Center for Human Genetics

Dissecting the Genetic Basis of Variation in Cocaine and Methamphetamine Consumption in *Drosophila melanogaster*

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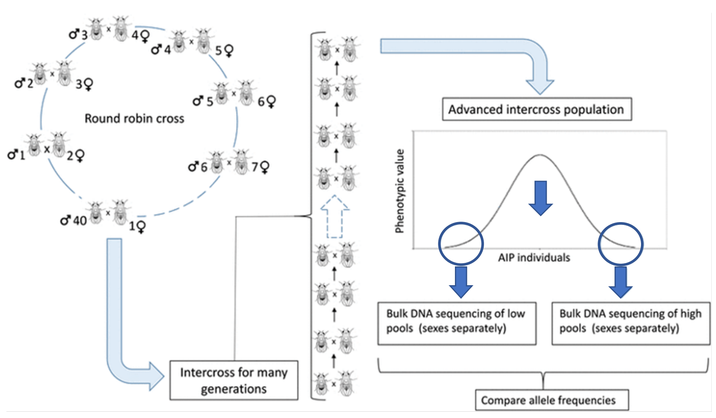
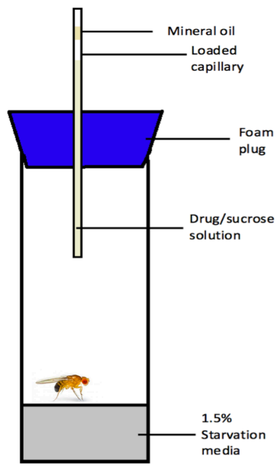
Studies on *Drosophila melanogaster* can identify genetic and transcriptional networks that underlie variation in voluntary consumption of cocaine and methamphetamine to serve as a blueprint for subsequent studies on humans. Exposure to these psychostimulants in flies results in behavioral and physiological effects that resemble those observed in humans. We derived an outbred advanced intercross population (AIP) from 37 of the sequenced inbred wild-derived lines of the *Drosophila melanogaster* Genetic Reference Panel (DGRP). These lines are maximally genetically divergent, have minimal residual heterozygosity, are not segregating for common inversions, and are not infected with *Wolbachia pipientis*. We assessed voluntary consumption of sucrose, methamphetamine-supplemented sucrose and cocaine-supplemented sucrose and found significant phenotypic variation in the AIP, in both sexes, for consumption of both drugs. We performed whole genome sequencing and extreme QTL mapping on the top 10% of consumers for each replicate, sex and condition, and an equal number of randomly selected flies. We evaluated changes in allele frequencies genome-wide among high consumers and the control flies and identified 3,033 variants associated with increased consumption that reside in 1,963 genes, enriched for genes associated with nervous system and mesoderm development. We assessed the effects of ubiquitous RNA interference (RNAi) on consumption for 22 candidate genes, of which 14 showed a significant increase or decrease in consumption. Extensive recombination in the AIP generates increased statistical power compared to genome-wide association analysis of the DGRP and illustrates the polygenic genetic architecture that underlies variation in cocaine and methamphetamine consumption. Supported by NIH grant U01DA041613.

Extreme QTL mapping with an Advanced Intercross Population



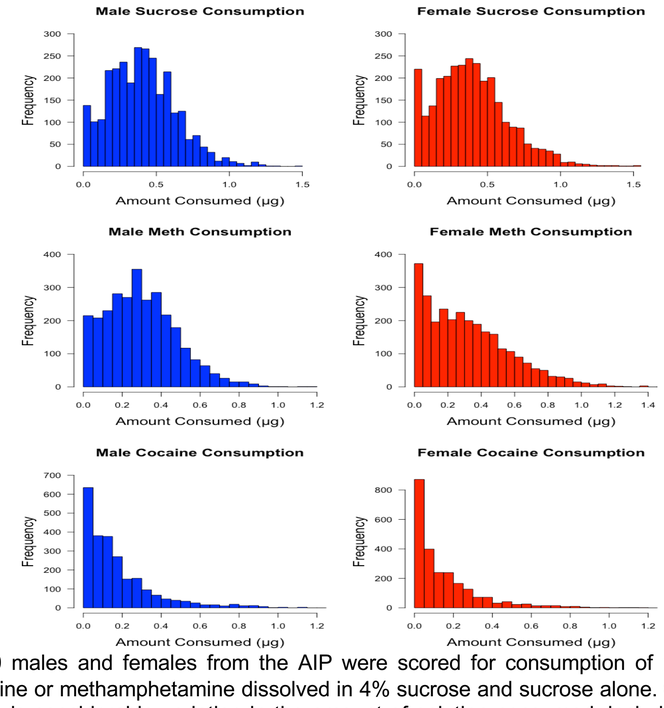
From Anholt RRH and Mackay TFC (2018) Mamm. Genome ***29:*** *5-23.*

Measuring drug consumption with a modified Capillary Feeder Assay



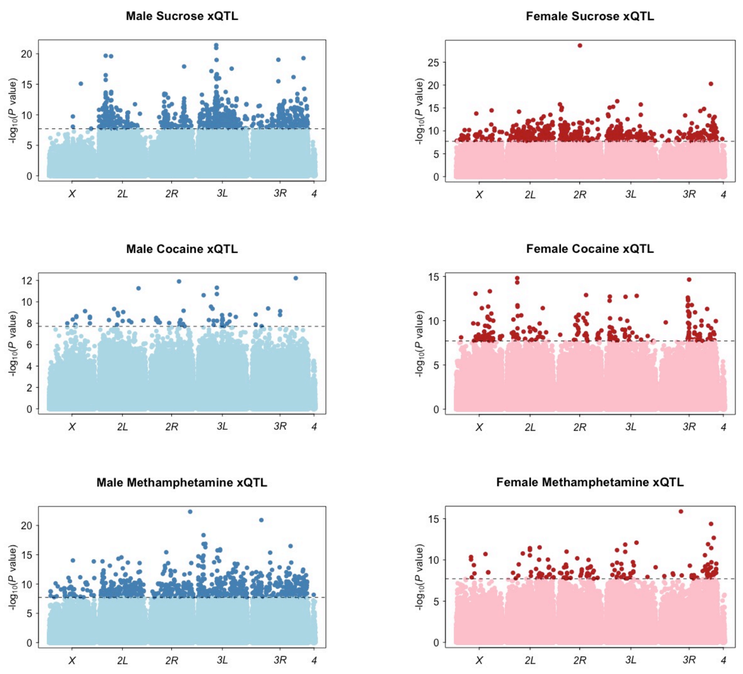
To assess consumption, we place individual 3-5 day old males or females in a fly vial with an agar solution at the bottom. The vial is plugged with a foam plug and a 5 µL capillary tube containing the appropriate solution is inserted through the plug. We mark the fluid level in each capillary tube at the beginning of the assay and then again after 18 hours of *ad libitum* feeding. Evaporation is controlled for by plugging the capillary tube with a drop of mineral oil and through the use of blank vials with the appropriate solution but no fly.

Variation in Drug Consumption in the AIP



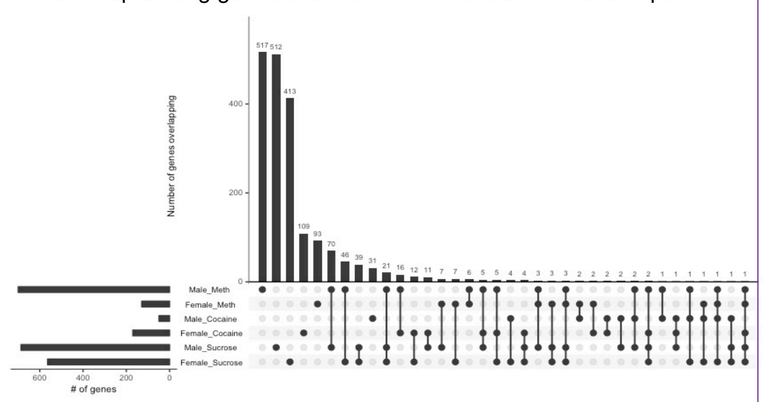
1500 males and females from the AIP were scored for consumption of 1 µg/µL cocaine or methamphetamine dissolved in 4% sucrose and sucrose alone. Overall, there is considerable variation in the amount of solution consumed, including flies that did not consume neither the cocaine nor methamphetamine solution

Extreme QTL Mapping



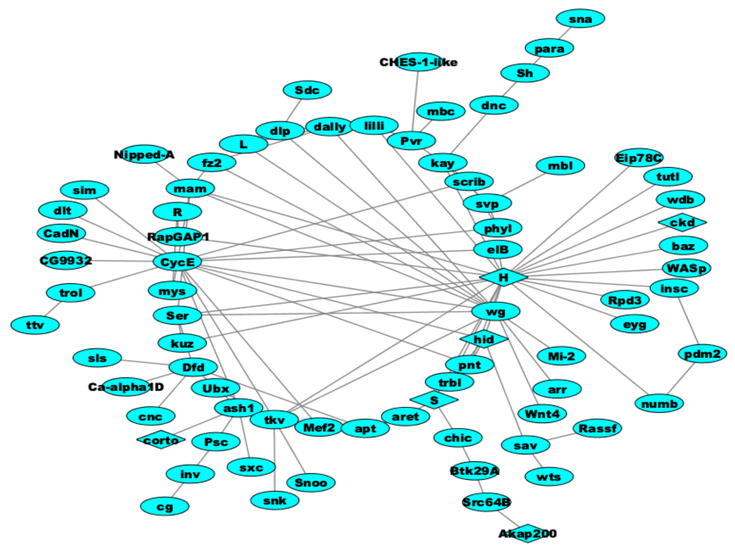
We compared allele frequencies between pools of high consumers and pools of random flies for each condition and sex. We identified 3,033 variants associated with increased consumption of sucrose, cocaine or methamphetamine.

Overlap among genes associated with variation in consumption



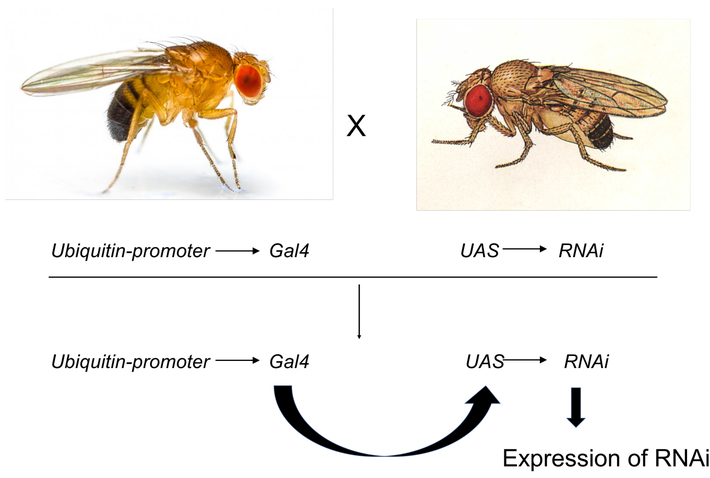
The 3,033 significant variants we identified map in or around 1963 unique genes. When looking across sexes and conditions, most genes are unique to each sex/treatment combination but significant overlap does exist when looking across two or more conditions.

Gene-gene Interaction Network

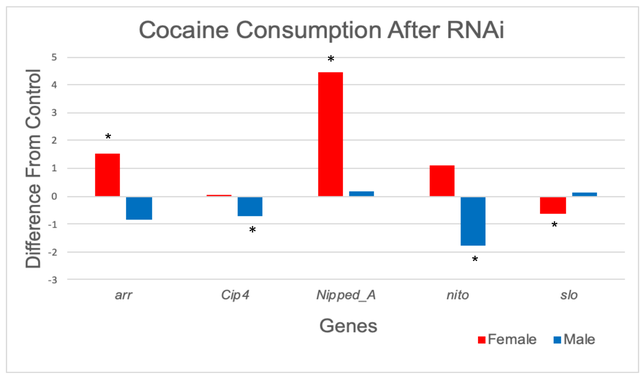


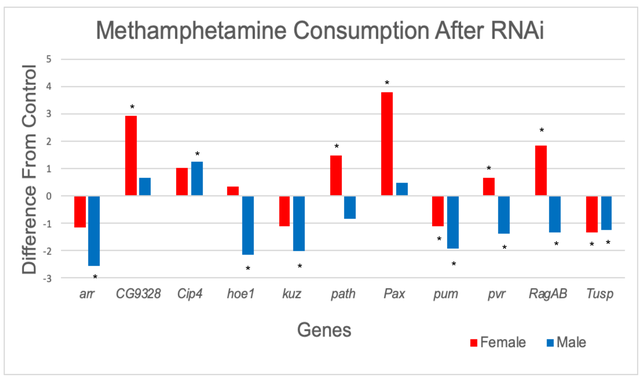
We assessed to what extent genes from all drug experiments could be assembled into a genetic interaction network utilizing known gene-gene interactions. 77 genes were mapped to the network. 92% have a human ortholog and 57% have a biological process involved in the nervous system.

Driving RNAi with the GAL4-UAS binary expression system



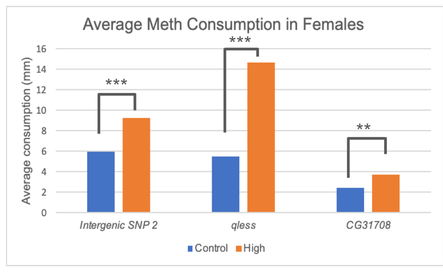
Functional validation of candidate genes using RNA interference

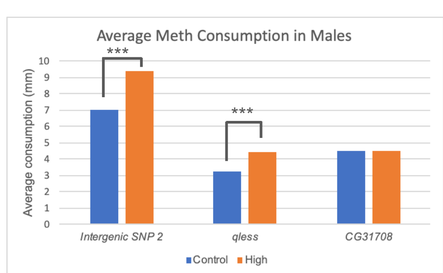


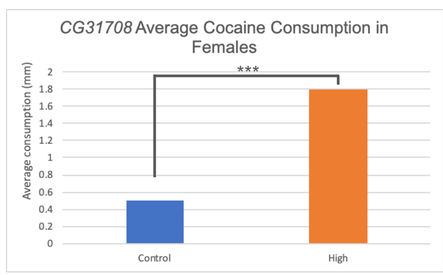


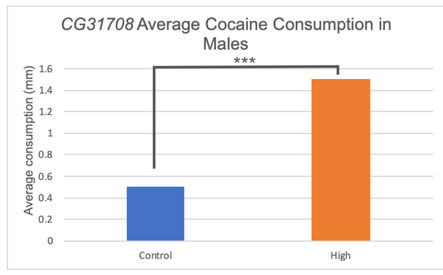
We utilized RNA interference to validate 22 candidate genes. Some genes such as *pum* and *Tusp* show the same direction of response in both sexes following knockdown. Other genes show sexual dimorphism such as *RagAB* and *pvr*.

DGRP-Based Validation of Variants









We constructed new AIPs for 5 significant intergenic SNPs and SNPs within 5 significant genes. For each SNP/gene, we selected 10 DGRP lines that contained the allele that was enriched in the high consuming pool ("high") and 10 lines with the alternate ("control") allele and made two separate populations using the round robin crossing scheme. After 5 generations of random mating, we tested the consumption of 150 flies/sex/ treatment to assess the population means. We then analyzed these data with the fixed effects ANOVA model Y = G + T + GxT + ε where G is genotype (high vs. control), T is treatment (cocaine, methamphetamine or sucrose) and GxT the interaction. We observed a significantly higher consumption of methamphetamine in the "high" populations for intergenic SNP 2, *qless* and *CG31708* in females and intergenic SNP 2 and *qless* in males. The population containing the "high" SNPs for *CG31708* also showed a higher consumption of cocaine than the population containing the "control" SNPs.

Conclusions

Using extreme QTL mapping with a DGRP-derived Advanced Intercross Population we identified genes associated with increased consumption of cocaine and/or methamphetamine at Bonferroni-corrected statistical significance.

We constructed a genetic network associated with increased consumption of cocaine or methamphetamine.

We functionally validated of 22 candidate genes with RNAi and 10 SNPs, 5 of which were intergenic, in AIPs containing alternative alleles in randomized genetic backgrounds revealing sexual dimorphism in consumption traits.

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