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Developmental Effects of a High-Fat, High-Sugar Diet on Delay Discounting for Food

by

Stephen H. Robertson

A dissertation

submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy in the Department of Psychology

Idaho State University

Spring 2017

To the Graduate Faculty:

The members of the committee appointed to examine the dissertation of Stephen H. Robertson find it satisfactory and recommend that it be accepted.

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Animal Welfare Research Committee Approval Page

November 3, 2015

Erin Rasmussen, Ph.D.
MS 8112
Psychology Department
Pocatello, ID 83209

RE: Your application dated 10/30/2015 regarding study number 737: Effects of high-fat diet on three measures of food reinforcer efficacy

Dear Dr. Rasmussen:

Thank you for your response to requests from a prior review of your application for the new study listed above. Your study is eligible for designated review.

This is to confirm that your application is now fully approved. The protocol is approved through11/3/2018.

You are granted permission to conduct your study as most recently described effective immediately. The study is subject to annual review on or before 11/3/2016, unless closed before that date.

Please note that any changes to the study as approved must be promptly reported and approved. Some changes may be approved by designated review; others require full board review. Contact me (208-282-2179; fax 208-282-4723; email: anmlcare@isu.edu) if you have any questions or require further information.

Sincerely,

Tom Bailey
IACUC Manager

Acknowledgements

First and foremost, I would like to thank my mentor, Dr. Erin Rasmussen. Dr. Rasmussen has had a profound effect on my professional and personal development. Under her tutelage, I have improved my understanding of using behavioral pharmacological techniques to investigate neuro-behavioral interactions, which has shaped my research interests. Dr. Rasmussen gave me the opportunity, feedback, and support to become a better researcher, teacher, and mentor. As such, her influence on my development cannot be overstated. Second, I would also like to thank my committee members, Dr. Brumley, Dr. Lawyer, Dr. Xu, and Dr. Thomas, who gave support and guidance throughout my time at Idaho State University that allowed me to refine my critical thinking skills and expand my research repertoire. Third, I would also like to thank Dante Kyne-Rucker, Bailey Perschon, Rebecca Rose, Andra Cates, Amber Wright, and Nick Burgett for helping me collect data during the project. Fourth, I would like to thank my parents for supporting me in various ways over the last decade or so as I found my path as a behavioral pharmacology researcher. Finally, I need to thank my dog, Micro, who served as an always available support system and audience for all of my presentations.

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Abstract

Diet induced obesity (DIO) is a laboratory procedure in which nonhuman animals are chronically exposed to a high-fat, high-sugar diet (i.e., cafeteria diet), which results in weight gain, altered sensitivity to reward, and alterations in the dopamine D_2 system. To date, few (if any) studies have examined age-related DIO effects in a rat model or have used an impulsive choice task to characterize diet-induced behavioral alterations in reward processes. In the current study, we exposed rats to a cafeteria-style diet for 8 weeks starting at age 21 days or 70 days. Following the diet exposures, the rats were tested on a delay discounting task – a measure of impulsive choice – in which preference for smaller, immediate vs. larger, delayed food reinforcers was assessed. Following stability, acute injections of haloperidol (0.03 – 0.3 mg/kg) were administered to assess the extent to which diet-induced changes in dopamine D_2 influence impulsive food choice. Across both age groups, rats fed a cafeteria diet gained the most weight and consumed significantly more calories than rats fed a standard diet. Adolescent rats showed the highest weight gain relative to all other groups. Rats fed a cafeteria diet showed lower food-related impulsivity than rats fed a standard diet under vehicle injections. Haloperidol dose-dependently reduced choice for the larger, later reinforcer. Rats fed a cafeteria diet showed a left-ward shift in the dose-response curve, suggesting heightened sensitivity to haloperidol, regardless of age, compared to rats fed a standard diet. As such, the results indicate that chronic exposure to a cafeteria diet resulted in changes in underlying dopamine D_2 that manifested as greater impulsivity independent of age at diet exposure.

Key words: cafeteria diet, delay discounting, diet-induced obesity, dopamine D_2 dysregulation, haloperidol, impulsivity

Effects of Cafeteria Diet on Delay Discounting in Adolescent and Adult Rats: Alterations on Dopaminergic Sensitivity

Rates of childhood obesity have been steadily increasing, such that 17% of children and adolescents in the United States are considered obese (Anderson & Butcher, 2006; Ogden et al., 2014). Childhood obesity is a significant public health concern because it is associated with lower physical and psychological health (Schwimmer et al., 2003) and predicts obesity in adulthood (Biro & Wein, 2010; Guo & Chumlea, 1999). As of 2014, 36.3% of adults in the United States were classified as obese (Ogden et al., 2015), which is problematic because obesity in adulthood is associated with a host of diseases (e.g., type-2 diabetes mellitus, gallbladder disease, coronary heart disease, high levels of blood cholesterol, high blood pressure and osteoarthritis) that lead to a shortened lifespan (Must et al., 1999). Correlational studies suggest that increases in obesity have been linked to a shift toward higher consumption of a diet high in fat and sugar (Nielsen et al., 2002; St-Onge et al., 2003; Dong et al., 2015).

Experimental research supports these correlational studies by establishing a causal link between prolonged consumption of a high-fat, high-sugar diet and adiposity (Johnson & Kenny, 2010; Kanarek & Orthen-Gambil, 1982; Rolls et al., 1980; Vucetic et al., 2012; Woods, et al., 2003). One relevant area of research is diet-induced obesity (DIO), which is an experimental preparation in which subjects (usually rodents) are given extended access to a high-fat, high-sugar diet and control animals are fed standard chow. One variation of the diet used in a DIO procedure is referred to as a cafeteria diet and consists of a variety of foods that are high in fat and refined carbohydrates that humans typically consume (e.g., bacon, sausage, cheesecake, frosting, etc.; Johnson & Kenny,

2010, Rolls et al., 1980). DIO results in hyperphagia in free-feeding environments (Johnson & Kenny, 2010; Kanarek & Orthen-Gambill, 1982; Rolls et al., 1980) and increases in body fat (Johnson & Kenny, 2010; Kanarek & Orthen-Gambil, 1982; Rolls et al., 1980; Vucetic et al., 2012; Woods, et al., 2003).

Several bodies of research have documented that DIO also alters dopaminergic function in the brain, especially in areas involved in reward. First, imaging studies show that animals fed a high-fat diet have lower activity in the ventral tegmental area (VTA) and nucleus accumbens (NAc) compared to those fed a control diet (Val-Lillet et al, 2011). Neural imaging research with obese humans also shows reduced striatal activity in response to consumption of a high-fat, high-sugar liquid compared to lean participants (Stice et al., 2009; Stice et al., 2009). Second, high-fat diet, compared to control diets, can directly alter dopaminergic brain reward areas, via downregulation of D_2 receptor densities in the striatum (Johnson & Kenny, 2010), reductions of DA in the NAc shell (Geiger et al, 2009), and lower DA D_2 receptor gene expression in the VTA (Vucetic et al, 2012). Third, high-fat diet can alter behavioral sensitivity to dopaminergic D_2 compounds in behavioral economic tasks that use food as a reward (Boomhower & Rasmussen, 2014; Robertson et al., 2017). Taken together, these studies demonstrate that diet can influence dopaminergic D_2 function in the brain, especially in areas involved in reward.

Researchers have identified delay discounting as a behavioral process that is influenced by DA-rich neural areas, such as the striatum (Bickel et al., 2011; Bickel et al., 2014). Delay discounting refers to the tendency for an outcome to become devalued as a function of the delay to its receipt. Behavior that is especially sensitive to delayed

outcomes is considered a facet of impulsivity. In nonhuman animals, delay discounting is assessed by arranging a series of choices between a single food pellet delivered immediately versus multiple food pellets delivered after a brief delay. According to one procedure (Evenden & Ryan, 1996; Boomhower & Rasmussen, 2014), the delay to the larger amount is increased systematically across the sessions until the animal shows a preference for the single-pellet delivered immediately for at least 50% of the 10 trials (indifference point). By establishing the delay at which an indifference point is found, researchers can then assess factors, such as diet or specific drugs (Evenden & Ryan, 1996; Koffarnus et al., 2011; Boomhower & Rasmussen, 2014) that alter impulsive choice patterns.

Preferences for more immediate food-related outcomes have been shown with obese humans (Rasmussen et al, 2010; Hendrickson & Rasmussen, 2013; Hendrickson & Rasmussen, 2016) and in some animal models of obesity (Boomhower et al., 2013). One animal model of obesity is the Zucker rat, in which obesity is the result of a homozygous *fa/fa* "fatty" allele pattern and lean Zucker rats (controls) have heterozygous *fa/Fa* or homozygous *Fa/Fa* alleles (Beck, 2000; Sahu, 2004; Zucker & Zucker, 1961). Boomhower et al. (2013) investigated impulsive choice patterns that underlie obesity using a delay discounting task in lean and obese Zucker rats. They found that obese Zucker rats were more sensitive to delays for the larger, later option (2 or 3 food pellets) relative to lean Zucker rats, such that they tolerated significantly shorter delays than lean Zucker rats. As such, a higher sensitivity to delayed outcomes may be one factor that promotes obesogenic patterns of behavior.

In addition, diet has also been shown to affect dopaminergic sensitivity during impulsive choice tasks. Boomhower and Rasmussen (2014) investigated the extent to which chronic exposure to a high-fat diet versus a standard diet altered impulsive food choice. Following three months of exposure to a high-fat or a standard diet, researchers tested rats on a delay discounting task. Researchers found no baseline differences in delay discounting as a function of diet; however, rats fed a high-fat diet were more sensitive to acute injections of haloperidol (a D_2 antagonist), such that they showed higher impulsivity under haloperidol than rats fed a standard diet. These changes are consistent with diet-related changes that affect D_2 structure and function in the brain (Geiger et al, 2009; Johnson & Kenny, 2010; Val-Lillet et al, 2011; Baladi, et al., 2012; Vucetic et al., 2012; Boomhower & Rasmussen, 2014; Robertson et al., 2017).

While adult animal models have been used to understand diet-related obesity, to our knowledge, no animal models have been used to study the behavioral effects of a high-fat, high-sugar diet in a developing rodent that would be analogous to a human in child and adolescent development (e.g., Ozane & Hales, 2004). Development is characterized by a myriad of neural changes. Relevant to the current report, neural areas related to self-control (e.g., prefrontal cortex) and reward (e.g., striatal regions) follow different developmental trajectories, such that regions involved in reward tend to mature earlier than the regions involved in self-control (Kalsebeek et al., 1988; Andersen, 2003; Gogtay et al., 2004; Casey et al., 2008), which results in relatively more influence from neural areas related to reward and may promote impulsive patterns of behavior. Indeed, researchers have documented that, in humans, children and adolescents tend to show

steeper patterns of delay discounting relative to adults (Green et al., 1994; Hendrickson & Rasmussen, 2016).

Because childhood obesity predicts adult obesity (Biro & Wein, 2010; Guo & Chumlea, 1999) and diet-induced disruptions of DA D_2 function leads to dysregulation of the neural reward systems, it is possible that early exposure to a cafeteria-style diet may promote persistent impulsive behavior patterns that may create long-term problems, such as obesity. Experiments focused on characterizing obesity in an animal model analogous to childhood and adolescent developmental stages should allow researchers to assess the extent to which development and dietary history may affect impulsive food choice.

The current study used a 2 X 2 experimental design to investigate the extent to which a cafeteria diet versus a standard diet interacted with development (21 days vs 73 days of age when dietary exposure begins) to influence rates of delay discounting and changes in DA D_2 function. The current study had two primary aims. First, we assessed the extent to which diet and age of diet onset resulted in differences in weight gain, caloric consumption, and baseline rates of delay discounting. Second, we assessed the extent to which diet and age resulted in changes in sensitivity to a DA D_2 antagonist, haloperidol.

Methods

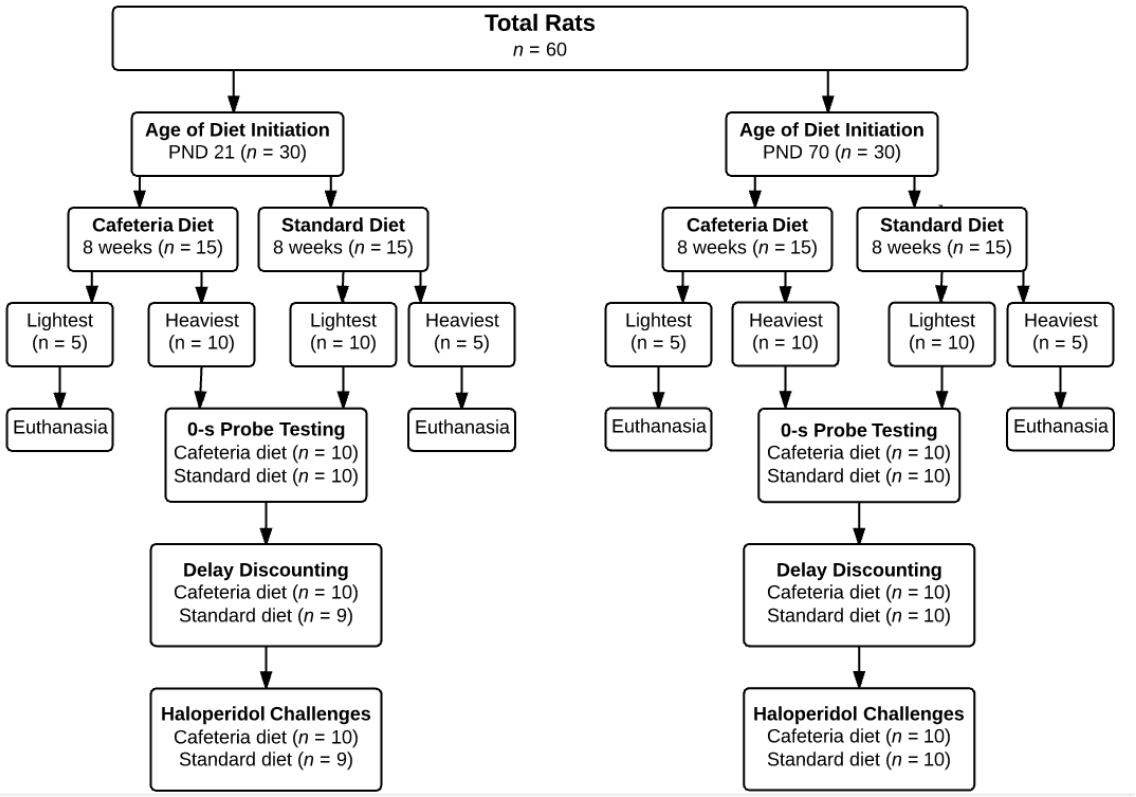


***Figure 1*. Diagram that shows progression of experimental conditions. Rats had to meet response criteria for each behavioral condition to move on to the next condition. As such, the number of session in each behavioral conditioned dependend on performance (outlined below). Analyses in the results show average number of sessions for baseline delay condition. The average number of 0-s probe sessions is shown in Figure 5.**

Subjects and Diets

Male Sprague-Dawley rats (n = 60), age 21 days (ADOL) or 70 days (ADULT), were obtained from a commercial breeder (Simonsen, Gilroy, CA) and individually housed in plexiglass shoebox cages with *ad libitum* access to water. Diet exposures began at either

21 days of age (n = 30) or 73 days of age (n = 30). The ages of these rats were selected to correspond to human development, respectively (Sengupta, 2013). By postnatal day (PND) 21, rats are equivalent to a 6-month-old human. By PND 42, rats are equivalent to a one-year-old human. By PND 50, rats reach puberty, which is equivalent to a human in early adolescence. By PND 70, rats are equivalent to a human adult. Further, the rat brain matures in a similar fashion as humans, such that systems involved in reward mature early in development (Andersen, 2003; Casey, Jones, & Hare, 2008); whereas, systems involved in self-control do not mature until adulthood (Gogtay, et al., 2004; Kalsebeek, Voorn, Buijs, Pool, & Uylings, 1988). Thus, these ages of the rats map on to human development and are developmentally appropriate for the research question, which makes them an excellent model to study the effects of diet-induced obesity on a developing system. Rats were house individually so that food intake can be monitored.

Each rat was randomly assigned to either a cafeteria-style diet or standard chow. Rats were individually housed so that food intake could be monitored. Rats fed a cafeteria diet received daily unlimited access to cooked sausage (3.17 kcal/g), cheesecake (4.23 kcal/g), potato chips (5.71 kcal/g), frosting (4.24 kcal/g), M&M's® (5.01 kcal/g), Twix® (5.01 kcal/g), and free access to standard chow (3.0 kcal/g). Rats exposed to the standard diet received unlimited access to standard chow. Rats were allowed 23 h access to their respective diets daily for 8 weeks. The 8 week diet exposure period was based on Johnson and Kenny (2010) and was used to produce sufficient differences in weight gain and reduction of DA D_2 receptor expression. Food was weighed prior to its placement in each rat's home cage and, after 23 hr, excess food was removed, weighed, and replaced with fresh food. After the diet exposures, 10 rats with the lowest body weight from the

standard chow group within each age group and 10 rats with the highest body weight from the cafeteria diet group within each age group were used for behavioral testing. Using the rats with the highest (DIO) and lowest (Standard Diet) body weights is a standard practice (e.g. Levin & Keesey, 1998; Huang et al., 2003; Huang et al., 2006; Johnson & Kenny, 2010; Boomhower & Rasmussen, 2014; Robertson et al., 2017) used to maximize the differences in weights and D_2 in the striatum between groups (Wang et al, 2001; Johnson & Kenny, 2010), as well as to ensure that the animals were a model of obesity, and not simply an evaluation of the effects of a cafeteria diet, per se. Weights of all animals are displayed in Table 1 and the weights of those only used for behavioral testing are shown in Figure 2. The Idaho State University Institutional Animal Care and Use Committee approved all procedures.

Apparatus

Seven Coulbourn® Habitest (Coulbourn Instruments, Whitehall, PA, USA) standard operant chambers individually placed in a sound-attenuating cubicle, equipped with two levers and stimulus lights, and a receptacle for reinforcer deliveries were used. Reinforcers were 45-mg grain-based Precision pellets (Bioserv, Frenchtown, NJ; 3.35 kcals/g). White noise was generated via a speaker situated on the top-right corner of the left wall. The chamber was ventilated via a 5 cm X 5 cm fan on the top-left corner of the left wall. Experimental events and data collection were controlled with a 0.01-s resolution using GraphicState® software (Coulbourn Instruments, Whitehall, PA, USA) on a Windows-based computer.

Behavioral Testing

Training

Following the 8-week diet exposure, rats were trained to lever press in a series of 3-h sessions. During this portion of the experiment, rats' weights were reduced to 85% of their free feeding body weight by restricting post-session access to their respective diets. Lever-press training for both levers was accomplished in a manner similar to other studies (see Boomhower et al., 2013 for details). After the rats successfully pressed each lever 60 times, they completed two additional sessions in which both levers were active to screen for side biases. Any side biases were corrected via shaping (i.e. reinforcing successive approximations) lever pressing to the unfavored lever. If the rats did not complete this sequence in 7 days, they were trained to lever press via shaping. After completing two sessions in which both levers were pressed 60 times, the rats were considered trained and delay discounting testing commenced.

Delay Discounting

During delay discounting testing, rats were maintained at 85% free feeding body weight to establish food as a reinforcer. Weights were maintained with food delivered during each session, as well as providing a supplemental amount of each rat's respective diet following the daily session (amount varied for each rat each day). Delay discounting sessions lasted 1.5 hours or until all trials were completed. The delay discounting procedure was a modified version of Evenden and Ryan's (1996) procedure used by Boomhower and Rasmussen (2014). Generally, the procedure was arranged such that a response on one lever resulted in the delivery of a single 45-mg grain-based pellet immediately and a response on the other lever resulted in the delivery three 45-mg grain-based pellets after a delay. Each session consisted of five blocks of discrete trials consisting of two forced-choice and 10 free-choice trials. For the forced choice trials,

rats experienced each contingency (i.e. one forced-choice trial for the single-pellet lever and one for the three-pellet option) in a randomly selected order. Following the forced choice trials, ten free choice trials commenced in which both levers were operational and rats could select either outcome. For each trial, the rat could make one of three choices: (a) select the one-pellet option (Smaller, Sooner; SS), (b) select the three-pellet option (Larger, Later; LL), or (c) make no response (an omission). If the rat chose the SS option, a single pellet was delivered immediately followed by the initiation of the intertrial interval (ITI). If the rat chose the LL option, three pellets were delivered following the specified delay, after which the ITI commenced. If the rat did not make a response after 30-s (an omission), the ITI commenced. During the ITI, all lights were extinguished and levers were not active. The duration of the ITI was such that each trial following a lever press or omission was held at 1 min. For instance, if the delay was 20-s, the rat would press the lever, wait 20-s for the pellet, and then a 40-s ITI commenced.

0-sec Probe Testing. Prior to experiencing delays, rats completed a series of tests that consisted of 5 blocks of 10 discrete choice trials as described above; however, both the one-pellet and three-pellet alternatives were delivered immediately. Each rat completed these tests until he pressed the three-pellet option 90% of the time in each block. This step is critical to demonstrate that the rat is sensitive to amount, prior to implementing delays.

Delay Discounting. Following the completion of 0-s probe testing, delays were introduced. The initial delay sequence consisted of blocks of delays of 0-, 1-, 2-, 4-, and

8-s. If responding for the LL alternative remained above 50% across all delay blocks, a second delay sequence consisting of blocks of delays of 0-, 2-, 4-, 8-, and 16-s was introduced. Again, if choice for the LL alternative remained above 50%, a final sequence of delays consisting of 0-, 5-, 10-, 20-, and 40-s was introduced. The order in which the delays were presented (i.e. ascending or descending) was counterbalanced across rats. Each delay sequence was in place for at least 5 sessions. These sessions were run 6 days a week. A 0-s probe test was conducted once weekly in order to check that the rats remained sensitive to amount.

Baseline behavior was considered stable when (a) the total responses for the LL option did not show an increasing or decreasing trend across the last five sessions, (b) the responses for the LL response for a given session did not vary by more than 20% of the grand mean of the previous five sessions, (c) choice for the 3-pellet alternative in the 0-s delay block was at least 90% across the last five sessions, and (d) the rat chose the 3-pellet option 90% in the 0-s probe session for that week (Boomhower & Rasmussen, 2014; Huskinson, Krebs, & Anderson, 2012). One rat (standard diet exposed at age 21) was excluded from analysis due to unstable baseline and was not moved to drug testing.

Haloperidol Challenges. After baseline responding was stable, acute haloperidol challenges commenced. Acute *i.p.* injections were administered 20 min prior to the experimental session. Injections (0, 0.03, 0.1, and 0.3 mg/kg) were administered once per week on the same day. The lower doses (0.03 and 0.1 mg/kg) were administered in a randomized order prior to the largest dose (0.3 mg/kg), which was administered last. Vehicle sessions were conducted the day before a haloperidol injection.

During haloperidol challenges, rats received 4 days of testing without drug (non-injection controls) and one day of 0-s probe testing without drug. All rats were required to pass the 0-s probe test in two sessions or less by selecting the three-pellet option for at least 90% of each trial in order to undergo the vehicle and drug testing for that week. One adult (Rat S28) completed some vehicle and drug testing sessions, though did not meet the 0-s probe criteria after repeated testing. This rat's data were included in the analysis because the pattern of results was the same with or without inclusion.

Drug

Haloperidol (Sigma-Aldrich, USA) was dissolved in a 1:1:18 vehicle solution of lactic acid (Sigma-Aldrich, USA), buffering agent, and saline (1 mL/kg). Both drug and vehicle solutions (which included the lactic acid, buffering agent, and saline) were held at a pH of 7 and were administer in a 1 mg/ml volume. Acute *i.p.* injections were delivered 20 min prior to the experimental session to ensure that the drug was behaviorally active.

Statistical Analyses

Statistical analyses were performed using IBM SPSS Version 23.0 (IBM SPSS Statistics for Macintosh, Version 23.0, Armonk, NY: IBM Corp.). For weight, weight gain and caloric consumption, data were averaged into weekly bins. Weight gain was calculated by subtracting the average weight for each week of diet exposure from the average weight for week one of diet exposure. Group differences in weight, weight gain and caloric consumption were assessed using mixed ANOVAs, with week as the repeated measure and diet (cafeteria vs. standard) and age (ADOL vs ADULT) as between-subject factors.

Baseline data. Delay discounting data were quantified in two manners. First, percent choice for the LL option from the last three sessions for each delay on the terminal delay sequence was averaged for each rat. Second, these means were plotted against delay for area under the curve (AUC) analysis, in which trapezoids are fitted to the area beneath the discounting curve and then the area of each trapezoid is summed (Myerson et al., 2001; Reed et al., 2012). Larger values indicate lower levels of delay discounting. Baseline percent LL choice were analyzed using a mixed ANOVA with delay block as the repeated measure and age at diet exposure and diet type as between-subject variables. AUC was analyzed using a 2 x 2 ANOVA, in which diet type and age were used as between-subject factors.

In addition, the mean number of 0-s probe sessions prior to implementing delay was assessed using a 2 x 2 ANOVA, with diet and age as between-subject factors. The number of sessions to reach stability and total number of 0-s probe sessions during the experiment were assessed using 2 x 2 x 2 ANOVAs with delay sequence (ascending vs. descending), age, and diet as between-subject factors. The mean number of responses made during the session was assessed using a 2 x 2 ANOVA with age and diet as between-subject factors. The mean number of responses made during each delay bin was assessed using a mixed ANOVA with delay as a repeated measure and age and diet as between-subject factors.

Drug data. The three sessions of vehicle data were averaged for each rat and then across each diet and age condition. Data for each dose of haloperidol are from a single session. Percent choice LL under drug conditions was analyzed using a mixed ANOVA with delay block for each dose as repeated measures and age at diet exposure and diet

type as between-subject variables. AUC was analyzed using a mixed ANOVA with dose as the repeated measure and age at diet exposure and diet as between subject factors. Finally, to measure drug sensitivity, haloperidol data were expressed as percent of vehicle for both percent choice LL and AUC and analyzed using a mixed ANOVA with dose as the repeated measure and diet and age at diet exposure as between-subject factors.

In addition, the mean total number of responses made during the session was assessed using a mixed ANOVA with dose as a repeated measure and diet and age as between-subject factors. The mean total number of responses made during each delay bin was assessed using a mixed ANOVA with delay and dose as a repeated-measure and diet and age as between-subject factors.

Results

Weight, Weight Gain and Food Intake

Figure 2 (top panel) shows weight across the dietary exposure period with rats in the behavioral portion of the study. Table 1 summarizes weight data for rats included in and excluded from behavioral testing and shows mean (SEM) and range for each subgroup of rats by the end of diet exposure. A mixed ANOVA (week as repeated measure, age and diet as between-subject factors) showed that weight increased across the diet exposure period, $F(1.64, 59.11) = 2411.84$, $p < 0.001$, $\eta_p = 0.99$. There was a main effect of diet, $F(1, 36) = 36.84$, $p < 0.001$, $\eta_p = 0.51$, in which DIO rats had higher body masses. There was a main effect of age, $F(1, 36) = 624.39$, $p < 0.001$, $\eta_p = 0.95$, such that adult rats had higher body masses. There was diet x age interaction, $F(1, 36) = 6.53$, $p = 0.015$, $\eta_p = 0.15$, such that adult rats fed a cafeteria diet showed the highest body masses. In addition, there was a week x diet interaction, $F(1.64, 59.11) = 25.38$, $p <$

0.001, $\eta_p = 0.41$, a week x age interaction, $F(1.64, 59.11) = 839.29$, $p < 0.001$, $\eta_p = 0.96$, and a week x diet x age interaction, $F(1.64, 59.11) = 4.64$, $p = 0.019$, $\eta_p = 0.11$.

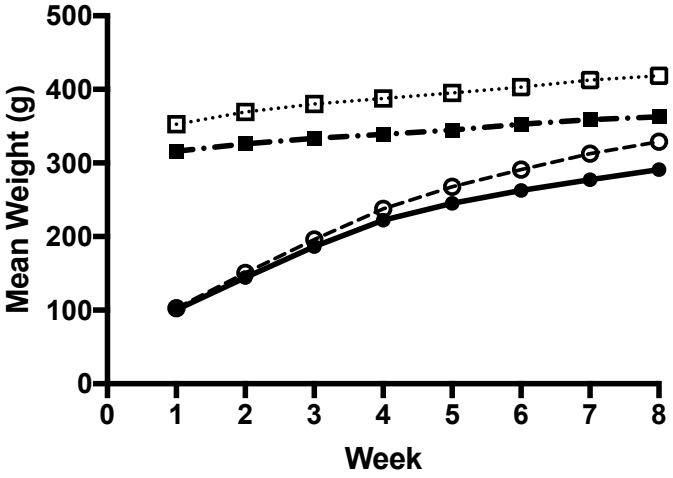
Figure 2 (middle panel) shows weight gain across the dietary exposure period. A mixed ANOVA (week as repeated measure, age and diet as between-subject factors) showed weight gain increased across week for all groups, $F(1.64, 59.11) = 2411.93$, $p < 0.001$, $\eta_p = 0.99$. There was also a main effect of diet, $F(1, 36) = 31.71$, $p < 0.001$, $\eta_p = 0.47$, in which DIO had higher weight gain, and age, $F(1, 36) = 1302.04$, $p < 0.001$, $\eta_p = 0.97$, in which adolescent rats had higher weight gain. In addition, there was a week x diet interaction, $F(1.64, 59.11) = 25.38$, $p < 0.001$, $\eta_p = 0.41$, a week x age interaction, $F(1.64, 59.11) = 839.32$, $p < 0.001$, $\eta_p = 0.96$, and a week x diet x age interaction, $F(1.64, 59.11) = 4.64$, $p = 0.02$, $\eta_p = 0.11$, such that adolescent rats with DIO exposure had the highest weight gain by the last week. There was no diet x age interaction ($p = 0.27$).

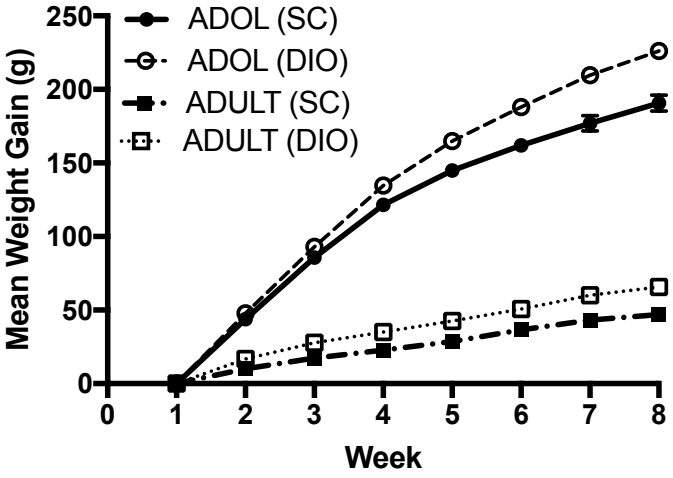
The bottom of Figure 2 shows mean weekly caloric intake across the diet exposure period. A mixed ANOVA revealed that across all groups, there was a main effect of week that trended toward significance, $F(7, 252) = 1.95$, $p = 0.06$, $\eta_p = 0.05$. There was also a main effect of diet, $F(1, 36) = 703.42$, $p < 0.001$, $\eta_p = 0.95$, in which DIO rats consumed the most kCals. There was also a trending effect of age, $F(1, 36) = 51.47$, $p = 0.06$, $\eta_p = 0.59$, in which adults consumed more kCals and a diet x age interaction, $F(1, 36) = 42.51$, $p < 0.001$, $\eta_p = 0.54$, such that adults in the DIO group had the highest consumption. There was also a week x diet interaction, $F(7, 252) = 8.99$, $p < 0.001$, $\eta_p = 0.20$, a week x age interaction, $F(7, 252) = 4.63$, $p < 0.001$, $\eta_p = 0.11$, and week x diet x age interaction, $F(7, 252) = 4.24$, $p < 0.001$, $\eta_p = 0.11$.

Table 1

Mean (SEM) and range for weights (g) following diet exposure for animals included in and excluded from behavioral testing

		Included		Excluded	
Diet	Age	*M* (*SEM*)	Range	*M* (*SEM*)	Range
Diet Induced Obesity	ADOL	313.52 (3.31)	301.70 – 329.50	281.20 (8.99)	247.00 – 298.00
	ADULT	419.10 (6.93)	392.40 – 465.40	371.46 (11.18)	341.90 – 394.60
Standard Diet	ADOL	278.33 (6.75)	238.40 – 294.60	316.12 (4.35)	307.30 – 330.00
	ADULT	365.31 (6.92)	332.00 – 389.10	426.22 (5.03)	398.50 – 466.70


Mean Weight (g)
500
400
300
200
100
0
0 1 2 3 4 5 6 7 8
Week


ADOL (SC)
ADOL (DIO)
ADULT (SC)
ADULT (DIO)
Mean Weight Gain (g)
250
200
150
100
50
0
0 1 2 3 4 5 6 7 8
Week

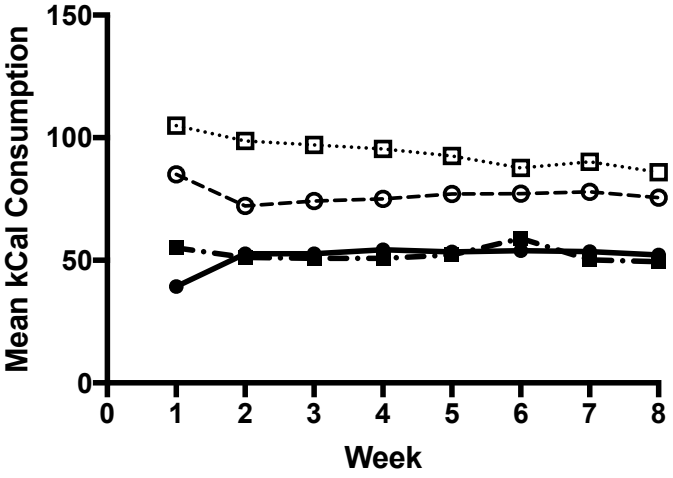

Mean kCal Consumption
150
100
50
0
0 1 2 3 4 5 6 7 8
Week

Figure 2. **Mean weight for rats that continued to behavioral testing (*n* = 40; top panel), mean weight gain for rats that continued to behavioral testing (*n* = 40; middle panel) and mean food consumption expressed in kCals (*n* = 40; bottom panel) across the 8-week diet exposure period for each group. Error bars represent 1 SEM. Some error bars are obscured by the data point.**

Baseline Delay Discounting

The number of 0-s probe sessions completed prior to commencing delay discounting was assessed using a 2 x 2 ANOVA (diet and age as between-subject factors). Figure 3 shows these data. There was a main effect of age, $F(1, 35) = 21.01$, $p < 0.001$, $\eta_p = 0.38$, such that animals exposed at day 73 completed the 0-s probe sequence in fewer sessions than rats exposed at day 21. There was no main effect of diet ($p = 0.83$) and no interaction ($p = 0.85$).

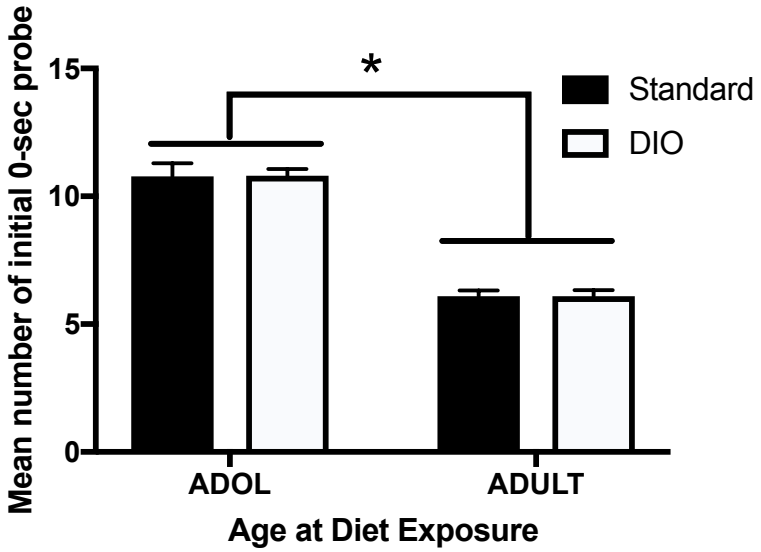


Figure 3. **Mean number of 0-s probe sessions completed prior to delay discounting. Error bars = 1 SEM.**

For baseline delay discounting (Figure 4), percent choice for the LL alternative decreased as a function of delay, $F(4, 140) = 250.\ 15$, $p < 0.001$, $\eta_p = 0.88$. There were no main effects of diet, age, or any interactions (p's > 0.20). For baseline levels of AUC, there were no main effects or interactions (p's > 0.20).

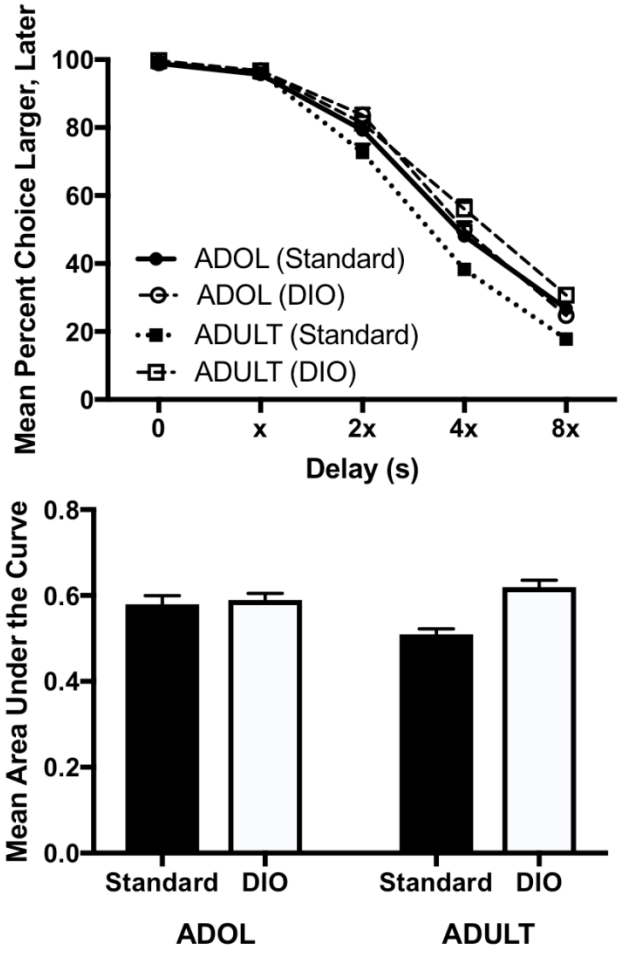


Figure 4. **The top panel shows rates of baseline percent choice for the three-pellet alternative across all delays tested on the terminal sequence for each group. The bottom panel shows area under the curve values for baseline delay discounting. Error bars represent 1 SEM. Some error bars are obscured by the data point.**

Because it is possible that delay sequence (ascending vs. descending) played a role in baseline delay discounting performance, a 2 x 2 x 2 ANOVA in which diet, age, and delay sequence were between-subject factors was used to investigate stable preference pattern. The was a main effect of age, $F(1, 31) = 8.53$, $p = 0.006$, $\eta_p = 0.008$, such that older animals reached stability sooner, which is shown in Figure 5 (top). There was a trending age x delay sequence interaction, $F(1, 35) = 3.61$, $p < 0.067$, $\eta_p = 0.104$, such that adolescent rats exposed to a descending delay sequence took more sessions to reach stability, which is shown in Figure 5 (bottom). There were no other main effects (p's = 0.16) and no other interactions (p's > 0.82).

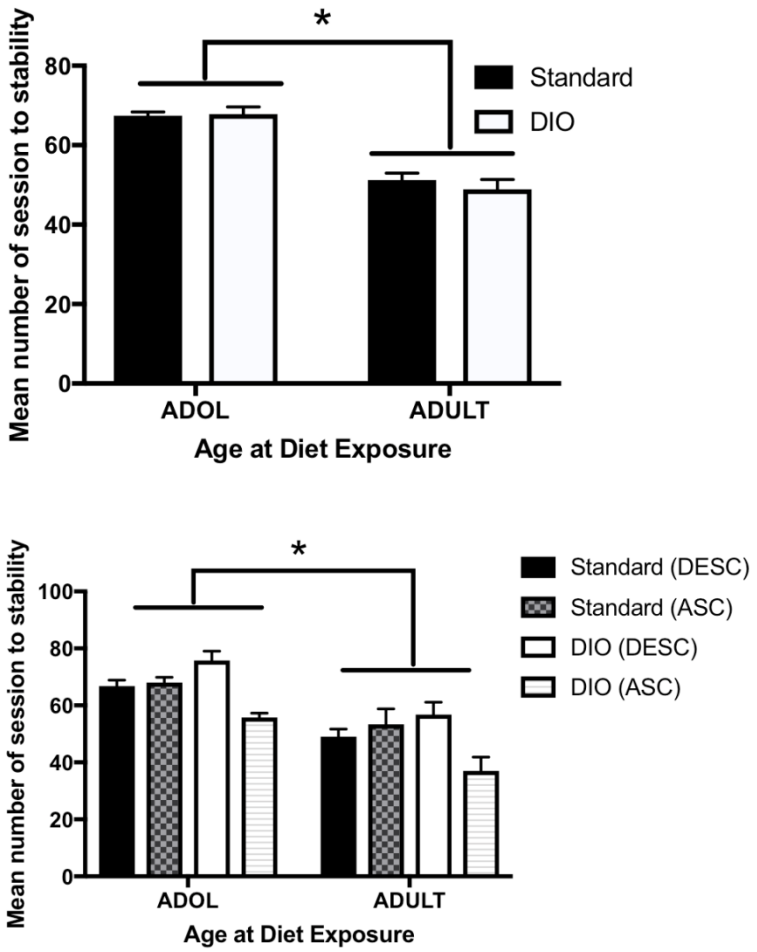


***Figure 5*. Mean number of sessions to reach stability as a function of age at exposure and diet type (top) and as a function of age at exposure, diet type, and delay sequence (bottom). Error bars = 1 SEM.**

The total number of responses made throughout the session during the final three stable sessions of baseline was assessed using 2 x 2 ANOVA (diet and age as between-subject factors; Figure 6). There were no main effects of diet, (p = 0.095), delay sequence (p = 0.69), age (p = 0.24), and no interactions (p's > 0.25)

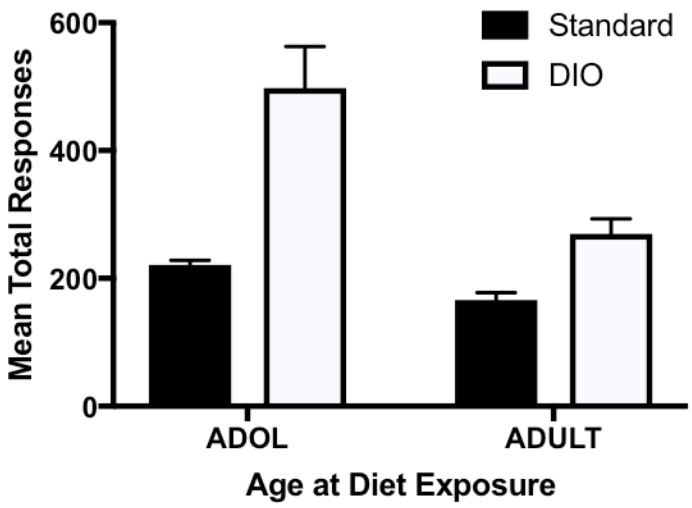


Figure 6. **Mean number of responses made during final three baseline sessions. Error bars = 1 SEM.**

To assess the number of responses made during the delay only, a mixed ANOVA (delay as a repeated measure and age and diet as between-subject factors; Figure 7) was used. Delay sequence was omitted from the analysis because it was not a significant factor in the initial analysis. There was a main effect of delay bin, $F(1.4, 50.1) = 7.33$, $p = 0.004$, $\eta_p = 0.173$, such that as the delay increased the number of responses emitted during the delay increased. A main effect of diet trended toward significance, $F(1, 35) = 3.16$, $p = 0.08$, $\eta_p = 0.083$, such that DIO rats made more responses that rats fed a standard diet. There were no other main effects or interactions (p 's > 0.12).

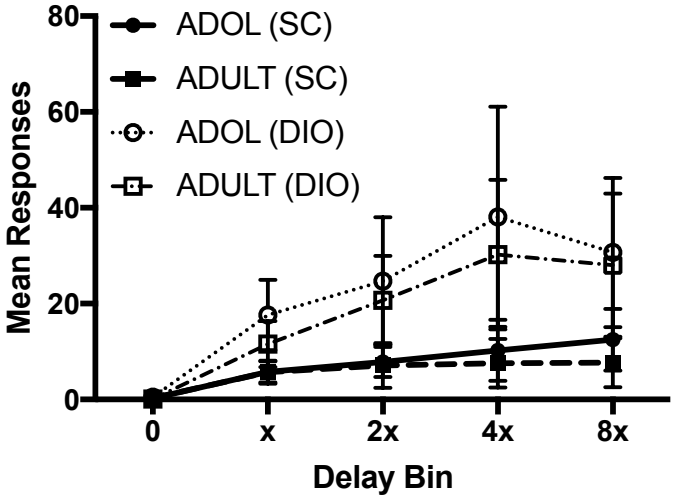


***Figure 7*.** **Mean responses made during delay only as a function of group. Error bars = 1 SEM. Some error bars may be obscured by the data point.**

Vehicle Delay Discounting

Data from the vehicle condition are presented in Figure 8. The top panel shows mean percent choice for the LL option as a function of delay. A mixed ANOVA revealed a main effect of delay, $F(4, 140) = 272.935$, $p < 0.001$, $\eta_p = 0.89$, such that percent LL choice decreases with delay. There was also a main effect of diet, $F(1, 35) = 6.88$, $p = 0.01$, $\eta_p = 0.16$, such that DIO rats showed higher percent LL choice. There was a delay x diet interaction, $F(4, 140) = 4.62$, $p = 0.002$, $\eta_p = 0.12$, such that rats fed a standard diet showed lower percent choice LL across the delays. There were no main effects of age ($p = 0.50$) and no other interactions (p's > 0.37). The bottom panel of Figure 8 shows the average AUC for vehicle injection sessions. An ANOVA revealed a main effect of diet, $F(1, 35) = 4.55$, $p = 0.04$, $\eta_p = 0.12$. There were no other main effects or interactions (p's > 0.65).

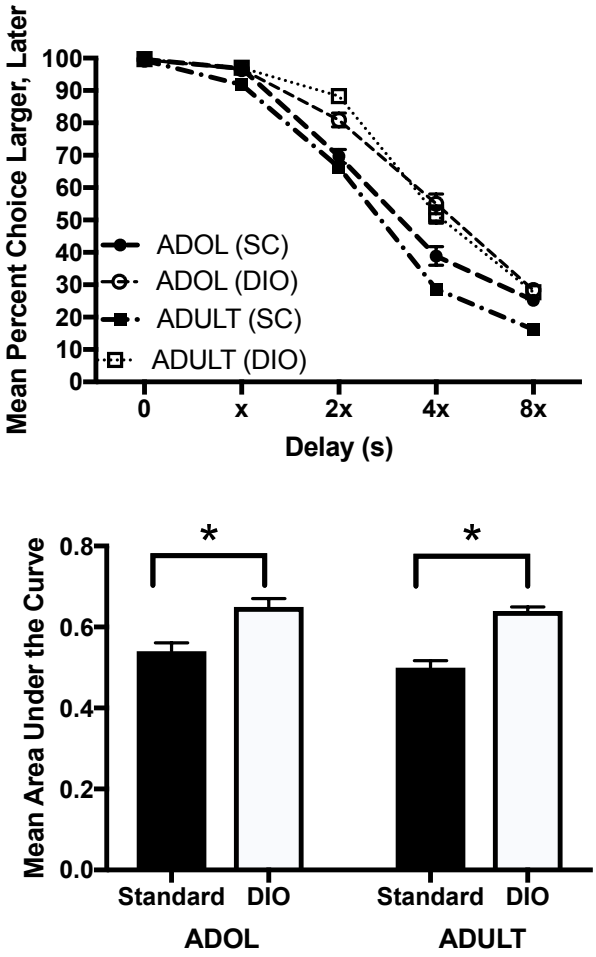


Figure 8. **Top panel shows mean percent choice for the LL alternative across all delays tested on the terminal sequence for each group following an injection of vehicle. Bottom panel shows mean AUC for each group following vehicle injections. Error bars represent 1 SEM. Some error bars are obscured by the data point.**

Acute Drug Challenges

Figure 9 shows mean percent choice for the LL option as a function of delay for each drug dose. Each panel represents a group, with adolescents in the top panels and adults in the bottom panels. Standard chow groups are represented on the left; cafeteria diet (DIO)

on the right. There was a main effect of delay, $F(4, 140) = 228.92$, $p < 0.001$, $\eta_p = 0.87$ and a main effect of dose, $F(3, 105) = 132.96$, $p < 0.001$, $\eta_p = 0.79$. While there was no main effect of diet ($p = 0.68$), there were several diet-related interactions. There was a delay x diet interaction, $F(4, 140) = 6.24$, $p < 0.001$, $\eta_p = 0.52$ and a drug x diet interaction, $F(3, 105) = 34.90$, $p = 0.03$, $\eta_p = 0.08$. In addition, there was a delay x dose interaction, $F(4.8, 168.04) = 26.70$, $p < 0.001$, $\eta_p = 0.43$. There was no main effect of age or any other interactions (p's > 0.20).

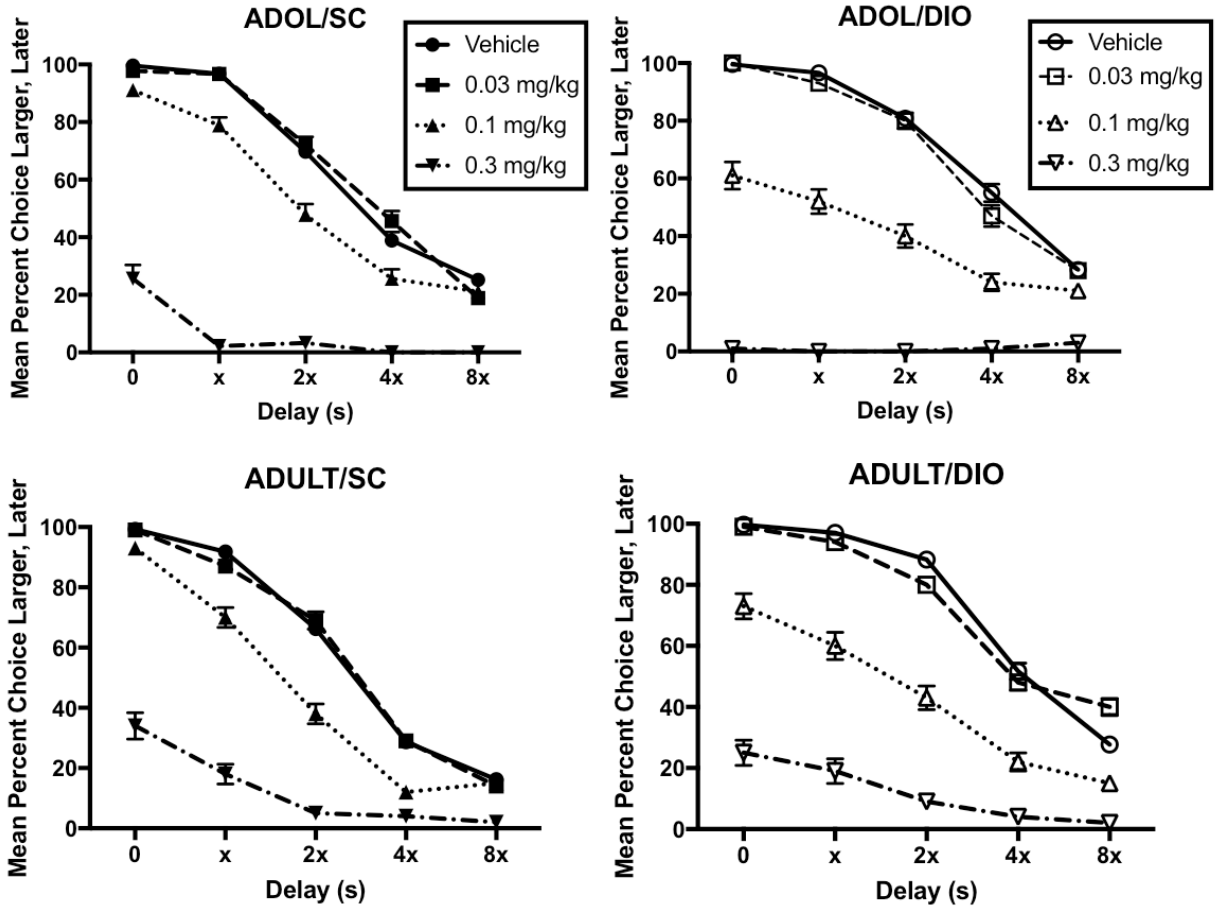


***Figure 9*. Percent choice for the three-pellet alternative as a function of delay following an injection of 0, 0.03 mg/kg, 0.1 mg/kg, and 0.3 mg/kg of haloperidol for**

each. Error bars represent 1 SEM. Some error bars are obscured by the data point.

AUC (Figure 10) values decreased dose-dependently, $F(3, 105) = 102.76$, $p < 0.001$, $\eta_p = 0.75$. A dose x diet interaction approached significance, $F(3, 105) = 2.30$, $p = 0.08$, $\eta_p = 0.06$. No interactions were revealed (p's > 0.41).

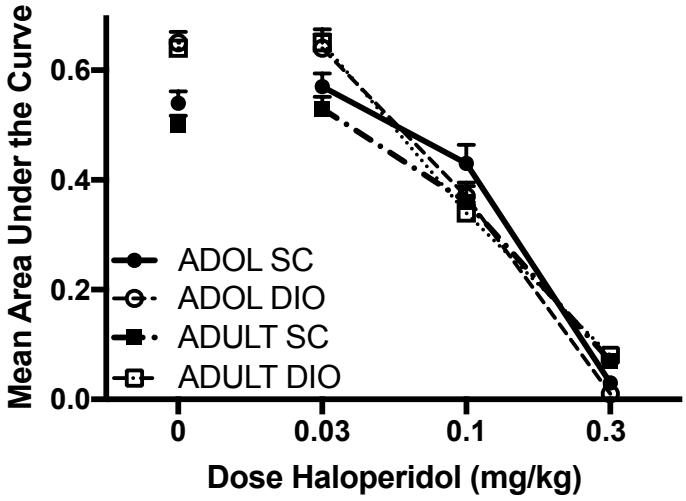


Figure 10. **Shows mean area under the curve following an injection of 0, 0.03 mg/kg, 0.1 mg/kg, and 0.3 mg/kg of haloperidol for each group. Error bars represent 1 SEM. Some error bars are obscured by the data point.**

Drug Data as Percent of Vehicle

Because there were differences in groups with the vehicle data (Fig. 8) and to more clearly show the diet x drug dose interactions from Figure 9, Figure 11 (top) shows data on drug sensitivity with mean percent LL choice under the terminal delay sequence as a function of dose. Data are expressed as percent of vehicle. Percent choice for the LL option decreased as a function of dose, $F(3, 105) = 145.17$, $p < 0.001$, $\eta_p = 0.81$.

There was also a main effect of diet, $F(1, 35) = 6.14$, $p < 0.02$, $\eta_p = 0.15$. A dose x diet interaction that approached significance was also revealed, $F(3, 105) = 2.57$, $p = 0.058$, $\eta_p = 0.07$, such that animals fed the cafeteria diet, regardless of age, demonstrated a sensitivity to the 0.1 mg/kg dose of haloperidol relative to animals fed a standard diet. No main effect of age or a diet x age interaction was found (p's > 0.55).

The bottom of Figure 11 shows mean AUC values expressed in terms of percent of vehicle. AUC decreased as a function of dose, $F(3, 105) = 145.10$, $p < 0.001$, $\eta_p = 0.81$. There was a main effect of diet, $F(1, 35) = 5.81$, $p < 0.02$, $\eta_p = 0.14$. In addition, there was a dose x diet interaction that approached significance, $F(3, 105) = 2.56$ $p = 0.059$, $\eta_p = 0.07$, such that rats fed a cafeteria diet showed greater sensitivity to the 0.1 mg/kg dose relative to animals fed a standard diet. No main effect of age or a diet x age interaction was apparent (p's > 0.54).

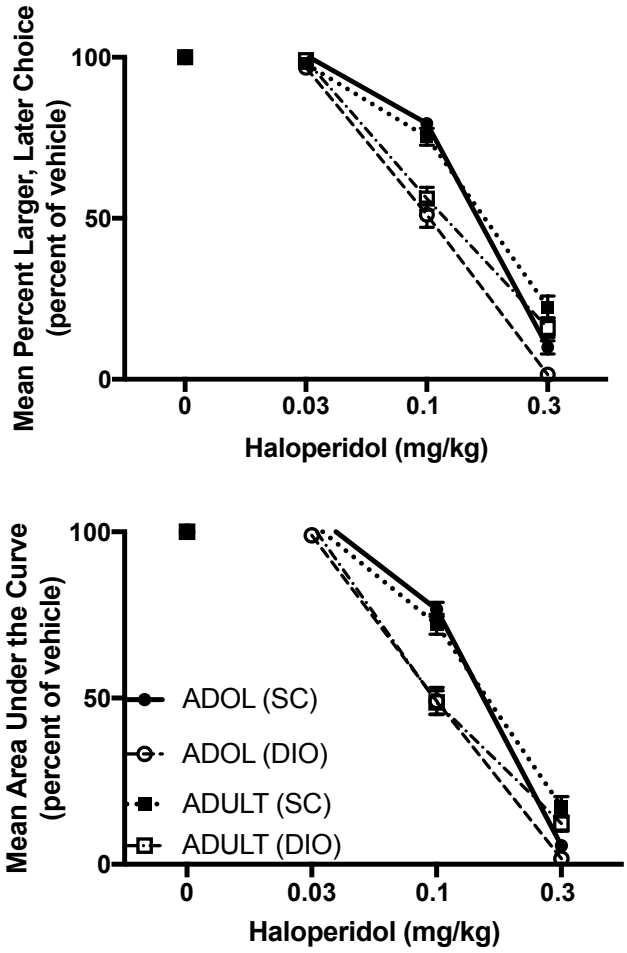


Figure 11. **Mean percent choice (percent of vehicle) for the three-pellet alternative (top) and mean AUC values (bottom) as a function of haloperidol dose. Error bars represent 1 SEM. Some error bars are obscured by the data point.**

Because there were interactions with diet, delay, and dose in the aforementioned analyses (Figures 9 and 11), we more carefully examined behavioral aspects related to amount and delay parameters. We started with the 0-sec delay, in which delay to both SS and LL alternatives were held constant at 0, but amount differed. This specific condition tested for sensitivity to amount. Figure 12 shows mean percent LL choice during the 0-s

block of the delay discounting task as a function of dose. A mixed ANOVA (dose as repeated measures and diet and age as between-subject factors) revealed a main effect of dose, $F(2.19, 76.79) = 89.65$, $p < 0.001$, $\eta_p = 0.72$. There was also a main effect of diet, $F(1, 35) = 5.81$, $p = 0.02$, $\eta_p = 0.14$, such that DIO rats showed a higher sensitivity to haloperidol. In addition, a dose x diet interaction trended toward significance, $F(2.19, 76.79) = 2.74$, $p = 0.07$, $\eta_p = 0.07$, such that DIO rats were particularly sensitive to the injections at the 0.1 (at both ages) and 0.3 (ADOL only) mg/kg doses. There were no other main effects or interactions revealed (p's > 0.18).

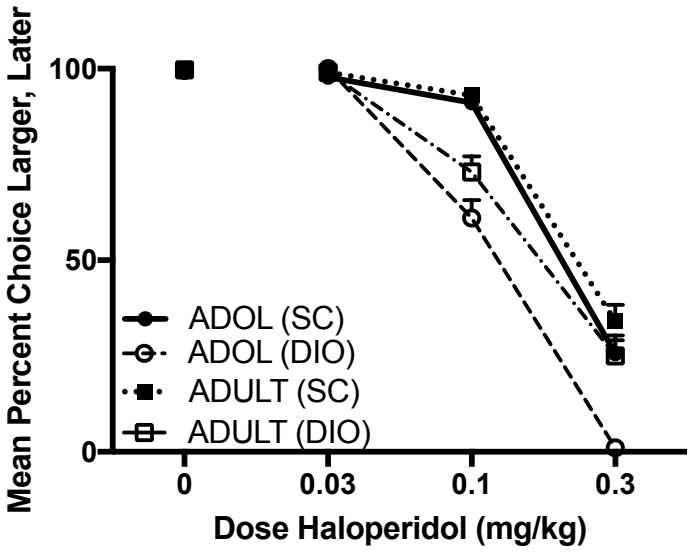


Figure 12. **Percent LL choice during the 0 second block of the delay discounting task as a function of dose for each group. Error bars represent 1 SEM. Some error bars are obscured by the data point.**

To more carefully examine aspects of delay sensitivity that may have played a role in DIO rats' sensitivity to haloperidol in the discounting data, we investigated the role of order (ascending or descending) of the delays imposed. Figure 13 (top) shows

percent LL choice as a function of delay for each diet group and whether the rats were assigned to the ascending or descending delay sequence. Age was collapsed across diet conditions due to the lack of differences observed in previous and present analyses. A mixed ANOVA (dose as the repeated measure and diet and delay sequence as between-subject factors) revealed that percent LL choice decreased as a function of increases in delay, $F(4, 140) = 77.98$, $p < 0.001$, $\eta_p = 0.69$. There was a delay x diet interaction, $F(4, 140) = 3.36$, $p = 0.01$, $\eta_p = 0.09$, such that rats fed a high-fat diet showed a lower preference for the LL alternative. There was a delay x sequence interaction, $F(4, 140) = 3.48$, $p = 0.01$, $\eta_p = 0.09$, such that lower delays in the descending sequence showed lower percent LL choice. Importantly, there was also a diet x sequence interaction that approached significance, $F(1, 35) = 3.76$, $p = 0.06$, $\eta_p = 0.10$, such that rats fed a cafeteria diet exposed to a descending sequence showed a larger reduction in percent LL choice. No other main effects or interactions were revealed (p's > 0.25).

One reason why DIO rats may have showed higher impulsivity in the descending delay sequence is because experiencing longer delays first may have made them especially sensitive to delay. Therefore, examining omissions made during these sessions would provide some additional information on this mechanism. Figure 13 (bottom) shows percent trials omitted following an injection of 0.1 mg/kg of haloperidol for each diet group exposed to an ascending vs descending delay sequence. A mixed ANOVA (dose as the repeated measure and diet and delay sequence as between-subject factors) revealed an delay x sequence interaction, $F(4, 140) = 4.26$, $p = 0.003$, $\eta_p = 0.11$, such that more omissions occurred at lower delays. We also found a diet x sequence interaction, $F(1, 35) = 4.23$, $p = 0.05$, $\eta_p = 0.11$, such that rats fed a cafeteria diet that were exposed

to a descending delay sequence showed the most omission. No other main effects or interactions were revealed (p's > 0.17).

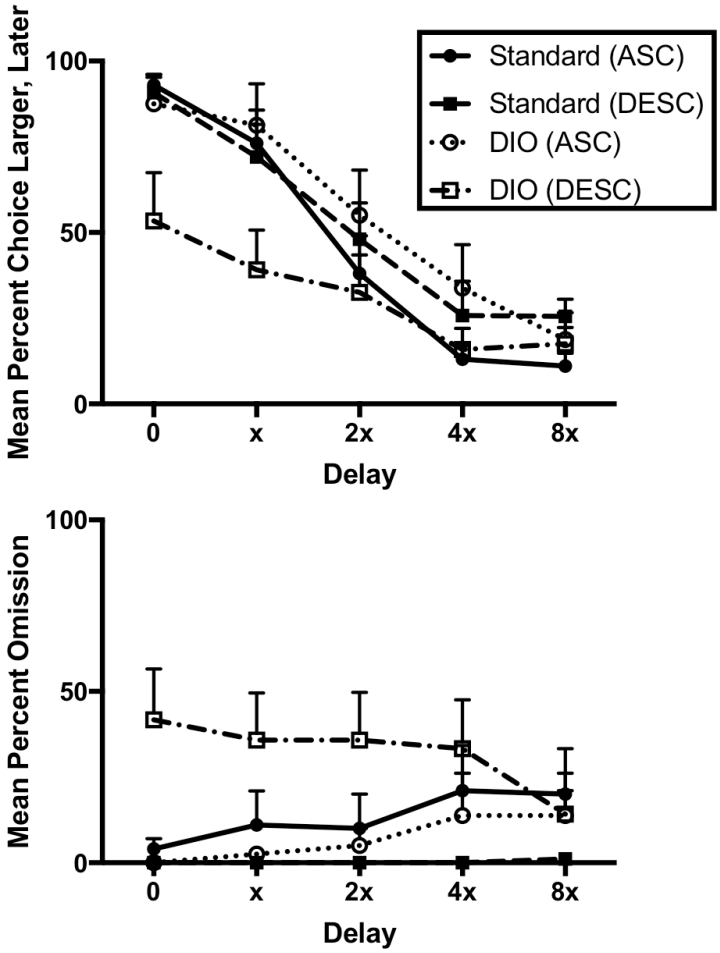


Figure 13. **Mean percent choice for LL (top) and mean percent omission (bottom) as a function of delay following an 0.1 mg/kg of haloperidol for each diet group displayed for ascending (ASC) or descending (DESC) delay sequences. Error bars represent 1 SEM. Some error bars are obscured by the data point.**

To determine the extent to which the changes in drug sensitivity were related to motor effects, the total number of responses emitted across each haloperidol session was

analyzed using a mixed ANOVA (dose as a repeated measure and diet and age as between-subject measures; Figure 14). A previous analysis included delay sequence but it did not reach statistical significance, so it was omitted from the analysis. There was a main effect of dose, $F(1.2, 43) = 12.74$, $p < 0.001$, $\eta_p = 0.27$, such that total responses emitted decreased dose-dependently. There was a trending dose x diet interaction, $F(1.23, 31) = 2.76$, $p < 0.09$, $\eta_p = 0.073$, such that DIO rats made more responses than rats fed a standard diet at most doses. No other main effects or interactions were revealed (p's > 0.20).

Figure 14. **Shows mean responses across session as a function of dose for each group (Standard diet = black circle; DIO = white circle). Error bars = 1 SEM.**

Responses during the delay only were also assessed using a mixed ANOVA (delay bin and dose as repeated-measures and diet and age as between-subject factors; Figure 15). Delay sequence was omitted from this analysis because it not did not reach statistical significance in a previous analysis. There was a main effect of delay, $F(1.25, 41.07) = 7.43$, $p = 0.006$, $\eta_p = 0.17$, such that longer delays were associated with more

responses emitted. There was also a main effect of dose, $F(1.11, 41.01) = 8.24$, $p = 0.005$, $\eta_p = 0.18$, such that responding during the delay decreased dose dependently. There was a delay x dose interaction, $F(2.29, 84.62) = 4.90$, $p = 0.007$, $\eta_p = 0.12$. There was also a trending dose x diet interaction, $F(1.1, 41.01) = 3.41$, $p < 0.068$, $\eta_p = 0.084$, such that DIO rats showed higher levels of responding than rats fed a standard diet. No other main effects or interactions were revealed (p's > 0.104).

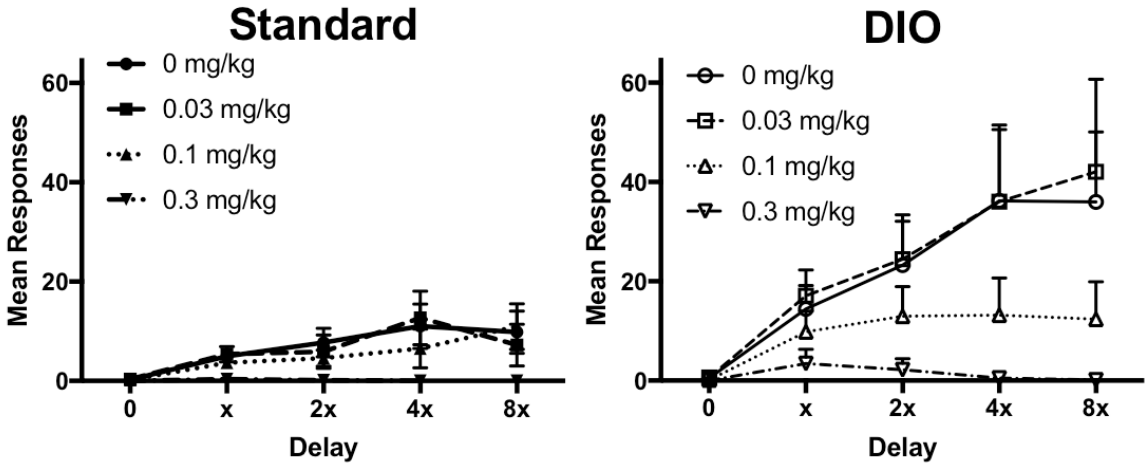


Figure 15. **Shows mean responses emitted during the delay only for rats fed a standard diet (left) and DIO rats (right). Error bars = 1 SEM. Some error bars may be obscured by the data point.**

Discussion

The current investigation assessed differences in body mass, caloric intake, delay discounting, and behavioral sensitivity to a DA D_2 antagonist as a function of diet and developmental timing of diet exposure. Rats given chronic access to a cafeteria diet showed a larger body masses and higher levels of weight gain compared to rats fed a standard diet within each age group. Rats that were exposed to their respective diets

starting at age 21 days showed greater weight gain across the diet exposure period than rats that started diet exposures at age 70 days. This is likely because, at age 21 days, rats have a much lower body mass than rats at age 70 days and are in a developmental period characterized by active growth (Sengupta, 2013). Within each age group, rats fed a cafeteria diet gain more weight relative to rats fed a standard diet. In general, these findings support literature that shows that DIO results in excessive weight gain relative to rats fed a standard diet, which is likely due to the observation that these rats consumed significantly more calories than rats exposed to a standard diet (Rolls, Rowe & Turner, 1980; Johnson & Kenny, 2010; Boomhower & Rasmussen, 2014; Robertson, Boomhower, & Rasmussen, 2017).

Baseline delay discounting data. We found that older rats reached stability on the initial 0-s probe session and baseline delay discounting in fewer sessions than younger rats. Thus, an age-related difference was apparent. Other research that compares acquisition of discounting with older rats shows that 24-months-old rats do not differ from 6-month old rats in terms of number of delay discounting sessions to stability or sensitivity to amount (Simon et al., 2010). It may be the case that once rats reach a particular age, their ability to acquire stable performance under delay discounting becomes similar. It should be mentioned, however, that we also found a trending effect of delay sequence that interacted with age, such that adolescent rats exposed to a descending delay sequence showed a higher number of sessions to reach stability than adult rats. Given that the descending sequence appears to be disruptive in terms of reaching a stable performance, future research should consider using all ascending delay sequence to reduce the influence of this variable. Conversely, researchers may also wish

to test both delay sequences across a larger range of different ages to further examine the extent to which age-related differences in acquisition interact with delay sequence.

We found no differences as a function of diet or age on baseline levels of delay discounting, which is consistent with a previous report on delay discounting following DIO (Boomhower and Rasmussen, 2014). However, the finding that rats fed a chronic high-fat, high-sugar diet showed lower rates of delay discounting following an injection of vehicle is notable, because it suggests differences between diet groups were apparent without drug on board. One interpretation of the difference between baseline and vehicle data is that a small perturbation, such as a vehicle injection, was enough to create small enough differences in the distributions that combined with dietary history to manifest as a diet-based difference. More research is needed to more carefully examine this. It was surprising, however, that rats exposed to the cafeteria diet were more self-controlled (greater preference for the larger-later outcome) than rats in the standard chow diet condition. There could be three reasons for this observation: an insensitivity to delay, a heightened sensitivity to amount, or both.

We also found a trending DIO effect on the number of responses emitted during the baseline session. This potential effect was largely driven by outliers in the DIO group, however. For instance, DIO rats exposed during adolescence showed a range of 92 – 1740 responses emitted (M = 497.72; SD = 652.02) and DIO rats exposed during adulthood showed a range of 75 – 861 responses emitted (M = 269.53; SD = 234.67). Adolescent rats on standard chow showed a range of 122 – 340 responses emitted (M = 221.15; SD= 62.55) and adult rats on standard chow showed a range of 73 – 449 responses emitted (M = 166.02; SD = 116.58). DIO rats, then, showed a much higher

level of responding during the sessions, which may be indicative of higher food-related food motivation relative to rats fed a standard diet.

Haloperidol data. Haloperidol dose-dependently reduced the percent choice for larger, later food outcomes; that is, the drug increased impulsive choice, for both diet and age conditions. This has been shown in other studies (Koffarnus et al, 2011; Boomhower & Rasmussen, 2014). Koffarnus et al. (2011) found that 0.1 mg/kg of haloperidol reduced preference for the LL alternative in standard laboratory rats using a delay discounting task with delays ranging from 0 - 60-sec. As such, the finding that all rats showed some shift in delay discounting following an injection of haloperidol is consistent with previous research.

Rats fed a cafeteria diet showed a higher behavioral sensitivity to haloperidol; that is, they became more impulsive for food than the rats fed the standard chow diet. Specifically, at the 0.1 mg/kg dose, rats fed a cafeteria diet showed a reduced tendency to select the LL option than the rats fed a standard diet, regardless of age. This finding is consistent with previous research that has demonstrated that adult rats fed a high-fat diet showed higher behavioral sensitivity to a 0.1 mg/kg dose than rats fed a standard rat chow diet (Boomhower & Rasmussen, 2014; Robertson et al, 2017). This also supports studies documenting diet-induced changes in dopaminergic D_2 that likely affect the reward areas of the brain (Geiger et al, 2009; Johnson and Kenny, 2010; Val-Lillet et al, 2011; Baladi, et al., 2012; Vucetic et al., 2012; Boomhower & Rasmussen, 2014; Robertson et al., 2017).

One possible behavioral mechanism for the observation that rats fed a cafeteria diet showed a lower preference for the LL alternative under haloperidol was, in part,

influenced by drug-induced changes in sensitivity to amount. Under the 0-sec block, in which there is no delay associated with either food option, haloperidol created a preference for the smaller, sooner option for some rats – and this was more pronounced in the DIO groups.

In addition to changes in sensitivity to amount, the diet-induced sensitivity to haloperidol can also be explained by a heightened sensitivity to delay. We controlled for order of delay values, in terms of ascending and descending order, by randomly assigning rats in each group to these two conditions. Rats exposed to standard chow did not differ in terms of discounting whether they received the delays in ascending or descending order. However, DIO rats that received the delays in descending order showed significantly higher impulsivity than those in the ascending delay condition; DIO rats exposed to the ascending delay sequence were statistically indistinguishable from the standard chow groups. A closer inspection of these data, in terms of omissions, suggest that DIO rats that experienced the delays in descending order exhibited a larger number of omissions – over 40% of the trials across delays, including short delays – than those in the other three groups. These omissions were likely not due to drug-related motor effects, because the DIO group that received the delays in ascending order (and the standard diet groups) did not show greater than 5 – 10% of omissions during these sessions – even at the larger delays. Thus, DIO animals exposed initially to the highest delays were more sensitive to delay, thereby enhancing sensitivity to the haloperidol-induced impulsivity effect. Because there is evidence for a cafeteria diet affecting both haloperidol-related sensitivity to amount *and* delay, future studies should attempt to parse these two mechanisms that potentially drive drug effects on impulsivity.

For response data, all rats showed a dose-dependent decrease in the total number of responses emitted across a session, with DIO rats showing a higher number of responses at the 0.1 mg/kg dose relative to rats fed a standard diet. In addition, DIO rats showed a higher number of responses emitted during each delay relative to rats fed a standard diet. These findings are important for several reasons. First, it supports other diet-related behavioral differences in haloperidol sensitivity that have already been discussed. Generally, DIO rats showed a higher sensitivity to haloperidol than rats fed a standard diet, which is evident in the ~60% reduction in the number of responses emitted at 0.1 mg/kg observed in the DIO group, but not the standard diet group, which did not show haloperidol-induced decrease in responses emitted at the same dose. Second, because responses during the delay do not have scheduled consequences, one interpretation of these responses might be a lack of behavioral inhibition for food (a separate mechanism of impulsivity) for DIO rats. Third, these data show that, despite the reductions, DIO rats were responding at a high level (about 260 responses, on average) – indeed, higher than the rats fed a standard diet (about 146 responses, on average) – across the session. As such, it gives support to the notion that the shift in impulsive choice exhibited by the DIO rats following an injection of haloperidol was not driven by motor impairment that interfered with responding during testing, but rather a higher allocation of behavior to the smaller, sooner food option.

One surprising finding was the consistent lack of age-related differences in the behavioral data. The rationale for investigating developmental aspects of DIO was influenced by the Competing Decision Systems Theory (Bickel et al., 2011; Bickel et al., 2014). According to this theory, DA-rich reward areas (e.g. striatal) and prefrontal

regions involved in self-control interact to influence impulsive choice patterns. The extent to which one area exerts more influence over the other, in part, determines the extent to which a pattern of impulsivity is evident. In theory, diet-induced changes in D_2 may lead to striatal regions overriding the influence of prefrontal regions. During development, the reward-related striatal regions develop prior to the prefrontal regions, which is thought to influence higher levels of impulsivity in childhood and adolescence (Galvan, 2010; Geier and Luna, 2009). We hypothesized that by using a high-fat, high-sugar diet to dysregulate DA D_2 systems during development, it is possible to disrupt the neural development of self-control and reward regions, which would result in a tendency to engage in impulsive patterns of food intake that would persist across development. However, we found no age effects in any of the data.

One possibility that could account for the lack of age differences in discounting is that studies have demonstrated human adolescents and adults differ in terms of delay discounting for money (Green et al., 1994; Hendrickson and Rasmussen, 2016) but not for food (Hendrickson and Rasmussen, 2016). This could reflect differences between discounting rates for secondary, non-consumable reinforcers vs. primary, consumable reinforcers, respectively. Perhaps discounting for food is something that remains stable across the lifespan. More research is needed in this area to determine the extent to which commodity-specific discounting differs across age.

One limitation of this study is that we only investigated a single pellet type (i.e., grain-based pellets). Grain-based pellets are similar in palatability and macronutrient content to the standard chow. In the current study, we opted to use grain-based pellets in order to control for introducing a pellet that differed in terms of palatability and

macronutrient content from the standard diet. However, it is possible that rats would show differences in delay discounting using pellets with different palatability and/or macronutrient content. In particular, given that rats fed a cafeteria diet had an extended dietary history of palatable food with a high caloric content from fat and sugar, it is possible that using a less palatable grain-based pellet is not sufficient to observe between-group differences in food impulsivity at baseline. A second limitation is that, although we observed differences in sensitivity to a D_2 antagonist, we are uncertain what brain regions and specific mechanisms are driving these effects. Some possibilities may be reductions in D_2 receptor expression in the striatum (Johnson and Kenny, 2010), reductions in D_2 receptor expression in the VTA (Vucetic et al, 2012), or lower availability of synaptic DA (Geiger et al, 2009). These considerations should be assessed in future studies.

Other limitations concern the nature of the cafeteria diet and obesity. First, as previously noted, the practice of selecting the heaviest rats from the cafeteria fed group and the standard diet fed group is conventional in DIO research (Levin & Keesey, 1998; Huang et al., 2003; Huang et al., 2006; Johnson & Kenny, 2010; Boomhower & Rasmussen, 2014; Robertson et al., 2017) and allows researchers to test rats that are more prone, rather than resistant, to DIO. This could be viewed as a limitation, though, for at least two reasons. First, the statistical differences between weight as a function of diet and age is due to only retaining animals that demonstrate the lowest weight gain in the standard chow group and the highest weight gain in the DIO group. Second, including animals that only showed the most extreme weight gain limits conclusions regarding the role of a HF, HS diet in promoting sensitivity to dopaminergic compounds. For instance, it is possible that only rats that are obesity-prone show sensitivity to dopaminergic

compounds prior to diet exposure, which has been demonstrated in a prior study (Vollbrecht, Nobile, Chadderdon, Jutkiewicz, & Ferrario, 2015). Given the methods utilized in the current study, we are not able to disentangle the factors that contribute to obesity-prone vs. obesity-resistant rats (e.g. pre-existing pharmacological sensitivities) and to be certain that sensitivity to the dopaminergic compound was specific to dietary exposure. Given that the study of obesity, per se, is a study of factors that underlie the development and maintenance of extreme body mass (Wang et al, 2001), these methods are ecologically valid and appropriate in the context of characterizing an animal model of obesity. Future studies, though, could parse the effects of diet and obesity-proneness in a more controlled manner.

A second issue concerns the degree of weight gain during the diet exposure condition. Adolescent rats fed a cafeteria diet showed levels of weight gain that are consistent with what would be expected given the literature (French et al., 1953; Kanarek & Marks-Kaufman, 1979; Kanarek & Orthen-Gambill, 1982). Adult rats fed a cafeteria diet, however, showed levels of weight gain that appeared lower than what is typically found following exposure to a cafeteria diet. For example, Johnson and Kenny (2010) found that adult rats gained around 175 g (75% more than standard chow controls), using a similar diet type and slightly shorter diet duration; whereas, in the current study, rats exposed to a cafeteria diet during adulthood gained, on average, only 65.85 g (40% more than controls) across the diet exposure period. One potential factor that likely contributed to the differences between the weight gain in adult rats in the current study versus Johnson and Kenny's study is the level of caloric consumption. Johnson and Kenny

found that rats consumed around 200 kcal daily, whereas in the current study rats consumed only around 100 kcal.

There were a number of procedural differences between the current study and Johnson and Kenny (2010) that may explain why there were differences in weight gain. For example, there were slight differences in cafeteria diet between studies that may have led to differences in consumption. Johnson and Kenny included bacon and pound cake, whereas the current study did not. In the current study, rats tended to prefer cheesecake, frosting, and sausage more than other foods; whereas, M&M's®, Twix®, and potato chips were associated with a fairly low rate of consumption. It is possible that by excluding these foods and replacing them with other HF, HS alternatives it might increase rates of consumption and overall caloric intake. Another noteworthy difference between the current study and Johnson and Kenny is that the, such that the current study used Spague Dawley rats and Johnson and Kenny used Wistar rats. It is possible that some strains of rats are more susceptible to DIO than others. More research is needed to determine what factors may influence differences in weight gain with a cafeteria diet across studies.

Despite lower diet-induced weight gain in rats in the present study, three pieces of evidence suggest that adult rats fed a cafeteria diet in the current study showed diet-induced neural changes. First, adult rats fed a cafeteria diet showed higher behavioral sensitivity to haloperidol relative to adult rats fed a standard diet. In addition, they showed similar behavioral sensitivity to haloperidol as the adolescent rats, which is noteworthy because adolescent rats showed expected levels of weight gain. Second, Johnson and Kenny found that, regardless of whether a rat was characterized as low or high weight in their respective dietary conditions (e.g. control rats versus rats given 18 –

23 hours of access to the cafeteria diet), they showed a similar level of reduction in D_2 receptor expression. Third, Colantuoni et al (2001) found reduced D_2 binding in the striatum and NAc in rats given access to a glucose solution relative to control animals – an effect observed prior to the development of obesity. As such, it seems reasonable to suspect that, despite the low levels of weight gain in adult rats fed a cafeteria diet, alterations in D_2 thought to underlie obesity likely occurred. More research in this area is needed.

Conclusion.

The current findings offer evidence that prolonged exposure to a cafeteria diet leads to subtle changes in behaviors that are unmasked by dopaminergic compounds, especially those that act on the D_2 receptor subtype. The findings also show that at least two specific behavioral mechanisms – sensitivity to delay and sensitivity to amount – drove the diet-induced changes in delay discounting. The study also supports diet-induced alterations in DA D_2 by extending neural endpoints involved in reward processes (e.g., Geiger et al, 2009; Johnson and Kenny, 2010; Val-Lillet et al, 2011; Baladi, et al., 2012; Vucetic et al., 2012; Boomhower and Rasmussen, 2014; Robertson et al., 2017) to more complex behavioral mechanisms such as food impulsivity. Future research should seek to characterize environmental factors that influence sensitivity to haloperidol following DIO, with a focus on disentangling the relative roles of sensitivity to delay and sensitivity to amount and to identify specific neural mechanisms that underlie these alterations.

Chapter 2: Comprehensive Literature Review

Developmental Effects of a High-Fat, High-Sugar Diet on Delay Discounting for Food

Since the early 1970's, rates of childhood obesity have steadily increased, such that 17% of children and adolescents are now considered obese (Anderson & Butcher, 2006; Ogden, Carroll, Kit, & Flegal, 2014). Childhood obesity is associated with a number of physical and psychological health conditions. It is estimated that 65% of obese children and adolescents report at least one physical disorder associated with obesity (e.g. hyperinsulemia, dyslipidemia) and report a lower quality of life compared to their normal-weight counterparts across physical, psychosocial, emotional, social, and school domains (Schwimmer, Burwinkle, & Varni, 2003).

Childhood obesity is a significant public health concern because it predicts obesity in adulthood (Biro & Wein, 2010; Guo & Chumlea, 1999). Adult obesity is associated with many chronic diseases that lead to shortened lifespans, such as type-2 diabetes mellitus, gallbladder disease, coronary heart disease, high levels of blood cholesterol, high blood pressure and osteoarthritis (Must, Spadano, Coakley, Field, Colditz, & Dietz, 1999). Given that obesity is a preventable condition that is a risk factor for many chronic, life-threatening diseases, it is important to understand factors that lead to and maintain obesity.

A number of studies link childhood obesity to chronic consumption of food that is high in fat and sugar (St-Onge, Keller, & Heymsfield, 2003). Since 1977, the composition of children's and adolescents' diets have shifted to include more high-sugar (HS) and high-fat (HF) foods and sugary beverages, such that from 1977 to 1996, fast food, snack, and pizza consumption increased by 32% and sugar-sweetened soft drink

consumption increased by 70% (Nielsen, Siega-Riz, & Popkin, 2002). In 1996, snack and pizza consumption accounted for 7.8% of daily energy intake and sugar-sweetened beverage consumption accounted for 8.6% of daily energy intake (Nielsen, Siega-Riz, & Popkin, 2002). Taken together, the shifts in diet have resulted in a mean increase of 118 kilocalories (kcal) per day in 1996 compared to 1977. More recently, one study estimated that children and adolescents who ate fast food consumed an average of 187 additional kcal per day, 9 more grams of fat per day, and 28 grams of sugar per day than children and adolescents who did not consume fast food (Bowman, Gortmaker, Ebbling, Pereira, & Ludwig, 2004). While these numbers may seem small, consider that these daily increases would allow an individual to gain 12 – 19.5 pounds per year.

Not surprisingly, this shift in exposure to food that is high in fat and sugar is associated with obesity. Nicklas and colleagues (2003) used a 24-hr diet recall method to assess the association between consumption of certain foods and overweight status in children. They found that overweight status in children was associated with snacking, drinking sugary beverages, and consumption of salty snacks, candy, desserts, and fats/oils. Dong and colleagues (2015) investigated weight gain in children and adolescents across a three-year period and found that excess weight gain was significantly associated with higher consumption of fat and sugar. In addition, healthy-weight children reported consuming fewer calories from these food items and as the number of calories consumed from these items increased, so did the likelihood of obesity

Slinging and Popkin (2013) reported that from 1994 to 2010, children and adolescents in the United States reported a modest decrease in fat and sugar intake. Specifically, in 1994, 39% of daily kcal consumption was from fat and sugar and in 2010

fat and sugar intake accounted for 33% of daily kcal consumption. Although these data suggest that at the national level, patterns of consumption of sugars and fats are generally decreasing, fat and sugar intake still exceeds daily recommended values by 18 to 28% (Poti, Slinging, & Popkin, 2013; Slinging & Popkin, 2013). As such, excessive intake of fat and sugar continues to be a problem in the United States (Poti, Slinging, & Popkin; Slinging & Popkin) and is associated with obesity in children (Dong, Bilger, van Dam, and Finkelstein, 2015; Nicklas, Yang, Baranowski, Zakeri, & Berenson, 2003).

Diet-induced obesity

While findings from studies with humans highlight an association between increases in childhood obesity and HF and HS diets, they are correlational and are therefore unable to establish causation. To establish causation, experiments in which diet is carefully controlled are necessary. Diet-induced obesity (DIO) is a laboratory procedure in which animals (typically rodents) are exposed to a HF and/or HS diet for an extended period of time. Control animals are fed *ad libitum* standard rat chow. Generally, there are three basic diets that are used to study DIO. First, using the cafeteria diet, animals are exposed to a variety of foods typically prepared for human consumption that are high in fat, such as bacon, sausage, cheesecake, frosting (Johnson & Kenny, 2010), as well as refined carbohydrates, such as, chocolate, potato chips, chocolate chip cookies, and cheese crackers (Rolls, Rowe & Turner, 1980). In the second method, researchers provide chronic access to a HF diet that is formulated specifically for lab use (Boomhower & Rasmussen, 2014; Woods, Seeley, Rushing, D'Alessio, & Tso, 2003; Vucetic, Carlin, Totoki, & Reyes, 2012). According to the third method, researchers provide chronic access to standard chow, water, and a sugar-water solution (Kanarek, & Orthen-Gambill,

1982; Lindvist, Baelemans, & Erlanson-Albertsson, 2008). Although the first method has high ecological validity given that it closely models typical human environments, the second and third methods allows one to more precisely control levels of fat or sugar intake, respectively, and to isolate the specific effects of a HF diet or HS diet rather than the combined effects of high levels of fat and refined carbohydrates.

As an example of DIO, Johnson and Kenny (2010) exposed rats to a standard chow or cafeteria diet. Researchers exposed rats to no (0 h), restricted (1 h), or extended (18 – 23 h) access to a cafeteria diet every day for 40 days consecutively, in addition to continuous access to standard chow. By the end of the 40-day exposure period, rats in the extended cafeteria diet condition gained twice as much weight on average relative to the rats in the restricted and control conditions. This is likely due to the finding that rats in the extended cafeteria diet condition consumed approximately double the number of total calories on average compared to the rats fed a restricted and control diet. In addition, rats in the restricted diet group consumed around five times the kilocalories (kcal) from fat compared to the control group, whereas the extended diet group consumed around 10 – 15x the kcal from fat compared to the control group. As such, rats exposed to extended, daily access to a HF, HS diet consume more calories and gain more weight than control rats.

Regardless of the type of diet used for DIO research, the behavioral and physiological outcomes tend to be similar and well-established. One consistent behavioral change is hyperphagia (heightened caloric consumption) in free-feeding environments (Johnson & Kenny, 2010; Kanarek & Orthen-Gambill, 1982; Rolls, Rowe, & Turner, 1980). Hyperphagia is accompanied by physiological and biochemical changes that include an

increase in body fat (Johnson & Kenny; Kanarek, & Orthen-Gambill; Rolls, Rowe, & Turner; Vucetic, Carlin, Totoki, & Reyes, 2012; Woods, Seeley, Rushing, D'Alessio, & Tso, 2003), elevated levels of glucose (Kanarek, & Orthen-Gambill; Rolls, Rowe, & Turner), elevated levels of insulin (Rolls, Rowe, & Turner), and elevated levels of leptin (Lindqvist, Baelemans, & Erlanson-Albertsson; Woods, Seeley, Rushing, D'Alessio, & Tso).

Neural alterations also result from diets high in sugar and fat. In particular, DIO results in decreased levels of dopamine (DA) in areas of the brain associated with the reward (Colantuoni et al., 2001; Geiger, Haburcak, Avena, Moyer, Hoebel, & Pathos, 2009; Johnson & Kenny, 2010; Vucetic, Carlin, Totoki & Reyes, 2012). Val-Lillet and colleagues (2011) investigated differences in brain activity between minipigs fed a standard diet or a HF diet for 10 weeks. At the end of the diet exposure, researchers used a single-photon emission computed tomography imaging technique to determine activity in the brain of each pig and found decreased activity in prefrontal cortex (PFC), ventral tegmental area (VTA), nucleus acumens (NAc), and nucleus pontis of obese compared to lean pigs. Of particular relevance, the VTA and NAc are neural structures implicated in reward function. As such, diet-induced alterations in DA occur in neural structures associated with reward.

Johnson and Kenny (2010) investigated the effects of prolonged HF diet on D_2 receptor density in the striatum, which is one primary neural structure implicated in reward and decision making (Balleine, Delagado, & Hikosaka, 2007). After 40 days of no, restricted, or extended access to a cafeteria diet, researchers found that D_2 receptor density in the striatum decreased; this was interpreted as a blunting of reward sensitivity.

In other words, extended exposure to a cafeteria diet was associated with a blunting of reward process.

Geiger and colleagues (2009) also reported neurochemical alterations related to reward in the NAc and that resulted from chronic exposure to a cafeteria diet. First, the researchers surgically implanted a cannula into the posterior shell of the NAc, which allowed them to sample concentrations of DA using a microdialysis probe. They found that animals exposed to the cafeteria diet had lower levels of DA in the NAc shell. Next, they allowed animals to eat either the cafeteria diet or standard chow and assessed DA activity as a function of dietary history. They found that animals exposed to the cafeteria diet showed an increase in DA activity in the NAc shell when given the cafeteria diet but not standard chow. This implies that continued access to the cafeteria diet is necessary to produce typical levels of DA signaling, which also support that high-fat diet may blunt reward processes.

A study by Colantuoni and colleagues (2001) also supports the notion that diets high in sugar alter DA, especially at the level of the D_2 receptor. Researchers gave rats 12 h continuous access to liquid glucose or water for 30 days. Using quantitative audioradiography, researchers quantified DA D_2 binding in the striatum and NAc. They found that, DA D_2 binding in the striatum and NAc decreased in animals given access to a glucose solution relative to control animals. Interestingly, these effects were apparent in the absence of any between-group differences in weight. This finding suggests that alterations in areas that underlie reward may *precede* the development of obesity. Thus, diet-induced neural alterations in D_2 may interfere with sensitivity to reinforcement and promote obesogenic patterns of eating behavior before weight gain is apparent. As such,

studies using a variety of different methods (i.e. imaging techniques, ex vivo measurement of DA receptors density, in vivo measurement of DA, quantitative audioradiography) have demonstrated that changes in D_2 occur after chronic exposure to a HF, HS diet.

Differential gene expression has been suggested as one mechanism that underlies diet-induced D_2 alterations. Vucetic, Carlin, Totoki, and Reyes (2012) demonstrated that genetic changes in DA-related genes occur as a function of prolonged exposure to a HF diet. After 17 weeks of *ad libitum* access to either the HF or standard diet, the researchers harvested the brains of the mice and assessed D_2-related gene expression in the hypothalamus, VTA, PFC and NAc using real-time PCR. D_2 receptor gene expression was down-regulated in the VTA, which would result in lower number of D_2 receptors in the VTA, an area associated with reward function.

Taken together, these studies demonstrate that extended exposure to a HF, HS, or cafeteria diet changes the D_2 receptor system, especially in areas of the brain involved with reward. One question that remains is the extent to which diet-induced changes in the neural areas implicated in reward actually leads to changes in sensitivity to reinforcement. Johnson and Kenny (2010) attempted to answer this question by demonstrating that changes in reward signaling that result from DIO lead to behavioral changes in *sensitivity to reinforcement*. In this study, the researchers used intracranial brain stimulation (ICBS), which is an experimental procedure that stimulates the release of DA via an electrical current applied directly to areas of the brain implicated in reward and is a type of reinforcer that rats readily self-administer (Owesson-White, Cheer, Beyene, Carelli, & Wightman, 2008). After determining baseline reward thresholds,

researchers exposed animals to their respective diets (i.e., control, restricted or extended cafeteria diet). After 40 days, the cafeteria diet was removed and rats had free access to standard chow only. The researchers noted that it took at least two weeks for the reward thresholds to return to baseline in rats with extended (i.e. 18 – 20 h) access to cafeteria diet. Thus, chronic exposure to a cafeteria diet results in reduced DA signaling, which interfered with sensitivity to reinforcement but abstaining from the cafeteria diet results in a return to typical levels of DA signaling and a typical level of sensitivity to reinforcement.

These studies demonstrate that DIO results in a variety of physiological changes. Of particular relevance are changes in neural function underlying reward. Taken together, these findings demonstrate that chronic exposure to a HF and/or HS diet leads to reductions in DA D_2 that result in diminished sensitivity to reinforcement. Changes in DA D_2 may contribute to obesity by interfering with typical neural reward processes and promoting continued intake of food that is high in fat and sugar. Although previous studies provide some evidence that DIO results in excessive food intake (Johnson & Kenny, 2010; Kanarek & Orthen-Gambill, 1982; Rolls, Rowe, & Turner, 1980), these studies were conducted in a free-feeding environment, which may overestimate food consumption because large quantities of food can be accessed immediately at a low response cost (Rasmussen, Robertson, Rodriguez, 2016). Indeed, it has been argued that to appropriately model obesity-related food consumption, two aspects of food availability need to be considered: effort involved in food procurement and delay to receipt of food (Rasmussen, Robertson, Rodriguez). Behavioral economic procedures offer researchers powerful tools that model these aspects of typical human food environments and can be

used to characterize behavioral outcomes that might result as a function of chronic exposure to a cafeteria-style diet. One such procedure, which considers sensitivity to delay to the receipt of food, is delay discounting.

Delay Discounting.

Delay discounting allows researchers to vary the accessibility of food by manipulating delay to receipt of food. According to a delay discounting procedure, researchers present subjects with a series of choices between a smaller reinforcer delivered immediately vs. a larger reinforcer delivered after a delay. This allows researchers to characterize the extent to which the subject shows a preference for the smaller, immediate outcome (i.e. impulsive choice pattern) or a preference for the larger, delayed outcome (i.e. self-controlled choice pattern). As such, delay discounting procedures allow researchers to investigate the extent to which delay influences food consumption.

Delay discounting is also considered a process in which the value of a reinforcer decreases as a function of the delay to receipt of a reward (Madden & Johnson, 2010). It is a phenomenon that has been documented across species (i.e. humans, pigeons, and rats; Vandervelt, Oliveria, & Green, 2016) and is thought to underlie problematic health-related behaviors, such that individuals who engage in behaviors that compromise physical health tend to discount delayed outcomes more steeply. Steeper delay discounting has been observed in cigarette smokers relative to non- and ex-smokers (Bickel, Odum, & Madden, 1999), individuals who are cocaine dependent relative to controls (Heil, Johnson, Higgins, & Bickel, 2006), individuals who are addicted to heroin relative to controls (Kirby, Petry, & Bickel, 1999), and obese individuals relative to

healthy weight individuals (Fields, Sabet, Peal, & Reynolds, 2011; Hendrickson & Rasmussen, 2013; Jarmolowicz, Cherry, Reed, Bruce, Crespi, Lusk, & Bruce, 2014; Rasmussen, Lawyer, & Reilly, 2010; Weller, Cook III, Avsar, & Cox, 2008). Given that excessive delay discounting is associated with a wide-range of health-related conditions, it is considered a trans-disease process (Bickel, Jarmolowicz, Mueller, Koffarnus, & Gatchalian, 2012; Bickel & Muller, 2009).

As mentioned, delay discounting procedures involve an individual making a series of choices between a small, immediate reward versus a relatively larger reward that is delayed. As the delay to the receipt of the larger reinforcer increases, the larger, delayed reinforcer becomes less valuable relative to the smaller, immediate reinforcer. Delay discounting procedures are designed to identify the point at which the smaller, sooner value and the larger, later value are chosen equally often, which is referred to as an indifference point. Indifference points are plotted as a function of delay. The hyperbolic equation describes the rate of discounting, in which the value (indifference point) of the larger, later reward decreases in a hyperbolic manner (see Figure 1).

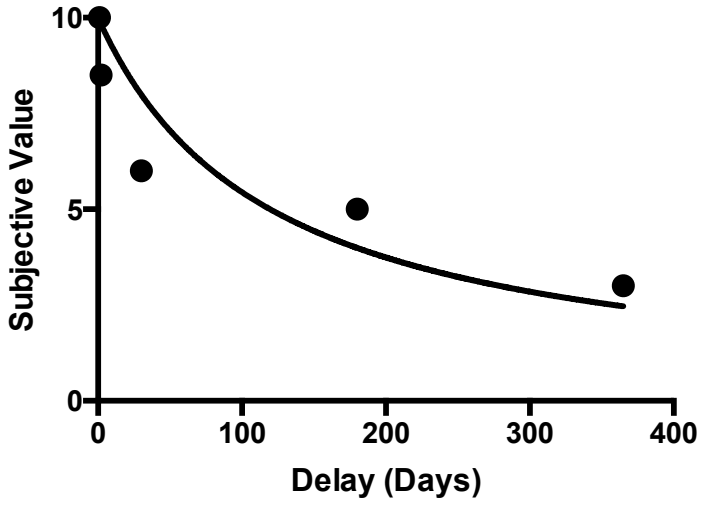


***Figure 16*. Hypothetical Delay Discounting Curve that shows subjective value decreases as a function of delay.**

The hyperbolic model, which characterizes discounting is:

$V = A/(1 + kD)$

(1)

where V is the subjective value of the larger, later outcome, A is the amount of the larger, later outcome, D is the delay to receipt of the larger, later outcome, and k is a free parameter that represents the rate of discounting (Madden & Johnson, 2010; Mazur, 1984, 1986). Larger k values indicate higher rates of discounting. Another method of quantifying discounting is to use area under the curve (AUC; Myerson, Green, & Warusawitharana, 2001). According to this method, the area of the graph under the indifference points is divided into trapezoids and the area of each trapezoid is quantified and summed to a value between 0 and 1. Larger AUC values indicate lower rates of discounting. Another accepted measure of delay discounting, usually done with nonhuman animals, is to quantify the percent choice of larger, later outcomes at each delay (Boomhower & Rasmussen, 2014; Huskinson, Krebs, & Anderson, 2012). According to this method, higher percent larger, later choice indicates lower levels of delay discounting.

Delay Discounting and Obesity.

Studies using human participants have demonstrated that obesity is associated with steeper delay discounting for hypothetical money relative to healthy-weight individuals. Weller, Cook, Avsar, and Cox (2008) investigated delay discounting in

obese and healthy-weight women. Using a delay discounting task for hypothetical monetary outcomes, researchers found that obese women (BMI> 30) showed a significantly steeper rate of discounting than healthy-weight women. Jarmolowicz et al. (2014) found that overweight and obese individuals discounted delayed monetary outcomes significantly steeper than underweight and normal weight individuals. As such, some findings support the notion that obese individuals show steeper rates of delay discounting for money. This is important in that it shows a general tendency for steeper delay discounting in obese individuals; however, because obesity is thought to result from an overconsumption of food, characterizing food-related impulsivity in obese individuals may allow researchers to better understand behavioral patterns associated with the specific reinforcer that contributes to the development and maintenance of obesity.

In order to characterize the association between food-related impulsivity and obesity, researchers have developed a delay discounting task for food-related outcomes (Rasmussen, Lawyer, & Reilly, 2010). According to the delay discounting task for food, participants are presented with a 5/8" cube that represents one standardized bite of food and make choices for hypothetical smaller, sooner bites of food versus larger, later bites of food. Rasmussen, Lawyer, and Reilly (2010) found a domain-specific association such that individuals with a high percent body fat discounted delayed food outcomes, but not monetary outcomes, much steeper than individuals with a low percent body fat. Hendrickson and Rasmussen (2013) and Hendrickson, Rasmussen, and Lawyer (2015), using the Food Choice Questionnaire, a paper-and-pencil delay discounting task,

replicated the finding that higher percent body fat predicted steeper discounting of delayed food items.

The findings from the Weller et al. (2008), Jarmolowicz et al. (2014), Rasmussen et al. (2010), and Hendrickson et al. (2013, 2015) studies are descriptive in that they allow researchers to make predictions about general patterns of behavior that are associated with obesity. They do not, however, necessarily demonstrate that higher rates of delay discounting are related to higher rates of food consumption. Appelhans et al. (2011) demonstrated that discounting rates interact with food reward sensitivity to predict food intake. Researchers assessed delay discounting using a computerized task that used $100 as the larger, later outcome and quantified delay discounting using AUC. Food reward sensitivity was assessed using a self-report measure (Power of Food Scale). Researchers measured food intake by offering participants access to a variety of palatable and unpalatable foods under the guise of a taste test. They found that women who showed high rates of discounting and high levels of food reward sensitivity showed the highest intake of palatable foods. Although the findings of this study would be more convincing if a healthy-weight control group was included, it nicely demonstrates higher rates of discounting interact with food reward sensitivity to predict actual caloric intake.

To date, researchers have demonstrated that obese individuals show steeper rates of delay discounting. In studies that have only investigated delay discounting using monetary outcomes, obese individuals show steeper rates of delay discounting than lean individuals (Weller et al.; 2008, Jarmolowicz et al., 2014). In studies that have investigated delay discounting for both food and money, individuals with a high percent body fat show a domain-specific effect, such that steeper rates of delay discounting were

documented for individuals with a higher percent body fat for food but not money (Hendrickson & Rasmussen, 2013; Hendrickson, Rasmussen, & Lawyer, 2015; Rasmussen, Lawyer, & Reilly, 2010). Further, research has also demonstrated that rates of delay discounting and food reward sensitivity can be used to predict actual food intake (Appelhans, Woolf, Pagoto, Schneider, Whited, & Liebman, 2011). Thus, although the delay discounting tasks use hypothetical outcomes, the choice patterns seem to have utility in predicting obesogenic patterns of eating. Thus far, the studies summarized have only considered delay discounting in lean and obese adults; however, research has demonstrated that children and adolescents show steeper delay discounting than adults, which is a factor that may contribute to the development of obesity in childhood.

Developmental Differences in Delay Discounting.

Children discount delayed monetary outcomes more so than adults (Green, Fry, & Myerson, 1994; Hendrickson & Rasmussen, 2016). Using larger later outcomes of $1000 and $10,000, Green, Fry, and Myerson (1994) showed that children (*M* = 12.1 years old) discounted delayed monetary outcomes much steeper than young adults (*M* = 20.3 years old) and older adults (*M* = 67.9 years old). Fields, Sabet, Peal, & Reynolds tested delay discounting for money in adolescent smokers (*M* = 17.19 years old). They found that obese adolescents showed much steeper discounting relative to health-weight adolescents. Hendrickson and Rasmussen (2016) investigated differences in delay discounting for food and money between adolescents and adults. They found that adolescents (*M* = 13.13 years old) discounted money significantly more than adults (M = 23.33 years old); however, there were no developmental differences in food discounting.

Thus, children and adolescents show steeper rates of delay discounting for money, but not food, compared to adults.

One issue that Hendrickson and Rasmussen (2016) broached in their study regarding the lack of developmental difference in food discounting is noteworthy: They did not use a measure of puberty in their study. Puberty is a time when childhood eating patterns start to become more adult-like, in terms of sheer amount. Moreover, preferences for different types of food widen (Nicklaus, Boggio, Chabanet, & Issanchou, 2004). Thus, it is possible that puberty may make food discounting more adult-like. Comparing food discounting in pre-pubescent children and adults may result in stronger differences in food discounting. More research is needed in this area.

Nonetheless, it is established that obesity in childhood predicts obesity in adulthood (Biro & Wein, 2010; Guo & Chumlea, 1999) and that sensitivity to delay for food may be involved. In other words, it is possible that impulsive food consumption patterns in childhood may continue across development. Indeed, Schlam, Wilson, Shoda, Mischel, and Ayduk (2013) demonstrated that the result of a delay to gratification task (using "the marshmallow task") during preschool predicted body mass index (BMI) 30 years later. Researchers instructed preschoolers that they could consume one edible whenever they wished, but the session would be over; however, if the child waited 15 or 20 minutes (i.e. delayed gratification), the researcher would give the child two of the edibles. Researchers found that children who did not delay gratification for food tended to have a higher BMI 30 years later than children who delayed gratification. Overweight children show higher patterns of delay discounting relative to their lean counter parts and these impulsive food choice patterns during childhood may continue throughout the

lifespan and lead to obesity as an adult. Thus, food-related impulsivity appears to be one possible behavioral mechanism of childhood obesity.

Generally, studies have demonstrated developmental differences in impulsivity and have documented that obesity is associated with higher rates of delay discounting in adolescent and adult populations; however, studies examining the relation between obesity and delay discounting in humans are limited in that they do not allow researchers to control dietary factors that may contribute to excessive discounting. Animal models of delay discounting allow researchers to investigate dietary, genetic, and neurochemical contributors to excessive delay discounting using animal models of obesity under highly-controlled conditions.

Animal Models of Delay Discounting

For rodents, delay discounting is assessed using an operant chamber in which responding on one lever results in the delivery of a single food pellet immediately and responding on the other lever results in the delivery of multiple food pellets after a delay (Madden & Johnson, 2010). It is customary to separate the experimental session into forced choice and free choice trials. During the forced choice trials, only one lever is active (i.e. either the lever associated with the smaller, sooner outcome or the lever associated with the larger, later outcome) and animals must make a response on this lever before progressing. This aspect of a delay discounting procedure allows each animal to be exposed to the contingencies associated with both the smaller, sooner option and the larger, later option. After exposure to the forced choice trials, animals complete a series of free choice trials in which both levers are active and the rat can select either lever (i.e. smaller, sooner outcome vs. larger, later). Generally, there are three types of delay

discounting procedures used with rodents: an adjusting amount procedure, an adjusting delay procedure, and the Evenden and Ryan (1996) procedure (Madden & Johnson, 2010). In an adjusting delay procedure, animals make a choice between a smaller, immediate reinforcer and a larger, delayed reinforcer and, based on the animal's responses, the delays are titrated up and down until an indifference point is reached (Madden & Johnson, 2010; Mazur, 1986). That is, if the animal chose the larger, delayed option during a block of trials, the delay for that same option would be increased on the next block of trials. If the animal chose the immediate option during a block of trials, the delay for the larger, later option would be shortened on the next block of trials. If the animal chose both standard and delayed options equally often (i.e. demonstrated indifference) during a block of trials, the delay did not change in then next block of trials. The adjusting amount procedure is similar to the adjusting delay procedure, except the amount of reinforcement (i.e. number of food pellets delivered) associated with the smaller, sooner lever is adjusted (instead of the delay to the larger, later) until an indifference point is reached (Madden & Johnson, 2010; Richards, Mitchell, De Wit, & Seiden, 1997).

The Evenden and Ryan (1996) procedure is similar to the other procedures in that animals respond between a smaller, sooner vs. larger, later option; however, according to this procedure, delays are systematically increased within each session independent of the animal's choices. The first block of trials starts with both the larger and smaller reinforcer options programmed at a 0-s delay and the delays are progressively increased across the session. For instance, in a procedure consisting of five blocks of trials, the delays may progress such that the first block is associated with a delay of 0-s, the second

block is a delay of 1-s, the third block is a delay of 2-s, the fourth block is a delay of 4-s, and the final block is a delay of 8-s. Across sessions, these delays will be increased until animals show indifference (50% preference for each option within a given block of delays) between the smaller, sooner outcome and the larger, delayed outcome. For instance, the terminal delay sequence may consist of blocks of 0-s, 5-s, 10-s, 20-s, and 40-s delays. This delay discounting procedure is favored by behavioral pharmacologists, because once baseline preference is stable, it allows researchers to test drug effects across a range of delays within a single session (Madden & Johnson, 2010).

Delay discounting has been used to characterize impulsive choice patterns in two rodent models of obesity: the obese Zucker rat, which is a genetic, single-trait rodent model of obesity, and the DIO model, which has been introduced previously. Boomhower, Rasmussen, and Doherty (2013) investigated delay discounting in lean and obese Zucker rats using an adjusting delay procedure, in which the standard delay associated with the smaller, sooner reinforcer (i.e., one sucrose pellet) was set at either one second or five seconds and the delay associated with the larger, later reinforcer (i.e., two or three sucrose pellets, depending on the condition) was titrated based on the animals' responses. For the two-pellet condition, obese rats had a significantly shorter adjusting delay for the 1-s standard delay condition than lean rats; however, both groups of rats had a similar adjusting delay for the 5-s condition. For the three-pellet condition, obese rats had a significantly shorter adjusting delay for both 1-s and 5-s standard delay conditions. Given that the obese rats had shorter adjusting delays than lean rats, this indicates a higher rate of discounting in obese Zucker rats compared to lean Zucker rats.

Boomhower and Rasmussen (2014) investigated the effects of a controlled HF diet on delay discounting in standard lab rats using a modified Evenden and Ryan (1996) procedure using sucrose (i.e. preferred) and carrot (i.e. non-preferred) pellets. No between-group differences in discounting were evident for either pellet condition. However, rats exposed to the high fat diet exhibited a higher sensitivity to acute injections of haloperidol, a D_2 antagonist, than rats fed standard chow. This sensitivity was evident in that DIO rats exhibited a more pronounced decrease than controls in the percent choice of the larger, later option at the highest dose of haloperidol (i.e. 0.1 mg/kg), which indicates differences in underlying DA activity, specifically at the D_2 receptor.

Studies with humans have shown that delay discounting is associated with obesity in that obese adults and adolescents show impulsive choice patterns for food (Hendrickson & Rasmussen, 2013; Hendrickson, Rasmussen, & Lawyer, 2015; Rasmussen, Lawyer, & Reilly, 2010); this finding also has been supported using a genetic animal model of obesity (i.e. the obese Zucker rat; Boomhower, Rasmussen, & Doherty, 2013). Therefore, *sensitivity to delayed food outcomes* may be one mechanism involved in obesity. Rats exposed to a HF diet also showed different patterns of discounting compared to control rats following a 0.1 mg/kg injection of haloperidol, which indicates that changes in DA activity may be one factor that influences delay discounting. Indeed, it has been suggested that delay discounting is influenced by the interaction between DA-rich areas related to reward and prefrontal areas that are thought to underlie self-control (Bickel, Jarmolowicz, Mueller, & Gatchalian, 2011; Bickel, Johnson, Koffarnus, MacKillop, & Murphy, 2014; Volkow Wang, Fowler, & Telang, 2008).

Neural Basis of Discounting

At the neural level, researchers have suggested that delay discounting is influenced by competing neural systems: one system involved in self-control (a preference for larger, delayed outcomes over smaller, immediate outcomes) and another system involved in impulsivity (a preference for smaller, immediate outcomes over larger, delayed outcomes; Bickel, Jarmolowicz, Mueller, & Gatchalian, 2011; Bickel, Johnson, Koffarnus, MacKillop, & Murphy, 2014). Generally, the primary area implicated in the "self-control" system is the prefrontal cortex (PFC) and the primary areas implicated in the "impulsive" system are the limbic and paralimbic systems (Bickel, Jarmolowicz, Mueller, & Gatchalian, 2011). According to the competing systems hypothesis, both of these systems influence aspects of choice. Excessive delay discounting may be the result of the hyperactive "impulsive" system overriding the "self-control" system, resulting in a tendency towards impulsive behavior.

Competing neural systems may play a role in obesity (Carr, Daniel, Lin, & Epstein, 2011). For instance, research has shown that areas implicated in self-control are hypofunctioning in obese individuals relative to lean individuals. Le et al. (2006) recruited lean and obese male participants. After participants fasted for 36 h, researchers obtained four PET scans (i.e. two baseline, two after administration of a liquid meal) from each participant. Researchers measured activity in the dorsolateral prefrontal cortex (DLPFC) in response to a liquid meal. Obese men showed less activation in the DLPFC after administration of the liquid meal relative to lean men. Researchers suggested that the lower activity observed in the DLPFC might indicate that neural mechanisms related to self-control are exerting less control on eating behavior in obese men relative to lean

men. Le et al. (2007) replicated and extended the findings from Le et al. (2006). In this study, the researchers measured activation in the DLPFC in response to a liquid meal in lean, obese, and formerly obese women. As in Le et al. (2006), participants fasted for 36 h prior to brain scans. Researchers then obtained two PET scans prior to administration of a liquid meal and two PET scans post-meal. They found differences in activation in the left DLPFC, such that lean and formerly obese women showed significantly more activation in this area than obese women. Two primary conclusions can be drawn from these results. First, given that obese women showed lower activation in the left DLPFC than the other groups, this area may play a role in inhibiting continued food consumption after satiation. Second, similar levels of activation in the left DLPFC were documented in lean women, as well as women who used to be obese but lost the weight, which suggests that changes in activity in neural areas that influence self-control might underlie healthier eating patterns. Generally, for both obese men and women, neural areas associated with self-control were less active following a meal, which may result in a tendency to overeat.

Kishnevsky and colleagues (2011) and Stoeckel and colleagues (2013) investigated patterns of neural activation in areas associated with self-control during a delay discounting task in obese women. Specifically, they examined activation in the lateral prefrontal cortex, inferior frontal gyrus (IFG), middle frontal gyrus (MFG), superior frontal gyrus (SFG), inferior parietal lobule (IPL), superior parietal lobule (SPL), and medial prefrontal cortex. Researchers used fMRI brain scans to create contrasts between neural activity during hard vs. easy trials. A hard trial was one in which the discrepancy between the smaller, sooner vs. larger, later options was small (i.e. $14 now

or $15 in a week), whereas an easy trial was one in which the discrepancy between the smaller, sooner vs. the larger, later option was large (i.e. $14 now or $50 in a week). Generally, they found that difficult choices resulted in greater activation in IFG, MFG, and medial prefrontal cortex, areas that might play a role in self-control. Interestingly, women who showed lower activation in the IFG, MFG, SFG, and IPL showed greater weight gain at a 1 – 3 year follow up. Thus, consistent with the competing neural systems hypothesis, lower activity in neural areas related to self-control predicted weight gain. In addition, participants who had higher impulsivity (*k*) values showed lower activation in areas of the MFG and right IPL. While this study has limitations (e.g. lack of control group, small sample size, inclusion only of women, use of money discounting task rather than food discounting task), these results support the idea that reduced activity in neural areas that underlie self-control are related to weight gain.

Taken together, Le et al. (2006, 2007), Kishnevsky et al. (2011), and Stoeckel et al. (2013) supports the notion that the PFC is hypofunctioning in obese individuals, areas of the PFC are involved in delay discounting, and hypofunction in these areas is predictive of weight gain. It is worth noting, however, that using methods that followed those of Le et al. (2006, 2007), Gautier et al. (2000) found greater activation in the PFC and lower activation in the limbic and paralimbic regions in obese men compared to lean men in response to a liquid meal, a finding that is clearly in opposition to the studies cited above. Future research is needed to characterize this discrepancy and clarify the role of PFC function in the development and maintenance of obesity.

Possible D2 receptor involvement with impulsivity. We already have discussed research in animal models that link HF and HS diet to obesity and alterations in D_2-

related reward function in the brain. Human studies also show that obese individuals have fewer D_2 receptors in the striatum compared to lean individuals (Wang et al., 2001; Volkow et al., 2008). In these studies, using PET, researchers injected lean and obese participants with a radioligand for the D_2 receptor in order to assess the relation between BMI and D_2 receptor availability in the striatum, an area that is associated with reward. They found that obese individuals had lower D_2 receptor availability in the striatum than lean individuals.

Obesity is also associated with a hypofunctioning striatum in obese relative to lean individuals in response to delivery of a HF, HS liquid (Stice, Spoor, Bohon, Veldhizen, & Small, 2009; Stice, Spoor, Ng, & Zald, 2009). Stice et al. (2009) administered either a tasteless solution, a HF, HS liquid, or no solution during brain scans in an fMRI scanner. Brain images were contrasts of activation during the delivery of the liquid vs. the delivery of the tasteless solution. Obese individuals showed lower activation of the striatum in response to the liquid food delivery relative to lean individuals. As such, studies have demonstrated that obesity is associated with lowered striatal activity and this difference may be one factor that contributes to an altered reward sensitivity in obese individuals.

The findings that obese individuals have a lower density of D_2 receptors in the striatum (Wang et al., 2001; Volkow et al., 2008), show reduced function in the striatum (Stice, Spoor, Bohon, Veldhizen, & Small, 2009; Stice, Spoor, Ng, & Zald, 2009), and that rats exposed to an extended high-fat, high-sugar diet show a decrease in D_2 receptors in the striatum (Johnson & Kenny, 2010) support the competing systems hypothesis, at least in part, by providing empirical evidence that obesity and consumption of a HF, HS

diet are associated with changes in neural activity in areas associated with reward relative to lean individuals. However, the competing systems hypothesis posits that the strength of the activation of the reward system is *greater* than the self-control system (Bickel, Jarmolowicz, Mueller, & Gatchalian, 2011; Bickel, Johnson, Koffarnus, MacKillop, & Murphy, 2014). Thus, according to the studies cited above, the direction of change (i.e., hypofunction) in function of the reward system is not consistent with predictions made from the competing systems hypothesis (i.e., hyperfunction).

Volkow, Wang, Fowler, and Telang (2008) accounted for the hypofunction-hyperfunction discrepancy by suggesting that the reward system and the self-control system may form a feedback loop. Here, activation in the reward system leads to activation in the self-control system, which should result in cessation of a bout of eating. The interplay between these two systems allows an individual to avoid overconsumption of food. However, when the number of D_2 receptors is reduced, reward may be blunted, which results in insufficient activity in the reward system to activate the self-control system. That is, extended exposure to a cafeteria-style diet leads to changes in sensitivity to reward. This reduced sensitivity then interferes with the initiation of neural areas that influence self-control. The end result is a hypofunctioning reward system, which leads to greater reward (food) seeking, that then leads to an under-functioning self-control system. The specific conditions under which, and the manner in which, the interaction between self-control and reward systems become dysregulated is still unclear and more research is needed to clarify this relation

Childhood and adolescence is a period of human development in which the reward system appears to exert more influence over the self-control system compared to

adults (Galvan, 2010; Geier & Luna, 2009). It is possible that, during this period of development, children and adolescents are likely to favor foods that are high in sugar and fat. Further, it is possible that chronic consumption of HF, HS foods will lead to persistent changes in reward and self-control, which may lead to chronic overeating and obesity.

Possible Developmental Effects of High-Fat, High-Sugar Diet

Researchers have noted that adolescents and adults differ in sensitivity to reward (Galvan, 2010; Geier & Luna, 2009). Although the specific mechanisms that underlie differences in sensitivity to reward during childhood and adolescence vs. adulthood are not fully understood, researchers have suggested that there are different developmental trajectories of neural areas related to self-control (i.e. PFC) and reward (i.e. striatum). Four lines of research are noteworthy. First, in humans, the prefrontal cortex matures around 20 years old (Diamond, 2002), whereas, reward-related areas in the limbic system (i.e. striatum, nucleus accumbens) mature much earlier (Casey, Jones, & Hare, 2008). This may result in reduced influence of the PFC relative to reward areas on behavior, which would result in increased manifestation of impulsivity relative to adults. The studies that show that children display steeper levels of delay discounting compared to adults support this area of research (Green, Fry, & Myerson, 1994; Hendrickson & Rasmussen, 2016).

Second, changes in density of white and grey matter may represent another developmental factor that contributes to differential sensitivity to reward across development. Grey matter density begins to decrease during adolescences and white matter tends to increase during early adulthood in the PFC (Giedd, 1996). White matter

density is thought to allow more influence of the self-control system by allowing complex neural circuits to form, which may allow the PFC to exert more influence during adulthood relative to childhood and adolescence (Tsujimoto, 2008). The idea is that, throughout development, gray matter decreases and white matter increases, which results in a reduction in the number of neurons (i.e. synaptic pruning occurs) but the connections between them are enhanced. That is, greater density of white matter is thought to be associated with greater myelination. Myelin enhances communication between neurons by speeding neural transmission. Of relevance to this review, the increases in white matter should facilitate communication between striatal and prefrontal regions by allowing these distant regions to communicate more efficiently and rapidly. As such, the prefrontal regions would be able to directly interact with the striatal regions more efficiently during adulthood than during development.

Third, DA activity changes across development (Geier & Luna, 2009). Seeman, et al. (1987) studied human post-mortem brains and found that D_2 receptor density in the striatum tends to spike during childhood, show a sharp decline around age 5, and gradually decrease (about 2.2% loss per decade) throughout the lifespan. In addition, tyrosine hydroxylase, an enzyme that limits the rate of production of DA, has been documented to show higher levels in the striatum during childhood relative to other developmental time points (Geier & Luna, 2009).

Finally, in rats, DA innervation of the PFC continues until PND 60, a developmental time period that corresponds to late adolescence in humans (Sengupta, 2013), which may result in relatively more influence being exerted by neural regions associated with self-control. As such, childhood is a developmental time period

characterized by a relatively underdeveloped PFC and a relatively matured reward system with high levels of DA activity, which might be responsible for differences observed between developing and adult humans in reward processes.

Given differences in sensitivity to reward in childhood and adolescence vs. adulthood, developing humans may be especially vulnerable to the onset of a pattern of excessive discounting for food. Developmental changes in structure and biochemical activity in areas related to reward processing and self-control may result in childhood and adolescence being a time period characterized by behaviors that maximize contact with reward (Galvan, 2010). Because HF, HS foods are widely available and easily accessible (Drewnowski & Darmon, 2005) and serve as powerful reinforcers (Epstein, Leddy, Temple, & Faith, 2007), this sort of food might be especially enticing for a developing human to seek and consume. Currently, the short- and long-term consequences of chronic consumption of a HF and HS diet on a developing neural system are not understood. One area of study, behavioral pharmacology, combines operant methods with pharmacological techniques that researchers use to explore changes in behavior and neurotransmission that occur as a result of DIO in intact organisms.

Behavioral Pharmacology and Obesity

Behavioral pharmacology techniques offer a powerful tool to investigate brain-behavior interactions. A number of studies show that high-fat diets can alter sensitivity to drugs that modify DA D_2 activity (Baladi, Daws, & France, 2012), which implicates diet-altered D_2 activity in animals exposed to a cafeteria diet. As such, one way to assess differences in underlying D_2 activity that may occur as a function of dietary exposure is

to test behavior after an injection of a drug that interacts with the D_2 system. One such drug is the D_2 receptor antagonist haloperidol.

Haloperidol has been used to characterize the involvement of D_2 in delay discounting (Koffarnus, Newman, Grundt, Rice, & Woods, 2011). Using a procedure in which rats choose between three sucrose pellets being delivered after 0-s, 10-s, 20-s, 40-s, or 60-s or one pellet delivered immediately, researchers found that 0.1 mg/kg of haloperidol significantly reduced percent of larger, later choices. As such, these studies demonstrate that at a sufficiently large dose (i.e. 0.1 mg/kg), standard laboratory rats tend to exhibit more impulsive behavior compared to vehicle. In an earlier study, however, Evenden and Ryan (1996) tested the effects of haloperidol on percent choice for larger, later (i.e. three pellets) versus smaller, sooner (i.e. one pellet) reinforcers; low doses of haloperidol (0.01 and 0.03 mg/kg) did not alter the percent choice of larger, later reinforcers. As such, these studies demonstrate that there is likely a dose-response relation with haloperidol's effects on delay discounting.

To date, one study has examined the differential effects of haloperidol on delay discounting using the same dose range as the aforementioned studies, though animals were also exposed to a controlled high-fat diet vs. a standard diet. Using a modified Evenden and Ryan (1996) procedure, Boomhower and Rasmussen (2013) reported haloperidol dose-dependently reduced preference for smaller, sooner outcomes (vs. larger, later outcomes) in rats fed a high-fat or standard diet. That is, a 0.01 mg/kg and a 0.03 mg/kg dos of haloperidol did not influence delay discounting in rats fed a high-fat diet or controls; however, a 0.1 mg/kg dose of haloperidol resulted in a greater preference shift for the smaller, immediate option in rats fed a high-fat diet compared to controls,

suggesting greater sensitivity to the drug. It can be inferred, then, that the high-fat diet altered the D_2 system in a manner that manifested as a greater sensitivity to the drug. Thus, administration of a D_2 compound allows researchers to investigate underlying neurotransmitter activity involved in impulsive choice, as well as uncover behavioral alterations that are induced by DIO.

The current study. Studies show that children tend to behave more impulsively than adults and that overweight children and adults tend to behave more impulsively for food than healthy weight individuals. These descriptive studies provide a basis for understanding behavioral processes related to obesity, but it is unknown what specific aspects of an individual's history contribute to the development or the persistence of behaviors that lead to and maintain impulsive choice patterns that may be promote obesity. Experiments using non-human animals allow researchers to directly manipulate and control aspects of behavioral and dietary history that may lead to problematic patterns of eating. To date, few animal models have been used to study the effects of diet on a developing rat that would be analogous to a human in childhood and adolescent developmental phases and of the studies that have been conducted, none have considered behavioral outcomes (e.g., Ozane & Hales, 2004). Experiments focused on an animal model of childhood obesity would allow researchers to characterize the extent to which dietary history and aspects of food availability, such as delay, interact and lead to problematic patterns of eating across development.

The proposed study is by a 2 x 2 design in which we examined the degree to which chronic exposure to a cafeteria diet and age (two different developmental periods

that correspond to human adolescence and adulthood) and their interactions altered impulsive food choice. We also examined underlying DA D_2 activity by administering acute injections of haloperidol before some experimental sessions. By comparing delay discounting and sensitivity to haloperidol from rats at differing developmental stages, we assessed diet- and age-related changes. **This study was the first to characterize diet-induced obesity in a model using developmental windows to investigate behavior and was the first to directly compare diet-induced changes in rats exposed to a cafeteria diet during adolescence vs. adulthood on discounting processes.** This study should have a positive impact by contributing to a growing literature on behavioral and neural mechanisms that underlie obesity, informing behavioral strategies used to improve health that can prevent long-term obesity.

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Appendix A

Average number of 0-s probe challenges completed across experiment

Average number of 0-s probe challenges was assessed using a 2 x 2 x 2 ANOVA (diet, age, and delay sequence as between-subject factors; Figure A1). There was a trending main effect of delay sequence, $F(1, 31) = 3.15$, $p < 0.086$, $\eta_p = 0.09$, such that rats assigned to a descending delay sequence had a higher average number of 0-s probe sessions completed (Figure A1 bottom). No other main effects or interactions (p's > 0.185)

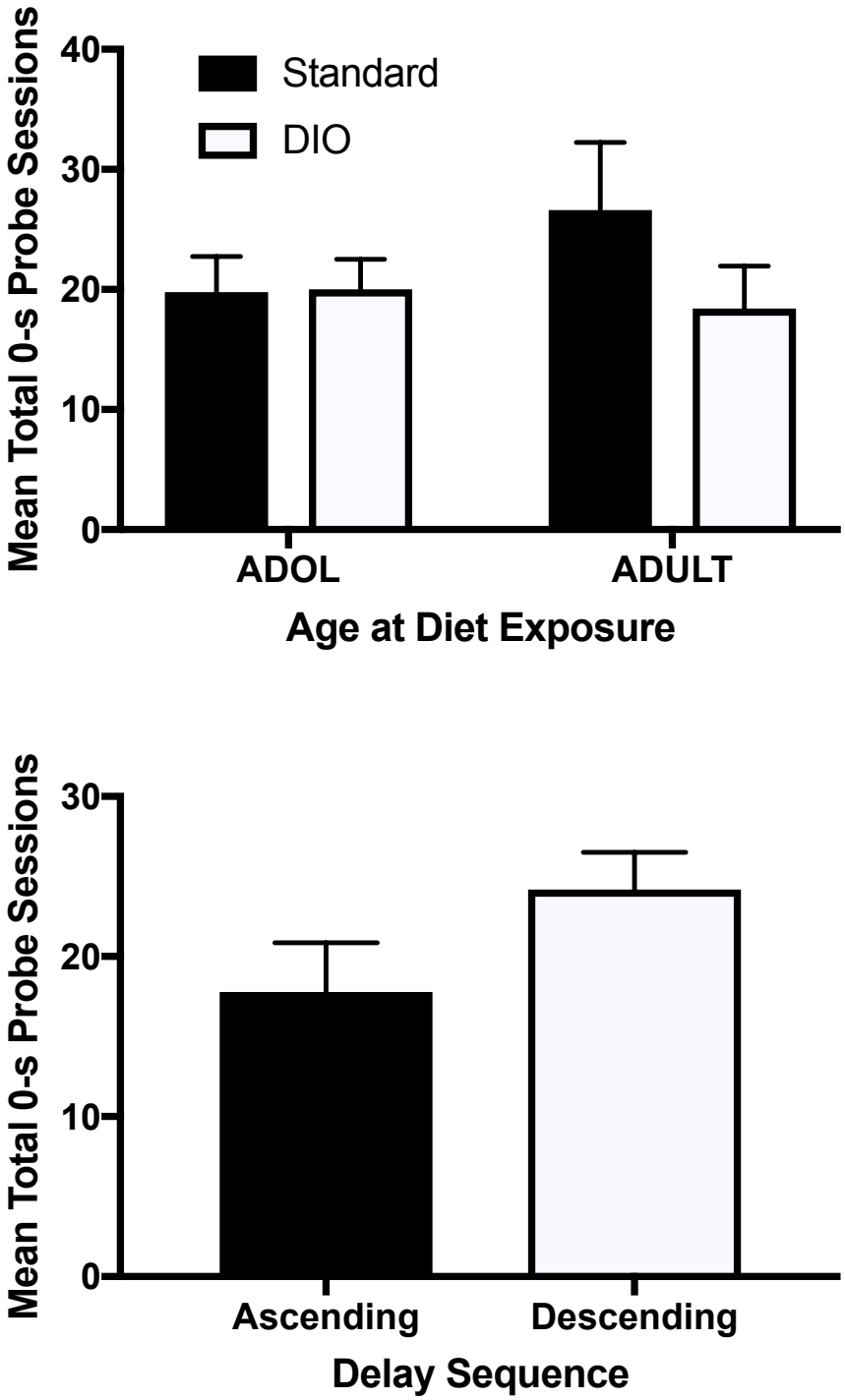

***Figure A1*.** **Shows mean total 0-s probe sessions completed across the experiment as a function of group (top) and as a function of delay sequence (bottom). Error bars = 1 SEM.**