From single flies to many genes: Using *Drosophila* to explore the genetics of psychostimulant consumption

Iris Titos[a,1] and Adrian Rothenfluh[a,b,1]

In our everyday life, we are often exposed to addictive agents like pain killers, tobacco, alcohol, even food, sex, or video games. Although the majority of people will not develop an addiction upon contact with those agents, a part of the population will. What determines whether a person is going to develop a pathological pattern upon exposure to an addictive agent? Family studies reveal a genetic influence in drug responses and addiction, and addictions are actually one of the most hereditable psychiatric disorders (1). Despite this, it has been challenging to identify specific genes that make an individual more susceptible to becoming addicted in human studies. The reasons for this include the need for large sample sizes due to the polygenic nature of addiction, where many genes have small effects. Furthermore, addiction is influenced by many environmental factors, such as socioeconomic status, childhood trauma, the availability/prevalence of the drug itself, and the cost (in time and money) to perform such large studies in the first place. Many of these limitations can be avoided by the use of model organisms, especially ones that allow for high-throughput assays like *Drosophila melanogaster*. This is what a recent PNAS paper by Baker et al. (2) tackles by assaying 18,000 single flies for their consumption of sucrose with, or without, the addition of the psychostimulants cocaine and methamphetamine (Fig. 1).

Drosophila melanogaster exhibit behaviors similar to humans when exposed to substances of abuse like alcohol, cocaine, and methamphetamine (3), and their genetic background and environment can be tightly controlled. Around 75% of disease-causing genes in humans are conserved in *Drosophila* (4), supporting the potential to translate fly discoveries to humans, as has been done repeatedly for alcohol addiction-related genes (5). During much of the past 100 y that vinegar flies have been a genetic model organism, researchers have studied *Drosophila* with Mendelian genetic approaches, where one gene is manipulated, and the resulting phenotype is studied. For the last two decades, the research group of Trudy Mackay and Robert Anholt has been pioneering a different approach: instead of searching for single genes with large phenotypic effects, they have been mapping many quantitative traits similar to human genome-wide association studies (GWASs). This was enabled by their development of the *Drosophila* Genetic Reference Panel (DGRP) (6), which consists of a population of ~200 wild-derived fly lines. Each of these lines has two identical alleles per gene—although with different alleles for all genes between them—and each line is fully sequenced at the genomic DNA level. The DGRP panel has been subjected to many phenotypic surveys followed by GWASs to identify variants that correlate with the phenotypes of interest (7). This has included their own study, where they phenotyped 46 DGRP lines for their cocaine and methamphetamine consumption preference and isolated 2,814 single-nucleotide polymorphisms (SNPs) that mapped to 1,358 genes (8).

DGRP strains are generally assayed in groups of (genetically identical) siblings, and thus the genetic variation is present in ~200 fixed strains and combinations. In reality, each fly, and each person (the occasional twin notwithstanding), is a unique combination of naturally occurring genetic SNP variants. To simulate this more natural genetic architecture of a population, Baker et al. (2) created an advanced intercross population (AIP) to study drug consumption. They generated the AIP from 37 highly diverse DGRP lines, which were randomly crossbred for over 50 generations, yielding a genetically diverse set of siblings (and progeny). Then, the authors performed single-fly feeding assays for 3,000 flies of each sex with sucrose, or with sucrose containing either cocaine or methamphetamine. After sequencing the top 10% of

[a]Molecular Medicine Program, University of Utah, Salt Lake City, UT 84112; and [b]Department of Psychiatry, Huntsman Mental Health Institute, University of Utah, Salt Lake City, UT 84112

Author contributions: I.T. and A.R. wrote the paper.

The authors declare no competing interest.

See companion article, "Genetic basis of variation in cocaine and methamphetamine consumption in outbred populations of *Drosophila melanogaster*," 10.1073/pnas.2104131118.

[1]To whom correspondence may be addressed. Email: iris.titos@utah.edu or adrian.rothenfluh@hsc.utah.edu.

Published July 27, 2021.

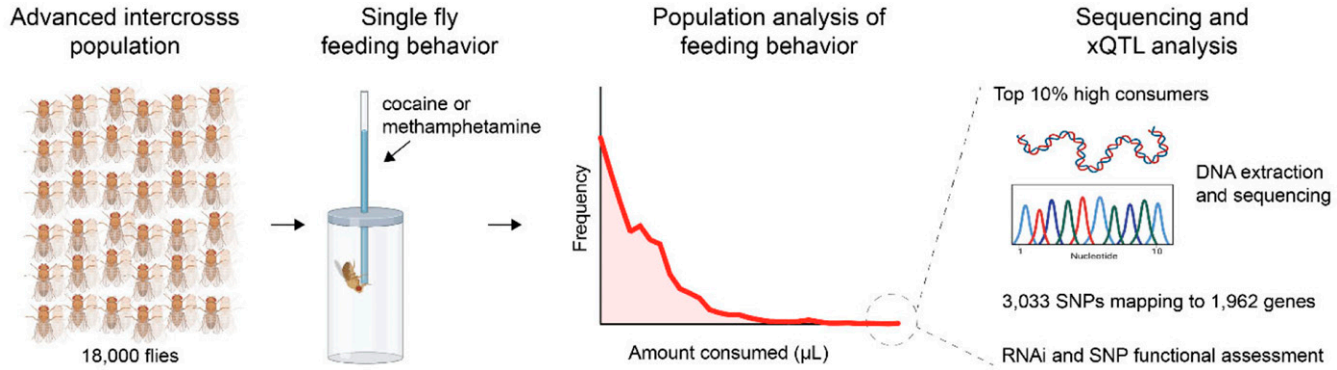

Fig. 1. Strategy used to identify SNPs involved in *Drosophila* cocaine and methamphetamine consumption. An advanced intercross population (AIP) containing 18,000 flies was subjected to single-fly feeding behavior of food containing only sucrose or sucrose supplemented with either cocaine or methamphetamine. From all the flies, the top 10% high consumers were selected and sequenced. The comparison of the top consumers sequence with that of a control population through extreme quantitative trait locus analysis (xQTL) allowed the identification of 3,033 single nucleotide polymorphisms (SNPs) that mapped to 1,962 genes that are involved in increased consumption in flies. Some of the genes and SNPs found in the screen were functionally assessed by RNA interference (RNAi) and allele-specific AIP libraries. Created with https://biorender.com/.

consumers for each condition and sex, they compared the DNA reads to the sequences of a random cohort of flies within the AIP population to determine the SNPs that were significantly enriched in the high consumers. Because of the considerable statistical power and the 2 sexes × 3 food conditions design, they found significant associations in SNPs mapping to over 1,900 genes. Half of these genes showed an association just with high amounts of sucrose consumption (in either males, or females, or both) and are therefore less relevant for the question of cocaine and methamphetamine consumption. However, even after discarding the genes that affect sucrose feeding only, the list of genes found associated with cocaine and methamphetamine consumption remains extensive. Can almost a thousand genes really be involved in drug responses? As of this writing, human studies have identified about 50 genes associated with cocaine use and dependence to varying degrees of statistical stringency (9–11). This disparity in numbers, from flies to humans, is likely due to the small sample size in the human studies at present, because GWASs performed for alcohol addiction with larger sample sizes have linked hundreds of human genes to alcohol consumption and dependence (12). Furthermore, the Mackay and Anholt group (13) also performed a GWAS on alcohol-related behaviors identifying 247 genes in *Drosophila*, which is on the same order of magnitude as human studies. Thus, while an association of hundreds of genes linked to *Drosophila* cocaine and methamphetamine consumption may seem large, it may well represent a situation similar to humans, once larger studies are completed with psychostimulant abuse.

Many of the SNPs implicated by Baker et al. in stimulant consumption showed effects in either males or females only. Sexual dimorphism is often not considered when studying behavior in flies, except if the behavior is of sexual nature itself, like courtship. In humans, sex differences are well-established in substance use disorder, ranging from the response to the drug itself, to the progressive changes that occur in the brain that lead to addiction (14). The authors confirmed a subset of the genes identified in their AIP screen using a gene knockdown approach and corroborated the many sexually dimorphic effects. Gene knockdown using RNA interference is useful to show causal involvement of the genes close to the SNPs identified, but it represents an artificial situation, as the identified SNPs are often outside of the protein-coding sequence, or even outside the transcribed gene region. The authors addressed this by constructing two fly lines for 10 different SNPs each, where the high-consuming allele and the other allele were crossed into diverse, randomized backgrounds. Nine out of these 10 SNPs tested led to significant cocaine or methamphetamine consumption phenotypes, again, in an often sexually dimorphic manner. Crucially, even all five SNPs that were intergenic, and not annotated to a specific gene, showed stimulant-consumption phenotypes. This is especially relevant since many human variants associated with addiction phenotypes are in intergenic regions (12), and the paper by Baker et al. thus convincingly shows that such variants can have phenotypic effects, even if their molecular mechanisms are far from obvious.

What are the mechanistic lessons then, that we can we extract from the large list of genes related to increased cocaine or methamphetamine consumption? Genetic interaction networks extracted from the list revealed an enrichment in genes associated with nervous system development. Given the vast knowledge on how drugs of abuse act on the nervous system, this is comforting, if not necessarily illuminating. A major challenge coming from this study is which criteria to use to select genes and gene networks for mechanistic follow-up studies and possible development of therapeutics. While the genetic interaction network extraction approach is intriguing, suggesting suites of cooperating genes involved, only a minority of genes implicated fell into such networks to begin with. Maybe the answer will lie in a systems' genetics integration of large datasets. For example, integrating the present study with their previous DGRP GWAS showed an overlap of 209 genes implicated. Another study involving the Mackay and Anholt group may also suggest a way. Huggett and colleagues (15) performed a transcriptomic analysis of human cocaine users and controls and then compared the observed changes to a database of transcriptional changes induced by a large panel of US Food and Drug Administration (FDA)-approved drugs. They found that ibrutinib, an inhibitor of Bruton's tyrosine kinase, caused opposite transcriptional changes to the ones induced by cocaine. Indeed, ibrutinib reduced cocaine-induced behavioral effects in *Drosophila*. The strength of this study lies in the integration of transcriptomics from postmortem tissue and a large FDA-approved therapeutics-induced transcriptomics database, followed by in vivo validation of a therapeutic intervention in *Drosophila*. Similarly, the large gene set implicated by the

recent PNAS study by Baker et al. might get integrated with their own single-cell transcriptomics data from fly brains exposed to cocaine (16), or the aforementioned human postmortem study (15), or even future human GWAS findings with larger samples of cocaine users. Either way, *Drosophila* remains a useful model organism in the study of complex disorders, because of their economy of scale in both hypothesis generation, be that via GWASs (2), or transcriptomics (16), as well as in hypothesis testing of candidate genes and SNPs (2) and therapeutic interventions (15). Neuropsychiatric disorders such as addiction are complex in their etiology, and the integration of many approaches, including model organisms, promises to get us closer to an understanding of what drives individuals from an initial drug experience to an obsessed-over preoccupation.

1 D. Goldman, G. Oroszi, F. Ducci, The genetics of addictions: Uncovering the genes. *Nat. Rev. Genet.* **6**, 521–532 (2005).
2 B. M. Baker, M. A. Carbone, W. Huang, R. R. H. Anholt, T. F. C. Mackay, Genetic basis of variation in cocaine and methamphetamine consumption in outbred populations of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2104131118 (2021).
3 K. R. Kaun, A. V. Devineni, U. Heberlein, *Drosophila melanogaster* as a model to study drug addiction. *Hum. Genet.* **131**, 959–975 (2012).
4 U. B. Pandey, C. D. Nichols, Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery. *Pharmacol. Rev.* **63**, 411–436 (2011).
5 D. R. Lathen, C. B. Merrill, A. Rothenfluh, Flying together: *Drosophila* as a tool to understand the genetics of human alcoholism. *Int. J. Mol. Sci.* **21**, 6649 (2020).
6 T. F. C. Mackay *et al.*, The *Drosophila melanogaster* Genetic Reference Panel. *Nature* **482**, 173–178 (2012).
7 T. F. C. Mackay, W. Huang, Charting the genotype-phenotype map: Lessons from the *Drosophila melanogaster* Genetic Reference Panel. *Wiley Interdiscip. Rev. Dev. Biol.* **7**, e289 (2018).
8 C. A. Highfill, B. M. Baker, S. D. Stevens, R. R. H. Anholt, T. F. C. Mackay, Genetics of cocaine and methamphetamine consumption and preference in *Drosophila melanogaster*. *PLoS Genet.* **15**, e1007834 (2019).
9 J. Gelernter *et al.*, Genome-wide association study of cocaine dependence and related traits: FAM53B identified as a risk gene. *Mol. Psychiatry* **19**, 717–723 (2014).
10 J. Cabana-Domínguez, A. Shivalikanjli, N. Fernàndez-Castillo, B. Cormand, Genome-wide association meta-analysis of cocaine dependence: Shared genetics with comorbid conditions. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **94**, 109667 (2019).
11 J. Sun, H. R. Kranzler, J. Gelernter, J. Bi, A genome-wide association study of cocaine use disorder accounting for phenotypic heterogeneity and gene–environment interaction. *J. Psychiatry Neurosci.* **45**, 34–44 (2020).
12 D. B. Hancock, C. A. Markunas, L. J. Bierut, E. O. Johnson, Human genetics of addiction: New insights and future directions. *Curr. Psychiatry Rep.* **20**, 8 (2018).
13 T. V. Morozova *et al.*, Polymorphisms in early neurodevelopmental genes affect natural variation in alcohol sensitivity in adult *Drosophila*. *BMC Genomics* **16**, 865 (2015).
14 J. B. Becker, M. L. McClellan, B. G. Reed, Sex differences, gender and addiction. *J. Neurosci. Res.* **95**, 136–147 (2017).
15 S. B. Huggett *et al.*, Ibrutinib as a potential therapeutic for cocaine use disorder. *medRxiv* [Preprint] (2021). https://doi.org/10.1101/2021.02.05.21251228 (Accessed 11 June 2021).
16 B. M. Baker *et al.*, The *Drosophila* brain on cocaine at single-cell resolution. *Genome Res.*, 10.1101/gr.268037.120 (2021).