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Common genes regulate food and ethanol intake in *Drosophila*

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ABSTRACT

The abuse liability of alcohol (ethanol) is believed to result in part from its actions on neurobiological substrates that underlie the motivation toward food and other natural reinforcers, and a growing body of evidence indicates that these substrates are broadly conserved among animal phyla. Understanding the extent to which the substrates regulating ethanol and food intake overlap is an important step toward developing therapeutics that selectively reduce ethanol intake. In the current experiments, we measured food and ethanol intake in Recombinant Inbred (RI) lines of *Drosophila melanogaster* using several assays, and then calculated genetic correlations to estimate the degree to which common genes might underlie behavior in these assays. We found that food intake and ethanol intake as measured in the capillary assay are genetically correlated traits in *D. melanogaster*, as well as in a panel of 11 *Drosophila* species that we tested subsequently. RI line differences in food intake in a dyed food assay were genetically unrelated to ethanol intake in the capillary assay or to ethanol preference measured using an olfactory trap apparatus. Using publicly available gene expression data, we found that expression profiles across the RI lines of a number of genes (including the D2-like dopamine receptor, DOPA decarboxylase, and fruitless) correlated with the RI line differences in food and ethanol intake we measured, while the expression profiles of other genes, including NPF, and the NPF and 5-HT2 receptors, correlated only with ethanol intake or preference. Our results suggest that food and ethanol intake are regulated by some common genes in *Drosophila*, but that other genes regulate ethanol intake independently of food intake. These results have implications toward the development of therapeutics that preferentially reduce ethanol intake.

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Background

Drugs of abuse act on neural circuits that evolved to motivate animals toward food and other natural reinforcers (see Kelley, Schiltz, & Landry, 2005; Robinson, Fischer, Ahuja, Lesser, & Maniates, 2015 for reviews). In rodents, the roles of the mesocorticolimbic dopamine system and the hypothalamus in regulating the intake of natural reinforcers and drugs of abuse, including alcohol (ethanol), have been extensively studied. Although there are differences between the ways that this circuitry regulates the intake of food, ethanol, and other drugs (Lajtha & Sershen, 2010; Nestler, 2005), there are also substantial similarities. For example, pharmacological blockade of a number of hypothalamic hormones, including NPY, galanin, orexin, and others can decrease both ethanol and food intake, as can site-specific administrations of dopaminergic antagonists (Barson, Morganstern, & Leibowitz, 2011, 2012; Leibowitz, 2007; Morganstern, Barson, & Leibowitz, 2011; Tabakoff & Hoffman, 2013). Similarly, the opioid receptor antagonist naloxone, which is widely used to treat alcohol dependence, can reduce both food and ethanol intake (see Berner, Bocarsly, Hoebel, & Avena, 2011 for review). A better understanding of how this circuitry differentially affects food and ethanol intake might lead to the development of therapeutics that preferentially decrease ethanol intake while leaving the motivation toward natural reinforcers intact.

Animal models used to study the relationship between the consumption of food and drugs of abuse are not limited to rodents. Ethanol is a common feature in the natural environment of *Drosophila melanogaster*, acting as an attractive cue for egg laying (David et al., 2004; McKenzie & McKechnie, 1979; Richmond & Gerking, 1979). These flies show a repertoire of ethanol-related behaviors that is remarkably similar to those seen in rodents, including locomotor stimulation at low doses, sedation at high doses, tolerance, and preferential ethanol intake, among others (Devineni & Heberlein, 2013; Kaun, Devineni, & Heberlein, 2012; Scholz & Mustard, 2013). Major neurochemical pathways and intracellular signaling cascades are conserved in these flies, and they function similarly to regulate ethanol-related behaviors (David et al., 2004; Kaun et al., 2012; Rodan & Rothenfluh, 2010; Scholz,

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2009). There is evidence that flies experience ethanol intoxication as rewarding (Kaun, Azanchi, Maung, Hirsh, & Heberlein, 2011) and that the ethanol preference they show is mediated by the pharmacological, rather than caloric, effects of ethanol (Devineni & Heberlein, 2009; Xu et al., 2012; but see Pohl et al., 2012 and Discussion section). Taken together, these results suggest that studies in flies can be used to elucidate conserved mechanisms that regulate ethanol-related behaviors.

The main goal of the current experiments was to determine the extent to which common genes regulate food and ethanol intake in *Drosophila*, and to identify genes that might regulate ethanol intake independently of food intake. An ancillary goal was to determine whether different behavioral indices of food and ethanol intake or preference are genetically correlated traits. We measured food and ethanol intake in Recombinant Inbred (RI) lines of *D. melanogaster* and in a panel of diverse *Drosophila* species using a capillary feeding assay. In separate experiments with the RI lines, we also assessed food by quantifying the amount of dyed food ingested and quantified ethanol preference using the olfactory trap assay, then compared RI line means from all behavioral assays to identify genetic correlations among the feeding and ethanol intake or preference assays. Finally, using our data from the RI lines, we queried a publicly available gene expression database to identify genes whose variation in expression across the RI lines was uniquely correlated with RI line differences in ethanol intake or preference, as well as genes that correlated with RI line differences in food and ethanol intake.

Materials and methods

Subjects

RI lines

A subset of RI lines from the Drosophila Genetic Reference Panel (DGRP; Mackay et al., 2012) was used in the current experiments. These lines were chosen for the current experiments because of the availability of whole genome expression data and because they represent a common allelic pool recently derived from wild-caught *D. melanogaster*. The RI lines were maintained in standard vials containing a potato/yeast medium consisting of 13.8% (w/w) potato flakes, 2.7% yeast, 1.7% glucose, 1.1% propionic acid, 0.1% methylparaben, and 80.6% water for at least two generations prior to the start of the experiments. All flies were housed at 25 °C on a 12/12-h light/dark cycle (lights on at 7:00 AM). Adult male flies (2–5-days-old) were collected using CO_2 anesthesia in all experiments. The sample sizes and numbers of lines tested for each assay varied, and are indicated in the description of each behavioral assay. Genetic correlations among the assays were estimated using data from 19 RI lines that were common to all assays. Gene-behavior correlations for each assay were estimated using the data from all lines tested in that assay.

Drosophila species

Drosophila erecta, *Drosophila hydei*, *Drosophila novamexicana*, *Drosophila persimilis*, *Drosophila pseudoobscura*, *Drosophila sechelia*, *Drosophila simulans*, *Drosophila virilis*, *Drosophila willistoni*, and *Drosophila yakuba*, were obtained from the University of California, San Diego Drosophila Species Center and maintained at Drake University for at least three generations prior to beginning these experiments. *D. melanogaster* (Canton S strain) was originally from the laboratory of Ulrike Heberlein (UCSF) and has been maintained at Drake University for 4 years. All species were maintained under the same experimental conditions used for the RI lines.

Behavioral assays

Blue food assay

A modification of the dyed-food intake procedure described by Deshpande et al. (2014) was used to quantify short-term food intake in food-deprived flies. Thirty to fifty adult male flies from each of 25 RI lines were collected into vials containing a filter paper soaked in water and were then food-deprived for 12–18 h. The flies were then flipped into vials containing standard food mixed with a blue dye, 0.1% (w/w) erioglaucine (Acros Organics, Morris Plains, NJ) for 30 min, then collected into microcentrifuge tubes and frozen at −20 °C prior to processing. The amount of dyed food ingested by flies increases linearly up to about 30 min, and is proportional to the number of proboscis extensions observed (Wong, Piper, Wertheim, & Partridge, 2009). The flies were weighed, homogenized in 1 mL of water using a polytron, and centrifuged for 15 min at 14,000 RPM. The optical density of 300 μL of supernatant was measured at 630 nm, the peak absorbance for erioglaucine, on a 96-well plate reader. Food intake is expressed as the optical absorbance divided by the total weight and fly count of each sample, normalized to the mean of each pass of the experiment. Final sample sizes for each of the 25 RI lines were 6–11 vials/line.

Olfactory trap assay

The olfactory trap assay (Woodard, Huang, Sun, Helfand, & Carlson, 1989) was used to measure initial ethanol preference. Following anesthesia with CO_2, 30–50 male fruit flies from each of 26 RI lines were placed in a Petri dish containing two traps manufactured from microcentrifuge tubes and 200-μL pipette tips. One trap was loaded with 20 μL of 20% (v/v) ethanol (Pharmco-Aaper, Shelbyville, KY) dispensed onto a piece of filter paper, while the second was loaded with 20 μL of water. The traps were placed into a 25 °C incubator for 48 h, during which time the flies entered either the water or the ethanol trap. Ethanol preference was calculated as the difference between the number of flies in the ethanol and water traps divided by the total number of flies in the assay. Final sample sizes for each of the 26 RI lines tested were 6–12. Results from the olfactory trap are referred to as 'ethanol preference' throughout the manuscript.

Capillary food and ethanol intake

The capillary feeding assay was conducted similarly to the procedure described by Xu et al. (2012). The capillary feeding apparatus consists of a standard vial into which two 200-μL pipette tips, cut at the small end to accommodate 5-μL glass capillary tubes (#2-000-001, Drummond Scientific Company, Broomall, PA) are inserted through the plug. One capillary tube was filled with a filtered liquid food solution (5% w/v yeast, 5% w/v sucrose) and the other was filled with the same liquid food solution to which 10% ethanol (v/v; Pharmco-Aaper, Brookfield CT) was added. This dose was chosen for the current studies because it is moderately preferred by wild-type flies (Devineni & Heberlein, 2009; Pohl et al., 2012; Xu et al., 2012), reducing the chances that RI line differences in ethanol intake might be obscured by floor or ceiling effects. To minimize evaporation from the capillaries, a mineral oil overlay was added to each capillary tube, a water-saturated buzz plug was placed at the bottom of each tube, and the vials were maintained in a large opaque plastic bin containing water-saturated sponges. The initial and final volumes of the solution in each capillary were measured to the nearest 0.1 mm daily using a digital micrometer. The intakes of food alone and ethanol prepared in food were measured for 3 days, with fresh capillary tubes used daily. Evaporative loss was estimated by measuring volume changes in control vials containing no flies, and these control values were subtracted from the recorded intakes of the experimental vials. The average intakes of food and ethanol prepared in food are

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