Supplementary Materials and Methods

Assessments and phenotype operationalization

The CATS SSAGA-OZ[1] provided DSM-IV diagnoses of illicit drug (opioid, cannabis, sedative, stimulant, and cocaine), alcohol, and nicotine dependence, and conduct disorder, major depressive disorder, and post-traumatic stress disorder. Similar diagnoses were obtained for CATS pilot project participants via the World Health Organization Composite International Diagnostic Interview.[2] A separate section queried the onset of heroin use milestones and characterized respondents' relationships to fellow users. Nearly all (99%) of CATS opioid dependent individuals reported being treated for heroin dependence (not surprising, since heroin is the primary opioid of abuse in New South Wales).

The illicit drug section of the interview contained questions about injection drug use, including querying the number of times used and whether the individual had a period of injecting daily. Since 94.1% of CATS opioid dependent participants reported a history of daily injection, we operationalized having had such a period as the opioid dependence endpoint (OD_E). Comparisons of phenotypic data from opioid dependent individuals who differed on daily injection status found that daily injectors more commonly met criteria for conduct disorder, and stimulant, cocaine, and alcohol dependence (**Supplementary Table 1**). They also had significantly earlier initial use of cannabis and opioids and were more likely to have relapsed after initial abstinence. Comparisons of dependent individuals who never injected daily to non-dependent opioid misusers revealed fewer significant differences. Interestingly, while the differences between mean initial use of cannabis and stimulants are surprisingly constant across groups, the transition to opioid use is earlier in OD_E group members.

Daily injection status in CATS also displayed a strong relationship with respondents' report of total lifetime opioid injections. In CATS daily injectors with data available (N=1153; 14 pilot project participants were not asked this question), 97.2% reported their total for lifetime injections as 100 or more times while 2.8% reported a total of 11-99 times. CATS opioid dependent individuals who denied a history of daily injection (N=73) had a wider range of responses: 46.6% never injected, 4.1% injected 1-2 times, 13.7% injected 3-10 times, 4.1% 11-99 times, and 31.5% injected 100 times or more. Thus, 98.0% of CATS opioid dependent individuals who reported having injected opioids 100 or more times had a history of daily injection.

The confirmation samples were drawn from genetic studies of substance dependence. While CATS focused solely on opioid dependence, the Yale-Penn studies also included individuals ascertained as dependent on other substances; in contrast, SAGE included no one ascertained on the basis of opioid dependence (**Supplementary Tables 2** and **3**). The three samples were thus derived from different aspects of the liability distribution for opioid dependence, varying from CATS (narrow range, most severe) to Yale-Penn (wider range) to SAGE (least severe). Additionally, differences in the assessments of these studies precluded examining identical phenotypes in each sample. We thus made *a priori* decisions regarding how best to characterize the OU_{IP} and OD_E groups for each sample (detailed below).

The Yale-Penn genetic studies of opioid, cocaine, and alcohol dependence sample is more heterogeneous in regards to its likely drug availability and use patterns. A total of 5390 subjects that included both small nuclear families and unrelated individuals had GWAS genotypic and interview data available. An electronic version of

the Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA) served as the assessment for these studies. Although this instrument is similar to the SSAGA (from which it was derived), differences in the assessment of opioid use and dependence prevented our defining identical phenotypes (**Supplementary Table 3**). Specifically, the SSADDA questions: (1) did not explicitly differentiate prescribed use of opioids from misuse (i.e., non-medical use); (2) asked respondents to identify the three opioids they most commonly used; and (3) queried the number of times opioids were injected lifetime without determining whether daily injecting had ever occurred. We addressed these respective differences by using an extreme discordant approach for the Yale-Penn studies sample that approximates severely dependent daily injectors and the non-dependent opioid misusers from the CATS sample. Specifically, we operationalized the OD_E group as opioid dependent individuals who reported substantial opioid injecting [i.e., who reported heroin as one of their three most often used opioids, had a period of daily or near daily opioid use, and had injected opioids at least 100 times lifetime (the largest quantity queried and, in CATS data, a reasonable proxy for daily injection)] and the OU_{IP} group as those reporting having a history of heroin use and no lifetime DSM-IV opioid dependence symptoms (i.e., individuals with a definitive history of non-medical opioid exposure without evidence of dependence).

Phenotypic data comparisons in the Yale-Penn confirmation sample (**Supplementary Table 2**) revealed fewer differences than in CATS and also demonstrated somewhat different substance use preferences in the two countries. The Yale-Penn OD_E group had significant higher prevalence only for nicotine and sedative dependence with the opposite relationship seen for alcohol dependence. Cocaine

dependence, much less common in Australia, was highly prevalent in both groups. A similar pattern in initial use was observed with a more rapid transition to opioids in the OD_E group.

SAGE cases and controls were selected for a GWAS of alcohol dependence from large investigations targeting other types of substance dependence including alcohol [Collaborative Study on the Genetics of Alcohol (COGA)], [3,4] nicotine [Collaborative Genetic Study of Nicotine Dependence (COGEND)], [5,6] and cocaine [Family Study of Cocaine Dependence (FSCD)]. [7] The assessments for the three component investigations (versions of the SSAGA) asked about non-medical opioid use, but did not determine which opioids were misused or whether opioid misusers had a history of daily injecting. In addition, there were missing data on drug injection history for some opioid-exposed COGEND participants whose report of opioid use exceeded the minimum level required to complete the full opioid assessment. In addition, some COGA subjects were not asked the number of times they had injected opioids. Since no SAGE participants were ascertained on the basis of opioid dependence, the sample included more individuals of unclear affection status (e.g., those who met DSM-IV criteria for opioid abuse or endorsed two DSM-IV dependence symptoms). We also had no information on whether these individuals had used, or even had access to, heroin or other commonly injected opioids. For SAGE, we decided *a priori* to operationalize OD_E as those meeting criteria for DSM-IV opioid dependence. We operationalized OU_{IP} as opioid misusers who did not meet DSM-IV criteria for opioid abuse and met at most one dependence criterion.

The OD_E group in the SAGE confirmation sample had significantly higher

prevalence of other forms of substance dependence and comorbid disorders examined (**Supplementary Table 2**). The relatively high prevalence of nicotine, alcohol, and cocaine dependence in the OU_{IP} group likely reflects ascertainment for these disorders in the SAGE component studies. Although the mean onset of cannabis use for each group in SAGE is nearly identical to the similar measure in CATS, it is surprising that the OD_E and OU_{IP} groups in SAGE do not differ in mean age of initial opioid use.

CATS genotypic data cleaning

Of the 559,348 SNPs for which genotyping was attempted, data were released for 557,425 (99.66%). Quality control measures were excellent, with concordance rates of 99.99% for blind duplicates and 99.77% for HapMap control samples. Further data cleaning included the application of standard filters for deviation from Hardy-Weinberg Equilibrium ($p>10^{-6}$), minor allele frequency (≥ 0.05), SNP call rate (>99%), sample genotyping (>99%), and GenomeStudio genotype quality score (≥ 0.7). Additional analyses were performed to detect cryptic relatedness using a pi-hat cut-off of 0.1; only a single sample from any identified related pair was retained. Principal components analysis (PCA) was performed with the SmartPCA program in the Eigensoft 3.0 package[8] to identify outliers of non-European ancestry. Principal components (PCs) were generated using data from additional samples including HapMap and a twin population of known Northern European and Scandinavian ancestry. Two PCs were also calculated for CATS samples based on this fitted model and all samples were then plotted with each PC serving as an axis. Outliers were identified based on their relative distance from the center of the northern European group; those more than 6 standard deviations from the PC1-PC2 centroid were removed.

CATS admixture analysis

Although CATS data reported here include only European ancestry participants, PCA was conducted using the SmartPCA program[8] to provide additional admixture correction for use in the association analyses. SNPs in LD with others in the panel with an r^2 of greater than 0.1 were removed resulting in the inclusion of data from 92,362 autosomal SNPs in the PCA. Three PCs were generated via PCA and included as covariates in the regression models [ANOVA statistics found no significant OD_E-OU_{IP} differences (p-values were 0.19, 0.75, and 0.81)].

Epigenetic annotation

Fetal brain histone H3K4me1 ChIP-seq, H3K4me3 ChIP-seq, and input data were aligned to human hg19 reference assembly using BWA,[9] and were pre-processed by using in-house program methylQA (available at http://methylqa.sourceforge.net/) to remove unmapped and redundant sequence. The default parameters were used to apply MACS2[10] to processed histone modification ChIP-seq data for the identification of peaks at a 1% false discovery rate. Motif finding analyses were performed using the FIMO tool[11] from the MEME suite and the HOMER tool suite,[12] and Transcription Factor Position Weight Matrix (TF PWM) was downloaded from JASPAR database.[13]

Duke Neurogenetics Study (DNS)

Participants

DNS exclusion criteria included: (1) medical diagnosis of cancer, stroke, diabetes requiring insulin treatment, chronic kidney or liver disease or lifetime psychotic symptoms; (2) use of psychotropic, glucocorticoid or hypolipidemic medication, and (3) conditions affecting cerebral blood flow and metabolism (e.g., hypertension). Current

DSM-IV Axis I and select Axis II disorders (Antisocial Personality Disorder and Borderline Personality Disorder) were assessed with the electronic Mini International Neuropsychiatric Interview[14] and Structured Clinical Interview for the DSM-IV Axis II (SCID-II),[15] respectively. These disorders are not exclusionary as the DNS seeks to establish broad variability in multiple behavioral phenotypes related to psychopathology.

On January 6th, 2014, 726 participants had overlapping fMRI and genetic data that was fully processed and used for these analyses. Of these participants, 71 were excluded due to scanner-related artifacts in fMRI data ($n = 6$), incidental structural brain abnormalities ($n = 2$), a large number of movement outliers in fMRI data ($n = 21$; see ART description below), inadequate signal in our amygdala regions of interest ($n = 14$; see coverage description below), poor behavioral performance ($n = 20$; accuracy lower than 75%), outlier status according to ancestrally-informative principal components ($n = 5$), scanner malfunctions ($n = 2$), and incomplete fMRI data collection ($n = 1$) leaving a sample of 655 participants and a final European-American subsample of 312 participants; for a full description of diagnoses present in the sample see **Supplementary Table 4**).

Genotyping

Human DNA from participants of the DNS cohort was isolated from saliva derived from Oragene DNA self-collection kits (DNA Genotek) customized for 23andMe (www.23andme.com). DNA extraction and genotyping were performed by the National Genetics Institute (NGI), a CLIA-certified clinical laboratory and subsidiary of Laboratory Corporation of America. The Illumina HumanOmniExpress BeadChips and a custom array containing an additional ~300,000 SNPs were used to provide genome-wide data.

DNS neuroimaging protocol BOLD fMRI paradigm

The paradigm consists of 4 task block requiring face-matching interleaved with 5 control blocks requiring shape-matching (see **Figure S1**). In each face-matching trial within a block, participants view a trio of faces derived from a standard set of facial affect pictures (expressing angry, fearful, surprised, or neutral emotions), and select which of 2 faces presented on the bottom row of the display matches the target stimulus presented on the top row. Each emotion-specific block (e.g., fearful facial expressions only) consists of 6 individual trials, balanced for gender of the face. Block order is pseudo-randomized across participants. Each of the 6 face trios is presented for 4 seconds with a variable inter-stimulus interval of 2-6 seconds; total block length is 48 seconds. In the shape-matching control blocks, participants view a trio of geometric shapes (i.e., circles, horizontal and vertical ellipses) and select which of 2 shapes displayed on the bottom matches the target shape presented on top. Each control block consists of 6 different shape trios presented for 4 seconds with a fixed inter-stimulus interval of 2 seconds, comprising a total block length of 36 seconds. The total paradigm is 390 seconds in duration. Reaction times and accuracy are recorded through an MR-compatible button box.

BOLD fMRI acquisition

Participants were scanned using a research-dedicated GE MR750 3T scanner equipped with high-power high-duty-cycle 50-mT/m gradients at 200 T/m/s slew rate, and an eight-channel head coil for parallel imaging at high bandwidth up to 1MHz at the Duke-UNC Brain Imaging and Analysis Center. A semi-automated high-order shimming program was used to ensure global field homogeneity. A series of 34 interleaved axial functional slices aligned with the anterior commissure-posterior commissure (AC-PC)

plane were acquired for full-brain coverage using an inverse-spiral pulse sequence to reduce susceptibility artifact (TR/TE/flip angle = 2000 ms / 30 ms / 60; FOV = 240 mm; 3.75 × 3.75 × 4 mm voxels (selected to provide whole brain coverage while maintaining adequate signal-to-noise and optimizing acquisition times); interslice skip = 0). Four initial RF excitations were performed (and discarded) to achieve steady-state equilibrium. To allow for spatial registration of each participant's data to a standard coordinate system, high-resolution three-dimensional structural images were acquired in 34 axial slices co-planar with the functional scans (TR/TE/flip angle = 7.7s / 3.0ms / 12; voxel size = 0.9 × 0.9 × 4 mm; FOV = 240 mm, interslice skip=0).

BOLD fMRI data analysis

The general linear model of Statistical Parametric Mapping 8 (SPM8) (http://www.fil.ion.ucl.ac.uk/spm) was used for whole-brain image analysis. Individual subject data were first realigned to the first volume in the time series to correct for head motion before being spatially normalized into the standard stereotactic space of the Montreal Neurological Institute (MNI) template using a 12-parameter affine model. Next, data were smoothed to minimize noise and residual differences in individual anatomy with a 6mm FWHM Gaussian filter. Voxel-wise signal intensities were ratio normalized to the whole-brain global mean. Then the ARTifact Detection Tool (ART; https://www.nitrc.org/docman/view.php/104/390/Artifact%20Detection%20Toolbox%20 Manual) was used to generate regressors accounting for images due to large motion (i.e., > 0.6 mm relative to the previous time frame) or spikes (i.e., global mean intensity 2.5 standard deviations from the entire time series). Participants for whom more than 5% of acquisition volumes were flagged by ART (n = 21) were removed from analyses.

An ROI mask (AAL atlas) from WFU pickatlas[16] was used to ensure adequate amygdala coverage for the face-matching and number-guessing tasks, respectively. Participants who had less than 90% coverage of the amygdala ($n = 14$) were excluded from analyses.

Following preprocessing steps outlined above, linear contrasts employing canonical hemodynamic response functions were used to estimate amygdala habituation as the linear decrease over successive face matching blocks (i.e., block 1 > block 2 > block 3 > block 4). Follow-up analyses evaluated amygdala response differences in block 1 across genotype groups for any significant effects that emerged. Individual contrast images (i.e., weighted sum of the beta images) were used in second-level random effects models accounting for scan-to-scan and participant-to-participant variability to determine mean contrast-specific responses using one-sample t-tests. A voxel-level statistical threshold of $P < 0.05$, family wise error corrected for multiple comparisons across the bilateral amygdala regions of interest, and a cluster-level extent threshold of 10 contiguous voxels was applied to these analyses. The bilateral amygdala regions of interest (ROI) were defined using the AAL template. BOLD parameter estimates from maximal voxels in the right and left amygdala ROI exhibiting a main effect for the amygdala habituation contrast were extracted using the VOI tool in SPM8 and exported for regression analyses in SPSS (v.22). Extracting parameter estimates from clusters activated by our fMRI paradigm, rather than those specifically correlated with our independent variables of interest, precludes the possibility of any correlation coefficient inflation that may result when an explanatory covariate is used to select a region of interest. We have successfully used this strategy in prior studies.

Genetic analysis of murine interstrain differences

Mice were housed four animals per cage and kept on 12 hour light and dark cycles. All experiments were performed during light periods. Morphine was administered subcutaneously using a twice daily regimen of escalating doses (10mg/kg on day 1, 20mg/kg on days 2-3, and 40 mg/kg on day 4) that has been demonstrated to achieve physical dependence.[17] Naloxone 10 mg/kg was injected 18 hours after the last dose of morphine to precipitate withdrawal. Mice were placed in clear plastic cylinders and the number of jumps made over the 15 minutes following this injection was counted as a measure of physical dependence. As per prior studies,[17] a total of eight mice from each strain were similarly tested. Randomization and blinding were not employed. Haplotype-based computational genetic mapping was performed as has been previously described[17-19] using a haplotype block map constructed from allelic data for the murine genome. Haplotype data were examined for seven genes: *CNIH3, CNIH2, GRIA1, GRIA2, CACNG8, GRIP1,* and *DLG4.*

Post-hoc comparisons to EA general population controls

The Twin Study of Mole Development in Adolescence,[20] an ongoing Australian investigation of melanocytic naevi, was used as a source of unrelated, EA population controls. Parents of adolescent twins were preferentially selected for inclusion. Alternatively, one twin was included when neither parent was available. Participants in this study were not assessed for opioid dependence. Genotyping of their DNA was conducted on either the Illumina 660W-Quad or the Illumina 610-Quad BeadChip. We conducted quality control using metrics identical to those performed in CATS including deviation from HWE, MAF, call rate, and GenomeStudio genotype quality score. Identical analyses were performed to detect cryptic relatedness among genotyped

participants (pi-hat cut-off of 0.1) and to identify outliers of non-European ancestry, based on their relative distance from the center of the northern European group. Principal components analyses were conducted using the SmartPCA program[8] with on combined data from these individuals and the OD_E and OU_{IP} groups from CATS. Separate analyses comparing these unrelated AU twin family members to the CATS OD_E and OU_{IP} groups were performed in a manner identical to the previously described CATS analyses with the sole exception being that only sex and PCs were included as covariates. For each comparison, PCs that differed significantly across the groups being compared were selected for inclusion in the respective analyses (e.g., 6 PCs were included as covariates in the comparison of AU twin family members and the CATS OD_E group).

Figure S1 DNS fMRI Task

Figure S2

Panel A The Q-Q Plot of Association Results for CATS Data

Panel B Manhattan Plot of Association Results for CATS Data

Panel C Linkage Disequilibrium Relationships of *CNIH3* SNPs in CATS Data

Panel D Regional Association Plot of Meta-analytic Results

Figure S3 Amygdala Activation across Blocks

Supplementary figure legends

Figure S1 Participants completed four expression-specific (Neutral, Angry, Fear, Surprise) face-matching task blocks interleaved with five sensorimotor shape-matching control blocks. Order for task blocks was counterbalanced across participants.

Figure S2 Panel A The quantile-quantile plot of association results for CATS data and the genomic inflation factor (λ=1.01) demonstrate an absence of inflation of the test statistic. Panel B The strongest evidence of association seen in the Manhattan plot of association results for CATS data includes a cluster of chromosome 1 SNPs located in *CNIH3*. Panel C The linkage disequilibrium relationships of *CNIH3* SNPs in CATS data indicates that the five SNPs other than rs1436175 are in fairly strong LD; rs1436175 is in moderate LD with these SNPs. Additional analyses conditioning on rs1436175 found p values for the other 5 SNPs ranging from .052-.067 indicating that the observed associations are almost entirely due to LD (i.e., they represent a single association signal). Similar analyses conditioning on rs1369846 found p values ranging from .016

for rs1436175 to >.5 for the remaining SNPs. Panel D The regional association plot of the meta-analytic results suggests that the 5 SNPs that reached genome-wide significance are all located in a single LD bin that does not include rs1436175.

Figure S3 Right amygdala (MNI coordinates x = 22 y = -6 z = -14) activation across blocks (i.e., Block 1 > Shapes, Block 2 > Shapes, Block 3 > Shapes, Block 4 > Shapes) stratified by rs10799590 genotype. In the context of evidence for significant amygdala habituation, there were no differences in activation by genotype across each block (Block 1, stand beta = 0.067, p = 0.24, Block 2, stand beta = -0.010, p = 0.86, Block 3, stand beta = -0.017, p = 0.77, Block 4, stand beta = -0.075, p = 0.19).
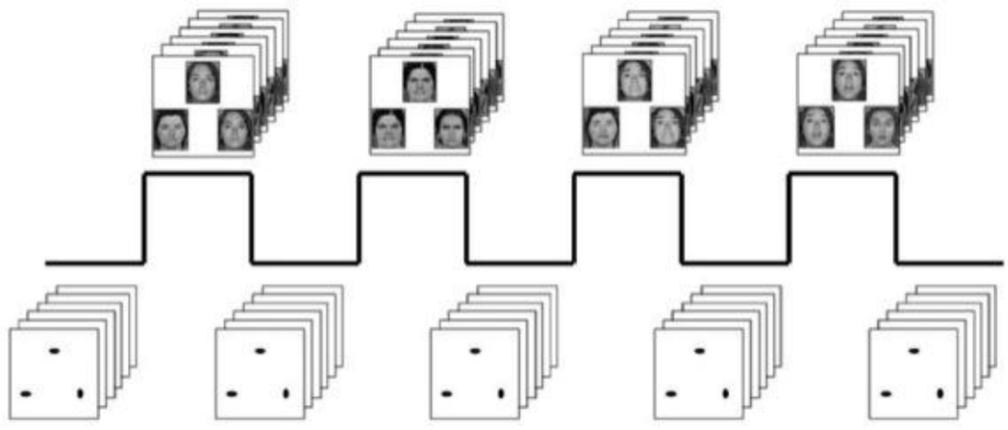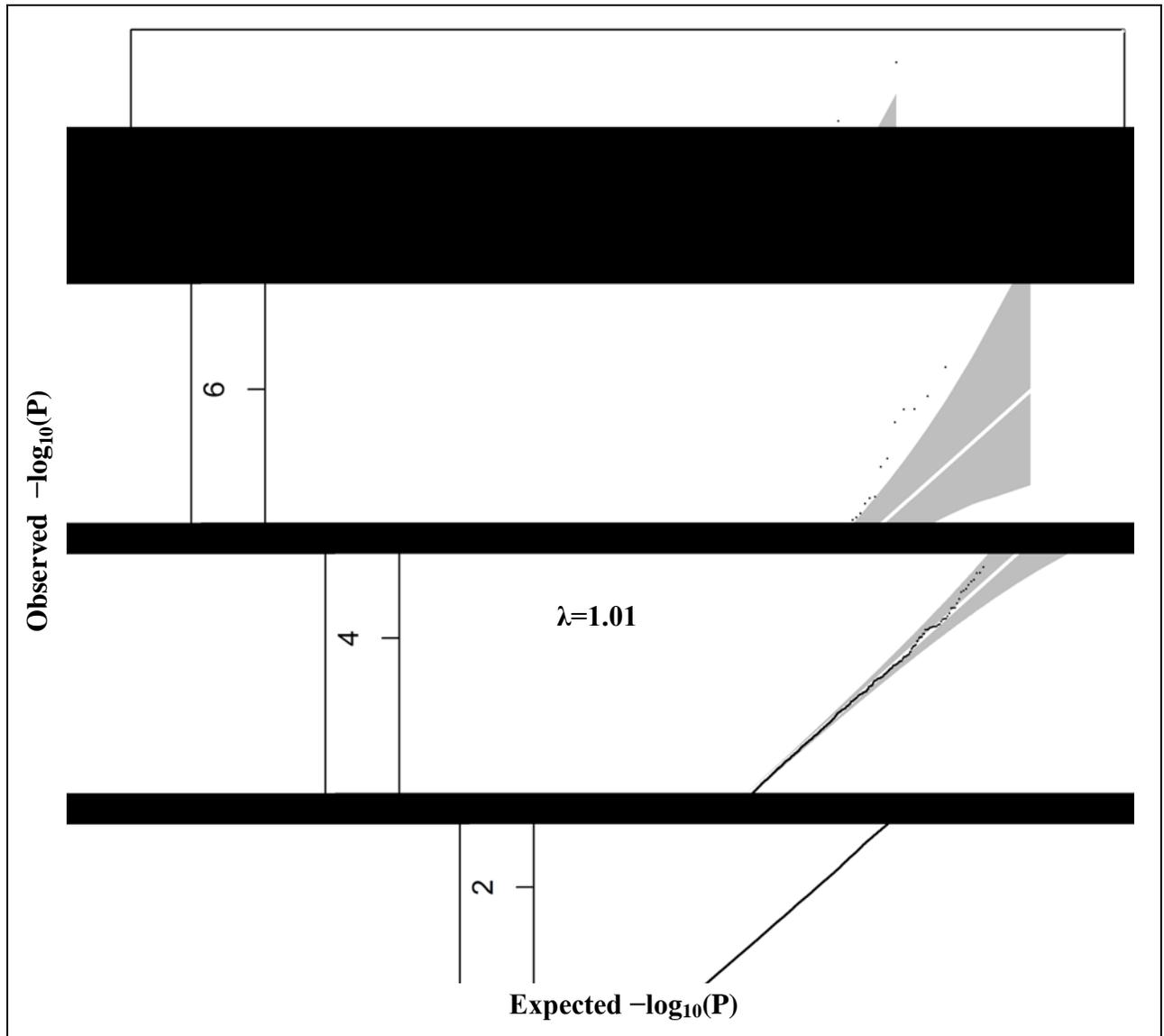
Figure S1 DNS fMRI Task

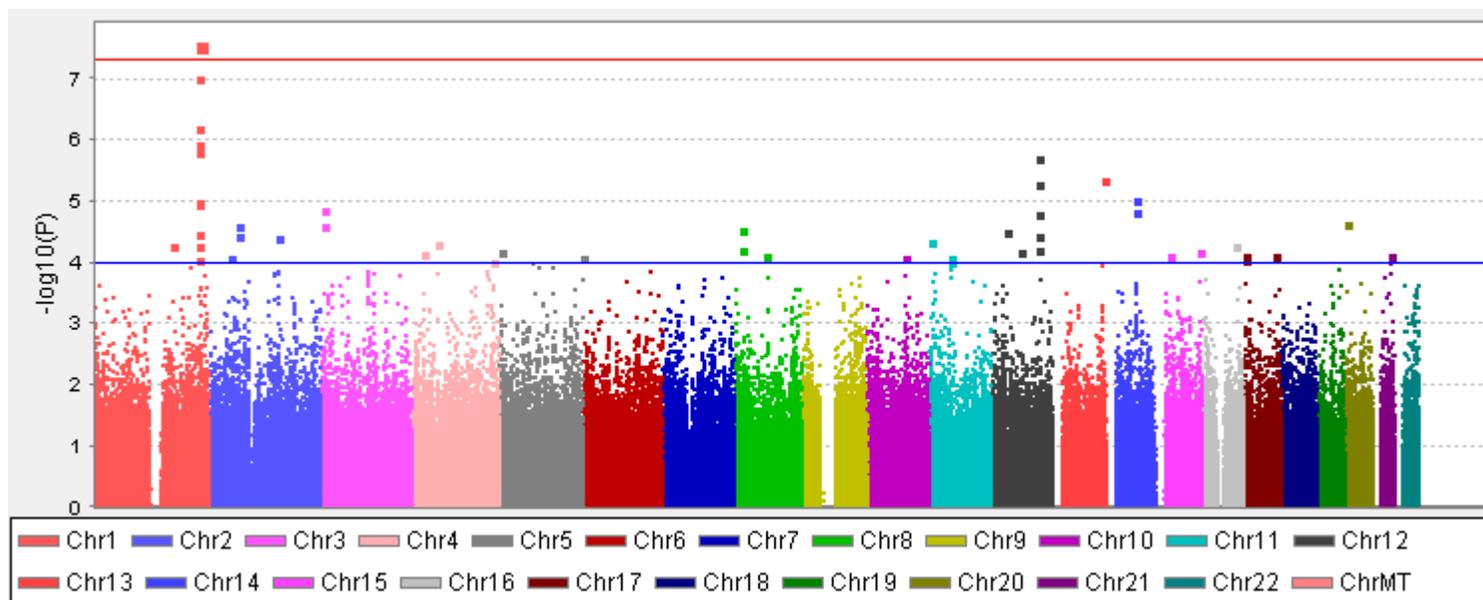**Figure S2, Panel A** The Q-Q Plot of Association Results for CATS Data

Figure S2, Panel B Manhattan Plot of Association Results for CATS Data
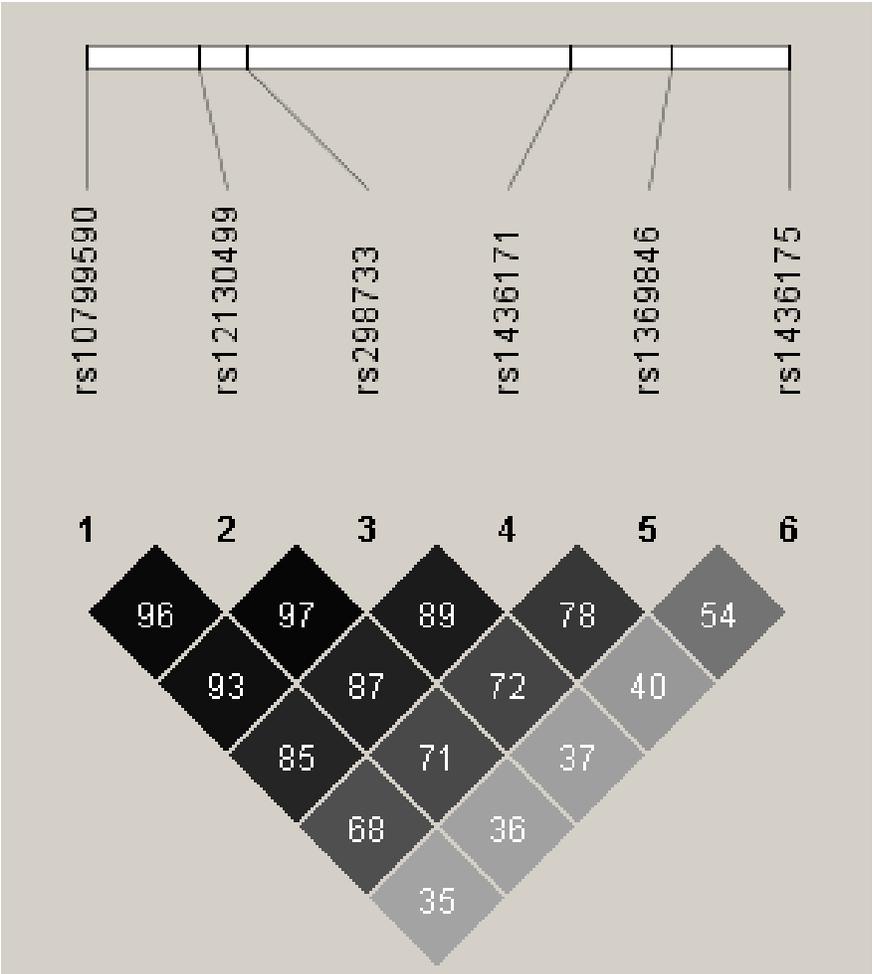
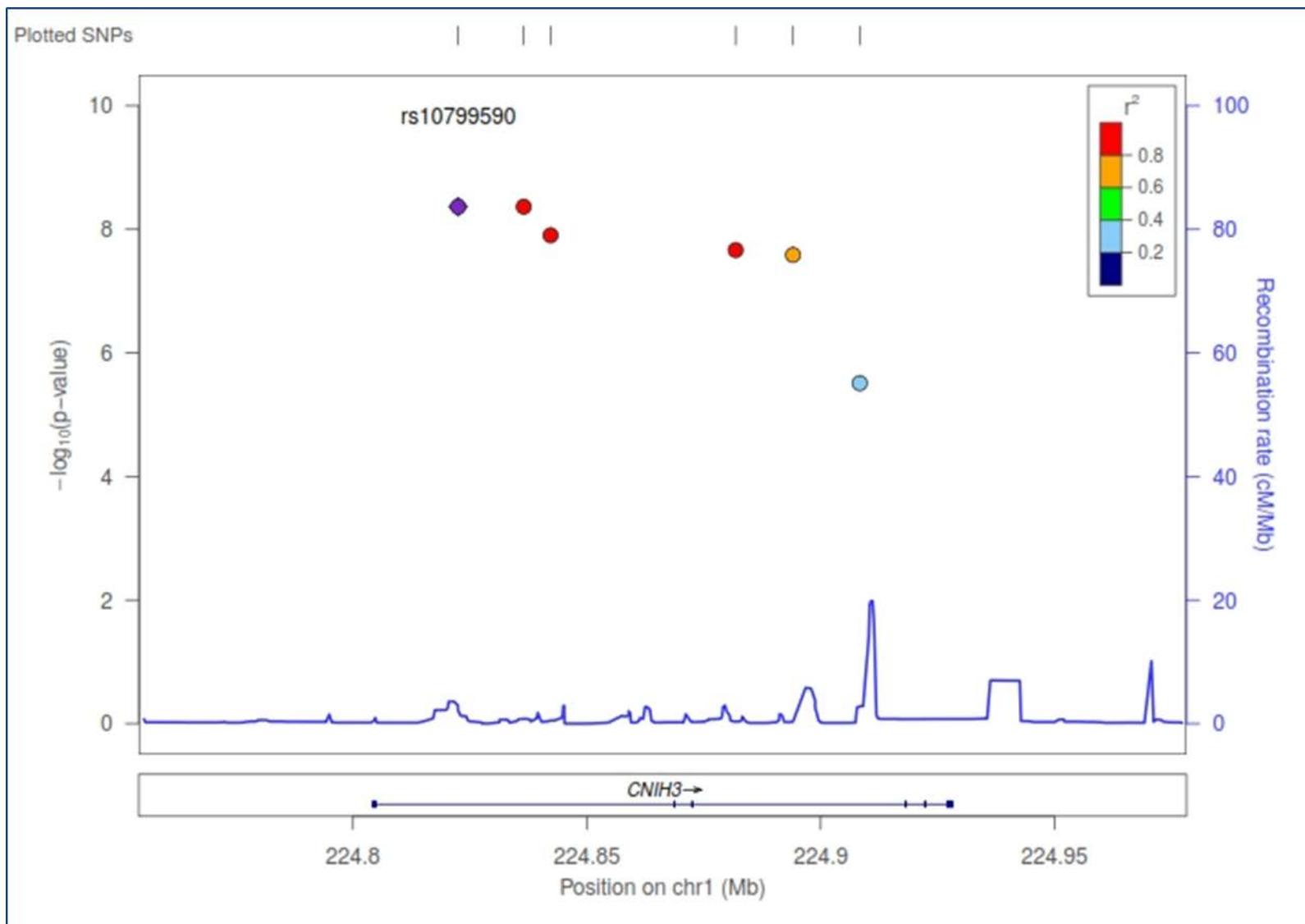**Figure S2, Panel C** Linkage Disequilibrium Relationships of

CNIH3 SNPs in CATS Data

Figure S2, Panel D Regional Association Plot of Meta-analytic Results
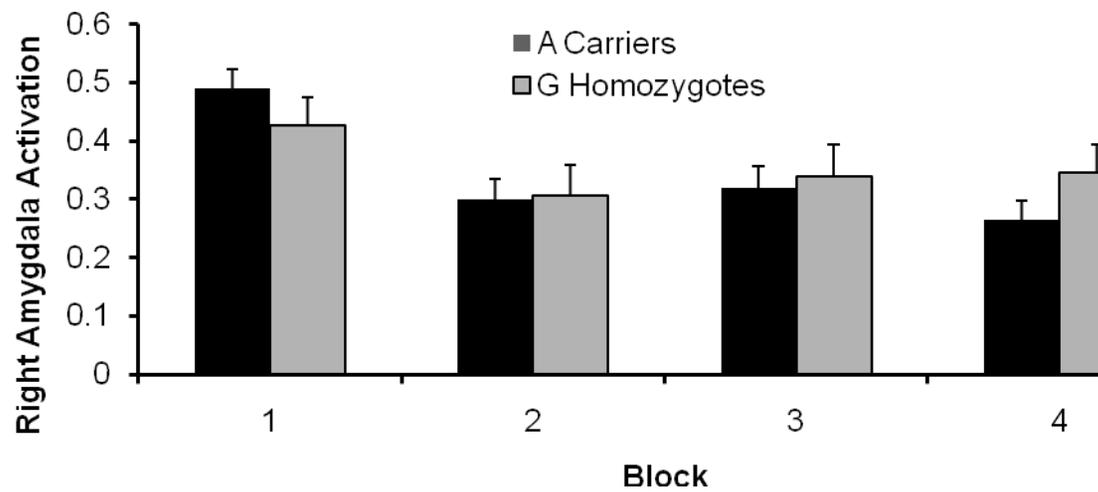
Figure S3. Amygdala Activation across Blocks

Supplementary Table 1 Phenotypic comparisons in CATS - Opioid dependent CATS participants with, and without, daily opioid injection and non-dependent, opioid-exposed neighborhood controls

Binary descriptive variables	OD$_E$ Group	OU$_{IP}$ Group	
	Opioid dependent daily injectors (N=1167)	Opioid dependent no history of daily injection (N=73)	Non-dependent, opioid-exposed individuals (N=88)
Male	60.1%	58.9%	53.4%
Alcohol dependence	42.1%	29.2%[a]	40.9%
Nicotine dependence	65.9%	61.1%	60.2%
Cannabis dependence	56.0%	52.1%	58.0%
Stimulant dependence	53.6%	31.5%[a]	36.4%[a]
Sedative dependence	38.8%	27.4%	5.7%[ab]
Cocaine dependence	32.1%	19.2%[a]	6.8%[ab]
Conduct disorder	48.8%	26.4%[a]	27.6%[a]
Post-traumatic stress disorder	39.2%	36.1%	17.1%[ab]
Major depressive disorder	61.0%	63.9%	67.1%
No relapse	15.3%	28.6%[a]	---
Continuous descriptive variables	**Mean age (Standard deviation)**		
Age	36.9 (8.4)	34.0 (8.9)[a]	34.0 (9.1)[a]
Age at first cannabis use*	14.1 (3.0)	14.9 (3.3)[a]	15.8 (2.4)[a]
Age at first stimulant use*	18.8 (5.5)	19.7 (6.2)	20.6 (5.4)[a]
Age at first opioid use	19.2 (5.3)	21.7 (8.0)[a]	23.5 (6.1)[a]

[a] Significantly different from opioid dependent daily injectors

[b] Significantly different from those opioid dependent individuals without a history of daily injection

* For those with a history with lifetime use

Supplementary Table 2 Phenotypic comparisons in confirmation study samples				
Binary descriptive variables	**Yale-Penn**		**SAGE**	
	OD_E **Group N=643**	OU_{IP} **Group N=157**	OD_E **Group N=190**	OU_{IP} **Group N=319**
Male	64.3%	69.4%	60.5%	70.2%[a]
Alcohol dependence	56.7%	70.1%[a]	97.9%	86.2%[a]
Nicotine dependence	84.9%	72.6%[a]	80.0%	66.8%[a]
Cannabis dependence	34.1%	35.7%	64.2%	44.8%[a]
Stimulant dependence	10.0%	11.5%	___	___
Sedative dependence	17.5%	3.8%[a]	___	___
Cocaine dependence	86.0%	85.3%	81.1%	50.5%[a]
Conduct disorder	4.2%	3.5%	58.4%	39.8%[a]
Post-traumatic stress disorder	15.1%	19.7%	24.2%	12.9%[a]
Major depressive disorder	18.7%	16.6%	61.1%	37.9%[a]
Continuous descriptive variables	**Mean age (Standard deviation)**			
Age	37.6 (9.3)	40.3 (9.1)[a]	35.8 (9.4)	36.1 (9.0)
Age at first cannabis use*	14.3 (5.9)	14.9 (5.1)	14.1 (2.8)	15.1 (3.4)[a]
Age at first stimulant use*	18.8 (11.2)	20.1 (13.1)	___	___
Age at first opioid use	20.1 (6.2)	25.9 (12.9)[a]	21.7 (7.9)	22.5 (7.5)
[a] Significantly different from OD_E group; * For those with a history with lifetime use				

Supplementary Table 3 Design & assessment of CATS & confirmation sample component studies							
Study (dbGaP accession number)	**CATS** **(phs000277)**	**Yale-Penn Genetic Studies** **(phs000425)**			**SAGE** **(phs000092.v1.p1)**		
Sources	**CATS**	**Opioid Dependence**	**Alcohol Dependence**	**Cocaine Dependence**	**COGA**	**COGEND**	**FSCD**
Basis of case ascertainment	opioid dependence	opioid dependence	alcohol dependence	cocaine dependence	alcohol dependence	nicotine dependence	cocaine dependence
Assessment							
Non-medical vs prescribed use differentiated	yes	no	no	No	yes	yes	yes
Individual opioids used recorded	separate heroin use section	three most commonly used opioids	three most commonly used opioids	three most commonly used opioids	no	no	no
Dependence assessed	opioid	opioid	opioid	Opioid	opioid	opioid	opioid
Injection drug use	yes	yes	yes	Yes	yes	yes	yes
Number of times injected – lifetime	yes	yes	yes	Yes	some, but not all participants	yes; some missing data	yes
Daily injection	yes	no	no	No	no	no	no

Supplementary Table 4 Psychiatric Diagnoses in Duke Neurogenetics Study (DNS) European-Amnericans	
Diagnosis	**Frequency***
Alcohol Abuse	23
Alcohol Dependence	19
Major Depressive Disorder	8
Marijuana Abuse	7
Generalized Anxiety Disorder	7
Social Anxiety Disorder	3
Agoraphobia w/o Panic Disorder	6
Bipolar Disorder NOS	6
Marijuana Dependence	5
Bipolar II	3
OCD	4
Bulimia Nervosa	2
Panic Disorder	2
Bipolar I	1
TOTAL	96
This table represents the number of current diagnoses across DNS participants. Some individuals presented with comorbid status.	

Supplementary Table 5 List of 23 strains included in the computational genetic analyses and mean number of jumps for each strain	
Strain name (JAX catalogue number)	Mean number of jumps (SEM)
BUB (653)	203.2 (12.5)
C57/B6 (664)	67.7 (6.9)
DBA (671)	23.8 (2.6)
129SV (2448)	2.5 (1.1)
AKR (648)	14.5 (1.9)
A/J (646)	47.4 (17.4)
Bc (651)	35.6 (5.2)
C3H (659)	20.4 (1.9)
MRL (486)	34.0 (1.7)
NZB (684)	55.0 (5.7)
B10 (461)	94.3 (14.0)
NZW (1058)	45.9 (4.7)
Lp (676)	2.9 (1.1)
LG (675)	2.0 (1.4)
SM (687)	0.25 (0.16)
FVB (1800)	33.6 (6.0)
NOD (1976)	27.8 (4.2)
SJL (686)	10.6 (3.1)
CBA (656)	32.0 (6.9)
MA (677)	7.2 (5.2)
NZO (2105)	10.6 (4.1)
SWR (689)	139.0 (8.5)

BTBR (2282)	110.4 (11.1)

Supplementary Table 6 Motif analysis of *CNIH3* SNPs						
SNP	**TF motif & location**	**Allele**	**Score**	**p-value**	**q-value**	**Motif**
rs10799590	MA0091.1_TAL1::TCF3	G	8.41	1.88E-04	0.00376	AGAACAGATGCA
	chr1,224822474,224822485,-	A	2.53	4.19E-03	0.0839	AGAATAGATGCA
rs298733	MA0084.1_SRY	C	NA	NA	NA	NA
	chr1,224842243,224842251,+	A	10.07	1.70E-04	0.00443	GTAGACAAT
rs298733	MA0078.1_Sox17	C	2.15	3.69E-03	0.096	ATCAGTGTC
	chr1,224842246,224842254,-	A	12.19	2.92E-05	0.00076	ATCATTGTC

Supplementary Table 7 Association with right amygdala habituation of SNPs in proximity to rs10799590*			
SNP	**Contrast**	**Stand Beta**	**P**
rs10799590	GG (n=102) > A Carrier (n=210)	0.147	0.009
rs1369848	GG (n=163) > A Carrier (n=148)	0.121	0.033
rs12730234	CC (n=98) > T Carrier (n=214)	-0.079	0.163
rs1965776	TT (n=101) > C Carrier (n=209)	0.132	0.019
*LD r^2 > 0.5 and each SNP tagging a distinct LD bin (r^2 > 0.8)			

Supplementary Table 8 Risk allele (RA) frequencies for *CNIH3* SNPs in the three samples and Australian European ancestry general population data and comparisons of the latter to CATS OD_E and OU_{IP} groups

SNP	RA	Risk allele frequency							CATS OD_E group vs AU twin families*		CATS OU_{IP} group vs AU twin families^	
		Yale-Penn		SAGE		CATS		AU twin families				
		OD_E	OU_{IP}	OD_E	OU_{IP}	OD_E	OU_{IP}		p value	OR (95%CI)	p value	OR (95%CI)
		N=643	N=157	N=190	N=319	N=1167	N=161	N=1933				
rs10799590	A	0.46	0.52	0.40	0.51	0.42	0.56	0.44	0.11	0.91 (0.82-1.02)	6.40E-5	1.62 (1.28-2.06)
rs12130499	T	0.44	0.50	0.41	0.51	0.42	0.56	0.44	0.11	0.92 (0.82-1.02)	2.95E-5	1.66 (1.31-2.10)
rs298733	A	0.47	0.53	0.41	0.51	0.42	0.57	0.45	0.11	0.92 (0.82-1.02)	4.06E-5	1.64 (1.30-2.09)
rs1436171	A	0.48	0.53	0.42	0.52	0.44	0.59	0.46	0.31	0.95 (0.85-1.05)	6.88E-6	1.73 (1.36-2.20)
rs1369846	C	0.43	0.47	0.37	0.46	0.38	0.54	0.41	0.023	0.88 (0.79-0.98)	1.08E-5	1.69 (1.34-2.14)
rs1436175	T	0.44	0.46	0.36	0.43	0.37	0.53	0.40	0.047	0.89 (0.80-1.00)	5.19E-6	1.72 (1.36-2.18)

*Analyses controlled for sex and 6 pcs; ^Analyses controlled for sex and 5 pcs

Supplementary Table 9 Estimates of the percentage of phenotypic variance explained by rs10799590	
Prevalence	Variance
.005	1.17%
.01	1.37%
.02	1.65%
.03	1.88%
.04	2.08%
.05	2.27%
.10	3.11%
.15	3.94%
.20	4.84%
.25	5.85%

Supplementary Table 10 Association of *CNIH3* SNPs in CATS with other phenotypes*												
Phenotype	rs10799590		rs12130499		rs298733		rs1436171		rs1369846		rs1436175	
	p val	OR	p val	OR	p val	OR	p val	OR	p val	OR	p val	OR
Alcohol dependence	0.13	1.14	0.21	1.11	0.21	1.11	0.35	1.08	0.99	1.00	0.22	0.90
Nicotine dependence	0.28	1.10	0.39	1.08	0.42	1.07	0.94	1.01	0.43	0.94	0.12	0.87
Cannabis dependence	0.84	0.98	0.62	0.96	0.76	0.98	0.75	0.97	0.27	0.91	0.002	0.77
Stimulant dependence	0.71	1.03	0.83	1.02	0.57	1.05	0.82	1.02	0.56	0.95	0.13	0.88
Sedative dependence	0.34	0.92	0.32	0.92	0.48	0.94	0.63	0.96	0.25	0.91	0.05	0.84
Cocaine dependence	0.32	0.92	0.14	0.88	0.20	0.89	0.21	0.90	0.12	0.87	0.02	0.82
Conduct disorder	0.64	0.96	0.70	0.97	0.82	0.98	0.57	0.95	0.81	0.98	0.28	0.91
Post-traumatic stress disorder	0.48	1.06	0.73	1.03	0.64	1.04	0.96	1.00	0.32	0.92	0.45	0.94
Major depressive disorder	0.85	0.98	0.78	0.98	0.96	1.00	0.71	0.97	0.30	0.92	0.40	0.93

*Additive models examining alternative phenotypes in the combined OD_E and OU_{IP} sample controlling for sex, age categories, and 3 PCs Key: p val = p value; OR = odds ratio

REFERENCES

1. Bucholz KK, Cadoret R, Cloninger CR, Dinwiddie SH, Hesselbrock VM, Nurnberger JI, Jr. *et al.* A new, semi-structured psychiatric interview for use in genetic linkage studies: a report on the reliability of the SSAGA. *J Stud Alcohol* 1994; **55:** 149-158.

2. Andrews G, Peters L. The psychometric properties of the Composite International Diagnostic Interview. *Soc Psychiatry Psychiatr Epidemiol* 1998; **33:** 80-88.

3. Reich T, Edenberg HJ, Goate A, Williams JT, Rice JP, Van Eerdewegh P *et al.* Genome-wide search for genes affecting the risk for alcohol dependence. *Am J Med Genet* 1998; **81:** 207-215.

4. Foroud T, Edenberg HJ, Goate A, Rice J, Flury L, Koller DL *et al.* Alcoholism susceptibility loci: confirmation studies in a replicate sample and further mapping. *Alcohol Clin Exp Res* 2000; **24:** 933-945.

5. Bierut LJ, Madden PAF, Breslau N, Johnson EO, Hatsukami D, Pomerleau OF *et al.* Novel genes identified in a high-density genome wide association study for nicotine dependence. *Hum Mol Genet* 2007; **16:** 24-35.

6. Saccone SF, Hinrichs AL, Saccone NL, Chase GA, Konvicka K, Madden PAF *et al.* Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. *Hum Mol Genet* 2007; **16:** 36-49.

7.   Bierut LJ, Strickland JR, Thompson JR, Afful SE, Cottler LB. Drug use and dependence in cocaine dependent subjects, community-based individuals, and their siblings. *Drug Alcohol Depend* 2008; **95:** 14-22.

8.   Patterson N, Price AL, Reich D. Population structure and eigenanalysis. *PLoS Genet* 2006; **2:** e190.

9.   Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009; **25:** 1754-1760.

10. Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE *et al.* Model-based analysis of ChIP-Seq (MACS). *Genome Biol* 2008; **9:** R137.

11. Grant CE, Bailey TL, Noble WS. FIMO: scanning for occurrences of a given motif. *Bioinformatics* 2011; **27:** 1017-1018.

12. Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P *et al.* Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell* 2010; **38:** 576-589.

13. Mathelier A, Zhao X, Zhang AW, Parcy F, Worsley-Hunt R, Arenillas DJ *et al.* JASPAR 2014: an extensively expanded and updated open-access database of transcription factor binding profiles. *Nucleic Acids Res* 2014; **42(Database issue):** D142-147. Epub 2013 Nov 4.

14. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E *et al.* The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* 1998; **59 Suppl 20:** 22-33;quiz 34-57.

15. First MB, Gibbon M, Spitzer RL, Williams JBW, Benjamin LS. *Structured Clinical Interview for DSM-IV Axis II Personality Disorders, (SCID-II).* American Psychiatric Press, Inc.: Washington, D.C., 1997.

16. So HC, Gui AH, Cherny SS, Sham PC. Evaluating the heritability explained by known susceptibility variants: a survey of ten complex diseases. *Genet Epidemiol* 2011; **35:** 310-317.

17. Chu LF, Liang DY, Li X, Sahbaie P, D'arcy N, Liao G *et al.* From mouse to man: the 5-HT3 receptor modulates physical dependence on opioid narcotics. *Pharmacogenet Genomics* 2009; **19:** 193-205.

18. Zheng M, Dill D, Peltz G. A better prognosis for genetic association studies in mice. *Trends Genet* 2012; **28:** 62-69. Epub 2011 Nov 24.

19. Liang DY, Zheng M, Sun Y, Sahbaie P, Low SA, Peltz G *et al.* The Netrin-1 receptor DCC is a regulator of maladaptive responses to chronic morphine administration. *BMC Genomics* 2014; **15:** 345.

20. Duffy DL, Iles MM, Glass D, Zhu G, Barrett JH, Höiom V *et al.* IRF4 variants have age-specific effects on nevus count and predispose to melanoma. *Am J Hum Genet* 2010; **87:** 6-16.