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Nociceptin receptor antagonist SB 612111 decreases high fat diet binge eating

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Abstract

Binge eating is a dysregulated form of feeding behavior that occurs in multiple eating disorders including binge-eating disorder, the most common eating disorder. Feeding is a complex behavioral program supported through the function of multiple brain regions and influenced by a diverse array of receptor signaling pathways. Previous studies have shown the overexpression of the opioid neuropeptide nociceptin (orphanin FQ, N/OFQ) can induce hyperphagia, but the role of endogenous nociceptin receptor (NOP) in naturally occurring palatability-induced hyperphagia is unknown. In this study we adapted a simple, replicable form of binge eating of high fat food (HFD). We found that male and female C57BL/6J mice provided with daily one-hour access sessions to HFD eat significantly more during this period than those provided with continuous 24 hour access. This form of feeding is rapid and entrained. Chronic intermittent HFD binge eating produced hyperactivity and increased light zone exploration in the open field and light-dark assays respectively. Treatment with the potent and selective NOP antagonist SB 612111 resulted in a significant dose-dependent reduction in binge intake in both male and female mice, and, unlike treatment with the serotonin selective reuptake inhibitor fluoxetine, produced no change in total 24-hour food intake. SB 612111 treatment also significantly decreased non-binge-like acute HFD consumption in male mice. These data are consistent with the hypothesis that high fat binge eating is modulated by NOP signaling and that the NOP system may represent a promising novel receptor to explore for the treatment of binge eating.

Keywords

nociceptin; high fat; binge eating; intermittent access; fluoxetine; hyperphagia

1. Introduction

Feeding is a critical survival behavior for animal species across phylogeny, including mammals. In humans, this behavior is subject to dysregulation in multiple psychiatric disorders including depression, anxiety disorders, and eating disorders. Binge eating is a form of feeding that is defined by consumption of a large amount of food in a short amount of time paired with a sense of loss of control^[1]. In humans, this pattern of feeding is accompanied by psychological components such as feelings of guilt or shame and is often preceded by anxiety or stress. The behavior of binge eating is moderately heritable (50–60%) and is observed across a range of eating disorders presentations including binge-eating disorder, bulimia nervosa, and a binge-purge subtype of anorexia nervosa^[2]. Genetic factors do not act alone, as environmental factors also contribute to liability^[3].

Pharmacotherapeutic treatment strategies for binge eating including serotonin selective reuptake inhibitors such as fluoxetine or citalopram and dopamine transporter-targeted compounds like lisdexamphetamine^[4,5]. Not all patients respond to these treatments, however, and side effects of these drugs limit their overall impact. Complementary pharmacological approaches are needed that target other signaling pathways engaged by binge eating, but our knowledge of the pathways that regulate binge eating behavior is incomplete.

Animal models have helped reveal complex neural networks and signaling pathways that act to increase or decrease feeding. Among the molecules that promote feeding are a host of neuropeptides including orexin, agouti related peptide, neuropeptide Y, melanin concentrating hormone, dynorphin, ghrelin, and the opioid-like molecule nociceptin^[6]. Nociceptin/Orphanin (N/OFQ) FQ binds a single opioid-like g-protein coupled receptor (NOP/ORL1) with no affinity for mu, kappa, or delta opioid receptors^[7–9]. It is a 17-amino acid peptide that it is encoded by the prepronociceptin gene^[10] that is expressed widely throughout the brain in mice, rats, and humans ^[11–14]. Early studies demonstrated that the overexpression of nociceptin or NOP agonists throughout the brain or in specific brain nuclei can produce hyperphagia ^[15–20]. These gain of function experiments point to the capacity of nociceptin signaling to produce feeding behavior, but the role of the endogenous nociceptin signaling pathway in behaviorally induced binge eating behavior is unclear.

Recently, several groups have developed highly potent and selective peptide and non-peptide-like NOP antagonists ^[17,19,21–24]. Early characterization demonstrated that the NOP antagonist SB 612111 could block the thermal hyperalgesic effects of nociceptin^[23]. Further characterization of this compound demonstrated that it had no effect on chow hyperphagia induced by food deprivation, though it was capable of blocking intracerebroventricular N/OFQ-induced hyperphagia. In this study we used adapted models of binge eating in rats and mice generated by intermittent access to high fat food to test if nociceptin signaling is required for this form of hyperphagia^[25,26]. Our results demonstrate that brief one-hour exposures to high fat containing food are sufficient to induce rapid and repetitive binge eating behavior in C57BL/6J mice; that chronic exposure to this form of binge eating is sufficient to induce changes in psychomotor behavior; and that intermittent high fat binge eating is modulated by nociceptin receptor signaling.

2. Materials and Methods

2.1 Animals

Adult C57BL/6J (n = 89 males, 24 females, Jackson Labs, Bar Harbor, ME) were group housed in ventilated cages (Tecniplast) in a colony room on a standard 12:12 h light-dark cycle with lights on at 7 AM. One animal with malocclusion was excluded from the vehicle group in Figure 3E. Unless otherwise noted animals had *ad libitum* access to food and water. For measurements of food intake and intermittent access binge eating, animals were singly housed. Mice were acclimated to single housing for a week prior to any measurements or experiments. Food hoppers were fitted with a custom made trapezoidal plexiglas divider to provide segregated access to multiple types of food at once. Cage changes were minimized during feeding measurements to limit stress effects on food consumption. All procedures were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill and performed in accordance with the National Institute of Health's guide for the care and use of laboratory animals.

2.2 Intermittent access high fat binge protocol

To limit the effects of food deprivation, stress, or novelty-induced suppression of feeding, we adapted our model from a previously published model by Czyzyk et al.^[25]. For our studies, individually housed mice were provided with either 1) *ad libitum* access to standard rodent chow (Harlan – 2020SX, caloric density = 3.1 kcal/gram) and high fat diet (HFD, Research Diets – 12492, 5.24 kcal/gram) or 2) *ad libitum* access to standard rodent chow and 1 hour daily intermittent access to HFD. Each day (11:00 AM, 4 hours into light cycle), body weights were measured and the overnight consumption of chow or HFD measured. Food was replenished daily for both food types and one-hour intake of both chow and HFD measured for both groups. At the end the hour, food weights were measured and the HFD removed from the intermittent access group. In this way we tracked consumption in the home cage during both intermittent and non-intermittent access sessions. All food consumption values are presented as normalized by the caloric density of the food divided by the body weight of the animal (kCal/g bodyweight).

2.3 Drugs and treatment design

SB 612111 (Tocris – cat no. 3573) was dissolved in DMSO and diluted in 0.9% sterile saline. Gentle heating at 45–50° C was used for 5 min after dissolution to ensure complete solubilization of the drug. 10 mg/kg stock was serially diluted in sterile saline to 3, 1, or 0.1 mg/kg. Fluoxetine (TCI chemicals – cat no. 56296) was dissolved directly in saline and injected at 30 mg/kg. All injections were intraperitoneal and injected at a volume of 10 ml/kg 30 min. prior to the onset of bingeing. SB 612111 drug treatments were performed in a modified Latin squares design to randomize drug order effects with 2 days of vehicle injections between drug treatment days. Animals were handled and injected with vehicle for two days prior to the first drug treatment. Because we observed a continuing gradual escalation of high fat food consumption over time, baseline binge consumption over the course of SB 612111 treatment as depicted in Fig. 3 were calculated from at least 2 vehicle treatment days interspersed between every drug dose. For tests of SB 612111 effects on the first HFD exposure (Figure 3E), animals were handled and injected with vehicle solution for

three separate days before treatment with SB 612111 and HFD exposure. Drug carryover effects between days were only observed for Fluoxetine. We used vehicle dimethyl sulfoxide (DMSO) concentrations up to 2.5% without observing any effects on high fat diet consumption.

2.4 Open Field Assay

Behavioral effects of intermittent access bingeing were assessed in an independent cohort of mice ($n = 10$ per group) after 3 weeks of chronic intermittent or continuous HFD exposure. No drug treatments were performed with these mice and an *ad libitum* chow only control group was included. In a previous study, the behavioral effects of intermittent access bingeing were assessed; however, the behavioral assays were performed 24 hours after the completion of a weekly binge cycle [25]. We reasoned that the timing of the behavioral assays relative their binge behavior may represent an important factor and hypothesized that the expectation of HFD access would produce changes in anxiety-like behavior. After three weeks of stable daily intermittent binge eating, we performed the behavioral assays during the time they would normally binge eat (11 am), and provided intermittent access to HFD following the completion of these assays (afternoon). We did not observe any changes in binge HFD consumption during this period.

Mice were placed in a square white plexiglas arena measuring 20.5 in. on each side and their movement recorded using a CCD camera. Mice were allowed to explore the box for 20 min. Behavior was tracked using Ethovision XT (Noldus Information Technology), where center was defined as the middle half of the box in both the X and Y planes.

2.5 Elevated Plus Maze

Mice were placed in a standard design elevated plus maze and allowed to explore the open and closed arms for 5 minutes. Exploratory behavior was tracked as previously described in the open field section.

2.6 Light/Dark Box Conflict Assay

Mice were placed in two-sided chambers containing a dark enclosed side and a brightly lit open side and allowed to explore both sides for 15 min. Behavior was tracked as previously described in the open field section.

2.7 Statistics

Statistical analyses were performed in Prism 6.0e (GraphPad; La Jolla, CA). Details of analyses are described in the figure legends or in the Results. In Figures 1 and 3, we did not hypothesize any sex differences so we did not assess those differences, but the data are presented together for illustrative purposes. Figures were assembled in Adobe Illustrator.

Results

3.1 Establishment of a model of high fat binge eating

To determine novel receptor signaling contributions to high fat binge eating, we developed a simple, replicable form of binge eating that limits the impact of stress and food deprivation.

We adapted our model from a previous study that provided access to high fat food on a weekly cycle [25,27]. We modified this model to promote daily hyperphagia by providing intermittent access to a palatable food containing 60% fat by weight in 1 hour sessions in the home cages during the light cycle and 24 hour access to standard rodent chow (Figure 1A). Under these conditions, we observe a highly significant increase in the amount of high fat diet (HFD) consumed during the 1 hour access periods in intermittent (**I-HFD**) animals relative to animals that receive 24 hour continuous (**C-HFD**) access (Figure 1B, **Males** - RM two-way ANOVA: group effect - $F(3, 75) = 163.8, p < 0.001$; time effect - $F(14, 1050) = 6.963, p < 0.0001$, group $\times$ time effect - $F(42, 1050) = 3.599, p < 0.0001$; **Females** - RM two-way ANOVA: group effect - $F(1, 22) = 398.4, p < 0.001$; time effect - $F(14, 308) = 3.79, p < 0.0001$; group $\times$ time effect - $F(14, 308) = 3.003, p < 0.0001$). Between group post hoc comparisons of these data reveal a highly significant difference between **I-HFD** and **C-HFD** mice even on the first day and each day thereafter during a two-week period in either male or female C57BL/6J mice. We observe no chow consumption during these one-hour access periods (data not shown). For the HFD consumed, within group post-hoc comparisons of the access days of the **I-HFD** group revealed a highly significant difference between the first day of HFD exposure and the subsequent days (Dunnett's multiple comparisons test, $p < 0.0001$ for Days 2-14 compared to Day 1 for males, $p < 0.05$ for Days 5-15 compared to Day 1 for females). We observed a concomitant reduction in home cage chow intake in male and female **I-HFD** mice (Figure 1C, **Males** - RM one-way ANOVA: $F(4.510, 148.8) = 3.63, p = 0.0054$; **Females** - RM one-way ANOVA: $F(4.05, 44.55) = 15.97, p < 0.0001$). Post hoc comparisons of the total chow intake reveal a significant difference between Day 1 and all subsequent days for female mice (Dunnett's multiple comparisons test of Day 2-14 to Day 1), but did not reach significance for males that may be due to the high variance on Day 1. When we considered how the intermittent HFD intake related to their total intake (Binge Index), we observed a highly significant increase in the Binge Index for both male and female **I-HFD** mice (Figure 1D, **Males** - RM One-way ANOVA: $F(5.298, 243.7) = 4.30, p = 0.0007$; **Females** - RM One-way ANOVA: $F(5.174, 56.92) = 5.566, p = 0.0003$). Post hoc comparisons of the Binge Index values over the first two weeks demonstrated a significant escalation for both male and female mice (Dunnett's multiple comparisons test of Days 2-14 to Day 1). Under these conditions, **C-HFD** mice (either male or female) preferred the HFD and not chow and demonstrate stable elevated caloric intake (see below). Thus brief, daily one hour exposures to HFD are sufficient to engender increases in food intake that escalate from the first day of exposure.

Following the acquisition of this form of hyperphagia on Day 1, we observed that **I-HFD** mice quickly orient to the placement of HFD in their home cage and rapidly consume it. To test whether these intermittent access periods of HFD produce rapid consumption, we shortened the access period to either 30 min. or 10 min. Reduction of the access period length to either 30 or 10 min. produced a highly significant reduction in the magnitude of binge consumption in both males (Figure 1F, baseline: 0.148 ± 0.0143 , 30 min: 0.0932 ± 0.00937 , 10 min: 0.0864 ± 0.0146 ; RM one-way ANOVA: $F(1.967, 17.70) = 10.06, p = 0.0013$) and females (baseline: 0.253 ± 0.0109 , 30 min: 0.203 ± 0.0112 , 10 min: 0.133 ± 0.0136 ; RM one-way ANOVA: $F(1.876, 20.64) = 41.44, p < 0.0001$). However, these data also demonstrate that in both male and female **I** mice, more than half of the HFD

consumption occurs rapidly within the first 10 min of the access period (Males – 58.42 +/- 14.41 % and Females – 52.63 +/- 7.17 %).

To determine the effect of chronic intermittent access HFD bingeing on body weight, we compared average body weights between **I-HFD** and **C-HFD** mice after 3 weeks. For both sexes, we observed that **C-HFD** mice exhibit a significant percent change elevation in body weight relative to **I-HFD** mice (Figure 1G, Unpaired student's *t* test, Males – $p = 0.0052$, Females – $p < 0.0001$). We also observed that the total intake in **C-HFD** mice was significantly elevated for both male and female mice (Figure 1E, Unpaired student's *t* test, Males – $t=2.965$, $df=38$, $p < 0.0001$; Females – $t=5.541$, $df=22$, $p = 0.0187$). Correlational assessments of home cage chow intake with binge HFD intake in the **I-HFD** mice revealed a significant inverse correlation between the two, with stronger binge eaters consuming less chow in their home cage over a 24-hour period (Figure 1H, slope significantly non-zero, $F = 6.949$, $p = 0.0158$).

Overall, we found in both male and female C57BL/6J mice that daily short 1-hour exposures to HFD produce significant increases in food intake that escalates over time concomitant with a decrease in home cage chow intake. **I-HFD** mice gain less weight and demonstrate a lower overall daily caloric intake than **C-HFD** mice.

3.2 Behavioral effects of chronic intermittent high fat binge eating

We hypothesized that chronic daily intermittent HFD binge eating might produce a state of expectant anxiety for the HFD. In a separate cohort of animals that had undergone 3 weeks of either *ad libitum* chow, continuous HFD, or intermittent HFD; we assessed anxiety-like behavior using three established locomotor assays that capitalize on mice innate preference for dark enclosed spaces: response to an open field, a light-dark box conflict assay, and an elevated plus maze.

In the open field assay (Figure 2A), we observed an overall main effect in distance traveled (Figure 2B, Chow: 9556 +/- 552 cm, **C-HFD** – 8576 +/- 299, **I-HFD** – 10492 +/- 450.4, one-way ANOVA, $F = 4.873$, $p = 0.0156$). Post hoc comparisons revealed a highly significant increase in distance traveled in **I-HFD** mice relative to **C-HFD** mice (Sidak's multiple comparisons test, $p = 0.0085$), but not between **I-HFD** and *ad lib* controls ($p = 0.2653$). We observed no significant overall effects in the time spent in the center (Figure 2C, Chow: 67.43 +/- 5.51, **C-HFD**: 79.58 +/- 7.391, **I-HFD**: 84.99 +/- 5.92, one-way ANOVA, $F = 2.021$, $p < 0.1520$), the number of center entries (Figure 2D, Chow: 49.20 +/- 3.809, **C-HFD**: 52.00 +/- 2.42, **I-HFD**: 61.80 +/- 6.189, one-way ANOVA, $F = 2.238$, $p = 0.1261$), or the latency to enter the center (data not shown).

In the light/dark conflict assay (Figure 2E), we observed an overall strong nonsignificant trend in the duration spent in the light side of the box (Figure 2F, Chow: 428 +/- 41.73 s, **C-HFD**: 354.7 +/- 32.72 s, **I-HFD**: 470.4 +/- 19.68 s, one-way ANOVA, $F = 3.216$, $p = 0.0559$), but post-hoc tests revealed a significant increase in the **I-HFD** group relative to the **C-HFD** group in light side duration ($p = 0.0387$) but not between **I-HFD** and chow controls. There was no significant effect in the latency to enter the light side (Figure 2G, Chow: 42.71 +/- 11.21 s, **C-HFD**: 37.46 +/- 13.15 s, **I-HFD**: 18.38 +/- 5.289 s, one-way ANOVA, $F =$

1.505, $p = 0.2401$) or the number of entries (Figure 2H, Chow: 25.70 $\pm$ 2.196 s, **C-HFD**: 24.90 $\pm$ 1.71 s, **I-HFD**: 26.40 $\pm$ 2.088 s one-way ANOVA, $F = 0.1394$, $p = 0.8705$).

In the elevated plus maze (Figure 2I), we observed no overall main effect on the time spent in distance traveled (Figure 2J, Chow: 1849 $\pm$ 113.3 cm (SEM), **C-HFD**: 1955 $\pm$ 101.7 cm, **I-HFD**: 1877 $\pm$ 102.3 cm, one-way ANOVA, $F = 0.26$, $p = 0.7730$), % open arm time (Figure 2K, Chow: 24.01 $\pm$ 1.906, **C-HFD**: 24.22 $\pm$ 2.647, **I-HFD**: 29.09 $\pm$ 3.172, one-way ANOVA, $F = 1.219$, $p = 0.3118$), or the probability of an open arm entry (Figure 2L, Chow: 0.2398 $\pm$ 0.02175, **C-HFD**: 0.2597 $\pm$ 0.01096, **I-HFD**: 0.2643 $\pm$ 0.03673, one-way ANOVA, $F = 0.2542$, $p = 0.7775$).

In total, we observed that chronic intermittent access to HFD produced significant overall effects in distance traveled in the open field driven by differences in **I-HFD** and **C-HFD** mice, but no effects on center time or entries. We observed no overall effects in the light-dark box or the elevated plus maze between *ad libitum* chow, **I-HFD**, or **C-HFD** mice.

3.3 Nociceptin receptor signaling contributions to intermittent HFD binge eating

Having established a simple, replicable model of HFD binge eating, we explored whether endogenous nociceptin receptor signaling modulates binge eating. We administered a potent, highly selective nociceptin receptor antagonist SB 612111 intraperitoneally in either **C-HFD** or **I-HFD** animals having established a stable baseline of HFD consumption. Although low doses of SB 612111 had no effect on binge consumption, we observed a significant overall effect of drug treatment and a significant drug by group interaction (RM two-way ANOVA; drug effect: $F(5, 90) = 18.99$, $p < 0.0001$; group effect: $F(1, 18) = 106.7$, $p < 0.0001$; group $\times$ drug effect: $F(5, 90) = 14.36$, $p < 0.0001$) in males (Figure 3A). Post-hoc comparisons revealed a significant reduction in binge consumption in **I-HFD** mice relative to baseline with 10 mg/kg SB 612111 (Dunnett's post test, $p = 0.0004$, 36.2% decrease, see inset). We observed no significant effect of SB 612111 in **C-HFD** mice. After a one-week period of washout and continued intermittent access HFD bingeing, we replicated the decrease in binge consumption with 10 mg/kg SB 612111 within this cohort of mice (data not shown) and in a separate cohort of untreated male **I-HFD** mice (data not shown). To determine whether SB 612111 has a differential effect on binge consumption during the acquisition of intermittent access binge eating behavior, we used a separate cohort of only **I-HFD** male mice receiving either vehicle or 10 mg/kg SB 612111 and observed a significant reduction in HFD consumption on their first day of HFD exposure (Figure 3E, Vehicle: 0.0804 $\pm$ 0.025, SB 612111: 0.0439 $\pm$ 0.0316; Unpaired Student's t test, $t = 3.054$, $df = 21$, $p = 0.006$).

In a separate cohort of female **I-HFD** and **C-HFD** mice, we observed a similar overall effect (Figure 3B, RM two-way ANOVA; drug effect: $F(5, 110) = 56.99$, $p < 0.0001$; group effect: $F(1, 22) = 325.7$, $p < 0.0001$; group $\times$ drug effect: $F(5, 110) = 49.97$, $p < 0.0001$) where 10 mg/kg SB 612111 produced a highly significant effect on binge HFD consumption (Dunnett's post test, Baseline: 0.253 $\pm$ 0.011 vs. 10 mg/kg SB 612111: 0.200 $\pm$ 0.014, $p = 0.0013$, 20% decrease see inset). As a positive control, we also tested the effect of 30 mg/kg of the serotonin selective reuptake inhibitor fluoxetine (FLX) in both male and female **I-HFD** and **C-HFD** mice, as this has been shown to robustly reduce binge feeding in

multiple preclinical studies and is commonly prescribed to treat binge-eating disorder [4,25]. Post tests revealed a highly significant and nearly complete reduction of binge consumption with FLX treatment (Males: Baseline = 0.14 $\pm$ 0.012 vs. FLX = 0.01 $\pm$ 0.007; Females: Baseline = 0.253 $\pm$ 0.011 vs FLX = 0.006 $\pm$ 0.002, Dunnett's post test $p < 0.0001$ for both groups).

These data demonstrate that SB 612111 and FLX both significantly reduce binge HFD consumption in **I-HFD** mice. We further explored the specificity of these effects within the context of total food intake. Analyses of total food intake in both **C-HFD** and **I-HFD** mice revealed a significant effect of drug and drug $\times$ group interaction in males (RM two-way ANOVA, drug effect: $F(5, 90) = 38.72$, $p < 0.0001$; group effect: $F(1, 18) = 2.614$, $p = 0.5392$; group $\times$ drug effect: $F(5, 90) = 11.03$, $p < 0.0001$) and significant effect of drug in females (RM two-way ANOVA, drug effect: $F(5, 110) = 22.87$, $p < 0.0001$; group effect: $F(1, 22) = 3.572$, $p = 0.0757$, drug $\times$ group effect: $F(5, 110) = 1.933$, $p = 0.0946$). However post-hoc analyses revealed that there was no significant effect of SB 612111 treatment on total intake relative to baseline in either **C-HFD** or **I-HFD** mice, but a highly significant reduction in total intake with FLX treatment in both males (Dunnett's post test; **I**: Baseline = 0.364 $\pm$ 0.009 vs. FLX = 0.276 $\pm$ 0.015; **C-HFD**: Baseline = 0.411 $\pm$ 0.007 vs. FLX = 0.117 $\pm$ 0.021, $p < 0.01$ in either post test) and females (Dunnett's post test; **I-HFD**: Baseline = 0.508 $\pm$ 0.01 vs. FLX = 0.346 $\pm$ 0.03; **C-HFD**: Baseline = 0.538 $\pm$ 0.018 vs. FLX = 0.298 $\pm$ 0.033, $p < 0.001$ in either post test). Furthermore, 30 mg/kg FLX produced acute reductions in body weight in both males and female mice (data not shown). These data demonstrate that SB 612111 treatment had no effect on total 24-hour food intake while FLX treatment produced a significant reduction in total 24-hour food intake.

In summary, the NOP/Opr11 antagonist, SB 612111, produced a significant, dose-dependent decrease in HFD binge consumption in the **I-HFD** group with no effect total intake. SB 612111 had no effect on the **C-HFD** group in either binge or total food intake. Treatment with the SSRI fluoxetine blocked binge consumption in the **I-HFD** group, while also reducing total food intake in both the **I-HFD** and **C-HFD**. Similar effects were observed in both male and female C57BL/6J mice. Additionally, we observed that SB 612111 treatment significantly decreases HFD consumption in acute intermittent access.

4. Discussion

Binge eating is a complex, multifaceted motivated behavior that is subserved by multiple neural circuits and receptor signaling pathways. One challenge associated with dissecting this complex signaling network is the dearth of simple behavioral models of binge eating that enable experimenters to capitalize on the development of highly specific and reversible pharmacological and neural circuit tools in genetically-modified laboratory mice. Additionally, many behavioral paradigms to dissect feeding or binge eating also suffer from unwanted floor effects through novelty-induced suppression of feeding by introducing the animal into novel testing arenas.

In this study, we adopted a simple, replicable model of home cage binge eating based on palatability-induced hyperphagia. Our data show that intermittent one-hour access to a high-

fat containing food produces a robust increase in intake even on the first day of access. This high fat binge eating escalates substantially after the first day and continues over multiple weeks. Combined with a concomitant decrease in home cage chow intake over this period, animals provided with chronic intermittent HFD intake will increase their one-hour binge intake during the first two weeks until it comprises more than 40% of their total daily caloric intake. Importantly, our data demonstrate that this form of food consumption is rapid and entrained similar to clinical definitions of binge eating in humans.

Multiple models of binge eating of sweet, fat, or sweet-fat food mixtures have been developed that incorporate different forms of diet cycling, access limitations, stress, or food deprivation [25,28–33]. Each of these models has unique strengths and weaknesses. In our study, we chose to capitalize on binge eating produced by providing intermittent access to palatable food that occurs independent of food restriction and stress (see chapter 4 Corwin *et al.* for review)[34] and has been observed in rats and mice [25]. Importantly, we used C57BL/6J mice; the most common strain of laboratory mice that is now a rich resource of genetically altered strains to map physiological and neural circuit contributions to behavior. This strain is so notoriously difficult to use in operant self-administration studies, so we chose to use the animal's home cage as the arena for our behavioral manipulations. Additionally, we chose not to incorporate food-deprivation because of its effects on gene expression of prepronociceptin and NOP[35] and because it has been demonstrated that SB 612111 has no effect on food-deprivation induced hyperphagia[23]. We hypothesized that although food-deprivation induced hyperphagia and palatability-induced hyperphagia are behaviors that share some common neural substrates, circuit-dependent fast-acting neurotransmission and neuromodulatory signaling mechanisms are discretely engaged through these forms of feeding. Additionally, many previous studies use food containing some amount of sucrose or even cafeteria diets [36–40]. We preferred a model that could selectively probe the receptor signaling pathways that are engaged by fat. Lastly, animal models of binge eating using both rats and mice incorporate multiple aspects of the behavioral phenomenon including stress, diet cycling, and palatable food access. Other cognitive and affective features of binge eating (e.g., guilt, distress), which vary considerably across humans, are less capturable by animal models. We posit that the primary utility of animal models is in generating time-locked, replicable binge-like hyperphagia, the core behavioral phenotype of binge eating in humans.

Using our model of chronic intermittent HFD binge eating, we determined whether this course of binge eating had any effects on anxiety like behavior. After three weeks of binge eating, we assayed performance in the open-field, elevated plus maze (EPM), and light-dark conflict assays. During this time, we maintained binge conditions following the behavioral assays to avoid possible withdrawal effects. We observed a significant increase in the distance traveled in the open field and an increase in the time spent in the light side of the light-dark box. The light-dark findings are broadly consistent with a previous study[31]; although, that study used rats and the light aversive side of the compartment was paired with the palatable binge food. We observed no significant effect on measures of anxiety with the EPM. Our data are consistent with a previous study[25] that used a different cycling of HFD access and took behavioral measures 24 hours after the last binge cycle. Overall the data did not support our hypothesis that chronic intermittent binge eating produces expectant anxiety

for the HFD, as we observed no decrease in open field center time, light side time or entries, or open arm time in the EPM. However, it is still possible that withdrawal from intermittent HFD binge eating is capable of producing anxiety.

One limitation of our study is that we cannot rule out that the effects we saw on locomotion in either the open field or light-dark box might be produced by changes in bodyweight in the C group. One study using C57BL/6J mice reported an increase in anxiety and depressive-like behavior after chronic HFD exposure vs. low-fat diet exposure; however, measures of locomotion like the distance traveled or velocity were not reported and there was no *ad lib* chow as in our study^[41]. A similar study in female rats exposed to chronic HFD with similar caveats was recently published^[42]. We explored the possibility of body weight effects on locomotion and observed no linear relationship between body weight and open field velocity (data not shown). Additionally, although there was a significant gain in body weight as expressed as percent change in the **C-HFD** group after 3 weeks, this effect is very subtle relative to common models of diet-induced obesity after 6–8 weeks [43]. Therefore we believe it unlikely that the differences between the I and C HFD groups in the open field and light dark assays derive from subtle changes in body weight produced by 3 weeks of intermittent or continuous HFD exposure. Lastly, we speculate that the timing of binge eating manipulations on mice and their effects on anxiety-like behavior may represent an important component in the experimental design. Future studies that integrate the binge eating and behavior into a shorter space of time are necessary to explore this hypothesis.

Anxiety disorders and eating disorders that include binge eating are highly comorbid [44,45], but the antecedent nature of these associations clinically are incompletely understood. Our data show that in mice, chronic maintained HFD binge eating does not produce anxiety-like behavior. Instead, we speculate that the subtle hyperactivity we observe in the **I-HFD** group is more consistent with elevated foraging behavior or behavioral disinhibition. Our data do not preclude the possibility that more chronic withdrawal from HFD can produce anxiety. Further studies in mice are necessary to explore the relationship between anxiety and binge eating.

Nociceptin is an opioid heptadecapeptide that binds a single g-protein coupled receptor (NOP/Opr11) with minimal affinity for μ , κ , δ opioid receptors. Central injections of this peptide or NOP agonists produces a dose-dependent increase in feeding behavior [15,17,19,20,46–48], and chronic infusions of this peptide are capable of producing changes in body weight [49]. Interestingly, three studies demonstrate that 1) N/OFQ induced hyperphagia is present only in Sprague-Dawley rats that have been previously established as “fat-preferring”; 2) NOP/Opr11 knockout mice consume significantly less high-fat containing food than their wildtype littermate controls, and 3) that a novel NOP antagonist LY2940094 increases lipid utilization metabolism in mice [50–52]. Taken together, we hypothesized that NOP/Opr11 signaling is recruited during and required for binge eating of high fat containing foods. Consistent with our hypothesis, we observed that treatment with the highly potent and selective NOP/Opr11 antagonist SB 612111 produced a dose-dependent decrease in intermittent HFD binge eating. We selected 10 mg/kg as our highest dose as this dose does not impact food-deprivation induced hyperphagia [23]; however, doses as high as 30 mg/kg are tolerated with no ill effects in C57BL/6 mice [53].

Importantly, we did not observe a change in the total 24-hour intake of mice treated with SB 612111 in either **I-HFD** or **C-HFD** mice. One reason for the lack of effect in **C-HFD** mice may be due to the pharmacokinetic properties of a single-dose of SB 612111 precluding its anorexigenic effect during the dark period where the majority of the HFD is consumed. In contrast, high doses of fluoxetine (FLX) almost completely blocked binge consumption with a concomitant reduction in the animal's 24-hour total intake. This is consistent with a previous study [25] and a recent study that used the 5HT_{2C} agonist mCPP [52]; however, we attribute this reduction in overall food intake to the potent acute anxiogenic capacity of these doses of FLX [54–56]. Although SSRIs like FLX are one of the most common drugs used to treat binge eating disorder [4] (Also see AHRQ Systematic Review - Management and Outcomes of Binge Eating Disorder), not all patient's symptoms improve with SSRI treatment [4]. Novel drugs with more selective modes of action and potential macronutrient selectivity might be more effective for those who binge eat on fat-rich foods.

At present, the exact physiological mechanism of SB 612111's effects are unclear, however present literature supports a model by which this drug acts on NOP/Opr1 in the central nervous system [9,48]. In the brain, there are several brain regions rich in NOP expression and that modulate binge eating that represent compelling targets for future investigation of SB 612111 effects including hypothalamic nuclei like the paraventricular, lateral, ventromedial and arcuate nuclei [46,57–61], midbrain monoaminergic nuclei like the ventral tegmental area and dorsal raphe nucleus [62–66], and extended amygdala structures such as the bed nucleus of the stria terminalis [67] and central amygdala [35]. As drugs that modulate 5HT signaling have been shown to have robust anorexigenic effects [68], an intriguing explanation for SB 612111's mechanism is through its interaction with the brain's serotonergic system. Future studies are necessary, however, to test these hypotheses.

Recently, a novel set of orally available highly selective NOP/Opr1 antagonists have been developed, and one of these compounds, LY2940094, has subnanomolar potency for human NOP, has sustained brain occupancy, and has been tested in rodent models of feeding [52,69]. This compound was found to reduce fasting-induced hyperphagia of chow in wildtype 129S6 mice, but not those with genetic deletion of the *Opr1* gene (NOP KO mice). Additionally, LY2940094 reduced the consumption of high fat food measured acutely over a 5-hour exposure and weight gain produced by 3 days of these exposures. They also showed that LY2940094 reduced intake of HFD in diet-induced obese models in rats and mice, and that this drug reduced the respiratory quotient in mice with access to HFD in cages that permit the measurement of O₂ utilization and CO₂ production. This last point reflects that NOP antagonism with LY2940094 promotes fat utilization in addition to reducing HFD intake. Our results provide convergent data that antagonism of NOP reduces HFD intake either in a single acute exposure or in a daily intermittent access schedule in both male and female C57BL/6J mice. Taken together, our two studies demonstrate that antagonism of NOP is a compelling target for the treatment of binge eating and obesity, and that further studies are needed to understand the hypophagic mechanism of action of NOP antagonists in the brain.

5. Conclusions

We conclude that short daily one-hour access periods to high fat containing food are sufficient to induce rapid, replicable binge-like consumption. Chronic intermittent binge eating produces changes in psychomotor behavior. Lastly, treatment with NOP/Opr1 antagonists reduced HFD binge eating in the intermittent access model.

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Figure 1. Intermittent daily access to HFD promotes large, rapid consumption

A. Schematic of home cage paradigm for continuous (left) vs intermittent (right) access to high fat food in C57BL/6J mice. Chow is *ad libitum*. Access to HFD was provided at 11 am.

B. Normalized consumption (kCal/ g bodyweight) of the HFD during the one-hour access period for male (black) and female (red) mice under continuous (closed symbols) or intermittent (open symbols) access conditions over the first two weeks. Within both the male and female group, data were analyzed by RM two-way ANOVA (n for each group: **I-HFD** male = 35, **C-HFD** male = 20, **I-HFD** female = 12, **C-HFD** female = 12, see Results for details). The **I-HFD** group consumed significantly more food during this access period than the **C-HFD** group for both male and female mice.

C. Normalized consumption of the home cage chow (kCal/ g bodyweight) for the male and female **I-HFD** groups over the first two weeks. Within both the male and female group, data were analyzed by RM one-way ANOVA (n for each group: **I-HFD** male = 35, **I-HFD** female = 12, see Results for details).

D. Fractional intake of the HFD for the male and female **HFD** group (Binge Index) during the first two weeks. Within both the male and female group, data were analyzed by RM one-way ANOVA (n for each group: **I-HFD** male = 35, **I-HFD** female = 12, see Results for details). We observed a significant escalation in the Binge Index for both groups.

E. Total caloric intake for male and female **C-HFD** and **I-HFD** groups. Data were analyzed by Unpaired student's *t* test (see Results for details). We observed a significant increase in caloric intake in both male and female **C-HFD** mice.

F. After establishing a stable baseline of consumption in the **I-HFD** group, we decreased the access period length for the intermittent group from 60 min. to 30 min and 10 min. Within both the male and female group, data were analyzed by one-way ANOVA (n = 10 males and 12 females; see text for details).

G. Percent change in bodyweight in males and females for **I** vs **C** mice. Continuous mice gain significantly more bodyweight than intermittent mice. Data were analyzed using an unpaired student's *t* test.

H. Correlational analyses of binge HFD intake vs home cage

chow intake in **I-HFD** mice. Male and Female animals were pooled. Data were fit using linear regression. Slope was significantly non-zero ($F = 6.949$, $p = 0.0158$). For all panels $* = p < 0.05$, $** = p < 0.01$, $*** = p < 0.001$, and $**** = p < 0.0001$.

Figure 2. Intermittent HFD binge effects on anxiety and psychomotor behavior

A. Open field apparatus. **B.** Distance traveled in the open field (cm) over 20 min. There is a significant overall effect of group and I mice travel significantly more than C mice. **C.** Time spent in the center of the open field. There is no significant effect on center time. **D.** Center entries. **E.** Light/Dark conflict box **F.** Time spent in the light side of the light/dark box. Significant overall group effect (see results) with a significant difference between I and C mice. **G.** Latency to enter the light side. No significant group difference **H.** Total number of entries into the light side. No significant group differences. **I.** Elevated Plus Maze (EPM). **J.** Distance traveled in the EPM. No significant group differences. **K.** % Open arm time in the EPM. No significant group differences. **L.** Probability of an open arm entry in the EPM. No significant group differences. For all measures, data were analyzed by one-way ANOVA with Sidak's multiple comparisons test. See Results for further information on the statistical analyses. $n = 10$ / group for each assay.

Figure 3. SB 612111 treatment reduces binge consumption, but not total 24 hour intake

A. Normalized binge intake in male mice treated with SB 612111 and 30 mg/kg fluoxetine (FLX). Drug Treatments were performed as described in the Methods. SB 612111 treatment produced a dose-dependent reduction in binge intake with 10 mg/kg SB 612111 producing a near 40% reduction in consumption intermittent mice (inset). There was no significant effect on continuous mice. **B.** Normalized binge intake in female mice treated with SB 612111 and 30 mg/kg FLX. SB 612111 produced a dose dependent reduction in binge intake with 10 mg/kg producing over 20% reduction in intake. There was no significant effect on continuous mice. **C.** Normalized 24 hour total intake in male mice treated with SB 612111 and 30 mg/kg fluoxetine (FLX). SB 612111 had no effect on total intake in either intermittent or continuous male mice. FLX produced a significant reduction in total intake in either group. **D.** Normalized 24 hour total intake in female mice treated with SB 612111 and 30 mg/kg fluoxetine (FLX). SB 612111 had no effect on total intake in either intermittent or continuous female mice. FLX produced a significant reduction in total intake in either group. For all panels, data were analyzed by a repeated measures two-way ANOVA with Dunnett's multiple comparison test to the baseline measurements of binge intake and total intake. **E.** HFD intake in first one-hour exposure to HFD in animals treated with vehicle or 10 mg/kg SB 612111 ($n = 12/\text{group}$). SB 612111 produced a highly significant reduction in HFD intake.