Cyfip1 haploinsufficiency increases compulsive-like behavior and paternally inherited palatable food intake: Implications for Prader-Willi Syndrome

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ABSTRACT

Binge eating (BE) is a heritable trait associated with eating disorders and involves consumption of a large quantity of food in a short time. We identified cytoplasmic FMRP-interacting protein 2 (*Cyfip2*) as a major genetic factor underlying BE and concomitant compulsive-like behaviors in mice. *CYFIP2* is a gene homolog of *CYFIP1* - one of four paternally deleted genes in patients with the more severe Type I Prader-Willi Syndrome (PWS). PWS is a neurodevelopmental genetic disorder where 70% of cases involve paternal deletion of 15q11-q13. PWS is defined phenotypically by the emergence of hyperphagia and obesity during childhood as well as cognitive deficits and obsessive-compulsive behaviors. We tested the hypothesis that *Cyfip1* haploinsufficiency would enhance premorbid compulsive-like behavior and palatable food (PF) consumption in a parent-of-origin-selective manner. Additionally, because our initial studies involved mice on a C57BL/6N background that possess the BE-associated missense mutation in *Cyfip2*, we tested mice on a mixed background where mice were homozygous for the C57BL/6J (B6J) allele at the *Cyfip2* locus. *Cyfip1* haploinsufficiency increased compulsive-like behavior on both backgrounds and PF consumption was greater with paternal inheritance. Gene expression in the hypothalamus revealed a paternal effect of *Cyfip1* deletion on transcription of *Cyfip1* but not *Cyfip2* or *Magel2*. To summarize, the selective increased compulsive-like behavior and PF consumption in paternally deleted *Cyfip1*$^{+/-}$ mice could translate to enhancing hyperphagia in a subset of individuals with neurodevelopmental disorders involving reduced expression or haploinsufficiency of *CYFIP1*, including PWS but also Fragile X Syndrome and 15q11.2 Microdeletion Syndrome.

INTRODUCTION

Binge eating (**BE**) refers to the rapid consumption of a large amount of food over a short time period and is accompanied by feelings of loss of control. Binge eating disorder (**BED**) is a psychiatric disorder with a lifetime prevalence of 3.5% in women and 2% in men (1). Both BED (2) and BE are heritable (3). However, genome-wide association studies have yet to identify genetic risk factors associated with BE (4). The first genome-wide significant loci for anorexia nervosa (comprising restricted eating) were recently identified (5) and genome-wide significant loci are expected to soon be uncovered for BE-associated disorders with increasing sample sizes and power (6).

We used quantitative trait locus (**QTL**) mapping and gene knockout in C57BL/6 mouse substrains to identify cytoplasmic FMR1-interacting protein 2 (*Cyfip2*) as a major genetic factor underlying BE and compulsive-like behaviors (7). The QTL capturing increased PF consumption mapped to a missense mutation in *Cyfip2* in the C57BL/6N strain (S968F; "*Cyfip2*^{M1N}") that is hypothesized to act as a gain-of-function mutation (8). Accordingly, mice with one copy of a null allele and one copy of the missense allele of *Cyfip2* showed a reduction in BE toward the phenotypic direction of the wild-type C57BL/6J level (7). This same missense SNP in *Cyfip2* was first associated with reduced behavioral sensitivity to cocaine (8), which could indicate a common neurobiological mechanism involving synaptic plasticity within the mesocorticolimbic dopamine reward pathway (9,10).

Cyfip2 and the gene homolog *Cyfip1* code for proteins that interact with the RNA binding protein Fragile X Mental Retardation Protein (**FMRP**) and are part of the canonical WAVE regulatory complex and transduce activity-dependent Rac signaling in regulating actin dynamics during neuronal development and synaptic plasticity (11). CYFIP1 expression is necessary for the maintenance and stabilization of neuronal dendritic arborization and morphological complexity (12). In humans, *CYFIP1* resides within a non-imprinted region on chromosome 15 (15q11.2) that contains four genes *TUBGCP5*, *NIPA1*, *NIPA2*, and *CYFIP1* (13). The syntenic region in mice is located on chromosome 7C (55.4 Mb - 56 Mb). Haploinsufficiency of 15q11.2 underlies Microdeletion Syndrome (**MDS**) which is characterized by developmental delay (speech, motor), reduced cognitive function, dysmorphic features, intellectual disability, autism, ADHD, obsessive-compulsive disorder, and schizophrenia (14).

At least one case study of 15q11.2 MDS reported hypotonia, increased food craving and obesity, and obsessive-compulsive disorder (15). *CYFIP1* haploinsufficiency is implicated in multiple symptoms of 15q11.2 MDS. Preclinical models of *Cyfip1* haploinsufficiency demonstrate perturbations in synaptic activity during neural development, activity-dependent plasticity, dendritic morphology, and fear learning (16-19).

The 15q11.2 region is also paternally deleted in a subset of individuals with a more severe form (Type I) of Prader-Willi Syndrome (**PWS**), a neurodevelopmental disorder defined genetically by paternal deletion of 15q11-q13 in a majority of cases (20). Extreme hyperphagia due to lack of satiety is the most defining, debilitating feature of PWS that is difficult to treat and emerges during childhood, leading to obesity. Obsessive-compulsive behaviors are frequent in PWS and can be focused on food and food-associated stimuli (e.g., food hoarding and stealing). Genetic deletion in PWS involves either the shorter paternal deletion (Type II) of 15q11-q13 or a larger, paternal Type I deletion that also includes the 15q11.2 MDS region comprising four genes: *TUBGCP5*, *NIPA1*, *NIPA2*, and *CYFIP1*. Type I PWS is associated with a more severe neurodevelopmental and neuropsychiatric profile, including reduced cognition, increased risk of autism and schizophrenia, and increased severity of obsessive-compulsive disorder (**OCD**) (15,21,22) that is highly correlated with decreased CYFIP1 expression (13).

Decreased CYFIP1 expression is also implicated in the Prader-Willi Phenotype (**PWP**) of a subset of individuals with Fragile-X Syndrome (**FXS**). FXS is the most common genetic cause of intellectual disability and autism and is caused by a CGG trinucleotide repeat expansion within the fragile X mental retardation 1 (*FMR1*) gene that codes for FMRP, a major interacting protein of CYFIP proteins (23). Interestingly, ten percent of FXS individuals also exhibit a PWP in the absence structural or imprinting differences in 15q11-q13. The PWP includes hallmark hyperphagia, lack of satiation, obesity, and more severe behavioral problems, such as obsessive-compulsive behaviors and an increased rate of autism (24,25). The cause of the PWP is unknown, although one logical candidate gene is *CYFIP1*, given its association with PWS and its interaction with FMRP (23). PWP-presenting individuals with FXS show a two-to four-fold decrease in CYFIP1 transcription compared to FXS

individuals without PWP (25). There was also a two-fold decrease in *Cyfip1* gene transcription in a mouse model of FXS (26).

Because of the association of the gene homolog *Cyfip2* with BE (7) and because both *CYFIP1* deletion and reduced CYFIP1 expression are associated with PWS (Type I) and hyperphagia in the PWP (FXS) respectively, here, we tested the hypothesis that *Cyfip1* haploinsufficiency would increase premorbid compulsive-like behavior and consumption of palatable food (**PF**) in our BE paradigm (7,27,28). We tested the effect of *Cyfip1* deletion on two different *Cyfip2* genetic backgrounds. Additionally, because a recent study demonstrated a parental origin (**PO**) effect of *Cyfip1* haploinsufficiency on hippocampal synaptic transmission, learning, and anxiety-like behavior (16) and because polymorphic genes residing in non-imprinted loci (e.g., *Cyfip1*) can potentially interact with imprinted loci (e.g., the PWS locus) to induce PO effects on multiple traits (29), we tested for a PO effect of *Cyfip1* deletion on compulsive-like behavior and PF consumption. To gain initial insight into the molecular mechanisms underlying the PO effect of *Cyfip1* deletion on PF consumption, we examined transcription of *Cyfip1*, *Cyfip2*, and *Magel2* - a nearby imprinted gene within the syntenic, canonical PWS region implicated in hyperphagia and obesity. Finally, because obsessions-compulsions are associated with BE (30-32) and hyperphagia in PWS (33), we assessed premorbid compulsive-like behaviors in a battery of anxiety-like and compulsive-like behaviors and post-BE training behaviors, including compulsive-like eating and concomitant behaviors in the light/dark conflict test (7) in *Cyfip1* haploinsufficient mice.

MATERIALS AND METHODS

Mice

All experiments were performed in accordance with the National Institutes of Health Guidelines for the Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Boston University. Mice were 50-100 days old at the first day of testing. A minimum sample size of N = 20 per Genotype per Treatment was employed for behavioral studies based on power analysis of PF consumption from the *Cyfip2* study (7) (see Supplementary Information for

additional details). Mice heterozygous for a null deletion in exons 4 through 6 of *Cyfip1* (*Cyfip1*$^{+/-}$) were propagated on two different C57BL/6 genetic backgrounds. First, *Cyfip1*$^{+/-}$ mice were re-derived using sperm from *Cyfip1*$^{+/-}$ mice that were generated by the International Knockout Mouse Consortium (34) from ES cells containing the "knockout first" allele C57BL/6N-Atm1Brd Cyfip1tm2a (EUCOMM)Wtsi/Wtsi; *Cyfip1*$^{N/-}$; MGI: 5002986) on the BE-prone C57BL/6N background (7). Sperm was obtained from The Jackson Laboratory (JAX; Bar Harbor, ME USA). *Cyfip1*$^{+/-}$ mice were bred to C57BL/6NJ mice to produce haploinsufficient mice (*Cyfip1*$^{N/-}$) and wild-type littermates (*Cyfip*$^{N/N}$). Mice homozygous for the null *Cyfip1* mutation are embryonic lethal (12,19).

Because the C57BL/6N-derived missense mutation (S968F) in the gene homolog *Cyfip2* is associated with increased PF consumption (7,8), we generated a second group of mice where we replaced the N alleles with C57BL/6J (J) alleles at the *Cyfip2* locus via backcrossing to the C57BL/6J strain for three generations. This approach drastically reduced the background level of PF consumption and ruled out a possible ceiling effect that could have prevented detection of the hypothesized increase in PF consumption in *Cyfip1*$^{+/-}$ mice. New C57BL/6J breeders were ordered from JAX at each generation of backcrossing. N1 offspring were first generated that were heterozygous for the *Cyfip1* J allele and null (-) allele and were heterozygous for the *Cyfip2* J allele and *Cyfip2* N allele. N1 mice were backcrossed to produce N2 mice. We picked N2 mice that were *Cyfip1*$^{J/-}$ and *Cyfip2*$^{J/J}$ and again backcrossed these mice to generate N3 mice that were either *Cyfip1*$^{J/J}$ (~50%) or *Cyfip1*$^{J/-}$ (~50%) and *Cyfip2*$^{J/J}$ (100%). Mice were housed in the vivarium adjacent to the testing room with *ad libitum* access to chow (Harlan® 2918) and water in the home cage. Behavioral testing was performed between 0730 h and 1300 h, during the light phase of a 12 h light/dark cycle (lights on at 0630). Cage mates were all assigned to the same treatment group within a cage. Details regarding *Cyfip1* and *Cyfip2* genotyping are provided in the Supplementary Information.

Premorbid anxiety- and compulsive-like behavioral battery

Because of the link between anxiety, compulsivity and pathological overeating (32) and because obsessive-compulsive behavior is associated with eating disorders (35,36), we incorporated a

behavioral battery to assess differences in premorbid anxiety-like and compulsive-like behaviors in experimentally naïve, *Cyfip1*$^{+/-}$ mice. Mice were tested in the behavioral battery and were either sacrificed afterward (mice on *Cyfip2*$^{N/N}$ background) or were subsequently trained for BE (mice on the *Cyfip2*$^{J/J}$ background). Mice were assayed in the battery with one test per day over five days in the following order: 1) open field; 2) elevated plus maze; 3) marble burying; 4) hole board; 5) mist-induced grooming. Procedural details are provided in the Supplementary Information. Testing was conducted between 0800 and 1300 h. The experimenters responsible for running the mice, video tracking, and data curation for analysis were blinded to Genotype throughout the course of the study.

Digging, burrowing, and burying of objects with bedding are highly correlated behaviors associated with survival. Inherent burying of non-aversive stimuli (marbles, food pellets) can be distinguished from defensive burying of aversive, noxious stimuli (shock grid, snakes) (37) and is not related to anxiety, stimulus novelty, or locomotor activity levels. Rather, inherent marble burying is an indirect measure of the natural tendency to dig (38-40). Marble burying represents persistent, repetitive behavior that is resistant to habituation and is proposed to model obsessive/compulsive behavior (39,41). This behavior is used to screen pharmacotherapeutics for OCD (42). Genetic variance in marble burying among inbred mouse strains is heritable but is genetically uncorrelated with anxiety-like behaviors and is thus, mediated by distinct genetic factors (39).

BE procedure

Mice were trained in an intermittent, limited access procedure shown to detect genetic differences in BE (7,27). We used a two-chambered conditioned place preference (**CPP**) apparatus, with differently textured floors in each chamber. The right and left sides were designated the food-paired (**FP**) and no-food-paired sides (**NFP**), respectively. Mice were trained and and video recorded in unlit sound-attenuating chambers (MedAssociates, St. Albans, VT USA). On Day 1, initial side preference was determined by placing each mouse on the NFP side with the divider containing an open entryway that provided free access to the both sides for 30 min. Clean, empty food bowls were placed in the far corners of each side. On Days 2, 4, 9, 11, 16, and 18, mice were confined to the FP side with

a closed divider that prevented access to the NFP side. Mice were provided either forty, 20 mg sweetened palatable food pellets (**PF**; TestDiet 5-TUL, St. Louis, MO USA) or home cage chow-like pellets (Chow; TestDiet 5BR3) in a non-porous porcelain dish in the far corner of the chamber. On Days 3, 5, 10, 12, 17, and 19, mice were confined to the NFP side with no access to the FP side. A clean, empty, and non-porous porcelain dish was placed in the far corner of the chamber during this time. On Day 22, side preference was again assessed with open access to both sides. No food was present in either bowl at this time. The experimenter was blinded to Genotype throughout data collection.

Light/dark conflict test of compulsive eating

In addition to premorbid anxiety- and compulsive-like behaviors, we also assessed post-training, compulsive-like eating and concomitant behaviors in the anxiety-provoking light/dark conflict test where rodents will avoid the aversive, light side (32). On Day 23, mice were assessed for behaviors as described (7,28). The light/dark box consists of a dark side, which is an enclosed black, opaque Plexiglas chamber, and a light side with clear Plexiglas. An open doorway allowed free access to both sides. A non-porous ceramic bowl containing forty, 20 mg PF pellets was placed in the center of the light side. Mice were initially placed on the light side facing both the food and the doorway and were video recorded for 30 min. Because the light side is aversive, increased behaviors in this environment were operationalized as compulsive-like, including compulsive-like eating (7,28).

Hypothalamus dissections

We chose a subset of Chow-trained, PF-naive mice (n = 7-9 per Genotype per PO; both sexes) on the *Cyfip2*$^{N/N}$ background or untrained, PF-naïve mice on a *Cyfip2*$^{J/J}$ background (n=7-9 per Genotype per PO per genetic background; both sexes) to examine baseline (PF-naive) differences in gene transcription between *Cyfip1*$^{N/N}$ versus *Cyfip1*$^{N/-}$ mice and the effect of PO. We examined *Cyfip1*, *Cyfip2*, and *Magel2* transcript levels in the hypothalamus, a brain region important for hyperphagia in PWS (33) and for the effects of deletion of *Magel2* (43,44) on eating behavior and body weight (45). Haploinsufficiency of *MAGEL2* is associated with PWS hyperphagia in humans (46).

On Day 24, 24 h post-assessment of compulsive-like eating and concomitant behaviors, brains from Chow-trained mice (*Cyfip2*$^{N/N}$ background) were harvested and the hypothalamus was free form dissected by pinching the entire structure from the ventral surface with forceps while using the anterior commissure and mammillary bodies as landmarks. Tissue was stored in RNAlater Solution (Invitrogen, Carlsbad, CA USA) at 4°C. After five days, the tissue was dried on a Kimwipe and transferred to a -80ºC freezer.

Real-time quantitative PCR (qPCR)

Total RNA from hypothalamus was extracted and processed for qPCR as described (7,27,47). Briefly, oligo-dT primers were used to synthesize cDNA. PCR reactions were conducted on the StepOne Plus 96-Well Real-Time PCR machine (Life Technologies, Foster City, CA, USA) in technical triplicates and averaged (SD < 0.5). Plates were balanced across Genotype, PO, and Sex. We report the difference in expression in KO relative to WT using the $2^{-(\Delta\Delta CT)}$ method (Schmittgen & Livak, 2008). Primer sequences are provided in the Supplementary Information.

Analysis

We analyzed food consumption in R (https://www.r-project.org/) using mixed model ANOVAs with Genotype, Treatment, and Sex as independent variables, and Day as a repeated measure. Additionally, we assessed the effect of PO (maternal, paternal) using mixed-model ANOVAs with Genotype, Treatment, and PO as independent variables and Day as a repeated measure. For the compulsive behavioral tests, unpaired t-tests were used to detect effects of Genotype for all behaviors except marble burying behaviors which were analyzed by non-parametric Mann-Whitney U tests. Slope analyses were conducted as previously described (7,48) using GraphPad Prism 7 (GraphPad Software, La Jolla, CA USA).

To control for differences in consumption due to the two different *Cyfip2* genetic backgrounds (7) and to increase our power to detect genotypic effects as a function of PO on behavior, we Z score-standardized datasets from each *Cyfip2* genetic background based on the average consumption across

all days (mean = 0, standard deviation = 1). We then combined datasets for analysis using mixed-model ANOVAs.

RESULTS

Cyfip1 haploinsufficiency increases compulsive-like marble burying

Sample sizes are listed in **Supplementary Table 1**. **Figure 1** illustrates the breeding scheme for *Cyfip1*$^{+/-}$ mice on two *Cyfip2* genetic backgrounds – either *Cyfip2*$^{N/N}$ or *Cyfip2*$^{J/J}$. Because symptomatic severity is worse in individuals with Type I PWS (which includes the *CYFIP1* deletion) and because compulsivity was negatively correlated with *CYFIP1* expression in PWS patients with Type I deletions (13), we hypothesized that *Cyfip1*$^{+/-}$ mice would exhibit greater compulsive-like behavior.

In the marble burying test, *Cyfip1*$^{N/-}$ mice on the *Cyfip2*$^{N/N}$ genetic background showed a greater number of marbles that were at least 50% buried and a greater average percentage of marbles buried than *Cyfip1*$^{N/N}$ mice (**Fig.2A-C**) which are commonly used measures of marble burying (49). A similar result was replicated in *Cyfip1*$^{J/-}$ on the *Cyfip2*$^{J/J}$ background (**Fig.2D,E**). *Cyfip1* deletion did not induce a change in any other behaviors within the battery on either *Cyfip2* genetic background (data not shown) or in the combined dataset (**Supplementary Table 2**, all ps > 0.05).

Because *CYFIP1* is paternally deleted in individuals with Type I PWS and because PO effects of *Cyfip1* deletion on synaptic transmission and behavior were reported (16), we next investigated the effect of PO of *Cyfip1* deletion on anxiety-like and compulsive-like behaviors. There was no effect of PO or interaction with *Cyfip1* Genotype on marble burying (**Fig.2F,G**) or any other behaviors within the battery (data not shown). To summarize, *Cyfip1* haploinsufficiency induced a selective increase in compulsive-like marble burying on two genetic backgrounds.

Cyfip1 haploinsufficiency increases PF consumption in an intermittent, limited access BE paradigm

In testing the hypothesis that *Cyfip1* haploinsufficiency increases PF consumption in our intermittent, limited access BE and CPP paradigm (**Fig.3A**), PF-trained mice of both genotypes

consumed more food than Chow-trained mice (**Fig.3B**) – this result was reflected by slopes of escalation that were significantly greater than zero in both PF-trained genotypes but not the Chow-trained genotypes (**Fig.3C**). As predicted, *Cyfip1*$^{N/-}$ mice consumed more PF than *Cyfip1*$^{N/N}$ mice, but not more Chow (**Fig.3B**). Finally, PF-trained *Cyfip1*$^{N/-}$ mice showed a greater y-intercept than all other groups (**Fig. 3C**), indicating an initial higher level of consumption that persisted throughout the study.

In examining the effect of *Cyfip1* haploinsufficiency on food consumption on a *Cyfip2*$^{J/J}$ genetic background, PF-trained mice again showed greater consumption than Chow-trained mice (**Fig.3D**). As predicted based on PF consumption on the *Cyfip2*$^{J/J}$ genetic background (7), none of these groups showed an escalation in consumption (**Fig.3D,E**). Furthermore, without including PO in the analysis, there was no difference in consumption between *Cyfip1*$^{J/-}$ versus *Cyfip1*$^{J/J}$ mice.

Paternal *Cyfip1* haploinsufficiency increases PF consumption

We next investigated the effect of PO of *Cyfip1* deletion on food consumption in light of a recent study demonstrating a PO effect of *Cyfip1* deletion on emotional learning and synaptic transmission (16). We focused on PF consumption rather than Chow based on the above results.

For mice on the *Cyfip2*$^{N/N}$ background, paternal versus maternal *Cyfip1* deletion induced greater PF consumption in all offspring (**Fig.4A**). Furthermore, paternally deleted *Cyfip1*$^{N/-}$ mice showed a greater y-intercept than both *Cyfip1*$^{N/N}$ groups (**Fig.4B**), indicating overall greater consumption across time. Wild-type *Cyfip1*$^{N/N}$ offspring derived from families with paternal versus maternal *Cyfip1* deletion also showed a greater y-intercept (**Fig.4B**), thus confirming an overall effect of parental *Cyfip1* genotype on long-term neurobehavioral development of all offspring within those families.

In examining on the *Cyfip2*$^{J/J}$ genetic background, there was less overall PF consumption, as expected (7). Additionally, there was no escalation in PF consumption for any of the groups (**Fig. 4C,D**). Slope analysis, however, showed an increase in y-intercept in mice with the paternally derived *Cyfip1* deletion compared to all other genotypes (**Fig. 4D**), indicating increased initial PF consumption early on during training.

To control for variance in consumption due to genetic background and to increase our power to detect a PO effect of *Cyfip1* deletion on PF consumption, we Z score-standardized each of the two datasets using the average PF consumption across Training Days for each genetic background and then combined these results into a single dataset. *Cyfip1*$^{+/-}$ mice with a paternal deletion by far exhibited the greatest amount of PF consumption (**Fig. 4E**). Slope analysis indicated that all groups *except for* paternally deleted *Cyfip1*$^{+/-}$ mice showed a significant escalation in PF consumption. Instead, paternally deleted *Cyfip1*$^{+/-}$ mice showed a significantly greater y-intercept compared to all other groups (**Fig. 4F**), thus reflecting an initially greater and persistent PF consumption across days.

Despite changes in PF consumption across Genotype and PO, we did not find any obvious evidence for differences in body weight that were associated with the results involving PF consumption. When the two genetic backgrounds were analyzed separately and the two treatments were collapsed to increase our power, there were still no differences in body weight as a function of Genotype or PO. When these two datasets were then combined, we detected a small but significant *decrease* in body weight in offspring of families with a paternal versus maternal deletion that did not depend on *Cyfip1* genotype (**Supplementary Figure 1**). To summarize, changes in body weight are unlikely to explain the increased PF consumption in paternally deleted *Cyifp1*$^{+/-}$ mice.

Conditioned food reward as measured via CPP in *Cyfip1* $^{+/-}$ mice

In examining CPP on the *Cyfip2*$^{N/N}$ genetic background, we did not detect any effect of *Cyfip1* Genotype or Treatment in the ANOVA model in mice from either genetic background (**Supplementary Figure 2A,B**). However, when PF treatment was considered alone, we observed increased PF-CPP in *Cyfip2*$^{N/-}$ versus *Cyfip2*$^{N/N}$ mice (**Supplementary Figure 2A**). In considering PO effects on PF-CPP, we did not detect any effect of Genotype, PO, or interaction (**Supplementary Figure 2C,D**).

Compulsive-like eating and concomitant behaviors in *Cyfip1*$^{+/-}$ mice in the light/dark conflict test

Because increased PF consumption can become compulsive-like (32), we next examined post-training compulsive-like eating and concomitant behaviors using the light/dark conflict test (7). There

was greater consumption of PF versus Chow and there was greater consumption in females versus males on both genetic backgrounds (**Supplementary Figure 3A,C**), as we have repeatedly observed under this paradigm (7,27,28). However, *Cyfip1* deletion had no effect on post-training, compulsive-like eating on either genetic background, regardless of PO (**Supplementary Figure 3B,D**). No genotypic differences or PO effects were observed for any other behaviors during the test for compulsive-like eating (7), including time spent on the light side, entries into the light side, or mean visit time in the light side (data not shown).

Reduction of *Cyfip1* but not *Cyfip2* or *Magel2* gene transcription in the hypothalamus of *Cyfip1*$^{N/-}$ mice

Because we observed a more pronounced increase in PF consumption following paternal *Cyfip1* deletion, we hypothesized that this result could involve a difference in hypothalamic gene transcription of *Cyfip1* and perhaps *Cyfip2* (7). Additionally, we examined hypothalamic gene transcription of *Magel2* which is a nearby imprinted gene that is located within the syntenic, canonical (Type II) PWS locus and has been implicated in changes in eating behavior in mouse models of PWS (45) and PWS-like hyperphagia in humans (46). **Table 1** lists the qPCR results as a function of both *Cyfip1* haploinsufficiency and PO. As expected, *Cyfip1* deletion significantly reduced gene transcription. When assessed on the *Cyfip2*$^{N/N}$ background, the reduction in *Cyfip1* transcription in *Cyfip1*$^{+/-}$ mice did not significantly depend on PO (**Table 1A,B**). However, in contrast to our prediction, when assessed on the *Cyfip2*$^{J/J}$ background, the decrease in *Cyfip1* expression was actually significantly *less* with paternal *Cyfip1*$^{J/-}$ allele (**Table 1C,D**); this result was also reflected in the combined dataset across *Cyfip2* genetic backgrounds (**Table 1E,F**). Finally, there was no effect of Genotype or PO on transcription of *Cyfip2* or *Magel2*.

DISCUSSION

Cyfip1 haploinsufficiency increased obsessive-compulsive-like behavior and PF consumption on two different *Cyfip2* genetic backgrounds, including the BE-prone *Cyfip2*$^{N/N}$ background and the BE-

resistant *Cyfip2*$^{J/J}$ background (**Figs. 1-3**) (7). Mice harboring the paternal *Cyfip1* deletion showed greater PF intake than mice harboring the maternal deletion (**Fig.4**), suggesting paternal *CYFIP1* deletion could influence eating in individuals with Type I PWS. The selective increase in sweetened PF but not Chow consumption is consistent with increased preference for sweetened PF in PWS (50). Together, these findings support the hypothesis that reduced expression of CYFIP1 could contribute to obsessive-compulsive like behaviors and enhance hyperphagia in Type II PWS or the PWP (FXS).

The selective increase in compulsive-like but not anxiety-like behavior on both *Cyfip2* genetic backgrounds following *Cyfip1* deletion (**Fig.2**; **Supplementary Table 2**) is consistent with a lack of genetic correlation between marble burying and anxiety (39). Marble burying is an obsessive-compulsive-like behavior in rodents that can predict a high level of BE (51,52). The increase in both premorbid marble burying and PF consumption in *Cyfip1*$^{+/-}$ mice is interesting because obsessive-compulsive disorder is a premorbid risk factor associated with eating disorders (35,36) and because obsessions-compulsions are associated with food in BE (30,31) and PWS hyperphagia (33). That further enhancement of marble burying in *Cyfip1*$^{+/-}$ mice was not observed following paternal deletion does not necessarily mean that it is a separable, unrelated phenotype from PF consumption. Rather, it could reflect a ceiling effect of Genotype on our measures of marble burying (53). Together, our results indicate that *CYFIP1* haploinsufficiency in humans could influence various, possibly biologically linked neurobehavioral symptoms in PWS and the PWP, namely obsessions-compulsions and increased food consumption.

As expected, mice on a *Cyfip2*$^{N/N}$ versus *Cyfip2*$^{J/J}$ background showed a greater increase in initial and escalated PF consumption in **(Fig.3B-E)** (54). The advantage of testing mice on the the BE-prone *Cyfip2*$^{N/N}$ background was that it revealed an increase in PF consumption across later training days in *Cyfip1*$^{N/-}$ mice and thus, an increase in slope of escalation (**Fig.3D,E**). On the other hand, testing mice on the BE-resistant *Cyfip2*$^{J/J}$ background revealed an early enhancement of initial PF consumption in *Cyfip1*$^{J/-}$ mice (**Fig.3D,E**) that was driven by paternal deletion of *Cyfip1* (**Fig.4C,D**)**.** Thus, the BE-prone and BE-resistant genetic backgrounds permitted detection of increased consumption at different time points in *Cyfip1*$^{+/-}$ mice. The overall increased PF consumption across

time with paternal *Cyfip1* deletion is clearly reflected in the combined standardized dataset that adjusted for differences in consumption due to genetic background (**Fig.4G-I**). These results highlight the utility of testing genetic mutations on multiple genetic backgrounds (55), in particular when large-effect loci are known *a priori* to influence a trait of interest (7).

Despite the paternally selective increase in PF consumption in *Cyfip1*$^{+/-}$ mice, there was no difference in body weight between genotypes (**Supplementary Figure 1**), suggesting that the increased PF consumption caused by *Cyfip1* deletion is not mediated by a homeostatic mechanism but rather, could be mediated by a difference in hedonic processing of sweetened PF (56) - the selective increase in PF consumption as well as the selective increase in PF conditioned reward in *Cyfip1*$^{+/-}$ mice (at least on the *Cyifp2*$^{N/N}$ genetic background; **Supplementary Figure 2A**) further supports the hedonic hypothesis that predicts enhanced preference for sweetened food in individuals with Type I PWS (50).

Recent studies indicate that CYFIP proteins could be critical for synaptic function within the mesolimbic dopamine pathway that is engaged by reinforcing stimuli such as drugs and PF. The *Cyfip2*$^{N/N}$ mutation is associated with cocaine neurobehavioral sensitivity and plasticity (8) and compulsive-like BE (7). Furthermore, differences in in *Cyfip2* mRNA expression are genetically correlated with differences in cocaine self-administration (57). Transcriptome analysis of the striatum from *Cyfip2*$^{N/-}$ versus N/N genotypes identified “morphine addiction” and “cocaine addiction” as three of the top five KEGG enrichment terms associated with *Cyfip2*$^{N/-}$ versus *Cyfip2*$^{N/N}$ mice (7). FMRP, a major interacting protein with CYFIP proteins, is involved in reward processing (58) and cocaine neurobehavioral plasticity (59). We hypothesize that *Cyfip1* deletion could enhance the hedonic effects of PF consumption which in turn, could interact with hypothalamic, homeostatic mechanisms of hyperphagia in PWS (60).

An important discussion point is the increase in PF consumption of all offspring from parents with the paternal *Cyfip1*$^{N/-}$ deletion (**Fig. 4A,B**). Maternal versus paternal *Cyfip1* deletion could affect social interactions with the dam and sire or with the pups, including maternal care and paternal pup retrieval. Of direct relevance to *Cyfip1*, maternal deletion of the *Fmr1* gene (coding for FMRP) can induce neurobehavioral phenotypes in wild-type offspring and enhance phenotypes in mutant offspring,

including locomotor hyperactivity, reduced behavioral response to D2 dopamine receptor activation, and enhanced behavioral response to GABA-B receptor activation (61). Additionally, male mice can demonstrate paternal pup retrieval (62) and thus, *Cyfip1* deletion could also affect sire-pup contact. Given the association between *CYFIP1* deletion and social deficits in both 15q11.2 MDS and PWS but also FXS, autism, and schizophrenia (11), it is plausible that *Cyfip1* deletion in mice affects social dynamics of the family in a PO-specific manner leading to long-term neurobehavioral effects in all offspring.

Despite paternal *Cyfip1* deletion inducing the greatest increase in PF consumption (**Fig.4**), this gene is not located within an imprinted locus (13) and indeed, we did not observe any further reduction of gene transcription as a consequence of paternal *Cyfip1* haploinsufficiency. In fact, we observed the opposite result, namely that there was *less* of a decrease in gene transcription associated with paternal *Cyfip1* deletion. This result was significant on the *Cyfip2*$^{J/J}$ genetic background but the same trend was also observed on the *Cyfip2*$^{N/N}$ background and the result remained significant when the datasets were combined (**Table 1**). On its surface, this result would seem to contradict the hypothesis that decreased *Cyfip1* expression is related to increased PF consumption. However, the relationship between decreased *Cyfip1* expression and increased PF consumption may not be linear and require only a small level of decreased *Cyfip1* function to interact with imprinted genes at the genomic level to affect behavior. I.e., while decreased *Cyfip1* transcription is presumably necessary for the paternal enhancement of PF consumption, the genomic perturbations induced by paternal *Cyfip1* deletion are more directly relevant to the neurobiological mechanism underlying behavior which is likely to ultimately involve changes in expression or function of genes within one or more imprinted loci throughout the genome. In support, a recent study of nearly 100 phenotypes showed that 1) most complex traits exhibit PO effects; 2) PO-specific QTLs can be identified in non-imprinted regions of the genome, and 3) non-imprinted KO alleles (e.g., here, *Cyfip1*) can induce extensive PO effects by interacting in *trans* with imprinted loci throughout the genome to affect network-level gene expression (29).

Our preclinical findings provide evidence that reduced expression of CYFIP1 could contribute to increased food consumption in PWS and the PWP in FXS. Future genomic studies of multiple brain

regions, cell types and developmental time points could identify gene networks modulated by paternal versus maternal *Cyfip1* deletion and the molecular mechanisms underlying the differences in eating behavior. Furthermore, PO effects of *Cyfip1* deletion could be tested for behavioral and genomic interactions with imprinted genes in PWS models of hyperphagia (e.g., deletion of *Magel2* or *Snord116*). Such efforts could inform pharmacotherapeutic treatment of eating behavior tailored to a particular subtype of neurodevelopmental syndrome.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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FIGURE LEGENDS

Figure 1: Generation of the *Cyfip1*$^{+/-}$ allele and breeding scheme for *Cyfip1*$^{+/-}$ mice on C57BL/6N and C57BL/6J backgrounds. (A): A schematic of the knockout first allele for KOMP generation of *Cyfip1*$^{N/-}$ mice (34) was obtained from the International Mouse Phenotyping Consortium (IMPC) website (http://www.mousephenotype.org/data/alleles/MGI:1338801/tm2a(EUCOMM)Wtsi). Mice containing floxed alleles flanking exons 4 through 6 were generated from embryonic stem cells on a C57BL/6N background by the International Knockout Mouse Consortium and were crossed to global Cre-expressing mice, yielding offspring heterozygous for deletions in exons 4 through 6. We derived mice heterozygous for the null deletion on a C57BL/6N background using sperm obtained from The Jackson Laboratory. **(B): Left panel:** In the first study, we re-derived *Cyfip1*$^{N/-}$ mice on an isogenic C57BL/6N background. **Right panel:** Mice were homozygous for the N allele (N/N) at *Cyfip2* which contains a missense mutation that we previously showed was associated with a marked enhancement of binge eating, accounting for approximately 50% of the genetic variance in parental strain binge eating (7). We maintained this colony on an isogenic C57BL/6N background by breeding *Cyfip1*$^{N/-}$ mice with C57BL/6NJ mice (black bars; N/N) ordered from The Jackson laboratory. **(C):** In the second study, we generated a second colony on a mixed background. The primary goal was to monitor and replace the binge-associated N/N *Cyfip2* alleles with C57BL/6J (J/J) alleles via backcrossing *Cyfip1*$^{N/-}$ mice to C57BL/6J (white bars; J/J) for three generations and assess the effect of *Cyfip1* deletion on binge eating on a mixed N3 background containing a fixed, binge-resistant, homozygous J/J genotype at *Cyfip2* (7). Mixed-color bars illustrate hypothetical recombination events that accumulate through backcrossing to C57BL/6J (white).

Figure 2. Premorbid compulsive-like marble burying in *Cyfip1*$^{N/-}$ and *Cyfip1*$^{J/-}$ mice. (A) Schematic of marble burying apparatus. **(B)** *Cyfip1*$^{N/-}$ mice buried more marbles with greater than 50% coverage of the marbles than wild-type *Cyfip1*$^{N/N}$ mice [U(102) = 1050; p = 0.031; two-tailed], and had a greater average percentage of burial across the marbles [**C**; F(1,96) = 7.1; p = 0.009]. **(D)** Similarly, J/- mice buried more marbles with greater than 50% coverage than J/J mice [U(89) = 754; p = 0.012, two-tailed],

and also trended toward a greater average percentage of burial (p = 0.083). **(E)** When the data were collapsed across genetic background, we found that *Cyfip1*$^{+/-}$ mice buried more marbles with greater than 50% coverage compared to Cyfip1 +/+ mice [**F;** U(203) = 3602; p = 0.0009; two-tailed] as well as a greater average percentage of burial [**G;** F(1,191) = 11.6; p = 0.0008].

Figure 3. PF consumption in *Cyfip1*$^{N/-}$ and *Cyfip1*$^{J/-}$ mice. (A) The conditioned place preference chamber has a smooth-textured non-food-paired side (left) and a rough-textured food-paired side. **(B)** Both wild-type *Cyfip1*$^{N/N}$ and haploinsufficient *Cyfip1*$^{N/-}$ mice trained with PF in the CPP chamber ate more food over time than Chow-trained mice [main effect of Treatment: F(1,932) = 274.7; p < 2x10^{-16}]. There was also a main effect of Sex [F(1,932) = 30.4; p = 4.5 x 10^{-8}], a Genotype x Treatment interaction [F(1,932) = 4.7; p = 0.03], and a Treatment x Sex interaction [F(1,932) = 22.3; p = 2.7 x 10^{-6}]. **(C)** Both PF-trained genotypes exhibited slopes that were significant from zero (*Cyfip1*$^{N/N}$: m = 0.009 ± 0.003, p = 0.024; *Cyfip1*$^{N/-}$: m = 0.005 ± 0.002, p = 0.045, respectively) indicating escalation in PF intake over time. However, neither Chow-trained group showed escalation. Moreover, PF-trained *Cyfip1*$^{N/-}$ mice showed a significantly greater y-intercept than all other groups ($: p < 0.008), indicating consistently greater overall food consumption throughout the study. **(D)** When we examined the same behaviors in *Cyfip1*$^{J/J}$ versus *Cyfip1*$^{J/-}$ mice, we observed main effects of Treatment [F(1,542) = 106.2; p = 2 x 10^{-16}] and Sex [F(1,542) = 27.0, p = 3.0 x 10^{-7}], but no effect of Day and no effect of Genotype (p = 0.10). **(E)** In examining escalation in food intake over time, none of the slopes were significantly different from zero (all p's > 0.06 vs. zero), indicating that none of the groups showed an escalation in intake.

Figure 4. Effect of parent-of-origin (PO) on PF consumption in *Cyfip1*$^{N/-}$ and *Cyfip1*$^{J/-}$ mice. (A): In considering mice on a *Cyfip2*$^{N/N}$ background, there was a main effect of Genotype [F(1,464) = 12.3; p = 0.0005], PO [F(1,464) = 9.0; p = 0.003], Sex [F(1,464) = 39.4; p = 8.0 x 10^{-10}], and Day [F(1,464) = 20.8, p = 6.5 x 10^{-6}]. The effect of Genotype was explained by *Cyfip1*$^{N/-}$ mice consuming more PF than *Cyfip1*$^{N/N}$ mice. The effect of Sex was explained by females consuming more PF than males (not

shown). The effect of PO was explained by offspring derived from parents with a paternal *Cyfip1* deletion consuming a greater amount of PF than offspring derived from parents with a maternal *Cyfip1* deletion. **(B):** No differences were observed among the groups in the slope of escalation in PF consumption [F(3,16) = 0.7 p = 0.56]; however, paternally deleted *Cyfip1*$^{N/-}$ mice showed a greater overall consumption than either of the N/N groups as indicated by a greater y-intercept ($: both p's < 0.02) and *Cyfip1*$^{N/N}$ mice derived from families with a paternal deletion showed a greater y-intercept than *Cyfip1*$^{N/N}$ mice with a maternal deletion (p = 0.046). **(C):** In considering mice on a *Cyfip2* J/J background, there was a main effect of PO [F(1,268) = 9.7; p = 0.002] in that offspring derived from parents with a paternal *Cyfip1* deletion ate more PF than offspring derived from parents with a maternal *Cyfip1* deletion. Importantly, there was a Genotype x PO interaction [F(1,268) = 8.9; p = 0.003] that reflected a generally greater PF consumption in *Cyfip1*$^{J/-}$ mice with a paternally versus maternally inherited deletion, in particular with the earlier time points. **(D):** The increase in PF consumption that occurred early on in paternally deleted *Cyfip1*$^{J/-}$ mice is reflected statistically by the significantly greater y-intercept compared to all other groups ($: p<0.0001). Nevertheless, there was no escalation in PF intake over time for any of the groups as none of them showed slopes that were significantly different from zero (all p's > 0.19). **(E):** Data were standardized for each genetic background using the average background consumption across Training Days in order to control for variance in PF consumption across the two genetic backgrounds and permit combining datasets to increase our power to detect effects of *Cyfip1* Genotype, PO, and their interaction (see Methods for additional details). Negative consumption values denote negative deviation from mean genetic background consumption across Training Days whereas positive consumption reflects positive deviation from mean genetic background consumption across Training Days (standardize mean = 0, standard deviation = 1). We found a main effect of Genotype [F(1,748) = 8.2; p = 0.004], PO [F(1,748) = 15.5; p = 8.9 x 10^{-5}], and Day [F(1,748) = 15.3; p = 0.0001], and a Genotype x PO interaction [F(1,748) = 4.1; p = 0.04]. **(F):** There was a significant escalation in consumption relative to zero in all groups (all p's < 0.03) except for the paternally deleted *Cyfip1*$^{+/-}$ group (p = 0.31) which exhibited a greater y-intercept than the other three groups [$: F(1,748) = 17.5; p < 0.0001]. Thus, in considering the combined data derived from both

Cyfip2 genetic backgrounds, there is an overall increase in PF consumption in *Cyfip1*$^{+/-}$ mice that depends on paternal inheritance of the *Cyfip1* deletion.

Table 1.

(A): *Cyfip2* N/N background (normalized to a single wild-type)			
	Maternal	Paternal	(Paternal vs. Maternal)
Cyfip1	**0.60 (0.04)**	**0.65 (0.04)**	t14<1
Cyfip2	1.11 (0.12)	1.08 (0.08)	t14<1
Magel2	0.81 (0.1)	1.01 (0.06)	t14=1.65; p=0.12

(B): *Cyfip2* N/N background (normalized to maternal and paternal wild-types)					
	Maternal	Paternal	(Paternal vs. Maternal)		
Cyfip1	**0.58 (0.04)**	**0.67 (0.04)**	t14=1.55; p = 0.14		
Cyfip2	1.11 (0.12)	1.11 (0.08)	t14<1		
Magel2	0.84 (0.12)	0.98 (0.09)	t14<1		

(C): *Cyfip2* J/J background (normalized to a single wild-type)			
	Maternal	Paternal	(Paternal vs. Maternal)
Cyfip1	**0.54 (0.03)**	**0.81* (0.09)**	***t12=2.95; p=0.012**
Cyfip2	0.87 (0.07)	1.06 (0.08)	t12=1.77; p=0.10
Magel2	0.96 (0.06)	0.91 (0.03)	t12<1

(D): *Cyfip2* J/J background (normalized to maternal and paternal wild-types)					
	Maternal	Paternal	(Paternal vs. Maternal)		
Cyfip1	**0.57(0.03)**	**0.8* (0.08)**	***t12=2.69; p=0.02**		
Cyfip2	0.85 (0.08)	1.06 (0.09)	t12=1.73; p=0.11		
Magel2	0.92 (0.06)	0.96 (0.04)	t12<1		

(E): Combined backgrounds (normalized to a single wild-type)			
	Maternal	Paternal	(Paternal vs. Maternal)
Cyfip1	**0.57 (0.03)**	**0.72* (0.05)**	t28=2.79; p=0.009
Cyfip2	1.00 (0.08)	1.07 (0.05)	t28<1
Magel2	0.88 (0.06)	0.96 (0.04)	t28=1.08; p=0.29

(F): Combined backgrounds (normalized to maternal and paternal wild-types)					
	Maternal	Paternal	(Paternal vs. Maternal)		
Cyfip1	**0.57 (0.02)**	**0.73* (0.05)**	t28=3.02; p=0.005		
Cyfip2	0.99 (0.08)	1.08 (0.06)	t28<1		
Magel2	0.87 (0.07)	0.99 (0.04)	t28=1.42; p=0.17		

Table 1. Changes in *Cyfip1*, *Cyfip2*, and *Magel2* expression in *Cyfip*$^{+/-}$ mice as a function of PO on either the *Cyfip2*$^{N/N}$ or *Cyfip2*$^{J/J}$ genetic background. All samples were harvested from mice that were naïve to PF training. Samples (n = 7-9 per Genotype per PO) were balanced across experimental cohorts and qPCR plates. Changes in gene expression in *Cyfip1*$^{+/-}$ versus wild-type *Cyfip1*$^{+/+}$ were calculated relative to either a single wild-type control (n=14-16) or relative to separate maternal and paternal wild-type controls. Data are shown as mean ± S.E.M. for fold-change in expression using the $2^{-\Delta\Delta CT}$ method. Bolded results indicate values that were significantly decreased relative to wild-type ($p < 0.05$; unpaired t-test). * indicates a significant difference between Paternal versus Maternal *Cyfip1*$^{+/-}$ groups ($p<0.05$; unpaired t-test).

Figure 1.

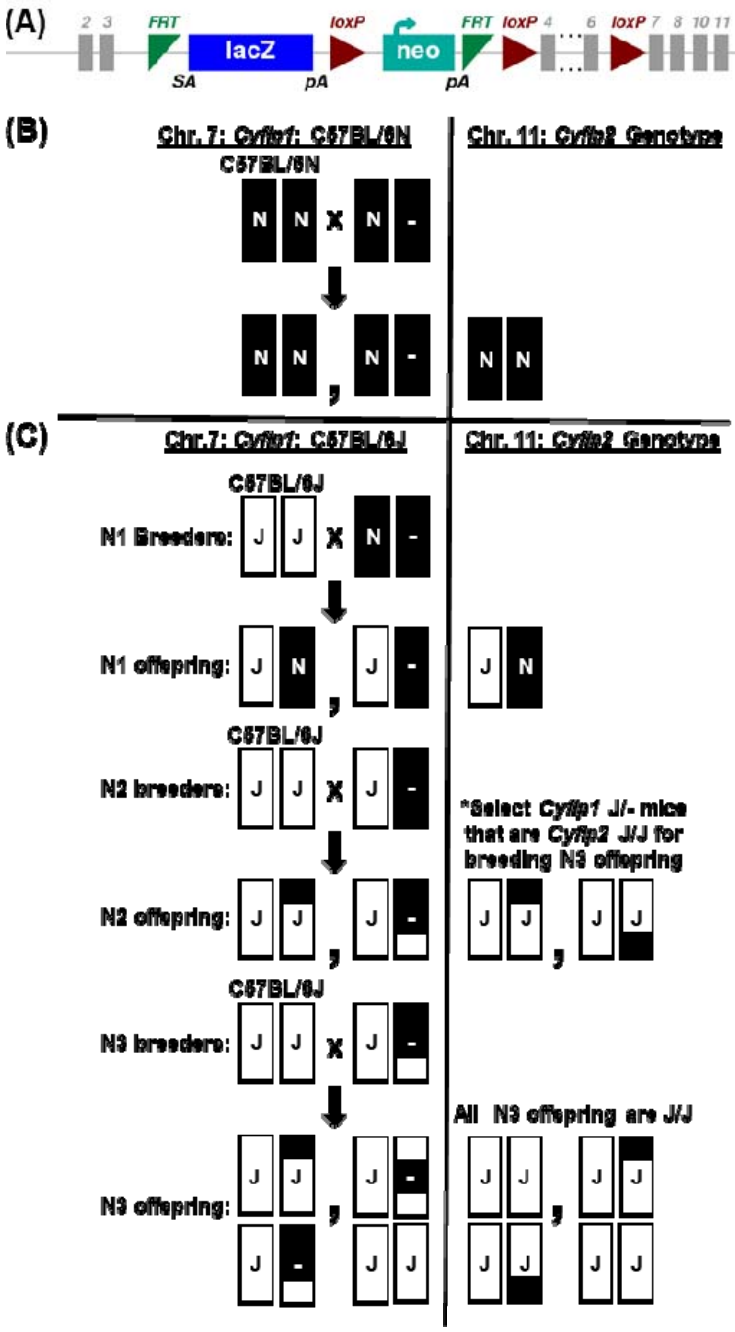

(A)
2 3
FRT
lacZ
loxP
neo
FRT
loxP
4
6
loxP
7 8 10 11
SA
pA
pA
(B)
Chr. 7: Cyflip1: C57BL/6N
Chr. 11: Cyflip2 Genotype
C57BL/6N
N N x N -
N N , N -
N N
(C)
Chr.7: Cyflip1: C57BL/6J
Chr. 11: Cyflip2 Genotype
C57BL/6J
N1 Breeders: J J x N -
N1 offspring: J N , J -
J N
C57BL/6J
N2 breeders: J J x J -
*Select Cyflip1 J/- mice that are Cyflip2 J/J for breeding N3 offspring
N2 offspring: J J , J -
J J , J J
C57BL/6J
N3 breeders: J J x J -
All N3 offspring are J/J
N3 offspring: J J , J -
J - J J
J J , J J
J J J J

Figure 2.

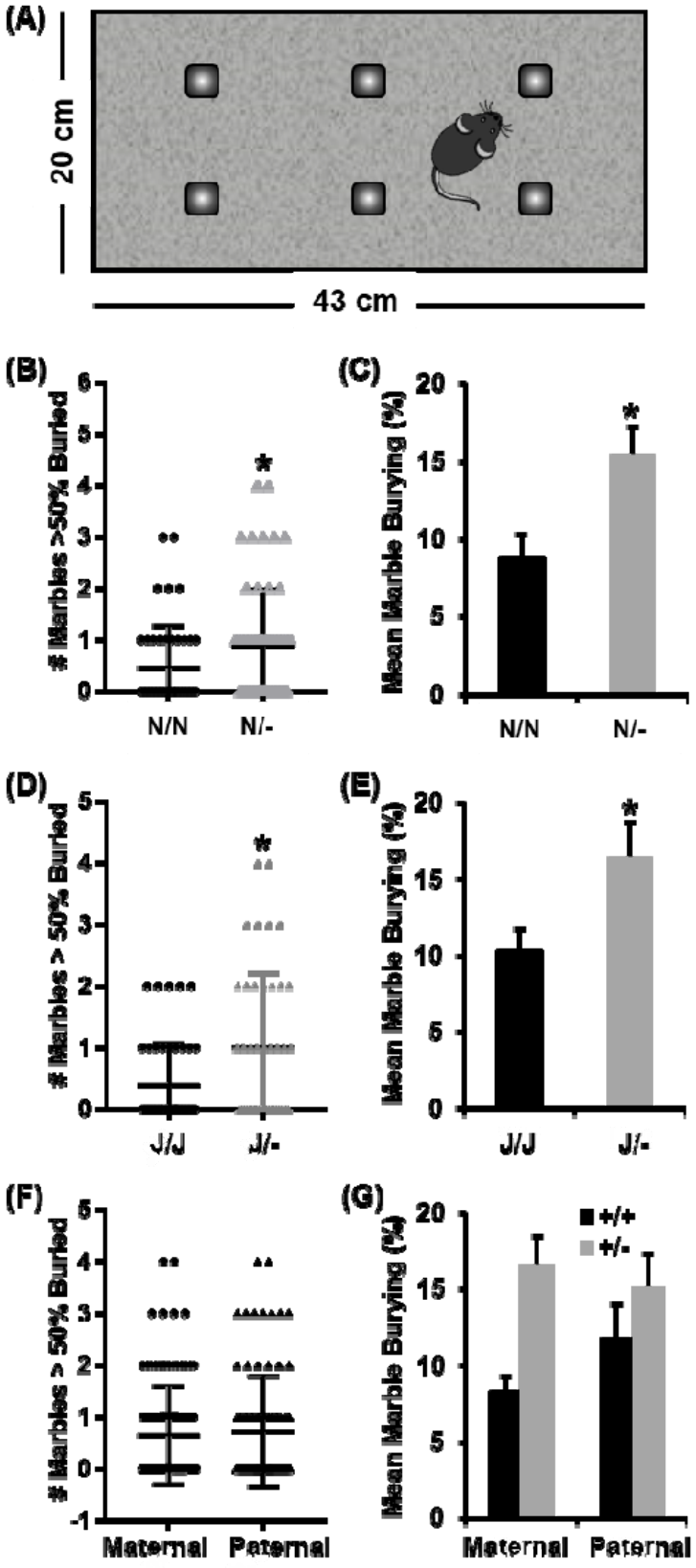

(A)
20 cm
43 cm
(B)
Marbles >50% Buried
N/N
N/-
(C)
Mean Marble Burying (%)
N/N
N/-
(D)
Marbles > 50% Buried
J/J
J/-
(E)
Mean Marble Burying (%)
J/J
J/-
(F)
Marbles > 50% Buried
Maternal
Paternal
(G)
Mean Marble Burying (%)
+/+
+/-
Maternal
Paternal

Figure 3.

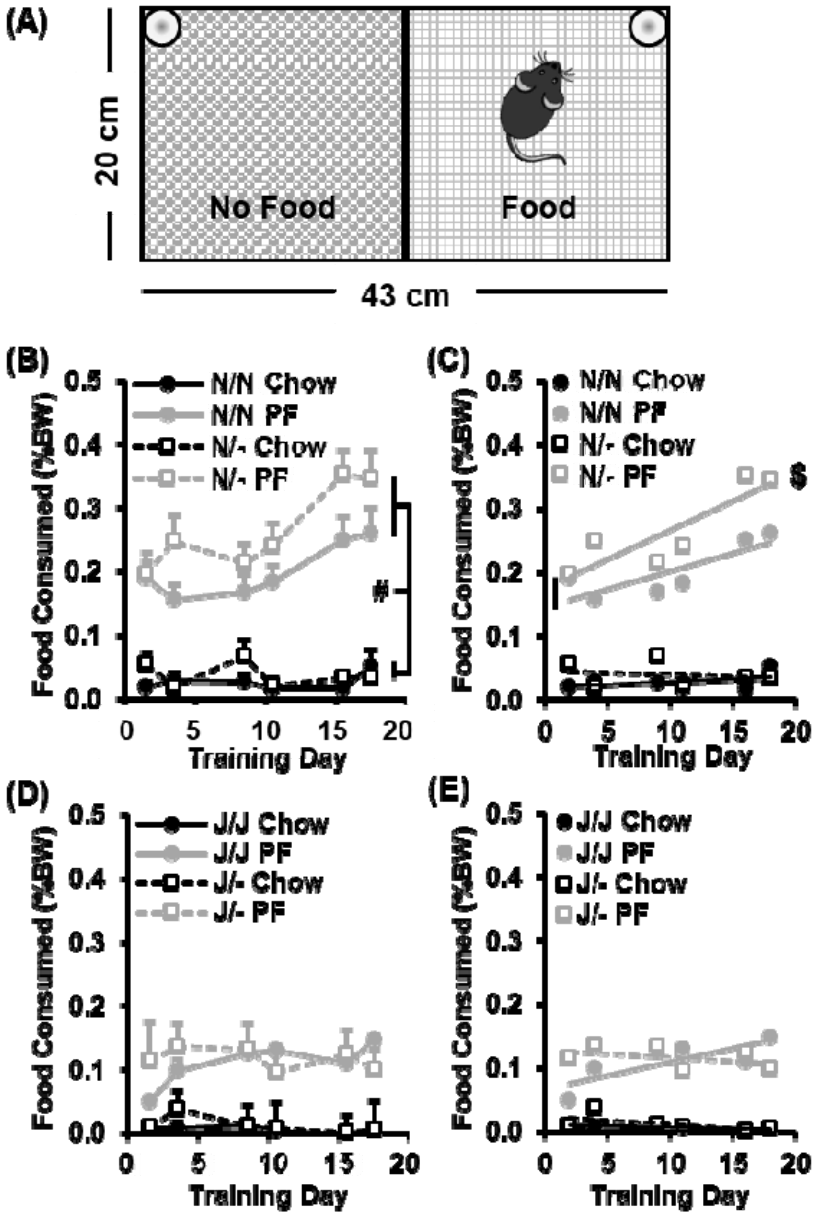
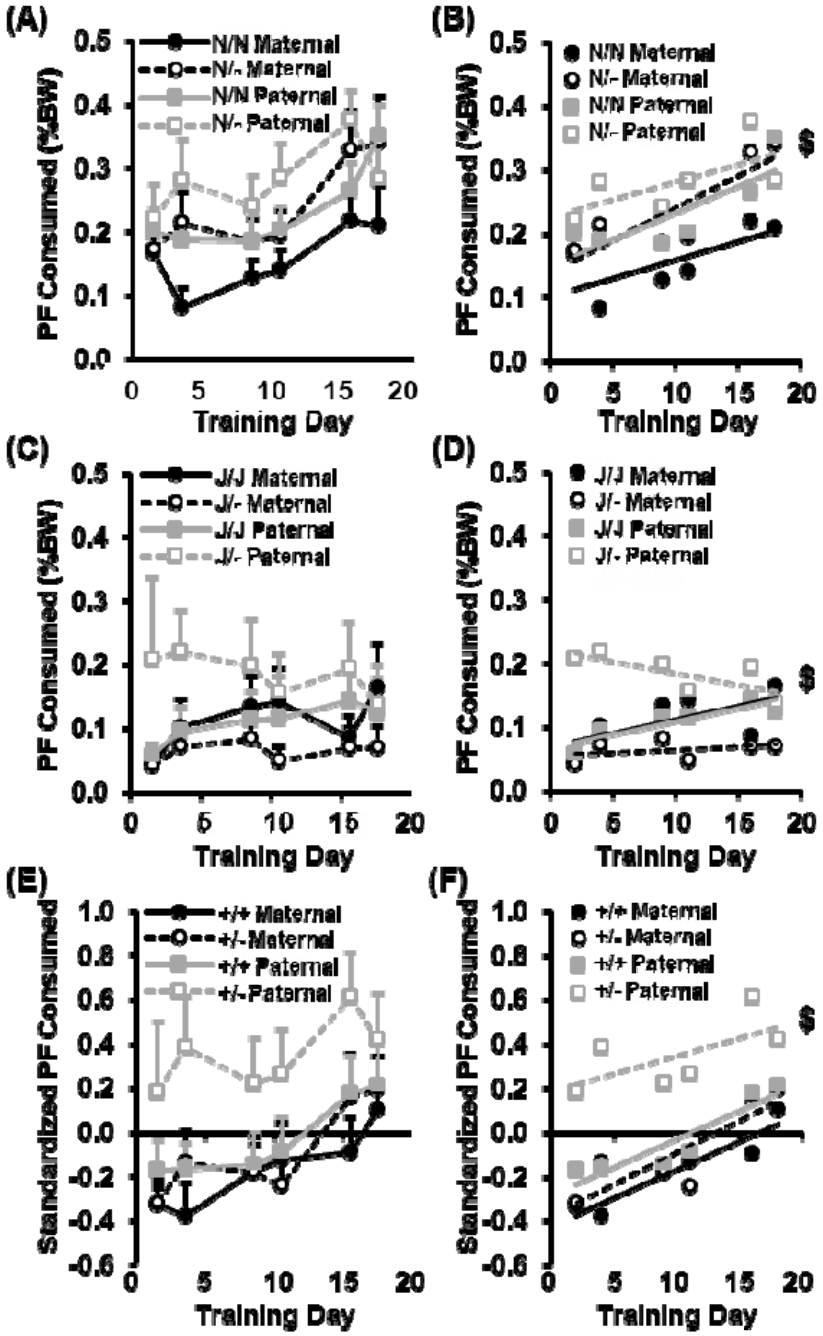

(A)
20 cm
No Food
Food
43 cm
(B)
N/N Chow
N/N PF
N/- Chow
N/- PF
Food Consumed (%BW)
Training Day
#
(C)
N/N Chow
N/N PF
N/- Chow
N/- PF
$
Food Consumed (%BW)
Training Day
(D)
J/J Chow
J/J PF
J/- Chow
J/- PF
Food Consumed (%BW)
Training Day
(E)
J/J Chow
J/J PF
J/- Chow
J/- PF
Food Consumed (%BW)
Training Day

Figure 4.


(A)
N/N Maternal
N/- Maternal
N/N Paternal
N/- Paternal
PF Consumed (%BW)
Training Day
(B)
N/N Maternal
N/- Maternal
N/N Paternal
N/- Paternal
$
PF Consumed (%BW)
Training Day
(C)
J/J Maternal
J/- Maternal
J/J Paternal
J/- Paternal
PF Consumed (%BW)
Training Day
(D)
J/J Maternal
J/- Maternal
J/J Paternal
J/- Paternal
$
PF Consumed (%BW)
Training Day
(E)
+/+ Maternal
+/- Maternal
+/+ Paternal
+/- Paternal
Standardized PF Consumed
Training Day
(F)
+/+ Maternal
+/- Maternal
+/+ Paternal
+/- Paternal
$
Standardized PF Consumed
Training Day

(A): *Cyfip2* N/N background (normalized to a single wild-type)			
	Maternal	Paternal	(Paternal vs. Maternal)
Cyfip1	**0.60 (0.04)**	**0.65 (0.04)**	t14<1
Cyfip2	1.11 (0.12)	1.08 (0.08)	t14<1
Magel2	0.81 (0.1)	1.01 (0.06)	t14=1.65; p=0.12

(B): *Cyfip2* N/N background (normalized to maternal and paternal wild-types)					
	Maternal	Paternal	(Paternal vs. Maternal)		
Cyfip1	**0.58 (0.04)**	**0.67 (0.04)**	t14=1.55; p = 0.14		
Cyfip2	1.11 (0.12)	1.11 (0.08)	t14<1		
Magel2	0.84 (0.12)	0.98 (0.09)	t14<1		

(C): *Cyfip2* J/J background (normalized to a single wild-type)			
	Maternal	Paternal	(Paternal vs. Maternal)
Cyfip1	**0.54 (0.03)**	**0.81* (0.09)**	***t12=2.95; p=0.012**
Cyfip2	0.87 (0.07)	1.06 (0.08)	t12=1.77; p=0.10
Magel2	0.96 (0.06)	0.91 (0.03)	t12<1

(D): *Cyfip2* J/J background (normalized to maternal and paternal wild-types)					
	Maternal	Paternal	(Paternal vs. Maternal)		
Cyfip1	**0.57(0.03)**	**0.8* (0.08)**	***t12=2.69; p=0.02**		
Cyfip2	0.85 (0.08)	1.06 (0.09)	t12=1.73; p=0.11		
Magel2	0.92 (0.06)	0.96 (0.04)	t12<1		

(E): Combined backgrounds (normalized to a single wild-type)			
	Maternal	Paternal	(Paternal vs. Maternal)
Cyfip1	**0.57 (0.03)**	**0.72* (0.05)**	t28=2.79; p=0.009
Cyfip2	1.00 (0.08)	1.07 (0.05)	t28<1
Magel2	0.88 (0.06)	0.96 (0.04)	t28=1.08; p=0.29

(F): Combined backgrounds (normalized to maternal and paternal wild-types)					
	Maternal	Paternal	(Paternal vs. Maternal)		
Cyfip1	**0.57 (0.02)**	**0.73* (0.05)**	t28=3.02; p=0.005		
Cyfip2	0.99 (0.08)	1.08 (0.06)	t28<1		
Magel2	0.87 (0.07)	0.99 (0.04)	t28=1.42; p=0.17		

Table 1. Changes in *Cyfip1*, *Cyfip2*, and *Magel2* expression in *Cyfip*$^{+/-}$ mice as a function of PO on either the *Cyfip2*$^{N/N}$ or *Cyfip2*$^{J/J}$ genetic background. All samples were harvested from mice that were naïve to PF training. Samples (n = 7-9 per Genotype per PO) were balanced across experimental cohorts and qPCR plates. Changes in gene expression in *Cyfip1*$^{+/-}$ versus wild-type *Cyfip1*$^{+/+}$ were calculated relative to either a single wild-type control (n=14-16) or relative to separate maternal and paternal wild-type controls. Data are shown as mean ± S.E.M. for fold-change in expression using the $2^{-\Delta\Delta CT}$ method. Bolded results indicate values that were significantly decreased relative to wild-type ($p < 0.05$; unpaired t-test). * indicates a significant difference between Paternal versus Maternal *Cyfip1*$^{+/-}$ groups ($p<0.05$; unpaired t-test).

Figure 1.

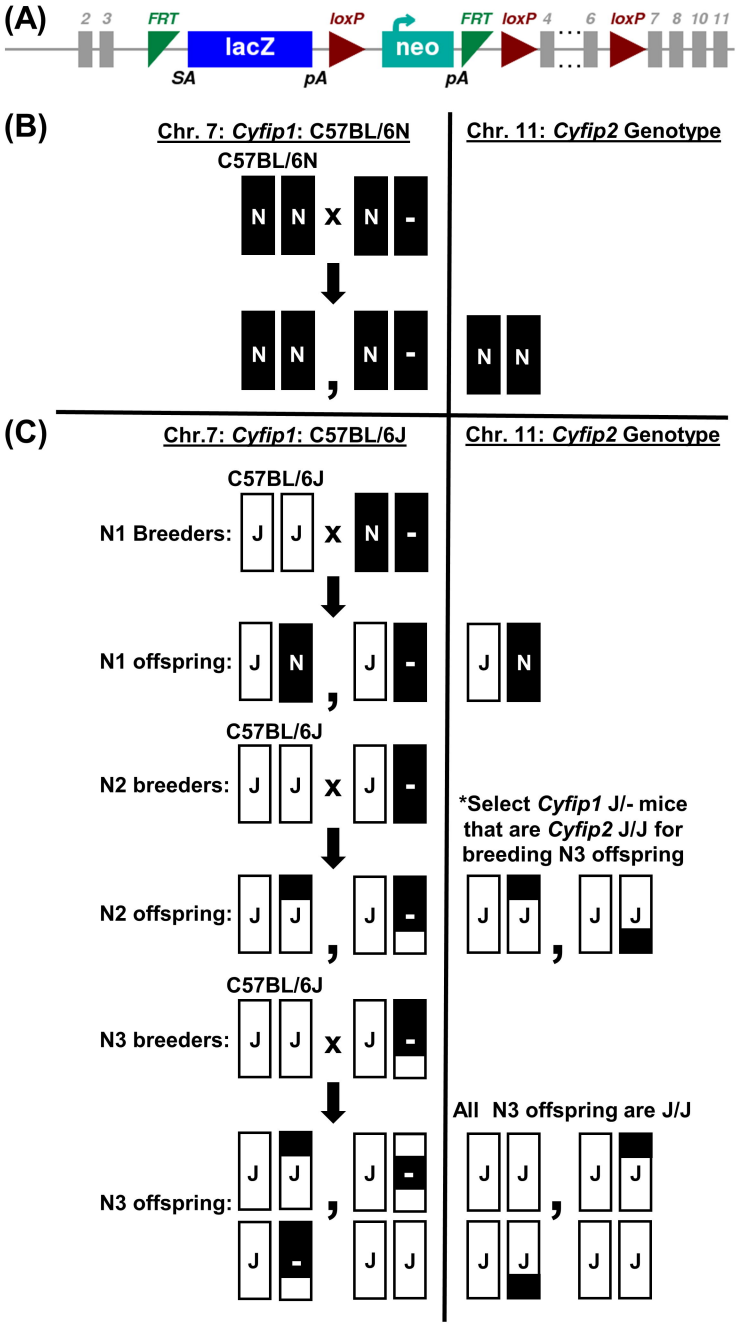

(A)
2 3
FRT
lacZ
SA
pA
loxP
neo
pA
FRT
loxP
4
6
loxP
7 8 10 11
(B)
Chr. 7: Cyfip1: C57BL/6N
C57BL/6N
N N x N -
N N , N -
Chr. 11: Cyfip2 Genotype
N N
(C)
Chr.7: Cyfip1: C57BL/6J
Chr. 11: Cyfip2 Genotype
C57BL/6J
N1 Breeders: J J x N -
N1 offspring: J N , J -
J N
C57BL/6J
N2 breeders: J J x J -
*Select Cyfip1 J/- mice that are Cyfip2 J/J for breeding N3 offspring
N2 offspring: J J , J -
J J , J J
C57BL/6J
N3 breeders: J J x J -
All N3 offspring are J/J
N3 offspring: J J , J -
J - J J
J J , J J
J J J J

Figure 2.

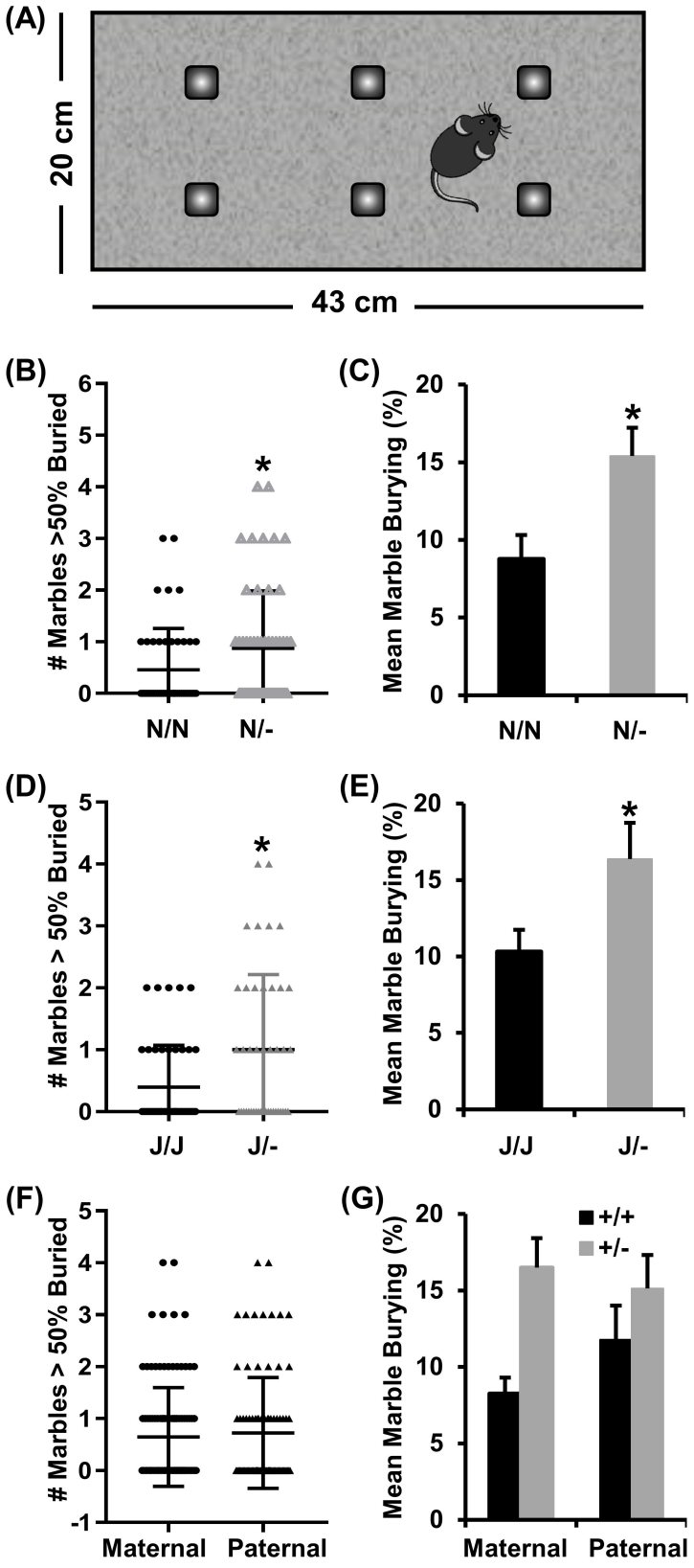

(A)
20 cm
43 cm
(B)
Marbles >50% Buried
N/N
N/-
(C)
Mean Marble Burying (%)
N/N
N/-
(D)
Marbles > 50% Buried
J/J
J/-
(E)
Mean Marble Burying (%)
J/J
J/-
(F)
Marbles > 50% Buried
Maternal
Paternal
(G)
Mean Marble Burying (%)
+/+
+/-
Maternal
Paternal

Figure 3.

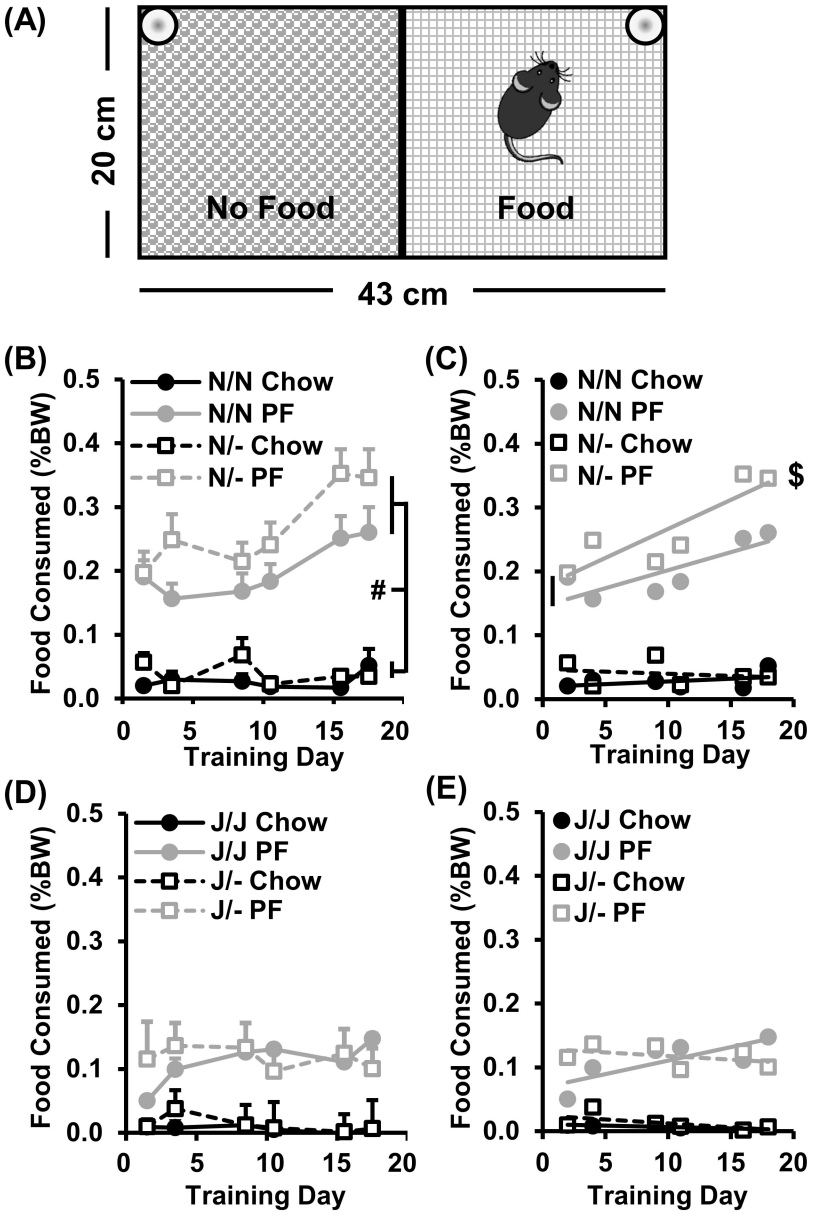
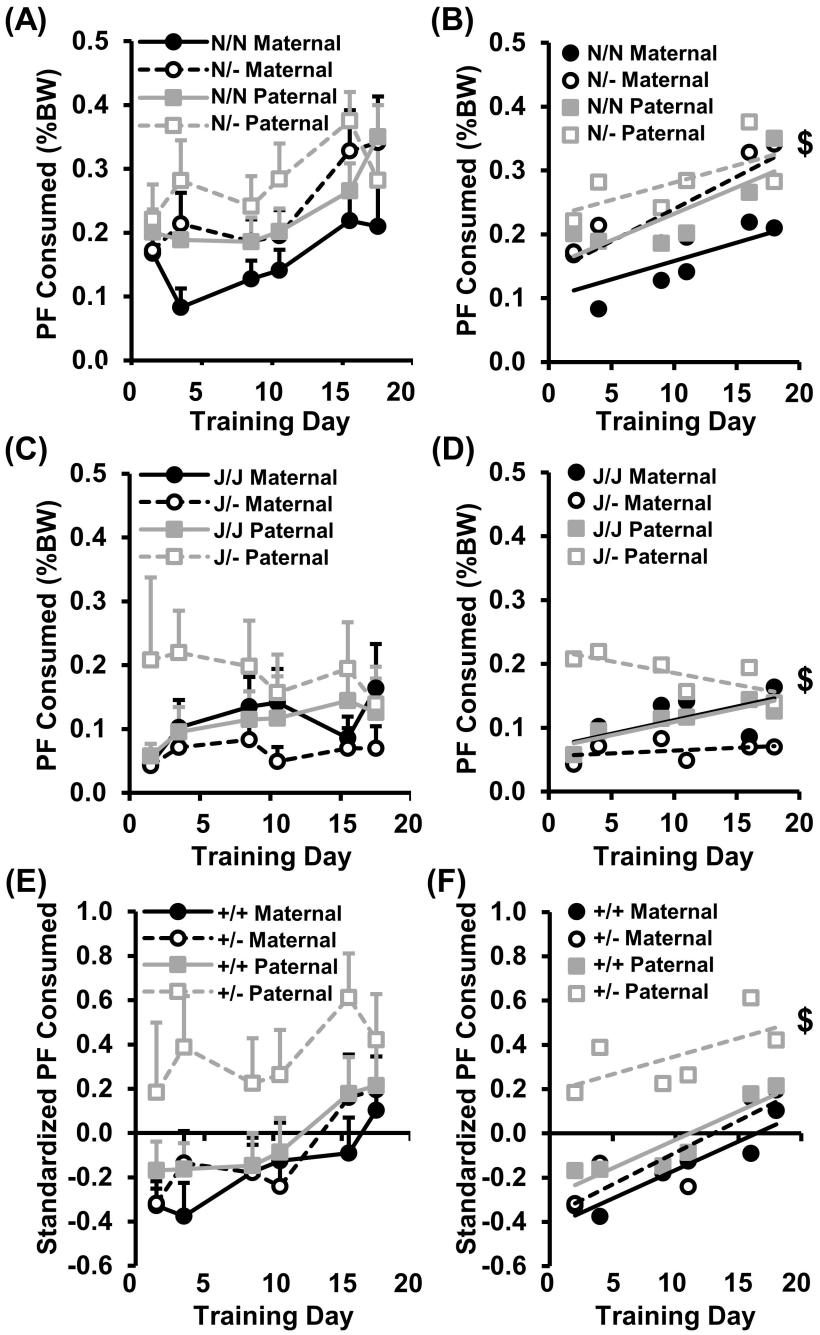

(A)
20 cm
No Food
Food
43 cm
(B)
N/N Chow
N/N PF
N/- Chow
N/- PF
Food Consumed (%BW)
Training Day
#
(C)
N/N Chow
N/N PF
N/- Chow
N/- PF
$
Food Consumed (%BW)
Training Day
(D)
J/J Chow
J/J PF
J/- Chow
J/- PF
Food Consumed (%BW)
Training Day
(E)
J/J Chow
J/J PF
J/- Chow
J/- PF
Food Consumed (%BW)
Training Day

Figure 4.


(A)
N/N Maternal
N/- Maternal
N/N Paternal
N/- Paternal
PF Consumed (%BW)
Training Day
(B)
N/N Maternal
N/- Maternal
N/N Paternal
N/- Paternal
$
PF Consumed (%BW)
Training Day
(C)
J/J Maternal
J/- Maternal
J/J Paternal
J/- Paternal
PF Consumed (%BW)
Training Day
(D)
J/J Maternal
J/- Maternal
J/J Paternal
J/- Paternal
$
PF Consumed (%BW)
Training Day
(E)
+/+ Maternal
+/- Maternal
+/+ Paternal
+/- Paternal
Standardized PF Consumed
Training Day
(F)
+/+ Maternal
+/- Maternal
+/+ Paternal
+/- Paternal
$
Standardized PF Consumed
Training Day