs. There were significant enrichments in both NAc and mFC. A total of 362 coincident *cis* eQTLs and ψQTLs were found in NAc from saline treated mice (odds ratio, OR, = 2.7, $P < 2.2 \times 10^{-16}$, Fisher's exact test), and 398 in mFC (OR = 2.6, $P < 2.2 \times 10^{-16}$). Similar results were found for the cocaine exposed samples [7], indicating this enrichment is not infusate specific.

An example of a coincident *cis* eQTL and *cis* ψQTL for exon 6 of *Tpgs2* in NAc of saline exposed mice is shown in Figure 2D. The peak SNP, rs31436205, was the same for both the *cis* eQTL and *cis* ψQTL (Figure S2). The C allele was associated with higher percent spliced in of exon 6 of *Tpgs2* and with lower transcript abundance, consistent with inclusion of this exon destabilizing the mRNA.

Editing QTLs

Sequence changes caused by RNA editing can alter transcript stability and abundance as well as changing coding sequence [29]. A total of 262 ± 31 *cis*-acting loci were identified that affect RNA editing efficiency (ϕQTLs) in saline-exposed mice averaged over the two brain regions [30]. Convincing quantitation of RNA editing event is more demanding than for transcript or spliceform abundance, since the editing occurs at single nucleotides. Editing events showed an ascertainment rate of 37 ± 0.5% across RNA-Seq samples from saline-exposed mice averaged over the two brain regions. The less than 100% detection rate implies decreased statistical power and indicates that the ϕQTLs should be treated with some caution.

We looked for statistically significant enrichment in coincident *cis* eQTLs and ϕQTLs to evaluate how often genetically determined variations in RNA editing can change transcript levels. In NAc from saline treated mice, there were 57 co-aligned *cis* eQTLs and ϕQTLs (odds ratio, OR, = 2.1, $P = 1.8 \times 10^{-6}$, Fisher's exact test) and 45 in mFC (OR = 1.8, $P = 4.7 \times 10^{-4}$, Fisher's exact test), both significant enrichments. Co-aligned *cis* ϕQTLs and *cis* eQTLs should show significantly increased numbers of editing sites located in the mature mRNA compared to intronic or intergenic regions, assuming that *cis* ϕQTLs regulate editing events which in turn alter transcript stability and result in *cis* eQTLs. Indeed, we found significantly increased numbers of editing sites in 5' untranslated, 3' untranslated and coding regions compared to intronic and intergenic regions for the coincident *cis* ϕQTLs and eQTLs in both NAc (odds ratio = 2.0, $P = 3.2 \times 10^{-3}$, Fisher's Exact Test) and mFC (odds ratio = 2.5, $P = 2.0 \times 10^{-4}$, Fisher's Exact Test) of saline treated mice.

Statistical enrichment of coincident *cis* eQTLs and ϕQTLs, as well as a corresponding enrichment of editing sites in 5' untranslated, 3' untranslated and coding regions was also found in cocaine exposed mice [7], indicating that these phenomenon are not infusate specific.

Transcriptome-wide association studies

To pinpoint individual genes for saline IVSA, we employed transcriptome-wide association studies (TWASs). This approach acknowledges that most complex trait loci act through variation in expression regulatory sequences, and hence transcript levels, rather than amino acid sequence changes [31]. A gene for a trait is identified by TWAS when the genetically-predicted part of the gene's expression is significantly correlated with the trait. TWAS enhances statistical power because the approach analyzes loci at the gene rather

than marker level, leading to decreased multiple hypothesis correction. FUSION and FOCUS packages were employed to perform TWAS, with FOCUS providing additional fine mapping compared to FUSION [17,18].

A total of 15 genes were uncovered using FUSION of saline exposed NAc, of which 9 were unique (Figure 3). Five genes were uncovered using saline exposed mFC, of which 4 were unique (Figure S3). There was no overlap between the NAc and mFC TWASs. A total of 5 unique genes were significant using FOCUS in NAc (posterior inclusion probability, pip > 0.8; active press: *Serpinb1a*, pip = 0.97; *Pcnp*, pip = 0.91; inactive press: *Cbx2*, pip = 0.94) and in mFC (infusions: *5031434O11Rik*, pip = 0.95; active press: *5031434O11Rik*, pip = 0.90; inactive press: *Tmem241*, pip = 0.94). Two genes, *5031434O11Rik* and *Serpinb1a*, were significant using both FUSION and FOCUS. We found no significant associations for phenotypes related to locomotor activity in a human GWAS catalog using genes identified in the TWASs [25].

FUSION identified *Zfp60*, a zinc finger gene, as significant for inactive lever presses in NAc of saline exposed mice (Figure 3). Decreased *Zfp60* expression was associated with increased inactive lever presses. This gene is expressed in olfactory bulb, hippocampus, cortical subplate and hypothalamus [32]. Consistent with the TWAS finding, a knockout of *Zfp60* in mice shows significant hyperactivity based on multiple endpoints of both the open-field and light-dark tests ($P = 7.9 \times 10^{-7}$ and 4.5×10^{-5}, respectively) [33].

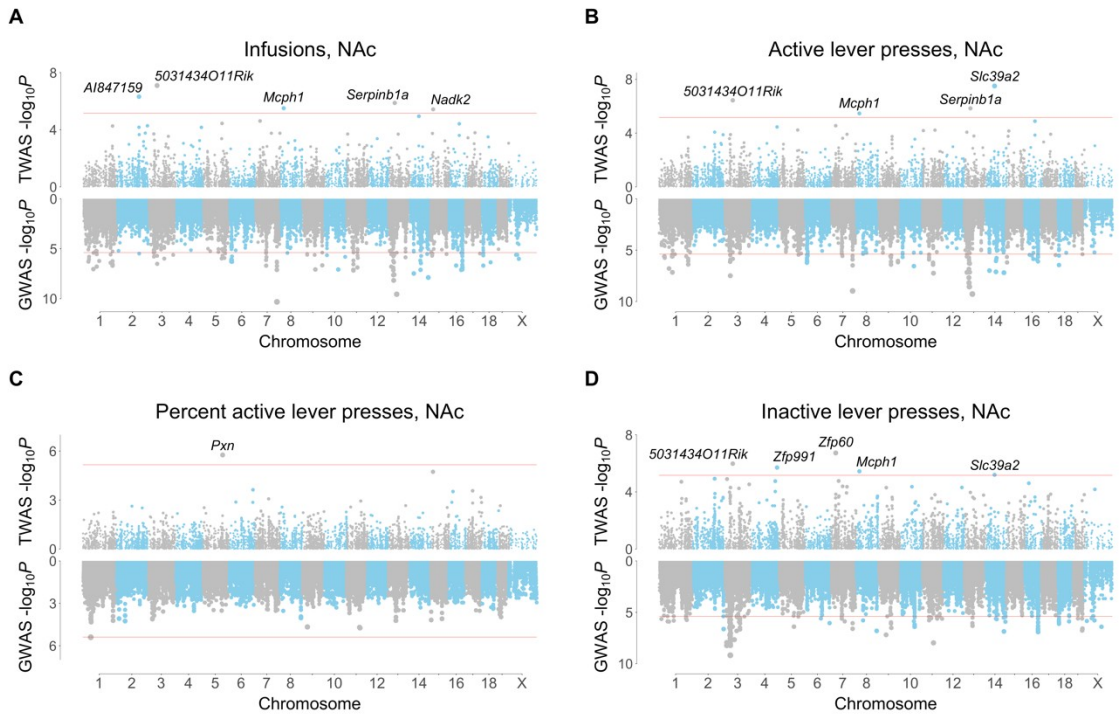

FIGURE 3 `FUSION` *TWASs for saline IVSA using NAc RNA-Seq. (A) Infusions. (B) Active lever presses. (C) Percent active lever presses. (D) Inactive lever presses. TWASs on top, GWASs on bottom.*

Another gene, *5031434O11Rik*, was significant for three out of four IVSA endpoints (infusions, active lever presses and inactive lever presses) using `FUSION` of saline exposed NAc (Figure 3). The gene was also significant for infusions (pip = 0.95, $-\log_{10}P = 14$) and active lever presses (pip = 0.90, $-\log_{10}P = 13$) using `FOCUS` of saline exposed mFC (Figure 4). Increased expression of *5031434O11Rik* was associated with increased lever presses.

The peak SNP for infusions near to *5031434O11Rik* on Chromosome 3 was rs49204785 at 52,563,394 bp, located 999,818 bp telomeric to the gene (Figure 5A). The C allele of rs49204785 was associated with significantly higher saline infusions compared to the T allele (effect size = 0.40 ± 0.08, $P = 3.1 \times 10^{-7}$) (Figure 5B). Consistent with the effect of *5031434O11Rik* being saline specific, there was no significant allelic difference of rs49204785 for cocaine.

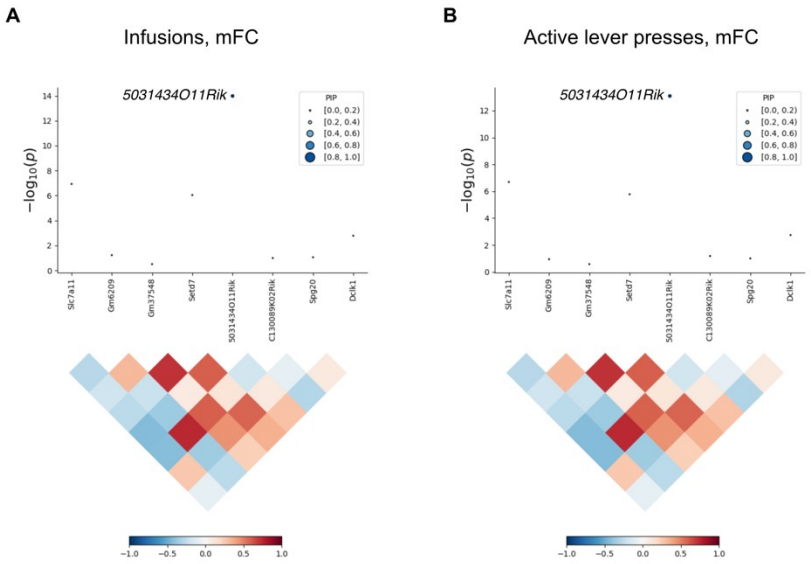

FIGURE 4 FOCUS of mFC in saline exposed mice. (A) FOCUS of 5031434O11Rik for infusions. Linkage disequilibrium map shown underneath. (B) FOCUS of 5031434O11Rik for active lever presses.

The peak SNP for the *5031434O11Rik cis* eQTL was rs45700288 (51,711,018 bp), which was in significant linkage disequilibrium with rs49204785 (D′ = 0.83, R^2 = 0.63, df = 475, $P < 2.2 \times 10^{-16}$) (Figures 5C,D). The T allele of rs49204785 showed significantly decreased saline infusions (linear mixed model: fixed effect, day of test; random intercept of strain; interaction between SNP and infusate: Z = 6.2, $P = 6.8 \times 10^{-10}$) (Figure 5E). In contrast, the effects of the C allele on saline infusions and either allele on cocaine infusions were not significantly different (linear mixed model: fixed effect, day of test; random intercept of strain; Z < 1.3, $P > 0.41$).

eCAVIAR

We used eCAVIAR to further evaluate the contribution of *5031434O11Rik* to saline IVSA. eCAVIAR accounts for the uncertainty introduced by linkage disequilibrium while evaluating the posterior probability that both GWAS and eQTLs are caused by the same SNP [19]. Support for the same causal variant is given by a colocalization posterior probability (CLPP) > 0.01.

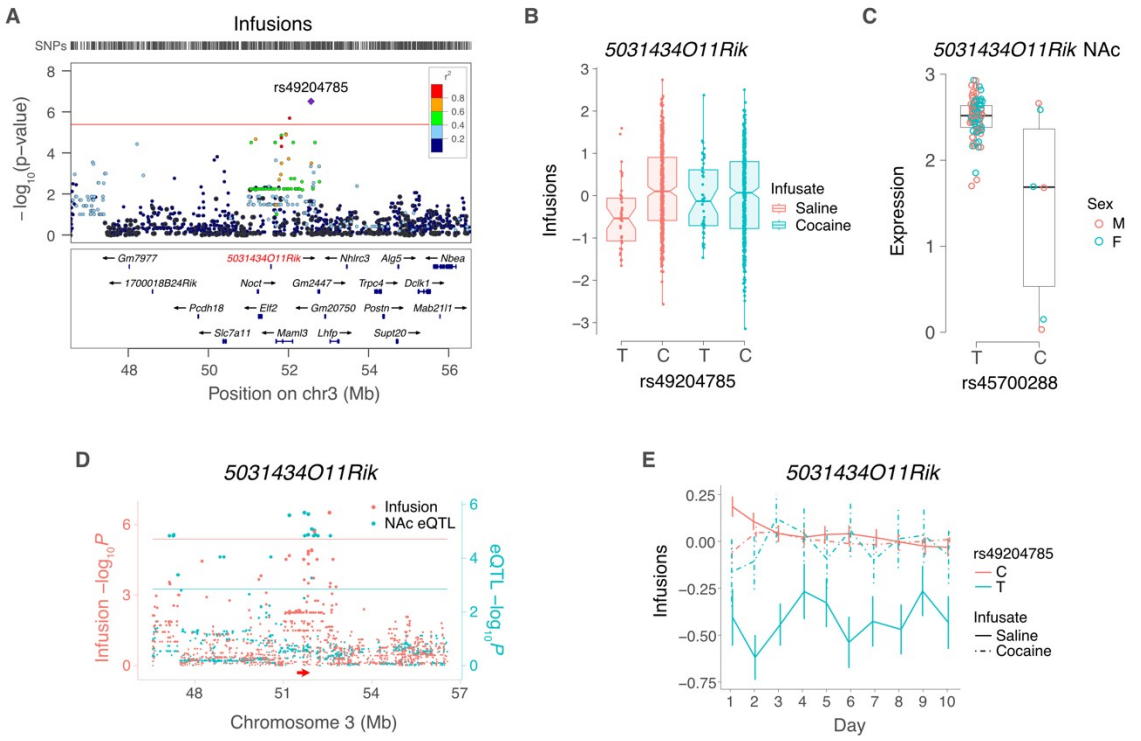

FIGURE 5 5031434O11Rik and saline infusions. (A) LocusZoom plot [34] *for saline IVSA infusions, showing locus harboring 5031434O11Rik (red). Peak SNP is rs49204785. R^2 values indicate linkage disequilibrium. (B) The peak behavior SNP shows significant allelic effect for saline but not cocaine infusions. (C) Expression of 5031434O11Rik in saline NAc. Peak eQTL for 5031434O11Rik is rs45700288. (D) Coincident loci for saline infusions and 5031434O11Rik cis eQTL in saline NAc. (E) Infusion time course for peak behavior SNP of 5031434O11Rik locus, rs49204785. Means ± s.e.m.*

Consistent with a causal role for saline IVSA suggested by the TWASs, `eCAVIAR` identified the peak SNP for the *5031434O11Rik cis* eQTL and the behavioral endpoints as rs48952099 (52,050,163 bp on Chromosome 3; infusions, NAc CLPP = 0.016; mFC CLPP = 0.018; active lever presses, NAc CLPP = 0.015; mFC CLPP = 0.016). This SNP was roughly half-way between the peak eQTL SNP (rs45700288; 51,711,018 bp) and the peak SNP for saline infusions (rs49204785; 52,563,394 bp).

5031434O11Rik

The *5031434O11Rik* gene encodes a long non-coding (lnc) RNA gene and is expressed in the olfactory region, cortex, hippocampus and cerebellum [32]. A recent study found that *5031434O11Rik* was the most strongly differentially expressed gene in the striatum of four mouse lines selectively bred for high voluntary wheel running compared to non-selected controls [35]. Increased expression of *5031434O11Rik* was associated with decreased activity, opposite to our TWAS results. The different genetic backgrounds in the wheel running and present studies may explain the contrasting relationship.

The authors of the wheel running study remarked that *5031434O11Rik* overlaps with, and is a potential antisense transcript to, the 5′ end of a neighboring gene, *Setd7*. Since *Setd7* is a histone lysine methyltransferase, *5031434O11Rik* may act by destabilizing the *Setd7* transcript and altering chromatin. However, we found no significant relationship between *5031434O11Rik* and *Setd7* transcript levels in the combined saline NAc and mFC samples analyzed using a linear mixed model that used tissue and strain as random effects ($t[1,108] = 1.5$, $P = 0.14$). Similarly, there was no significant correlation between *5031434O11Rik* and *Setd7* transcript levels in an RNA-Seq atlas of mouse tissues using tissue as a random effect ($t[1,32] = 1.9$, $P = 0.07$) [36]. These observations suggest that *5031434O11Rik* may regulate locomotor activity by a mechanism independent of chromatin or, if the gene does modify chromatin, it does not act by changing *Setd7* transcript levels.

Saline IVSA and open field

The endpoint of percent active lever presses was an attempt to normalize IVSA based on locomotor activity. Of the 145 unique behavioral loci for saline IVSA, only one was significant for percent active lever presses. In addition, only one gene was significant for percent lever presses using `FUSION`, `FOCUS` and `eCAVIAR`. These observations suggest that the loci for the other saline IVSA endpoints may be detecting genetic effects for phenotypes related to spontaneous locomotor activity. Contrary to this conclusion, there was no significant relationship between the saline IVSA endpoints and distance traveled in the open field for combined data from inbred and BXD recombinant inbred strains ($R < 0.12$, df =65, $P > 0.34$) [12–14].

DISCUSSION

We previously used longitudinal linear mixed models to identify 17 behavioral loci for cocaine IVSA in the HMDP [6,7]. In this study, we used the same approach to analyze a parallel group of control mice that had undergone saline IVSA. We found 145 loci for the

saline IVSA, only one of which overlapped with cocaine. The much larger number of loci for saline compared to cocaine is consistent with the higher broad and narrow sense heritabilities of saline IVSA, and suggests a simpler genetic architecture for saline than cocaine.

Four potential genes emerging from the saline IVSA analyses, *Myh4*, *Npc1*, *Zfp60* and *5031434O11Rik*, were implicated in locomotor activity by previous studies. One region on Chromosome 11 harbored three loci that regulated saline infusions, active lever presses and inactive lever presses. In the middle of this region was *Myh4*, a myosin heavy chain gene. In a previous study, an intronic SNP in *Myh4* was positively selected in mouse lines bred for voluntary wheel running. This SNP causes the mini-muscle phenotype, which results in decreased muscle mass and faster but decreased duration of wheel running. The genetic regulation of saline IVSA could thus be due to non-neural as well as neural effects. *Npc1* possessed a *cis* eQTL that was coincident with a QTL for saline infusions, suggesting a possible role for the gene in this behavior. *Npc1* was also significant in a human GWAS study for walking pace.

The lncRNA gene, *5031434O11Rik*, was implicated in saline infusions, active lever presses and inactive lever presses using `FUSION` TWAS, and in infusions and active lever presses using `FOCUS` TWAS and `eCAVIAR`. *5031434O11Rik* was also the most strongly differentially expressed gene in lines of mice selectively bred for wheel running. Another gene emerging from the TWASs, the zinc finger gene, *Zfp60*, was found to regulate locomotor activity in a human GWAS.

Compared to protein coding genes, our understanding of lncRNA genes is poor, although a role in brain development is emerging [37,38]. Many lncRNA genes regulate chromatin, supporting the idea that *5031434O11Rik* may be an antisense transcript that destabilizes the overlapping mRNA of its neighboring gene, *Setd7* [35]. However, we found no significant relationship between *5031434O11Rik* and *Setd7* transcript levels, suggesting that if *5031434O11Rik* acts by regulating chromatin it uses a mechanism other than through destabilization of *Setd7* transcripts.

Only one locus was significant for percent active lever presses out of the 145 saline loci and only one gene was significant using `FUSION`, `FOCUS` and `eCAVIAR`. Since percent lever presses are a measure of saline IVSA normalized to locomotor activity, the lack of loci for this endpoint are consistent with the other endpoints being surrogate measures of locomotion. Arguing against this conclusion, there was no significant relationship between

saline IVSA and distance traveled in the open field. The relative absence of significant loci for percent active lever presses also suggests that the loci for the other three endpoints are unlikely to be due to noise, and that the *P* value threshold is appropriate for genome-wide association.

Significant enrichment of ψQTLs and ϕQTLs with their co-aligned *cis* eQTLs was found in both saline exposed NAc and mFC. In addition, coincident *cis* ϕQTLs and eQTLs showed significant enrichment of editing sites in untranslated regions and coding regions compared to intronic or intergenic regions. Splicing or RNA editing can thus alter transcript abundance by changing mRNA stability. Similar results were found in the cocaine IVSA study, suggesting that this phenomenon is general.

Our saline IVSA study was designed as a control for cocaine and to dissect the genetic similarities and differences between these behaviors. Indeed our results indicate distinct genetic foundations for the two traits. However, the physiological relevance of saline IVSA is unknown. In outbred rats that successfully acquired cocaine self-administration, operant responding reinforced only by a visual stimulus was correlated with subsequent lever pressing for cocaine [39]. However, our results suggest that reinforcement supplied by the saline infusion and flashing of the house light is regulated by partially different genetic pathways than that provided by cocaine. Notably, pressing the inactive lever does not flash the house light, and the large number of loci for inactive lever presses, some in common with infusions and active lever presses, suggests that the flashing is not the only motivating factor contributing to active lever pressing in saline IVSA.

Although some genes for saline IVSA also regulated locomotor activity, the connection between these traits is clouded by contradictory evidence in our study. Nevertheless, our work establishes that a widely used control infusate for self-administration has minimal genetic overlap with an important drug of addiction, and raises interesting questions about what exactly is being measured by saline IVSA.

AUTHOR CONTRIBUTIONS

Conceived and designed the study: EE, JDJ, DJS. Acquired the data: AHK, JRB. Analyzed the data: AHK, JRB, NL, CG-F, TCS, MC, XX, EE, JDJ, DJS. Wrote the paper: AHK, JRB, JDJ, DJS.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The sequencing data generated in this study can be downloaded from the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/) under accession number PRJNA755328. Data and code are also available from figshare (https://figshare.com/; doi: 10.6084/m9.figshare.27958836).

ETHICS STATEMENT

Animal experiments were approved by the Binghamton University Institutional Animal Care and Use Committee. All procedures were conducted in accordance with guides on the humane care and use of laboratory animals issued by the National Institute of Health and the American Association for Accreditation of Laboratory Animal Care.

ADDITIONAL INFORMATION

Supplementary information is available.

REFERENCES

1. Deak JD, Johnson EC. Genetics of substance use disorders: a review. *Psychol Med*. 2021;51(13):2189-2200. doi:10.1017/S0033291721000969
2. Fernàndez-Castillo N, Cabana-Domínguez J, Corominas R, Cormand B. Molecular genetics of cocaine use disorders in humans. *Mol Psychiatry*. 2022;27(1):624-639. doi:10.1038/s41380-021-01256-1
3. Hedegaard H, Minino AM, Warner M. Drug overdose deaths in the United States, 1999–2018. *Natl Cent Health Stat Data Briefs*. 2020;356:1-7.

4. Palmer RHC, Johnson EC, Won H, et al. Integration of evidence across human and model organism studies: A meeting report. *Genes Brain Behav*. 2021;20(6):e12738. doi:10.1111/gbb.12738
5. Pierce RC, Fant B, Swinford-Jackson SE, Heller EA, Berrettini WH, Wimmer ME. Environmental, genetic and epigenetic contributions to cocaine addiction. *Neuropsychopharmacology*. 2018;43(7):1471-1480. doi:10.1038/s41386-018-0008-x
6. Bagley JR, Khan AH, Smith DJ, Jentsch JD. Extreme phenotypic diversity in operant response to intravenous cocaine or saline infusion in the hybrid mouse diversity panel. *Addict Biol*. 2022;27(3):e13162. doi:10.1111/adb.13162
7. Khan AH, Bagley JR, LaPierre N, et al. Genetic pathways regulating the longitudinal acquisition of cocaine self-administration in a panel of inbred and recombinant inbred mice. *Cell Rep*. 2023;42(8):112856. doi:10.1016/j.celrep.2023.112856
8. Kendler KS, Myers J, Prescott CA. Specificity of genetic and environmental risk factors for symptoms of cannabis, cocaine, alcohol, caffeine, and nicotine dependence. *Arch Gen Psychiatry*. 2007;64(11):1313. doi:10.1001/archpsyc.64.11.1313
9. Chen H, Wang C, Conomos MP, et al. Control for population structure and relatedness for binary traits in genetic association studies via logistic mixed models. *Am J Hum Genet*. 2016;98(4):653-666. doi:10.1016/j.ajhg.2016.02.012
10. Rau CD, Parks B, Wang Y, et al. High-density genotypes of inbred mouse strains: Improved power and precision of association mapping. *G3 GenesGenomesGenetics*. 2015;5(10):2021-2026. doi:10.1534/g3.115.020784
11. Kent WJ, Sugnet CW, Furey TS, et al. The human genome browser at UCSC. *Genome Res*. 2002;12(6):996-1006. doi:10.1101/gr.229102
12. Bogue MA, Ball RL, Philip VM, et al. Mouse Phenome Database: towards a more FAIR-compliant and TRUST-worthy data repository and tool suite for phenotypes and genotypes. *Nucleic Acids Res*. 2023;51(D1):D1067-D1074. doi:10.1093/nar/gkac1007
13. Geuther BQ, Deats SP, Fox KJ, et al. Robust mouse tracking in complex environments using neural networks. *Commun Biol*. 2019;2:124. doi:10.1038/s42003-019-0362-1
14. Delprato A, Algéo M -P., Bonheur B, et al. QTL and systems genetics analysis of mouse grooming and behavioral responses to novelty in an open field. *Genes Brain Behav*. 2017;16(8):790-799. doi:10.1111/gbb.12392
15. Hasin-Brumshtein Y, Khan AH, Hormozdiari F, et al. Hypothalamic transcriptomes of 99 mouse strains reveal trans eQTL hotspots, splicing QTLs and novel non-coding genes. *eLife*. 2016;5:e15614. doi:10.7554/eLife.15614
16. Lippert C, Listgarten J, Liu Y, Kadie CM, Davidson RI, Heckerman D. FaST linear mixed models for genome-wide association studies. *Nat Methods*. 2011;8(10):833-835. doi:10.1038/nmeth.1681

17. Gusev A, Ko A, Shi H, et al. Integrative approaches for large-scale transcriptome-wide association studies. *Nat Genet*. 2016;48(3):245-252. doi:10.1038/ng.3506
18. Mancuso N, Freund MK, Johnson R, et al. Probabilistic fine-mapping of transcriptome-wide association studies. *Nat Genet*. 2019;51(4):675-682. doi:10.1038/s41588-019-0367-1
19. Hormozdiari F, van de Bunt M, Segrè AV, et al. Colocalization of GWAS and eQTL signals detects target genes. *Am J Hum Genet*. 2016;99(6):1245-1260. doi:10.1016/j.ajhg.2016.10.003
20. Talmadge RJ, Acosta W, Garland T. Myosin heavy chain isoform expression in adult and juvenile mini-muscle mice bred for high-voluntary wheel running. *Mech Dev*. 2014;134:16-30. doi:10.1016/j.mod.2014.08.004
21. Bennett BJ, Farber CR, Orozco L, et al. A high-resolution association mapping panel for the dissection of complex traits in mice. *Genome Res*. 2010;20(2):281-290. doi:10.1101/gr.099234.109
22. Ghazalpour A, Rau CD, Farber CR, et al. Hybrid mouse diversity panel: a panel of inbred mouse strains suitable for analysis of complex genetic traits. *Mamm Genome*. 2012;23(9-10):680-692. doi:10.1007/s00335-012-9411-5
23. Lusis AJ, Seldin MM, Allayee H, et al. The Hybrid Mouse Diversity Panel: A resource for systems genetics analyses of metabolic and cardiovascular traits. *J Lipid Res*. 2016;57(6):925-942. doi:10.1194/jlr.R066944
24. Park CC, Gale GD, de Jong S, et al. Gene networks associated with conditional fear in mice identified using a systems genetics approach. *BMC Syst Biol*. 2011;5(1):43. doi:10.1186/1752-0509-5-43
25. Sollis E, Mosaku A, Abid A, et al. The NHGRI-EBI GWAS Catalog: knowledgebase and deposition resource. *Nucleic Acids Res*. 2023;51(D1):D977-D985. doi:10.1093/nar/gkac1010
26. Timmins IR, Zaccardi F, Nelson CP, Franks PW, Yates T, Dudbridge F. Genome-wide association study of self-reported walking pace suggests beneficial effects of brisk walking on health and survival. *Commun Biol*. 2020;3(1):634. doi:10.1038/s42003-020-01357-7
27. Mockenhaupt S, Makeyev EV. Non-coding functions of alternative pre-mRNA splicing in development. *Semin Cell Dev Biol*. 2015;47-48:32-39. doi:10.1016/j.semcdb.2015.10.018
28. Titus MB, Chang AW, Olesnicky EC. Exploring the diverse functional and regulatory consequences of alternative splicing in development and disease. *Front Genet*. 2021;12:775395. doi:10.3389/fgene.2021.775395

29. Brümmer A, Yang Y, Chan TW, Xiao X. Structure-mediated modulation of mRNA abundance by A-to-I editing. *Nat Commun*. 2017;8(1):1255. doi:10.1038/s41467-017-01459-7
30. Li Q, Gloudemans MJ, Geisinger JM, et al. RNA editing underlies genetic risk of common inflammatory diseases. *Nature*. 2022;608(7923):569-577. doi:10.1038/s41586-022-05052-x
31. Cano-Gamez E, Trynka G. From GWAS to function: Using functional genomics to identify the mechanisms underlying complex diseases. *Front Genet*. 2020;11:424. doi:10.3389/fgene.2020.00424
32. Lein ES, Hawrylycz MJ, Ao N, et al. Genome-wide atlas of gene expression in the adult mouse brain. *Nature*. 2007;445(7124):168-176. doi:10.1038/nature05453
33. Groza T, Gomez FL, Mashhadi HH, et al. The International Mouse Phenotyping Consortium: comprehensive knockout phenotyping underpinning the study of human disease. *Nucleic Acids Res*. 2023;51(D1):D1038-D1045. doi:10.1093/nar/gkac972
34. Pruim RJ, Welch RP, Sanna S, et al. LocusZoom: Regional visualization of genome-wide association scan results. *Bioinformatics*. 2010;26(18):2336-2337. doi:10.1093/bioinformatics/btq419
35. Saul MC, Majdak P, Perez S, Reilly M, Garland T, Rhodes JS. High motivation for exercise is associated with altered chromatin regulators of monoamine receptor gene expression in the striatum of selectively bred mice. *Genes Brain Behav*. 2017;16(3):328-341. doi:10.1111/gbb.12347
36. Bastian FB, Roux J, Niknejad A, et al. The Bgee suite: integrated curated expression atlas and comparative transcriptomics in animals. *Nucleic Acids Res*. 2021;49(D1):D831-D847. doi:10.1093/nar/gkaa793
37. Mattick JS, Amaral PP, Carninci P, et al. Long non-coding RNAs: definitions, functions, challenges and recommendations. *Nat Rev Mol Cell Biol*. 2023;24(6):430-447. doi:10.1038/s41580-022-00566-8
38. Srinivas T, Mathias C, Oliveira-Mateos C, Guil S. Roles of lncRNAs in brain development and pathogenesis: Emerging therapeutic opportunities. *Mol Ther*. 2023;31(6):1550-1561. doi:10.1016/j.ymthe.2023.02.008
39. Gancarz AM, Robble MA, Kausch MA, Lloyd DR, Richards JB. Sensory reinforcement as a predictor of cocaine and water self-administration in rats. *Psychopharmacology (Berl)*. 2013;226(2):335-346. doi:10.1007/s00213-012-2907-6

FIGURE LEGENDS

FIGURE 1 Longitudinal genome scans for saline IVSA. (A) Infusions. (B) Active lever presses. (C) Percent active lever presses, (D) Inactive lever presses. Red horizontal line, family-wise error rate = 5%.

FIGURE 2 Regulation of gene expression in NAc and mFC of saline-exposed mice. (A) *Myh4* is close to loci for three different saline IVSA endpoints. (B) Co-aligned NAc eQTL hotspot and *Zfp473 cis* eQTL. Red arrow, location of *Zfp473*. Blue horizontal line, eQTL hotspot significance threshold, FDR < 0.05. Red horizontal line, *cis* eQTL significance threshold. (C) Coincident loci for saline infusions and *Npc1 cis* eQTL in mFC. (D) Coincident NAc *cis* ψQTL for exon 6 and eQTL for *Tpgs2*. Peak marker rs31436205 for both QTLs. Blue and red horizontal lines, respective significance thresholds.

FIGURE 3 `FUSION` TWASs for saline IVSA using NAc RNA-Seq. (A) Infusions. (B) Active lever presses. (C) Percent active lever presses. (D) Inactive lever presses. TWASs on top, GWASs on bottom.

FIGURE 4 `FOCUS` of mFC in saline exposed mice. (A) `FOCUS` of *5031434O11Rik* for infusions. Linkage disequilibrium map shown underneath. (B) `FOCUS` of *5031434O11Rik* for active lever presses.

FIGURE 5 *5031434O11Rik* and saline infusions. (A) LocusZoom plot [34] for saline IVSA infusions, showing locus harboring *5031434O11Rik* (red). Peak SNP is rs49204785. R^2 values indicate linkage disequilibrium. (B) The peak behavior SNP shows significant allelic effect for saline but not cocaine infusions. (C) Expression of *5031434O11Rik* in saline NAc. Peak eQTL for *5031434O11Rik* is rs45700288. (D) Coincident loci for saline infusions and *5031434O11Rik cis* eQTL in saline NAc. (E) Infusion time course for peak behavior SNP of *5031434O11Rik* locus, rs49204785. Means ± s.e.m.