THE EFFECTS OF DIET AND SOCIAL ISOLATION AS EARLY-LIFE STRESSORS DURING DEVELOPMENT: A RODENT MODEL OF ANXIETY

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ABSTRACT

Anxiety is one of the most prevalent mental health disorders both globally and in the United States. Early-life stressors such as poor socialization and isolation, as well as diets high in fats and sugars have been shown to increase levels of anxiety and play a detrimental role on mental health. The present study sought to analyze the effects of both poor nutritional diet and social isolation on anxiety levels using an animal model. Eighty male Sprague-Dawley rodents at age five weeks at the beginning of testing were used to measure anxiety behaviors on the Elevated Plus Maze (EPM). Rodents were placed into one of four testing groups to assess anxiety: a control group, a high-fat and high-sucrose diet group, a socially isolated group, and an experimental condition in which social isolation and a high-fat, high-sucrose diet was given. It was hypothesized that Sprague-Dawley rodents living socially isolated and receiving the high-fat, high-sucrose diet would experience the high levels of anxiety, rodents living socially isolated would experience more anxiety than those receiving only the high-fat, high-sucrose diet, and rodents receiving the high-fat, high-sucrose diet would have higher levels of anxiety than the control group. Results showed that overall poor nutritional diet was not contributing to increased levels of anxiety in young male rodents, while social isolation was partially contributing to increased anxiety levels. Although many non-significant results were found, the importance of socialization and proper nutritional diet is explored, as well as the possible resiliency of young rodents to early-life stress.

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CHAPTER 1

INTRODUCTION

What we do and are exposed to during childhood can have a large impact on our health and well-being in adolescence and adulthood (McCormick & Matthews, 2007). The present study explores the relationship between the development of anxiety and stress from two early life stressors that can alter our physiology: poor nutritional diet consisting of foods high in fat and sugar content, and social isolation during the early developmental period.

The American Diet

In Western cultures such as the United States, a balanced diet is considered to consist of vegetables, fruits, whole grains, dairy, proteins, and healthy oils (American Heart Association, 2017). However, "healthy eating" is often seen as an expensive and time-consuming alternative that not all individuals or families are willing to make, and the sale of unhealthy food items can be viewed as a cheaper and faster alternative (Patsch, Smith, Liebert, Behrens, & Charles, 2016). As products such as these can be expensive to purchase, many families opt to consume their meals through fast-food chains selling burgers or chicken nuggets that are generally overly-processed with excess amounts of fat (Bauer et al., 2012). In addition to over-processed and fatty meats, these fast-food meals are usually eaten with large fountain drinks high in sugar, and another side of an overly processed and fatty food such as French fries. Not only that, but many fast food

organizations add copious amounts of sugar to their products to make them more palatable to consumers. Even outside of fast-food chains, Americans consume large quantities of foods high in fat such as red meat and poultry, with desserts and drinks high in sugar content such as bottled sodas, juice, ice cream, cookies, and candy (Schlosser, 2012). Much of the American diet has transitioned into a normalization of consuming foods that are high in both fat and sugar and making this acceptable as part of one's day-to-day routine. With more families consuming these types of foods and at younger and younger ages, the rate of disorders and diseases in America, associated with with both mental and physical health, continues to climb (Adair & Popkin, 2005).

In the United States today, the leading causes of death are chronic diseases such as heart disease, cancer, chronic lower respiratory diseases, and stroke, and the top worldwide causes of death are ischemic heart disease, stroke, and chronic obstructive pulmonary disease (Taylor, 2017). Although it is generally celebrated that society has moved away from infectious diseases being the leading causes of death, and that chronic diseases are considered the result of living longer, there is more than enough evidence to conclude that diet is connected to many of these chronic diseases. In today's society, nearly 2/3 of Americans (both adults and children) are overweight or obese, 10% of Americans have diabetes as of 2015 (not including those in the pre-diabetes stage), and one in every four deaths are due to heart disease (Centers for Disease Control and Prevention, 2017). The spike in these chronic diseases is partly due to an increase in life span from medical advances, but also from a shift in the way we consume our food. In 2004, fast food accounted for 15% of American's daily energy intake, 28% of Americans consumed fast food at least twice a week, and 40% of high-school aged adolescents ate

some form of fast food every day (Bauer et al., 2012). These numbers are likely even higher now, almost fifteen years later, however no newer data is currently in publication.

The fast food diet is notorious for being extremely high in saturated fats, excessive calories, sugar, and processed meats. Diets high in fat have been correlated with illnesses such as obesity, cognitive decline, Alzheimer's disease, neurodegenerative disorders, and higher levels of anxiety (Kielbinski et al., 2016). Omega-6 fatty acids in particular have been linked to a wide variety of health issues such as an increase in chronic inflammatory diseases like fatty liver disease, cardiovascular disease, and obesity (Patterson, Wall, Fitzgerald, Ross, & Stanton, 2012), as well as in the development of mental health disorders such as depression (Su, Huang, Chiu, & Shen, 2003). As Omega-6 fatty acids are the predominant fat source in many Western foods (Simopoulos, 2008), this emphasizes how high-fat diets may be contributing to decreased overall health. Diets high in sugar have also been correlated with obesity, metabolic disorders and changes in glucose metabolism (Fleur et al., 2014). Sugar addiction is also a point of concern and can keep us wanting more and more sugary food, which leads to consuming empty calories. In animal studies, rodents who were given the choice between cocaine and saccharine solutions had a strong preference for the sweet fluids over cocaine (Cantin et al., 2010). It is evident that not only is diet affecting our health, it is affecting our health behavior and possibly even altering the foods that we seek out. The more we seek out these fatty and sugary foods, the worse our physical and mental health may become.

Diet and Anxiety

Our diets have clearly been linked to diseases and leading causes of death in the United States such as cardiovascular disease and diabetes, however the connection

between diet and mental health is of concern as well. Anxiety and mood disorders are mainly thought of as genetically influenced or triggered by negative environmental events that occur in our lives (Smoller, 2017). More recently, however, environmental influences of anxiety are being examined, as anxiety and mental health are becoming an ever-increasing area of concern. The prevalence of anxiety disorders among adults has been increasing over the last several decades, affecting 10% of western cultures and 7% of the global population (Cohen-Cline, Turkheimer, & Duncan, 2015). Anxiety among children is also rising, and developing anxiety disorders at a younger age can have detrimental effects on development and learning. Anxiety sensitivity in children is associated with negative affect (Viana et al., 2015), indicating increased levels of anxiety during the developmental period also fosters more negative attitudes later in life. In addition, children are especially susceptible to increased harm from anxiety and stress in childhood due to disruption in physiological homeostasis (McCormick & Matthews, 2007). As neuronal circuits are developing in children, increased prevalence of anxiety can disrupt the developmental process resulting in complications such as behavioral issues and metabolic instabilities in adolescence and adulthood (Charmandari & Souvatzoglou, 2003).

A link between mental health in children and diets poor in nutritional value has also been found. There is a known additive effect when a child experiences both acute and chronic stress, and a nutritionally unbalanced diet; mood disorders, over-activation of the hypothalamus-pituitary-adrenal (HPA) axis, and obesity are among the main concerns when considering the interaction between diet and stress during childhood development (van Reedt Dortland et al., 2013).

Apprehensions regarding diet content are of great concern for developing children because those exposed to diets with poor nutritional content during developmental years are more likely to experience both physical and mental health issues in adulthood. (Lesser, Arroyo-Ramirez, Mi, & Robinson, 2017). Aside from the detrimental effects to mental health, nutritionally unbalanced diets have damaging effects on the physical development and wellbeing of children as well. Over-activation of the Enteric Nervous System through the intestine-brain-connection stimulated by excessive lipid consumption excites insulin sensitivity and circulates a surplus of nutrients (Wang et al., 2008), and diets high in fat and sugar increase fat storage (Appelhans et al., 2013), both of which increase the risk of obesity and diabetes (Bluher, 2013). Children of lower socioeconomic status (SES) could be at an even greater potential risk due to fewer resources and decreased access to nutritionally balanced food (Shih, Dumke, Goran, & Simon, 2013).

Research in Animal Models

Even with the current research on how diet is affecting our rates of disease and mental health, formulating studies and conducting experiments on human participants is highly unethical and violates natural human rights. In order to conduct further research on these matters, animal models using rodents are often implemented as a substitution to using human participants. Sprague-Dawley rodents have been used as animal models for dietary research as they share many similar dietary responses to sugars and fats that have been shown in humans. The Sprague-Dawley rodent is affected by a diet high in fat and sugar in much the same way as humans and has been shown to develop similar rates of obesity when given only these diets in comparison to control groups (Adam & Epel, 2007). Sprague-Dawley rodents are also highly social mammals and prefer to be housed

with other rodents, which makes their similarity to humans a more accurate model for use in research as well.

Research on high-fat diets in Sprague-Dawley rodents shows many of the same results as in humans; it results in higher rates of obesity, increased cognitive decline, impaired memory, increased rates of Alzheimer's Disease, and a higher prevalence of neurodegenerative disorders (Arcego et al., 2016). Rodents exposed to a diet high in sugar also show nearly identical dietary responses as in humans; higher rates of obesity, addictive-like behavior and preference for sucrose water, and increased weight gain. However, when Sprague-Dawley rodents on diets high in fat and sugar are assessed on their levels of anxiety, the findings are mixed as to if the levels increase or decrease. Maniam, Antoniadis, Le, and Morris (2016) found that rodents who were exposed to early life stress by limited nesting and given a high-fat, high-sugar diet after the weaning period showed a reduction in anxiety rates. Yet, another study showed that a high-fat, high-sugar diet reduced anxiety-like behavior in male rodents but not females (Lesser, Arroyo-Ramirez, & Robinson, 2017). Recent research appears to indicate a possible connection between decreased anxiety levels in Sprague-Dawley rodents and diets high in fat and sugar, which is contrary to current research exploring anxiety behavior and associations in humans. If the human model holds true for rodents, we would expect to see increased levels of anxiety when exposed to a nutritionally unbalanced diet comprised of high fats and sugars.

Stress During the Developmental Period

Although diet plays a large role in stress and anxiety levels in humans, there are other environmental factors that influence our levels of anxiety as well. Early life stress

such as social isolation is another predictor of increased risk for the development of anxiety disorders and mental health issues. Social isolation can lead to both anatomical and neurochemical changes in adulthood when experienced as a stressor during childhood development (Weiss et al., 2004), which in turn leads to increased prevalence of mental health issues, particularly anxiety disorders.

Primarily studied in rodent animal models (due to ethical reasons), isolation from social groups in adolescent rats is detrimental to developing and maintaining the ability to understand communication signals (Meaney & Stewart, 1981). The damaging effects of social isolation occur predominantly during the developmental period in rats from weaning to early adolescence (Arakawa, 2005). A rat isolated from its social counterparts during this developmental phase shows a reduction in the above-mentioned communication mechanisms and never regains these abilities even after being re-socialized and housed with other rodents for the remainder of its lifetime (Rosa et al., 2005). In addition, rats raised socially isolated also experience increased anxiety and aggression and decreased spatial memory (Einon & Morgan, 1977). These life-long changes resulting from deficits during pre-pubescent development in rodents can provide important insight into the risks human children may face growing up without sufficient access to social groups. Research on developing rodents living isolated from litter mates or other cage partners can provide important insight on how developing children struggling to create social groups may be affected later on during adolescence and into adulthood.

Social isolation has also been studied among humans as well as rodents, and research reveals many of the same effects found in rodents (Rubin, Coplan, & Bowker,

2009). Early life stressors from situations such as decreased social interaction can have detrimental effects on a developing child through disruption in the development of the nervous system and in alterations in neuroendocrine and behavioral processes (Hoeijmakers, Lucassen, & Korosi, 2015). Research also shows that high levels of stress during pre-puberty and in the adolescent years significantly altered stress responses and increased sensitivity to stressful stimuli (McCormick & Matthews, 2007). Social isolation is aversive, and therefore a stressor, and can alter our physiological responses in the same ways as mentioned above when experienced during primary developmental years.

Social isolation in humans is generally seen in the form of exclusions from social media, political activities, and cultural and economic groups, (Burchardt, 2000). The link between decreased social activity and increased mental health issues is well known, especially its correlation with increased rates of anxiolytic responses (Thapa, 2015). This connection is intuitive to most people, as everyone can likely relate to a time when fear of social abandonment created a great sense of stress and anxiety in one's life. Humans are social creatures, and naturally experience a great deal of stress when one's identity to a particular social group or even one's family is threatened.

Unfortunately, however, not everyone experiences these instances of social out-casting or isolation in the same rate. Groups identifying as non-white or those economically disadvantaged (such as low SES) have a larger reduction in intergroup relations which leads to increased rates of mental health issues among these groups, particularly higher levels of anxiety and increased frequency of anxiety disorders (Sonpar, 2015). Incidentally, low SES families and those of non-white ethnicity are also the most likely to choose fast food restaurants over a home-cooked meal, mainly due to

decreased environmental access to resources that could provide the means to increase consumption of healthier foods (Taylor, 2017). Grocery stores carrying higher quality foods are less likely to be found in low SES communities, but fast food restaurants can be accessed nearly everywhere. Stores that supply these higher quality foods such as Whole Foods are also perceived as pricier, and therefore less likely to be shopped at by low-income families. These low SES families and communities are regrettably the most at risk for the developmental detriments that can befall children who are not exposed to nutritionally balanced food and intergroup relations during childhood (Hughes et al., 2015). The combination of diets high in fat and sugar along with decreased social interaction is more than likely related to a detriment in psychological development. This in turn leads to an increase in anxiety levels leading up to adulthood and once adulthood is reached, especially in those populations most at risk for these factors.

While it is inherently important to work toward providing foods that create a well-balanced diet and opportunities for socialization to all developing children, how much diet and socialization contribute to these developmental deficiencies is in question. It is not known how long one must be socially isolated before these developmental changes begin, or how long a child must be exposed to high amounts of fat and sugar before the effects of elevated anxiety becomes observable. There are also implications of how both poor diet and decreased socialization lead to increased mental health issues in adulthood and future risk of anxiety disorders, but it is unclear whether one plays a larger role in the development of mental health issues, if diet or isolation is more prevalent among this risk factor, or if there is a synergistic effect between the two. The present study aims to begin to answer some of these questions.

The Present Study

The goal of the present study is to examine the relationship between social isolation and diets high in both fat and sugar content to determine their individual and additive effects on anxiety during early development. Both social isolation and poor diet have been shown to increase prevalence of anxiety among humans and rodents (Lukkes, Mokin, Scholl, & Forster, 2009; Mariam, Antoniadis, & Morris, 2015); however, they have not been explored together in a research setting. To examine both components jointly Sprague-Dawley rats will be used. Although a quasi-experimental design using human participants could be used to look at the relationship between isolation and diet, only correlational associations could be made between the two and no further research into which effect is more prevalent would be established. A laboratory setting with controlled conditions allows for better understanding of the causational relationship between socialization and diet and can provide more valuable conclusions than a correlational study in this context. As Sprague-Dawley rats have similar dietary responses to high sugar and fat content and are highly social animals (Adam & Epel, 2007), an animal model is appropriate for testing a subject area that would be unethical with human participants.

Although both diet and social isolation have been shown to increase mental health disorders and rates of anxiety, it is predicted that social isolation will have a stronger effect during the developmental period on anxiety than diet. To test this, Sprague-Dawley rodents will be placed into four groups: one group will receive a nutritionally balanced diet while being group housed with other rodents, a second group will also receive the nutritionally balanced diet but the rodents will be housed individually (in isolation), a

third group will receive a diet high in fat and sucrose while being group housed among other rodents, and a fourth group will receive the diet high in fat and sucrose but the rodents will be housed individually. I hypothesize that 1) rodents housed with a cage partner and receiving a nutritionally balanced diet will have the lowest anxiety of all the groups; 2) rodents housed without a cage partner and receiving a nutritionally balanced diet will have higher anxiety than the control group; 3) rodents housed with a cage partner and receiving a high-fat/high-sucrose (HFHS) diet will have more anxiety than the control group; and 4) rodents housed without a cage partner received the HFHS diet will have the highest anxiety levels.

CHAPTER 2

METHODS

Eighty male Sprague-Dawley rodents (Simonsen Laboratories, CA; 80—100 grams (g) at the start of testing) will be divided into four experimental groups: (1) the control group will consist of 20 rodents receiving the nutritionally balanced lab-chow diet, and will be group housed with other rodents; (2) the social isolation control group will consist of 20 rodents receiving the high-fat, high-sucrose (HFHS) diet, and will be group housed with other rodents; (3) the diet control group will consist of 20 rodents receiving the nutritionally balanced lab-chow diet, and will be housed in isolation; (4) the experimental group will consist of 20 rodents receiving the HFHS diet, and will be housed in isolation. Group housing conditions will consist of two rats per enclosure. Single housing conditions will consist of one rat per enclosure. Only male rats will be used in this study to reduce the variance associated with sex differences and hormonal fluctuations due to female rats' estrus cycles.

Animals will be housed in the California State University, Fullerton (CSUF) Humanities vivarium in room H-620 (John Chappell, Director of Animal Care), and cared for according to CSUF IACUC guidelines. Animal rooms are on a 12-hour light cycle, with lights on at 0700. Animals are provided with food and water *ad lib*, and are also inspected upon cage cleanings for any medical issues that may arise (e.g., tumors, external bleeding, etc.). Animals will be weighed weekly to ensure proper growth rates

and guarantee no animal falls below the average weekly growth rate (provided in the table below) produced by Charles River Laboratories

Table 1. Average Sprague-Dawley Rodent Growth Rates

Mean Weight	
Age (weeks)	Average Weight (g)
3	50
5	125
7	225
9	300
11	350
13	400
15	450

Procedure

The experimental procedure will involve randomly assigning Sprague-Dawley rats to one of the four conditions: 1) group housing with a nutritionally balanced diet; 2) group housing with a HFHS diet; 3) social isolation housing with a nutritionally balanced diet; 4) social isolation housing with a HFHS diet. A 2 x2 between-subjects factorial design will be used, and subsequent baseline activity rates and pre-test anxiety measures of each subject using the Open Field test will be established. All animals were five weeks old at the start of the study. The study will be 30 days in length, in which each subject is exposed to their grouping and diet environments for 30 days after having been tested for baseline activity rates. After 30 days, each rodent will be tested for anxiety levels using the Elevated Plus Maze (EPM). Video recording using a 360-degree high-definition camcorder will be utilized for both the OF Test and the EPM, so that assessments of

behavioral measures for each respective anxiety test can be scored both live in the lab as testing occurs, and post-testing to establish adequate reliability of scoring. Inter-rater reliability was established for research assistants scoring behavioral measures on both the OF Test and EPM. Cronbach's alpha for all scorers on each behavioral measure was found to be high for all raters ($\alpha > .95$). Each video was scored two times by two separate raters, all of whom had met adequate reliability before scoring any videos. Each scorer was trained by receiving a one-hour training session on proper assessment of behavioral measures for both the Open Field test and the Elevated Plus Maze.

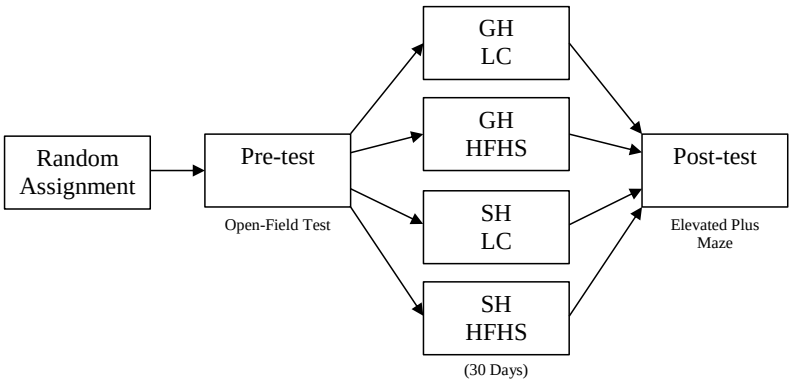

Figure 1. Experimental procedure and design. Group housed is notated as GH, single housed as SH, nutritionally balanced lab chow diet as LC, and high-fat, high-sucrose diet as HFHS.

Behavioral Assessment Measures

Open-Field Test

The Open Field Test consists of a square field with walls to prevent animal escape but no ceiling to observe animal behavior. The field is marked with 9" x 9" grid marks

with red waterproof tape on the bottom to create equal sized square crossings. The open field test will be used to assess anxiety on three behavioral measures: grid line crossings (the frequency with which the rodent crosses a grid line with all four paws), the total time spent in the center (the amount of time the rodent spends in the center squares of the field), and rearing (the frequency with which the rodent stands on hind legs with front paws completely lifted off the ground in any part of the open field). An increase in each of these behavioral measures is indicative of decreased anxiety (Goma & Tebeña, 1978). Each subject will be gently placed into the lower right corner of the field and videotaped for five minutes to assess the behavioral measures. The field will be cleaned with 10% ethanol in between each test to remove the smell and any residual fur of previous animals.

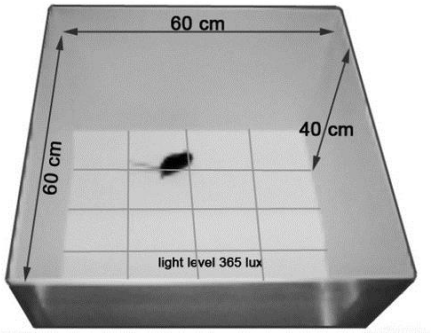

Figure 2. Open-Field Test diagram.

Elevated Plus Maze

The Elevated Plus Maze (EPM) is a plus-shaped apparatus elevated off the ground with two open arms and two equally sized enclosed arms, connected by a central zone. The EPM will be used to assess anxiety on four behavioral measures: frequency of open

arm entries, frequency of enclosed arm entries, time spent in open arms, and time spent in enclosed arms. All entries are defined by the subject placing all four paws into one arm of the maze. Increased frequency of exploration into open arm entries is indicative of decreased anxiety, while remaining in closed arm entries is a behavioral indication of increased anxiety (Hogg, 1996). Subjects will gently be placed into the central zone facing an open arm to begin each trial and videotaped for five minutes to assess the behavioral measures. The EPM will be cleaned with 10% ethanol in between each test to remove the smell of previous animals.

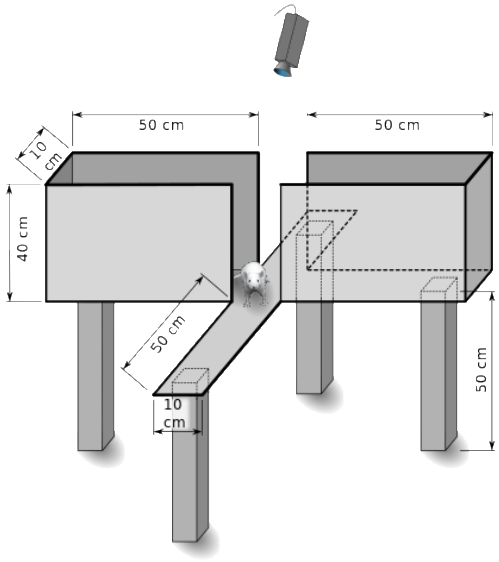

Figure 3. Elevated Plus Maze diagram.

Testing Conditions

Diet

Two different diets were utilized in the present study. The diet to emulate the western American diet is comprised of saturated fatty acids with 30% total fat, of which 15% is saturated fat, and 35% sucrose (pellet code: D11112201, Research Diets, Inc.). Rodents exposed to the HFHS diet with group housing, and the HFHS diet with social isolation housing received this diet. The standard lab chow diet is nutritionally balanced and comprised of 23% crude protein and 4.5% crude fat (pellet code: 5001, Lab Diets, MO). Rodents exposed to the nutritionally balanced diet and group housing condition, and the nutritionally balanced with social isolation housing condition received this diet.

All animals were fed on a daily *ad lib* schedule and allowed to eat as much as was desired. Restrictions on food access were not put into place as young rodents need to increase their food consumption as they grow. To ensure that all animals were receiving enough food to meet average weights, an *ad lib* feeding schedule was determined to be the best choice. This diet also makes the most sense in reproducing similar effects to that of the American diet, in which most parents allow their children to eat as much as desired until satiated levels of hunger have occurred.

Isolated vs. Group Housing

Rodents housed in both the group and socially isolated conditions were kept in separate rooms in the animal vivarium. Animals housed together with another rodent were kept in one room with all other animals also being group housed, regardless of their diet condition. Animals in the socially isolated conditions were all also kept in one room together, however the housing containers were opaque, and all animals could not see one

another. All socially isolated animals were also kept in the same room together regardless of their diet condition. Other than instances in which cages needed to be cleaned, or during the daily feeding and replenishing of water supply, socially isolated rodents did not have contact with other animals or humans. Group housed rodents were not exposed to any additional socialization other than their rodent cage partner.

All rodents over the course of the testing period were found to be in generally good health and experienced no health problems. All rodents maintained appropriate weights for their growth weights. Although experiencing the early-life stressors of social isolation, poor nutritional diet, or both, rodent weights were not found to exceed or fall below the expected averages and did not greatly vary from the weights of the rodents in the control group. Rodents in all groups generally gained weight at the same rate and were on target with the recommended growth weights.

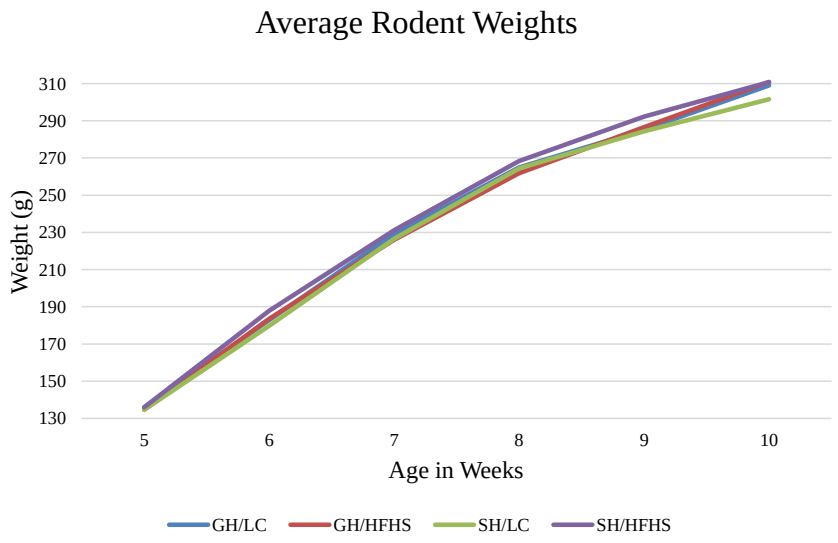

Figure 4. Average rodent weights in grams by week of age.

CHAPTER 3

RESULTS

A 2 x 2 between-subjects multivariate analysis of variance (MANOVA) was performed on four dependent behavioral assessments, measured from the EPM: the number of open arm entries made, the number of closed arm entries made, the total time spent (in seconds) in the open arms, and the total time spent (in seconds) in the closed arms. Independent variables were diet (nutritionally balanced and HFHS), and isolation housing (no isolation and isolation). The Roy-Bargman Stepdown Analysis was performed in conjunction with the MANOVA analysis to account for shared variance across the dependent variables. A multivariate analysis of covariance (MANCOVA) was also performed on the above-mentioned independent and dependent variables, with the addition of each of the behavioral measures assessed during baseline anxiety testing using the Open Field Test: the total number of rears, the total number of gridline crosses, and the total time spent in the center squares of the field.

MANOVA Procedure

Normality Assumptions

IBM SPSS MANOVA was used for the analyses with the sequential adjustment for nonorthogonality. Order of entry of independent variables was isolation, then diet. The total sample *N* was 80, with 20 rodents in each of the four experimental conditions.

Following the guidelines outlined for MANOVA in Tabachnik and Fidell (2012), both univariate and multivariate assumptions of normality were assessed.

Univariate normality was assessed by examining the distributions of histograms, Q-Q plots, detrended normal Q-Q plots, and boxplots. Some issues of normality were found in the number of open arm entries, the amount of time spent in the open arms, and the amount of time spent in the closed arms. However, to fully capture any and all effects of anxiety across all groups, no transformations were performed to correct skewed distributions. Q-Q plots and detrended normal Q-Q plots indicated that all distributions were generally linearly associated. Univariate outliers were assessed examining z-score frequency tables, upon which outliers were found in the distributions for the number of open arm entries, the amount of time spent in open arms, and the amount of time spent inside the closed arms. Outliers were found in the same distributions as those that were also found to violate normality, possibly due to differences in group anxiety levels. No correction to outliers were made to retain any effects of group differences in anxiety levels.

Multivariate normality analyses were also performed, upon which a possible singularity issue was found in the amount of time spent inside the closed arms. Multivariate outliers were also found, most likely because no corrections to univariate normality and outliers were made. Homogeneity of the variance-covariance matrices was met, Box's M Test, $p = .075$. Linearity between dependent variables was found to be met after assessing scatterplots and Q-Q plots. The absence of multicollinearity and singularity was tested for using a correlation matrix and collinearity diagnostics. The time spent in open arms correlates at a rate higher than 0.9 with the number of open arm

entries, indicating a possible singularity issue. However, the MANOVA analysis works optimally with highly correlated dependent variables and negative correlations, which are present in the current data, so this assumption is generally found to be met. It should also be noted that although several violations of normality were reported, the MANOVA is a fairly robust procedure controlling highly for Type I error rate, so use of the statistical test is still effective (Grimm & Yarnold, 1995).

Table 2. Correlations Between Dependent Measures on EPM

Condition	1	2	3	4
1. Open Arm Entries	1.0			
2. Closed Arm Entries	0.333**	1.0		
3. Time in Open Arms	0.926**	0.289**	1.0	
4. Time in Closed Arms	-0.773**	-0.497**	-0.812**	1.0

Note: All correlations are based on $N = 80$.
**$p < .01$.

Univariate Analysis

The test of Between-Subjects Effects revealed several statistically significant univariate main effects for the type of housing (isolation vs. group housed): the number of open arm entries, $F(1, 76) = 8.369$, $p = .005$, $\eta^2 = .097$, the time spent inside the open arms, $F(1, 76) = 9.643$, $p = .003$, $\eta^2 = .111$, and the time spent inside the closed arms, $F(1, 76) = 6.251$, $p = .015$, $\eta^2 = .073$, all had significant univariate main effects, whereas the number of closed-arm entries was not found to have a statistically significant main effect, $F(1, 76) = .751$, $p = .389$, $\eta^2 = .009$.

The type of diet given (nutritionally balanced vs. HFHS) was found to have only one statistically significant univariate main effect, the total number of closed arm entries

made, $F(1, 76) = 6.429$, $p = .013$, $\eta^2 = .077$. The total number of open arm entries made, $F(1, 76) = 1.281$, $p = .261$, $\eta^2 = .015$, the total time spent inside the open arms, $F(1, 76) = .882$, $p = .351$, $\eta^2 = .010$, and the time spent inside the closed arms, $F(1, 76) = .034$, $p = .077$, $\eta^2 = .038$, were all found to be non-significant main effects.

Table 3. Univariate Results

Between-Subjects	Within-Subjects	DF	F	*η2*	*p*-value
Isolation	Open Arm Entries	1, 76	8.369	.097	.005**
	Closed Arm Entries	1, 76	.751	.009	.389
	Time in Open Arms	1, 76	9.643	.111	.003**
	Time in Closed Arms	1, 76	.251	.073	.015*
Diet	Open Arm Entries	1, 76	1.281	.015	.261
	Closed Arm Entries	1, 76	6.429	.077	.013*
	Time in Open Arms	1, 76	.882	.010	.351
	Time in Closed Arms	1, 76	3.207	.038	.077

Note. $N = 80$. DF = degrees of freedom.
*$p < .05$, **$p < .01$

Simple Comparisons Testing

In order to evaluate the statistically significant univariate main effects further, simple comparisons testing was conducted in order to determine which groups were contributing the most to the univariate main effects. Group means were evaluated for the total number of open arm entries made, the time spent in the open arms, and the time spent inside the closed arms to evaluate which group was contributing to the univariate effects. Rodents who were group housed made significantly more open arm entries ($M = .80$, 95% CI [.45, 1.15], $p = .005$), than rodents who were housed socially isolated ($M = .23$, $SD = 36.12$), and also spent significantly more time in the open arms ($M = 8.59$, 95%

CI [4.78, 12.40], p = .003) than rodents who were socially isolated (M = 2.11, SD = 5.52). This data supports Hypothesis 2 that socially isolated animals would display higher levels of anxiety than rodents who were group housed. In addition, socially isolated animals spent more time inside the closed arms (M = 283.09, 95% CI [277.99, 288.20], p = .003), than animals that were group housed (M = 271.02, SD = 26.37), which also supports Hypothesis 2.

To evaluate the statistically significant main effect of total closed arm entries made on diet, simple comparisons testing was also performed. Rodents who were given the HFHS diet made significantly more closed arm entries (M = 7.33, 95% CI [6.24, 8.41], p = .013), than rodents who were given the nutritionally balanced diet (M = 5.35, SD = 3.53), which does not support Hypothesis 3, that rodents given the HFHS diet would experience higher levels of anxiety than those given the nutritionally balanced diet.

Interactions

There were no statistically significant interactions found between isolation housing and diet on behavioral measures of the EPM. The total number of open arm entries, F(1, 76) = .396, p = .531, η^2 = .005, the total number of closed arm entries, F(1, 76) = .050, p = .823, η^2 = .001, the time spent in the open arms, F(1, 76) = .172, p = .679, η^2 = .002, and the time spent inside the closed arms, F(1, 76) = .034, p = .854, η^2 <.001, were all found to be statistically non-significant. This finding does not support Hypothesis 4, which predicted that rodents exposed to both the socially isolated housing in combination with the HFHS diet would show the highest levels of anxiety when compared across all groups.

Table 4. Simple Comparisons Testing Results

Between-Subjects	Group	Within-Subjects	DF	Univariate F	*η2*	*p*
Isolation	GH	Open Arm Entries	1, 76	8.369	.097	.005**
	GH	Time in Open Arms	1, 76	9.643	.111	.003**
	SH	Time in Closed Arms	1, 76	.251	.073	.015*
Diet	HFHS	Closed Arm Entries	1, 76	6.429	.077	.013*

Note. Group housed is notated as GH, Single housed is notated as SH, and high-fat, high-sucrose diet is notated as HFHS, DF = degrees of freedom.
*p <. 05, **p < .01.

MANOVA Analysis

Although several univariate main effects were found, further analysis of the univariate *F* tests must be performed because of the high correlations among dependent variables (Tabachnik & Fidell, 2012). A Roy-Bargmann Stepdown Analysis was performed to separate the shared variance between the dependent variables. All dependent variables were ordered into the model in descending order of importance: the time spent in the open arms, the total number of open arm entries, the time spent inside the closed arms, and the total number of closed arm entries. In the stepdown analysis each dependent variable was analyzed, in turn, with higher priority dependent variables treated as covariates and with the highest-priority dependent variable tested in a univariate ANOVA.

None of the dependent variables were found to make a unique contribution to predicting differences between rodents given both a nutritionally balanced diet and those given a HFHS diet. There were no group differences between the total time spent in the open arms, stepdown $F(1, 76) = .882$, $p = .351$, Pillais' Trace = .155. the number of open

arm entries made, stepdown $F(1, 75) = .448$, $p = .505$, or the total amount of time spent inside the closed arms, stepdown $F(1, 74) = 2.67$, $p = .106$. These findings are consistent with the univariate results reported above and are not surprising. However, when analyzed with the stepdown analysis, the significant univariate main effect for an increase in the number of closed arm entries made when given a HFHS diet is no longer present, stepdown $F(1, 73) = 2.75$, $p = .101$. Although there was a significant univariate difference between diet groups for the number of closed arm entries made, this finding was likely attributed to the shared variances across the dependent variables and is not a statistically significant finding once all of the shared variance is accounted for.

When examining the effects of the stepdown analysis on the isolation condition, only one of the dependent variables was found to be making a unique contribution to predicting group differences between rodents housed with other rodents, and those housed socially isolated. After the stepdown analysis, the total time spent in the open arms remained a statistically significant main effect, stepdown $F(1, 76) = 9.64$, $p = .003$, Pillais' Trace = .065, with group housed rodents spending significantly more time in in the open arms ($M = 8.59$, $SD = 11.91$) than those who were housed socially isolated ($M = 2.11$, $SD = 5.52$). However, the total time spent in the open arms became non-significant, stepdown $F(1, 75) = .012$, $p = .911$, as well as the total time spent inside the closed arms, stepdown $F(1, 74) = .001$, $p = .977$, and the total number of closed arm entries made, stepdown $F(1, 73) = .001$, $p = .970$. This is also contrary to the univariate findings in which the total number of open arm entries and the time spent inside the closed arms was statistically significant. After accounting for the shared variance across the dependent

variables, only the total time spent inside the open arms had a statistically significant effect on differences in anxiety between housing conditions.

Similar to the univariate findings, the stepdown analysis also shows no statistically significant differences for interactions between diet and social isolation. The total time spent in the open arms, stepdown $F(1, 76) = .173$, $p = .679$, the number of open arm entries, stepdown $F(1, 75) = .379$, $p = .540$, the total time spent inside the closed arms, stepdown $F(1, 74) = .807$, $p = .372$, and the total number of closed arm entries made, stepdown $F(1, 73) = .004$, $p = .950$, where all statistically non-significant findings. This finding in the stepdown test is not surprising, as we would expect non-significant univariate findings to remain non-significant when all shared variance is accounted for.

Table 5. Roy-Bargmann Stepdown Analysis Results

IV	DV	DF	Stepdown F	*p*-value
Isolation	Time in Open Arms	1, 76	9.642	.003**
	Open Arm Entries	1, 75	0.012	.911
	Time in Closed Arms	1, 74	0.001	.977
	Closed Arm Entries	1, 73	0.001	.97
Diet	Time in Open Arms	1, 76	0.882	.351
	Open Arm Entries	1, 75	0.448	.505
	Time in Closed Arms	1, 74	2.67	.106
	Closed Arm Entries	1, 73	2.75	.101
Isolation* Diet	Time in Open Arms	1, 76	0.172	.679
	Open Arm Entries	1, 75	0.379	.54
	Time in Closed Arms	1, 74	0.807	.372
	Closed Arm Entries	1, 73	0.004	.95

Note. IV = independent variable. DV = dependent variable. DF = degrees of freedom.
**$p < .01$.

MANCOVA

In addition to the MANOVA analysis, a MANCOVA was also performed using the behavioral measures from the Open-Field Test for baseline anxiety rates as a covariate. Both univariate and multivariate assumptions of normality were assessed, in which all dependent measures of the OF Test were found to be met.

The addition of a covariate to the model did not produce any changes in significant univariate main effects or any new additions of statistically significant interactions. All three behavioral measures from the Open-Field Test (the total number of rears, the total number of gridline crosses, and the total time spent in the center squares produced only slight changes in the *p*-values. All significant univariate main effects remained statistically significant, and all non-significant main effects remained statistically non-significant. This suggests that the Open-Field Test may not be an accurate measure for baseline anxiety to be used as a covariate. The use of the Roy-Bargman stepdown analysis was not employed because there were no additional findings reported in the MANCOVA that warranted further follow-up. As the results in the univariate MANOVA analysis did not differ greatly from the univariate MANCOVA analysis, the Roy-Bargman stepdown analysis was not needed.

CHAPTER 4

DISCUSSION

Overview of Hypotheses

Hypothesis 1

In general, the results of the present study showed that Hypothesis 1 was generally supported. Rodents who were exposed to both the nutritionally balanced diet and group housing with other rodents had the lowest levels of anxiety in comparison to all other groups, particularly when comparing socially isolated rodents with group housed rodents. When examining trends in group differences, rodents that were group housed overall spent more time in the open arms of the EPM and made more open arm and closed arm entries. Even though the control group was not demonstrating a significantly lower amount of anxiety than the treatment groups on all dependent measures of anxiety, overall results showed the control group had slightly lower anxiety levels than all other groups.

Hypothesis 2

Hypothesis 2 was found to be partially supported, with support found on one behavioral measure of the EPM. Socially isolated rodents spent significantly less time inside the open arms than group houses rodents, indicating that rodents spent fewer instances exploring in an exposed area, indicating higher levels of anxiety. Rodents who explore more their environments more are said to feel less anxious and experience less

stress, and the results reporting that socially isolated rodents spent less time engaging in exploratory behavior into the open arms also aligns with previous research and supports the hypothesis.

There were no significant differences among socially isolated and group housed rodents on measures of the total amount of open arm entries made, although in general socially isolated rodents made fewer open arm entries than group housed rodents. Rodents who explore more in their environment are said to feel less anxious and experience less stress, which makes sense knowing that overall socially isolated rodents spent less time exploring the open arms. Although group housed rodents did make more overall open arm entries than socially isolated rodents, the results implicating that these group differences are non-significant is surprising and does not support Hypothesis 2. Socially isolated rodents also spent more overall time inside the closed arms than group housed rodents, however this finding was also non-significant and does not support Hypothesis 2. It was believed that this finding would be significant because more time spent in the closed arms of the EPM is indicative of less exploratory behavior which indicates greater levels of anxiety. However, the results of the study found that even though socially isolated rodents spent more time inside the closed arms, group differences were not significant, and therefore Hypothesis 2 was not supported by this behavioral measure.

There were also no significant differences found among socially isolated and group housed rodents on scores of the total number of closed arm entries made, although group housed rodents overall made slightly more closed arm entries than those who were living socially isolated. It was expected that socially isolated rodents would make more

closed arm entries than group housed rodents, which the results did not show. Although statistically there were no significant differences between the number of entries made, socially isolated rodents averaged fewer closed arm entries than the group housed rodents. One reason this might appear in the findings is because socially isolated rodents were spending significantly more time inside the closed arms, and likely not moving as frequently from one closed arm to the other, but rather staying in one arm for the length of the EPM trial. This finding would make sense in accordance with what is known about rodents who freeze when they are fearful, stressed, or anxious (Shumake & Monfils, 2015). Socially isolated rodents may have been experiencing high levels of anxiety that caused them to go into the closed arms and spend the majority of their time there, with little exploration into other areas of the EPM. If that is truly the case, then this finding would support Hypothesis 2, because socially isolated rodents were displaying higher levels of anxiety than group housed rodents by spending more time inside the closed arms and spending less time exploring the maze.

Hypothesis 3

Hypothesis 3 was found to be unsupported by the results of the study. Rodents exposed to the HFHS diet did not significantly differ from those given the nutritionally balanced diet on any of the dependent measures. Although non-significant, the rodents on the high-fat, high-sucrose diet actually made more overall entries into the closed arms than those on the nutritionally balanced diet, which is contrary to the research expectations, as previous research shows that a high-fat and high-sugar diet should increase anxiety and decrease the number of entries made into the closed arms. This finding is possibly explained by the fact that the diet these rodents were exposed to

contained 35% sucrose, and excessive sucrose consumption has been linked to higher levels of activity in rodents (Franklin, Wearne, Homewood, & Cornish, 2017). Although increased sugar in the diet leads to neuroinflammation which in turn also leads to higher levels of anxiety, it is possible that the effects of the inflammation may take longer than the 30 days the rodents were exposed to the diet to appear, and that increased activity from sugar is a more immediate and observable effect. It is possible that any observable effect of anxiety was not readily apparent due to excessive activity levels from the high degree of sugar in the diet.

Rodents exposed to the high-fat, high-sucrose diet did not vary significantly in comparison to those on the nutritionally balanced diet, although overall they tended to make more open arm entries, spend more time in the open arms, and spend less time in the closed arms. These findings also do not align with the predicted outcome of Hypothesis 3, although they may also be explained by the increased activity due to increased sugar consumption as noted above. The high-fat, high-sucrose diet rodents possibly had overall grater exploratory behavior than those given the nutritionally balanced diet, and thus made more overall arm entries and spent more time in either of the open or closed arms, as opposed to having a preference for one or the other.

Hypothesis 4

Hypothesis 4 was also not found to be supported by the data, as there were no significant interactions between housing and diet effects found. This finding is surprising and contrary to previous research. It is known that both social isolation and poor diet are associated with greater levels of anxiety, so it is expected that rodents exposed to both of these conditions would have significantly greater levels of anxiety than other groups

exposed to only one or none of these conditions. One possible explanation for this may be that social isolation produces more immediate effects of anxiety, especially for social creatures such as rodents, while the anxiolytic effects of poor diet may take longer to be expressed and were not captured within the time frame of the present study. It is possible that rodents exposed to both social isolation and a HFHS diet for longer than the experimental 30 days would begin to display a larger synergistic effect of anxiety.

Limitations

The present study has several limitations, one of which was scoring the results of the EPM and Open Field Test without the simultaneous video-tracking and recording system that is used in more advanced research laboratory settings. Although all behavioral measures on each of the tests were scored by raters who were approved with acceptable inter-rater reliability, there will always be some human error that is unavoidable. It is possible that with the use of an advanced video tracking system that tracks and assesses animal movement and does not rely on semi-subjective human decision making, some results in the study would have been more refined and possibly more accurate than in the current research.

Another limitation to the present study is that rodents who were socially isolated were all kept in the same room together, and although the isolation housing containers were opaque, and the rodents could not see each other, they were still able to hear other rodents' movements and smell their scents in the same room. In addition, socially isolated rodents were also exposed daily to the laboratory technician responsible for ensuring adequate food and water levels in each cage. In more ideal conditions, rodents exposed to isolation housing would be separated into individual rooms where all isolated

rodents would not be able to sense any other animals nearby. Space in the animal vivarium was limited to only specific areas and being able to sense other rodents nearby may have slightly reduced the effects of the isolation condition.

Another limitation to the study was not being able to expose the young rodents to the testing conditions immediately after being weaned from their mothers. Rodents had to be shipped to the university vivarium in groups before any testing could begin, and thus rodents were exposed to one another and were able to socialize with other rodents after the weaning period. This early socialization, even though brief, may have played a role in some of the non-significant findings of isolation housing effects. In more ideal conditions, colonies of rodents would be born inside the lab, and testing conditions could occur immediately after young rodents were weaned from their mothers.

One final limitation to the present study was any possible human error that was present in the laboratory testing room during the Open Field and EPM testing. Under ideal conditions, all animals would be placed into their respective testing fields, and experimenters would leave the room to reduce any human bias, and animals would be scored on behavioral measures with a video-tracking system. However, due to financial restrictions, this set-up was not possible, and experimenters were required to stay inside the testing room while rodents were undergoing their tests inside the apparatus. Any slight noises or movements seen or witnessed by the animal could have introduced bias into the results of the study. Although all experimenters were properly trained on appropriate behavior while inside the testing room, some variance in results associated with human error is unavoidable.

Future Considerations

Several considerations regarding the current study could be made in the future to help better understand the results and further pull apart the effects of isolation and poor nutritional diet. The first modification could be to further break down the effects of the high-fat, high-sucrose diet used, and instead have two separate diet groups, one which receives the high-sucrose diet and the other which receives the high-fat diet. This would help further break down the effects of how poor nutritional diet contributes to mental health and doing so may explain why no significant effects of diet on anxiety were found. Current research has studied the effects of how diets high in both fats and sugars are detrimental to physiological and mental health (Francis & Stevenson, 2011), and how diets high in fat may be more detrimental to mental health and anxiety disorders than diets high in sugars (Sasaki, de Vega, St-Cyr, Pan, & McGowan, 2013). Having side by side comparisons of these effects in animals would allow for further separation between dietary effects and their consequences on mental health. It is possible that if one nutritional component is contributing to decreased anxiety and mental health more than the other, than the effects of this were not evident in the current study because high fat and high sugar components were combined. Separating these two factors would allow for a more meaningful analysis and deeper understanding of how diet may be affecting overall health and anxiety.

More longitudinal research should also be done on how the effects of social isolation and diet contribute to levels of anxiety and rates of other mental illnesses later on in life. There is some evidence to suggest that the childhood and adolescent years may be a window of vulnerability in terms of being susceptible to certain environmental

stimuli that may increase overall prevalence of mental illness (Baker, Loughman, Spencer, & Reichelt, 2017). The current study was only 30 days in length, and therefore long-term effects of the isolation and diet manipulations in these animals is currently not known. Future research dealing with early-life stressors should follow up at varying time intervals later on in life to determine whether or not levels of anxiety or other mental illnesses have increased, decreased, or remained the same.

There is also some evidence to suggest that young rodents may be more resilient to the effects of early-life stress from social isolation, which can be affected by the young rodent's experience of maternal behavior (Beery & Kaufer, 2015). As the current study had no contact with rodent mothers and the nurturing bond between the mother and her pups was unobservable, this is one factor that cannot be accounted for in the current study but may be contributing to the non-significant findings of social isolation. If the young rodents had an especially nurturing bond with their mother, it is possible they may be more resilient to early-life stress and anxiety associated with social isolation. If this is the case, it would explain why almost all effects of anxiety from social isolation were non-significant. This however has valuable insight if the same concept is applicable toward human children. If creating a more nurturing environment and bond between mothers and children fosters resilience to early-life stress, this is valuable information to research. The opposite would also be true; it could be valuable to understand whether a non-nurturing mother-child bond may increase the susceptibility of a child to early-life stress and possibly contribute to higher levels of anxiety and mental health issues.

Future research should also examine the effects of socialization and poor diet across both male and female rodents. The current study used only male rodents to control

for the variability of female rodents' estrus cycles; however, having data that compares anxiety levels between both males and females would be valuable, as there is some evidence to suggest that there are gender differences in how males and females are affected by these conditions. There is evidence to suggest that diets high in fat affect hippocampal neurogenesis in male rodents but not females (Lindqvist et al., 2006), which would suggest that male rodents' memory may be more affected by a high-fat diet rather than anxiety levels, and females less so. Other research also points to evidence that females may be especially vulnerable in comparison to males to changes in neuroendocrine responses from social isolation, which lead to higher rates of depression (Grippo et al., 2007), which is correlated with anxiety as well. Understanding the sex differences between how males and females may be affected differently by both diet and socialization may lead to better understanding of how these underlying mental health issues can be prevented in the future.

One final consideration for future research is to increase the time interval that rodents spend in each of the conditions beyond the 30 days studied in the current analysis. This would be beneficial to further examine the effects of how poor nutritional diet and isolation may be contributing to increased levels of anxiety. There is current research to suggest that anxiety related to social stress appears fairly quickly (Shetty & Sadananda, 2017), but research studying whether anxiety continues to increase the longer the social isolation period goes on has not been researched fully. This may explain why some significant effects of isolation were present in the study, but not all. An increase in the length of social isolation would bring further insight into whether the stressful effects of social isolation increase over time, or if they produce a ceiling effect.

Time intervals of manifestations of anxiety from dietary effects seems to be less consistent and less researched as well. Studying how early-life stress is affected when exposed to the stressor for longer and longer periods of time would be valuable information to understanding if increased anxiety and poor mental health is positively associated with the length of time one maintains a poor nutritional diet. This would give better insight into whether chronic diets high in fats and sugars can produce detrimental mental health effects only up to a certain point, or if they can continue to have deteriorating effects over time.

Conclusions

Overall, the present study found that social isolation increased rates of anxiety somewhat in comparison to rodents who were group housed, whereas a diet high in fats and sugars did not appear to contribute to increased rates of anxiety behavior in rodents. Although the effect of social isolation on anxiety was only partially significant, the importance of isolation as a form of early-life stress remains. The results of this study reported only on how social isolation was affecting young rodents, and the implication of social isolation in adulthood and disadvantaged populations remain prevalent in current research. As previously mentioned, it is also possible that younger rodents may be more resilient to early-life stressors to begin with, but the full detrimental effects do not become apparent until later on in life. If this is the case, society may be misinterpreting early-life stressors on children as not having any sort of damaging effect, when in reality these effects may show up later in life, during adolescent years when the vast majority of symptoms of mood and mental health disorders truly begin to present themselves (Merikangas et al., 2010). Preventative programs to ensure social isolation is experienced

as little as possible by children should continue to be put into place, such as after-school programs, anti-bullying seminars, increased funding for the arts and sciences to create inclusive clubs and organizations, and teaching general acceptance and inclusion of students and peers. Although preventing early-life social stress from social exclusion is not likely to be completely preventable, increasing prevention strategies and targeting groups that may be more susceptible is increasingly beneficial.

Despite finding no significant effect for diets high in fats and sugars increasing anxiety, it is important to speak to the effect that diet can have on general health, which in turn can inadvertently affect mental health as well. The promotion of healthier diets is a societal step that needs to occur, especially as the rate of obesity and cardiovascular health risks continue to climb. The leading causes of death in the United States continue to be diseases such as Coronary Heart Disease (Taylor, 2017) that are preventable when consuming nutritionally balanced diets low in fats and sugars, which contribute to prevention of clogged arteries that lead to heart disease. Health issues related to poor diet also cause more frequent hospitalizations and doctor trips (Syddall, 2016), which can decrease the mental well-being of the person undergoing ill health (Celano, Villegas, Albanese, & Huffman, 2016). Although in the present study poor nutritional diet was not directly affecting anxiety levels, there is something to be said about how poor diet can contribute to overall decreased health, which leads to a greater number of doctor visits and hospitalizations, which can lead to decreased well-being and mental health. Individuals who frequent doctor's offices due to decreased health experience more negative affect and are more pessimistic, which has been tied to decreased mental well-being (Plomin, et al., 1992). Thus, diet is indirectly affecting mental health and anxiety

rates as well. By being conscientious about the nutritional value of food being consumed, early prevention of health risks like heart disease associated with poor dietary choices can lead to fewer adverse health conditions to feel anxious about.

In conclusion, although social isolation and poor nutritional diet do not appear to be directly increasing levels of anxiety in young rodents, both early-life stressors still have important roles to play in American society and may still play an important preventative role in protecting against future increases in anxiety disorders and to decrease mental health disorders. Further research can be done in both areas to fully pull apart the effects of socialization and diets with poor nutritional content and refine any effects they may be having which could lead to a better understanding of how to treat and prevent mental illness.

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