Addiction-like behavior in Drosophila

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Alcohol abuse is a pervasive problem known to be influenced by genetic factors, yet our understanding of the mechanisms underlying alcohol addiction is far from complete. Drosophila melanogaster has been established as a model for studying the molecular mechanisms that mediate the acute and chronic effects of alcohol. However, the Drosophila model has not yet been extended to include more complex alcohol-related behaviors such as self-administration. We recently established a paradigm to characterize ethanol consumption and preference in flies. We demonstrated that flies prefer to consume ethanol-containing food over regular food, and this preference exhibits several features of alcohol addiction: flies increase ethanol consumption over time, they consume ethanol to pharmacologically relevant concentrations, they will overcome an aversive stimulus in order to consume ethanol, and they exhibit relapse after a period of ethanol deprivation. Thus, ethanol preference in flies provides a new model for studying important aspects of addiction and their underlying mechanisms. One mutant that displayed decreased ethanol preference, krassavieti, may represent a first step toward uncovering those mechanisms.

Alcohol abuse is a widespread medical and societal problem, with an estimated prevalence of 8.5% in the US.1 Genetic factors contribute to one’s risk for alcoholism, but few specific genes have been identified.2 Although alcoholism is an exclusively human phenomenon (for example, the DSM-IV criteria for alcohol abuse refer to legal, social and interpersonal problems related to alcohol3), rodent models that represent specific aspects of alcohol addiction have provided valuable insight. The two-bottle choice paradigm, perhaps the most common ethanol assay used in rodents, measures an animal’s voluntary intake of ethanol solution relative to water.4 Other assays such as conditioned place preference and operant conditioning measure the rewarding and reinforcing properties of ethanol, respectively.4 Selective breeding,5 pharmacological manipulations,6 and reverse genetic approaches7 using rodents have made significant contributions to our understanding of the mechanisms underlying ethanol preference. However, rodents are not the ideal model organism for unbiased, forward genetic approaches to identify novel genes involved in ethanol preference due to the significant time and expense required for genetic screening.

In contrast, Drosophila melanogaster is one of the most genetically accessible model organisms. Behavioral responses to ethanol are conserved between flies and mammals: both exhibit locomotor stimulation at low doses and motor incoordination and sedation at high doses.9 Flies also exhibit tolerance with repeated ethanol exposures9,10 Importantly, several molecular pathways shown to mediate acute responses to ethanol in flies, such as the cAMP11, neuropeptide F (neuropeptide Y in mammals),12 and EGFR13 pathways, also regulate mammalian ethanol responses.13-15

In addition to displaying mammalian-like responses to acute ethanol exposure, flies exhibit preference for ethanol in a number of measures: (1) females prefer to lay eggs on ethanol-containing substrates,16 (2) larvae spend more time on ethanol-containing agar17 and (3) flies preferentially consume ethanol-containing food.18
These preference behaviors likely reflect the evolutionary history of the fruit fly. In its natural environment, *Drosophila melanogaster* frequently encounters significant levels of ethanol produced by fermenting plant materials. Flies have evolved mechanisms to process the ethanol they ingest, by efficiently degrading it for use as an energy source or a precursor for lipid biosynthesis.

Despite the significant progress in using flies to study the molecular mechanisms that mediate ethanol intoxication, little is known about the mechanisms underlying ethanol preference behaviors, in particular voluntary ethanol consumption. Ethanol consumption is a more complex behavior than ethanol intoxication or tolerance, as it is not simply a physiological response but requires an animal to make a choice. Furthermore, the genes that influence ethanol intoxication and ethanol consumption are likely to be overlapping, but not identical. We thus sought to establish a paradigm for ethanol consumption in flies that would model at least some of the key features of alcohol addiction.

In our study, we used a two-choice feeding assay similar to the two-bottle choice assay used in rodent studies of ethanol consumption. Liquid food was presented in capillary tubes, and flies chose between food containing either 0% or 15% ethanol. Flies exhibited a robust, dose-dependent preference for the ethanol-containing food. While this preference was initially highly variable, ethanol preference was established within 24 hours and increased over a 5-day period. Voluntary ethanol consumption led to pharmacologically relevant ethanol concentrations in flies.

We characterized the sensory inputs that influence ethanol preference, and showed that while flies are attracted to the smell of ethanol, they are averse to its taste. However, olfactory attraction alone could not explain the preference for consuming ethanol, nor could attraction to the calories present in ethanol. These results suggested that flies might be attracted to ethanol as a drug, in addition to its role as a food source. To support this notion, we demonstrated that flies exhibit addiction-like behaviors when consuming ethanol. First, flies were willing to overcome an aversive stimulus, quinine, in order to consume ethanol. Second, flies rapidly returned to high levels of ethanol consumption after a 1-3 day period of ethanol deprivation, modeling a relapse-like effect.

We speculate that ethanol preference in flies is likely to be influenced by a complex set of internal and external factors (Fig. 1). We have studied the roles of olfactory and gustatory inputs in influencing preference for ethanol and implicated the potential importance of its pharmacological effects. In addition to these factors, the increase in ethanol preference over time may reflect learning and memory processes that allow flies to reliably discriminate which capillaries contain the ethanol food. It is also possible that flies leave chemical cues such as aggregation pheromones to aid in this discrimination and reinforce their preference. The complexity of factors influencing ethanol preference may account for the initially high individual variation in this behavior.

Our study demonstrates that ethanol preference in *Drosophila* models characteristics of alcohol addiction. Like nearly all rodent models, this fly model does not encompass the entire spectrum of addiction behaviors. Our paradigm meets three of the six criteria classically proposed for an animal model of alcoholism (voluntary consumption, pharmacologically relevant ethanol levels, and consumption that is not dependent on caloric or sensory effects) as well as a more recently proposed criterion (relapse), but we have not yet demonstrated the other three criteria (tolerance, willingness to “work” for ethanol, and withdrawal symptoms when ethanol is removed). Future work may shed light on whether these phenomena are also associated with ethanol consumption in flies. Regardless, with this model in hand, the molecular and neural mechanisms underlying addiction-like behaviors in flies can now be investigated.

To begin to investigate the molecular pathways that influence ethanol preference, we identified one mutant, *kra* (also known as *exba/eIF5c*), that had decreased ethanol preference. *kra* also exhibited decreased ethanol sensitivity and strong defects in ethanol tolerance, suggesting that ethanol sensitivity and/or tolerance may influence ethanol preference. *Kra* is an evolutionarily conserved predicted translation initiation factor that inhibits translation in vitro. *Kra* has been shown to interact with Short stop (Shot), a cytoskeletal crosslinking protein, to regulate filopodia formation and midline axon guidance. Both protein synthesis and cytoskeletal dynamics play important roles in cellular plasticity underlying drug addiction, and abused drugs have been reported to alter the expression of axon guidance molecules in the adult rodent brain, making *kra* a tempting focus of study.

In addition to studying *kra*, unbiased genetic screens can identify novel genes.
that influence ethanol preference, which can then be tested in mammalian models. It will also be interesting to identify the neural circuitry underlying ethanol preference in flies and to determine whether it overlaps with the circuits known to mediate feeding (such as the hugin neurons\textsuperscript{28}) or reward (such as the dopamine\textsuperscript{29} and octopamine systems\textsuperscript{30}). The availability of tools in Drosophila for manipulating both genes and neural circuits opens the door for studying the genetic and neural pathways involved in drug reward.

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