UNIVERSITY OF CALIFORNIA, SAN DIEGO

Rodent Models for Compulsive Binge-Like Eating; Pharmacological Effect of Sigma-1 and Mu-Opioid Receptor Ligands

A thesis submitted in partial satisfaction of the
requirements for the degree of Master of Science

in

Biology

by

Belinda Elin Hui

Committee in charge:

Professor Byungkook Lim, Chair
Professor Brenda Bloodgood
Professor James Nieh
Professor Eric Zorrilla

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The Thesis of Belinda Hui is approved, and it is acceptable
in quality and form for publication on microfilm and electronically:

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Chair

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DEDICATION

To Mom and Dad

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ABSTRACT OF THE THESIS

Rodent Models for Compulsive Binge-Like Eating; Pharmacological Effect of Sigma-1 and Mu-Opioid Receptor Ligands

by

Belinda Hui

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Professor Byungkook Lim, Chair

In modern society, an increased availability of palatable food has promoted obesity, leading some to diet and restrict oneself from certain "desired" foods. This restriction leads to under-acceptance of alternative diets, followed by an overconsumption of palatable food when one can no longer withstand dieting. Here, we modeled this behavior of "fasting" and "binging" in mice, based off our previous free-

feeding rat model. In this mouse model, female mice were assigned one of three diet groups (ad libitum chow diet, ad libitum palatable, sucrose-rich, chocolate-flavored diet, and intermittent access to chocolate diet). To assess binge eating behavior, the mice in these three diet groups were compared on operant-self administration (fixed/progressive ratio), and daily energy consumption. Operant session results revealed that intermittent mice had a significantly higher self-administration of palatable diet, and were significantly more motivated and compulsive than ad libitum mice, and demonstrated significant rejection of non-palatable chow diet. All of these results showed that intermittent access to a palatable diet promotes compulsive eating behavior, allowing for the establishment of a mouse binge eating model. Binge eating has been shown by various studies to cause neurological changes in the brain, similar those caused by drug addiction. The following studies on the mouse model tested the hypothesis that compulsive eating may alter various neurological mechanisms in a similar manner as drugs of addiction. BBP-02, a sigma-1 receptor antagonist, was treated in mice but showed no effect on self-administration, suggesting that sigma-1 by itself may not play a role in the modulation of compulsive eating. Another drug, 14-methyoxymetoponin, a mu-opioid receptor agonist, resulted in a significant decrease in self administration with intermittent mice at the two highest doses, suggesting that the opioid system may be altered in mice with compulsive eating behavior.

CHAPTER 1: INTRODUCTION

Fifteen million Americans today suffer from binge eating disorder (1), and with the increasing prevalence of palatable food, this number is only on the rise, and the cost analysis of binge eating yields a cost of $3,500 a year, and up to a $14,000 a year for those seeking treatment (2). Binge eating disorder is a life-threatening disease characterized by the consumption large amount of foods in a short amount of time, following periods of self-restriction (3). With dieting, there are systematic differences in our environment and palatability of food, with people trying to abstain from "forbidden foods", and this time of "fasting" has been hypothesized to be involved in the development of binge eating and/or obesity. It has been suggested that binge eating is involved in the development of obesity, but these two are distinct concepts, and one can be present without the other. It has been suggested in many studies that food addiction share the same stress/reward pathways as those of addictive substances (such as alcohol, cocaine, nicotine, etc.), but there is still a lot unknown about what specific pathways are directly linked to binge eating (4). There is currently a lack of animal models for binge-eating, and of those models that are currently established, very few are mouse models, even though mice have the largest number of genetic tools available. The aim of this study is to create a mouse model for binge eating to allow for better understanding of the roles of different neuronal and genetic bases that govern binge-eating behavior.

CHAPTER 2: FREE FEEDING RAT MODEL FOR BINGE EATING

Many Western weight loss diets involve abstinence from certain "illicit" foods due to their "richness," macronutrient composition (e.g., sugary/starchy or fatty), or caloric-density (9–12). Instead, dieters limit themselves to nominally "healthier" foods that may be characteristically less palatable, and limiting access may increase the reinforcing value of "illicit" foods while devaluing alternatives, thereby counterproductively promoting subsequent consumption of the "illicit" food (13–18). Perhaps accordingly, dieting often leads to intake "cycling" and is a putative causal factor in binge eating disorders (19–21), weight gain (22–24), and adverse long-term metabolic outcomes (25) (but see (23)).

Cyclic overeating vs. dieting from illicit foods may be homologous to the alternating cycles of drug use vs. abstinence that promote the transition from drug use to dependence (26; 27). Abstinence, in this view, leads to an aversive emotional state that promotes escalating use via negative reinforcement mechanisms (28–30; 56). Rodent models suggest that greater drug access contributes to the etiology of addiction (31); rodents with extended, daily access to cocaine (32), heroin (33), and methamphetamine (34) escalate their drug intake and show signs of addiction, whereas those with brief access do not.

Whether greater (vs. more restricted) durations of access to palatable food comparably influence pathological eating is unclear. As with drugs of abuse (35–37), intermittent access to palatable food leads to greater rates of intake than continuous access; highly limited palatable food access (e.g., 10min -2hr/day) can drive so-called

binge-like intake (18; 38–42). On the other hand, rats with extended (18-23hr/day), but not restricted (1hr/day), daily access to a "cafeteria diet" develop deficits in brain reward function and become obese (43). Existing models have not differentiated the contributions of duration vs. intermittency of access to the development of abnormal eating. The role of extended access to food vs. the resulting obesity also have been conflated. Finally, few have emphasized the lasting reduction in intake of less preferred, but otherwise acceptable, food that occurs following access to palatable food (15; 43; 44). This "finickiness" may reflect adjustments in food reward, analogous to the hypohedonia of previously rewarding stimuli common in addiction (45).

To test the hypothesis that intermittent, extended access to palatable food drives intake cycling, young, female rats were given either continuous or intermittent (3x/week) access to a palatable, sucrose-rich, chocolate-flavored diet. Intermittent access groups received long (24 hours) access on access days. Based on the addiction literature, we hypothesized that rats given intermittent long access to palatable food would escalate their palatable food intake and reject their less preferred, but otherwise acceptable, food to a greater degree than those with intermittent short or continuous access to highly palatable food.

2.1 METHODS

Subjects

Young adult (125-150g), female Wistar rats (Charles River, *n*=32) were pair-housed in wire-topped plastic cages in a temperature- (22 °C) and humidity- (60%) controlled vivarium (12:12 h reverse-light cycle). Before experiments, rats had chow *ad libitum* (Harlan 7012 Teklad LM-485, Indianapolis, IN). Water was always available.

Body weights and intake were recorded daily for 2-4 days before experiments. Procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Care and Use Committee of The Scripps Research Institute.

Feeding Schedule

The "palatable" diet was chocolate-flavored, sucrose-rich, nutritionally-complete 45-mg pellets ("CHOC") (5TUL, Test Diets, St. Louis, MO) with similar macronutrient composition (~67% carbohydrates, 21% protein, and 13% fat by kcal) and caloric density (~3.44 kcal/g), but 90% 24h preference ratio, vs. chow (3.10 kcal/g) (15). Rats were assigned to one of four groups (*n*=10/group), matching for body weights and 24-h chow intake: **Chow (**continuous chow access), **Chocolate Choice** (continuous CHOC and chow access), **Intermittent-Long Choice** (Int-Long; 24h CHOC and chow access on MWF/24h chow access on TThSatSun). Cage mates were assigned to the same group, separated by clear plastic dividers to allow individual measurements. CHOC was presented at dark onset. MWF are analyzed as "access days," Tu-Th as "non-access days," and weekends separately, due to consecutive non-access days. Continuous chow access was given, even when CHOC was present. Intake and weight gain were defined as the difference between initial and subsequent weights via a 0.1 g precision scale. At dark onset, food was weighed daily Monday-Saturday (yielding daily weekday intake and 48h weekend intake), and body weight weekly, on Thursdays. Feed efficiency was defined as g body weight gained/100 kcal consumed.

Data analysis

Effects of diet schedules on dependent variables were determined using mixed-design ANOVAs; the within-subject factor was Time (week, day, or weekend) and the between-subjects factor was diet Group. In cases where a significant Time x Group interaction occurred, between-group pairwise comparisons were performed at each timepoint using Fisher's protected LSD tests (46). Differences were considered significant when $p<.05$. *F* values and *df*s were adjusted for sphericity violations using Greenhouse-Geisser correction if epsilon$_b$ > 0.75.

2.2 RESULTS

24h weekday energy intake:

Access days. In (Fig 1; $F(9,108)=2.6$, $p=.01$), Group x Time interactions reflected that Chocolate Choice rats initially ate more on access days than the stable, comparable intake of Chow and Int-Short (Choice) rats, but then normalized their access day energy intake by weeks 3-4. Int-Long and Int-Long Choice rats, instead, consistently overate

Non-access days. Group x Time interactions $F(9,108)=3.1$, $p<.01$) reflected that Int-Long Choice rats progressively ate less on non-access days than other groups and that the initial overeating of Chocolate and Chocolate Choice rats normalized from week 1 to week 2.

2.3 DISCUSSION

Intermittency promoted greater daily (24-h) palatable diet intake; Int-Long rats ate ~2-fold more than continuous rats on access days. Whereas restrictedness drove bingeing, longer durations of access promoted more lasting rejection of otherwise acceptable

alternatives [see also (18; 38–40; 47)]. Intermittent, long access rats uniquely continued to under-eat chow on non-access days and through weekends.

Overeating of palatable food vs. undereating of chow were dissociable, meaning overeating of palatable food is not driven by undereating of chow food. Almost maximal daily overeating of palatable food occurred on the first day of access (**Fig. 1**), whereas undereating chow took days to develop and grew across 2+ weeks. Also, within Int-Long rats, the degree of overeating palatable food did not correlate with the degree of undereating chow on non-access days.

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CHAPTER 3: MOUSE MODEL FOR BINGE EATING

Following the development of the free feeding model of ad lib and intermittent access to a palatable diet for rats, we decided to incorporate operant chamber sessions into this model in order to measure motivated and compulsive behavior and better understand, to what degree, these rats are willing to work for their food based on their diet schedule assignments. We established a model first in rats, and the rat model for binge eating revealed that the rats given intermittent access to palatable chocolate diet are compulsive, and are shown to "binge" on days they are given access to chocolate (57). With the establishment of this model, we were able study various brain reward mechanisms with respect to compulsive eating (61). There are still many different paths that can be explored with the rat binge eating model, but we are also interested in better understanding the relationship between compulsive eating on a genetic basis, which can be accomplished by the creation of a mouse model for binge eating. The mouse genome has great flexibility for genetic manipulations and gene knockouts, which will allow for studying an array of different genes and allow for comparing compulsive behavior of transgenic and gene-knockout mice to those of the binge model. However, before we are even able to use all the genetic tools available in mice, we must establish a mouse model for compulsive, binge-like eating, and identify a system that may be linked to binge eating that we can focus on studying.

In this chapter, we outline the development of the mouse operant binge eating model. We predicted that the mice will exhibit similar behavior as the rats in the rat operant binge eating model, which showed the Int-Long diet group to show a cycling of

binging during access days and fasting during non-access days, to have significantly higher level of self-administration in operant chambers, and to be significantly more compulsive in operant chambers than ad lib Choc and Chow mice.

3.1 METHODS

Subjects

Twenty wild-type C57BL/6J mice were obtained from Jackson Laboratory at six weeks old. The animals were pair-housed in a controlled 12-hour light, 12-hour dark cycle at all times (lights on at 09:00 pm) with access to chow and water *ad-libitum*. Body weight and food intake were measured daily until start of experiment.

Mice were trained to use operant nose-poke chambers, and then sorted into three diet groups by balancing z-scores for weight, performance in operant chamber, percent body fat, and daily food consumption. At 9 weeks old, diet assignments were first introduced in a 1-hour operant chamber fixed ratio session, and the six-week mouse model began.

Feeding Schedule

The "palatable" diet was a chocolate-flavored, sucrose-rich, nutritionally-complete 20-mg pellets ("CHOC") (5TUL, Test Diets, St. Louis, MO) with similar macronutrient composition (~67% carbohydrates, 21% protein, and 13% fat by kcal) and caloric density (~3.44 kcal/g) vs. chow (5TUM, Test Diets, St. Louis, MO, 3.30 kcal/g). Mice were assigned to one of three groups, matching for body weights, performance in operant sessions, 24-h chow intake, and % body lean: **Chow** (Chow, continuous chow access, n=6), **Chocolate** (Choc, continuous CHOC access, n=6), **Intermittent-Long** (Int-

Long, 24h CHOC/0h chow access on MWF, n=8). Cage mates were assigned to the same group, separated by clear plastic dividers to allow for individual measurements. CHOC was presented at onset of dark cycle for Int-Long mice. MWF are analyzed as "access days", and TuTh as "non-access days", and weekends separately, due to consecutive non-access days. Chow was never available when CHOC was present, but otherwise available. On the onset of the dark cycle on access days, all mice were placed in operant chambers under a fixed ratio schedule, which required reinforcers for a pellet reward. Intake and weight gain were defined as the difference between initial and subsequent weights via a 0.1g precision scale. At dark cycle onset, food was weighed daily Monday-Saturday (yielding daily weekday intake and 48h weekend intake), and body weight was taken on Tuesdays and Fridays prior to dark cycle.

Operant Chambers

In order to measure the degree of self-administration and binge behavior, mice were placed in operant chambers during the onset of their dark cycle during access days (MWF). In these operant chambers, mice have to poke their nose in the active nose-poke hole a set amount of times in order to receive a reward pellet. A control nose-poke hole in the chamber was used as a measure to assure the mice could distinguish between the holes and knew the active nose-poke hole administered a pellet.

Training

Before the mouse model began, mice had to be trained to associate a nose-poke with a pellet reinforcer. Mice were first placed in operant chambers for 24 hours on a FR1 schedule (1 nose-poke=1 reinforcer), and ratio of active nose-poke to control nose-

poke hole was 50:50. As mice began to learn, the ratio increased, and training sessions were shortened to 6 hours and fixed ratio was increased. By the end of the training period, 90% of nose-pokes in operant chambers were for the active-nose poke hole, concluding that the mice have learned to associate the active-nose poke hole to the reinforcer. During training sessions, some mice were dispensing more pellets than they consumed due to natural explorative "nose-poking" behavior in mice, so fixed ratio was increased to 5 nose-pokes to one reinforcer (FR5) to eliminate any non-food directed nose-pokes.

Measuring Self-Administration with Fixed Ratio

Self-administration of pellet diet was measured using a fixed ratio schedule. Mice were placed into operant chambers at the onset of their dark cycle for one hour during access days in over the span the six-week mouse model. In the fixed ratio, mice had to nose poke 5 times in order to receive one reward pellet.

Measuring Compulsivity with Progressive Ratio

Degree of compulsivity was measured using a progressive ratio schedule. Mice were placed into operant chambers at the onset of their dark cycle for a maximum duration of three hours. In progressive ratio, the requirements for reinforcement are increased systematically after each reinforcer. The number of reinforcers in progressive ratio is a measurement of how hard the mice will work, despite not receiving a reward, thus correlating with compulsivity.

Measuring Seeking Behavior with Extinction Sessions

At the end of the study, mice were given an extinction session, where they were placed in an 1 hour FR5 session, but no reinforcer pellet was given for any amount of responses in the session. This session was given in place of a regular 1 hour FR5 session.

Measurement of body composition

Animal body composition was measured with nuclear magnetic resonance imaging (EchoMRI-1100, software version 2008.01.18M, Echo Medical Systems, Houston, TX).

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using SPSS data analysis packages. A mixed ANOVA analyzing for repeated measures between subject groups was used, followed by pairwise comparisons, involved Fisher's protected LSD tests.

3.2 RESULTS

Daily food energy consumption on diet schedule

At approximately 9 weeks of age, mice were assigned to diet groups and food intake was measured daily before the onset of the dark cycle, for six weeks. There is significance between diet group and energy consumption, primarily comparing Chow and Chocolate mice versus Int-Long mice. **Figure 2A** shows the daily intake, where Int-Long mice have a cycling of over-eating on access days and under-eating on non-access days, whereas the Choc and Chow mice are consuming relatively the same amount day by day. As shown in **Figure 2B**, after week 1 of the feeding schedule, Int-Long mice significantly

over-ate on access days and under-ate on non-access days ($p<.05$), while Choc and Chow mice relatively consumed the same amount on access and non-access days. In **Figure 2C**, there was a between subject (group) effect ($F(2,4)=20.748$, $p<0.001$), with Int-Long consuming significantly less on weekends than Chow and Choc ($p<.05$), which did not differ in intake except for during week 2, where Choc was consuming less than Chow ($p<.05$), but no group x week interaction. Follow up Post Hoc (LSD) was conducted for main effect, revealing a group difference in Int-Long mice from all other groups ($p<.002$).

Self-Administration in Operant Chamber Fixed Ratio Sessions

Mice were placed into operant chambers at the onset of their dark cycles on access days. They were given a fixed ratio schedule, in which they had to nose-poke a fixed amount of times (5) in order to receive a reward pellet. In **Figure 3A** there is a significant time effect ($F(5,165)=3.924$, $p<.005$), showing a decrease in reinforcers over time, but not a significant time x group interaction. There was also a significance between subject effect ($F(2,33)=14.294$, $p<.001$), with Int-Long having significantly more reinforcers than Chow and Choc ($p<.05$). A Post Hoc (LSD) showed Int-Long mice was significantly different than both Chow and Choc ($p<.001$), with no significant difference between Choc and Chow. Number of pellets consumed is shown in **Figure 3B**, with a within-subject week effect ($F(5,165)=7.305$, $p<.001$), but no significant week x group effect. Post Hoc (LSD) revealed that Int-Long is significantly different than Choc and Chow ($p<.001$), and there is no significant difference between Choc and Chow. Int-Long is consuming significantly less than Chow and Choc for all weeks, and Chow consuming significantly more than Choc in weeks 2 and 3. **Figure 3C** shows the proportion of

pellets consumed in operant chambers during FR5 session. There is a within subject effect for week ($F(5,165)=5.388$, $p<.001$), with a significant week x group interaction ($F(10, 165)=3.003$, $p<.005$). Post Hoc (LSD) was conducted, revealing that Int-Long is significantly different from both Chow and Choc ($p<.001$), with no difference between Chow and Choc.

Latency before first reinforcer in these sessions was also measured and shown in **Figure 4A**. The between subject effect was significant ($F(2,92)=7.196$, $p<.001$), with Int-Long having the shortest latency period (92.1 seconds), compared to Chow (373.3 seconds) and Chocolate (203.2 seconds). All groups were significantly different from one another (Choc and Chow: $p<.005$, Choc/Chow and Int-Long: $p<.001$)

Figure 4B shows that the % of reinforcers in 10 minutes, with a significant between-subject effect ($F(2,92)=7.196$, $p<.001$). This value was highest for Int-Long (28.1%), following with Choc (22.7%), and Chow (8.2%). Both Choc ($p<.05$) and Int-Long ($p<.001$) were significantly different from Chow. The % for active reinforcers in 30 minutes was not significantly different between the three groups ($F(2,92)=1.292$, $p=.28$).

Number of Responses/Breakpoints in Operant Chamber Progressive Ratio Sessions

One progressive ratio session was conducted, with results shown in **Figure 5**. **Figure 5A** shows a between subject effect ($F(2,16)=7.131$, $p<.01$), with Int-Long (600.9, $p<.02$) having a significantly higher number of responses than Choc (68.6) and Chow (162.8). **Figure 5B** shows the break point for number of responses in PR, shows a between subject effect ($F(2,16)=7.580$, $p<.01$) with Int-Long (102.1, $p<.02$) having a significantly higher break point compared to Choc (33.0), and Chow (17.2).

Extinction Session

Figure 6A shows an extinction session x group effect ($F(2,16)=36.741$, $p<.01$) and a between subject effect ($F(1,16)=13.558$, $p<.001$), with Chow ($p<.05$) and Int-Long ($p<.001$) having a significantly different number of responses in extinction compared to pre-extinction. **Figure 6B** shows the change in responses from pre-extinction to extinction, with a between group effect ($F(2,18)=7.227$, $p<.01$), and a Post Hoc (LSD) showed a significant difference between Int-Long with Chow and Chocolate ($p<.01$).

Body Composition/Weight

Figure 7A shows % body fat of mice before and after diet schedules. There is a multivariate difference ($F(2,14)=244.340$, $p<.001$), with a significant between subject effect ($F(1,4)=78.704$, $p<.001$) with Choc gaining a significant % body fat at the end of the study ($p<.02$). **Figure 7B** shows change in weight from the start of diet schedule to the end of the study. A one-way ANOVA showed a between group effect ($F(2,17)=10.326$, with $p<.005$), and Choc with a significantly higher change in fat than Chow and Int-Long ($p<.005$), and no difference between Chow and Int-Long. There was no between-group significance for change in lean. Choc showed a significantly higher change in weight compared to Chow and Int-Long, with a between-group significance ($F(2,17)=9.518$, $p<.005$), and there was no difference between Int-Long and Chow.

3.3 DISCUSSION

In order to establish a binge eating model in mice, mice were assigned one of three diet groups: ad lib Chow, ad lib Choc, and Int-Long. Daily food intake was

measured to quantify fasting and binging, and operant sessions of fixed ratio (for a measure of self-administration), progressive ratio, and extinction session (for a measure of compulsivity) were performed. Physiological characteristics of body composition were analyzed to see if our binge eating model was associated with obesity. This was done so by comparing pre-diet and end of study weight and body composition.

Int-Long mice over-ate on access days, and under-ate on non-access days

The results from 24-hour home-cage intake are consistent with our hypothesis, which suggests that the intermittency and durations of past access to palatable food have dissociable influences on its intake and that of alternatives. Int-Long developed cyclic intake and body weight, with daily overeating of the palatable food and undereating of the otherwise acceptable alternative, compared to ad lib Chow and Choc mice whose daily energy intake was relatively the same on a day to day **(Fig 2)**. The results are consistent with those seen in the free-feeding rat model **(Fig 1)**, and in other similar studies conducted in our lab (57). This overconsumption during access days to a palatable diet and under consumption during non-access days to normal chow diet can be compared to the human dieting culture, where one refrains from eating a palatable food, but after a given time, can no longer fight the urge to resist the palatable food, and ends up eating more than he/she would normally eat.

Int-Long mice showed increased self-administration and "urgency" to obtain palatable diet in operant chamber sessions

The results are consistent with the hypothesis that intermittent long mice will show an increased self-administration in operant session and more motivated and compulsive behavior, as measured in operant chambers through fixed ratio and progressive ratio sessions.

Significantly higher self-administration in Int-Long mice during FR5 sessions (**Fig 3A**) reveals a desire to obtain palatable diet even though work must be done to obtain the diet. This suggests that intermittency to a palatable diet drives increased self-administration. **Figure 3B** shows that Int-Long mice are consistently consuming most of their pellet reinforcers**,** while Choc and Chow mice began by consuming most pellet reinforcers, but amount consumed goes down over time, showing that the Int-Long mice, in a sense, are "cleaning their plates", while the Choc and Chow may be nose-poking for the "action" inside of the operant chambers rather than for the desire to eat. Latency periods before first reinforcer (**Fig 4A**) reveal level of urgency of the mice have to obtain the diet. The latency for Int-Long is four-fold shorter than Chow, which reveals a great level of urgency to obtain the palatable diet. This level of urgency is further supported by Int-Long mice having a significantly high percent of reinforcers earned within 10 minutes of FR5 (**Fig 4B)**, which further supports "urgency" to obtain palatable diet. Interestingly, Choc mice also had a significantly shorter latency period than Chow, and has a significantly higher percent of reinforcers in earned 10 minutes. Int-Long is expected to have a sense of "urgency" since the FR session follows a 24-hour deprivation of the palatable diet for the Int-Long mice. However, it was not expected that the Choc mice have this sense of "urgency" as well, since they have ad lib access to the diet. This behavior is possibly due to some component of the palatable diet that makes it more

motivating to consume than the chow diet that is independent of intermittency (3). This further supports the idea that the chocolate-flavored high sucrose diet is, in fact, palatable. This is similar to human behavior: for example, the Int-Long group would be a binge eater will restrict himself from eating junk food, throwing away all the junk food he has at home. This person, after a period of time of restriction, can no longer resist, and runs to the grocery store, buys bags of chips and cookies, and eats all of it in an hour. The Choc group, conversely, would be a person who always has a fridge full of junk food (can eat whenever he pleases). One day, he goes to the fridge or grocery store and it was unexpectedly completely empty, and he would probably start vigorously trying to figure out what's happening and get frustrated, as the Choc mice, confused and frustrated when the diet is no longer freely presented to them, will be aroused, and starts nose-poking to figure out where the diet went. The Chow mice would also have some degree of arousal, but less than the Choc due to the palatability of the CHOC diet.

Int-Long mice showed increased responses in PR and extinction session, suggesting compulsive behavior.

In our experiment, progressive ratio sessions serve as a measure of reinforcement efficacy (63), which in this case, is an indication of value of the food pellet for the mice. Motivated behavior, by nature, is not detrimental; all animals and people should be motivated to eat to meet a caloric need. However, addictive disorders like compulsive eating, take motivation to get something pleasurable (food, drug, alcohol) to a level beyond satisfying a need, which then becomes compulsive behavior. The component of compulsive behavior is crucial for defining binge-like eating and not just over-eating, because in a presence of a great challenge, these mice still want to obtain the desired

food. At high break points in PR sessions, these mice are tapping into compulsivity because the work expended is disproportionate to the objective value of the reinforcer. In the progressive ratio session (**Fig 5A**), the intermittent long mice nose poked significantly more than the ad libs: ~9 fold the amount of Chow ($p<.01$), and ~4 fold the amount of Choc ($p<.02$). These intermittent mice who are continually nose-poking in progressive ratio are not even working to satisfy a caloric need anymore because they are not being rewarded, and the intermittent mice, which continue to nose-poke despite no lack of reinforcer, are considered compulsive. The breakpoint **(Fig 5B)** for number of nose pokes per reinforcer for Int-Long was ~3 fold higher than Choc, and ~6 fold higher than Chow. The Int-Long mice did not stop until they were responding an average of about 100 nose pokes for a single pellet (with one mouse as high as 240 nose pokes/pellet), showing persistent action despite the amount of work expended being disproportionate to objective value of the reinforcer. It can also be noted that these Int-Long mice have significantly higher break points than Choc mice, who are responding for the same palatable, chocolate-flavored pellet. In our PR model with rats, classical punishment-resistant responding was used to correlate compulsive behavior, which corresponds with PR in the rat model, thus supporting the idea that at high break points, we are tapping in to compulsive behavior (6, 57).

An extinction session (**Fig 6**) goes a level beyond PR sessions, in that mice now no longer receive a reinforcer for any amount of work done. In extinction sessions, mice eventually learn to dissociate a previously learned rule (in this case, nose poke results in reinforcer). In this study, we are looking at the first extinction session only, so we expected to see an “extinction burst” which is defined as an initial elevation in

responding relative to reinforced responding (64). This burst will be greater for more highly valued things, due to discrepancy between expected reward value and ongoing reward (a negative reward prediction error). The extinction session, thus, serves as a measurement of the degree of seeking behavior for a diet that has been removed during operant sessions, and is an additional measurement of compulsivity. In this extinction session, Int-Long mice have significantly more responses in the extinction session compared normal FR5 sessions, which shows an extinction burst in these mice, suggesting that they value the reinforcer more than the Chow and Choc mice. The high response rate suggests that the Int-Long mice also exhibit compulsive "seeking" behavior, where the compulsive behavior perseveres and intensifies, despite no indication of reinforcer. There is also a significant increase in responses for Chow mice from normal FR5 session to the extinction session, but since there is no significant difference between Chow and Choc responding and that the two are significantly lower than Int-Long responding in the extinction session, this increase of responses in extinction session is likely due to confusion from the previous association where five nose pokes amounted to one pellet reinforcer. An example of this in humans is a child who usually screams and throws a tantrum to get attention from his parents, who will come running to him to calm him down. However, if his parents stop giving him attention, this is not what he expects, and as a result, his screams and tantrum will intensify and become more frequent due to a lack of usual, expected response (his parents giving him attention).

Ad lib chow mice exhibit significant fat and weight gain at end of session due to high-sucrose diet

Choc mice were the only group that gained a significant % of body fat and a significant amount of weight (**Fig 7**), which shows that a diet composing of just the sucrose-rich chocolate flavored diet leads to obesity. Even though Int-Long mice had intermittent access to the same palatable diet (and overate on these days), they did not gain a significant amount of body fat, and their weight gain over time was comparable as Chow. It is possible that there is no significant body fat change or weight increase due to the significant under-consumption of these mice during non-access days, which balances out their overconsumption on access days. This lack of change in body composition is also comparable to human binge eaters. Although many binge eaters are overweight, binge eating disorder and obesity are distinct concepts, and one can be present without the other. Many people that are diagnosed with binge eating disorder are not overweight or obese; more importantly, the disorder may cause many detrimental health issues, psychologically and physiologically, with a prime example being bulimia nervosa. Also, in a rat model measuring metabolic rates, Int-Long rats had significant differences in metabolism action and rate of fuel substrate consumption that are suggested to be detrimental to long-term health dysfunction (58). The body composition data of these mice suggests that this mouse binge-eating model is not associated with obesity, but it is important to note that obesity is just one of many diseases that binge eating disorder is associated with, and there are many other detrimental health effects linked to this disease.

Establishment of Mouse Model for Binge-Eating

With the mouse binge eating model established, we can now explore how genetic bases can play a role in modulating binge eating behavior. However, we first need to identify a system that is linked to binge eating and will permit the use of genetic tools. In

the next chapter, we explore roles of different receptors to learn more about their role in binge-eating

CHAPTER 4: PHARMALOGICAL DRUG TREATMENT (SIGMA-1 AND MU-OPIOID LIGANDS)

In this chapter, we examined the roles of sigma-1 receptor and mu-opioid receptor on modulating compulsive eating behavior through the administration of the BBP-02 and 14-MM drug. We predicted that both the sigma receptor antagonist and opioid receptor agonist would decrease compulsive eating behavior.

Following the development of the mouse model, mice were treated with two pharmacological drugs that targeted receptors that were previously shown to alter feeding behavior. The purpose of these treatments was to investigate how certain receptors and mechanisms in the brain may be linked to compulsive eating.

4.1 INTRODUCTION: SIGMA 1 RECEPTOR DRUG (BBP-02)

The first receptor that was investigated was the **σ1** receptor. The **σ** receptor in the brain is known to play a role in addictive mechanisms, and has two primary binding sites: **σ1 and σ2.** The **σ1 receptor** is a transmembrane protein concentrated in certain regions of the central nervous system, and modulates Ca^{2+} signaling, by acting as a chaperone that shuttles between mitochondrial endoplasmic reticulum and extracellular membrane. The sigma-1 receptor is also implicated in animal models of neurodegeneration, motor dysfunction, and addiction. In a study previously conducted in the Zorrilla Lab, a *σ*-1 receptor antagonist, BD-1063, was shown to decrease ethanol intake and reinforcement in a rat model of excessive drinking (66), and block compulsive-like eating (65).

The **σ1 antagoinst** BD-1063 used our prior studies had a moderately bind high affinity to **σ1,** but we used BBP-02, another sigma drug with a much higher binding affinity and much greater selectivity for sigma-1 than BD-1063 (59). This high selectivity and binding affinity means that BBP-02 more specifically targets sigma-1, and because of prior downregulation of alcohol and palatable diet self-administration, we hypothesize that BBP-02 will reduce self-administration and decrease compulsive binge-like eating in mice.

4.2 METHODS: SIGMA 1 RECEPTOR DRUG (BBP-02)

Directly following the establishment of the mouse model in which behavior and intake of the three groups of mice was measured for 6 weeks, the drug treatment of BBP-02 was administered to all mice. Drug doses of 1mg/kg, 10 mg/kg, and 40 mg/kg were administered to mice, with saline as a vehicle for control. Dose concentrations were determined by using similar doses that yielded altered behavior in the prior studies (65,66, 67). Drug dose assignments were based on a randomized Latin Square Design. Before the onset of the dark cycle, mice were given intraperitoneal injections (.01ml/g) with a pre-treatment period of 30 minutes before being placed in operant chamber. Mice were placed in FR 5 sessions for one hour, and responses were recorded. On the access days following a drug injection session, mice were given a normal FR5 operant session to eliminate any residual effect of drug and to check that mice behavior in operant chambers has returned to normal.

4.3 RESULTS: SIGMA 1 RECEPTOR DRUG (BBP-02)

Figure 8A shows that there was no significant difference in operant behavior between the groups for any of the three doses of drugs compared to vehicle, and **Figure 8B** shows

there was no significant difference in pellet consumption in these operant sessions. The home-cage intake was recorded. **Figure 8C** shows consumption in the 24 hours following drug administration (operant + home cage intake) which had a significant within-subject effect of dose ($F(3,48)=3.409$, $p<.05$), with the Chow mice consuming significantly less in the 24 hours following administration of the highest dose of 30 mg/kg BBP-02 ($p<.02$). Looking at just the home cage (23hr) intake in **Figure 8D**, a similar result can be seen, with a significant within-subject dose effect ($F(3,48)=3.645$, $p<.02$)), with Chow mice consuming significantly less in the remaining 23 hours following the 30 mg/kg administration of BBP-02 ($p<.02$).

4.4 DISCUSSION: SIGMA 1 RECEPTOR DRUG (BBP-02):

Sigma-1 Receptor may not be involved in Driving Down Self Administration

There is no BBP-02 drug effect on operant behavior (**Fig 8A, 8B**), which suggests that the sigma-1 receptor may not be what is specifically driving down compulsive behavior. This result is not what was expected, and does not correspond to the conclusion of the previous study using BD-1063, but since BBP-02 is much more potent and selective for sigma-1, which suggests that the sigma-1 receptor, alone, may not be responsible for regulating compulsive behavior. The BD-1063 receptor may be binding additional sites aside from sigma-1, which is driving down compulsivity in the rats in the study with Sabino et al. and Cottone et al. However, it must be taken into account that there are a few key differences between this study and the studies previously listed. These key differences include the use of a mouse model instead of a rat model, the use of an intermittent long access group instead of an intermittent short access group, and

intraperitoneal drug injection instead of a subcutaneous drug injection, which may have caused the drug to lose potency (since IP injections go to the liver before entering in the bloodstream). Because of these key differences between the two studies, we may want to repeat this study using an intermittent short group and administering the drug subcutaneously in order to more accurately draw conclusions from the results of the two experiments.

Delayed Undereating of Chow mice after BBP-02 treatment

Although there was no significant effect of self-administration in the first hour in which BBP-02 was administered, Chow mice were significantly undereating in the 23 hours following self-administration after receiving treatment to the highest dose (30 mg/kg), as shown in **Figure 8C and D**. A possible explanation of this undereating could be that there is a delayed pharmakinetic effect of BBP-02, and that it takes longer for the drug to accumulate in the brain. However, the PET study (67) argues against the phenomenon, because the study showed that BBP-02 treatment resulted in immediate reaction. A possible explanation for why an under-consumption can only be seen in the Chow group is because the schedules of access makes mice who are under the palatable diet less sensitive to the anorectic effect of the drug.

4.5 INTRODUCTION: MU OPIOID RECEPTOR DRUG (14 MM)

The second receptor that is investigated in this study is the mu-opioid receptor. In broad terms, opioid receptors control pain, reward, and addictive behaviors, with opioids being substances that act on these receptors to release endorphins. Opioid receptors have been previously shown to play a role in food intake and binge-eating behavior, and

studies show that rats with intermittent access to a sucrose-rich diet had alterations in opioid systems and showed signs of opioid withdrawal (68-70). The mu-opioid receptor initially mediates positive reinforcement following direct and indirect activation, and has been studied with many drugs of addiction. Some have conceptualized that compulsive eating results in similar psychological and physiological changes as drug addiction (addiction-like behavior), and as such, the "dark side of addiction" hypothesis can be suggested to explain behavior in binge eating mice. The "dark side of addiction" hypothesis states that the body adapts to disturbances of homeostasis, and that if a certain pathway in the body is activated (i.e. mu-opioid activation), the body recruits opponent anti-reward processes to downregulate the effects of pathway activation, resulting in a diminished opioid response. This results in needing more opioid to achieve pleasurable feeling, thus an increase in self administration. However, when the mu-opioid is gone, there is no more activation and no more reward processes activated, but the anti-reward processes are still present, creating a deficit and withdrawal state, which motivates resumed and escalating drug use (71). With this hypothesis applied to this study, when the Int-Long mice receive no palatable food, they are in a deficit state because of opponent processes still present. However, there are two types of drugs that can temporarily relieve opioid deficiency: agonists and antagonists. Agonists relieve opioid deficit situations developed in a context of withdrawal by taking the place of the drug and gives the user the same feeling he or she would receive if they were taking an opiate, causing the release of endorphins. The μ-Opioid receptor mediates positive reinforcement following direct (morphine) or indirect (addictive substance/drug) activation, and the drug administered to the mice was 14-methoxymetopon (14-MM), a very potent μ-Opioid

receptor agonist. 14-MM is not being tested as potential drug treatment for binge eating, but rather, as a way to conceptually assess if there is a different level of mu opioid expression in binging mice versus control mice. There have been many studies showing the modification of opioid systems in animals with different feeding diets, showing that palatable diets may create effects similar to a drug of abuse (60). Drugs of addiction attach to mu opioid receptors and the linkage of these chemicals triggers the same biochemical brain processes that reward people with feelings of pleasure, generally for basic life functions. Repeated exposure to these opioids alters the brain, including opioid tolerance, which requires a need to take a higher dosage of the drug to achieve the same effect, and withdrawal during abstinence (71).

When an agonist is introduced, it can either facilitate positive reinforcement, creating pleasurable feeling and making the subject want more of the pleasurable feeling, thus increasing self-administration. Conversely, an agonist can act as a substitute to the opioid. The subject feels enough of the pleasurable feeling from the agonist, and in turn, decreases self-administration. Thus, we have two competing alternate hypotheses: the agonist 14-MM treatment will cause either an increase in self-administration or decrease in self-administration, selectively in the intermittent mice, due to the compulsive nature of these mice that may play a role in altering their opioid system.

4.6 METHODS: MU OPIOID RECEPTOR DRUG (14 MM)

14 MM was solubilized in isotonic saline. Drug doses of 6.4 ug/kg, 16 ug/kg, and 40 ug/kg were prepared, with saline as a vehicle. Dose concentrations were determined through a previous study (59), with highest dose falling below the drug concentration that

was shown to cause motor impairment, but still within range of analgesia. Drug dose assignments were based on a randomized Latin Square Design. Before the onset of the dark cycle, mice were given subcutaneous injections (0.1 ml/1 g) with a treatment period of 30 minutes before being placed in operant chambers, where intermittent mice were given first exposure to palatable diets. Mice were placed in FR 5 sessions for one hour, and responses were recorded. On the access day following drug injection, mice were given a normal FR5 operant session to eliminate any residual effect of drug and to ensure that mice behavior in operant chambers has returned to normal.

4.7 RESULTS: MU OPIOID RECEPTOR DRUG (14 MM)

The results of a mixed ANOVA for repeated measures indicated a significant dose effect for treatment, shown in **Figure 9A.** However, there is not a significant dose x group interaction. Follow-up comparisons indicated that there was a significant pairwise difference between vehicle and the 6.4 dose ($p=0.051$), and vehicle and the 40 dose ($p=0.005$) for the intermittent mice only. There was also a linear contrast of dose effect shown in the intermittent group. **Figure 9B** shows a significant dose effect on operant pellet consumption ($F(3,14)=5.699$, $p<.01$), with Int-Long mice consuming significantly less at the highest dose (14 ug/mL, $p<.002$).

4.8 DISCUSSION: MU OPIOID RECEPTOR DRUG (14-MM)

Intermittent mice have a downregulation of self-administration with 14-MM treatment

There is downregulation of self-administration in intermittent mice at the two highest doses of 14-MM drug for intermittent mice only **(Fig 9A)**, which suggests that the opioid

system in the intermittent mice may be altered due to compulsive eating behavior. Altered opioid systems have been previously seen in rodent models of sucrose-rich diets: Rats that had excessive sugar intake causing an altered binding to mu-opioid receptors in the brain (60), rats given intermittent access to a high sugar diet showing neurochemical signs of opioid withdrawal (60). The results of this treatment are similar to those seen in the studies mentioned above (68-70), which showed altered opioid systems in rats. However, these were showing decreased intake in free feeding; and our results are novel in correlating an alteration of the opioid system with self-administration in binge-eating mice.

CHAPTER 5: CONCLUSIONS

The goal of this project was to create a mouse model for compulsive binge-like eating, and to see the pharmacological effect of sigma-1 and mu-opioid receptor ligands on binge-eating. While there is a research being conducted about what causes obesity, there is little known about binge eating specifically, and how binge eating behavior is connected to different neuronal and genetic bases. There is a lack of animal models for binge eating, and with the models that currently exist, there are very few that are mouse models, even though mice have the most genetic tools available. The establishment of a mouse binge model will allow for further studies that will help better understand what may cause binge eating and how binge eating can alter/be influenced by biological functions, on both the neurological and genetic level. The establishment of the mouse model followed with two pharmacological drug treatments will allow for much more to be learned about the bases that govern binge-eating.

The studies in Chapter 2 describe the rat free feeding model, which was the first study conducted in this lab to better understand binge eating, and this study was used as a basis to create a model with the same feeding schedules, but with the addition of operant chambers to allow for a better understanding of behavior, and the switch to a mouse model to allow for more versatility in future experiments, opening the door for gene studies in addition to neuronal studies.

The studies in Chapter 3 describe the establishment of a mouse binge eating model. Intermittent mice developed binge-like behavior by exhibiting cyclic over-eating and under-eating, increased level of self-administration and urgency to receive reinforcer in FR5 sessions, and demonstrated compulsive behavior through a significantly high

amount of responses during PR sessions and “seeking” behavior shown in extinction sessions. The development of the mouse binge-eating model allowed for the testing of pharmaceutical drugs on the mouse model and how binge-eating behavior may alter, or be altered by various mechanisms.

Chapter 4 describes the two drug treatments administered to the mice to better understand the relationship between binge eating behavior and neuronal mechanism. These drug treatments revealed that sigma-1 by itself may not be involved in regulation of binge eating, as found in a previous study, and that the opiate system is altered in mice that have binge behavior. However, future studies are needed to ensure these differences are not due to different experimental settings and intermittency durations.

Future Studies

Future studies may further examine the applicability of therapeutic-like opioid receptor ligands such as suboxone (a combination of buprenorphine and naloxone). Suboxone is a partial opioid antagonist, so it will not have as strong of a stimulation in the opioid pathway as 14-MM, thus having less addiction liability. This makes suboxone a more suitable as a drug for treating binge-like eating disorders.

With the mouse model of binge eating established, many future studies can be conducted using the numerous genetic tools available in mice to better understand the relationship between genes and binge eating. Specifically, Cre-Lox recombination, a site-specific recombinase technology, can be used to carry out deletions and insertions at very

specific sites in DNA of cells, specifically different neural circuits and receptors, such as the mu opioid receptor gene.

FIGURES

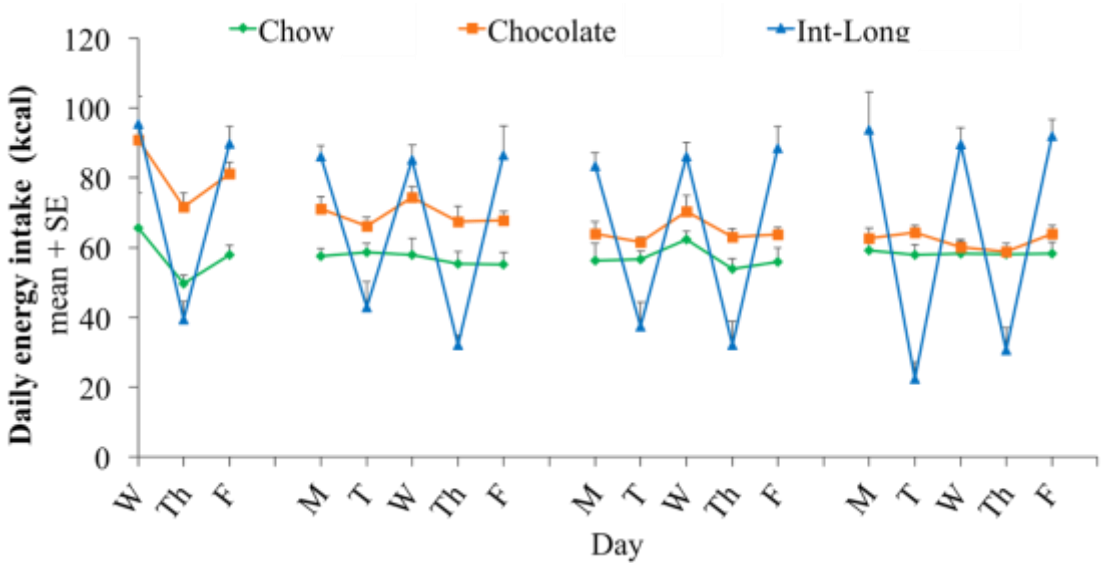

Figure 1. Intermittent rats in free feeding model show cycling of fasting on chow diet and binging on palatable diet. Daily (24hr) weekday energy intake for free-feeding rats plotted across 4 weeks of the study

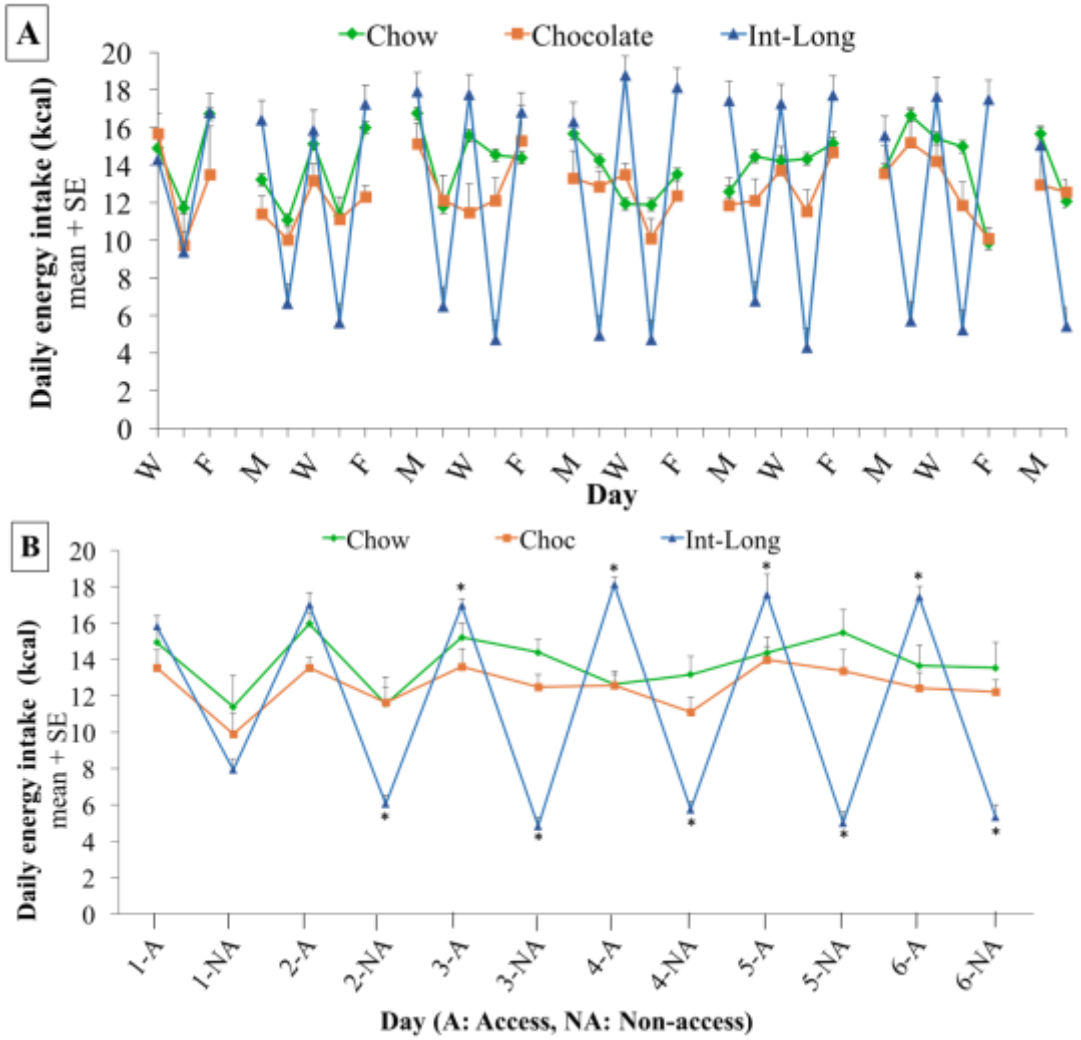

Figure 2. Intermittent mice in mice binge eating model show cycling of fasting on chow diet and binging on palatable diet. Daily (24hr) weekday energy intake for mice plotted across the 6 weeks of the study in **A**, and in **B**, is shown as weekly averages of access and non-access days across 6 weeks. **C** shows weekend (48hr) energy intake for mice across the 6 weeks. **Different from all other groups that week ($p<.05$)*

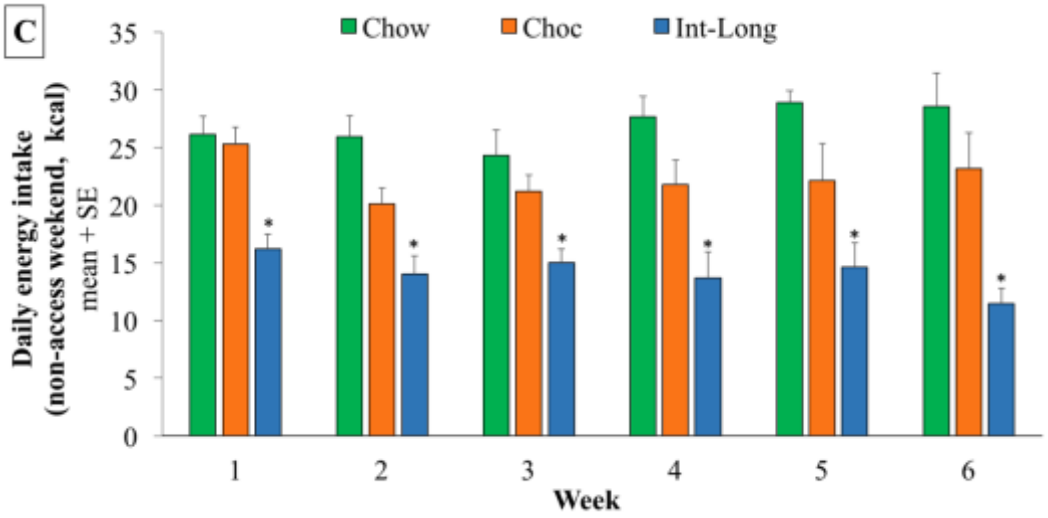

Figure 2. ***continued***

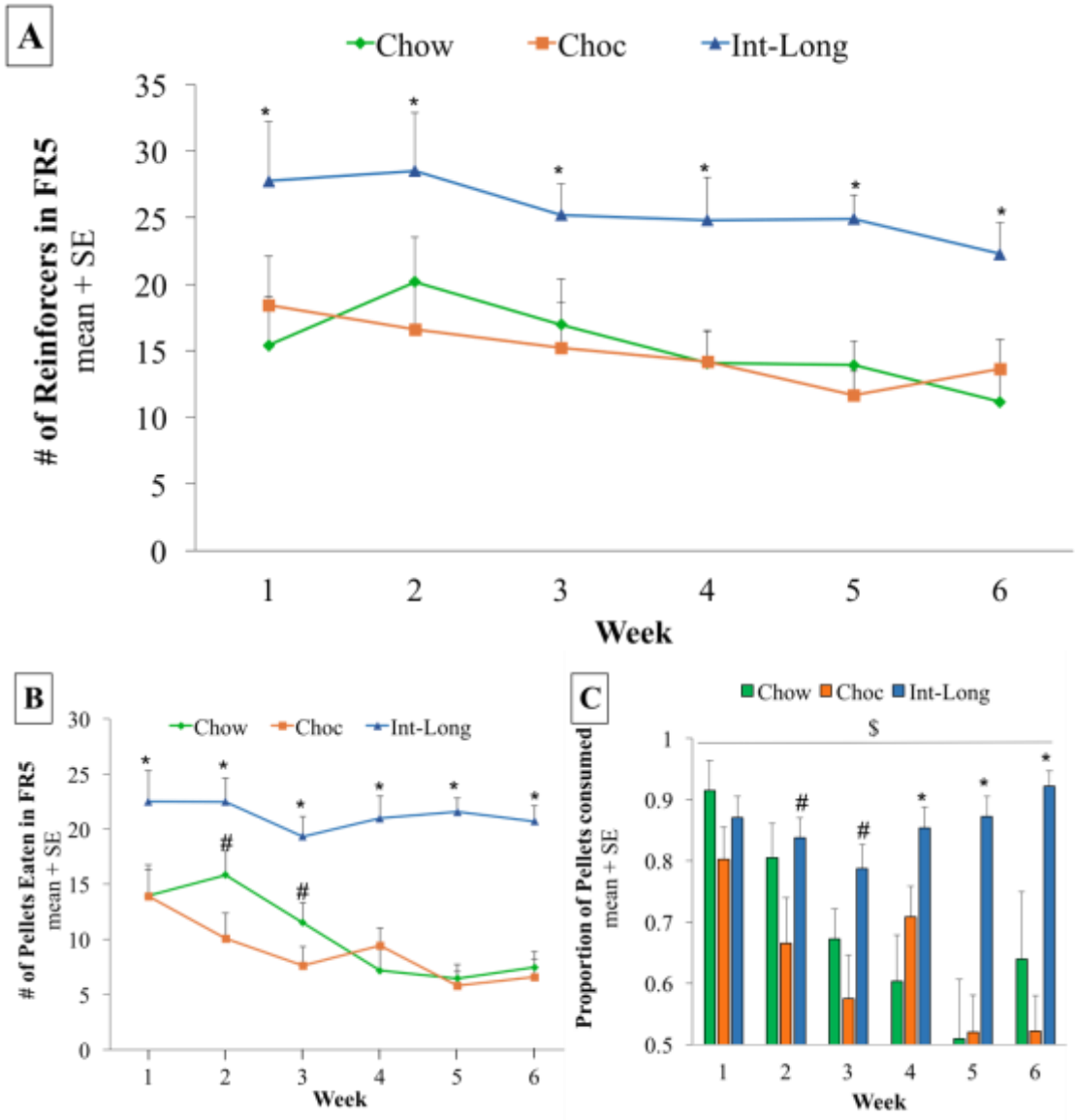

Figure 3. Intermittent mice show significantly higher self-administration and pellet consumption in fixed ratio operant sessions. Weekly average of reinforcers in 1 hour fixed ratio 5 (FR5) sessions in **A**, and number of pellets consumed in 1hr FR5 sessions in **B**, and percent of pellets consumed in FR5 session in **C**. **Different from all, #Different from Choc p<.05, $Significant group x week interaction (p<.05)*

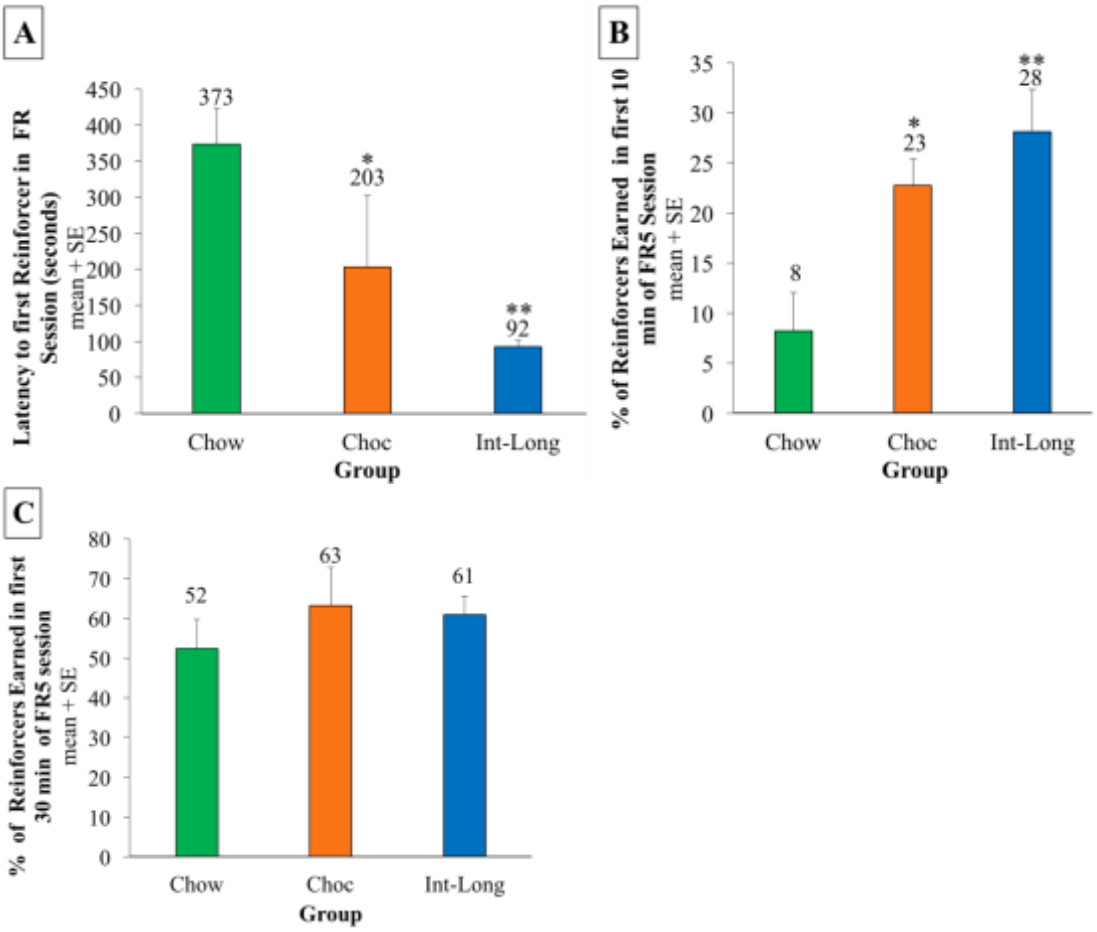

Figure 4. Intermittent mice have the shortest latency period before first reinforcer and greatest percentage of reinforcers in the first 10 minutes of FR5 sessions. Latency period before first reinforcer in 1hr FR5 session in **A**, and percent of reinforcers earned in first 10 minutes (**B**) and in first 30 minutes (**C**) of 1hr FR5. **Different from Chow ($p<.05$); **Different from Chow($p<.001$)*

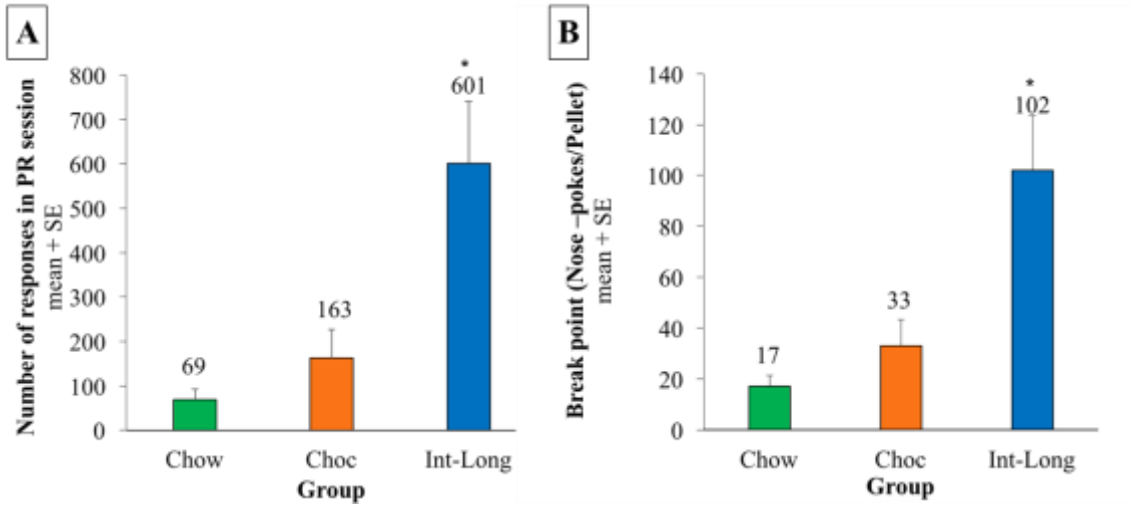

Figure 5. Intermittent mice have significantly higher amount of responses and breakpoint for reinforcers during progressive ratio session. Number of responses in progressive ratio (PR) session in **A**, and breakpoint for number of responses in PR session in **B**. **Different from all other groups ($p<.05$)*

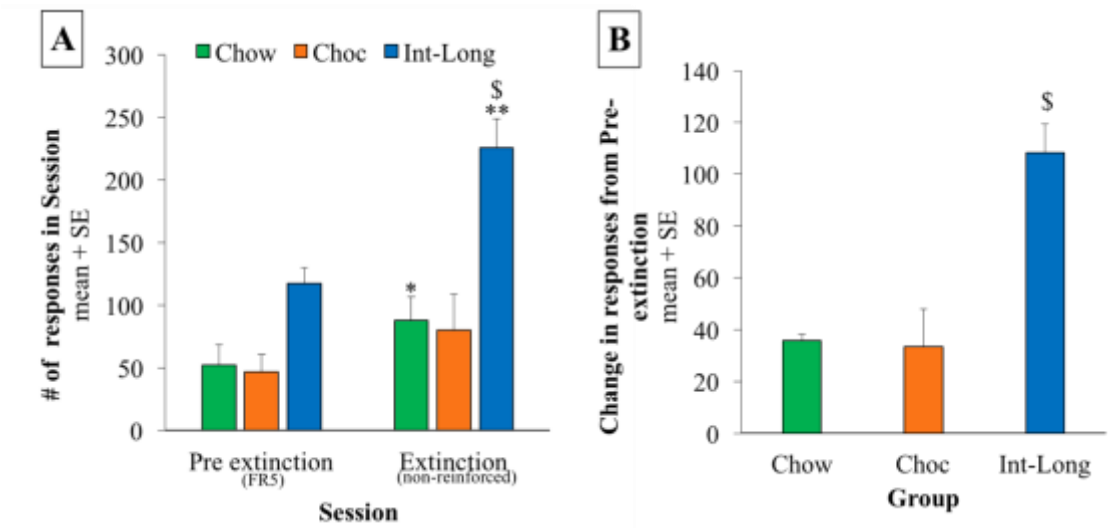

Figure 6. Intermittent mice have a significantly higher extinction responding than all other groups. Number of responses in 1hr FR5 Pre-Extinction (average of 3 sessions before extinction session), compared to extinction session shown in **A**. Change in responses from pre-extinction is shown in **B**. **Different from Pre-Extinction (p<.02); **Different from Pre-Extinction (p<.001); $Different from all groups (p<.05)*

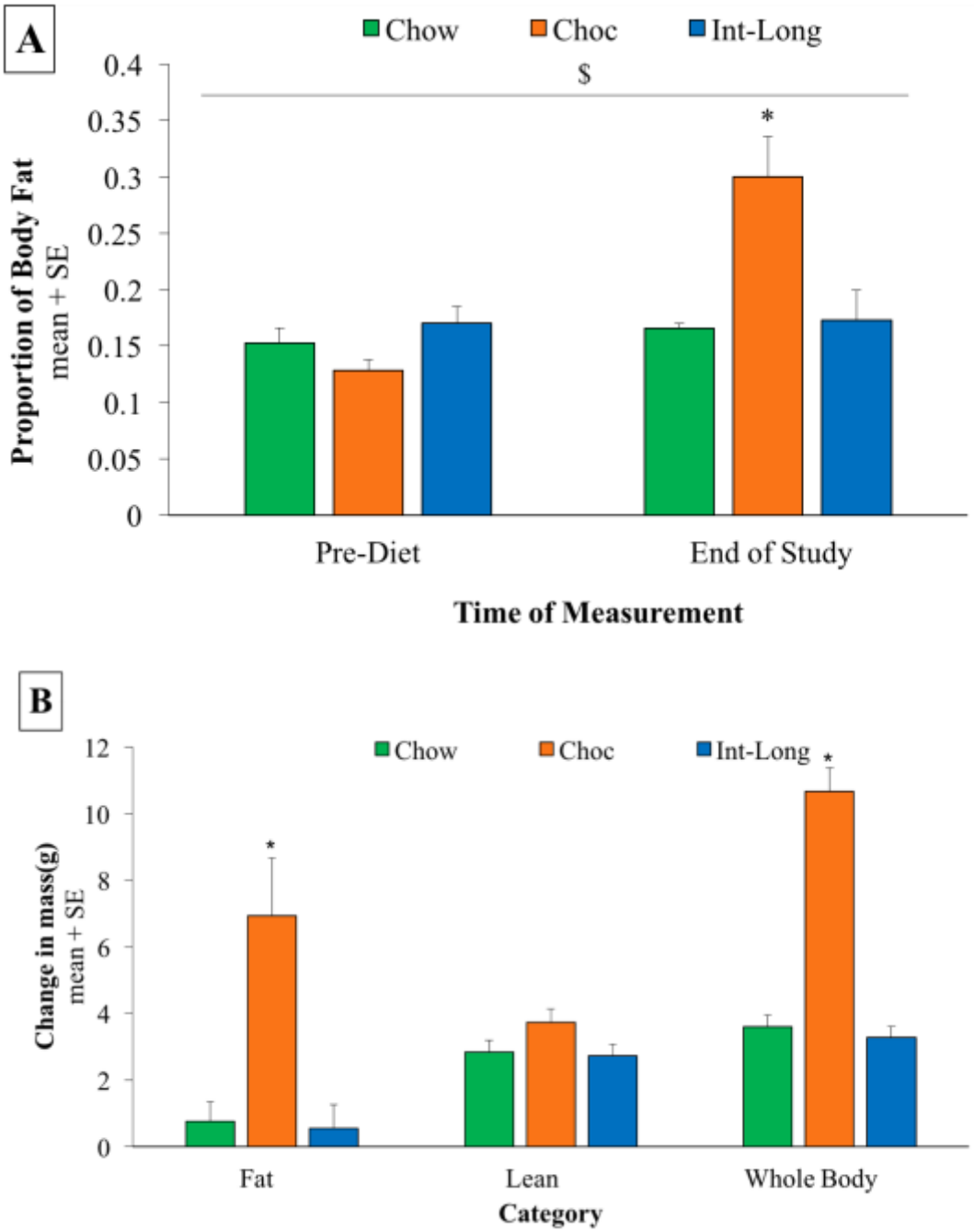

Figure 7. Ad lib Choc mice had significant increase in body fat composition and weight gain. Percent of fat weight in mice before diet schedules compared to at the end of study in **A**. **Different from Pre-Diet weight (p<.02) $Significant group x time interaction (p<.01)*
Change in weight from start of diet schedules to end of study in **B**. **Different from all groups (p<.05)*

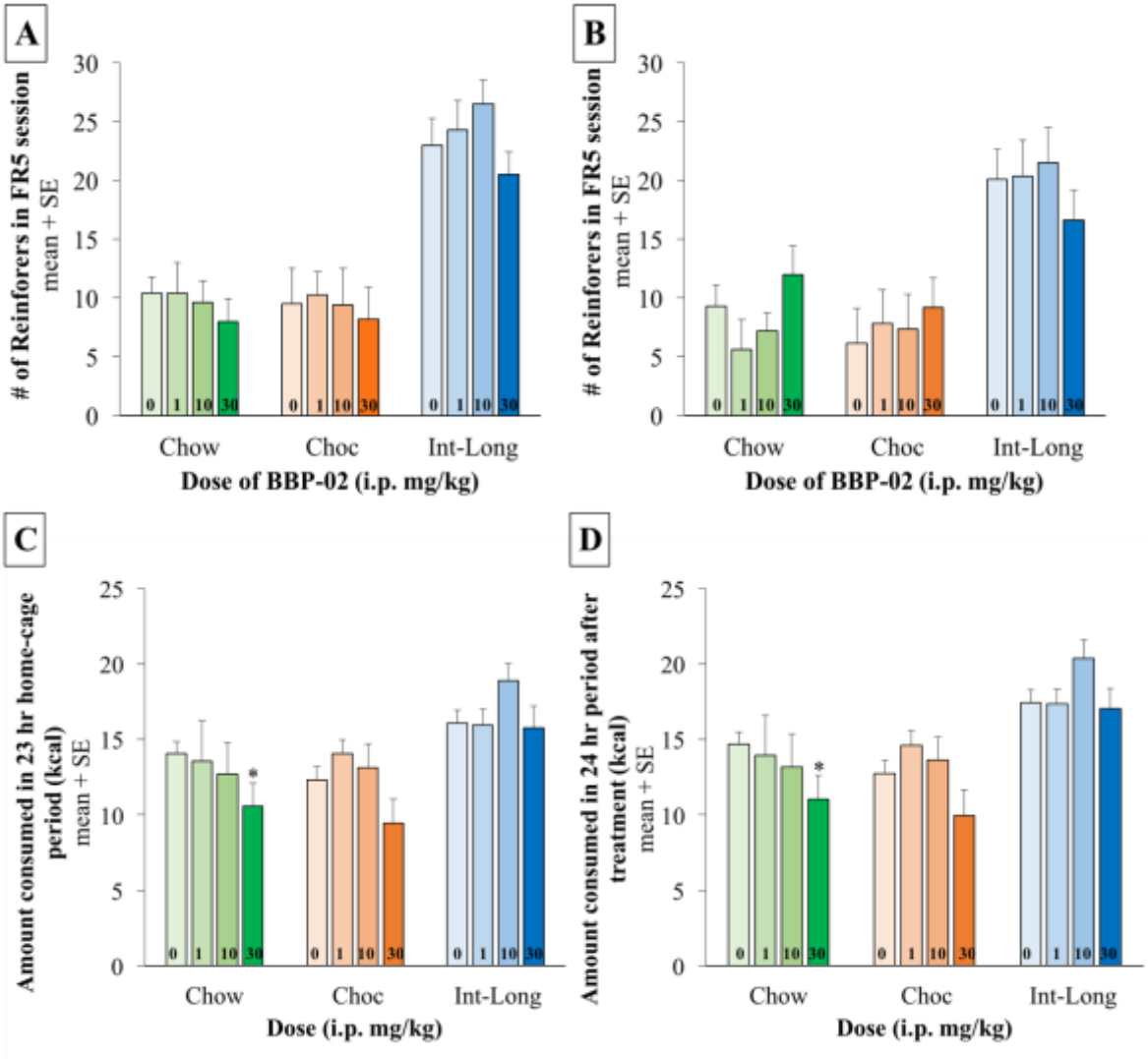

Figure 8. No change in self administration with BBP-02 drug treatment, but delayed undereating of home cage diet for Chow mice in the 23 hours following drug administration. Number of reinforcers in 1hr FR5 session following administration of different doses of BBP-02 in **A,** and number of pellets consumed in these sessions in **B.** Intake during 24 hours following BBP-02 administration in **C**, and during 23 hours in home cage (after 1hr operant session)in **D**. **Different from vehicle (p<.02)*

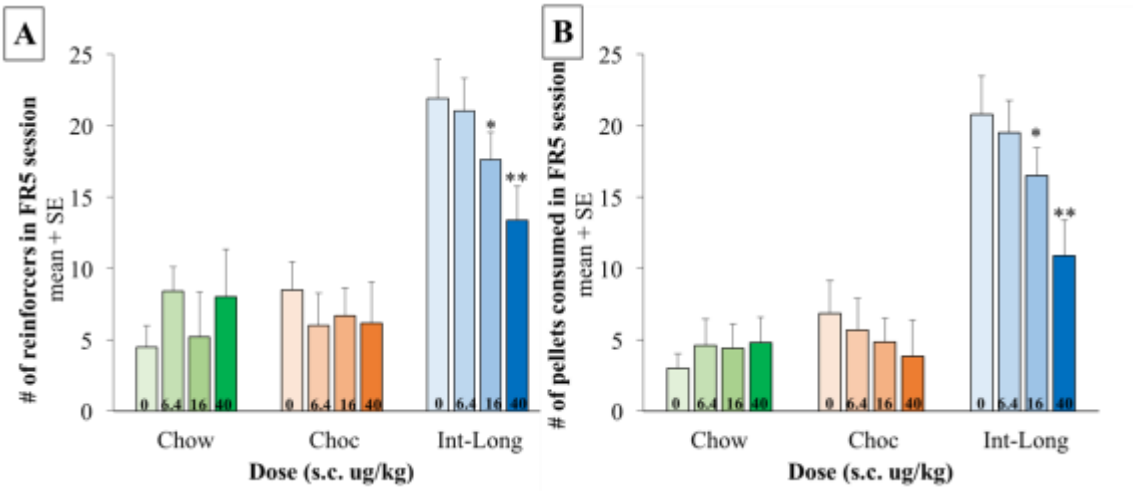

Figure 9. Decrease of self-administration and pellet consumption for Intermittent mice after administration of highest doses of 14-MM. Number of reinforcers in 1hr FR5 session following administration of different doses of 14-MM in **A,** and number of pellets consumed in these sessions in **B**. **Different from vehicle (p=.051,.054), **Different from vehicle (p<.01)*

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