Title

Behavioral Economic Analysis of Binge Eating in Female Rats; Glucocorticoid Receptor Expression Level in the Central Amygdala after Intermittent Access to Palatable Food

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Behavioral Economic Analysis of Binge Eating in Female Rats; Glucocorticoid Receptor Expression Level in the Central Amygdala after Intermittent Access to Palatable Food

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

in

Biology

by

Jiayuan L. Murphy

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Professor Christina Gremel, Chair
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Professor James Nieh
Professor Eric Zorrilla

2020

The Thesis of Jiayuan L. Murphy is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California San Diego

2020

DEDICATION

I dedicate this thesis to my Mom and Dad, Yang and Charlie, as well as my Grandma and Grandpa, Huixian and Weixin.

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ABSTRACT OF THE THESIS

Behavioral Economic Analysis of Binge Eating in Female Rats; Glucocorticoid Receptor Expression Level in the Central Amygdala after Intermittent Access to Palatable Food

by

Jiayuan L. Murphy

Master of Science in Biology

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Professor Christina Gremel, Chair

Professor Byungkook Lim, Co-Chair

Binge eating is characterized by eating a large amount of food within a short period of time with the feeling of loss of control. We applied the principles of behavioral economics, which separate consumption into unconstrained demand (Q_0) and elasticity of demand (α) to

study binge eating. We found that rats with intermittent access to palatable diet (INT) had increased Q_0 and decreased α comparing to rats with ad-libitum chow (CHOW) or palatable diet (CHOC), which correlated with greater palatable food intake and consumption during self-administration. Within INT group, Q_0 positively correlated with fixed ratio self-administration responses. Initial chow intake, initial body weight, and first-day palatable diet intake predicted higher α. Results show behavioral economic measures have predictive values in assessing compulsive eating.

Drug dependence has been implicated with the change in glucocorticoid receptor (GR) levels in the central amygdala (CeA), where its elevation relates to negative affect upon abstinence, increasing likelihood of relapse through negative reinforcement. Here we accessed change in GR mRNA expression in rats after intermittent access to palatable diet. Results showed that female, but not male, INT rats had higher expression comparing controls; female INT rats had higher expression than male counterparts; and female INT rats with substantially high progressive-ratio response showed significantly higher expression comparing to controls, while other female INT rats did not. Results show sex and individual-level differences in the development of compulsive eating, suggesting differences in neuroadaptation in response to intermittent access diet schedule.

CHAPTER 1: INTRODUCTION

Substance use disorders are characterized by behavioral and pharmacological symptoms such as tolerance, withdrawal, an increase of time and effort in drug-seeking and use, repeated unsuccessful effort to cut down use, and continued use despite negative outcomes [1]. At the same time, palatable foods that are calorically dense have gained increasing attention as sharing characteristics of substances of abuse with addiction potential [2-7]. This concept of "food addiction" has been operationalized by the Yale Food Addiction Scale, in which individuals are assessed on personal behaviors towards food that highly resemble the pathological measures used to diagnose substance use disorders. Some examples of such behaviors include the inability to stop consuming certain foods despite being full, eating to relieve negative emotions, repeated attempts to cut down the intake of certain foods, and significant physical and psychological distress due to food consumption [8].

One hallmark of addiction is the loss of control over intake [1]. In the context of food addiction, binge eating is defined as eating an abnormally large amount of food within a short period of time, where the individual loses control over the amount of food being consumed [1]. Binge eating is a major behavioral symptom for binge-related eating disorders and is also closely associated with obesity, as 40%-70% of people with binge eating disorder are obese [9-12]. Regarding what could elicit binge eating behaviors, current understandings point towards dieting and the intentional abstinence from certain foods, which usually are foods self-labeled as "unhealthy" but are preferred for their sensory qualities (e.g., taste, smell, etc.) [17-20]. Limiting oneself from consuming such foods has been proposed to lead to a withdrawal-like state with strong cravings and negative emotions, that in turn, may make an individual more prone to

engage in compulsive-like binge eating on the foods they were not allowed to have [14, 21, 22]. This cycle of restricting and binge eating is given the name “yo-yo” dieting and is commonly present among the dieting population [23-24].

Because binge eating is closely associated with eating disorders, obesity, and other health conditions that negatively impact affected individuals and societal health economics [25-29], it is critical to understand its development, mechanism, and progression in order to develop treatments. Therefore, this study aims to contribute to a better understanding of the behavioral and neurological aspects of binge eating.

CHAPTER 2: BEHAVIORAL ECONOMIC ANALYSIS OF BINGE EATING IN FEMALE RATS

The concept of behavioral economics refers to the assessment of the relation between cost and demand of a certain substance [30]. In the case of self-administration models of drug or food, this refers to the relation between the unit price of the substance (ratio-requirement) and the subject's total consumption at each unit price [30, 31]. A demand curve can be generated by increasing the number of responses required to receive each unit of reinforcer across fixed-ratio (FR) sessions, or a between-session progression. The mathematical function fit to the demand curve provides two important pieces of information: 1.) the level of demand when the cost is at zero (Q_0), which is the approximated level of consumption if no effort is needed to obtain the substance and 2.) the elasticity of demand (α), which is how sensitive the consumption is to the increasing cost of obtaining it. In addition to those two parameters, O_{max} and P_{max} values, which are derived from α and k (a scaling constant used to represent data range), can also be obtained from demand curve analysis. O_{max} is defined as the maximum level of work output across the range of increasing unit costs, and P_{max} is the estimated unit cost (i.e., ratio-requirement "price") at which the maximum total work output is seen [30].

Demand curve measures have been shown to predict addiction-like behaviors in both animals and humans. Bentzley et al. reported that the elasticity measure, α, predicted a greater degree of drug-seeking behaviors in rats during abstinence from cocaine [32]. They also found that α and Q_0 both predicted punishment-resistance cocaine intake in a rat self-administration model [32]. At the same time, the translational values of behavioral economic studies have been demonstrated by performing drug purchasing tasks in human subjects, in which the individual

hypothetically reports the amount of drug that he or she would purchase at different prices. Studies done investigating cocaine, heroin, alcohol, and nicotine have reported consistent correlation of elasticity or unconstrained demand with real-world substance use [33-37]. Murphy et al. have shown that behavioral economic measurements predicted the level of alcohol consumption in undergraduate drinkers who have gone through a brief intervention [37]. Bruner et al. reported correlations between the hypothetical consumption under a range of prices for cocaine in addicted individuals with cocaine use in real-life [35]. Murphy et al. also reported that individuals' Cigarette Purchasing Task performance had a valid correlation with real-world nicotine dependence and use [36].

Demand curve analysis allows us to dissect consumption into two factors contributing to it: the unconstrained demand and the elasticity of demand, in which either factor could independently correlate with compulsive-like behavior, motivation, and/or consumption [30, 345]. In this case, compulsivity can be defined as the persistence of a behavior despite incorrect or aversive outcomes [38]. Behavioral economic measures can potentially quantify and assess proxy measures associated with the compulsivity of substance use on an individual level. These could be taken into account in designing more effective treatment plans that target unconstrained or inelastic aspects of demand, as appropriate [35].

Behavioral economic studies also have been conducted in the context of assessing the reinforcing values of food. For example, Epstein et al. have reported a positive correlation between demand intensity (Q_0) for energy-dense foods and BMI in females [39]. However, behavioral economic studies have not been performed with the purpose of studying compulsive-like, binge eating.

Evidence has shown that intermittent access to palatable food contributes to the development of addiction-like properties towards the palatable diet [40, 41]. For example, animals that have time-constrained access to a preferred diet develop cyclic intake behaviors, where they binge eat on days they have access to the preferred diet and under-eat on days with access only to the diet that is less palatable. Previous studies have found that rats receiving intermittent 24-hour access to a sucrose-rich, chocolate-flavored diet alternated with regular chow diet developed tolerance towards food reward as they escalated intake towards the sucrose-rich diet and rejected chow (which was accompanied by weight-cycling), showed withdrawal-like, irritable behaviors, increased effort exerted during progressive-ratio self-administration sessions to obtain the preferred diet, had increased fixed-ratio session time-out responses, and continued to eat despite negative outcomes as a mild foot-shock was paired with every pellet earned [40, 41].

To identify possible predictors of compulsive-like behaviors in binge eating, we will use the established rodent model for binge eating to construct demand curves for each animal and each diet group, respectively. The topics we aim to address are 1.) the effect of intermittent access to palatable food on the four key behavioral economics parameters: α, , O_{max} and P_{max} 2.) the correlations between these parameters and self-administration operant sessions across all diet groups 3.) the correlations between these parameters and individual differences in the severity of compulsive-like adaptations within INT subjects 4.) baseline measures that predict development of individual differences in the degree of compulsive-like behaviors within INT subjects. We hypothesize that upon an intermittent access diet schedule, animals would have significantly lower α and higher Q_0, O_{max}, and P_{max}; lower α and higher Q_0

would predict greater operant session responses and number of reinforcers earned across all diet groups; lower α and higher Q_0 would also correlate with greater operant session responses and number of reinforcers earned within INT animals; finally, greater body weight, palatable food intake, and chow rejection would predict lower α and higher Q_0 within INT animals.

2.1 METHODS

Animals

Female Wistar rats (*n*=24) were received around 6-7 weeks old. Upon arrival, they were given a wheat-based, extruded diet (Teklad global 18% protein, 2018, Envigo, Madison, WI) for a total of 2 days before the start of the experiment. Rats had free access to water at all times.

Rats were pair-housed with a clear divider placed between cage-mates to ensure individual measurement of food intake while avoiding total social isolation. Animals were placed in a vivarium which was under a 12-hour light/dark cycle, with lights on at 9:00 AM and off at 9:00 PM. Procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by The Scripps Research Institute's Institutional Care and Use Committee.

Feeding schedule

Two types of 45-mg pellet diets were used for this study. One was a corn-based, chow diet ("chow") (5TUM, Test Diets, St. Louis, MO), and the other was a chocolate-flavored, sucrose-rich, nutritionally-complete diet ("choc") (5TUL, Test Diets, St. Louis, MO) that was highly palatable. Compared to chow, choc was had roughly similar energy density (3.44 kcal vs. 3.30 kcal per gram) and macronutrient proportions (choc: 12.7% energy from fat, 66.7% from carbohydrate, 20.6% from protein; chow: 10.4% from fat, 65.5 from carbohydrate, 24.1% from protein), but was much higher in its sucrose content (49.60% vs. 3.8%) and preferredness (91.2±3.7% preference ratio vs. chow) [43].

Two weeks upon arrival, animals were matched based on baseline measurements and assigned to one of the 3 diet groups. The CHOW group had *ad libitum* access to chow only, the CHOC group had *ad libitum* access to choc only, and the INT group had 24-hour access to choc only starting at dark onset of every Monday, Wednesday, and Friday, with access to chow only on the other days (Fig 1).

Intake was measured daily with the exception of the 24 hours between Saturday to Sunday. Diet switching/renewal was done shortly (0-60 min) prior to dark onset. Mondays, Wednesdays, and Fridays will be referred to as "access days" as the INT animals receive choc; Tuesdays, Thursdays, Saturdays, and Sundays will be referred to as "non-access days" as the INT animals receive chow.

Self-administration operant sessions

Animals received 2 weeks of self-administration fixed-ratio (FR1) operant training before the start of the study. All subjects responded for chow pellets during this training period. Each FR1 session took place approximately one hour before the dark onset of an access day. Each operant chamber had one active lever, which in FR1, each press outside the time-out period would elicit the dispense of a pellet; and an inactive lever, which pressing would not produce any scheduled consequences. Following each pellet being dispensed, there was a 3.75-second time-out period where further lever press responses were recorded but had no scheduled consequences. Lights were turned off for each session (consistent with the dark cycle) to encourage feeding behaviors. Water was always available via a sipper tube during operant

sessions. The criteria for successfully learning to self-administer pellets were >75% active lever responses vs. inactive lever responses and receiving at least 10 pellets. During the training period, rats started with a 24-hour FR1 session paired together with their cagemate in the same operant box to potentially facilitate learning by observation. From this session onwards, rats always had an operant session individually, without their cagemate. Then rats were given an 8-hour FR1 session, and any rat that did not meet the criteria for learning continued with the session for a total of 24 hours. Afterward, rats had two 30-min FR1 sessions that were 2 hours apart from each other for a total of 4 days. Rats that did not meet the criteria for learning continued with the 2×30-min schedule for the following 2 days, with the ones still did not meet the criteria remaining in session for 24 hours.

After training, rats received a 30-minute FR1 session on each access day, hence 3 times a week. These continued for 6 weeks to allow for stabilization of their operant performance (when their active responses, pellets earned, and time-out responses did not escalate further). For the purpose of constructing demand curves, the number of responses to obtain each pellet increased across each week (3 sessions total of FR3 during Week 7, 3 sessions of FR6 during Week 8, 3 sessions of FR12 during Week 9, and so on). The sessions for constructing demand curves concluded when rats reached FR 96 (Week 12) as the reinforcer received diminished to nearly zero for all groups. Because of this, only 1 FR96 session was performed. [30]

Following the conclusion of FR96 testing, rats received 5 regular FR1 sessions to re-establish stable self-administration performance and then were assessed in a progressive-ratio (PR) session. In a PR session, the number of responses for a pellet increased exponentially within the session, following the formula: response ratio = $[4 \cdot (e^{(\#\text{ of reinforcer} * 0.075))} - 3.8]$ as

described by Kresler et al. and Cottone et al [40, 43]. Three active lever presses were needed to obtain the first reinforcer in order to prevent accidentally initiating sessions. If no response was made in 14 minutes, the session was automatically terminated. The time limit was 2 hours for a PR session (Fig 2) [43].

Demand Curve Analysis

The software used for demand curve fitting was Demand Curve Analyzer (SQAB, 2018) developed by Gilroy et. al. The parameters α, Q_0, O_{max}, P_{max}, and *k* were obtained from the exponentiated demand function [44].

$$Q = Q_0 * 10^{k(e^{(-\alpha * Q0 * C)}-1)}$$

The exponentiated function was used rather than the exponential function because we anticipated having values that were zero [44, 45].

Statistical analysis

Intake and FR session performance measures were analyzed using mixed-design 2-way ANOVA. Group was the between-subject factor, and Time was the within-subject factor. Fisher's LSD test was performed for interpreting significant Group and Group × Time effects. Paired sample *t*-test was used to compare weekly performance within groups. Demand curve parameters (log transformed α, Q_0, O_{max}, P_{max}) were analyzed using 1-way ANOVA followed by Fisher's LSD. The value of α was log-transformed for analysis and illustration to satisfy assumptions of

parametric analysis. Because of the unequal variance (confirmed by Levene's test), PR performance measures were analyzed using Kruskal-Wallis test followed by the Mann–Whitney *U* test. Spearman's correlation was used to assess correlations between demand curve parameters (α, Q_0) with intake measures, operant session performance, or baseline predictors on the other. Partial correlation was performed to assess any independent correlations between demand curve parameters and intake measures as well as operant session measures. All statistical analysis was done using IBM SPSS Statistics v24 (IBM, Armonk, NY).

2.2 RESULTS

Replicating the previous rodent binge eating model

The model used for this study was established previously by Kreisler and Spierling et al. and demonstrated robust consistency with previous descriptions [32, 33]. Most measures stated below are from the average of Week 4-6, by when animals' behaviors stabilized.

Daily intake

Examining the total daily intake (intake during FR1 sessions combined with daily homecage intake after the session, INT rats ate significantly more on access days ($F(2, 21)=44.29$, $p<0.001$) and under-ate on non-access days ($F(2, 21)=86.05$, $p<0.001$) when compared to the intake of CHOW and CHOC rats (Fig 3). This was reflected in significantly greater intake in INT rats on access day vs. non-access days ($F(2, 21)=93.52$, $p<0.001$). CHOW and CHOC rats did not differ in daily intake from one another nor between access and non-access days. Looking at homecage intake alone (without intake during sessions), the effects remained as INT ate significantly more on access-days ($F(2, 21)=11.55$, $p<0.001$) than both controls.

Fixed-ratio operant sessions

During FR1 sessions, INT rats had ~5 fold higher active lever press responses ($F(2, 21)=39.39$, $p<0.001$) (Fig 4A), ~4 fold greater number of pellets earned ($F(2, 21)=69.48$, $p<0.001$) (Fig 4B), and ~8 fold higher time-out responses ($F(2, 21)=18.50$, $p<0.001$) (Fig 4C) as compared with both CHOW and CHOC rats. INT rats also had a disproportionately higher ratio

of time out responses vs. pellets earned (~3 fold higher) ($F(2, 21)= 8.88$, $p<0.01$) than CHOW rats ($p<0.001$) but not CHOC (Fig 4D).

Progressive-ratio operant sessions

INT rats had a ~6 fold increase in PR active responses ($H=15.57$, $p<0.001$) (Fig 5A), ~2 fold increase in pellets earned ($H=14.663$, $p<0.001$) (Fig 5B), and ~4 fold increase in PR breakpoint than CHOW and CHOC rats ($H=14.625$, $p<0.001$) (Fig 5C).

Demand curve measures

For purposes of illustration, an aggregate demand curve was generated for each diet group (Fig 6A), fit to the observed average intake at each unit cost [Fig 6A]. A work function graph also was plotted for each group based on the observed total work output at each unit cost (Fig 6B). Note, however, that the below behavioral economics measures and analyses were derived from individual demand curve functions fit to each subject's data). INT rats showed an 11-fold decrease in raw α ($F(2, 21)=44.07$, $p<0.001$) as compared with CHOW and CHOC rats (Fig 7A). Int rats also showed a 3-fold increase in Q_0 ($F(2, 21)=19.60$, $p<0.001$) (Fig 7B), higher O_{max} ($F(2, 21))=38.37$, $p<0.001$) (Fig 7C) and higher P_{max} ($F(2, 21)=11.23$, $p<0.001$) (Fig 7D). CHOW and CHOC did not differ from one another in any of these parameters. The average maximum work exerted by INT rats in this range of unit prices (O_{max}) was 467 lever presses, compared to 51 for CHOW and 49 for CHOC. Correspondingly, at their maximum output, the unit price (P_{max}) was 8.2 responses, compared to 3.7 for CHOW and 1.8 for CHOC. As another way of illustrating inelasticity, INT animals also had a higher maximum output vs. regular FR1

responses ratio at a ~1.6 fold increase from FR1 active lever presses to their O_{max} than both CHOW (~1.1 fold change) and CHOC (~0.7 fold change).

Correlations of demand curve parameters with intake and operant session measures

Demand curve parameters vs. intake measures

Results showed that across subject α had a strong negative correlation with total access-day intake (ρ=-0.79, p<0.001, n=24) (Fig 8A). Opposite to α, Q_0 had a strong positive correlation with total access-day intake (ρ=0.83, p<0.001, n=24) (Fig 8B).

Demand curve parameters vs. FR1 session measures

Across subjects, α had a strong negative correlation with the number of pellets self-administered during FR1 sessions (ρ=-0.85, p<0.001, n=24) (Fig 9A), while Q_0 had a strong positive correlation to it (ρ=0.96, p<0.001, n=24) (Fig 9B). When controlling for α, Q_0 still strongly positively correlated with pellets received (ρ=0.87, p<0.001, n=24) while the correlation between α and pellets received diminished when controlling for Q_0 (ρ=-0.36, p=0.097, n=24), suggesting that higher Q_0 independently and uniquely predicts greater number of reinforcers earned during FR1 session.

Demand curve parameters vs. PR session measures

Similar to correlations with FR performance, across subjects, α had a strong negative correlation with the number of pellets received under a PR schedule (ρ=-0.75, p<0.001, n=24)

(Fig 9C), while Q_0 had a strong positive correlation (ρ=0.75, p<0.001, N=24) (Fig 9D). When controlling for either α or Q_0, the correlations diminished, suggesting that neither α or Q_0 independently predict consumption during PR session.

Individual differences within INT animals

Correlates of demand measures

Within the INT group, Q_0 showed a significant positive correlation with the number of pellets received during FR1 sessions (ρ=0.84, p<0.001, n=12), a relationship not seen with α (Fig 10). When controlling for α, Q_0 still strongly correlated with pellets received (ρ=.84, p=0.001), n=24), suggesting that higher Q_0 independently predicts greater number of reinforcers self-administered during FR1 session. Neither measures significantly correlated with individual differences amongst INT subjects in PR performances or intake.

Antecedents of demand measures

On the other hand, baseline chow intake (ρ=0.68, p<0.05, n=12) and body weight (ρ=0.62, p<0.05, n=12) (both measured after the training period and before the first day of the diet schedule), as well as intake of choc on the first day of access (ρ= .62, p<0.05, n=12), predicted the subsequent development of higher α. These correlations were not seen with Q_0 (ρ= 0.27 for baseline chow intake, ρ=0.30 for initial body weight, and ρ=0.44 for first access day intake) and also were not seen in *ad libitum*-fed CHOC or CHOW rats (ρ=-0.37 for baseline

chow intake, ρ=0.30 for initial body weight, and ρ=0.44 for first access day intake), indicating that they were not pre-existing relationships.

2.3 DISCUSSION

Previous evidence has suggested that behavioral economics studies provide promising measures of reinforcing efficacy that distinguishes unconstrained demand vs. inelastic of demand. These measures appear to have predictive validity for real-world consumption of nicotine, alcohol, and many illicit drugs in both animals and humans [46, 47]. Studies also have applied behavioral economic analysis to assess the abuse potential as well as to compare the reinforcing values of drugs [48, 49]. Regarding food consumption, previous studies have compared the reinforcing values of foods that were high and low in its energy density and examined the relations between the reinforcing values of those foods and BMI in humans [39]. Researchers have also studied the effect of genetic knockouts that induced obesity in mice on their demand elasticity towards food [50]. However, behavioral economic analysis has not been applied to study intermittent-access induced compulsive-like eating. Therefore, the purpose of this study is to examine changes in behavioral economic parameters induced by intermittency in diet as well as to identify relations between demand curve measures with food intake and self-administration performances across all diet groups and within the intermittent-access group only.

Replication of previously established binge eating model

Following the rodent binge eating model established by Kreisler and Spierling et al., intermittent, 24-hour access to the sucrose-rich diet induced escalation of daily intake and operant self-administration, rejection of the otherwise acceptable, but less preferred diet alternative, weight cycling, an increase in FR timeout responses, and elevation of PR breakpoint. These changes were not seen in subjects with continuous access to the palatable diet. Replicating

findings of Kreisler et al., and Spierling et al., results show that intermittent extended access produces robust binge-like eating with consummatory, motivational and compulsive-like behavioral adaptations [41, 42].

INT animals developed greater inelasticity to increasing cost and greater unconstrained demand

Consistent with our hypothesis, INT rats developed significant differences in behavioral economic measures compared to control animals. Their elasticity to the increase in cost (α) was on average ~11 fold lower than either CHOW or CHOC, indicating their demand for consumption was much less sensitive to the increase in the effort needed to obtain each reinforcer. The number of reinforcer that the animal would consume when at a minimal price (Q_0) also significantly increased in INT rats, indicating greater demand for choc if free intake was allowed (183 pellets vs. 45 pellets for chow and 90 pellets for choc). Significantly increased inelasticity or unconstrained demand were not seen in rats with *ad libitum* access to the palatable diet, indicating the essential role of intermittent access in observed changes.

One hallmark of addiction and the transition from casual drug use to dependence is the increased motivation in drug-seeking, which might result from changes in sensitivity to increases in price or changes in unconstrained demand [30, 35]. INT animals demonstrate an increase in both factors by showing relatively inelastic demand in response to the increased price, indicating an elevated essential value of palatable food and their willingness to work hard to obtain it, as well as showing an elevated level of intake when the cost is minimal. These two changes were both results of the intermittency in access towards the palatable diet.

Inelasticity and large unconstrained demand towards reinforcers predicted greater intake and self-administration responses

Consistent with our predictions, α and Q_0 strongly correlated with intake measures and consumption during FR, PR sessions. The drastically decreased demand elasticity and increased unconstrained demand for reinforcers elicited by diet intermittency align with INT animals' cyclic intake behaviors and escalated FR and PR responses. When compared to animals with no intermittent diet exposures, INT animals' demand curve measures strongly associated with overeating of choc and rejection to chow, as well as binge-like intake during self-administration sessions.

Partial correlations controlling for either α or Q_0 revealed an unique relation between Q_0 and pellets earned during FR sessions: when controlling for α, Q_0 still strongly correlated with FR pellets while α, indicating Q_0 was an independent predictor of consumption during FR session. On the other hand, α did not show independent correlations with pellets earned during FR or PR sessions. Q_0 did not independently predict consumption during PR session. This result is consistent with the fact that the FR session is a construct which minimal effort is needed for a reinforcer, hence reflecting the amount of reinforcers self-administered during "free-intake", a measure approximated by Q_0.

Individual differences predicted by demand curve measures within INT group

Predictors of demand parameter

When analyzing demand curve parameters' correlations within the INT group, we have hypothesized that lower α and higher Q_0 would correlate with compulsive-like characteristics. Indeed, we found that higher Q_0 was strongly correlated with more pellets on average earned from FR sessions. This finding was consistent with the hypothesis that greater unconstrained demand would predict greater consumption at a minimal, near-zero cost (1 lever press).

We have previously demonstrated that Q_0 is strongly correlated with FR pellets self-administered across all diet groups. Yet still, this effect is strongly persistent when examining INT group only, which had significantly elevated their unconstrained demand due to the intermittent access to palatable food. Because Q_0 is correlated with FR pellets self-administered during sessions but not overall access day intake, this result suggest that higher Q_0 is related to the increase in urgency to consume palatable food, which resembles a greater degree of binge-like eating. Q_0 potentially provides an effective way to normalize the individual differences in the reinforcing properties of substances under the same conditions; at the same time, it also has been reported to on its own predict real-world drug use in human studies [35]. It is possible that intermittent access to palatable food induces varying neurological responses in individuals, which may be a major contributor to individual vulnerabilities in the development of binge eating [38].

Antecedent of demand parameter

Chow intake and body weight immediately prior to the start of their diet schedules (at the end of the training period) as well as choc intake on the first day of access correlated with higher α in INT animals. Because these effects were not seen in control animals, the findings suggest that lower initial food intake and body weight can be antecedents to the development of inelasticity. Lower intake and weight initially could have been predictors of insensitivity towards increasing cost of obtaining choc pellets. On the other hand, animals with higher initial intake and body weight had more elasticity of demand. This could potentially be due to the higher body weights of those animals allowing them to wait longer for food intake compared to their lower body weight counterparts. Consistent observations have been found in a study by Belke et al, where the rats' demand for sucrose became more inelastic in response to a decrease in body weight through caloric restriction [51]. Other factors that are related to body weight could potentially also affect the elasticity of demand. For example, a study by Blaisdell et al. reported diet-induced obesity decreased PR responding in mice potentially due to an increase in circulating leptin level that leads to a decrease in dopamine turnover rate in the mesolimbic system, thus reducing their motivation for self-administration [52].

Contrary to our prediction, higher choc intake on the initial day of access predicted higher elasticity. This effect might be due to the degree of acceptance towards a novel form of diet. The animals who later showed more sensitivity (higher α) towards increase in unit cost might have been more flexible in approaching a new diet compared to animals that had low sensitivity (lower α).

CHAPTER 3: GLUCOCORTICOID RECEPTOR MRNA EXPRESSION LEVEL AFTER INTERMITTENT ACCESS TO PALATABLE FOOD

Glucocorticoid receptor mRNA expression level in the central nucleus of amygdala

The amygdala has been proposed to be a key region involved in the development of escalation of intake or self-administration, compulsive-like behaviors, and loss of control in addiction [30, 53-55]. Studies have shown that there is an increase in amygdala response in individuals with substance-related addictive disorders. For example, Deborah et al. has reported a fMRI study showed increased activity of the amygdala when nicotine-dependent individuals were exposed to smoking-cues [56]. Another fMRI study by Goudriaan et al. also revealed problem gamblers had higher amygdala activation when shown pictures related to gambling [57]. Due et al. also reported higher amygdala activation when exposed to alcohol oder in alcohol dependent individuals, and the over-activation was diminished after therapeutic interventions [58]. Similar observations were found regarding binge-related disordered eating. Ely et al. reported that, in females without a history of eating disorder, the amygdala was shown to decrease in response towards sucrose tasting in a fed state (vs. hungry state); however, this decrease in response was not seen in women recovering from bulimia nervosa, a disorder involving episodes of binge eating with loss of control over intake [59]. Gearhardt et al. reported the degree of amygdala activation when presented with images of milkshakes was positively correlated with food addiction score in females [60]. Bohon et al. reported an increase in connectivity of amygdala to the left putamen and insula during anticipation for a milkshake in individuals with bulimia nervosa when compared to healthy controls [61].

At the same time, the glucocorticoid receptor (GR) molecule, which is involved in stress response, has been shown to have altered activation levels particularly in the central nucleus of amygdala (CeA) in subjects with substance use disorder [62-65]. Vendruscolo et al. reported increased functional sensitivity of GR in the CeA in rats during a period of acute withdrawal (6-8 hours abstinence) as well as increased mRNA expression level during protracted withdrawal (3 weeks abstinence) from alcohol. Vendruscolo et al. also reported that the GR antagonist mifepristone had inhibitory effect in the development of alcohol dependence in rats, and it also reduced alcohol seeking behaviors in dependent rats during protracted withdrawal, but not in non-dependent rats [65]. In a clinical study, mifepristone also reduced alcohol craving as well as the numbers of drinks consumed in a week following the mifepristone treatment in alcohol-dependent human subjects [64]. A similar effect was seen in the current model of compulsive, binge-like eating by Kreisler et al., who reported that GR antagonists (both mifepristone and the highly selective Corcept drug candidate, Compound 13) have an effect in decreasing fixed-ratio self-administration responses of palatable food in rats that had long (24-hour) intermittent access to a sucrose-rich, palatable diet and showed compulsive-like binge eating behaviors, but not in rats that had continuous access to either chow or palatable diet, neither in rats that had short (30-min) intermittent access to the palatable diet [66]. However, the effect of intermittency access to palatable diet on changes in mRNA expression level of GR in the CeA has not been investigated. In this study, we will analyze and compare the mRNA expression level of GR in the CeA among rats that had continuous access to chow (CHOW), continuous access to palatable diet (CHOC) and intermittent access to palatable diet (INT). We hypothesize that the INT group would exhibit

an elevated level of GR expression in the CeA comparing to both continuous access groups (CHOW and CHOC) in both males and females.

Spierling et al. previously reported that within the INT group, sex differences as well as individual level differences existed in the escalation of compulsive-like behaviors. Female INT rats overate on access day of palatable diet to a greater extent than male INT rats, comparing to their respective CHOW and CHOC animals; After normalizing their body weights, female INT rats had higher fixed-ratio and progressive ratio self-administration (PR) responses comparing to male INT rats; female INT rats' weight gain after access day accelerated throughout the study, a phenomenon not observed in male INT rats [41]. Altogether, these evidences and the disproportional occurrence of binge-related eating disorders as well as self-reported binge-eating tendencies in females comparing to males suggest there might be neurobiological differences between males and females, which potentially underly the sex differences in the development of compulsive-like eating [67-69]. Therefore, we hypothesize that relative to their controls, female INT animals would exhibit a higher level of CeA GR mRNA comparing to male INT animals.

On an individual level, Spierling et al reported the observation that a subset of INT animals had exacerbated elevation in PR responding. These animals were classified as INT-HIGH animals by the criteria of two standard deviations greater than the mean of control animals' PR responding, and INT animals that did not reach this criteria were classified as INT-LOW animals. Because INT-HIGH animals did not show significant differences in PR responding during the first week of intermittent access diet schedule, the elevated PR responses later in the study were likely caused by individual differences in susceptibility to the development of compulsive-like behaviors, which was captured in PR responding [41]. It is

possible that the degree of elevation in CeA GR expression is a part of the neurological differences that account for the existing individual differences in PR responding. Therefore, we hypothesize that INT-HIGH animals exhibit a higher level of CeA GR mRNA comparing to INT-LOW animals in both sexes.

Glucocorticoid receptor mRNA expression level in the anterior insular cortex

The anterior insular cortex (AIC), as a paralimbic cortical structure, is generally being recognized as a brain region that regulates bodily homeostasis, interoceptive awareness, and emotions [70-72]. In recent years, the AIC also has been receiving increasing attention for its association with psychiatric morbidities [30, 73, 74]. Based on imaging studies and analysis, AIC was shown to be involved in various stages of disordered eating [75-80]. Spierling et al. has recently reported optoinhibition of glutamatergic projection from the anterior insula to the nucleus accumbens specifically decreased PR responding in INT-HIGH rats [75]. In human neuroimaging studies, Ellison et al. reported higher activity in females with anorexia nervosa when shown pictures of caloric beverages comparing to controls [76]; Boutelle et al. reported increased insula reactivity in obese children than normal weight controls when sated [77]. Wonderlich et al. reported positive correlation between insula activity with food craving prior to binge eating in women with bulimia nervosa [78]. These evidence suggest there is functional adaptations of the insula involved in non-homeostatic, disordered eating.

It has been proposed that the activation of the HPA axis has a modulatory role in increasing the activity of the insula [79]. At the same time, glucocorticoid antagonist (mifepristone) has been examined for its clinical potential in treating patients with anorexia

nervosa through regulating the HPA axis [80]. It has also been shown that administering synthetic glucocorticoid (prednisolone) increased the activity of the insula when subjects were shown food pictures [80]. Although little is known about the site-specific role of glucocorticoid receptor in the anterior insula cortex, the change in GR level in the AIC could potentially be involved in the development of compulsive eating. Therefore, we will also analyze and compare the expression level of GR in the AIC between groups in both male and females to reveal potential sex differences; we will also examine possible differences on the individual level. Based on the observations of group, sex, and individual differences as stated above, we hypothesize that 1.) the INT group would exhibit an elevated level of GR expression in the AIC comparing to both CHOW and CHOC 2.) relative to their controls, female INT animals would have a higher level of AIC GR mRNA expression comparing to male INT animals 3.) INT-HIGH animals would have a higher level of AIC GR mRNA expression comparing to INT-LOW animals in both sexes.

3.1 METHODS

Animals

As described by Spierling et al, 32 female and 24 male Wistar rats (Charles River) were received around 6-8 weeks old. Upon arrival, they were given the 45-mg pellet chow diet (5TUM TestDiet, St. Louis MO) before the start of the experiment. Rats had free access to water at all times. [32].

Rats were pair-housed (same sex) with a clear divider placed between cage-mates to ensure individual measurement of food intake while avoiding total social isolation. Animals were placed in a vivarium under a 12-hour light/dark cycle [41].

Procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by The Scripps Research Institute's Institutional Care and Use Committee.

Diet schedules

Two types of 45-mg pellet diets were used for this study. One was a corn-based, chow diet ("chow") (5TUM, Test Diets, St. Louis, MO), and the other was a chocolate-flavored, sucrose-rich, nutritionally-complete diet ("choc") (5TUL, Test Diets, St. Louis, MO) that was highly palatable. Compared to chow, choc was had roughly similar energy density (3.44 kcal vs. 3.30 kcal per gram) and macronutrient proportions (choc: 12.7% energy from fat, 66.7% from carbohydrate, 20.6% from protein; chow: 10.4% from fat, 65.5 from carbohydrate, 24.1% from protein), but was much higher in its sucrose content (49.60% vs. 3.8%) and preferredness (91.2±3.7% preference ratio vs. chow) [43].

Two weeks upon arrival, animals were matched based on baseline measurements and assigned to one of the 3 diet groups. The CHOW group had *ad libitum* access to chow only, the CHOC group had *ad libitum* access to choc only, and the INT group had 24-hour access to choc only starting at dark onset of every Monday, Wednesday, and Friday, with access to chow only on the other days.

Intake was measured daily with the exception of the 24 hours between Saturday to Sunday. Diet switching/renewal was done shortly (0-60 min) prior to dark onset. Mondays, Wednesdays, and Fridays will be referred to as "access days" as the INT animals receive choc; Tuesdays, Thursdays, Saturdays, and Sundays will be referred to as "non-access days" as the INT animals receive chow [41].

Self-administration operant sessions

Animals received 3 weeks of self-administration fixed-ratio (FR1) operant training before the start of the study. All subjects responded for chow pellets during this training period. Each FR1 session took place approximately one hour before the dark onset of an access day. Each operant chamber had one active lever, which in FR1, each press outside the time-out period would elicit the dispense of a pellet; and an inactive lever, which pressing would not produce any scheduled consequences. Following each pellet being dispensed, there was a 3.75-second time-out period where further lever press responses were recorded but had no scheduled consequences. Lights were turned off for each session (consistent with the dark cycle) to encourage feeding behaviors. Water was always available via a sipper tube during operant sessions. The criteria for successfully learning to self-administer pellets were >75% active lever

responses vs. inactive lever responses and receiving at least 10 pellets. During the training period, rats started with a 24-hour FR1 session paired together with their cagemate in the same operant box to potentially facilitate learning by observation. From this session onwards, rats always had an operant session individually, without their cagemate. For the rest of the training period, rats were subsequently individually given one 12-hour FR1 session, one 3-hour FR1 session, five 30-min FR1 sessions, and one progressive-ratio (PR) session. In a PR session, the number of responses for a pellet increased exponentially within the session, following the formula: response ratio = $[4 \cdot (e^{(\#\ \text{of reinforcer} * 0.075))} - 3.8]$ as described by Cottone et al [33]. Three active lever presses were needed to obtain the first reinforcer in order to prevent accidentally initiated sessions. If no response was made in 14 minutes, the session was automatically terminated. The time limit was 2 hours for a PR session [33].

After training, rats maintained on one 30-minute FR1 session and one PR session before access day each week (hence, 2 access days per week will be preceded with either a FR or PR session) for 6 weeks to allow for stabilization of their operant performances (when their active responses, pellets earned, and time-out responses did not escalate further). During Week 7-11, rats proceeded with FR and PR sessions, but with a more staggered schedule due to calorimetry measurements occurring concurrently [41].

Characterization of high vs. low responders

INT rats were classified as high responders (INT-HIGH) if during Week 4-6, they have average number of active responses in PR self-administration sessions that were 2 standard

deviations above the chow controls. Otherwise, they were classified as low responders (LO-INT) [41]. In males, 4 out of 11 INT rats were INT-HIGHs. In females, 5 out of 9 were INT-HIGHs.

Quantifying expression of GR

After tissue sample collection, tissues of the CeA and the AIC were homogenized, RNA extracted, DNase I treated, and reverse transcribed using SuperScript III First Strand Synthesis Kit, followed by Taqman qPCR using StepOne software. GAPDH was used as a control to compare expressions.

For analyzing GR expression in the CeA, 22 female samples were used for qPCR (CHOC: *n*=8; CHOW: *n*=5; INT: *n*=9). 23 male samples were used (CHOC: *n*=6; CHOW: *n*=6; INT: *n*=11).

For analyzing GR expression in the AIC, 28 female samples were used for qPCR (CHOC: *n*=8; CHOW: *n*=9; INT: *n*=11). 12 male samples were used (CHOC: *n*=3; CHOW: *n*=3; INT: *n*=6).

Data analysis

Separate univariate ANOVAS were performed on $2^{(-\Delta\Delta Ct)}$ to compare the 3 diet groups within each sex as well as comparing INT-HIGH and INT-LOW separately vs. the 2 control groups. Fisher's LSD was performed for pairwise comparison. Independent-sample t-test was used to compare between male and female of the same diet group.

3.2 RESULTS

Glucocorticoid receptor mRNA expression level in the central nucleus of amygdala

Diet schedule group differences in both male and female animals

Females had a group effect of $F(2, 19)=3.47$, $p=0.05$, with pairwise comparisons revealing INT having significantly higher CeA GR expression than both CHOW and CHOC ($p<0.05$) (Fig 12). CHOW and CHOC did not differ in their expression levels ($p=0.92$).

In contrast to females, there was no group difference in CeA GR mRNA expression levels in males comparing by diet schedule ($F(2, 20)=0.59$, $p=0.56$).

Sex differences comparing male and female animals of the same diet group

Female INT animals had significantly higher level of CeA GR expression than male INT animals ($p<0.05$). When analyzed by INT-HIGH and INT-LOW separately, female INT-HIGH animals still had significantly higher expression level comparing to male INT-HIGH animals ($p<0.05$). The expression level for female INT-LOW animals was descriptively higher than that of male INT-LOW animals, but did not reach a significant level ($p=0.056$).

No significant difference in CeA GR mRNA expression between males and females was seen when comparing male vs. female CHOW animals ($p=0.61$) and CHOC animals ($p=0.49$).

Individual differences in both male and female animals

When comparing INT-HIGH and INT-LOW rats vs. both *ad libitum* fed groups, pairwise comparison revealed that INT-HIGH had significantly higher CeA GR expression than both CHOW and CHOC ($p<0.05$) (Fig 13). In contrast, INT-LOW did not show significant differences

from either CHOW (p=0.26) or CHOC (p=0.25). INT-HIGH rats had descriptively higher levels than INT-LOW rats, but not significantly (p=0.30).

In contrast to females, there was no individual difference in CeA GR mRNA levels in male rats comparing by high vs. low PR distinction (F(3, 19)=0.53, p=0.67).

Glucocorticoid receptor mRNA expression level in the anterior insular cortex

Diet schedule group differences in both male and female animals

In contrast to GR level in CeA, there was no significant group difference in normalized GR mRNA levels within the AIC in either females (F(2, 25)=0.22, p=0.80) or males (F(2, 9)=0.53, p=0.32) comparing by diet schedule.

Sex differences comparing male and female animals of the same diet group

No significant difference in AIC GR mRNA expression between males and females was seen when comparing CHOW animals (p=0.91), CHOC animals (p=0.37), or INT animals (p=0.38). Also no significant differences was seen when comparing INT-HIGH (p=0.13) or INT-LOW (p=0.126) between males and females.

3.3 DISCUSSION

Elevation of CeA GR mRNA expression in female animals after intermittent access diet schedule

Here, we examined the expression level of GR in the CeA , which could potentially be a site-specific mediator of compulsive eating through negative reinforcement during abstinence [30]. We hypothesized that the INT group would exhibit an elevated level of GR expression in the CeA comparing to both CHOW and CHOC groups in both males and females. After comparing across diet groups, our result shows that GR expression level in the CeA did not differ between the control groups of either sex. On the other hand, after 11 weeks of intermittent access to the palatable diet, female INTs had elevated GR expression in the CeA compared to controls, a phenomenon not seen in male INTs with the respective control animals.

This elevation of GR CeA that is specific to females potentially could correlate with a greater degree of negative emotions, such as stress, irritability, and anhedonia experienced during abstinence from palatable foods. It may be a contributing factor to more females engaging in compulsive eating to use food as a source to relieve negative emotions.

Female INT animals had higher GR mRNA expression than male INT animals after intermittent access diet schedule

We hypothesized that relative to their controls, female INT animals would have a higher level of CeA GR mRNA comparing to male INT animals. When comparing male and female animals of the same diet group, both control groups did not differ in their expression levels. At

the same time, female INT animals had significantly higher GR mRNA expression level comparing to male INT animals. This is another evidence suggesting that the intermittent access to palatable food uniquely elevated GR activity in the CeA in female animals, potentially due to inherent genetical and neurobiological predispositions which are still to be discovered. Spierling et al. have reported in her paper investigating sex differences and individual vulnerability for binge eating, that under the same intermittent-access regime to palatable food, comparing to male INT rats, female INTs developed greater access day intake as well as greater degree of rejection towards chow on non-access days. Female INT animals also uniquely escalated in the degree of weight cycling. Also only observed in female animals, CHOC significantly gained more weight compared to CHOW [41]. These findings are highly consistent with the disproportional prevalence of binge-related eating disorders in females than in males [1, 67-69]. Sinclair et al. have proposed that there are neurological differences in the rewarding effect of highly palatable food to be a contributor to this phenomenon, based on the finding that females had significantly higher response at the mesocorticolimbic reward circuitry immediately after access to a high-sugar, high-fat diet [81]. Here, we have also demonstrated that females may be more susceptible to compulsive eating behaviors due to the sex differences in neurological changes that may contribute to the development of negative reinforcement.

Differences in GR expression elevation for INT-HIGH vs. INT-LOW animals and comparison between males vs. females

Previous study by Spierling et al. reported that INT-HIGH rats, comparing to INT-LOW, had greater intake cycling, FR responses, and showed unique metabolic characteristics such as mainly utilized carbohydrates as energy source and potentially had a tendency to "spared" their fat storage [41]. These characteristics may indicate that INT-HIGHs have underlying neurological predisposition for individual vulnerability in developing compulsivity towards palatable food.

We hypothesize that INT-HIGH animals have higher level of CeA GR mRNA comparing to INT-LOW animals in both sexes. Our results based on pairwise comparison between female CHOW, CHOC, INT-HIGH, and INT-LOW rats have revealed the significant increase of CeA GR in INT-HIGH only, comparing to both controls. At the same time, when comparing INT-HIGH and INT-LOW animals from males and females separately, female INT-HIGH animals had significantly increased expression level, while female INT-LOW animals trended towards an increase in expression, but they did not reach significant level comparing to their male counterparts.

The significant up-regulation of GR in this subset of animals could potentially drive substance seeking behaviors through negative reinforcement, such as the escalated self-administration responses. One possibility is that the animals that were classified as INT-HIGH have predisposed sensitivity towards the intermittency in access to palatable food, leading to a significant increase in expression level of GR in the CeA and hence, a greater degree of negative affect and the greater urgency to relieve it. As reported by Vendruscolo et al., the up-regulation of

CeA GR mRNA level was observed during protracted abstinence in alcohol-dependent rats comparing to controls, and antagonizing GR receptors could both block escalation of drinking (if antagonizer was put in place before ethanol vapor exposure) and block excess self-administration of alcohol during protracted abinstinence (if the antagonizer was put in place shortly after abstinence) [65]. At the same time, Kreisler et al. reported that antagonizing GR systemically significantly decreased FR self-administration responses to choc specifically in long-access INT animals, and not in CHOW, CHOC, or short-access INT animals [66]. These evidences, together with the finding from this study suggest that consistently with alcohol dependence, the intermittency in access to palatable food could also induce an elevation in the level of GR mRNA expression in the CeA, which potentially accounts for the escalated seeking behaviors during self-administration sessions through negative reinforcement mechanisms. At the same time, the degree of elevation of GR mRNA expression in the CeA varies between male and females, with females being susceptible to developing this elevation comparing to males; the degree of elevation in females is also different on an individual level, which potentially predict higher degree of palatable food seeking behaviors and negative affect driven motivation. The genetic and neurological basis of the sex and individual differences observed in this study remain a direction for future research.

Site-specificity in the increase of GR expression

At the same time, tissue samples from the anterior insula cortex (AIC) was also collected, and the expression level of GR was measured. Similar hypotheses with GR expression level in the CeA were made regarding the changes in expression level of GR to animals after intermittent access diet schedule, that we would observe higher expression of GR mRNA in INT rats comparing to controls in both male and female, higher expression in female INT animals comparing to male counterparts, and higher expression in INT-HIGH animals comparing to INT-LOW animals. However, we did not observe any group or sex differences in GR level in the AIC.

This result suggests that the elevation of GR expression has brain site-specificity, that GR mRNA expression level has been significantly increased in female INT animals, especially female INT-HIGH animals in the CeA. However, this did not occur in the AIC. At the same time, we cannot indicate that the GR activity in the AIC was changed or unchanged as a response to intermittent access diet based on this result. There are other mechanisms through which GR activity can be altered. For example, GR molecule activity can be changed by site-specific phosphorylation, such as phosphorylation of Ser232 [64]. The interaction between GR and AIC is an area for further study and investigations.

CHAPTER FOUR: CONCLUSION

Binge eating shares many characteristics with substance-related and addictive disorders, such as reward tolerance, escalation of intake, loss of control over consumption, increased effort in seeking behaviors, consumption despite negative consequences, and withdrawal-like negative affect during periods of abstinence [30, 41]. In Chapter 2, we utilized the concept of behavioral economic analysis, a method used to access abuse potential of substances and its parameters had reported predictive values for real-world substance use, to analyze how intermittent access to palatable diet induce changes in behavioral economic parameters and their correlations with intake measures and self-administration responses. Rats were divided into three diet groups: 1.) CHOW (continuous access to regular chow) (n=6) 2.) CHOC (continuous access to palatable diet, choc) (n=6) and 3.) INT (intermittent access to choc) (n=12). Animals were trained on fixed-ratio (FR) self-administration operant sessions. Demand curves were conducted for each individual animal by increasing in the number of active lever presses needed in order to receive one pellet of food across each week of FR sessions (FR3, FR6, FR12,...FR96). Demand curve parameters were retrieved by curve fitting. Results showed that INT rats had significantly lower elasticity of demand to the increase in cost (α) and higher unconstrained demand (Q_0) compared to control groups. Demand curve measures also correlated with greater access day intake, lower non-access day intake, and higher self-administration responses. Within the INT group, unconstrained demand (Q_0) was significantly correlated with the number of pellets received during FR1 sessions. Initial intake, initial body weight, and first-day choc intake predicted higher elasticity (α) within INT animals.

Glucocorticoid receptor (GR) expression in the central amygdala (CeA) has been implicated in negative affect during drug withdrawal [30, 65, 66]. Its antagonization has reported to be effective in reducing self-administration in alcohol dependent rats as well as rats on the intermittent access diet schedule [65, 66] In this study, we quantified the expression of GR in INT and control animals from both sex and found that females INTs had significantly increased GR expression comparing to control, while male INTs did not. The subgroup of female INTs that had high PR responses separately had significantly higher GR expression, while the subgroup with low PR responses did not. These findings suggest that GR mRNA expression level changes potentially implicate sex and individual vulnerability and neurological predisposition in the development of binge eating behaviors. We also assessed the GR mRNA expression level in the anterior insular cortex (AIC), an area shown to be implicated in disordered eating. However, we did not observe differences in expression among diet groups, males vs. females, or high vs. low PR responders, suggesting the brain site-specific elevation in GR mRNA express upon intermittent access diet schedule.

Future directions may include examining how drug treatments for binge-related eating disorders affect the change in behavioral economic measures in INT animals. Sex differences in behavioral economic parameters also remains to be a topic for future studies. Also, examining the expression level as well as functional changes in GR activity in the CeA at different time point of abstinence from palatable diet may provide more insight on the development of compulsive eating behaviors potentially accounted for by GR.

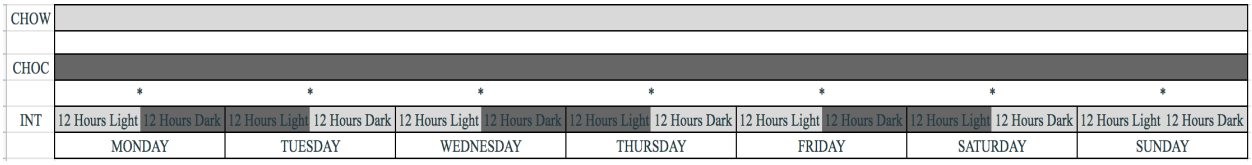

Figure 1: Diet schedule. Rats were divided into 3 diet groups: CHOW (*n*=6), CHOC (*n*=6) and INT (*n*=12). Figure above presents their respective diet schedule throughout the week. Lighter grey represents receiving chow, while darker grey represents receiving choc. Each asterisk represent dark onset and the change of diet for INT rats.

		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14	
FR1 self-administration training week	FR1 self-administration training week	FR1						FR3	FR6	FR12	FR24	FR48	FR96	FR1	PR	

Figure 2: Experimental design. Two weeks of fixed-ratio self administration (FR1) training preceded the start of Week 1, when animals were divided into diet groups and maintained on FR1 sessions at the beginning of each access day throughout Week 1-6. Starting Week 7, the number FR responses needed to earn a pellet (45 mg) was increased each week, ending at Week 12, FR96. Rats returned to FR1 schedules until Week 14, followed by a single PR session.

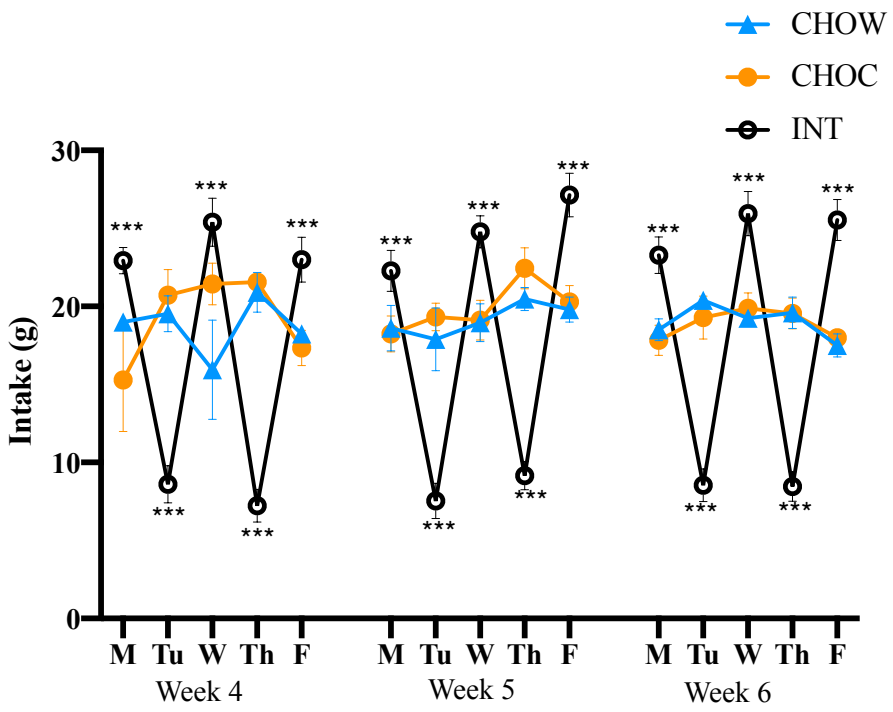

Figure 3: Intake cycling in INT rats comparing to CHOW and CHOC. Figure shows the average intake measures of each diet group during Week 4-6. Based on mixed-design 2-way ANOVA and post-hoc Fisher's LSD, INT rats (n=12) showed elevated intake on access days to choc and underconsumption of chow on non-access days comparing to both CHOW (n=6) and CHOC (n=6). CHOW and CHOC did not differ from each another. Data show M+SEM. ***: p<0.001 and indicates significantly different from all other groups.

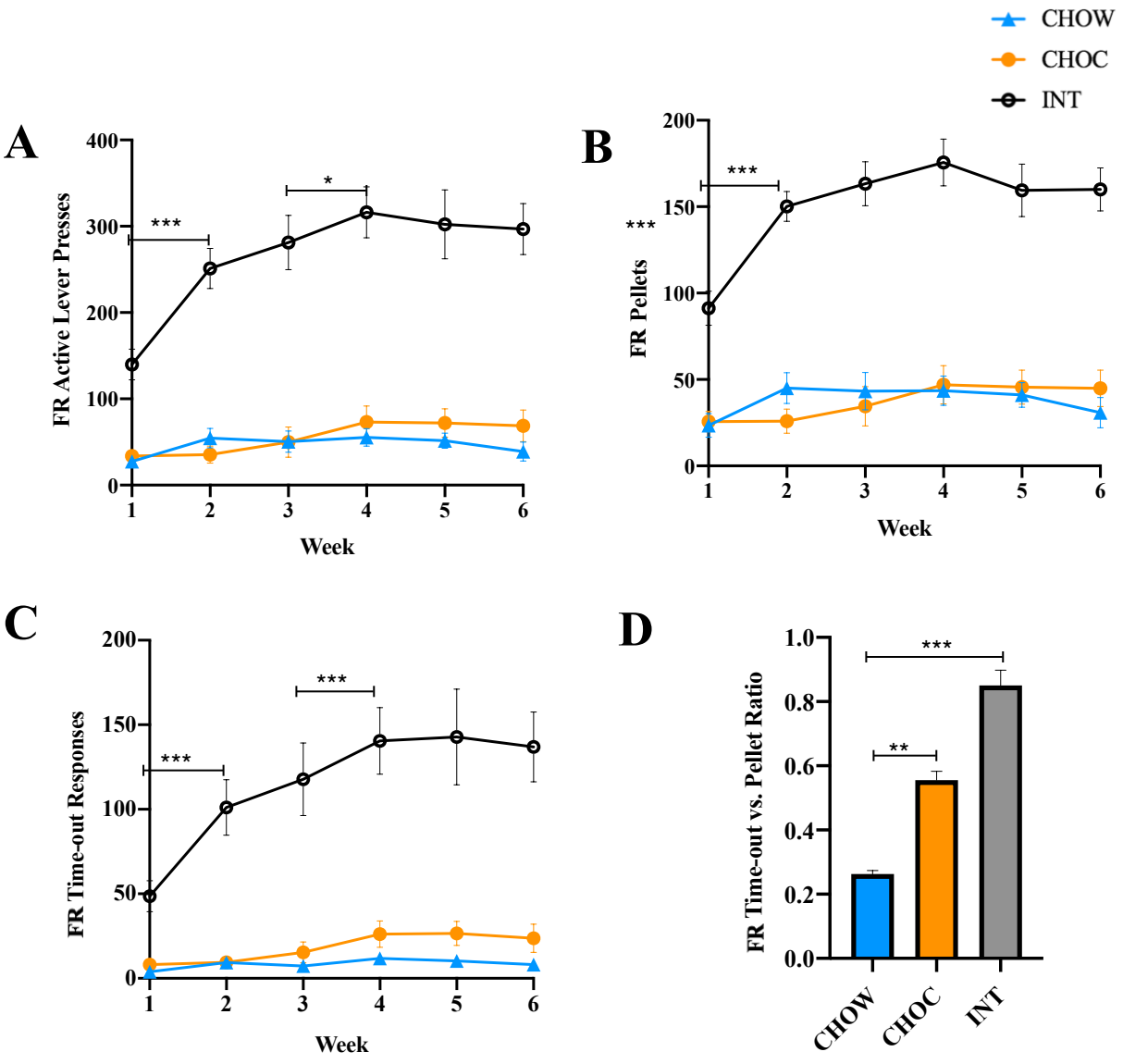

Figure 4: Elevated fixed-ratio (FR) self-administration measures in INT animals. Using mixed-design 2-way ANOVA and post-hoc Fisher's LSD, results showed starting from Week 1, INT (n=12) animals developed higher **A)** active lever pressing responses, **B)** (45-mg) pellets earned, and **C)** time-out responses than both control groups (CHOW: n=6; CHOC: n=6) during FR1 sessions. Paired sample t-test showed that INT animals' FR1 responses did not escalate further starting from Week 4. **D)** INT rats had a higher time-out responses vs. pellet ratio than CHOW only. A, B and C show M+SEM. *: p<0.05 **: p<0.01 ***: p<0.001.

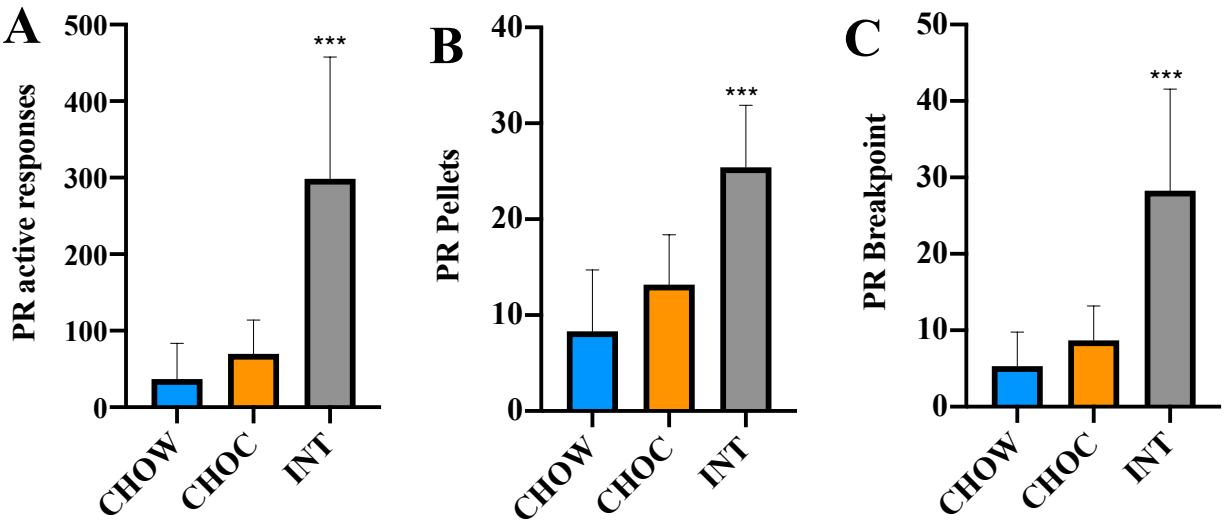

Figure 5: Elevated progressive-ratio (PR) self-administration sessions measures in INT animals. Kruskal-Wallis test followed by the Mann Whitney *U* test showed INT (*n*=12) rats had significantly higher **A)** active lever responses **B)** breakpoint and **C)** number of 45-mg pellets earned comparing to CHOW (*n*=6) and CHOC (*n*=6). ***$p<0.001$ and indicates significantly different from all other groups.

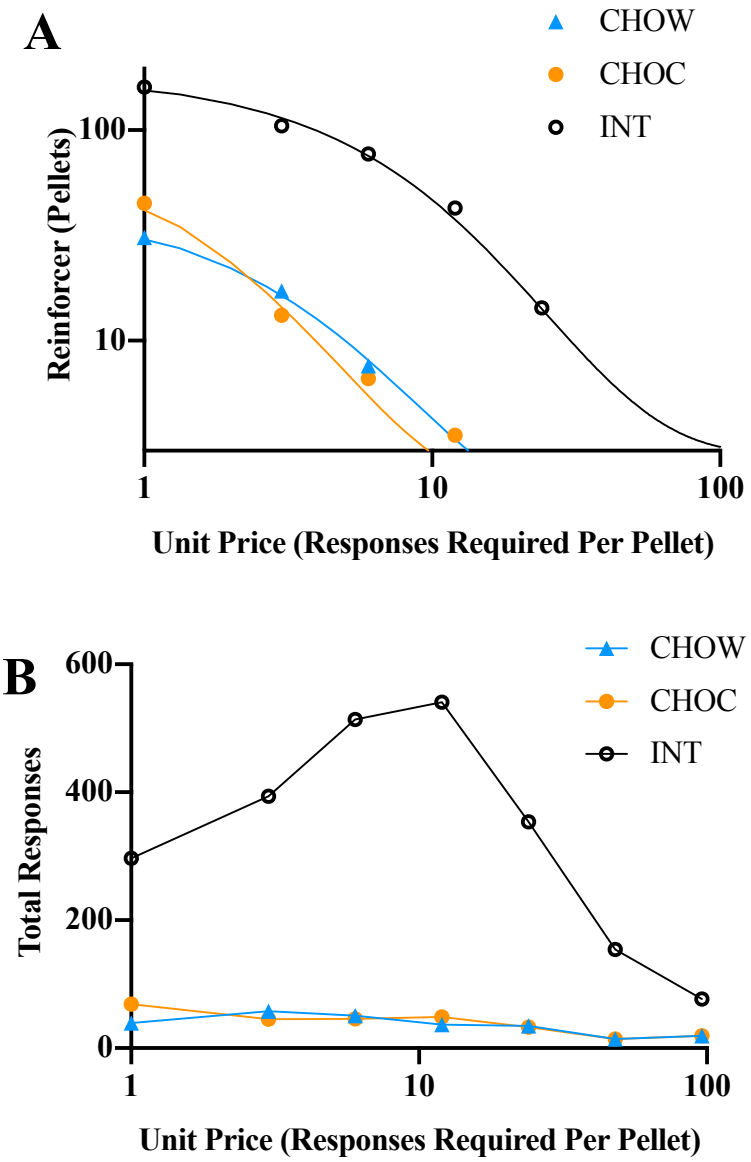

Figure 6: The fitted demand curves and work function graphs for each diet group. A) Demand curves for CHOW, CHOC, INT groups. Symbols represent observed average reinforcer received at each parametric cost. Exponentiated demand function

$$Q = Q_0 * 10^{k(e^{(-\alpha * Q0 * C)} - 1)}$$

was used for curve fitting. **B)** The plotted work function line graphs for each diet group respectively. Each symbol represent group average of numbers of active responses made at each price.

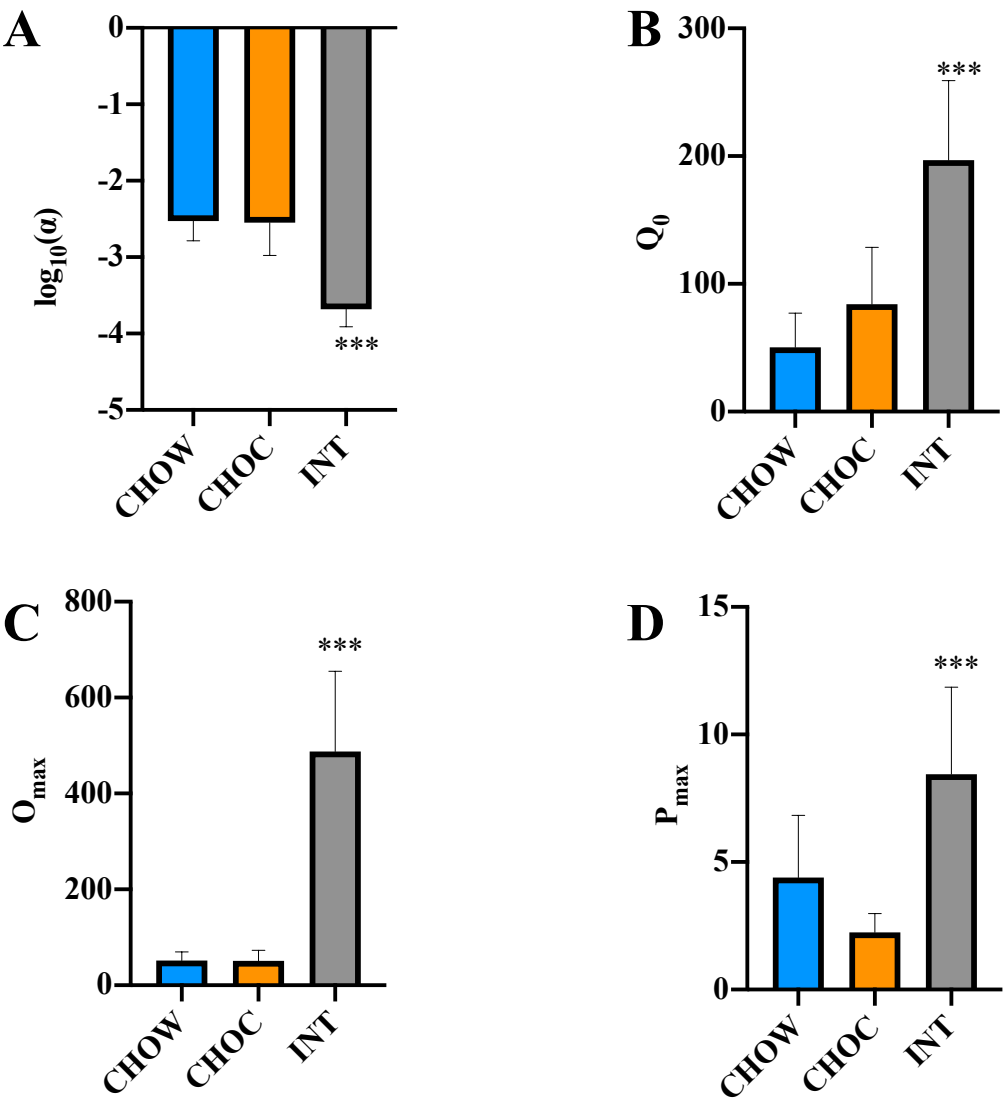

Figure 7: Demand curve measures of CHOW, CHOC, and INTS. Demand curve parameters were retrieved from curve fitting using the exponentiated demand function. INT animals (n=12) showed significantly **A)** lower α (more inelasticity of demand) **B)** higher Q_0 (greater unconstrained demand) **C)** higher O_{max} (greater maximum work output) **D)** higher P_{max} (higher unit price corresponding to maximum work output) than CHOW (n=6) and CHOC (n=6). ***p<0.001 and indicates significantly different from all other groups.

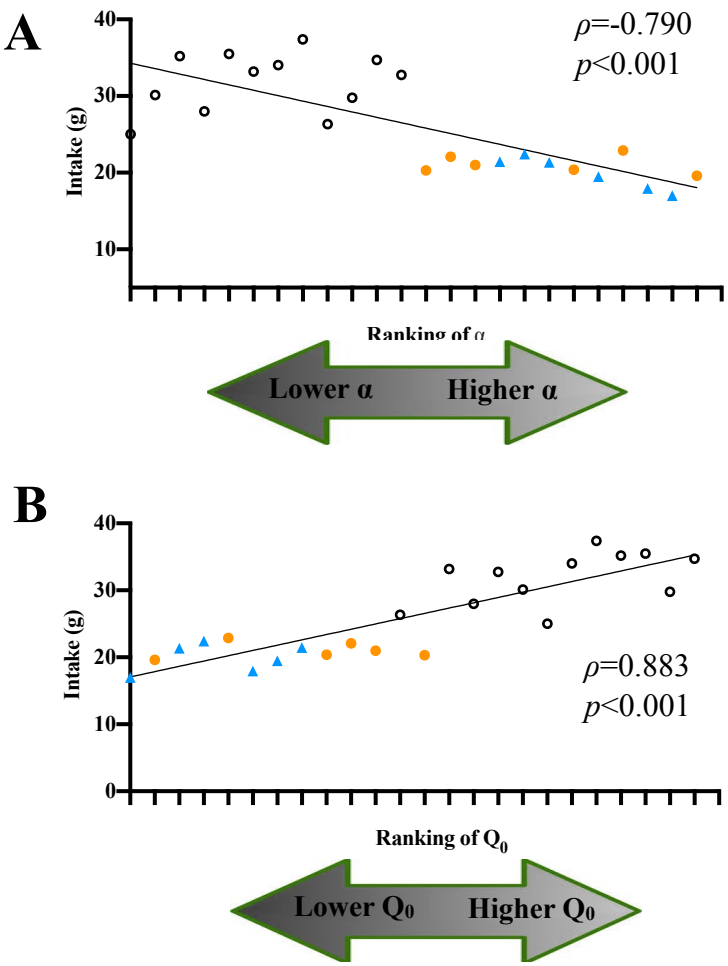

Figure 8: Correlation of α and Q_0 with access-day intake. Based on Spearman correlations, lower α predicted greater access-day intake. On the other hand, higher Q_0 predicted greater greater access-day intake. CHOC: *n*=6. CHOW: *n*=6. INT: *n*=12.

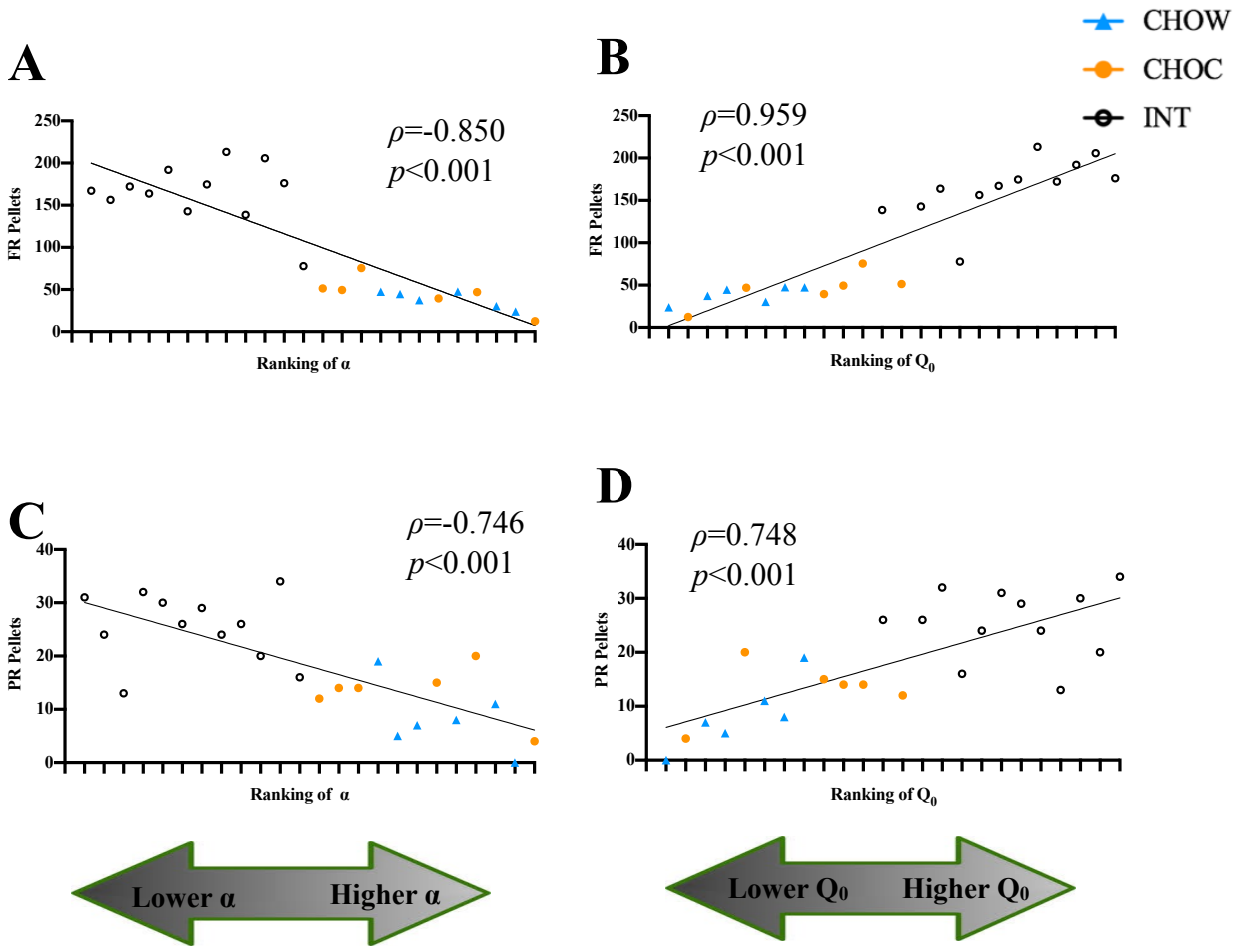

Figure 9: Correlation of α and Q_0 with operant session performances. Based on Spearman correlations, lower α predicted higher numbers of reinforcers (45-mg pellets) received during **A)** FR sessions and **C)** PR session. On the other hand, higher Q_0 predicted greater number of pellets received during **B)** FR sessions and **D)** PR session. CHOC: n=6. CHOW: n=6. INT: n=12.

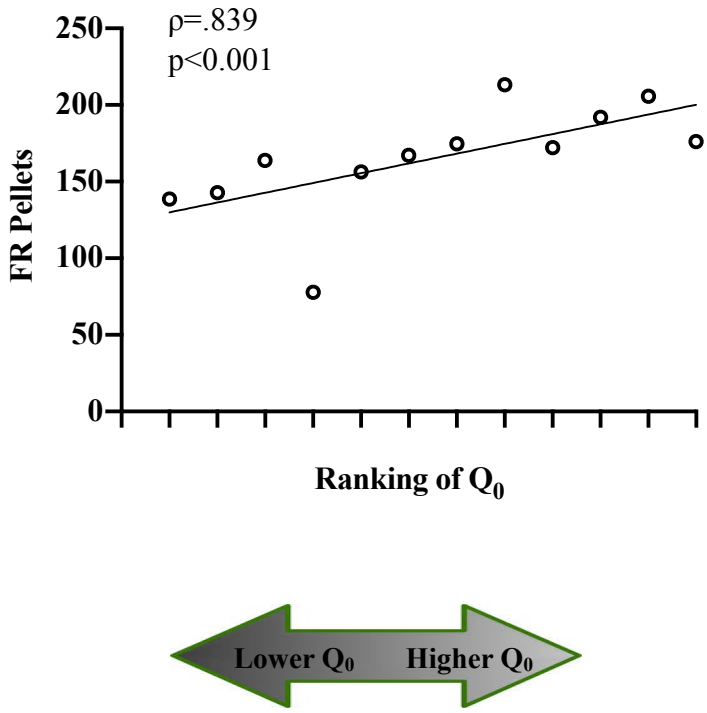

Figure 10: Higher Q_0 correlates with more pellets earned during FR1 sessions in INT animals. Spearman correlation showed that within INT animals only, Q_0 positively correlated with reinforcers (45-mg) pellets earned during FR1 sessions from Week 4-6 (*n*=12)

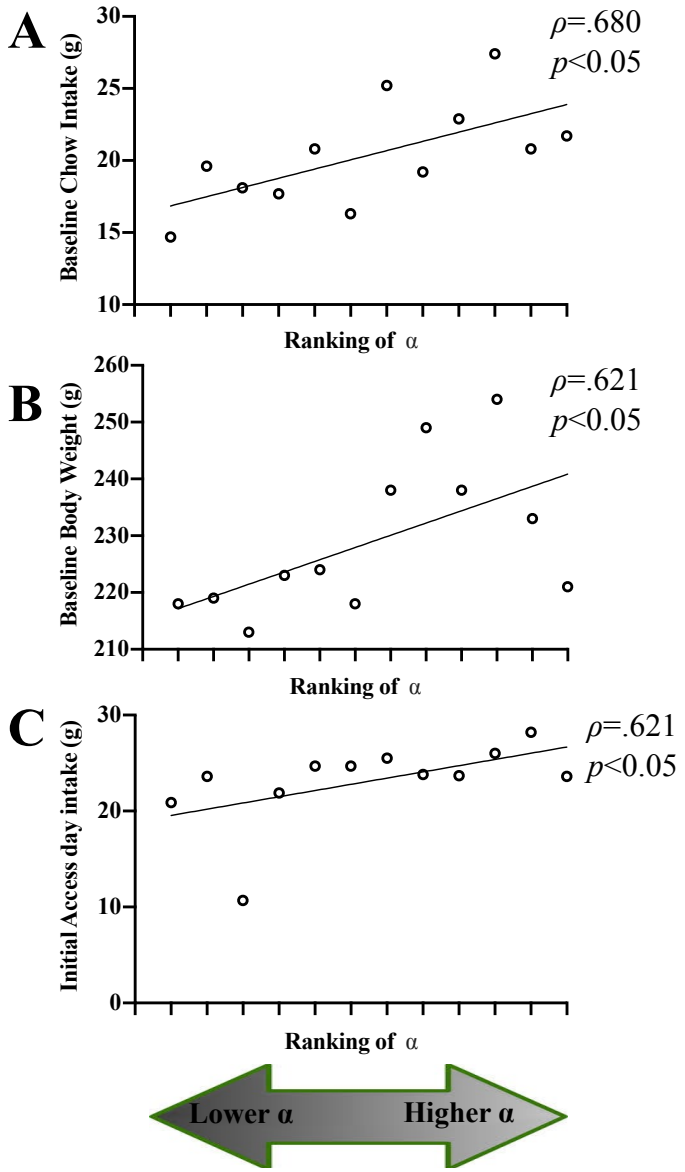

Figure 11: Antecedent predictors of α within INT group. Spearman correlation showed that within the INT group, **A)** chow intake and **B)** body weight at the end of the training period before the start of the diet schedules and **C)** choc intake on the first day of access predicted higher α (*n*=12).

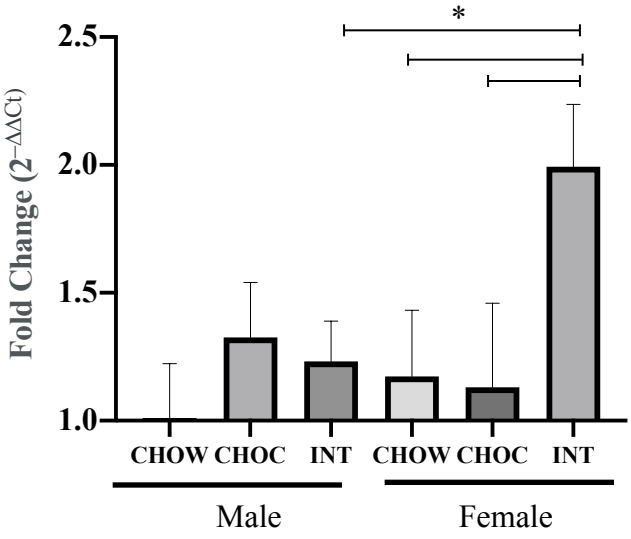

Figure 12: Diet group and sex differences in the elevation of CeA GR level. Based univariate analysis and Fisher's LSD test, qPCR results showed that female INTs had significantly increased GR level in the CeA comparing to CHOW and CHOC, a phenomenon not seen in males. Female INT animals also had significantly higher expression level than male INT animals. For males (n=23), CHOC: *n*=6; CHOW: *n*=6; INT: *n*=11. For females (*n*=22), CHOC: *n*=8; CHOW: *n*=5; INT: *n*=9. *: $p<0.05$.

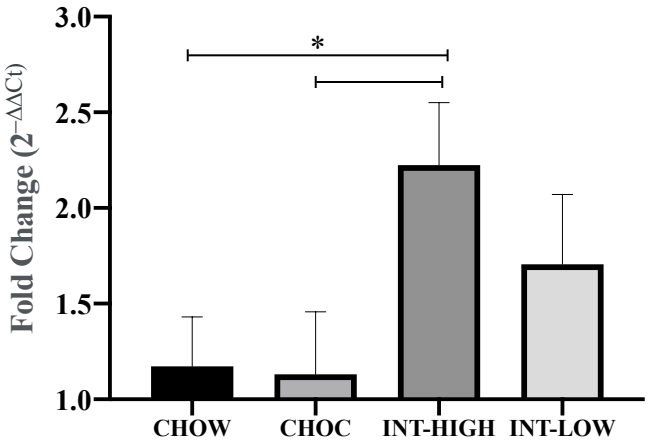

Figure 13: Differences in GR level increase between female INT-HIGH and INT-LOW animals. Based univariate analysis and Fisher's LSD test, qPCR results showed that female INT-HIGH animals had significantly higher GR mRNA expression comparing to both controls, while INT-LOW did not differ significantly. CHOC: *n*=8; CHOW: *n*=5; INT-HIGHT: *n*=5; INT-LOW: *n*=4. *: $p<0.05$.

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