Quantitative Trait Locus Mapping of Oxycodone Reward and Naloxone Aversion in C57BL/6 Substrains

Lisa R. Goldberg[1], Stacey L. Kirkpatrick[1], Neema Yazdani[1], Olga Lacki[1], Megan K. Mulligan[2], and Camron D. Bryant[1]

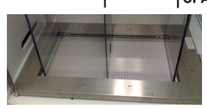
1. Laboratory of Addiction Genetics, Department of Pharmacology and Experimental Therapeutics and Psychiatry, Boston University School of Medicine
2. Department of Anatomy and Neurobiology, University of Tennessee Health Science Center

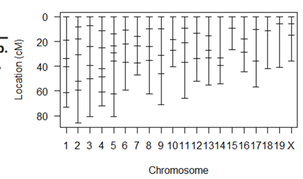
ABSTRACT

Opioid addiction is a heritable substance abuse disorder whose genetic basis remains poorly understood. Mammalian model organisms are valuable for identifying novel genetic factors that contribute to variation in addiction-relevant behaviors, including opioid-induced psychomotor stimulation and reward. C57BL/6J (B6J) and C57BL/6NJ (B6NJ) strains show robust strain differences in several addiction traits, including ethanol consumption, psychostimulant behaviors, and naloxone conditioned place aversion (Bryant et al., 2008; Kirkpatrick and Bryant, 2014). Quantitative Trait Locus (QTL) mapping in a cross between these closely related substrains drastically reduces the number of genetic variants from millions (e.g., B6J vs. DBA/2J) to thousands, accelerating the rate at which we can identify the underlying quantitative trait genes (QTGs). We conducted QTL mapping for oxycodone conditioned place preference (OXY-CPP; N=133) and naloxone conditioned place aversion (NAL-CPA; N=151), along with saline controls (SAL; N=140). We utilized a 9 day CPP/CPA protocol; 24 hours post-assessment of initial preference on Day 1 (D1), mice received an i.p. injection of drug (1.25 mg/kg OXY, 4 mg/kg NAL, or SAL) on D2 and D4 on the drug-paired side, and SAL (i.p.) on the SAL-paired side on D3 and D5. Mice were assessed for drug-free CPP/CPA (D8) and drug state-dependent CPP/CPA (D9) under the influence of drug. We identified NAL- specific QTLs associated with freezing during training and testing on chromosomes 10 and 8. We also identified OXY - specific QTLs for OXY-induced locomotor behaviors during training (rotations, distance traveled; chr. 1), OXY-CPP (chr. 9), and conditioned behaviors during OXY reward assessment (distance and entries into OXY-paired side; chr. 5). We are currently increasing our sample size to N=200 per drug treatment and will use genome editing and transcriptome analysis of the mesocorticolimbic circuitry to validate novel genetic factors and neurobiological mechanisms underlying the motivational properties of opioids.

1. Place conditioning protocol and and genetic marker map

D1	D2	D3	D4	D5	D6	D7	D8	D9
SAL	DRUG	SAL	DRUG	SAL	-	-	SAL	DRUG
Initial Pref.	Training				Consolid.		CPP/ CPA	State-Dep. CPP/CPA

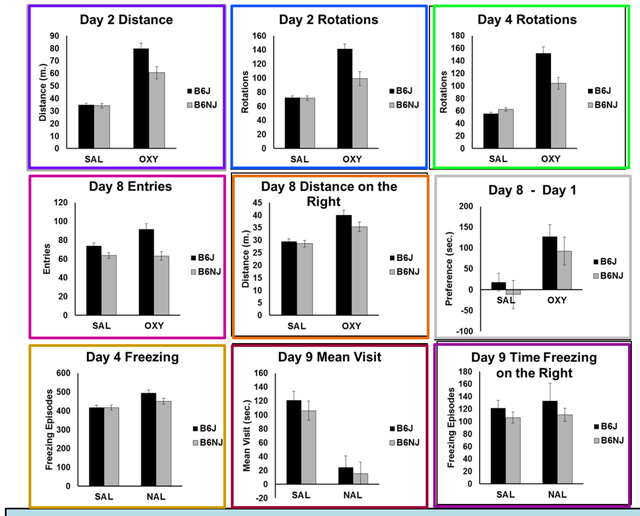




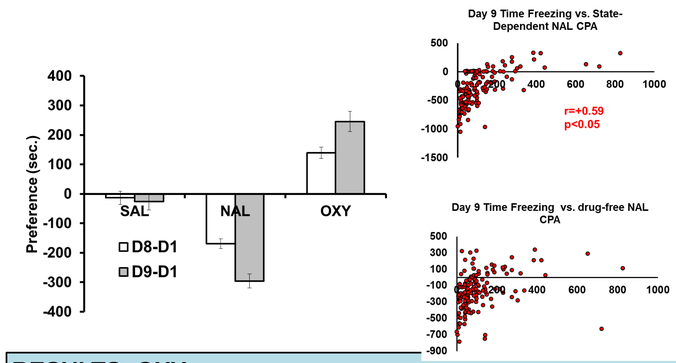
MATERIALS & METHODS

Female C57BL/6J (B6J) and male C57BL/6NJ (B6NJ) mice were ordered from the Jackson Laboratory (Bar Harbor, ME, USA) and were paired as F1 breeders. Non-sibling B6J x B6NJ F1 offspring were paired to generate B6J x B6NJ F2 mice. Briefly, we utilized a 9-day protocol with 30-min training and test sessions. On Day 1 (D1), mice received an i.p. injection of saline and initial preference for the drug-paired side was assessedby allowing mice free access to both sides. On training days (D2-D5), mice received an i.p. injection of either drug (4 mg/kg NAL, 1.25 mg/kg OXY; D2, D4) or SAL (D3, D5) and were confined to either the drug-paired or SAL-paired side, respectively. Mice were left undisturbed in their home cages on D6 and D7. On D8, final preference for the drug-paired side was assessed, as on D1. On D9, state-dependent preference was assessed under the influence of the drug. All individuals were genotyped using a custom DELTAgene panel (Fluidigm 96x96 SNPtype assay) consisting of 96 SNP markers spaced approximately 30 megabases apart. QTL analysis was performed using the R package R/qtl (n.perm = 1,000, p<0.15). Separate QTL scans were conducted for each treatment group (NAL, SAL, OXY) and the genomic locations of the suggestive peaks were compared to determine whether the QTLs were treatment-specific.

2. B6J and B6NJ parental strain differences in OXY- and NAL-induced behaviors



3. OXY-CPP and NAL-CPA behaviors in F2 mice



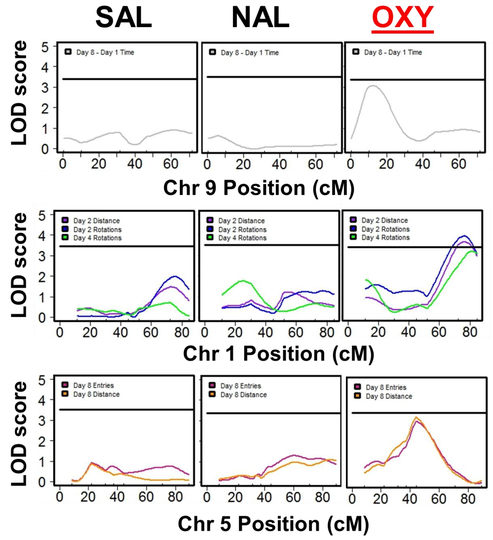
RESULTS: OXY

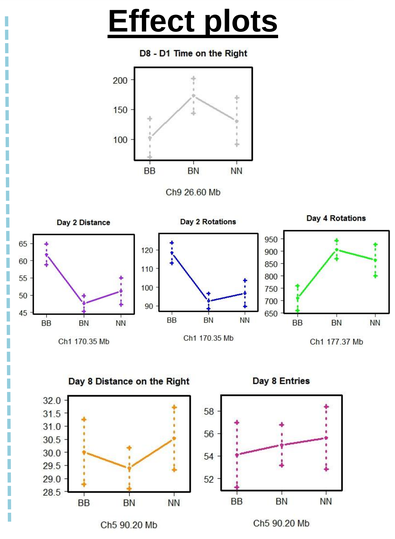
We identified overlapping OXY-CPP specific QTL for drug-free distance traveled on the drug-paired side and entries into the drug-paired side on chromosome 5 (pos=44.34; LOD=3.06, 3.26; p<0.15). We also identified a OXY-CPP-specific QTL for drug-free OXY-CPP on chromosome 9 (pos=26.60 Mb, LOD=3.06, p<0.15), where a missense SNP in *Herpud2* maps to a nearby locus(pos=24.94 Mb), indicating a potential candidate gene for opioid reward. We also identified overlapping OXY-CPP specific QTL for day 2 locomotor activity, day 2 rotations, and day 4 rotations on chromosome 1 (pos=76.33, 76.33, 81.33; LOD= 3.65, 3.94, 3.20; p<0.05, p<0.05, p<0.10).

RESULTS: NAL

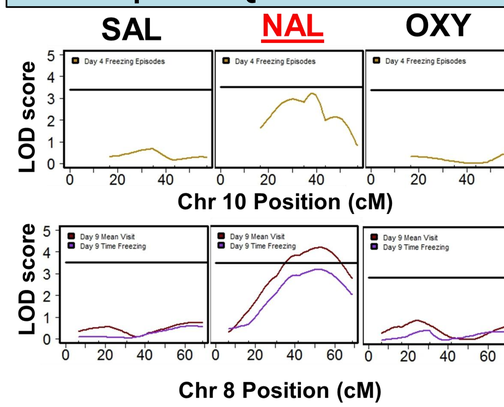
We identified a NAL-CPA specific QTL for Day 4 freezing episodes on chromosome 10 (pos= 75.55 Mb; LOD=3.22; p<0.1). We also identified overlapping NAL-CPA specific QTL for state-dependent (Day 9) mean visit on the drug-paired side and state-dependent (Day 9) freezing on the drug-paired side on chromosome 8 (pos=52.54, 51.54; LOD= 4.19, 3.19; p<0.05, p<0.10).

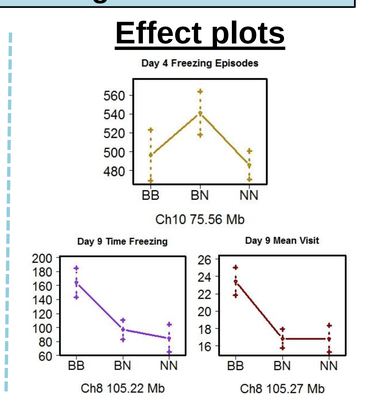
4. OXY-specific QTLs associated with psychomotor activity, Pavlovian-conditioned locomotor, approach, and reward behaviors





5. NAL-specific QTLs associated with freezing and aversion





FUTURE DIRECTIONS

- We will increase our sample size to N = 200 mice which will provide us with additional power to assess the reliability of our current QTLs and possibly detect additional smaller effect loci.
- We will utilize known variants between the J and NJ strains to prioritize candidate genes within the QTLs.
- In addition, we will complete mRNA sequencing analysis of striatal samples from a subset of F2 mice, chosen based on genotypes at the closest SNP marker and phenotype (tail-ends of the distribution) to possibly assist in the choice of candidate genes as well as aid in ascribing neurobiological mechanisms.
- We will use genome editing to create null mutants and knock-in mice containing predicted functional B6NJ alleles on a B6J background and vice versa.

FUNDING: R00DA029635, T32GM0086541, Transformative Training Program in Addiction Science (Burroughs Wellcome 9550300872), R03DA038287, R21DA038738, R01DA039168