Quantitative trait locus mapping of binge-like eating and its motivational components in a reduced complexity cross: Implications for genome-wide studies of food "addiction" and eating disorder traits

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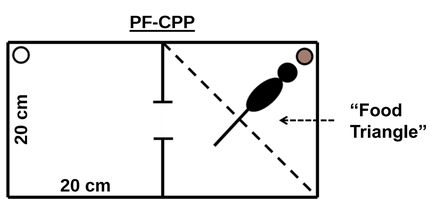
Background: Eating disorders, including Binge Eating Disorder, are highly lethal psychiatric conditions that exhibit a lifetime prevalence of 1 to 3%. Although they are heritable, genome-wide association studies in humans have yet to identify the causal genetic factors. Mammalian model organisms offer a powerful approach to studying the genetic basis of heritable traits that define eating disorders, including binge eating and its motivational components. Here, we wished to develop a forward genetic mouse model of binge-like eating with the goal of discovering novel genetic factors that contribute to this clinically important trait. We used C57BL/6 (B6) inbred substrains which have proven to be extremely useful in identifying novel quantitative trait genes for complex traits such as locomotor sensitization to cocaine (Kumar et al., *Science*, 2013, 342: 1508-12). C57BL/6J (B6J) and C57BL/6NJ (B6NJ) show robust strain differences in several behavioral traits, yet they contain only approximately 10,000 genetic variants. Thus, B6 substrains contain a markedly reduced genetic complexity compared to other laboratory inbred strains that typically possess millions of SNPs. Notably, in addition to cocaine behavioral traits, B6 substrains demonstrate differences in anxiety-like behavior – because both substance abuse and anxiety are co-morbid with binge eating, a cross between B6 substrains permits the ability to determine whether there is a shared genetic basis.

Methods: We used a conditioned place preference (CPP) procedure that allowed us to measure both consumption and conditioned reward for palatable food (PF). Outbred CFW mice, B6J mice, B6NJ mice, B6J x B6NJ-F_1 mice, and -F_2 mice (N=125) were assessed for initial preference for the palatable food-paired side in a two-chamber design on Day 1. On Training Days 2, 4, 9, 11, 16, 18, and 23, mice were provided limited access to a porcelain dish containing palatable food pellets (5-TUL, Test Diet®, St. Louis, MO) for 30 min. On Training Days 3, 5, 10, 12, 17, and 19, mice were provided a clean, empty porcelain dish with no food for 30 min. Mice were assessed weekly for conditioned place preference for the palatable food-paired side (PF-CPP) on Days 8, 15, and 22. On Day 23, mice were assessed once again for binge-like eating. B6J, B6NJ, and F_2 mice were also assessed for anxiety-like behavior in the elevated plus maze (EPM). All behavioral data were video recorded and tracked using AnyMaze software (Stoelting Co., Wood Dale, IL). Quantitative trait locus (QTL) mapping was conducted for palatable food consumption, PF-CPP, and EPM behavior in R/qtl using 96 informative markers (1000 permutations; $p < 0.05$).

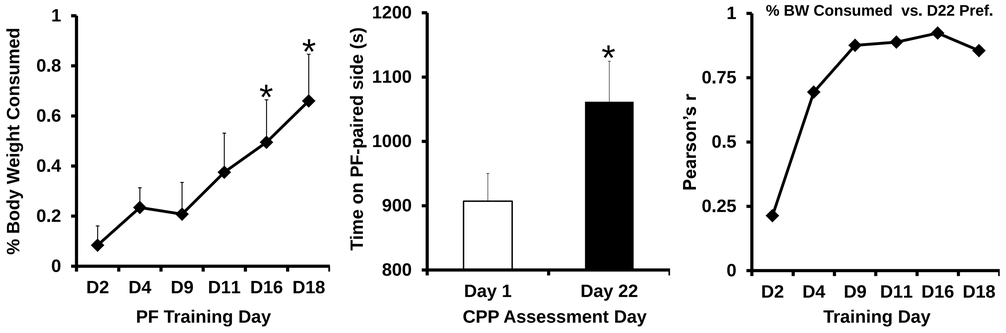
Results: Outbred CFW mice exhibited a nine-fold escalation in PF consumption that was accompanied by PF-CPP. Strikingly, the escalation in consumption coincided with an escalating, nearly perfect correlation with PF-CPP ($r = 0.95$), thus assigning increasing motivational value behind each binge episode. The B6NJ strain showed robust binge-like eating that was accompanied by PF-CPP and conditioned locomotor activity whereas the closely related C57BL/6J substrain (B6J) did not show either behavior. Interestingly, B6NJ also showed a three-fold increase in anxiety-like behavior relative to B6J, even prior to palatable food training, supporting the hypothesis that anxiety is a risk factor for binge eating. Importantly, we identified a single genome-wide significant QTL on chromosome 11 that was responsible for differences in both palatable food consumption (LOD = 3.6-5.8; peak = 24-34 Mb) and conditioned food reward (LOD = 4.0; peak = 39 Mb; B6NJ allele > B6NJ allele for both traits). Finally, we identified a second, independent QTL on chromosome 11 (LOD = 3.5; peak marker = 82 Mb) that influenced anxiety-like behavior.

Discussion: Outbred CFW and B6NJ inbred mice showed binge-like eating and conditioned food reward whereas B6J mice did not. We identified a single QTL on chromosome 11 that influenced both the consummatory and motivational properties of palatable food consumption, indicating that binge eating and conditioned food reward are mediated by the same genetic factor(s). Interestingly, nearly the same locus was also identified for cocaine-induced locomotor sensitization suggesting a common underlying genetic basis. The identification of a second, distinct locus on chromosome 11 that influenced anxiety-like behavior indicates a separate genetic mechanism for this motivational trait. The reduced genetic complexity of this cross will greatly accelerate the identification of QTL genes. Future directions will include mapping expression QTLs (eQTLs) from striatal tissue and using CRISPR/Cas9 to genome edit the candidate, quantitative trait nucleotides that underlie the QTLs. Lastly, we will use the CFW outbred strain and other high resolution, genetically diverse mapping populations to enhance our understanding of the genetic architecture of binge eating. Our results could inform translational genetic studies and novel pharmacotherapeutic development for treating binge eating in humans.

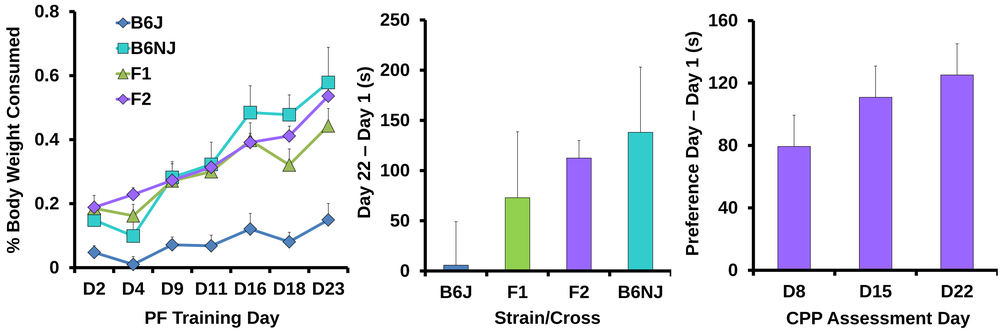
Preference Days	Training Days						
	D1	D2	D3	D4	D5	D6	D7
Week 1	open access	food right	no food left	food right	no food left	home cage	home cage
	D8	D9	D10	D11	D12	D13	D14
Week 2	open access	food right	no food left	food right	no food left	home cage	home cage
	D15	D16	D17	D18	D19	D20	D21
Week 3	open access	food right	no food left	food right	no food left	home cage	home cage
	D22	D23	D24				
Week 4	open access	food right	EPM				



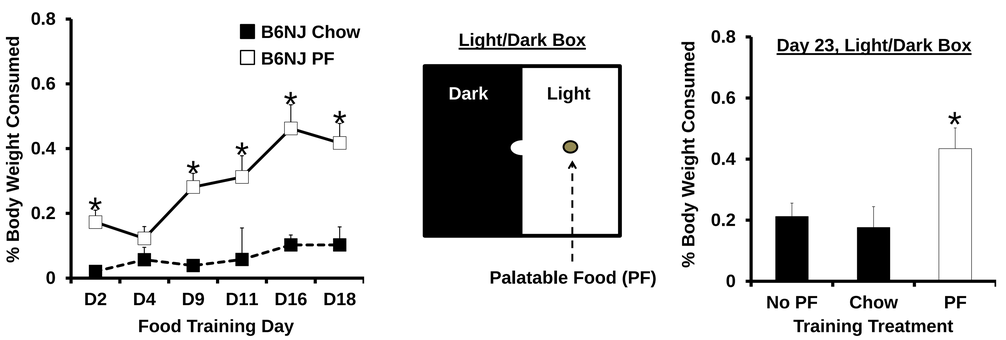
1. Binge eating and PF-CPP in outbred CFW mice



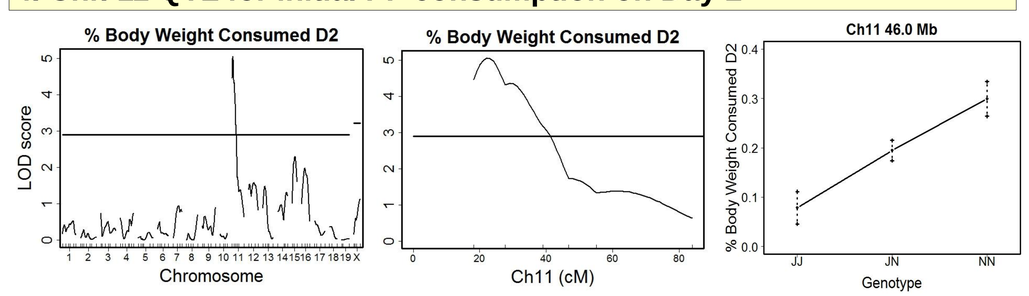
2. Binge eating and PF-CPP in B6J, B6NJ, F_1, and 122 F_2 mice



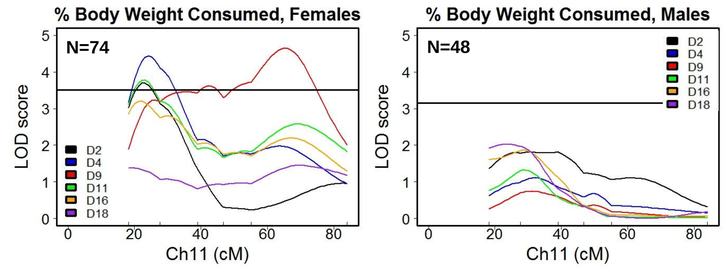
3. Binge eating of PF *but not chow* in binge-prone B6NJ mice



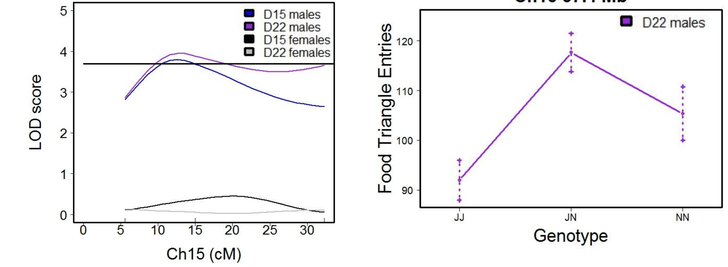
4. Chr. 11 QTL for initial PF consumption on Day 2



5. Chr. 11 QTL for PF consumption is female-selective and varies across PF training days



6. Male-selective Chr. 15 QTL for PF seeking behavior in the food triangle



7. Summary and Future Directions

- We developed a forward genetic model of binge eating and its motivational components in mice using the closely related B6J and B6NJ substrains.
- We identified a female-selective chr. 11 QTL (46 Mb) for initial PF consumption and a male-selective chr. 15 QTL for entries into the food triangle on D15 and D22.
- Interestingly, the chr. 11 QTL that we identified for PF consumption maps to the same region as a QTL influencing cocaine-induced locomotor sensitization *in the same genetic cross* (48 Mb Kumar et al., 2013; *Science*; 342(6165): 1508-12).
- We will measure the premorbid transcriptome and PF-induced transcriptome in parental strains to aid in identifying quantitative trait genes and ascribing a neurobiological mechanism.
- We will use genome editing via TALENs or CRISPR/Cas9 to rapidly validate functional variants responsible for initial and escalated PF consumption and the supporting motivational behaviors.
- We are also examining genetic differences in the hedonic properties of a sweetened sucrose solution in B6 substrains using a lickometer.

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