Consideration of genetic and sex effects in mice enhances consilience with human addiction studies

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Abstract

Concerns about external validity of rodent models and translation of findings across species are often based on narrow investigations of populations with limited diversity. Sources of individual variation – including genetics and sex – are only infrequently encompassed in model organism studies. As with most complex diseases, risk for cocaine use disorder is subject to considerable inter-individual variation. Explicit inclusion of individual differences in rodent research may reveal conserved phenotypes and molecular systems relevant to human addiction. We surveyed cocaine-related traits in both males and females of eight inbred mouse strains whose genomes collectively capture 90% of the genetic diversity of the mouse species. Across these strains, individual differences explained a substantial proportion of variance in cocaine-responsive or cocaine response-predictive behavioral and physiological phenotypes. Wild-derived mouse strains often extended the phenotypic ranges of these behaviors beyond what is observed in conventional laboratory strains. Striatum transcriptional responses to cocaine were also highly dependent upon strain and sex differences; most cocaine-responsive genes were differentially expressed in a manner moderated by strain, sex, or their combination. We compared the strain- and sex-mediated transcriptional responses to cocaine in mice to transcriptomic analysis of people with cocaine use disorder and found that mouse similarity to humans was highly dependent upon mouse genetic background and sex. Specifically, male WSB/EiJ mice and female NOD/ShiLtJ mice exhibited the greatest degree of neural transcriptional consilience with humans with cocaine use disorder. Model organism diversity thus represents a crucial source of biological information that can substantially improve external validity of neuropsychiatric research.

23 Significance Statement

24 Laboratory mice are widely used in research on neurobiological mechanisms of addiction, but
 25 most studies use a single strain and often sex of mice. To assess how individual differences in
 26 mice modulate addiction-related traits and how this impacts comparative analysis with
 27 humans, we studied cocaine-relevant behaviors and brain molecular correlates in both males
 28 and females of genetically diverse mouse strains. In this population, individual differences
 29 related to sex and/or genetics explain large proportions of differences in cocaine-related traits.
 30 Importantly, brain gene expression data demonstrated that some strains mimic human
 31 genomic states more readily than others. Individual differences thus represent a crucial and
 32 underdeveloped source of biological information about addiction mechanisms that may
 33 influence the translational utility of such studies.

Introduction

Complex diseases such as neuropsychiatric disorders are typically characterized by the contributions of many genes (Hyman, 2018). Behavioral genetics in non-human animals aims to establish consilience with psychiatrically relevant human systems by studying conserved behaviors and their conserved underlying neural molecular substrates. However, despite mounting evidence of deep conservation in the complex gene systems driving behavior (Saul et al., 2019a; Sinha et al., 2020; Young et al., 2019), uncertainty about the psychiatric relevance of non-human animal systems abounds in the psychiatric genetics community (National Advisory Mental Health Council Workgroup on Genomics, 2018). These doubts likely arise from longstanding and well-characterized difficulties in replicating non-human animal behavioral findings (Crabbe et al., 1999) as well as the failure to translate psychiatric genetics results from any species into the clinic (Hyman, 2012). A critical reassessment of non-human animal behavioral genetics is needed for non-human animals to contribute to the human psychiatric literature.

To simplify experimental design, rodent behavioral and genomic studies of behavioral traits, though often quite detailed in their behavioral and biological scope, most often focus their efforts on identifying individual differences within a single inbred mouse strain for practical reasons (Pascoli et al., 2018; Walker et al., 2018) or within outbred rat populations that often ship from multiple vendors (Fitzpatrick et al., 2013), each with their own genetic bottlenecks. Sex differences are frequently ignored in both human and non-human animal research (Datta et al., 2020). Experimental methodologies agnostic to these known sources of individual differences can produce valuable biological insights in some circumstances, but the omission of sex and genetic variation in these studies limits generalizability without necessarily reducing experimental noise (Prendergast et al., 2014; Tuttle et al., 2018). Further, using a single inbred genetic background can suppress behavioral effects observed in even highly penetrant knockout alleles (Sittig et al., 2016). On the other hand, systematic inclusion of tractable genetic diversity may allow rodents to not just better emulate clinically relevant characteristics, but to contribute new genetic paradigms of understanding neuropsychiatric disorders (Neuner et al., 2019). These important advances in model organism genetics are often ignored in discussions

of how to best model neuropsychiatric phenotypes in non-human animals (e.g. Nestler and Hyman, 2010).

As highly prevalent behavioral disorders, substance use disorders drive a public health crisis associated with substantial morbidity and mortality. Illicit substance use disorders afflict approximately 1 in 14 young adults in the United States (SAMHSA, 2017) and drug overdoses are now the leading cause of accidental death among American adults under 55 (Kochanek et al., 2017). Genetic variation and sex differences are both known to influence addiction vulnerability; cocaine use disorder is highly heritable ($H^2 \approx 0.71$) (Goldman et al., 2005) and substance use behaviors show sex differences in both humans and other animals (Becker et al., 2012; Becker and Chartoff, 2019). Consequently, the neurobiology underlying addiction cannot be fully understood without consideration of genetic background and sex in both humans and non-human animals.

Given the genetic diversity in the population of inbred mouse strains (Beck et al., 2000) and the multitude of derived mouse recombinant inbred and heterogeneous stock populations useful for genetic mapping and trait correlation work (Chesler et al., 2005; Logan et al., 2013; Philip et al., 2011), it is possible to sample over diverse genotypes and identify strains and sexes that best mimic the human disease state. Such a strategy can improve translational relevance while surveying these populations for heritable drivers of unidentified genetic mechanisms of disease susceptibility. Though rodent genetic variation does not capture precise human variants, it can be exploited to determine underlying mechanisms in addiction-relevant processes (Bogenpohl et al., 2017; Huggett et al., 2020; Palmer et al., 2019). As a further benefit, genotypic and phenotypic precision, along with high minor allele frequencies and in many cases, a well-randomized population structure, allows genetics studies in rodents to be performed at orders of magnitude lower cost than human GWAS.

To assess the influence of genetic variation, sex, and their potential interactions on cocaine-related phenotypes in mice, we undertook a large-scale evaluation of behavioral, physiological, and brain transcriptomic measures in both male and female mice from the eight inbred founder strains of the Diversity Outbred (DO) mouse heterogeneous stock (Saul et al., 2019b). Because these strains include wild-derived inbred strains from the three dominant subspecies of mice,

their genomes together capture approximately 90% of the genetic diversity in the species *Mus musculus* (Roberts et al., 2007). We further assessed how strain and sex differences drive consilience with human addiction, identifying which strains and sexes capture significant overlapping brain molecular correlates with human cocaine use disorder.

In the eight founder strains for the DO, we surveyed behavioral and physiological correlates of vulnerability to cocaine use – including multiple novelty response behaviors, circadian molecular rhythm phenotypes, and reversal learning as a measure of reward learning and impulsivity. We directly measured cocaine-related behaviors such as initial locomotor sensitivity to cocaine and intravenous self-administration (IVSA) of cocaine. Finally, using RNA sequencing (RNAseq), we measured the striatum transcriptome response to cocaine in all eight founder strains and compared them to a meta-analysis of postmortem brain tissue from cocaine use disorder patients. Our work represents the first time that many of these behavioral, physiological, and molecular traits have been studied using methods powered to detect sex and genotype effects and the extent to which these interact to moderate one another's effects.

Results and Discussion

We first examined heritability, sex differences, and genetic differences moderated by sex among the founders of the DO. Significant effects are reported in the text; all tests are reported in **Supplementary Table S1**.

Response to novelty predicts psychostimulant addiction-related phenotypes in both humans (Ersche et al., 2010) and mice (Dickson et al., 2015). We first assessed differences in behavioral traits related to exploration and response to novelty using the open field, light-dark box, hole board and novel place preference tests. Though novel place preference heritability was moderately weak ($H^2 = 0.16$), the other three phenotypes displayed strong to very strong heritability ($H^2 = 0.42-0.55$, **Figure 1A-D**). Further, for the phenotype of total entries in the hole board test, an exploratory behavior, there was a significant sex difference ($F_{1,597} = 10.69$, $p = 0.0014$), which was apparently driven by lower hole board exploration in males than females for all strains but PWK/PhJ. We detected strain-by-sex interactions in the total entries in the hole board ($F_{7,597} = 2.74$, $p = 0.0083$) and in the proportion of distance traveled in the center of

the open field ($F_{7,684} = 2.09$, $p = 0.043$), indicating that sex differences in these novelty response traits are moderated by genetic background. Total entries in the hole board (**Figure 1A**) and novel place preference (**Figure 1B**) exhibited extended range due to the inclusion of wild-derived strains, demonstrating that wild-derived genetic variability functions in defining an expanded phenotypic range (Wahlsten et al., 2003). Transitions between the sides of the light-dark box also showed phenotypes toward the extreme in wild-derived mouse strains along with NOD/ShiLtJ mice (**Figure 1D**).

Circadian rhythm and reward-related behavioral phenotypes are co-inherited and rhythm disruptions are linked to development and progression of substance use disorders (Logan et al.,

2014). Further, the molecular clock system directly influences the expression of dopamine receptors in the striatum involved in the modulation of cocaine reward-related behaviors (Ozburn et al., 2015). A cell-based assay on fibroblasts derived from each of the founder strains in which a *Bmal1-dLuc* reporter was utilized for circadian measurement of luciferase bioluminescence to assess differences in circadian rhythmicity (Kim et al., 2016; Ramanathan et al., 2014). We found very high heritability of the amplitude of these rhythmic patterns ($H^2 = 0.59$, **Figure 1E**), but no significant sex differences or strain x sex interactions. These results suggest that genetic differences in the molecular clock are one potential mechanism for individual differences in addiction-related phenotypes.

Reversal learning tasks evaluate impulsive and compulsive behaviors that predict addiction liability (Izquierdo and Jentsch, 2012). Within reversal learning paradigms, one measure of impulsivity is the number of premature responses produced during the reversal phase of the task. This phenotype is similar to an impulsivity-related measure implemented in the five choice serial reaction time task (Dalley et al., 2007). Premature reversal responses in our study showed moderate heritability across our eight strains of mice ($H^2 = 0.29$, **Figure 1F**), but no significant sex differences or strain x sex interactions. We observed the lowest rate of premature responding in the wild-derived PWK/PhJ strain.

We next sought to assess individual differences in initial locomotor response to cocaine, a behavioral phenotype that predicts subsequent drug use in humans (de Wit and Phillips, 2012). In these diverse mouse strains, initial cocaine sensitivity showed strong heritability ($H^2 = 0.48$ for initial sensitivity, **Figure 1G**). Wild-derived strains, particularly WSB/EiJ and PWK/PhJ, exhibited the highest initial sensitivity to cocaine.

Operant drug self-administration procedures directly quantify reinforced responding for drug (Dickson et al., 2015) and produce profound transcriptional responses in inbred strain (Walker et al., 2018). Heritability of cocaine IVSA traits in the founders of the DO was strong ($H^2 = 0.47$ for sessions to acquisition of IVSA, **Figure 1H**) to very strong ($H^2 = 0.60$ for total infusions at FR-1 1.0 mg/kg, **Figure 1I**). The high heritability of infusions earned is similar in magnitude to the observed heritability of human cocaine use disorder (Goldman et al., 2005). Of note, 129S1/SvImJ mice do not acquire IVSA and do not take any cocaine during the acquisition

phase. The largest phenotypic range manifests in wild-derived mice; PWK/PhJ mice acquired IVSA behavior very quickly and self-administered the most infusions of cocaine at FR-1 1.0 mg/kg – about four times as much as C57BL/6J – while WSB/EiJ mice self-administered the fewest infusions of cocaine relative to the other strains that do acquire IVSA behavior. The broad phenotypic range of volitional cocaine-taking behaviors in these genetically diverse mouse strains represents a clear opportunity to study mechanisms underlying variation in the initiation of cocaine of addiction. Because addiction-related phenotypes are highly heritable and sometimes exhibit sex differences moderated by genetics, we next sought to assess heritable differences in the molecular response to cocaine. Male and female mice of the eight DO founder strains were given multiple injections of cocaine or saline over 19 days. Samples for bulk RNAseq were collected from striatum 24-48 hours after the final injection of repeated administration of either cocaine or vehicle (sham). First, we documented that in the absence of cocaine, most transcripts exhibited expression that was moderate to strong at baseline in the founder strains (median $H^2 = 0.29$) and that

subspecies of origin explains the greatest amount of variance in expression (**Figure 2A**). Using a linear modeling approach (Chen et al., 2014; Phipson et al., 2016), we found few expression differences attributable solely to the effect of cocaine treatment. Instead, significant effects of cocaine arose in interaction with individual differences such as genetic background, sex, and their interaction. The greatest quantity of differentially expressed transcripts was observed in the strain-by-sex-by-drug treatment three-way interaction (1,282 genes at $q < 0.01$, **Figure 2B**, **Supplementary Table S2**). Some strains showed stronger cocaine effects as measured by number of differentially expressed genes than others; 129S1/SvImJ and PWK/PhJ had many genes whose expression is influenced by cocaine and sex-by-cocaine interactions (PWK/PhJ: 89 genes at $q < 0.01$, **Figure 2C**) while the commonly used C57BL/6J strain had very few genes influenced by cocaine (four genes at $q < 0.01$, **Figure 2C**). Because statistical power was approximately equal for all strains involved in this study, these differences likely reflect real individual differences in the brain's sex-specific responses to cocaine.

This genetics- and sex-inclusive approach identified many more changes in gene expression following repeated cocaine exposure than had we used only a single sex in a single inbred strain. For instance, we identified differential expression of the serotonin receptor 1D gene *Htr1d* in the strain-by-sex-by-cocaine interaction factor. This gene, while not altered in C57BL/6J animals, is upregulated in males of 129S1/SvImJ and PWK/PhJ strains, but downregulated in males of the CAST/EiJ strain (**Figure 2D**).

Some of the heritable transcriptome differences we observed corroborate previous work showing heritable differences in pharmacokinetics of cocaine (Wiltshire et al., 2015) – for example, PWD/PhJ mice – closely related to the high cocaine taking PWK/PhJ strain – are known to have some of the highest brain concentrations of cocaine shortly after injection. These pharmacokinetic differences do not account for the strong differences between strains such as 129S1/SvImJ and A/J, whose brain cocaine pharmacokinetic profiles are very similar (Wiltshire et al., 2015). Further research on highly diverse mice may resolve the mechanisms driving individual differences between strains with closely matched pharmacokinetic profiles.

We assessed to what degree strain and sex combinations mimic human genomic findings to understand how individual differences' influence on consilience with human research. We used

the MSET method (Eisinger et al., 2013) to identify statistically significant overlaps of orthologous genes between a human cocaine use disorder brain transcriptome dataset and analogously analyzed cocaine response transcriptomes within each individual strain and sex combination (Huggett et al., 2020). The magnitude of overlap was highly variable; the strongest overlaps were in NOD/ShiLtJ female and WSB/EiJ male mice, though the NOD/ShiLtJ female finding is not robust to different significance thresholds and does not survive stringent multiple testing correction (Figure 3A, Supplementary Table S3. Other strains, including the most widely used C57BL/6J, do not strongly mimic cocaine use disorder brain transcriptomes, a finding consistent with previous observations of C57BL/6J males (Huggett et al., 2020). When we examined the directionality of differential expression overlaps in NOD/ShiLtJ females and in WSB/EiJ males, we found concordance in the upregulated and downregulated genes between human and mouse data; in this experiment, WSB/EiJ males mostly captured downregulated aspects of expression while NOD/ShiLtJ females mostly captured upregulated aspects (Figure

3B). Genes within the NOD/ShiLtJ female set include directional matches in immediate early genes such as *Arc*, and *Junb* while genes downregulated in WSB/EiJ males include multiple oxidative phosphorylation-related genes such as *Ndufa5*, *Cox6c2*, and *Ndufaf2*. The presence of two mitochondrial Complex I subunit genes is particularly interesting; cocaine treatment disrupts Complex I in the rat brain (Cunha-Oliveira et al., 2013) and a Complex I subunit gene is associated with cocaine use disorder in humans (Huggett and Stallings, 2020a).

To describe the molecular systems dysregulated after repeated cocaine exposure in these strains, we performed separate Gene Ontology Biological Process (GO BP) analysis of the genes shared between human cocaine use disorder and WSB/EiJ male or the NOD/ShiLtJ female differentially expressed gene sets. WSB/EiJ male differentially expressed genes were enriched mostly for RNA binding and mRNA processing while NOD/ShiLtJ female differentially expressed genes were enriched for nucleobase-related metabolic processes (**Supplementary Table S5**. Together, these results imply that similarities mostly capture alterations in transcriptional regulatory components.

Because the transcriptome results derive from the same group of mice as cocaine locomotor sensitivity, it is of particular note that WSB/EiJ mice show the strongest behavioral response in cocaine-induced locomotor sensitivity of all the strains tested (**Figure 1G**). Variants that alter initial sensitivity to drugs are among the best supported and described addiction candidates in humans, for example, nicotinic acetylcholine receptor polymorphisms associated with smoking alter the receptor's sensitivity to nicotinic agonists (Bierut et al., 2008). This result implies that WSB/EiJ male mice experience human-relevant alterations in their neurobiological states due to differential cocaine sensitivity after repeated cocaine injection. However, WSB/EiJ mice take fewer infusions of cocaine at FR-1 1.0 mg/kg than any other strain that reliably acquires IVSA (**Figure 1H**). Though the finding of higher consilience associated with lower cocaine-taking behavior appears to contradict the conventional interpretation of cocaine self-administration, where higher cocaine intake indicates higher addiction vulnerability (Piazza et al., 2000), we note that experimenter-administered cocaine affects neural signaling differently than self-administered cocaine (McCutcheon et al., 2011). Consequently, this finding should demonstrate that individual differences are highly important to consider for translational research using non-

human animals, but should not be taken as a specific recommendation of WSB/EiJ males as a model for human cocaine use disorder phenotypes.

The translational relevance of non-human animals – often represented by mice – is the subject of substantial discussion (Seok et al., 2013; Takao and Miyakawa, 2015). Within addiction biology, a particularly active source of ferment is the question of how rodents may best recapitulate aspects of addictive behaviors such as compulsive use and reinstatement (Ahmed, 2012). Here, we further this discussion by demonstrating that similarity of rodent molecular systems to those involved in human neuropsychiatric-traits are heterogeneous and contingent upon the specific rodents chosen. Within conventional inbred mouse strains, NOD/ShiLtJ females and WSB/EiJ males showed the highest consilience with human cocaine phenotypes in the brain as measured by similarity in genomic response. This finding has broad implications for translational work. In many cases, the study of a single strain and/or only one sex of mice within an experiment may limit the generalizability of the data and diminish potential relevance for human addiction. Rather, it may be advantageous to explicitly take individual differences of the type reported here into account. Strategies for querying diversity include identification of specific strains that exhibit characteristics of the disease-susceptible population at a behavioral or molecular level or surveys of a diverse population to ensure that natural variation is represented, which allows queries of known biological sources of variation in addiction vulnerability.

An important aspect of experimental rigor is the external validity of model organism research paradigms. Here we show that this validity is in part driven by the organism under investigation in addition to other characteristics of the research paradigm. Genetic variation is a valuable resource for the discovery of biological mechanisms of addiction (Kumar et al., 2013; Ruan et al., 2020). Similar to humans, individual differences among mice greatly influence behavioral, physiological, and transcriptomic cocaine-related traits. For many of these traits, individual differences explain a substantial proportion of the variation. This variation can be exploited to enhance consilience of biological systems under study, or to discover the underlying biological mechanisms of vulnerability to disease. Individual variation in addiction-related traits is a largely untapped resource that can be exploited to improve and accelerate discovery of

neurobiological and genetic mechanisms related to risk for addiction as well as other complex diseases.

Methods

Standard Operating Procedures

All methodologies used in this work are documented in depth in the Center for Systems Neurogenetics of Addiction's Standard Operating Procedures (SOPs) (<https://www.jax.org/research-and-faculty/research-centers/systems-neurogenetics/data-resources>). The brief methods appearing below summarize these SOPs and reference specific SOPs.

Animals

Mice from the following strains were surveyed in these experiments: A/J (JAX stock #000646), C57BL/6J (JAX stock #000664), 129S1/SvImJ (JAX stock #002448), NOD/ShiLtJ (JAX stock #001976), NZO/HILtJ (JAX stock #002105), CAST/EiJ (JAX stock #000928), PWK/PhJ (JAX stock #003715), and WSB/EiJ (JAX stock #001145). These strains are the founders of the DO heterogeneous stock and CC recombinant inbred strains. Surveys of these eight strains can demonstrate statistical heritability patterns that justify further dissection using the derived resources (Saul et al., 2019b). The mice used in the Research Animal Facility at The Jackson Laboratory came from breeding colonies maintained in the Research Animal Facility. These colonies were derived from production colonies at The Jackson Laboratory and breeders were replaced with animals from The Jackson Laboratory's production colony at least every five generations. The mice used in the Jentsch Lab at Binghamton University were shipped to the Jentsch Lab from either the Research Animal Facility colonies or from production colonies in The Jackson Laboratory. The studies described utilized a total of 1,085 mice (**Supplementary Table S4**). All procedures were approved by the Jackson Laboratory of Mammalian Genetics or Binghamton University institutional animal care and use committees.

Mouse Husbandry and Housing

Mice were housed according to the CSNA animal housing SOP (<https://www.jax.org/-/media/jaxweb/files/research-and-faculty/tools-and-resources/system-neurogenetics/csna-animal-housing.pdf>).

Cocaine

Cocaine hydrochloride was provided by the National Institute on Drug Abuse Drug Supply Program Division of Therapeutics and Medical Consequences (catalog number: 9041-001). Cocaine was stored in powder form at room temperature until it was formulated into 0.9% Saline (100 mg/mL clear solution) in various concentrations specific to each experiment according to their individual SOPs.

Novelty Response Behavioral Phenotypes

Open field, light-dark box, hole board, and novel place preference behavioral paradigms were conducted in this order on consecutive days over the course of a week during the light phase of the light:dark cycle. Open field data were collected for 60 minutes according to the SOP (<https://www.jax.org/-/media/jaxweb/files/research-and-faculty/tools-and-resources/system-neurogenetics/open-field-assay.pdf?la=en&hash=32DDAFF2B17B2D4961C136C5616C4982AC23EC3B>). Light-dark data were collected for 20 minutes with the mouse starting in the light side of the chamber facing the dark side according to the SOP (<https://www.jax.org/-/media/jaxweb/files/research-and-faculty/tools-and-resources/system-neurogenetics/light-dark-assay.pdf?la=en&hash=A63CF8D22EB7936CF6C69A3178373981F4016675>). Hole board data were collected for 20 minutes according to the SOP (<https://www.jax.org/-/media/jaxweb/files/research-and-faculty/tools-and-resources/system-neurogenetics/hole-board-assay.pdf?la=en&hash=EC343A797D37209CF64D34E6031608A511D8E15D>). Novel place preference included a five minute acclimation period to a center chamber, a 10 minute exposure period to a randomized exposure side, a five minute acclimation period, and a final test period consisting of a five minute habituation period again to the center and a 20 minutes preference assessment for which both the novel side and the initial familiar exposed side were

accessible according to the SOP (<https://www.jax.org/-/media/jaxweb/files/research-and-faculty/tools-and-resources/system-neurogenetics/novelty-place-preference-assay.pdf?la=en&hash=B5D2D0FC9028B408E84729C0C8832C580AB8E039>). All mice from the Center for Systems Neurogenetics of Addiction were tested through this novelty pipeline prior to any other test and were then randomized and assigned into either reversal learning, cocaine locomotor sensitization, or cocaine intravenous self-administration. The novelty study produced observations from a total of 783 mice.

Bmal1-dLuc Circadian Rhythm Data

Data for circadian rhythm were measured in primary fibroblast cultures generated from skin biopsies in the founders. To isolate fibroblasts, ear biopsies (one mm in diameter) were digested in Dulbecco's Modified Eagle's Medium (DMEM, HyClone) containing 2.5 mg/ml collagenase D (Gibco) and 1.25 mg/ml pronase (Millipore) for 90 mins and then plated in DMEM growth media containing 10% Fetal Bovine Serum (FBS, HyClone), 292 µg/ml L-glutamine (HyClone), 100 units/ml penicillin (Hyclone) and 100 µg/ml streptomycin (HyClone). *Bmal1-dLuc* reporter was delivered to fibroblasts by lentiviral-mediated gene delivery (VectorBuilder). Following synchronization of rhythms by 15 µM forskolin (Sigma) for two hours, the temporal patterns of *Bmal1-dLuc* bioluminescence was recorded for ~70 seconds at intervals of 10 minutes over six to seven days from fibroblast cultures in DMEM recording media containing 15 µM forskolin, 25 mM HEPES (Gibco), 292 µg/ml L-glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, and 10 µM luciferin (Promega) by an automated 32-channel luminometer (Lumicycle, ActiMetrics) in a standard tissue culture incubator at 32°C. The amplitude of bioluminescence rhythms was determined from baseline-subtracted data using the damped sine fit and Levenberg-Marquardt algorithm (Izumo et al., 2003). The circadian study produced observations from a total of 56 mice.

Reversal Learning

Data for reversal learning were collected at both JAX and Binghamton University using the SOP (<https://www.jax.org/-/media/jaxweb/files/research-and-faculty/tools-and-resources/system-neurogenetics/reversal-learning->

[assay.pdf?la=en&hash=8484E47B170462960E11C1FAEEE6FF3CE6FDFC08](https://www.jax.org/-/media/jaxweb/files/research-and-faculty/tools-and-resources/system-neurogenetics/cocaine-locomotor-sensitization-assay.pdf?la=en&hash=8484E47B170462960E11C1FAEEE6FF3CE6FDFC08)). The reversal learning data produced observations from a total of 202 mice.

Initial Locomotor Sensitivity to Cocaine

Data for initial locomotor sensitivity were collected as described previously (Schoenrock et al., 2020) using data from days 1-3 in the SOP for locomotor behavioral sensitization to cocaine (<https://www.jax.org/-/media/jaxweb/files/research-and-faculty/tools-and-resources/system-neurogenetics/cocaine-locomotor-sensitization-assay.pdf?la=en&hash=9E5D4C248C3BCCAD947C164AE81663C13A77EB0D>). Briefly, mice were placed into the open field arena for 30 minutes, removed, and injected i.p. with either saline (days 1-2) or 10 mg/kg cocaine (day 3) and returned to the open field arena for 60 minutes. Distance moved after injection on day 3 minus day 2 was used as a measure of initial locomotor sensitivity to cocaine. The sensitization study produced observations from a total of 230 mice.

Cocaine Intravenous Self-Administration

Prior to cocaine intravenous self-administration, mice were implanted with a jugular catheter and allowed a minimum of 10 days for post-operative recovery. In an operant conditioning paradigm, mice were allowed to acquire cocaine self-administration at 1.0 mg/kg, then evaluated for dose-response effects at eight different doses. After a stabilizing dose at 1.8 mg/kg, extinction-related responses during seven days of withdrawal were recorded. Finally, cued reinstatement was recorded for two days. Self-administration in these eight mouse strains was performed according to v1.0 of the CSNA's SOP (<https://www.jax.org/-/media/jaxweb/files/research-and-faculty/tools-and-resources/system-neurogenetics/intravenous-self-administration-ivsa-paradigm.pdf?la=en&hash=FA64135F219C7DF65937A1CF9270301B0E771836>). The intravenous self-administration study produced observations from a total of 217 mice.

Data Deposit

Data for each phenotype will be deposited in the Mouse Phenome Database (MPD) (Bogue et al., 2019) upon publication.

Heritability Calculations

For each trait, heritability was calculated from linear models using the isogenic strain as the independent categorical variable using the following equation:

$$h^2 = \frac{MS_{strain}}{MS_{strain} + (n_{mean} - 1) * MS_{resid}}$$

where MS_{strain} is the mean square of the strain effect, n_{mean} is the mean number of samples within each strain, and MS_{resid} is the mean square of the residuals. For the reversal learning data, an additional additive covariate of site was included in the model to account for inter-lab variation. This term was not utilized in the heritability calculation.

For some traits such as number of infusions at FR-1 1.0 mg/kg cocaine self-administration, a single strain such as 129S1/SvImJ showed little to no variation, which may upwardly bias heritability calculations. For these traits, heritability was calculated both with and without the low variance strain. The data reported in the paper rely upon the inclusive calculation, but results of both methods of calculation are reported for completeness (see **Supplementary Table S1**).

RNAseq

Striatum tissue was collected during the light stage of the light:dark cycle between 24 and 48 hours after the final injection in the cocaine behavioral sensitization protocol according to the SOP (<https://www.jax.org/-/media/jaxweb/files/research-and-faculty/tools-and-resources/system-neurogenetics/post-sensitization-tissue-collection.pdf?la=en&hash=9E6CD8DEB39606B791A5D25F6CD0611EF14D96A7>). Tissue was collected for both sexes of each founder strain exposed to either sham (saline) or 10 mg/kg IP cocaine.

RNA was isolated from striatum tissue using the MagMAX mirVana Total RNA Isolation Kit (ThermoFisher) and the KingFisher Flex purification system (ThermoFisher). Tissues were lysed and homogenized in TRIzol Reagent (ThermoFisher). After the addition of chloroform, the RNA-containing aqueous layer was removed for RNA isolation according to the manufacturer's protocol, beginning with the RNA bead binding step.

RNA concentration and quality were assessed using the Nanodrop 2000 spectrophotometer (Thermo Scientific) and the RNA Total RNA Nano assay (Agilent Technologies). 2µl of diluted 1:1000 diluted ERCC Spike-in Control Mix 1 (Ambion by Life Technologies) was added to 100ng of each RNA sample prior to library construction. Libraries were prepared by the Genome Technologies core service at The Jackson Laboratory using the KAPA RNA Hyper Prep Kit with RiboErase (HMR) (KAPA Biosystems), according to the manufacturer's instructions. Briefly, the protocol entails depletion of ribosomal RNA (rRNA), RNA fragmentation, first and second strand cDNA synthesis, ligation of Illumina-specific adapters containing a unique barcode sequence for each library, magnetic bead size selection, and PCR amplification. Libraries were checked for quality and concentration using the D5000 ScreenTape assay (Agilent Technologies) and quantitative PCR (KAPA Biosystems), according to the manufacturers' instructions.

RNAseq libraries were pooled and sequenced by Novogene in 150 bp paired-end format on an Illumina NovaSeq 6000 sequencer targeting 90 million read pairs per sample. Sequencing achieved a median read depth of 132 million reads. The resultant reads were determined to be of consistently high quality using fastqc v0.11.3 and MultiQC v1.2.

Reads were generated from raw data and demultiplexed using BCL2Fastq v2.18.0.12, concatenated by sample, and aligned with the STAR aligner v2.6.1 (Dobin et al., 2013) to the GRCm38 mouse reference genome with v94 of the Ensembl transcriptome. Transcript-level quantification was estimated using RSEM v1.3.0 (Li and Dewey, 2011) on a transcriptome BAM file produced as an output of this alignment. The data were imported into R v3.5.1 and summarized to the gene level using tximport v1.10.1 (Soneson et al., 2016), TMM-normalized using edgeR v3.24.3 (Chen et al., 2014), and imported into limma v3.38.3 (Ritchie et al., 2015) using the log₂-transformation function voom. We compared multivariate approaches modeling with interaction factors between edgeR and voom+limma approaches and found that voom+limma performs better than edgeR for controlling false negatives. Upon initial examination of the findings, we identified intermittent contamination with choroid plexus, which potentially derives from the ventricular aspect of the dorsal striatum. Correcting for this contamination necessitated an additive covariate for choroid plexus consisting of log-mean CPM values of *Kl* and *Ttr* expression, unambiguous markers for choroid plexus (Sathyanesan et al.,

2012). These values were $\log_2$ transformed for work in limma. For An overall model for all strains included this choroid plexus factor as a nuisance variable plus the main effects of strain, sex, and cocaine injection and all of their interactions. Individual models included the choroid plexus nuisance variable plus sex, cocaine injection, and sex:cocaine injection interaction. Correction for local false discovery rates utilized the qvalue package in R v2.14.1 (Storey and Tibshirani, 2003). Because brain transcriptional changes are subtle (Hitzemann et al., 2014), all results reported are at $q < 0.01$ with no fold-change cutoff (**Supplementary Table S2**).

Raw data and transcript-level expression estimates will be deposited in the Gene Expression Omnibus (Barrett et al., 2012) upon publication (accession number: GSEXXXXX).

Cross-Species Gene List Comparison

To assess the molecular correspondence of mouse cocaine self-administration with human cocaine use, we compared the results of the current study to differentially expressed genes (BH-FDR < 0.05) associated with cocaine use disorder (CUD) in the midbrain ($n = 20$, 50% CUD, $M_{AGE} = 49.2$, s.d. = 3.9; (Bannon et al., 2014), microarray), hippocampus ($n = 15$, 46.7% CUD, $M_{AGE} = 39.4$, s.d._{AGE} = 39.4; Huggett and Stallings, 2020a, 2020b, RNA-sequencing) and dIPFC neurons ($n = 36$, 52.7% CUD, $M_{AGE} = 35.0$, s.d._{AGE} = 11.0; (Huggett and Stallings, 2020a); neuron-specific RNA-sequencing). The aforementioned studies utilized methods that maximized power for identifying differentially expressed genes and used case/control analyses that compared individuals with CUD to matched cocaine free controls. A list of all the differentially expressed genes can be found on GeneWeaver (<https://www.geneweaver.org/>; GS398242).

A key of 1:1 orthologs between humans and mice was generated from the MGI Vertebrate Homology indices (accessed 2020-05-18). This key was used to compare the human gene set to contrast tables within strain and sex combinations. To perform these comparisons, we used the MSET algorithm (Eisinger et al., 2013) as contained in v1.16.6 of the msaul/msaul R package (<https://github.com/msaul/msaul>). MSET p-values were corrected for multiple comparisons using the qvalue package in R v2.14.1 (Storey and Tibshirani, 2003). Gene Ontology Biological Process (GO BP) analysis on overlapping genes was performed on mouse Ensembl identifiers using the AmiGO v2 web tool (accessed 2020-05-28) on GO Ontology Database

doi:10.5281/zenodo.3727280 with Fisher's exact tests and Bonferroni correction settings (Carbon et al., 2009).

Supplemental Material

Supplementary Table S1: Statistical test results for heritability and genotype-by-sex interactions.

Supplementary Table S2: Differential expression results for all tests discussed.

Supplementary Table S3: Quantification of overlap significance between human cocaine use disorder transcriptome and individual strain and sex cocaine transcriptomes.

Supplementary Table S4: Sample sizes for all strain and sex combinations for studies reported.

Supplementary Table S5: Gene Ontology Biological Process results for WSB/EiJ male and NOD/ShiLtJ female overlapping gene sets.

Raw behavioral data will be deposited in the Mouse Phenome Database upon publication.

Gene expression data will be deposited in the Gene Expression Omnibus upon publication.

All scripts, code, and metadata used for analysis are deposited in GitHub (repository: github.com/msaul/csna_founders_survey_2020)

Author Contributions

EJC, PED, LMT, SAS, JDJ, RWL, CAM, LGR, VMP, and SJSR conceived the studies. SJSR, PED, JRB, LSB, SAS, RD, ML, AO, TR, TW, and LHG designed and implemented the behavioral experiments. SMK designed and implemented the circadian transcriptional experiment. JRB, LSB, UD, PED, ML, SMK, AO, TR, SAS, TW, LHG, VMP, and MCS analyzed the behavioral data. SMK, MCS, and VMP analyzed the circadian transcriptional data. MCS, VMK, and VMP analyzed the RNAseq data. MCS, UD, PED, SBH, JRB, LSB, SAS, TW, LHG, VMP, and EJC interpreted the behavioral results. SMK, MCS, and VMP interpreted the results of the circadian transcriptional experiment. MCS, VMK, VMP, and EJC interpreted the RNAseq results. MCS, SBH, RHCP, and EJC conceived, designed, analyzed, and interpreted the comparison between mouse and human gene sets. MCS and EJC wrote the manuscript.

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Literature Cited

- Ahmed SH. 2012. The science of making drug-addicted animals. *Neuroscience* **211**:107–125. doi:10.1016/j.neuroscience.2011.08.014
- Bannon MJ, Johnson MM, Michelhaugh SK, Hartley ZJ, Halter SD, David JA, Kapatos G, Schmidt CJ. 2014. A Molecular Profile of Cocaine Abuse Includes the Differential Expression of Genes that Regulate Transcription, Chromatin, and Dopamine Cell Phenotype. *Neuropsychopharmacology* **39**:2191–2199. doi:10.1038/npp.2014.70
- Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, Yefanov A, Lee H, Zhang N, Robertson CL, Serova N, Davis S, Soboleva A. 2012. NCBI GEO: archive for functional genomics data sets—update. *Nucleic Acids Res* **41**:D991–D995. doi:10.1093/nar/gks1193
- Beck JA, Lloyd S, Hafezparast M, Lennon-Pierce M, Eppig JT, Festing MF, Fisher EM. 2000. Genealogies of mouse inbred strains. *Nat Genet* **24**:23–25. doi:10.1038/71641
- Becker JB, Chartoff E. 2019. Sex differences in neural mechanisms mediating reward and addiction. *Neuropsychopharmacology* **44**:166–183. doi:10.1038/s41386-018-0125-6
- Becker JB, Perry AN, Westenbroek C. 2012. Sex differences in the neural mechanisms mediating addiction: a new synthesis and hypothesis. *Biol Sex Differ* **3**:14. doi:10.1186/2042-6410-3-14
- Bierut LJ, Stitzel JA, Wang JC, Hinrichs AL, Gruzza RA, Xuei X, Saccone NL, Saccone SF, Bertelsen S, Fox L, Horton WJ, Breslau N, Budde J, Cloninger CR, Dick DM, Foroud T, Hatsukami D, Hesselbrock V, Johnson EO, Kramer J, Kuperman S, Madden PAF, Mayo K, Nurnberger J, Pomerleau O, Porjesz B, Reyes O, Schuckit M, Swan G, Tischfield JA, Edenberg HJ, Rice JP, Goate AM. 2008. Variants in Nicotinic Receptors and Risk for Nicotine Dependence. *Am J Psychiatry* **165**:1163–1171. doi:10.1176/appi.ajp.2008.07111711

- 539 Bogenpohl JW, Mignogna KM, Smith ML, Miles MF. 2017. Integrative Analysis of Genetic,
540 Genomic, and Phenotypic Data for Ethanol Behaviors: A Network-Based Pipeline for
541 Identifying Mechanisms and Potential Drug Targets In: Schughart K, Williams RW,
542 editors. *Systems Genetics, Methods in Molecular Biology*. New York, NY: Springer New
543 York. pp. 531–549. doi:10.1007/978-1-4939-6427-7_26
- 544 Bogue MA, Philip VM, Walton DO, Grubb SC, Dunn MH, Kolishovski G, Emerson J, Mukherjee G,
545 Stearns T, He H, Sinha V, Kadakkuzha B, Kunde-Ramamoorthy G, Chesler EJ. 2019.
546 Mouse Phenome Database: a data repository and analysis suite for curated primary
547 mouse phenotype data. *Nucleic Acids Res* gkz1032. doi:10.1093/nar/gkz1032
- 548 Carbon S, Ireland A, Mungall CJ, Shu S, Marshall B, Lewis S, the AmiGO Hub, the Web Presence
549 Working Group. 2009. AmiGO: online access to ontology and annotation data.
550 *Bioinformatics* **25**:288–289. doi:10.1093/bioinformatics/btn615
- 551 Chen Y, Lun ATL, Smyth GK. 2014. Differential Expression Analysis of Complex RNA-seq
552 Experiments Using edgeR In: Datta S, Nettleton D, editors. *Statistical Analysis of Next
553 Generation Sequencing Data*. Cham: Springer International Publishing. pp. 51–74.
554 doi:10.1007/978-3-319-07212-8_3
- 555 Chesler EJ, Lu L, Shou S, Qu Y, Gu J, Wang J, Hsu HC, Mountz JD, Baldwin NE, Langston MA,
556 Threadgill DW, Manly KF, Williams RW. 2005. Complex trait analysis of gene expression
557 uncovers polygenic and pleiotropic networks that modulate nervous system function.
558 *Nat Genet* **37**:233–242. doi:10.1038/ng1518
- 559 Crabbe JC, Wahlsten D, Dudek BC. 1999. Genetics of mouse behavior: interactions with
560 laboratory environment. *Science* **284**:1670–1672.
- 561 Cunha-Oliveira T, Silva L, Silva AM, Moreno AJ, Oliveira CR, Santos MS. 2013. Mitochondrial
562 complex I dysfunction induced by cocaine and cocaine plus morphine in brain and liver
563 mitochondria. *Toxicol Lett* **219**:298–306. doi:10.1016/j.toxlet.2013.03.025
- 564 Dalley JW, Fryer TD, Brichard L, Robinson ES, Theobald DE, Lääne K, Peña Y, Murphy ER, Shah Y,
565 Probst K, Abakumova I, Aigbirhio FI, Richards HK, Hong Y, Baron J-C, Everitt BJ, Robbins
566 TW. 2007. Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine
567 reinforcement. *Science* **315**:1267–1270.
- 568 Datta U, Schoenrock SE, Bubier JA, Bogue MA, Jentsch JD, Logan RW, Tarantino LM, Chesler EJ.
569 2020. Prospects for finding the mechanisms of sex differences in addiction with human
570 and model organism genetic analysis. *Genes Brain Behav* **19**. doi:10.1111/gbb.12645
- 571 de Wit H, Phillips TJ. 2012. Do initial responses to drugs predict future use or abuse? *Neurosci
572 Biobehav Rev* **36**:1565–1576. doi:10.1016/j.neubiorev.2012.04.005
- 573 Dickson PE, Ndukum J, Wilcox T, Clark J, Roy B, Zhang L, Li Y, Lin D-T, Chesler EJ. 2015.
574 Association of novelty-related behaviors and intravenous cocaine self-administration in
575 Diversity Outbred mice. *Psychopharmacology (Berl)* **232**:1011–1024.

576 Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR.
577 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**:15–21.
578 doi:10.1093/bioinformatics/bts635

579 Eisinger BE, Saul MC, Driessen TM, Gammie SC. 2013. Development of a versatile enrichment
580 analysis tool reveals associations between the maternal brain and mental health
581 disorders, including autism. *BMC Neurosci* **14**:147. doi:10.1186/1471-2202-14-147

582 Ersche KD, Turton AJ, Pradhan S, Bullmore ET, Robbins TW. 2010. Drug addiction
583 endophenotypes: impulsive versus sensation-seeking personality traits. *Biol Psychiatry*
584 **68**:770–773.

585 Fitzpatrick CJ, Gopalakrishnan S, Cogan ES, Yager LM, Meyer PJ, Lovic V, Saunders BT, Parker CC,
586 Gonzales NM, Aryee E, Flagel SB, Palmer AA, Robinson TE, Morrow JD. 2013. Variation in
587 the Form of Pavlovian Conditioned Approach Behavior among Outbred Male Sprague-
588 Dawley Rats from Different Vendors and Colonies: Sign-Tracking vs. Goal-Tracking. *PLoS*
589 *ONE* **8**:e75042. doi:10.1371/journal.pone.0075042

590 Goldman D, Oroszi G, Ducci F. 2005. The genetics of addictions: uncovering the genes. *Nat Rev*
591 *Genet* **6**:521–532.

592 Hitzemann R, Darakjian P, Walter N, Dan Iancu O, Searles R, McWeeney S. 2014. Introduction to
593 Sequencing the Brain Transcriptome. *International Review of Neurobiology*. Elsevier. pp.
594 1–19. doi:10.1016/B978-0-12-801105-8.00001-1

595 Huggett SB, Bubier JA, Chesler EJ, Palmer RC. 2020. Meso-limbic Gene Expression Findings from
596 Mouse Cocaine Self-Administration Recapitulate Human Cocaine Use Disorder
597 (preprint). *Bioinformatics*. doi:10.1101/2020.01.31.929406

598 Huggett SB, Stallings MC. 2020a. Genetic Architecture and Molecular Neuropathology of Human
599 Cocaine Addiction. *J Neurosci* JN-RM-2879-19. doi:10.1523/JNEUROSCI.2879-19.2020

600 Huggett SB, Stallings MC. 2020b. Cocaine’omics: Genome-wide and transcriptome-wide
601 analyses provide biological insight into cocaine use and dependence. *Addict Biol*
602 **25**:e12719. doi:10.1111/adb.12719

603 Hyman SE. 2018. The daunting polygenicity of mental illness: making a new map. *Philos Trans R*
604 *Soc B Biol Sci* **373**:20170031. doi:10.1098/rstb.2017.0031

605 Hyman SE. 2012. Revolution Stalled. *Sci Transl Med* **4**:155cm11-155cm11.
606 doi:10.1126/scitranslmed.3003142

607 Izquierdo A, Jentsch JD. 2012. Reversal learning as a measure of impulsive and compulsive
608 behavior in addictions. *Psychopharmacology (Berl)* **219**:607–620.

609 Izumo M, Johnson CH, Yamazaki S. 2003. Circadian gene expression in mammalian fibroblasts
610 revealed by real-time luminescence reporting: Temperature compensation and
611 damping. *Proc Natl Acad Sci* **100**:16089–16094. doi:10.1073/pnas.2536313100

612 Kim S-M, Neuendorff N, Chapkin RS, Earnest DJ. 2016. Role of Inflammatory Signaling in the
613 Differential Effects of Saturated and Poly-unsaturated Fatty Acids on Peripheral
614 Circadian Clocks. *EBioMedicine* **7**:100–111. doi:10.1016/j.ebiom.2016.03.037

615 Kochanek KD, Murphy SL, Xu J, Arias E. 2017. Deaths: Final Data for 2017. *Natl Vital Stat Rep*
616 **68**:1–77.

617 Kumar V, Kim K, Joseph C, Kourrich S, Yoo S-H, Huang HC, Vitaterna MH, de Villena FP-M,
618 Churchill G, Bonci A, Takahashi JS. 2013. C57BL/6N mutation in cytoplasmic FMRP
619 interacting protein 2 regulates cocaine response. *Science* **342**:1508–1512.
620 doi:10.1126/science.1245503

621 Li B, Dewey CN. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or
622 without a reference genome 16.

623 Logan RW, Robledo RF, Recla JM, Philip VM, Bubier JA, Jay JJ, Harwood C, Wilcox T, Gatti DM,
624 Bult CJ, Churchill GA, Chesler EJ. 2013. High-precision genetic mapping of behavioral
625 traits in the diversity outbred mouse population: Genetic mapping of behavioral traits in
626 the outbred mouse. *Genes Brain Behav* **12**:424–437. doi:10.1111/gbb.12029

627 Logan RW, Williams III WP, McClung CA. 2014. Circadian rhythms and addiction: Mechanistic
628 insights and future directions. *Behav Neurosci* **128**:387–412.

629 McCutcheon JE, Wang X, Tseng KY, Wolf ME, Marinelli M. 2011. Calcium-Permeable AMPA
630 Receptors Are Present in Nucleus Accumbens Synapses after Prolonged Withdrawal
631 from Cocaine Self-Administration But Not Experimenter-Administered Cocaine. *J*
632 *Neurosci* **31**:5737–5743. doi:10.1523/JNEUROSCI.0350-11.2011

633 National Advisory Mental Health Council Workgroup on Genomics. 2018. Report of the National
634 Advisory Mental Health Council Workgroup on Genomics.

635 Nestler EJ, Hyman SE. 2010. Animal models of neuropsychiatric disorders. *Nat Neurosci*
636 **13**:1161–1169. doi:10.1038/nn.2647

637 Neuner SM, Heuer SE, Huentelman MJ, O’Connell KMS, Kaczorowski CC. 2019. Harnessing
638 Genetic Complexity to Enhance Translatability of Alzheimer’s Disease Mouse Models: A
639 Path toward Precision Medicine. *Neuron* **101**:399–411.e5.
640 doi:10.1016/j.neuron.2018.11.040

641 Ozburn AR, Falcon E, Twaddle A, Nugent AL, Gillman AG, Spencer SM, Arey RN, Mukherjee S,
642 Lyons-Weiler J, Self DW, McClung CA. 2015. Direct Regulation of Diurnal Drd3 Expression
643 and Cocaine Reward by NPAS2. *Biol Psychiatry* **77**:425–433.
644 doi:10.1016/j.biopsych.2014.07.030

645 Palmer RHC, Benca-Bachman CE, Bubier JA, McGeary JE, Ramgiri N, Srijeanthan J, Huggett SB,
646 Yang Jingjing, Visscher P, Yang Jian, Knopik V, Chesler EJ. 2019. Cross-Species Integration
647 of Transcriptomic Effects of Tobacco and Nicotine Exposure Helps to Prioritize Genetic
648 Effects on Human Tobacco Consumption | bioRxiv. *bioRxiv*.

649 Pascoli V, Hiver A, Van Zessen R, Loureiro M, Achargui R, Harada M, Flakowski J, Lüscher C.
650 2018. Stochastic synaptic plasticity underlying compulsion in a model of addiction.
651 *Nature* **564**:366–371. doi:10.1038/s41586-018-0789-4

652 Philip VM, Sokoloff G, Ackert-Bicknell CL, Striz M, Branstetter L, Beckmann MA, Spence JS,
653 Jackson BL, Galloway LD, Barker P, and Wymore AM, Hunsicker PR, Durtschi DC, Shaw
654 GS, Shinpock S, Manly KF, Miller DR, Donohue KD, Culiati CT, Churchill GA, Lariviere WR,
655 Palmer AA, O'Hara BF, Voy BH, Chesler EJ. 2011. Genetic analysis in the Collaborative
656 Cross breeding population. *Genome Res* **21**:1223–1238.

657 Phipson B, Lee S, Majewski IJ, Alexander WS, Smyth GK. 2016. Robust Hyperparameter
658 Estimation Protects Against Hypervariable Genes and Improves Power to Detect
659 Differential Expression. *Ann Appl Stat* **10**:946–963. doi:10.1214/16-AOAS920

660 Piazza PV, Deroche-Gamonet V, Rouge-Pont F, Le Moal M. 2000. Vertical Shifts in Self-
661 Administration Dose–Response Functions Predict a Drug-Vulnerable Phenotype
662 Predisposed to Addiction. *J Neurosci* **20**:4226–4232. doi:10.1523/JNEUROSCI.20-11-
663 04226.2000

664 Prendergast BJ, Onishi KG, Zucker I. 2014. Female mice liberated for inclusion in neuroscience
665 and biomedical research. *Neurosci Biobehav Rev* **40**:1–5.

666 Ramanathan C, Xu H, Khan SK, Shen Y, Gitis PJ, Welsh DK, Hogenesch JB, Liu AC. 2014. Cell Type-
667 Specific Functions of Period Genes Revealed by Novel Adipocyte and Hepatocyte
668 Circadian Clock Models. *PLoS Genet* **10**:e1004244. doi:10.1371/journal.pgen.1004244

669 Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. 2015. limma powers differential
670 expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*
671 **43**:e47–e47. doi:10.1093/nar/gkv007

672 Roberts A, De Villena FP-M, Wang W, McMillan L, Threadgill DW. 2007. The polymorphism
673 architecture of mouse genetic resources elucidated using genome-wide resequencing
674 data: implications for QTL discovery and systems genetics. *Mamm Genome* **18**:473–481.

675 Ruan QT, Yazdani N, Blum BC, Beierle JA, Lin W, Coelho MA, Fultz EK, Healy AF, Shahin JR,
676 Kandola AK, Luttik KP, Zheng K, Smith NJ, Cheung J, Mortazavi F, Apicco DJ, Ragu Varman
677 D, Ramamoorthy S, Ash PEA, Rosene DL, Emili A, Wolozin B, Szumlinski KK, Bryant CD.
678 2020. A Mutation in Hnrnp1 That Decreases Methamphetamine-Induced
679 Reinforcement, Reward, and Dopamine Release and Increases Synaptosomal hnRNP H
680 and Mitochondrial Proteins. *J Neurosci* **40**:107–130. doi:10.1523/JNEUROSCI.1808-
681 19.2019

682 SAMHSA. 2017. The 2017 National Survey on Drug Use and Health (NSDUH).

683 Sathyanesan M, Girgenti MJ, Banasr M, Stone K, Bruce C, Guilchicek E, Wilczak-Havill K, Nairn A,
684 Williams K, Sass S, Duman JG, Newton SS. 2012. A molecular characterization of the
685 choroid plexus and stress-induced gene regulation. *Transl Psychiatry* **2**:e139.
686 doi:10.1038/tp.2012.64

687 Saul MC, Blatti C, Yang W, Bukhari SA, Shpigler HY, Troy JM, Seward CH, Sloofman L,
688 Chandrasekaran S, Bell AM, Stubbs L, Robinson GE, Zhao SD, Sinha S. 2019a. Cross-
689 species systems analysis of evolutionary toolkits of neurogenomic response to social
690 challenge. *Genes Brain Behav* **18**:e12502. doi:10.1111/gbb.12502

691 Saul MC, Philip VM, Reinholdt LG, Chesler EJ. 2019b. High-Diversity Mouse Populations for
692 Complex Traits. *Trends Genet* **0**. doi:10.1016/j.tig.2019.04.003

693 Schoenrock SA, Kumar P, Gómez-A A, Dickson PE, Kim S-M, Bailey L, Neira S, Riker KD,
694 Farrington J, Gaines CH, Khan S, Wilcox TD, Roy TA, Leonardo MR, Olson AA, Gagnon LH,
695 Philip VM, Valdar W, de Villena FP-M, Jentsch JD, Logan RW, McClung CA, Robinson DL,
696 Chesler EJ, Tarantino LM. 2020. Characterization of genetically complex Collaborative
697 Cross mouse strains that model divergent locomotor activating and reinforcing
698 properties of cocaine. *Psychopharmacology (Berl)*. doi:10.1007/s00213-019-05429-3

699 Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith
700 GP, Gao H, Hennessy L, others. 2013. Genomic responses in mouse models poorly mimic
701 human inflammatory diseases. *Proc Natl Acad Sci* **110**:3507–3512.

702 Sinha S, Jones BM, Traniello IM, Bukhari SA, Halfon MS, Hofmann HA, Huang S, Katz PS, Keagy J,
703 Lynch VJ, Sokolowski MB, Stubbs LJ, Tabe-Bordbar S, Wolfner MF, Robinson GE. 2020.
704 Behavior-related gene regulatory networks: A new level of organization in the brain.
705 *Proc Natl Acad Sci* 201921625. doi:10.1073/pnas.1921625117

706 Sittig LJ, Carbonetto P, Engel KA, Krauss KS, Barrios-Camacho CM, Palmer AA. 2016. Genetic
707 Background Limits Generalizability of Genotype-Phenotype Relationships. *Neuron*
708 **91**:1253–1259. doi:10.1016/j.neuron.2016.08.013

709 Sonesson C, Love M, Robinson M. 2016. Differential analyses for RNA-seq: transcript-level
710 estimates improve gene-level inferences. *F1000Research* **4**.
711 doi:10.12688/f1000research.7563.2

712 Storey JD, Tibshirani R. 2003. Statistical significance for genomewide studies. *Proc Natl Acad Sci*
713 **100**:9440–9445. doi:10.1073/pnas.1530509100

714 Takao K, Miyakawa T. 2015. Genomic responses in mouse models greatly mimic human
715 inflammatory diseases. *Proc Natl Acad Sci* **112**:1167–1172.

716 Tuttle AH, Philip VM, Chesler EJ, Mogil JS. 2018. Comparing phenotypic variation between
717 inbred and outbred mice. *Nat Methods* **15**:994–996.

718 Wahlsten D, Metten P, Crabbe J. 2003. A rating scale for wildness and ease of handling
719 laboratory mice: results for 21 inbred strains tested in two laboratories. *Genes Brain*
720 *Behav* **2**:71–79.

721 Walker DM, Cates HM, Loh Y-HE, Purushothaman I, Ramakrishnan A, Cahill KM, Lardner CK,
722 Godino A, Kronman HG, Rabkin J, Lorsch ZS, Mews P, Doyle MA, Feng J, Labonté B, Koo
723 JW, Bagot RC, Logan RW, Seny ML, Calipari ES, Shen L, Nestler EJ. 2018. Cocaine self-
724 administration alters transcriptome-wide responses in the brain's reward circuitry. *Biol*
725 *Psychiatry*.

726 Wiltshire T, Ervin RB, Duan H, Bogue MA, Zamboni WC, Cook S, Chung W, Zou F, Tarantino LM.
727 2015. Initial locomotor sensitivity to cocaine varies widely among inbred mouse strains:
728 Initial locomotor sensitivity to cocaine in inbred mice. *Genes Brain Behav* **14**:271–280.
729 doi:10.1111/gbb.12209

730 Young RL, Ferkin MH, Ockendon-Powell NF, Orr VN, Phelps SM, Pogány Á, Richards-Zawacki CL,
731 Summers K, Székely T, Trainor BC, Urrutia AO, Zachar G, O’Connell LA, Hofmann HA.
732 2019. Conserved transcriptomic profiles underpin monogamy across vertebrates. *Proc*
733 *Natl Acad Sci* **116**:1331–1336. doi:10.1073/pnas.1813775116

734