Genetic differences in the behavioral organization of binge eating, conditioned food reward, and compulsive-like eating in C57BL/6J and DBA/2J strains

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ABSTRACT

Binge eating (BE) is a heritable symptom of eating disorders that carries significant health costs, including anxiety and depression, malnutrition, and sometimes obesity. The only FDA-approved drug for BE is an amphetamine-like stimulant; thus, the development of new therapeutics is critical for improving treatment outcome and minimizing side effects for patients. Genome-wide analysis of BE could lead to safer and more efficacious therapeutics. Here, we used our intermittent, limited access paradigm to examine strain differences in BE of palatable food, conditioned food reward, and compulsive-like eating between C57BL/6J (B6J) and DBA/2J (D2J) inbred mouse strains. We identified a robust strain difference in BE, with D2J showing escalation in consumption relative to B6J. Furthermore, D2J, but not B6J, showed a conditioned place preference for the food-paired side. D2J also showed compulsive-like eating in an anxiety-provoking environment, while simultaneously showing avoidance of the environment. Slope analysis of consumption in B6JxD2J F1-mice indicated different modes of inheritance for acute versus escalated food intake, suggesting different genetic factors underlying distinct components of BE. Heritability estimates of BE and compulsive-like eating were 56 and 73 percent, indicating that these traits are amenable to quantitative trait locus mapping. Finally, mice carrying one copy of a deletion in the first coding exon of *Hnrnph1*, a gene important for methamphetamine sensitivity, showed increased food consumption that was driven primarily by females. To summarize, we established a genetic model of BE and concomitant behaviors that will be useful in a systems genetics approach to understand BE.

INTRODUCTION

Eating disorders (**ED**) are the most lethal of all psychiatric disorders, with high mortality rates and lifetime prevalence rates ranging from 1-3% (Smink et al., 2012). ED and related behaviors, including binge eating (**BE**) are heritable; however, the genetic basis of ED and BE remain largely unknown (Yilmaz et al., 2015). BE can be observed in all three major EDs, including Binge Eating Disorder (**BED**), Bulimia Nervosa (**BN**), and Anorexia Nervosa (**AN**) (Casper et al., 1980; Wardle and Beinart, 1981). BE is defined by the compulsive/unrestrained consumption of an extraordinarily large amount of food (typically palatable food with a high caloric content) within a brief time period and is accompanied by a sense of loss of control as well as feelings of uncomfortable fullness, distress, disgust, anxiety, depression, guilt, and remorse (Wolfe et al., 2009). BE is associated with multiple health issues, including mood dysfunction, aberrant eating patterns and cycles of BE and food restriction, malnutrition, and obesity-related health issues (Razzoli et al., 2017). BE can be extinguished in humans and in preclinical models (Bosch et al., 2008; de Jong et al., 2013); however, there is a high rate of rapid and long-term relapse (Fairburn et al., 2000; Olmsted et al., 2015).

Potential pharmacotherapeutic treatment options for BE have had limited success. The only FDA-approved drug for the treatment of moderate-to-severe BED is Lisdexamfetamine dimesylate, an amphetamine prodrug with limited, long-term efficacy and a high risk of abuse potential and adverse cardiovascular events (Jasinski and Krishnan, 2009; Reas and Grilo, 2015). Accordingly, treating BE-presenting patients with amphetamines could be problematic, given the high degree of comorbidity and shared neurobiological mechanisms between BE and substance use disorders (Schreiber et al., 2013; Schulte et al., 2016). Clearly, more efficacious treatments with fewer side effects are needed.

Very few genome-wide forward genetic (linkage or GWAS) studies have been conducted for BE or BED in humans (Davis, 2015) or in preclinical models (Kirkpatrick et al., 2017). In rodents, BE in non-stressed, non-food-deprived animals is most readily achieved under a

specific set of conditions including 1) presentation of palatable food (e.g., sweetened or high fat), 2) limited access (e.g., 0-2 h), and 3) intermittent access (Corwin, 2006; Corwin and Babbs, 2012). We recently utilized these conditions to conduct a genome-wide quantitative trait locus (QTL) study of BE between closely related C57BL/6 substrains and mapped and validated cytoplasmic FMR1-interacting protein 2 (*Cyfip2*) as a major genetic factor underlying BE between these strains (Kirkpatrick et al., 2017). Owing to the drastically reduced genetic complexity of this cross, BE exhibited a simple Mendelian inheritance, yielding only one locus explaining the parental strain (genetic) variance in BE (Kirkpatrick et al., 2017). This highlights both a main advantage as well as a main disadvantage of utilizing reduced complexity crosses for forward genetic studies of complex traits. One the one hand, reduced genetic complexity can quickly lead to identification of the underlying genetic factors responsible for trait variation (Bryant, 2011; Kirkpatrick et al., 2017; Kumar et al., 2013). On the other hand, complex traits in a reduced complexity cross are expected to segregate phenotypic variance in a near-Mendelian fashion and thus, only one major locus/gene is likely to account for most of the genetic variation in a complex trait, (Kirkpatrick et al., 2017; Kumar et al., 2013). Ultimately, populations of increasing complexity must be employed to understand the genetic basis of complex traits such as BE.

C57BL/6J (B6J or B) and DBA/2J (D2J or D) are two of the most widely used inbred strains in forward genetic studies of complex traits, largely because they are the original parental strains of one of the largest and most widely used set of murine recombinant inbred (**RI**) strains for dissecting the genetic basis of neurobehavioral traits - the BXD RI panel (Philip et al., 2010). A heritable strain difference in BE and associated behaviors between B6J and D2J provides the foundation for future use of the BXD RI panel in a systems genetics approach to advance our knowledge regarding the preclinical genetic and neurobiological basis of BE and ultimately translate these findings to humans.

To facilitate the long-term goal of conducting genetic mapping studies in additional crosses and panels, in the present study, we assessed strain differences in BE of sweetened palatable food (**PF**) and concomitant behaviors between the BE-resistant B6J strain (Goldberg et al., 2017; Kirkpatrick et al., 2017) and the D2J strain which has previously been shown to exhibit either less or more consumption of sweetened solutions relative to the B6J strain, depending on the duration of access and the concentration of solution (Blizard et al., 1999; Dym et al., 2007; Dym et al., 2009; Lewis et al., 2005; Pinhas et al., 2012; Reed et al., 2004). Additionally, to better understand the modes of inheritance of the complex BE phenotype, we tested a group of B6JxD2J-F_1 mice in the same BE paradigm. Using the data obtained from these studies, we then estimated narrow-sense heritability of 15 BE-related traits. Finally, because of the comorbidity between BE and substance use disorders, we tested mice carrying a small deletion in the first coding exon of *Hnrnph1* that show reduced behavioral sensitivity to methamphetamine (Yazdani et al., 2015).

METHODS AND MATERIALS

Mice

Experiments were conducted in accordance with the NIH Guidelines for the Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Boston University. Seven-week-old C57BL/6J (B6J), DBA/2J (D2J) and B6JxD2J-F_1 mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and habituated to the vivarium one week before testing in a separate room.

Hnrnph1$^{+/-}$ (H1$^{+/-}$) mice were generated on an isogenic B6J background (Yazdani et al., 2015). Briefly, TALENs vectors coding for either the right or left arm of the TALE effector targeted the first coding exon of *Hnrnph1* (Cellectis Bioresearch, Paris, France). These vectors were injected into B6J embryos (The Jackson Laboratory, Bar Harbor, ME, USA) at the BUMC

Transgenic Core facility (http://www.bumc.bu.edu/transgenic/). Founder $H1^{+/-}$ mice were then crossed with wild-type (WT) B6J mice to generate a colony of $H1^{+/-}$ offspring and WT littermates.

Mice were maintained on a 12 h/12 h light/dark cycle (lights on at 0630 h) and housed two to four animals per cage in same-sex cages. Laboratory chow (Teklad 18% Protein Diet, Envigo, Indianapolis, IN, USA) and water were available *ad libitum* in home cages. Testing was conducted during the light phase.

BE, CPP, and the light/dark test of compulsive-like eating

Twenty-four hours after 30-min side preference assessment on day 1 (D1), mice were restricted to the right side of the apparatus (pointed floor texture) and given access to 40 sweetened PF pellets (5TUL purified rodent tablets, Test Diet, MO, USA) or 40 unsweetened Chow pellets (5BR3 Test Diet, MO, USA) of a similar size and weight for 30 min on D2, D4, D9, D11, D16, and D18. In contrast, mice were restricted to the left side (smooth-textured floor) on D3, D5, D10, D12, D17, and D19 with an empty bowl for 30 min. On D22, mice were assessed for final side preference.

On D23, a light/dark test was employed (Kirkpatrick et al., 2017) to assess compulsive-like eating and concomitant behaviors. Briefly, light/dark boxes had two chambers (20 cm x 40 cm) connected by a small entryway. The dark, normally preferred chamber had black, opaque sides and bordered the clear, light-exposed, aversive side. A white porcelain bowl containing PF was secured to the middle of the light side floor. Because rodents are naturally light averse, compulsive-like eating was operationalized by the consumption of PF in the light side. Additional non-ingestive compulsive behaviors were recorded using AnyMaze video tracking software (Stoelting, Wood Dale, IL, USA). Only a subset of data from the Chow-trained D2J group on D23 were available for analysis (n=12/23 total).

Behavioral analysis of BE in B6J, D2J, B6JxD2J-F_1, and *Hnrnph1*$^{+/-}$ mice

B6J and D2J mice were tested in a 2 x 2 factorial design to examine PF or chow consumption as a function of Genotype. $H1^{+/-}$ mice were tested with WT littermates to assess

the effect of *Hnrnph1* on PF consumption. Data analysis was performed in R (https://www.r-project.org/). Consumption (normalized to body weight), CPP (time spent on the food side), compulsive-like eating (normalized PF consumption on D23), and behavior (percent time spent in light, number of entries to light, number of freezing episodes in light, and mean visit time) were analyzed using mixed-model ANOVA (Genotype, Treatment, and Sex as independent variables; Day as a repeated measure). Data analyses for $H1^{+/-}$ mice were conducted similarly but without Treatment since all mice were trained with PF. Post hoc one- or two-way ANOVA and Welch's unequal variances *t* tests ($p < 0.05$, Bonferroni-corrected) were also conducted. Slope analysis of normalized food consumption across the food training days was conducted to quantify escalation (Babbs et al., 2013; Kirkpatrick et al., 2017). Due to a video recording failure caused by insufficient computer storage space, non-ingestive behaviors on D23 were not obtained in 8 of the 16 mice of the PF-trained F_1 mice. Therefore, data from only 8 F_1 PF-trained mice are reported for the non-ingestive D23 phenotypes.

Heritability estimates of BE and concomitant phenotypes

Narrow-sense heritability (h^2) was estimated using the between-strain variance (genetic; V_G) and the average within-strain variance (environmental; V_E) of the parental strains for each phenotype and then calculating the percentage of the total variance (V_G+V_E) accounted for by V_G (i.e., heritability) according to the following formula: $h^2 = V_G/(V_G+V_E) *100$ (Falconer and Mackay, 1996; Hegmann and Possidente, 1981).

Correlation matrices and exploratory factor analysis

To examine the relationship among the major behavioral phenotypes, correlation matrices of variables collected in PF-trained B6J and D2J mice were generated in R using the "rcorr" function from the package "Hmisc." The function employed Spearman's rank correlations to compare 15 behavioral variables (see Tables 1 and S1) in a pairwise fashion. To reduce the data and summarize the relationships among behaviors more efficiently, these variables were then used in an exploratory factor analysis (Kirkpatrick and Bryant, 2014). This analysis was

conducted using the R package “psych” using the “factanal” function. We used the “Varimax” function for orthogonal rotation of the matrix, yielding a simple structure that most efficiently loads each variable onto as few factors as possible. Factors with eigenvalues greater than one were included in the analysis, and variables with loadings of 0.5 or greater were reported. Chi square tests were used to determine the ideal number of factors to explain the variance.

RESULTS

BE and CPP in B6J and D2J strains and in B6J x D2J-F_1 mice

In examining strain differences in BE behaviors in a CPP paradigm (**Fig.1A**), we found that D2J mice trained with PF (*n* = 24; 14 females/10 males) displayed significantly more food intake than D2J Chow (*n* = 23; 9 females/14 males), B6J PF (*n* = 24; 12 females/12 males), and B6J Chow groups (*n* = 24; 12 females/12 males; **Fig.1B**). Both D2J and B6J trained with PF consumed more food than either Chow group on all food days except day (D) 9 for B6J (**Fig.1B**). Importantly, D2J and B6J Chow groups did not exhibit differences in normalized food intake on any food day in the study (**Fig.1B**). D2J females exhibited an overall higher summed normalized food intake than males, regardless of the food type (**Fig.1C**). Examination of the slopes of escalation in normalized food intake over the course of the study revealed that the B6J PF, D2J PF, and D2J chow groups all escalated their intake (**Fig.1D**). Notably, PF-trained D2J mice exhibited the most robust escalation in consumption by far (i.e. greater slope than any of the other three groups) (**Fig.1D**).

The relationship between consumption of a particular diet and changes in BW across Training Days was complex. The D2J Chow group displayed the highest percent change in body weight compared to all other groups (**Fig.1E**) yet displayed much less food consumption than the D2J PF group during BE training (**Fig.1C**). Notably, although PF-trained mice consumed more food during the BE training sessions, they gained less BW than those trained with Chow. This finding could result from decreased home cage chow consumption resulting in

decreased caloric intake, perhaps in anticipation of the more preferred PF, as has been previously reported (Berner et al., 2008; Corwin, 2006; Cottone et al., 2008). Finally, although females showed the greatest amount of BE, they actually gained much less weight than males (**Fig.1C,E**).

To examine genetic differences in food-seeking behavior, we compared the time spent on the food-paired side of the CPP box during open access on D22 with the initial time spent on D1. Corresponding with the increased BE observed in PF-trained D2J mice (**Fig.1B**), these mice also displayed an increase in time spent on the food-paired side of the CPP box on D22 versus D1 (**Fig.1F**). Thus, D2J mice show increased BE and increased conditioned reward for PF.

Following assessment of food seeking behavior, on D23 we assessed compulsive-like food seeking in the light/dark conflict test whereby all groups were provided access to PF located in the normally aversive light side of the light/dark box (**Fig.2A**). PF-trained D2J mice exhibited greater compulsive-like eating than all other groups (**Fig.2B**), consistent with an association between BE and compulsive-like eating. Despite the increase in compulsive eating, D2J mice actually spent *less* time in the light side of the light/dark box (**Fig.2C**), and had *fewer* entries into the light side (**Fig.2D**), as well as an increase in the number of freezing episodes (**Fig.2E**), and a shorter mean visit time in the light side (**Fig.2F**) than B6J mice. This behavioral repertoire was in stark contrast to a recent genetic model of BE that we reported, where we found a positive, rather than negative, relationship between compulsive eating and concomitant compulsive behaviors in the BE-prone C57BL/6NJ strain (Kirkpatrick et al., 2017). Thus, despite the motivation to venture into the light side and obtain the PF, the D2J PF group presumably retreated to the dark side to consume the PF. Furthermore, because D2J mice made so few visits, they must have retrieved large amounts of food per visit. No differences were found between treatment groups within each strain (**Fig.2C-F**), indicating that these avoidance behaviors are driven by stable, pre-existing genetic differences between strains. Of note, the

DBA/2 strain is known to exhibit a high level of anxiety-like behavior in multiple assays, including light/dark, zero maze, and open field (Milner and Crabbe, 2008) and QTLs have been identified for anxiety-related traits in B6JxD2J F2 and BXD RI strains (Philip et al., 2010; Sokoloff et al., 2011) indicating that strain differences in anxiety-like traits are heritable and controlled by genetic factors.

In order to better understand mode of inheritance of strain differences in BE, compulsive eating, and associated behaviors, we next tested a group of B6 x D2-F_1 hybrid mice trained with either PF (*n* = 16; 8 females/8 males) or Chow (*n* = 15; 7 females/8 males). As observed in D2J mice, PF-trained F_1 mice consumed more food over the course of the study than Chow-trained F_1s (**Fig.3A**), in particular on D2, 11, 16, and 18. Moreover, the PF group consumed more total food than the Chow group (**Fig.3B**) and showed a greater escalation in intake (**Fig.3C**). In addition, we observed a Sex-by-Treatment interaction (**Fig.3D**) whereby female but not male F_1 mice trained with PF showed significantly less increase in BW compared to their female Chow-trained counterparts. Despite differences in consumption between PF and Chow, there was no effect of Treatment on change in time spent on the food-paired side (**Fig.3E**).

Interestingly, when we compared the behavior of the F_1 PF group with the B6J and D2J groups, we found a similar initial intake to B6J mice on the first food day (**Fig.S1A**) that was significantly less than D2J. However, the F_1 mice showed a slope of escalation in PF intake over time that was similar to D2J and greater than B6J, (**Fig.S1B**). This result was ultimately driven by a level of consumption that eventually became intermediate of B6J and D2J on the final training day (D18; **Fig.S1A**). Nevertheless, F_1 mice did not show any difference in summed PF consumption relative to B6J (**Fig.S1C**). To summarize, The B6J allele was dominant with regard to initial PF consumption, the D2J allele was dominant with regard to escalation in PF consumption, and the mode of inheritance was additive with regard to D18 consumption. These observations provide evidence that different genetic factors could mediate various aspects of BE.

In examining compulsive-like eating in F_1 mice in the light/dark apparatus, PF-trained F_1 mice ate more PF than Chow-trained F_1 mice (**Fig.4A**) and this level was similar to B6J mice (**Fig.2B**). PF-trained mice also showed a greater number of freezing episodes (**Fig.4D**). However, there was no difference in the percentage of time spent in the light (**Fig.4B**), the number of entries (**Fig.4C**), or the average time per visit (**Fig.4E**).

Heritability estimates of BE and concomitant behaviors

We next sought to estimate heritability (h^2) of 15 BE- and CPP-related variables collected from PF-trained mice in these two studies (**Table 1**). Generally, the most heritable phenotypes were related to PF intake. Specifically, normalized PF intake on D2, D18, and on D23 in the light/dark apparatus exhibited heritability estimates of 34%, 51%, and 73%, respectively. Conversely, non-ingestive behaviors in the light/dark apparatus were the least heritable, with time in the light, number of entries into the light, and distance in the light exhibiting heritability estimates of 11%, 0.8%, and 10%, respectively. Intermediately heritable phenotypes included those related to conditioned locomotor behaviors in the PF-paired side of the CPP apparatus, including D22 distance on the food side (28%), D22 entries into the food side (18%), and D22 entries into the food triangle (22%).

Factor analysis of BE and compulsive eating in B6J and D2J mice

To better understand relationships among the 15 BE- and CPP-related variables, we conducted exploratory factor analysis for both the B6J and D2J mice (**Table 1**). For B6J, three factors explained 55% of the total variance [χ^2 (88, N = 24) = 158.8; p = 5.7 x 10^{-6}]. The first factor, which explained 19% of the variance, was termed “Conditioned Place Preference”, as it encompassed four variables related to the CPP assessment day (D22 Entries to the Food Side, D22 Distance on the Food Side, D22 Entries to the Food Triangle, and D22 Distance in the Food Triangle). A second factor comprised PF intake on D2, D18, and on D23 in the light/dark test, and was termed “PF Seeking & Consumption.” The third factor encompassed the three compulsive behavior variables in the light/dark box and was termed “Compulsive Behavior.”

For D2J, three factors also explained a substantial amount of the variance [χ^2 (88, *N* = 24) = 155.2; p = 1.3 x 10^{-5}]. The first factor, termed "Binge Eating & Compulsive Behavior" accounted for 23% of the total variance. However, this factor was quite different from the first factor in B6J mice because in this case, both BE and compulsive eating behaviors loaded onto one factor, including D18 Intake, escalation slope, and the three non-ingestive compulsive-like behaviors in the light/dark box. The second factor contained D22 variables distance on the right side, time in the food triangle, and distance in the food triangle, and was named "Conditioned Place Preference" to reflect these behaviors. The third factor encompassed variables pertaining to the food side and the food triangle during PF training and CPP testing and was called "PF Seeking".

BE and compulsive-like behaviors in *Hnrnph1*$^{+/-}$ mice

We previously identified *Hnrnph1* (H1) as a quantitative trait gene for methamphetamine stimulant sensitivity that was originally identified in crosses and congenics comprising B6J and D2J alleles (Yazdani et al., 2015). Because of the comorbidity between ED comprising BE and substance use disorders (Schreiber et al., 2013), we sought to investigate possible pleiotropic effects of H1 on BE. Therefore, we tested H1$^{+/-}$ mice and WT littermates in our PF BE paradigm. A schematic of H1$^{+/-}$ is shown in **Fig. 5A**. There was greater PF intake in H1$^{+/-}$ mice compared to WT mice across multiple training days (**Fig.5B**). H1$^{+/-}$ females showed greater PF consumption than H1$^{+/-}$ males (**Fig.5B**), consistent with our recent studies indicating increased BE in females (Kirkpatrick et al., 2017; Goldberg et al., 2017). For summed PF intake, H1$^{+/-}$ mice consumed more PF than WT mice, with H1$^{+/-}$ females consuming more PF overall than males (**Fig.5C**). Interestingly, none of the groups showed escalated consumption; however, H1$^{+/-}$ females displayed a greater y-intercept, indicating consistently greater intake (**Fig.5D**). In contrast to the effects on PF consumption, there were no effects of Genotype or Sex on change in BW (**Fig.5E**) or change in time spent on the food-paired side (**Fig.5F**), indicating no difference in PF-CPP. In

examining compulsive behavior in the light/dark apparatus, there were no effects of Genotype or Sex on any of these measures (**Fig.S3)**.

DISCUSSION

We identified robust strain differences in BE, with D2J but not B6J exhibiting a dramatic escalation in PF consumption that was accompanied by CPP for the PF-paired environment (**Fig.1**) as well as compulsive-like eating (**Fig.2**). Consistent with our previous studies examining BE in B6J mice (Kirkpatrick et al., 2017) or WT littermates on a B6J background (Goldberg et al., 2017), B6J mice showed little evidence of BE in our intermittent, limited access paradigm and correspondingly, showed no difference in food reward or compulsive-like eating (**Fig.1,2**). Interestingly, we found evidence for different modes of inheritance in B6J x D2J-F_1 mice. The lack of increased consumption in F_1 mice is similar to the parental B6J phenotype, indicating dominant inheritance with respect to the B6J allele (**Fig.3; S1**). On the other hand, F_1 mice showed a slope in escalation of PF consumption that was similar to D2J (**Fig.S1**), indicating a dominant mode of inheritance with respect to the D2J allele. For the final Training Day (D18), F_1 mice showed an intermediate phenotype (**Fig.S1A**), indicating an additive mode of inheritance. Finally, compulsive-like intake in F_1 mice appeared to be semi-dominant with respect to the B6J allele, with F_1 mice consuming only slightly more PF than B6J mice, but significantly more than their Chow-trained counterparts. The combined observations suggest a complex genetic architecture of BE between B6J and D2J, with multiple alleles contributing to different behavioral components of this phenotype.

Exploratory factor analysis of BE behaviors identified distinct factors for BE-prone D2J versus the BE-resistant B6J mice. Importantly, for D2J, phenotypes directly relevant to BE (slope of escalation in consumption, D18 consumption) loaded onto the same factor as compulsive/anxiety-like behaviors (time spent, entries, and distance traveled in the light side) – a factor that we termed “Binge Eating and Compulsive Behavior”. In contrast, with B6J,

consummatory behaviors versus light/dark behaviors loaded onto two separate factors (Table 1). One factor in B6J mice that we termed “PF Seeking & Consumption” encompassed initial (D2) PF consumption, D18 PF consumption, and compulsive-like consumption on D23 (**Table 1**). The other B6J factor that we termed “Compulsive Behavior” contained the compulsive/anxiety-like behaviors in the light/dark box. Together, these findings suggest that in D2J mice, compulsive-/anxiety-like behavior could represent a pre-existing, stable affective trait that drives propensity to BE, whereas in B6J mice, initial intake is more predictive of future intake and that this reduced intake remains far more stable than the intake exhibited by D2J.

Strain differences in BE could not solely be explained by initial differences in body weight as there was no significant difference in body weight on the first day of the training session (**Fig.1E**), yet there was already a marked increase in PF consumption in D2J mice (**Fig.1B**). Furthermore, the subsequent increase in PF consumption was accompanied by *reduced* weight gain relative to Chow-trained mice (**Fig.S2**), which suggests that if anything, BE was associated with the consumption of fewer overall calories. The observation that BE is associated with *less* percent change in BW is further supported when the same data are broken down by Sex where, e.g., D2J females showed more than a three-fold decrease in the % increase in BW relative to D2J males (**Fig.1C**) yet they showed more than a 1.5-fold increase in %BW consumed relative to D2J males (**Fig.1C**). Specifically, female D2J mice showed greater BE as expected based on our previous findings (Kirkpatrick et al., 2017; Goldberg et al., 2017) yet they showed more than a three-fold lower percent increase in BW relative to male mice (**Fig.1E**). For F_1 mice, the relationship between consumption and body weight was less clear as body weight was unaffected by Treatment or Sex.

It is interesting to note that despite D2J exhibiting compulsive-like consumption of PF relative to the control D2J Chow counterparts and relative to B6J mice (**Fig.2B**) they nevertheless continued to exhibit marked avoidance/aversion for the light side of the light/dark box, showing less time, fewer entries, and a shorter average visit time that was similar to Chow-

trained D2J mice (**Fig.2**). This is in stark contrast to the BE-prone B6NJ strain (closely related substrain of B6J) that exhibited compulsive-like eating that was associated with a decreased avoidance behavior (increased time in the light side), an increase in visit time in the light side, and a concomitant fewer number of entries into the light side (Kirkpatrick et al., 2017). Clearly, the propensity to consume PF does not compete with innate avoidance behavior in D2J mice (**Fig.2C-F**) and thus, D2J mice manage to avoid the light side while at the same time, presumably transport large amounts of PF to the safer environment where they can consume it. This behavior is in some ways reminiscent of hoarding behavior exhibited by many patients with BE (Novara et al., 2016; Raines et al., 2015), including hoarding of food (Hagan et al., 2002) with more severe hoarding behavior being associated with the presence of ED, in particular with females (Wheaton et al., 2008). Hoarding may represent a behavioral strategy in addition to BE that could serve to alleviate negative emotions (Raines et al., 2015). In the case of humans, hoarding of food for BE consumption is an adaptive behavior that serves to avoid the aversive social stigma associated with BE (Smith and Robbins, 2013). In D2J mice, this hoarding-like behavior is also adaptive since it permits efficient intake of the PF without any sacrifice in avoidance behavior of the aversive light environment.

We are not aware of any other preclinical, forward genetic studies of BE besides our recent study (Kirkpatrick et al., 2017). However, there is an extensive literature on forward genetic studies of PF consumption (sucrose, fat, etc.) in rodents and other animal species. Because we utilized the 5-TUL diet in which sucrose is the primary palatable ingredient, a brief discussion is warranted on the literature involving differences in sucrose consumption between B6J and D2J (Blizard et al., 1999; Dym et al., 2007; Dym et al., 2009; Glendinning et al., 2005; Lewis et al., 2005; Pinhas et al., 2012; Reed et al., 2004). An important observation is that the length of access to sucrose can determine the directionality of strain difference in sucrose consumption between B6J and D2J. Short-term access (2h or less) induced greater consumption in D2J (Dym et al., 2007) and longer-term access (6h or greater) induced greater

consumption in B6J (Blizard et al., 1999; Glendinning et al., 2005; Kotlus and Blizard, 1998). For the long-term access (B6J > D2J; the opposite direction of our results), a QTL on distal chromosome 4 was identified (Blizard et al,. 1999) that has been hypothesized to be mediated by one or more polymorphisms in the *Tas13R* gene (Glendinning et al., 2005; Montmayeur et al., 2001; Reed et al., 2004). However, because there is no genetic correlation between short- versus long-term consumption among inbred strains (Dym et al., 2007), this provides evidence that long-term and short-term consumption are mediated by separate genetic factors. We hypothesize that the strain difference in short-term sucrose consumption (D2J > B6J), which is not mediated by variation at the *Tas13R* locus, could contribute to at least one component of BE in our model, namely initial differences in PF consumption on D2 (D2J > B6J). Future mapping studies will determine whether distinct loci contribute to short-term sucrose and PF consumption and whether these loci are largely separable from other behavioral components comprising our BE model.

Because substance use disorders and BE are comorbid (Schreiber et al., 2013) and because we originally identified *Hnrnph1* (H1) as a quantitative trait gene underlying methamphetamine stimulant sensitivity using genetic crosses and fine mapping between B6J and D2J alleles (Yazdani et al., 2015), we tested $H1^{+/-}$ mice in our BE paradigm and found that $H1^{+/-}$ mice showed greater PF intake over time than WT counterparts. Additionally, females binged more than males, which is in line with our findings in D2J mice and our recent studies (Kirkpatrick et al., 2017; Goldberg et al., 2017). Specifically, female $H1^{+/-}$ mice consistently showed the greatest intake over the course of the study (**Fig.5**). Despite the effect of $H1^{+/-}$ on BE, we did not observe any effects of H1 genotype on compulsive-like eating or concomitant behaviors (**Fig.S3**), further supporting the idea that various components of the BE phenotype are genetically separable, with different genetic factors contributing to the acute PF response versus PF escalation versus the compulsive-like behaviors that support it.

To summarize, we identified a genetic model of BE that lays the groundwork for a future large-scale forward genetic study in mice. The clear advantages offered by the genetic tools comprising alleles from the B6J and D2J parental strains, including the BXD RI panel (Philip et al., 2010) interval-specific congenics for fine mapping (Iakoubova et al., 2001), and the wealth of phenotypic data on GeneNetwork (Chesler et al., 2005; Chesler et al., 2003) ensure that a systems genetic approach with these powerful tools will likely yield new insight into the genetic, genomic, and neurobiological mechanisms of not only risk for BE and comorbid traits but also the neurobiological adaptations underlying the establishment of and recovery from BE which will inform new treatment strategies.

ACKNOWLEDGEMENTS

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AUTHOR CONTRIBUTIONS

CDB conceptualized and designed the experiments. RKB, JCK, and JS collected data from the food experiments. NY designed and generated the $H1^{+/-}$ mice. RKB and JCK analyzed the data. RKB and CDB drafted the manuscript.

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Figure Legends.

Figure 1. Genetic differences between B6J and D2J strains in BE and PF-CPP. A) The conditioned place preference (CPP) chamber is illustrated as seen from above with the video recording camera. **B)** A mixed effects three-way ANOVA (Genotype, Treatment, Day) found that D2J mice trained with PF displayed significantly more food intake than all other groups (* denotes difference vs. all other groups; # denotes difference vs. B6J Chow). There was a main effect of Genotype [$F_{1,540}$ = 269.8, $p < 2 \times 10^{-16}$] and of Treatment [$F_{1,540}$ = 321.7, $p < 2 \times 10^{-16}$] on food intake. There was also a Genotype-by-Day interaction [$F_{1,540}$ = 8.1, $p = 2.2 \times 10^{-7}$], a Day-by-Treatment interaction [$F_{1,540}$ = 6.2, $p = 1.2 \times 10^{-5}$], and a three-way interaction between Genotype, Day, and Treatment [$F_{1,540}$ = 112.7, $p < 2 \times 10^{-16}$] on food intake. **C)** Females exhibited a higher summed normalized food intake than males overall [$F_{1,87}$ = 20.4, $p = 2.0 \times 10^{-5}$; * difference vs. male counterparts; # difference vs. all other groups]. **D)** There was an escalation of food intake over the course of the study in B6J PF, D2J PF, and D2J Chow groups (ps = 0.0003, 0.002, and 0.0002 vs. 0, respectively). Mice in the D2J PF group showed the most robust escalation in consumption as indicated by the greatest slope ($F_{3,16}$ = 33.3, p < 0.0001). **E)** The D2J Chow group gained more weight than all other groups (main effect of Genotype [$F_{1,87}$ = 53.3, $p = 1.29 \times 10^{-10}$], main effect of Treatment [$F_{1,87}$ = 8.3, p = 0.005], main effect of Sex [$F_{1,87}$ = 47.8, $p = 7.6 \times 10^{-10}$], interaction between Genotype and Sex [$F_{1,87}$ = 26.4, $p = 1.7 \times 10^{-6}$], interaction between Genotype, Treatment, and Sex [$F_{1,87}$ = 7.1, p = 0.009]). **F)** Only the D2J PF group displayed an increase in time spent on the food side (right side) of the CPP box on D22 versus D1 (interaction between Genotype and Day [$F_{1,166}$ = 7.5, p = 0.007]; * difference D22 vs. D1).

Figure 2. Genetic differences between B6J and D2J strains in compulsive-like eating and concomitant behaviors in the light/dark conflict test. A) Illustration of the light/dark conflict apparatus. **B)** A two-way ANOVA (Genotype, Treatment) found that the D2J PF group showed

greater PF intake than all other groups (main effect of Genotype [$F_{1,43}$ = 25.3, p = 9.2 x 10^{-6}], main effect of Treatment [$F_{1,43}$ = 40.9, p = 9.8 x 10^{-8}], interaction between Genotype and Treatment [$F_{1,43}$ = 17.6, p = 0.0001; *difference vs. both Chow groups, # difference vs. B6J PF]. D2J mice also spent less time in the light side of the light/dark box (**C**; main effect of Genotype [$F_{1,43}$ = 35.6, p = 4.1 x 10^{-7}; * B6J > D2J]), showed fewer entries into the light side (**D**; main effect of Genotype [$F_{1,43}$ = 14.8, p = 0.0004; * B6J > D2J), a higher number of freezing episodes (**E**; main effect of Genotype [$F_{1,43}$ = 7.2, p = 0.01; * D2J > B6J], and a shorter mean visit time in the light side (**F**; main effect of Genotype [$F_{1,43}$ = 11.7, p = 0.001; *B6J > D2J] than B6J mice.

Figure 3. BE and PF-CPP in B6J x D2J-F_1 mice. A) A mixed effects three-way ANOVA (Treatment, Day, Sex) found a main effect of Treatment ($F_{1,178}$ = 43.5, p = 4.6 x 10^{-10}; * difference vs. Chow). PF-trained F_1 mice consumed more over the course of the study. We also observed an effect of Day ($F_{1,178}$ = 34.3, p = 2.2 x 10^{-8}) and of Sex ($F_{1,178}$ = 1.6 x 10^{-8}). PF-trained F_1 mice ate significantly more total food than Chow-trained F_1 mice (**B**, t_{29} = 2.9, p = 0.007; difference vs. Chow) and had greater escalation in intake over time (**C**, $F_{1,8}$ = 16.5, p = 0.004). The level of BE on D18 was intermediate between the parental strains (**Fig.S1**), thus providing evidence for an additive mode of inheritance. We also observed a Treatment x Sex interaction for body weight change, in that males gained weight (p =7.5 x 10^{-7}), while females lost weight (p = 0.005) (**D**, $F_{1,27}$ = 8.6, p = 0.007; * significant interaction). There was no difference in time spent on the food-paired side when preference was tested in the absence of food (**E**, all ANOVA ps > 0.1).

Figure 4. Compulsive-like eating and concomitant behaviors in the light/dark conflict test in B6J x D2J-F_1 mice. F_1 mice trained with PF ate significantly more food in the light/dark conflict test (**A**, t = 3.8, p = 0.0007; * difference vs. Chow). However, we did not detect any effect of Treatment on time spent on the light side (**B**), the number of entries into the light side

(**C**) or the average visit time to the light side (**E**, all ps > 0.15). In contrast, F_1 mice trained with PF exhibited a significantly greater number of freezing episodes on the light side (**D**, $t_{29} = 2.2$, p = 0.04; difference vs Chow).

Figure 5. BE and PF-CPP in *Hnrnph1*$^{+/-}$ mice. A) Illustration of the gene-edited chromosomal region in *Hnrnph1* haploinsufficient (H1$^{+/-}$) mice. This schematic is an abbreviated version of a previously published schematic (Yazdani et al., 2015) **B)** A mixed effects 3-way ANOVA (Sex, Genotype, Day) revealed greater PF intake over time in H1$^{+/-}$ mice compared to WT mice and in females compared to males. There was a main effect of Sex ($F_{1,258} = 35.7$, $p = 7.5 \times 10^{-9}$), Genotype ($F_{1,258} = 43.8$, $p = 2.1 \times 10^{-10}$, and an interaction ($F_{1,258} = 10.4$, p = 0.001). **C)** Similarly, there was an effect of both Sex ($F_{1,43} = 5.6$, p = 0.02; # difference vs. males) and Genotype ($F_{1,43} = 18.2$, p = 0.0001; difference vs. WT) for summed intake as well. **D)** When comparing escalation in BE over time, we found that none of the groups showed significantly non-zero slopes, indicating no escalation (all ps > 0.08). However, female H1$^{+/-}$ mice showed a greater y-intercept than any of the other three groups ($F_{3,19} = 29.0$, p < 0.0001), indicating enhanced acute PF consumption. There was no effect of Genotype or Sex on change in body weight **(E)** or for time spent on the food-paired side, although mice as a whole did learn to prefer this side (**F**, Main effect of Day: $F_{1,86} = 7.7$, p = 0.007).

Figure 1.

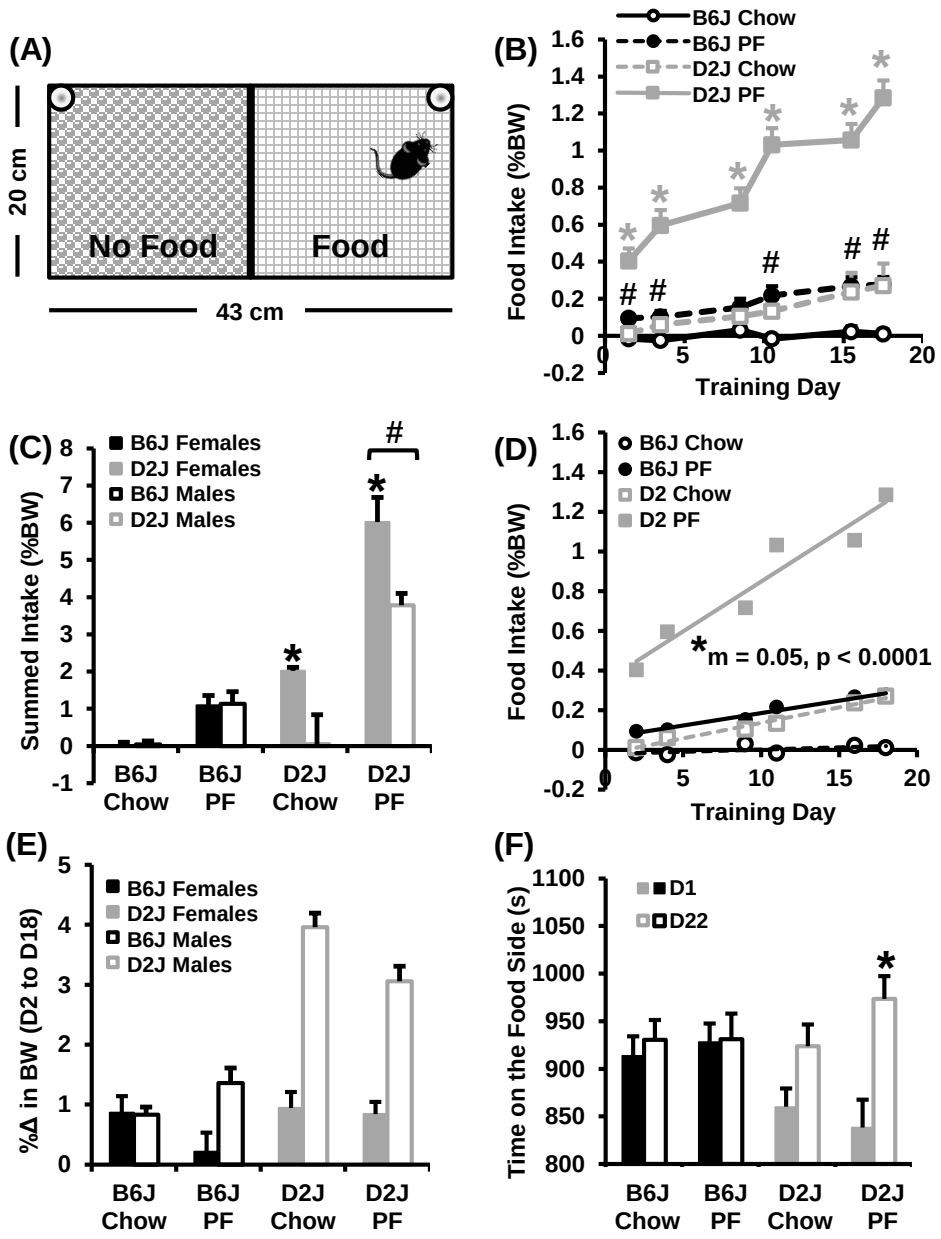
(A)
20 cm
No Food
Food
43 cm
(B)
B6J Chow
B6J PF
D2J Chow
D2J PF
Food Intake (%BW)
Training Day
(C)
B6J Females
D2J Females
B6J Males
D2J Males
Summed Intake (%BW)
B6J Chow
B6J PF
D2J Chow
D2J PF
(D)
B6J Chow
B6J PF
D2 Chow
D2 PF
Food Intake (%BW)
*m = 0.05, p < 0.0001
Training Day
(E)
B6J Females
D2J Females
B6J Males
D2J Males
%Δ in BW (D2 to D18)
B6J Chow
B6J PF
D2J Chow
D2J PF
(F)
D1
D22
Time on the Food Side (s)
B6J Chow
B6J PF
D2J Chow
D2J PF

Figure 2.

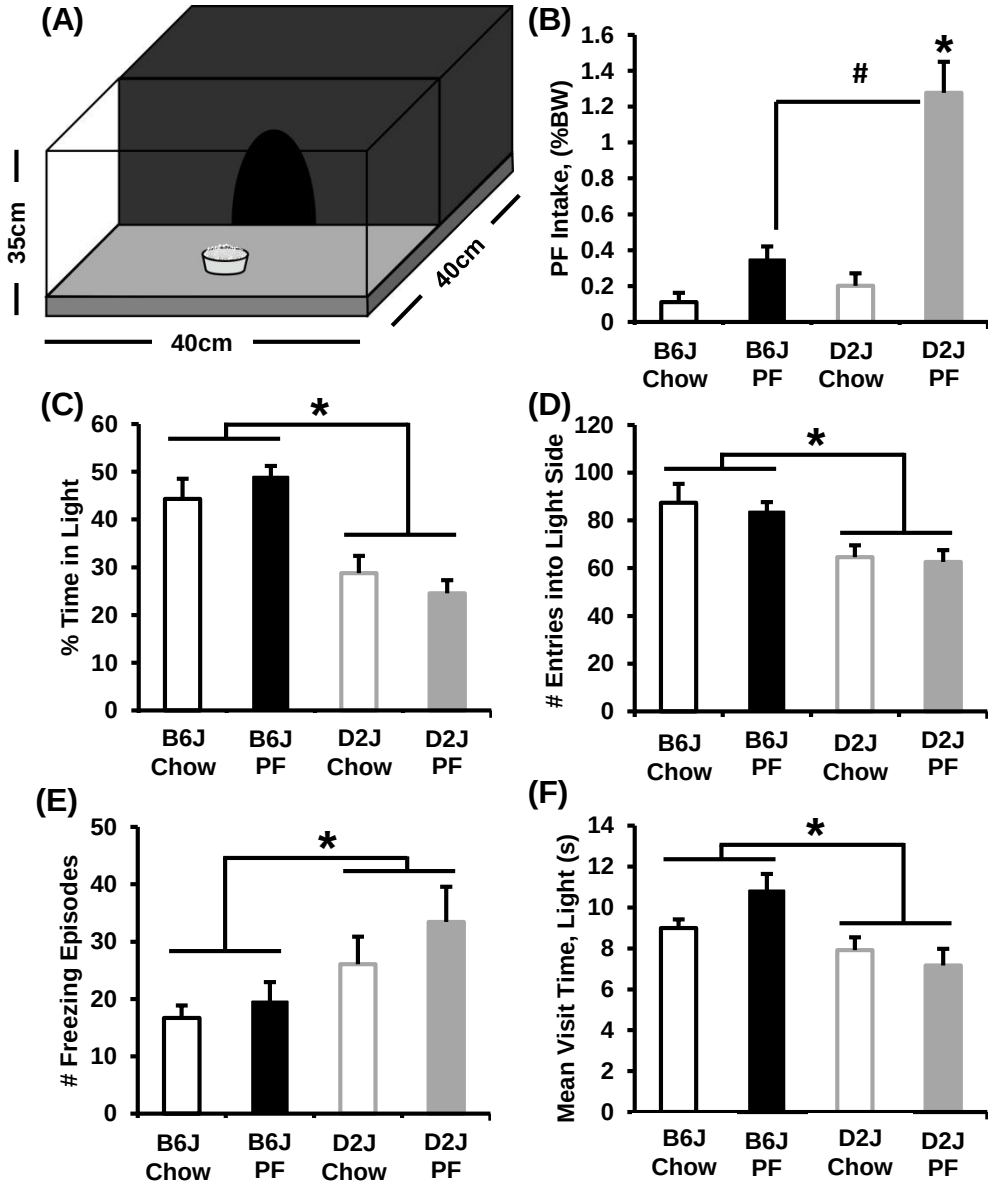
(A)
35cm
40cm
40cm
(B)
PF Intake, (%BW)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
#
*
B6J Chow
B6J PF
D2J Chow
D2J PF
(C)
% Time in Light
60
50
40
30
20
10
0
*
B6J Chow
B6J PF
D2J Chow
D2J PF
(D)
Entries into Light Side
120
100
80
60
40
20
0
*
B6J Chow
B6J PF
D2J Chow
D2J PF
(E)
Freezing Episodes
50
40
30
20
10
0
*
B6J Chow
B6J PF
D2J Chow
D2J PF
(F)
Mean Visit Time, Light (s)
14
12
10
8
6
4
2
0
*
B6J Chow
B6J PF
D2J Chow
D2J PF

Figure 3.

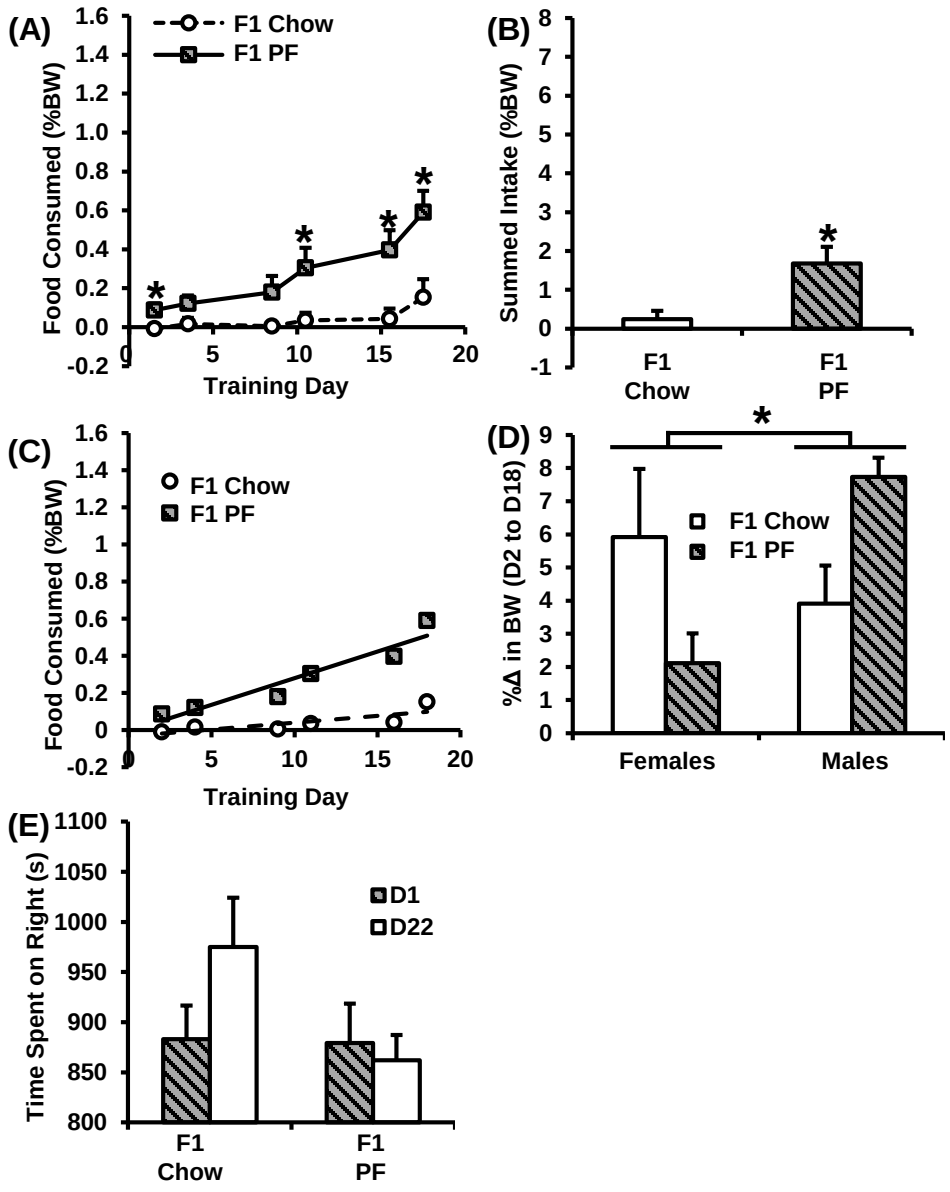
(A)
F1 Chow
F1 PF
Food Consumed (%BW)
Training Day
(B)
Summed Intake (%BW)
F1 Chow
F1 PF
(C)
F1 Chow
F1 PF
Food Consumed (%BW)
Training Day
(D)
%Δ in BW (D2 to D18)
F1 Chow
F1 PF
Females
Males
(E)
Time Spent on Right (s)
D1
D22
F1 Chow
F1 PF

Figure 4.

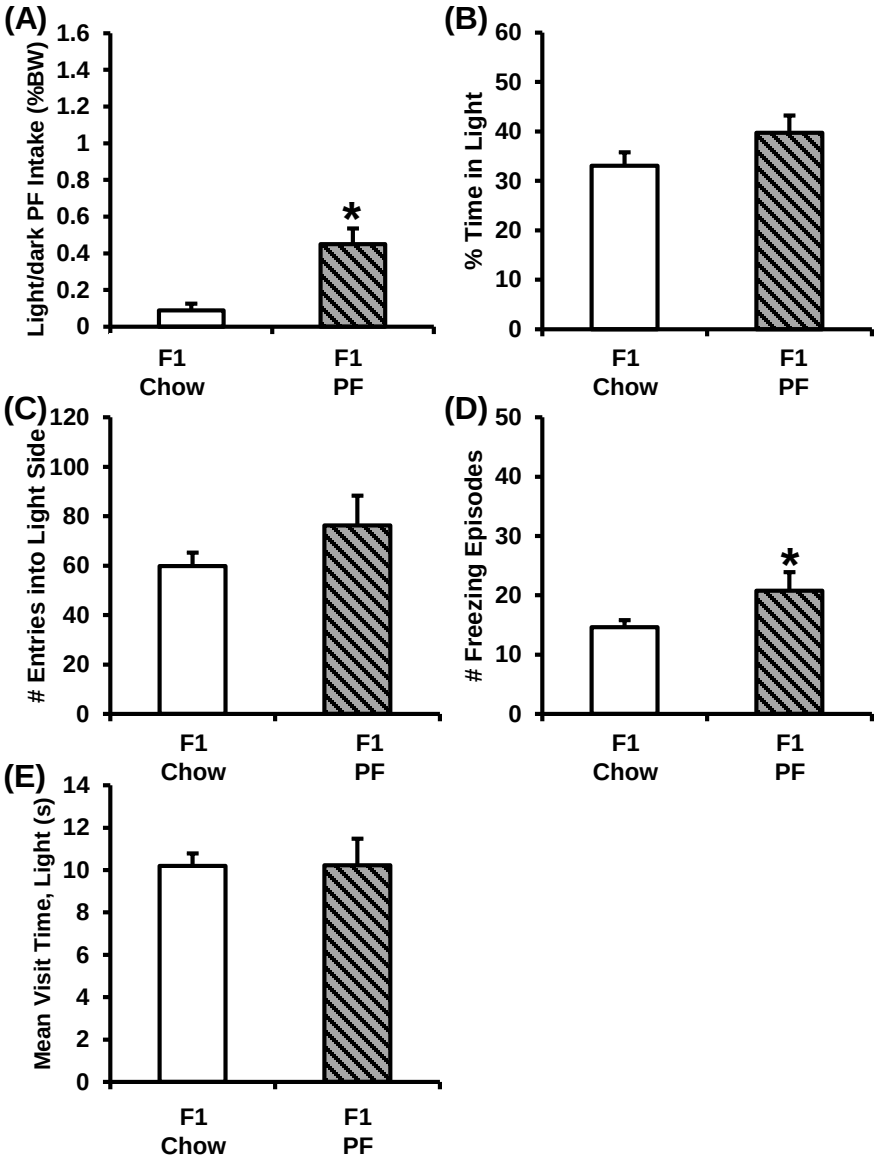
(A)
Light/dark PF Intake (%BW)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
F1 Chow
F1 PF
(B)
% Time in Light
60
50
40
30
20
10
0
F1 Chow
F1 PF
(C)
Entries into Light Side
120
100
80
60
40
20
0
F1 Chow
F1 PF
(D)
Freezing Episodes
50
40
30
20
10
0
*
F1 Chow
F1 PF
(E)
Mean Visit Time, Light (s)
14
12
10
8
6
4
2
0
F1 Chow
F1 PF

Figure 5.

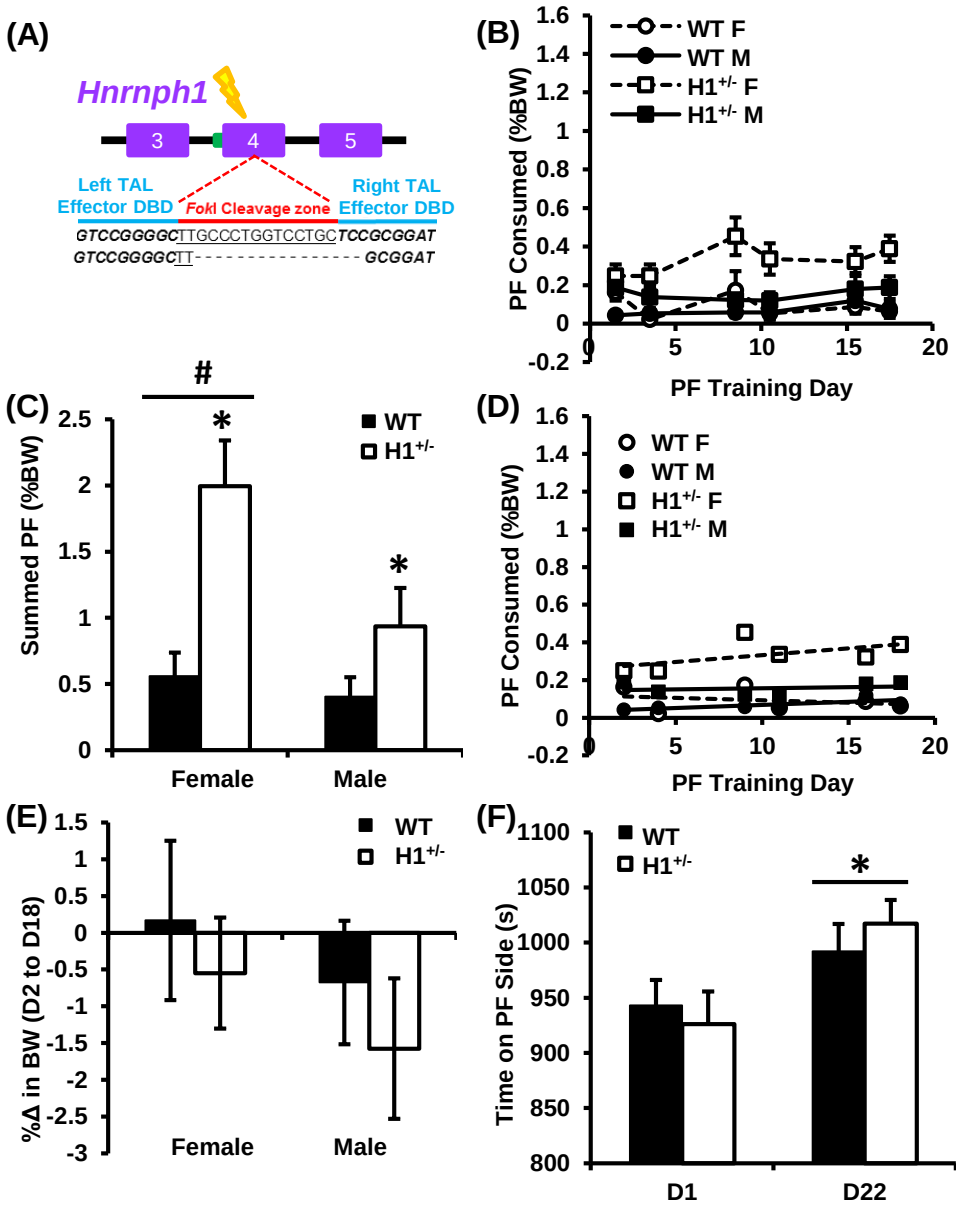
(A)
Hnrnph1
3
4
5
Left TAL Effector DBD
FokI Cleavage zone
Right TAL Effector DBD
GTCCGGGGCTTGCCCTGGTCCTGCTCCGCGGAT
GTCCGGGGCTT- - - - - - - - - - - - - - - - GCGGAT
(B)
WT F
WT M
H1+/- F
H1+/- M
PF Consumed (%BW)
PF Training Day
(C)
#
*
*
WT
H1+/-
Summed PF (%BW)
Female
Male
(D)
WT F
WT M
H1+/- F
H1+/- M
PF Consumed (%BW)
PF Training Day
(E)
WT
H1+/-
%Δ in BW (D2 to D18)
Female
Male
(F)
WT
H1+/-
*
Time on PF Side (s)
D1
D22

Table 1. Exploratory factor analysis of BE, conditioned behaviors, and compulsive-like eating of PF in B6J versus D2J strains.

B6J variables	Heritability (h^2)	Conditioned Place Preference	PF Seeking & Consumption	Compulsive Behavior
% Variance		19	19	17
D2 PF Intake (%BW)	34%		0.67	
D18 PF Intake (%BW)	56%		0.74	
D18 Time in Food Triangle (s)	27%			
D18 Entries to Food Triangle	30%			
D18 Distance in Food Triangle (m)	51%		0.58	
Escalation Slope	34%			
D22 Entries to Right Side	18%	0.60	-0.50	
D22 Time on the Right Side (s)	12%			
D22 Distance on Right Side (m)	28%	0.99		
D22 Entries to Food Triangle	22%	0.79		
D22 Time in Food Triangle (s)	2%			
D22 Distance in Food Triangle (m)	17%	0.88		
Light/dark PF Intake (%BW)	73%		0.78	
Light/dark Time on Light Side (s)	11%			0.89
Light/dark Entries Into Light Side	1%			0.69
Light/dark Distance on Light Side (m)	10%			0.95

D2J variables	Heritability (h^2)	Binge eating & Compulsive Behavior	Conditioned Place Preference	PF Seeking
% Variance		23	19	15
D2 PF Intake (%BW)	34%			
D18 PF Intake (%BW)	56%	0.68		
D18 Time in Food Triangle (s)	27%			
D18 Entries to Food Triangle	30%			0.62
D18 Distance in Food Triangle (m)	51%			
Escalation Slope	34%	0.54		
D22 Entries to Right Side	18%			0.71
D22 Time on the Right Side (s)	12%			-0.65
D22 Distance on Right Side (m)	28%		0.94	
D22 Entries to Food Triangle	22%		0.81	
D22 Time in Food Triangle (s)	2%			-0.95
D22 Distance in Food Triangle (m)	17%		0.95	
Light/dark PF Intake (%BW)	73%			
Light/dark Time on Light Side (s)	11%	0.91		
Light/dark Entries Into Light Side	1%	0.81		
Light/dark Distance on Light Side (m)	10%	0.96		