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The Effects of Short-Term Consumption of a Western-Style Junk-Food Diet in Rats

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THE EFFECTS OF SHORT-TERM CONSUMPTION OF A WESTERN-STYLE JUNK-FOOD DIET IN RATS

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by

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ABSTRACT

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THE EFFECTS OF SHORT-TERM CONSUMPTION OF

A WESTERN-STYLE JUNK-FOOD DIET IN RATS

Advisor's name: Dr. Ashley Fricks-Gleason

Reader's name: Dr. Mark Basham

Obesity is an epidemic in our Western culture and is becoming more and more prevalent throughout the world. In fact, it is estimated that 2 billion of the world's population is overweight, with one-third being classified as obese (Jacob & Jutka, 2015). Obesity has been linked to a variety of health conditions including stroke, hypertension, and type 2 Diabetes (WHO, 2009). It is imperative that we begin to further our understanding of the dangers that can result from excessive consumption of these foods. In this way, we can begin to educate our society about the impacts that our dietary choices can have. The current study investigated the effects of consuming a western-style junk-food diet for a short period of 28 days and also the impact of diet removal. After 14 days on the diet the experimental group outperformed the control group on a spatial learning task, which opposed the original prediction ($F(1,22) = 3.043$, $p = 0.095$). Further, upon removing access to the high-fat, high-sugar (HFHS) diet, the experimental group was significantly more anxious and depressed than the control group ($F(1,8) = 11.661$, $p = 0.009$; $F(1,10) = 37.866$, $p = 0.0001$, respectively).

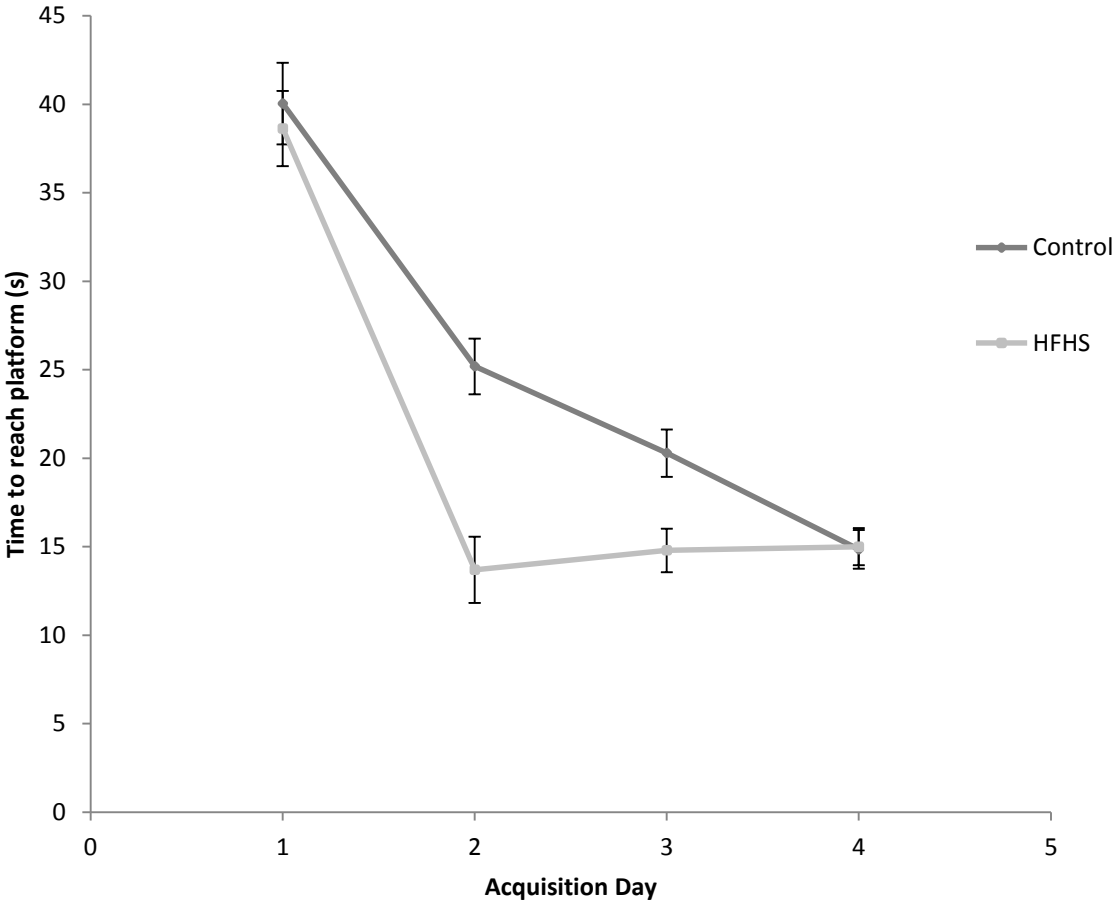

Figure 1. Spatial learning was assessed using a Morris water maze. This figure illustrates the difference in spatial learning abilities between groups over the four acquisition days. Repeated measures ANOVA revealed a marginal main effect of diet ($F(1,22) = 3.043$, $p = .095$) and a significant main effect of day ($F(3, 20) = 64.651$, $p < .0001$). There was also a marginally significant interaction of diet*day, ($F(3,20) = 2.784$, $p = .0674$).

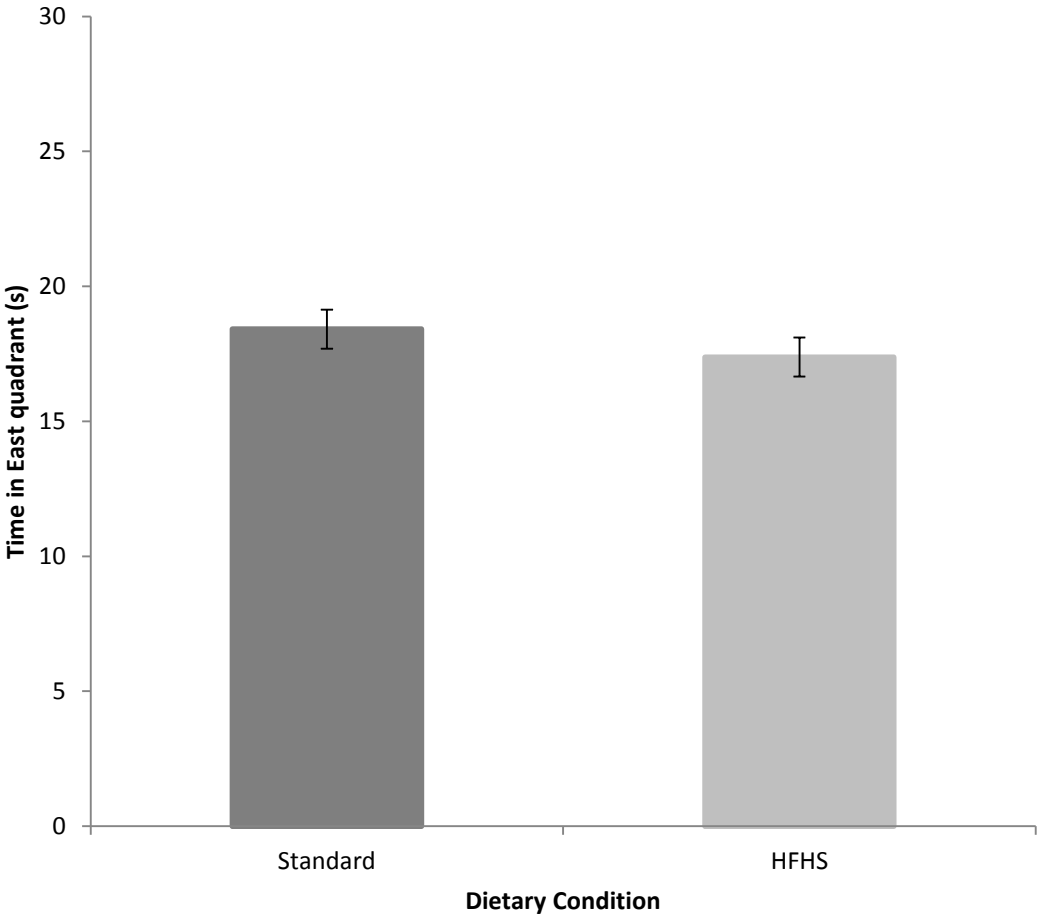

Figure 2. Short-term memory was assessed using the Morris water maze. There was no significant difference in the amount of time spent in the east quadrant during the spatial memory probe trial, ($F(1,20) = .4921$, $p = .491$).

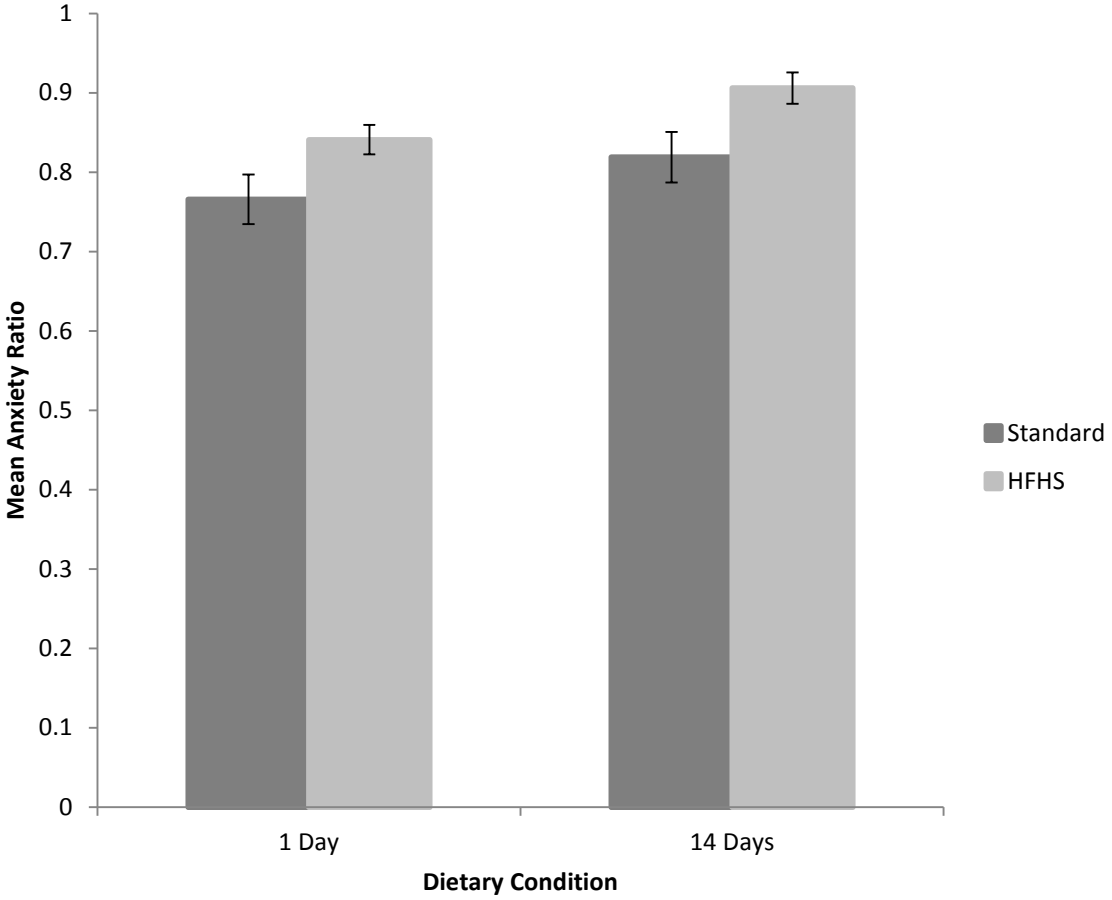

Figure 3. Anxiety was measured using an elevated plus maze. This figure illustrates the difference in mean anxiety ratio (time in closed arm/total time) between the two groups both 1 day and 14 days following diet removal. Repeated measures ANOVA revealed a significant main effect of diet on anxiety ratio ($F(1,8) = 11.661$, $p = .0092$). However, there was no significant main effect of day on anxiety ratio or diet*day interaction ($F(1,8) = 0.049$, $p = 0.830$).

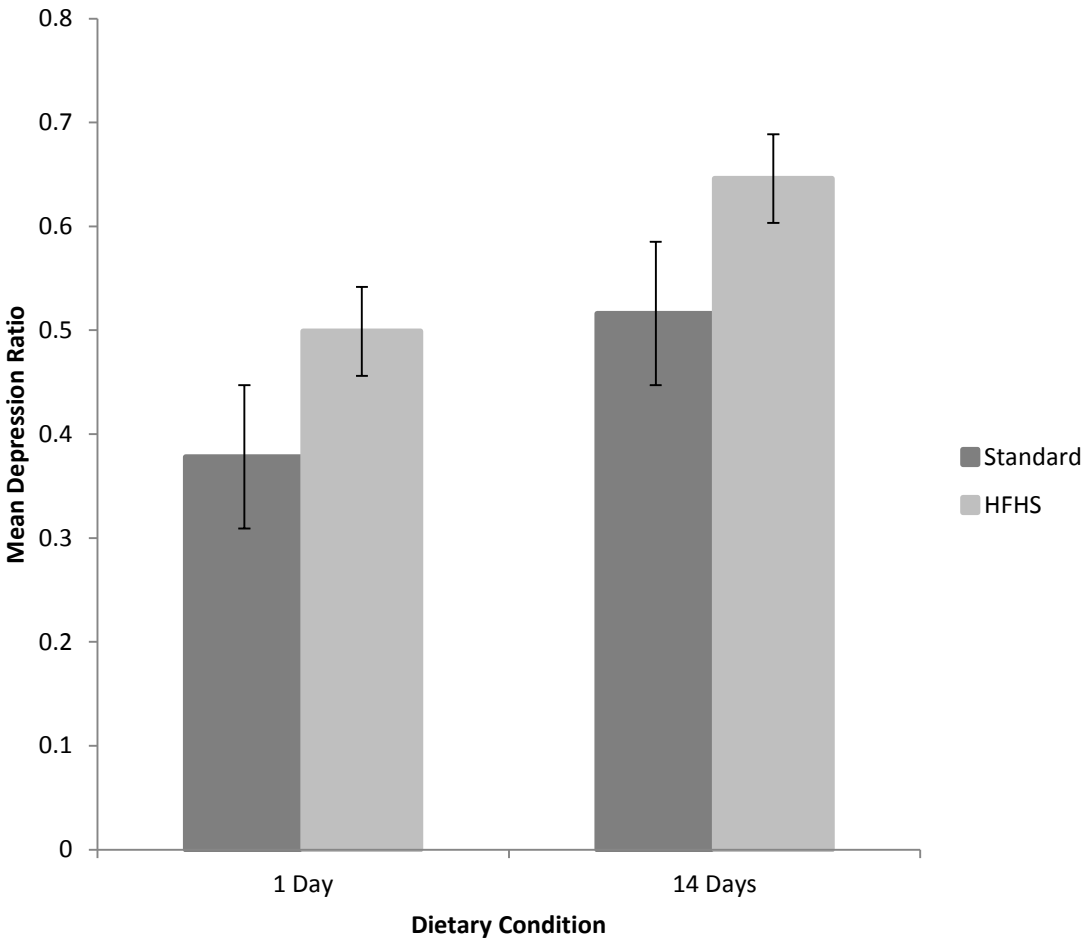

Figure 4. Depression was measured using a forced swim test. This figure illustrates the mean difference in depression ratio (time spent immobile/total time) between the two groups at 1 day and 14 days following diet removal. Repeated measured ANOVA revealed a significant main effect of diet ($F(1,10) = 37.866$, $p = .0001$) and day ($F(1,10) = 4.9254$, $p = .05$) on depression ratio. There was no significant interaction between day and diet ($F(1,10) = 0.0063$, $p = .9382$).

THE EFFECTS OF SHORT-TERM CONSUMPTION OF A WESTERN-STYLE JUNK-FOOD DIET IN RATS

Timothy Lackner & Ashley Fricks-Gleason, Ph.D

Abstract: Obesity is an epidemic in our Western culture and is becoming more and more prevalent throughout the world. In fact, it is estimated that 2 billion of the world's population is overweight, with one-third being classified as obese (Jacob & Jutka, 2015). Obesity has been linked to a variety of health conditions including stroke, hypertension, and type 2 Diabetes (WHO, 2009). It is imperative that we begin to further our understanding of the dangers that can result from excessive consumption of these foods. In this way, we can begin to educate our society about the impacts that our dietary choices can have. The current study investigated the effects of consuming a western-style junk-food diet for a short period of 28 days and also the impact of diet removal. After 14 days on the diet the experimental group outperformed the control group on a spatial learning task, which opposed the original prediction ($F(1,22) = 3.043$, $p = 0.095$). Further, upon removing access to the high-fat, high-sugar (HFHS) diet, the experimental group was significantly more anxious and depressed than the control group ($F(1,8) = 11.661$, $p = 0.009$; $F(1,10) = 37.866$, $p = 0.0001$, respectively).

Key words: *obesity, high-fat, high-sugar diet, HFHS, junk-food diet, dopamine, D1-receptor, D2-receptor,*

1. Introduction

Obesity is an epidemic in our Western culture and is becoming more and more prevalent throughout the world. It is estimated that 2 billion of the world's population is overweight, with one-third being classified as obese (Jacob & Jutka, 2015). Many of our diets are saturated with sugars and fats that cause bodily harm when ingested in excess. Unfortunately, many people are either unaware of the dangers of consuming these foods or are unwilling to change their dietary

habits, regardless of these dangers. By furthering our understanding of the dangers that can result from excessive consumption of these foods, we can begin to educate our society about the impacts that our diets can have.

Obesity is linked to a variety of health conditions including stroke, hypertension, cardiovascular diseases, type 2 Diabetes, and also increased risk for cancers of the breast, colon, prostate and more (WHO, 2009). According to the World Health Organization, 19% of deaths worldwide are attributable to diet-related factors including high cholesterol, high blood glucose, obesity and overweightness, and high blood pressure (WHO, 2009). Unfortunately, consumption of a Western-style junk-food diet, high in sugar and fat, has been implicated in the development of each of these risk factors (Nakatsuji et al., 2010).

Obesity is directly correlated with increased consumption of sugars and fats (Malik & Hu, 2012). As we consume more sugars and fats, our bodies undergo physiological alterations including decreased insulin response and changes in glucose metabolism, which have been implicated in a variety of health-related problems (Francis & Stevenson, 2013; Wang et al., 2014). One major risk is the development of cognitive deficits related to impaired hippocampal function. The hippocampus requires insulin to effectively process information, but it becomes less and less efficient as we develop insulin resistance (Liu et al., 2015; Kanoski & Davidson, 2010).

Therefore, the first aim of this experiment is to investigate the effects of short-term consumption of a HFHS junk-food diet on the cognitive abilities of rats, specifically spatial learning and memory. I believe that a two-week exposure to a Western-style junk-food diet will cause deficits in the ability to learn the Morris Water Maze task when compared to the group maintained on a diet of standard laboratory chow. Additionally, I hypothesize that rats maintained on a Western-style diet will be impaired in their memory of the location of the escape platform 10 days after learning the maze.

However, obesity carries far more consequences than just cognitive impairments. Obesity has also been implicated in the alteration of brain chemistry, specifically at the level of the dopamine receptors in the brain's reward pathways (Huang et al., 2001; Alsio et al., 2010; Alsio et al., 2014; Johnson & Kenny, 2010). Huang et al. (2001) found that severely obese individuals showed a

significant reduction in D2 dopamine receptor availability in specific nuclei associated with both addiction and reward. Further, Alsio et al. (2010) observed the down-regulation of both D1 and D2 receptor expression in the nucleus accumbens after long-term exposure to a palatable food diet. D1 and D2 receptors are both highly abundant in the reward areas of the brain, such as the substantia nigra and striatum, and are involved in a variety of functions including addiction, mediating various behavioral responses, and regulating neuronal growth (Amador et al., 2014; Georges et al., 1999; Spangler et al., 2004). Interestingly, individuals addicted to cocaine, alcohol, and opiates have also shown similar decreases in the brain's reward regions (Turchan et al., 1997; Johnson & Kenny, 2010; Spangler et al., 2004). Therefore, down-regulation of both the dopamine D2 receptors and D1 receptors appear to be associated with a variety of addictive behaviors. The current study intends to draw out the similarities between addiction-related changes in brain chemistry and those observed in a rat model of Western-style diet consumption.

Further, this study will attempt to identify the causal nature of consuming a Western-style diet in regards to dopamine receptor expression. An individual's genetic makeup may cause a down – regulation of dopamine receptors, predisposing them to the overconsumption of unhealthy foods. On the other hand, alterations in the brain's dopamine pathways may be an effect of Western-style diet consumption. This style of dietary consumption results in the chronic release of dopamine into the brain's reward pathway (Sharma et al., 2013; Spangler et al., 2003). Therefore, the down-regulation of dopamine receptors might be a compensatory mechanism for chronic overstimulation. I hypothesize that a 4-week exposure to a Western-style diet will cause down-regulation of both D1 and D2 receptor expression in the dorsal and ventral striatum. Perhaps, the down-regulation of D1 and D2 gene expression perpetuates the addictive nature of eating disorders, promoting the overconsumption of rewarding foods as a method of compensating for reduced activity in the brain's reward pathways (Spangler et al., 2004).

As more and more research reaches a public audience regarding the negative impacts of consuming a Western-style junk-food diet, one might assume that the logical response would be to cease

consumption. However, researchers have illustrated using a rat model that restricting access to a rewarding diet after extended exposure can result in the development of negative psychological symptoms (Pickering, 2009). Removal of a HFHS diet after 4 weeks of access resulted in withdrawal-like symptoms, including increased anxiety (Pickering, 2009). The obesity prone (OP) rats in Pickering's study showed increased craving and reduced center-activity in an open field test following removal of the HFHS diet (Pickering, 2009). These behavioral results are consistent with withdrawal symptoms induced by addictive drugs (Rothwell, 2009). The current study aims to identify the psychological symptoms that may appear after removal of a Western-style junk-food diet following 28 days of unrestricted access.

This study will focus on the short-term effects of Western-style diet consumption. I believe that removing the junk-food diet after 28 days will cause the subjects to exhibit both depressive and anxious symptoms up to 14 days after diet removal. I predict that the subjects maintained on a junk-food diet and then withdrawn from that diet will show more anxiety than the subjects maintained on the standard laboratory chow diet. I also predict that the experimental group will show more symptoms of depression compared to the control group once the junk-food diet is removed.

Overall, this study has the potential to substantiate the claims regarding the negative impacts of consuming a Western-style, junk-food diet. Previous studies of a similar nature have lacked a comprehensive approach. However, the current study plans to identify the physiological, cognitive, and behavioral effects of consuming this style of diet. Further, many researchers have focused specifically on long-term consumption, but this research will specifically investigate short-term effects.

2. Method

All procedures were carried out under strict compliance with ethical principles and guidelines of the OLAW Guide for the Care and Use of Laboratory Animals. All testing procedures and dietary conditions were approved by the Institutional Animal Care and Use Committee of Regis University in Denver, Colorado.

2.1. Animals

Twenty-four adult, male Sprague-Dawley rats were used throughout the study. The rats weighed between 140 and 190 grams at the time of arrival. The rats were individually housed in Regis University's Animal Care Facility on a 12:12 light/dark cycle (06:00 – 18:00 light) with unrestricted access to food and water. Cage bedding was changed every 2-3 days.

2.2. Diet

The subjects were randomly assigned to two groups: control animals (n=12) receiving *ad libitum* access to standard laboratory chow and experimental animals (n=12) receiving *ad libitum* access to both standard laboratory chow and a high-fat, high sugar (HFHS) junk-food diet. The HFHS junk-food diet consisted of Nestle Chocolate Powder, Chips Ahoy cookies, Jif Peanut Butter, Ruffles potato chips, and standard laboratory chow (200 g = 19.6% fat, 14% protein, 25.7% sugar 58.13 carbs; 4.5 kcal/g). Body weight and food consumption were monitored throughout the experiment.

2.3. Experiment 1

Experiment 1 consisted of two parts. Part 1 involved the use of a Morris water maze (MWM) to assess spatial learning. Part 2 was used to assess the spatial memory of the subjects.

2.3.1. Morris Water Maze

A circular pool (1.6m) was filled with water (0.5m) and divided into four quadrants: north, south, east and west. Powdered milk was added to the pool to cloud the animals' vision in the water. A GoPro video camera was mounted directly above the pool to record behavior. A square escape platform (12 cm) was placed in the East quadrant 18 cm from the edge of the maze and 2 cm below the water's surface. The escape platform remained in the same position for the duration of the experiment.

2.3.2 Spatial Learning

Spatial learning was assessed using the Morris water maze (MWM). The subjects were tested in the MWM after 14 days of exposure to the dietary conditions. Acquisition training consisted of 4 days in which the subjects completed 4 trials per day. Each subject was placed in the water at the perimeter of the pool with its head facing the wall and given 60 seconds to reach the escape platform. The subjects were released

from each of the four starting quadrants daily, with the first trial beginning in the north quadrant. The order of starting positions remained constant for the duration of acquisition training: (N), (W), (S), (E). Once the platform was reached, the subjects remained there for 10 seconds. If the platform was not reached in 60 seconds, the subjects were gently guided to the escape platform and left there for 10 seconds. The subjects were then removed from the pool, gently towel-dried, and returned to their home cages for a 30-minute intertrial interval. The amount of time it took each subject to reach the escape platform was recorded.

2.3.3 Spatial Memory

A probe trial was conducted ten days following the final acquisition trial to assess memory for the spatial learning task. The escape platform was removed from the MWM. Subjects were released from the north (N) quadrant and allowed 60 seconds to swim. Subjects were then removed from the pool, gently towel-dried, and returned to their home cages. The amount of time spent in each of the four quadrants was recorded.

2.4. Euthanasia 1

After 28 days of exposure to the dietary conditions, half of the subjects from each group were euthanized using an overdose of Euthasol (120 mg/kg, i.p.). After the brains were quickly removed, they were stored in 4% paraformaldehyde at 4 °C for 24 hours and then dehydrated in 30% sucrose in 0.1M PBS at 4 °C until sectioning.

2.5. Experiment 2

Experiment 2 assessed the behavioral effects of removing access to a high-fat, high-sugar junk-food diet. Psychological symptoms of anxiety and depression were measured using an elevated plus maze and a forced swim test, respectively. The subjects' behavior was assessed 24hrs and 2 weeks following diet removal. Each assessment period was split into 2 days: on the first day, half of the subjects from each group completed the forced swim test and the other half completed the elevated plus maze. The following day, each subject completed the opposite test. The elevated plus maze (EPM) and the forced swim test (FST) are widely accepted measures of anxiety and depression, respectively.

2.5.1. Diet Removal

After 28 days of exposure to the dietary conditions, the junk-food component was removed from the experimental diet so that the remaining subjects only had access to standard laboratory chow. The control diet remained the same.

2.5.2. Elevated Plus Maze

The elevated plus maze consists of two open arms and two closed arms that form a plus shape and are elevated (0.75m) off of the ground. The subjects were placed where the four arms meet, the neutral zone, and allowed 6 minutes to explore. The amount of time spent in the open arms, closed arms, and neutral zone was recorded. The number of entries into the closed and open arms was also recorded. An anxiety ratio was calculated by dividing the time spent in the open arms by the total time.

2.5.3. Forced Swim Test

A clear, 5-gallon water basin was filled with water to a depth of 40 cm. A GoPro video camera was mounted directly above the basin to record behavior. The subjects were gently placed in the center of the basin, allowed 6 minutes to swim, and were then removed from the basin, gently towel-dried, and returned to their home cages. The amount of time each subject spent mobile and immobile was recorded. A depression ratio was calculated by dividing the time spent immobile by the total time.

2.6 Euthanasia 2

6 hours after completion of the elevated plus maze and forced swim tests, the remaining subjects were euthanized using an overdose of Euthasol (120 mg/kg, i.p.). After the brains were quickly removed, they were stored in 4% paraformaldehyde at 4 °C for 24 hours and then dehydrated in 30% sucrose in 0.1M PBS at 4 °C until sectioning.

2.7 Sectioning

Coronal brains sections 30μm thick were taken from the substantia nigra and striatum. Brain sections were placed into wells containing 0.1M PBS and 1mg/kg sodium azide and stored at 4 °C.

2.7.1 Histology: Staining

D1 Staining Procedure:

All steps were completed under constant agitation in free-floating, 2 mL wells. All solutions

were made in 0.1M PBS. Endogenous peroxidases were blocked with 3% H_2O_2 for 10 minutes. The tissues were rinsed and moved into non-specific block (5% NGS; 0.2% Triton-X) for 60 minutes. The tissues were then incubated in primary antibody solution (2% NGS; 0.2% Triton-X; 1:5000 primary (Millipore, Catalog # 324390, Lot 2697726)). The reaction was jump-started on the bench for 1 hour and then incubated overnight at 4 C. The tissues were then moved into the secondary antibody (2% NGS; 0.2% Triton-X; 1:200 biotinylated goat anti-rabbit (Vector, Catalog # BA-1000; Lot # Z1203)) to incubate for 60 minutes. The signal was amplified using ABC solution (ABC Elite Kit, Vector, Catalog # PK-6100) for 30 minutes before being visualized with DAB (DAB Peroxidase Substrate Kit, Vector, Catalog # SK-4100) was used to visualize the reaction. The tissues were moved into the DAB solution for 3 minutes and were then rinsed and mounted onto gelatin-coated slides.

2.7.2 Microscopy & Image Analysis

Low power images were captured using the 10x objective of the RoachScope (Backyard Brains). Although not yet completed, the brain images will be quantified for D1 & D2 dopamine receptor expression using ImageJ analysis software.

2.8 Statistical Analysis

A repeated measures ANOVA was used to compare the differences between groups using the statistical program JMP12. Outliers were identified using boxplots generated in SPSS.

3. Results

3.1. Experiment 1 - Effect of a junk-food diet on spatial learning and memory.

The amount of time it took each rat to reach the escape platform decreased over the 4 days of acquisition (Repeated measures ANOVA, $F(3, 20) = 64.651$, $p < 0.0001$). The rats in the experimental group found the escape platform faster than the control rats, indicated by a marginal main effect of diet, ($F(1,22) = 3.043$, $p = 0.095$). There was also a marginally significant diet*day interaction ($F(3,20) = 2.784$, $p = 0.0674$; Figure 1).

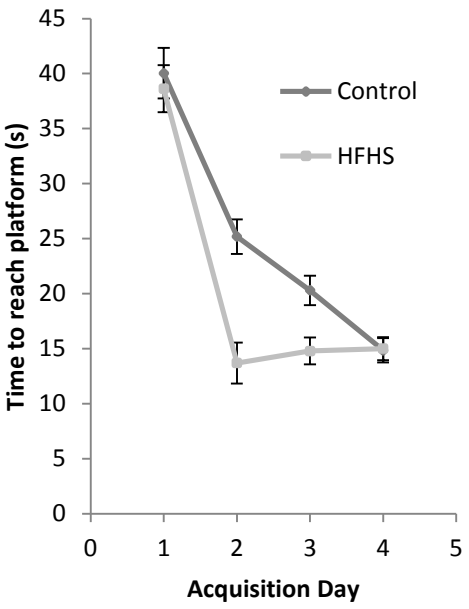

Figure 1. Spatial learning was assessed using a Morris water maze. This figure illustrates the difference in spatial learning abilities between groups over the four acquisition days. Repeated measures ANOVA revealed a marginal main effect of diet ($F(1,22) = 3.043$, $p = 0.095$) and a significant main effect of day ($F(3, 20) = 64.651$, $p < 0.0001$). There was also a marginally significant interaction of diet*day, ($F(3,20) = 2.784$, $p = 0.0674$).

During the probe trial, the mean amount of time spent in the east quadrant was greater in the control group than the experimental group (18.42 ± 4.17; 17.38 ± 2.29), however, this difference was not significant (ANOVA, $F(1,20) = 0.4921$, $p = 0.491$; Figure 2). Using a box plot distribution, 2 outliers were detected in the experimental group (R535 & R554) and removed from all statistical analyses.

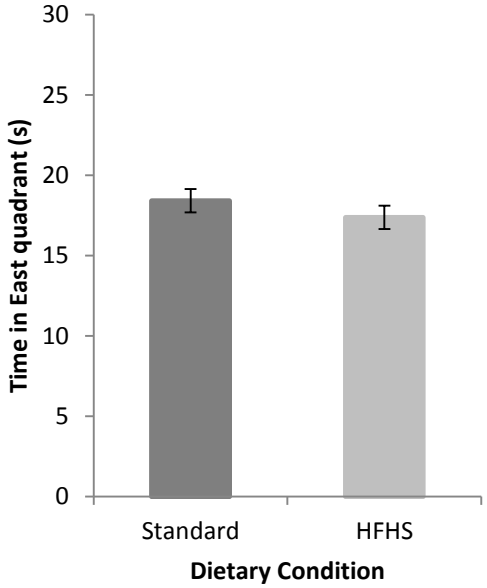

Figure 2. Short-term memory was assessed using the Morris water maze. There was no significant difference in the amount of time spent in the east quadrant during the spatial memory probe trial, ($F(1,20) = 0.4921$, $p = 0.491$).

Brain slices were collected and stained for the D1 & D2 dopamine receptors. Although this data is not yet fully quantified, Figures 3 & 4 provide representative images illustrating the neurochemical differences between the control and experimental groups in the striatum and substantia nigra regions.

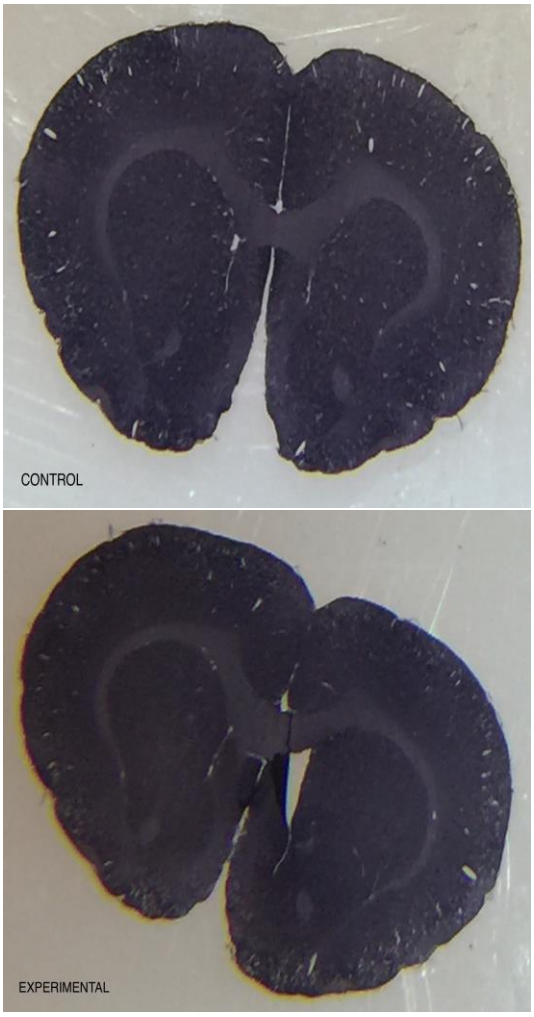

Figure 3. IHC was used to stain for dopamine receptor expression. This figure illustrates the differences between D-1 dopamine receptor expression in the dorsal and ventral striatum in the experimental and control groups.

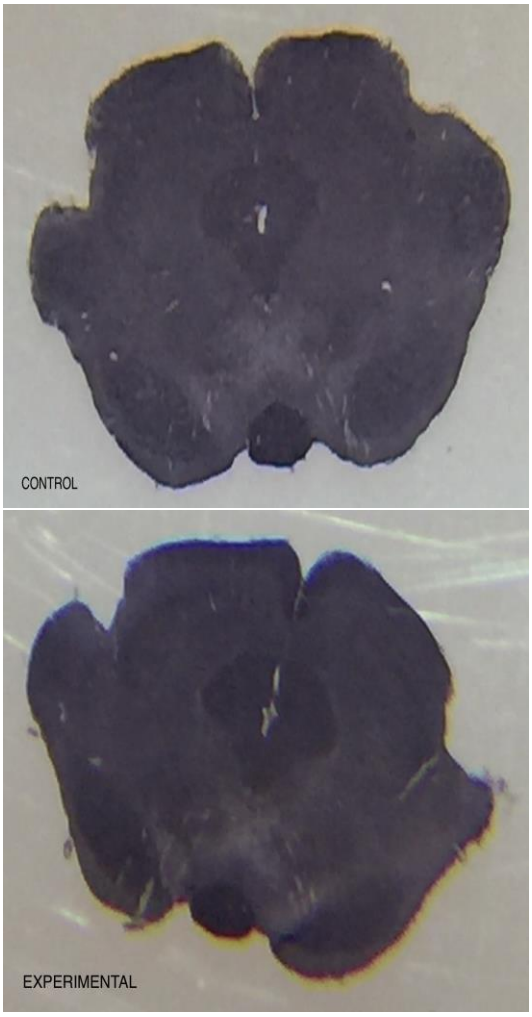

Figure 4. Dopamine receptor expression was visualized using IHC. This figure illustrates the differences between D-1 dopamine receptor expression in the substantia nigra in the experimental and control groups.

3.2. Experiment 2 - Effect of diet-removal on anxious and depressive symptoms.

The mean anxiety ratio was higher in the HFHS group than the control group 24 hours after diet removal (0.841 ± 0.041; 0.765 ± 0.075)

and this ratio increased in both groups after 14 days (0.906 ± 0.044; 0.818 ± 0.081). Repeated measures ANOVA revealed a significant main effect of diet removal on anxiety ratio ($F(1,8) = 11.661$, $p = 0.0092$; Figure 5). However, there was no significant main effect of day on anxiety ratio or diet*day interaction ($F(1,8) = 2.99$, $p = 0.122$; $F(1,8) = 0.049$, $p = 0.830$). A box plot distribution identified two extreme outliers in the experimental group (R540 & R543) who were removed from statistical analyses.

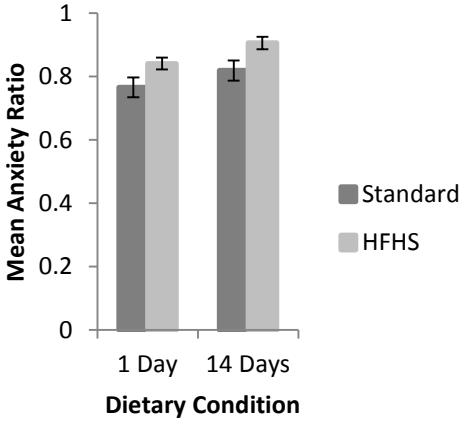

Figure 5. Anxiety was measured using an elevated plus maze. This figure illustrates the difference in mean anxiety ratio (time in closed arm/total time) between the two groups both 1 day and 14 days following diet removal. Repeated measures ANOVA revealed a significant main effect of diet on anxiety ratio ($F(1,8) = 11.661$, $p = 0.0092$). However, there was no significant main effect of day on anxiety ratio or diet*day interaction ($F(1,8) = 0.049$, $p = 0.830$).

The mean immobility ratio was higher in the experimental group than the control group 24 hours after diet removal (0.516 ± 0.105; 0.378 ± 0.143) and this ratio increased in both groups after 14 days (0.646 ± 0.073; 0.499 ± 0.093). Repeated measures ANOVA revealed a significant main effect of day on depression ($F(1,10) = 4.9254$, $p = 0.05$), as well as a significant main effect of diet removal on depression ratio ($F(1,10) = 37.866$, $p = 0.0001$; Figure 6). However, there was no significant interaction between day and diet ($F(1,8) = 0.049$, $p = 0.830$).

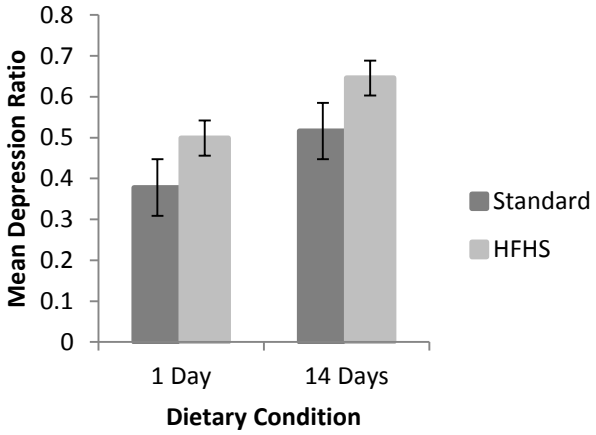

Figure 6. Depression was measured using a forced swim test. This figure illustrates the mean difference in depression ratio (time spent immobile/total time) between the two groups at 1 day and 14 days following diet removal. Repeated measured ANOVA revealed a significant main effect of diet ($F(1,10) = 37.866$, $p = .0001$) and day ($F(1,10) = 4.9254$, $p = 0.05$) on depression ratio. There was no significant interaction between day and diet ($F(1,10) = 0.0063$, $p = 0.9382$).

4. Discussion

4.1. Discussion of results found in Experiment 1

The Morris water maze test for spatial learning abilities indicated a main effect of diet on learning performance. However, this effect was in the opposite direction of what was originally predicted. I hypothesized that the control group, maintained on the standard laboratory chow diet, would be able to find the escape platform faster, and therefore, demonstrate superior learning abilities, but this was not the case. Interestingly, the experimental group, maintained on a diet high in fats and sugars, was able to learn the location of the escape platform significantly faster than the control rats. These results indicate that short-term consumption of a high-fat, high-sugar diet might be beneficial when completing tasks involving learning processes.

The results from the memory probe trial indicated no difference between the two groups. After being maintained on the junk-food diet for 28 days, both groups still spent the same amount of time in the east quadrant, indicating that neither group had a superior memory of the learning task. It was originally predicted that the control group would outperform the experimental group. However, it appears that 28-day access to the HFHS diet was not sufficient to disrupt spatial memory processes. Most previous research that has found diet-induced deficits in spatial memory fed their rats the respective diets for at least 8 weeks (Greenwood & Winocur, 2005; Molteni et al., 2002; Valladolid-Acebes et al., 2011; Winocur & Greenwood, 2005). The current study was investigating the effects of short-term consumption; it appears that 4-week exposure to the HFHS diet was not long enough to induce the predicted cognitive deficits.

4.2. Discussion of results found in Experiment 2

The results from the elevated plus maze and forced swim tests indicated that removal of the junk-food diet after 28-day access is sufficient to cause symptoms of anxiety and depression up to 14 days after removal. In both cases, significant main effects of diet removal on behavioral symptoms were found: these data imply that removing access to the junk-food diet in the experimental animals led them to be consistently more anxious and depressed at both time points, compared to the control rats. Further, for the forced swim test, a

significant main effect of day on depression ratio was found, which suggests that the depression ratio of both groups, regardless of diet, increased over time. Overall, the results of experiment 2 indicate that removal of a rewarding junk-food diet after short-term access can lead to symptoms of anxiety and depression up to 14 days after removal, which carries implication for how our society deals with dietary changes.

5. General Discussion

The results of the current study leave room for interesting interpretation: on one hand, they indicate that consumption of junk-food might be beneficial in the short-term while on the other hand, it appears that restricting access to this diet after short-term exposure can lead to negative psychological symptoms.

As I mentioned earlier, the hippocampus requires insulin to effectively process information and that it becomes less efficient as we develop a resistance to insulin; unfortunately, insulin resistance is a common byproduct of obesity (Francis & Stevenson, 2013; Leboucher et al., 2013; Wang et al., 2015). Although not tested herein, it is likely that the experimental rats were not exposed to the HFHS diet long enough to develop insulin resistance and the associated disruption in hippocampal function. It appears that the experimental rats were still responding to insulin properly and, therefore, allowing the elevated levels of glucose circulating in their bloodstreams to be absorbed. The experimental rats may have had an advantage from consuming the junk-food diet, seeing as learning tasks quickly deplete the brain's glucose reserves. Perhaps, the HFHS foods may have served as additional "fuel" to improve the experimental group's learning abilities (Benton, 2005; Christie & Schrater, 2015). In the short term, consumption of foods high in fats and sugars might provide a cognitive boost leading to increased performance. However, it is difficult to pinpoint where exactly this threshold between improvement and impairment lies. I would anticipate that this threshold is different for each individual and also that there are healthier alternatives to junk food that could provide a similar cognitive boost.

On the other hand, the behavioral results illustrate that removing access to a junk-food diet can lead to the development of psychological symptoms. It appears that we become dependent on the rewarding experience of consuming

unhealthy foods, such that removing them from our diets becomes extremely difficult (Sharma et al., 2013). In our American society, we often place too much emphasis on instant gratification so, perhaps, this experience of anxiety and depression may be clouding our vision of the long-term benefits of diet change. Had I been able to continue my study, I would have liked to see how long it would take for the symptoms of depression and anxiety to return to a "normal" level in the experimental rats.

Overall, research studies like mine are becoming more important because they have the potential to educate the public about diet and its adverse effects. Diet has been linked to Type 2 Diabetes, stroke, CV disease, cancer, and even neurodegenerative disorders like Alzheimer's disease, yet foods saturated in sugars and fat still plague our diets (Hargrave, 2015; Hsu, 2014; Trivedi, 2012; WHO, 2009). It is clear we must become more conscious of the foods we choose to fuel our bodies with and the effects these choices can have. Unfortunately, diet consumption is often overlooked as a contributing factor to our general wellbeing. However, it is becoming clearer that the foods we consume have a monumental impact on our health, cognitions, behavior, mood, and feelings (Francis & Stevenson, 2013; Trivedi, 2012). The current study illustrates that consuming unhealthy foods can carry both positive and negative consequences. Perhaps, in moderation, these types of food may not be detrimental to our health, but as a society we seem to have great difficulty in limiting our consumption. Many of the foods we eat are manufactured to ensure that customers will continue coming back for more (Peretti, 2013). Once we begin traveling down this "rewarding" path of consuming sugary, fat-filled foods, we don't want to turn back. This may be one of the reasons that many of our attempts to stick to a healthy diet fail. When we cut unhealthy foods from our diet, we are likely to experience feelings of anxiety and depression, which makes sticking to a diet increasingly more difficult. However, if we can create a way to incrementally remove unhealthy foods from our diet while also making diet-related research more accessible to the general public, perhaps, we can begin to catalyze a lasting change.

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Regis University Animal Study Proposal

Please Leave Blank
Proposal #:
Approval Date:

Date: 10/15/2015

ADMINISTRATIVE DATA

Department: Neuroscience & Psychology

Principal investigator: Timothy Joseph Lackner

Campus address: 3333 Regis Boulevard, Denver, CO 80221

Phone: 402-350-6156 E-mail: tlackner@regis.edu

Project title: THE EFFECTS OF SHORT-TERM CONSUMPTION OF A WESTERN-STYLE JUNK-FOOD DIET IN RATS

Type of submission: Proposal for New Research

List the names of all individuals authorized to conduct procedures involving animals under this proposal and identify key personnel *[e.g., co-investigator(s)]*, providing their department and e-mail:

Timothy Lackner	tlackner@regis.edu	Psychology and Neuroscience
Ashley Fricks-Gleason	africksgleason@regis.edu	Psychology and Neuroscience
Mark E. Basham, Ph.D.	mbasham@regis.edu	Psychology and Neuroscience

ANIMAL REQUIREMENTS

Species: Rat

Strain, subspecies, or breed: *[e.g., C57BL/6] Sprague Dawley*

Approximate age, weight or size: 250 g

Sex: Male

Source(s): Charles River Regis colony

Location(s) where manipulation will be conducted: SCI 114 & Regis Animal Facility

Number of animals to be used: 24

STUDY OBJECTIVES

Briefly explain the aim of the study and why the study is important to human or animal health, the advancement of knowledge, or the good of society in language that a layperson can understand. Please comment on whether the study unnecessarily duplicates other studies.

Obesity is an epidemic in our Western culture and is becoming more and more prevalent throughout the world. Many of our diets are saturated with sugars and fats that cause bodily harm when ingested in excess. Obesity-inducing diets cause a variety of illnesses including strokes, cardiovascular disease, and Type II Diabetes (Francis, 2013). Furthering our understanding of the neurological effects caused by exposure to a high-fat high-sugar (HFHS) diet can allow us insight into the underlying mechanisms of diet-induced obesity. Investigating the role of environmental conditions like exposure to a HFHS diet can enable us to develop interventions to counteract the negative side effects of obesity. It is also important to analyze the behavioral and cognitive effects that result from a HFHS diet so that we can understand the implications of the physiological alterations. Research on diet-induced obesity is often focused on long-term implications, but I wish to investigate whether or not the subjects will exhibit neurological changes, alterations in behavior, and cognitive deficits over a 4-week exposure to a HFHS diet. There is also research being conducted to assess the

similarities between exposure to HFHS diets and morphine exposure so I plan on comparing my data to previously published data regarding the neurological changes and withdrawal-like behaviors that result from morphine addiction to determine any similarities.

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RATIONALE FOR ANIMAL USE

1. Explain your rationale for animal use. *[The rationale should include reasons why it is necessary to use animal models.]*

Investigating the negative side effects of high-fat high-sugar diets in humans is extremely difficult. However, animal models have been an efficient method of furthering our understanding of the physiological, behavioral, and cognitive changes.

2. Justify the appropriateness of the species selected. *[The species selected should be the lowest possible on the phylogenetic scale.]*

Rat models of diet-induced obesity resulting from high-fat high-sugar diets have been well accepted in the scientific community. These rats respond well to the stated tests and procedures and are easy to handle.

3. Justify the number of animals to be used. *[The number of animals should be the minimum number required to obtain statistically valid results. Include justification for group size through a power analysis when possible.]*

24 subjects is the minimal number used by previous researchers to obtain statistically significant results in studies of a similar nature.

DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES

- Briefly explain the experimental design and specify all animal procedures. All procedures to be employed in the study must be described. This description should allow the IACUC to understand the experimental course of an animal from its entry into the experiment to the endpoint of the study. A flowchart may be an effective presentation of the planned procedure.

  Include the following specific information, if applicable:

  - **Experimental injections or inoculations** *[substances, e.g., infectious agents, adjuvants, etc.; dose, sites, volume, route, and schedule]*.
  - **Blood withdrawals** *[volume, frequency, withdrawal site, and methodology]*.
  - **Food or fluid restriction** If food, or fluid, or both food and fluid, will be restricted, describe method for assessing the health and wellbeing of the animals. If you are seeking a departure from the recommendations of the *Guide*, provide a scientific justification.
  - **Pharmaceutical-grade and Non-pharmaceutical-grade Compounds** Identify any drugs, biologics, or reagents that will be administered to animals. If these agents are not human or veterinary pharmaceutical-grade substances, provide a scientific justification for their use and describe methods that will be used to ensure appropriate preparation and administration.
  - **Other procedures** *[e.g., survival studies, tail biopsies]*.
  - **Resultant effects**, if any, that the animals are expected to experience *[e.g., pain or distress, ascites production, etc.]*.
  - **Other potential stressors** *[e.g., noxious stimuli, environmental stress]* **and procedures to monitor and minimize distress**.
  - **Experimental endpoint criteria** *[e.g., tumor size, percentage body weight gain or loss, inability to eat or drink, behavioral abnormalities, clinical symptomatology, or signs of toxicity]* must be specified when the administration of tumor cells, biologics, infectious agents, radiation or toxic chemicals are expected to cause significant symptomatology or are potentially lethal. List the criteria that will be used to determine when euthanasia is to be performed. Death as an endpoint must be scientifically justified.
  - **Veterinary care** Indicate the plan of action in case of animal illness *[e.g., initiate treatment, call investigator prior to initiating treatment, euthanize]*.

1. 24 rats will be randomly assigned to experimental (n=12) and control groups (n=12).
2. The control group will have ad libitum access to a standard laboratory diet of chow and water for the duration of the experiment. The experimental group will have ad libitum access to both the standard laboratory diet and a high-fat high-sugar diet (Cafeteria-based diet consisting of: chips ahoy cookies (130g), classic ruffles (40g), Jif peanut butter (130g), Nesquik classic chocolate powder (130g), standard laboratory rat chow (200g) and 175ml of water) for 28 days.
3. Spatial learning and memory will be recorded from Day 14 to Day 28. Days 14-17 will involve 4 acquisition trials in which the subjects are familiarized with the Morris water maze (MWM) apparatus. The escape platform and the visual references will remain in the same locations for the duration of testing. The amount of time it takes the subject to reach the platform will be recorded for each trial. 10 days after the final acquisition trial, the escape platform will be removed to assess spatial memory. The amount of time the subject spends in each of the four quadrants will be recorded.
4. After 28 days, 6 rats from each group will be euthanized with an overdose of euthasol (120mg/kg) and transcardially perfused with 4% paraformaldehyde. After perfusions, the brains will be immersed in RNALater and stored at -20C. Any neurological changes that have occurred will be analyzed using *immunohistochemistry* techniques.
5. *Immunohistochemistry* will be used to measure dopamine D2 receptor expression and encephalin and dynonorphin opioid gene expression in several brain regions (substantia nigra, dorsal striatum (caudate putamen), and ventral striatum (nucleus accumbens)).
6. After 28 days, the experimental group will have the high-fat high-sugar diet removed while the standard laboratory diet will remain.
7. On day 29, I will begin to measure any differences in behavioral symptoms. Withdrawal-based anxiety will be observed using an elevated plus maze and depressive behaviors will be measured via a forced swim test. Each of the tests will be administered every 4^{th} day for a total of 22 days. On day 29, half of the subjects from each group will complete the MWM and half will complete the elevated plus maze to assure there are no confounds as a result of competing either of the tests first. The following day (day 30), the subjects will complete whichever test they did not complete the day prior and this pattern will continue for the duration of the experiment.

8. Upon completing the experiment on day 50, the remaining rats will be euthanized using an overdose of euthasol (120mg/kg) and transcardially perfused with 4% paraformaldehyde.

SURGERY

If surgery is proposed, complete the following:

1. Identify and describe the surgical procedure(s) to be performed. Include preoperative procedures *[e.g., fasting, analgesic loading]*, and monitoring and supportive care during surgery. Include the aseptic methods to be used.

   The subjects will be perfused following an overdose of euthasol (120mg/kg). The subjects will first be perfused with saline and then with 4% paraformaldehyde for 4-5 minutes.

2. Identify the individual(s) that will perform surgery and their qualifications, training, and/or experience.

   Timothy Lackner will perform the surgical procedure under the direct supervision of Dr. Fricks-Gleason. Timothy Lackner has gained experiencing performing perfusions from Advanced Research Methods in Neuroscience (NS401).

3. Identify the location where surgery will be performed. *[building(s) and room(s)]*

   All procedures will be performed in the Regis University Animal Facility Surgical Suite.

4. If survival surgery, describe postoperative care that will be provided and frequency of observation. Identify the responsible individual(s) and location(s) where care will be provided. *[building(s) and room(s)]*

5. If non-survival surgery, describe how euthanasia will be provided

   Euthanasia will be provided using an overdose of euthasol (120mg/kg).

7. Has major or minor survival surgery been performed on any animal prior to being placed on this study? *[Major survival surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions or involves extensive tissue dissection or transection (such as laparotomy, thoracotomy, craniotomy, joint replacement, or limb amputation)]*. If yes, please explain.

   No.

8. Will more than one survival surgery be performed on an animal while on this study?
   If yes, please justify.

   No.

METHOD OF EUTHANASIA OR DISPOSITION OF ANIMALS AT END OF STUDY

Indicate the proposed method of euthanasia.

The subjects will be euthanized with an overdose of euthasol (120mg/kg).

FIELD STUDIES

If animals in the wild will be used, describe how they will be observed, any interactions with the animals, whether the animals will be disturbed or affected, and any special procedures anticipated. Indicate if federal, state, and/or local permits are required and whether they have been obtained.

SPECIAL CONCERNS OR REQUIREMENTS OF THE STUDY

List any special housing, equipment, animal care or any departures from the *Guide [e.g., special caging, water, feed, waste disposal, environmental enrichment, etc.]*.

Experimental rats will be placed on a high-fat high-sugar diet (Cafeteria-based diet consisting of: chips ahoy cookies (130g), classic ruffles (40g), Jif peanut butter (130g), Nesquik classic chocolate powder (130g), standard laboratory rat chow (200g) and 175ml of water) for 4 weeks. Many researchers have successfully utilized cafeteria-based diets to mimic high-fat high-sugar diets in rats. Beilharz, Maniam, & Morris (2014) successfully utilized a cafeteria-based diet consisting of lard, sucrose, and a selection of biscuits and cakes to demonstrate cognitive impairments in place and object recognition that resulted from elevated fat and sugar intake. Other researchers have implemented cafeteria-based diets consisting of 100% lard (Pickering, Alsiö, J. Hulting, & Schiöth, 2009), a combination of traditional Crisco and 32% sucrose (Jurdak, Lichtenstein, & Kanarek, 2008), and also a mixture of condensed milk, bread, chocolate, biscuits, dried coconut, cheese, and boiled potatoes (Kumar & Alagawadi, 2013) to assess the physiological, behavioral, and cognitive effects of consuming high-fat and/or high-sugar diets.

References

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PRINCIPAL INVESTIGATOR CERTIFICATIONS

1. I certify that I have determined that the research proposed herein is not unnecessarily duplicative of previously reported research.

2. I certify that the individuals listed in Section A. are authorized to conduct procedures involving animals under this proposal, and have received training in: the biology, handling, and care of this species; aseptic surgical methods and techniques (if necessary); the concept, availability, and use of research or testing methods that limit the use of animals or minimize distress; the proper use of anesthetics, analgesics, and tranquilizers (if necessary); and procedures for reporting animal welfare concerns.

3. I certify that I will obtain approval from the IACUC before initiating any significant changes in this study.

4. I certify that I will notify the IACUC regarding any unexpected study results that impact the animals. Any unanticipated pain or distress, morbidity or mortality will be reported to the attending veterinarian and the IACUC.

5. I certify that I am familiar with and will comply with all pertinent institutional, state, and federal rules and policies.

Principal Investigator

Name: Tim Lackner Signature: Timothy Joseph Lackner Date: 11-26-15

FINAL APPROVAL

Certification of review and approval by the Institutional Animal Care and Use Committee:

Name: Signature: Date:

List any attachments here: