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The Effects of a High Fat Diet and Lithium on Social and Anxiety-like Behaviors in Bipolar and Autistic Mouse Strains

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Submitted in Partial Completion of the
Requirements for Commonwealth Honors in Biology

Bridgewater State University

May 9, 2017

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The Effects of a High Fat Diet and Lithium on Social and Anxiety-like Behaviors in both Bipolar and Autistic Mouse Strains

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Honors Thesis

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May 9, 2017

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1. Abstract

Bipolar Disorder (BD), or manic-depressive illness, is a psychiatric disease in which an individual experiences sudden changes in mood, activity, and overall energy. Individuals with the disorder switch between excessive joy and excitement, and extremely sad and hopeless episodes. A common treatment of BD is lithium, a mood stabilizer that helps to relieve manic symptoms. Lithium has been known to contribute to the onset of diabetic-like symptoms, in addition to a large correlation, up to three times higher risk, of developing Type II Diabetes in individuals with BD. In general, individuals who consume a high fat diet (HF) are often seen to have an increased possibility of developing Type 2 Diabetes Mellitus. This type of diabetes affects the way one's body is able to modulate blood glucose levels, which can lead to hyperglycemia and weight gain. Another mood disorder, Autism Spectrum Disorder (ASD), is characterized by a lack of social behaviors and often includes symptoms of anxiety, depression, and obsessive-compulsive disorder. Often treated using similar methods to that of BD, similar comparisons can be made due to the exposure of a high fat diet and lithium. This thesis tested the effects of a high fat diet and lithium consumption on anxiety, social behavior, and diabetic symptoms in both Black Swiss mice, a strain that mimics the manic episodes of BD, and Balb/cJ mice, a strain that mimics behavior associated with ASD. The Balb/cJ mice became less social when on lithium and diet had no effect. They also were resistant to diabetic symptoms as the HF did not make them gain weight nor did it alter glucose tolerance or insulin levels. The Black Swiss had no differences based on liquid or diet socially, but did exhibit decreased anxiety when on lithium. They had increased insulin and cholesterol levels however, no increase to body weight, glucose or triglyceride levels. The two strains were affected differently by alterations to diet and liquid, but since they lack a genetic connection, cannot be compared closer.

2. Introduction

2.1 Bipolar Disorder

Bipolar Disorder (BD), or manic-depressive illness, is a psychiatric disease affecting 1% of the population in which an individual experiences sudden changes in mood, activity, and overall energy (NIMH 2015). Those with the disorder alternate between manic and depressive episodes. During a manic episode, an individual experiences excessive energy, increased activity and risk-taking behavior, difficulty sleeping, extreme irritability, increased sociability, and a "high" feeling (Girardi et al. 2016). A depressive episode is much the opposite in which individuals have decreased energy, trouble concentrating, worried and empty feelings, increased sleeping, and overall sad, hopeless, and suicidal thoughts. These episodes can also happen simultaneously which is categorized as a "mixed" episode (Girardi et al. 2016).

There are three types of the disorder. Bipolar I Disorder is defined by full blown manic episodes that last at least a week or requires hospitalization due to severe manic symptoms. Depressive episodes are always accompanied and are present for at least two weeks. Bipolar II Disorder is an alternation between depressive and hypomanic, less severe manic, episodes. This is better characterized by major depression with at least one episode of such hypomania. Cyclothymic Disorder, or rapid-cycling BD, is defined by numerous periods of hypomanic symptoms as well as numerous depressive symptoms stretching to at least two years. The difference is that the symptoms never reach the qualifications to become an episode (Girardi et al. 2016; NIMH 2015). As the disorder is not yet curable, one's only hope is a reduction and control of symptoms. While some undergo psychotherapy and even electroconvulsive therapy, others respond better to medications that may serve as mood stabilizers.

2.1.1 Lithium

Lithium is a substance that is one of the oldest used drugs in psychiatric treatments. Serving as a mood stabilizer, its used in patients to manage both manic and depressive episodes of BD (Girardi et al. 2016). It is used as a mood stabilizer due to its ability to modify serotonergic mechanisms and allow the processing, turnover, and release of serotonin (Pandey et al. 2003). It also modulates many intricate regulatory networks including receptor mediated neurotransmitter processes and along with serotonin, lithium can modify dopamine, glutamate and GABA production and turnover (Malhi et al. 2013). It works to lower resting membrane potentials, which reduces neuronal excitability. Overall, it attenuates excessive activity along with contributing to stabilization of neuronal activity, stress resilience, improved neuronal plasticity and regulation of chronobiological processes (Alda 2015).

Individuals receiving lithium for a variety of reasons have been shown to develop nephrogenic diabetes insipidus or NDI (Rej et al. 2014). Although not a true form of diabetes, it presents the same symptoms of diabetes including excessive urination and drinking (NORD 2016). Lithium treatment has also been shown to cause weight gain in both humans and rats (Praharaj 2016; Levine and Saltzman 2006). Thyroid function and disruption is one of the most frequent adverse effects reported in long-term lithium treatment with increased levels of TSH (Grandjean and Aubry 2009; Girardi et al. 2016). It can be predicted that since BD patients are often treated with lithium, they are at increased risk of developing the disorder or other diabetic symptoms.

Patients receiving lithium treatment had elevated vasopressin levels in comparison to healthy control and patients not on lithium (Watson et al. 2007). Studies done on healthy volunteers with long-term lithium administration, reported negative effects on learning, memory,

alertness, and vigilance. Similar studies done with BD patients reported mental slowness as well as a negative effect on memory and information processing (Grandjean and Aubry 2009; Girardi et al. 2016). Although lithium has long been the first choice of treatment for patients with BD, between 30-40% of patients fail to respond. In those that do respond, there is a high association between such and a family history of BD (Serretti et al. 2004).

2.1.2 BDNF

Brain-derived neurotropic factor (BDNF) is a protein found in its greatest concentration in the brain playing large roles in learning and memory. Being released by both nerves and neuronal support cells, its larger function includes playing a role in the maturation, growth, differentiation, migration, and survival of neurons (Maisonpierre et al. 1990). BDNF has also been suspected to play a role in Bipolar Disorder as studies done post-mortem of BD patients have shown decreased BDNF levels (Knable et al. 2004; Rakofsky et al. 2012). Looking at late stage BD patients, serum BDNF levels are lower. Lithium on the other hand has been shown to increase such levels of BDNF (Rakofsky et al. 2012). During mood episodes, there is a fluctuation seen in BDNF levels indicative of its role in regulation of mood (Rakofsky et al. 2012).

2.1.3 Vasopressin

Vasopressin or arginine vasopressin (AVP), also known as antidiuretic hormone (ADH) is a hormone responsible for regulating plasma osmolality and volume (Sharman et al. 2008). Being mainly synthesized in the magnocellular cells of the hypothalamic supraoptic (SON) and paraventricular nuclei (PVN), such is released upon stimulation to act on the kidneys and blood vessels specifically. It is also produced within smaller populations of the PVN, ones that do not leave the brain (Caldwell 2008). Found in the body often as argenine-vasopressin (AVP), the

hormone has also been linked to playing a role in social behaviors (Aspé-Sánchez et al. 2016; Meyer-Lindenberg et al. 2011) while also having increased levels being present in varying mood disorders (Dempster et al. 2009). Although social behavior has many factors both environmentally and genetically that contribute to its development, vasopressin receptor genes have been linked to such in which mutations to these genes increase susceptbility to mood disorders and a fluctuation in social behavior (Wu et al. 2015). A study done with rats in which vasopressin was used as a therapeutic via nasal administration showed increased sociability in those after receiving the administration (Ramos et al. 2014). Knockout studies have also been done in which AVP is involved in the regulation of various social behaviors such as the regulation of aggression, social recognition, and social bonding (Caldwell 2008).

2.1.5 Mouse Model

Mice are a commonly used animal model for the studying of physiological and neurological conditions and behaviors. Specifically for the studying of BD, the Black Swiss mouse strain (BLS) is a strain that has been known to mimic the manic state symptoms of BD. A study done using BLS and C57BL/6 (B6) mice showed that BLS mice showed increased manic behavior including risk-taking and reward-seeking behavior, which has implications to increased motivation, decision making, and addiction (Hiscock et al. 2007). When BLS mice are treated with lithium, the normalization of certain manic-like behaviors occurred including reduced sucrose preference however having no effect on hyperactivity or anxiety (Logan and McClung 2016). Although given its implications in Bipolar Disorder, it was found that no significant differences in hippocampal and frontal cortex levels of BDNF occurred in BLS mice when compared to three other strains, B6, A/J, and CBA/J (Hannah-Poquette et al. 2011).

2.2 Autism Spectrum Disorder

Autism or Autism Spectrum Disorder (ASD) is a range of neurodevelopment disorders characterized by specific models of behavior and one's inability to partake in social behaviors such as interaction and communication (Taurines et al. 2012). It is estimated that 1 in every 68 children has ASD (Christensen 2016). As implied by the name, there is a wide variety and severity associated with the disorder. With the most extreme cases, symptoms include little to no social interaction with peers, avoidance of eye contact, inability to comprehend and interpret other's feelings and social cues, lack of seeking to share enjoyment or achievements, lack of emotional reciprocity, and failure to develop peer relationships, as well as awkward, inappropriate, and even a complete lacking of speech (NINDS 2015, APA 1994). More behavioral symptoms include repetitive or unusual movements, the need for routine behaviors, and emotional outbursts in overly stimulating environments (Taurines et al. 2012).

Although the causes of ASD are greatly unknown, it is believed to be the result of both genetics and environment as a number of genes has been associated with ASD. Disruptions in brain development may be due to defects in genes that control such development and early brain cell communication (NINDS 2015). Due to the timing of ASD, the most constructive from of treatment includes early intervention to better assist in the development of such lacking social behaviors and language skills. While medication is not a first line of treatment, ASD symptoms such as anxiety, depression, and obsessive-compulsive disorder may be treated with mood stabilizers and antipsychotic medications (NINDS 2015).

2.2.1 BDNF

Behavior patterns such as core symptoms of deficits in social communication and interactions are said to be a result of neurodevelopmental abnormalities. Neurotrophins, crucial

moderators of neuroplasticity, are also said to be specifically involved in the development of ASD. A most prominent neurotrophin is BDNF, and there has been decreased levels of such found in ASD patients compared to those without it (Taurines et al. 2014). BDNF knockout mice displayed impaired cognitive function, and increased levels of aggression, anxiety, and motor activities, all of which are those that are seen in ASD (Kernie et al. 2000; Ito et al. 2011; Taurines et al. 2014). Conflicting results have presented increased BDNF levels in autistic children compared to non-autistic control (Correia et al. 2010). A study looking into why these differences existed, as well as sex differences, found decreased BDNF levels only in female children, and not males, when compared to non-diagnosed controls (Ricci et al. 2013; Kasarpalkar et al. 2014). Conclusions made from this study were that the severity of autism played a role in BDNF expression. When splitting the study group into atypical autistic subjects, of those with a milder phenotype, serum BDNF levels were found to be higher than controls. The other group, typical autistic subjects, or more severe phenotypes, showed no differences from the controls (Kasarpalkar et al. 2014). Lower BDNF levels were attributed to impairment in neuroprotective mechanisms, whereas higher levels were attributed to a certain immune response designed to increase neuroprotective mechanisms.

2.2.2 Insulin

Although the reason for the relationship is unknown, individuals with ASD have shown a higher incidence of developing Type 2 Diabetes Mellitus (T2DM) as well as an increased susceptibility in developing T2DM symptoms such as obesity, hepertension, and dyslipidemia (Chen et al. 2016). Although there is also a higher prevalence of T2DM in ASD individuals due to the use of medications such as antipsychotics, when adjusted, the association was still present indicating that the use of antipsychotics was not the only link between ASD and T2DM. Since

this connection is established, it has been hypothesized as to how and why this connection occurs. One suggestion is the role that insulin signaling plays in the development of autism and that it can contribute to ASD in genetically susceptible individuals via the PI3K/Tor pathway. This pathway is one that affects a form of synaptic plasticity implicated in autism and can be activated by insulin (Stern 2011). If this connection is true, one can argue that increased insulin levels may be seen in ASD individuals.

2.2.3 Mouse Model

Balb/cJ mice are a model strain often associated with Autism as they exhibit decreases in social behavior, a major indicator that an individual has Autism (Brodkin 2007). When compared to Swiss Webster controls, Balb/c mice have displayed decreased social behavior coupled with increased social avoidance, indicative of their lack of sociability (Jacome et al. 2011). These mice also exhibit increased anxiety and aggressive behaviors, larger brain sizes, underdeveloped corpus callosums, and decreased levels of serotonin (Brodkin 2007). BTBR mice, another strain used to study ASD exhibit decreased social and explorative behaviors as well as lower BDNF levels, mirroring findings of BDNF variations present in ASD (Scattoni et al. 2012).

2.3 Obesity and Diabetes

As of 2014, there were 21.0 million people in the United States diagnosed with diabetes and it was estimated that 8.1 million people were undiagnosed (CDC 2014). Diabetes is a disease characterized by abnormally high blood glucose levels (NIDDK 2014). Symptoms of the disease include increased thirst and hunger, rapid weight loss, tiredness, and even loss of feeling in extremities. There are three main types of diabetes including Gestational, Type I, and Type II. Gestational diabetes can occur when a woman is pregnant and the excess hormones lead to insulin resistance. Such usually goes away following birth of the child. Type I develops when one's body no longer makes any or enough insulin. This is relatively unpreventable as it occurs when one's own immune system destroy the insulin producing cells of the body (NIDDK 2014).

Type II Diabetes is developed due to diet and exercise issues and impairs one's ability to regulate blood glucose levels through the inability of insulin to perform properly and the development of insulin resistance (Leahy 2005). Diet induced Type II Diabetes Mellitus (T2DM) is responsible for around 95% of reported cases of diabetes (CDC 2014). There are many environmental factors that contribute to the development of T2DM and the stressing of the glucose homeostatic system.

2.3.1 High Fat Diet

Consistently consuming a high-fat diet (HFD) can lead to obesity and diabetic symptoms. Such has been demonstrated using the C57BL/6J (B6) mouse strain, one that is genetically predisposed to develop diabetes (Surwit et al. 1988). Behaviorally, consuming high levels of fats can alter one's ability to function cognitively and increase anxious behavior. One study suggested that a HFD can increase depressive-like states and that anxiety is likely one of the first impairments to arise after such metabolic distress (Zemdegs et al. 2015). Another study

specifically looking at BD patients showed that those with a BMI in the obese range were associated with worse cognitive functioning than those with a normal BMI (Depp et al. 2014). In that study alone, three in every four patients had a BMI in the obese range. As a HFD causes obesity which in turn can cause a person to develop T2DM, it can be predicted that BD patients on a HFD will exhibit declined behavior and worsening of BD symptoms, specifically being less sociable.

2.3.2 Insulin

Insulin is a hormone involved in the body's regulation of glucose homeostasis. It is secreted by pancreatic beta cells during times of increased glucose to help regulate blood glucose levels (Leahy 2005). Individuals on a HFD compensate by producing large amounts of insulin but often develop a resistance, meaning that the insulin produced does not work, causing poor regulation of blood glucose levels. Obesity-induced inflammation, caused by accumulation of fats from a HFD also contributes to insulin resistance and T2DM (Bhattacharya et. al 2016). Along with insulin resistance, other markers are often used as indicators of T2DM. Two key metabolic abnormalities associated with insulin resistance are increased triglyceride levels and lower levels of high-density lipoprotein cholesterol. A ratio between the two, TG/HDL-C, is a marker or such resistance and a predictor of diabetes (McLaughlin et al. 2003). Hypertriglyceridaemia is found in 40-70% of T2DM patients and is promoted by insulin resistance (Howard 1987; Arca 2015).

2.3.3 BDNF

BDNF has also been shown to play roles in the regulation of food intake. A study done in which BDNF was removed from the brain resulted in mice that had higher levels of anxiety as well as an onset of obesity. This onset was demonstrated by increased body weight, as well as

elevated serum levels of insulin, glucose, leptin, and cholesterol (Rios et al. 2001). Also when on a HFD, male mice have been shown to have lower BDNF levels. This in turn increased the drive to eat and is thought to contribute to diet-induced obesity in males and in this study, T2DM (Liu et al. 2014). Rats have demonstrated similar findings when on a diet high in saturated fat and refined sugar. Such a diet led to reduced BDNF mRNA and protein just after two months on the diet, and such was related to deficiency in learning and memory (Molteni et al. 2002). Cognitive tests in general have showed impaired attention, speed, and mood when on a HFD, suggesting such has an overall detrimental effect on brain health (Holloway et al. 2011; Sommerfield et al. 2004).

2.4 Study Design

Those with BD are also known to develop other heath issues due to the many symptoms. These issues include, but are not limited to, anxiety, substance abuse, cardiovascular disease, and even obesity (NIMH 2015). The development of obesity specifically can be due to unhealthy lifestyles of bipolar patients along with medication effects (Depp et al. 2014). It is also said to be linked due to increased adiposity, which in turn contributes to inflammation seen in mood disorders which has negative effects on brain structure and function (McElroy et al. 2016). Because of the increased susceptibility in developing obesity, there is also an increased chance of developing diabetes, specifically there is about a two to three times increased risk of BD patients developing T2DM (Calkin et al. 2013). Studies have actually found correlations between patients of BD and diabetes, with BD being more common among those with T2DM. Glucose and fats are often dysregulated in BD patients due again to either a sedentary lifestyle or medication side effects. It has also been suggested that there may be disease mechanisms shared between the two (Charles et al. 2016).

Similar links have also been made between individuals with ASD and their susceptibility in developing T2DM. There are many behavioral factors that are believed to be reasons as to why this is so. Children with ASD lack motivation to participate in things such as physical activities with other children and structured meal times which both promote a healthier well-being (Lee et al. 2008). It is also the case that many parents use food as incentives to complete tasks and such often lacks nutrition as they settle for anything the child will eat (Zimmer et al. 2012; Evans et al. 2012). Since these factors are mostly related to humans and behavioral issues, it can be hard to see similar results in a lab setting where the HFD is the only controlled factor. This study will focus on the association with poor eating habits, ASD and BD symptoms, and the possible development of T2DM symptoms.

3. Methodology

3.1 Experiment 1: The Effects of a High Fat Diet and Lithium on Diabetes Symptoms, Anxiety, and Sociability in Balb/cJ Mice.

3.1.1 Statement on animal care

All animal studies were carried out with the approval from Bridgewater State University's Institutional Animal Care and Use Committee *(IACUC)*.

3.1.2 Animal Protocol

36 male BALB/cJ mice, one that mimics ASD, were purchased from Jackson Laboratories (Bar Harbor, ME) and individually housed upon arrival into the lab when they were six weeks old. The mice were kept in the same room under the same conditions and a 12:12 LD cycle. Mice were subdivided into different food and drinking groups. As for drinking, there was water (H_2O) as the control and 10 mM lithium chloride (LiCl) as the experimental. For food, there were three different food types: High Fat (HF, 5.10 kcals per gram with kcal percentages 61.6% fat, 18.1% protein, and 20.3% carbohydrate, TestDiet 58Y1, St. Louis, MO), 10% Fat (TEN, 3.76 kcals per gram with kcal percentages 10.2% fat, 18.0% protein, and 71.8% carbohydrate, TestDiet 58Y2), and Regular Chow (RC, 3.36 kcals per gram with kcal percentages 13.4% fat, 29.8% protein, and 56.8% carbohydrate, LabDiet 5001, St. Louis, MO). The TEN food served as a second control when studying liquid consumption behaviors as it has been found previously that RC fed mice significantly drink more liquid than HF due to food consistency (Hicks et al. 2016). This experiment had 6 groups each with n=6: 1) HF/H_2O, 2) HF/LiCl, 3) RC/H_2O, 4) RC/LiCl, 5) TEN/H_2O, 6) TEN/LiCl. Weekly measurements of body weight, and food and drink intake were recorded from aged-week 8-15 (7 weeks of study total). Food consumption was converted into kilocalories (kcals) per week consumed.

3.1.3 Behavior

Multiple behavioral tests were conducted during weeks five through seven of the study (aged-week 12 through 14) including a light-dark box, and open field, and a social test. When performing assays, mice were individually placed, for a 10-minute duration, into an empty cage surrounded by a SmartCage™ . The SmartCage™ contains IR beams, which record the animals' movements and allow for analysis of such activity. A light-dark box test was conducted during week 12 to test specifically for anxiety. A red box, which is a box that is dark to the mice since they are unable to see red, is placed on one side of the empty cage. If a mouse is more anxious, it will spend an increased amount of time in the dark box. They will also enter the box more, and right away, after being placed into the light side of the cage at the start of the assay. Specific variables looked at were dark occupancy, dark latency, and dark entries. Other variables included some used in an open field test such as distance traveled, velocity, and rears in the dark and light zones respectively, for comparison of the type of activity present within each zone.

During the last week of study, week 14, a sociability test was conducted after allowing the mice to become acclimated to the cage via an open field test. The mice were placed into the empty cage and allowed to explore it for 10 minutes before the addition of a Swiss Webster companion mouse to test the sociable behavior of the mice towards the new mouse. Open field test variables such as distance traveled and velocity will be tracked for exploration and anxiety indicators. After the acclimation period and while the experimental mouse is in the open cage, a non-experimental mouse is placed in a small raised box on one side of the cage. Analysis was done to see whether the experimental mouse, which has full access to the cage, spent more time on the side with the companion mouse thus being social, or whether they preferred the empty side. A calculation was done to compare if the same mouse changed their preference of spending

time on a specific side of the cage from before to after the addition of the companion mouse. Mice were also observed for any aggressive or social behavior, in the form of biting and sniffing respectively, towards the companion mouse.

3.1.4 BDNF

Brains were collected following euthanasia and split into the frontal cortex and hypothalamus and then immediately stored in -80°C for later testing of BDNF using ELISA kits. A protease/lysis buffer cocktail was prepared for protection of the degradation of proteins while also allowing the lysing of the cells. The cocktail was prepared using 100 μl of protease inhibitor (Halt Protease Inhibitor Single-Use Cocktail EDTA-Free 100x, ThermoScientific) for every 10 ml of Pierce IP Lysis buffer (ThermoScientific, Rockford, IL). For the frontal lobe, 200 μl of cocktail was added for each 0.1 gram of respective homogenized brain tissue along with an additional 100 μl of cocktail. For the hypothalamus, 200 μl of cocktail, made as stated above, was added to each homogenized brain sample. A low target concentration (working dilution 1:2) of sample and sample diluent buffer was created and then used in a BDNF ELISA (Mouse BDNF PicoKine ELISA Kit, Boster Biological Technology Co., Pleasanton, CA).

3.1.5 Glucose Tolerance and Insulin

Intraperitoneal glucose tolerance tests (GTTs) were conducted during week 13 of the study. Food was removed 12 hours prior to the test. Baseline blood glucose will be measured at Time 0 (fasting glucose), using a One-Touch Ultra-2 Glucose Monitor, and subsequently measured at time 30, 60, and 120 minutes post-injection of 2g/kg glucose. On the last day of study, trunk blood was collected following euthanasia to test for insulin levels. After clotting, samples were centrifuged at 4°C for 20 minutes at 2,000 x g to separate serum to be used in an ELISA (Ultra Sensitive Mouse Insulin ELISA Kit, Crystal Chem Inc., Downers Grove, IL).

3.1.6 Statistical Analysis

A Two-way ANOVA with Tukey HSD post-hoc pairwise comparisons using Systat statistical programming was done to analyze differences in weight, eating, and drinking amounts for all weeks between the six experimental groups (based on food and liquid type). Such was also done to determine differences in glucose tolerance, insulin, and BDNF levels as well as all variables within all behavior tests (light-dark box, open field, and sociability). Specifically for the sociability test, calculations for zone 1 and 4 were done to look at time spent in each respective zone (after-before).

3.2 Experiment 2: The Effects of High Fat Diet and Lithium on Diabetes Symptoms, Anxiety, and Sociability in Black Swiss Mice

3.2.1 Statement on animal care

All animal studies were carried out with the approval from Bridgewater State University's Institutional Animal Care and Use Committee *(IACUC)*.

3.2.2 Animal Protocol

36 male Black Swiss mice, one that mimics the manic stage of BD, were purchased from Charles River Laboratory (Shrewsbury, MA) and individually housed upon arrival into the lab when they were six weeks old. The mice were kept in the same room under the same conditions and a 12:12 LD cycle. Mice were subdivided into different food and drinking groups after 4-5 days of acclimation. As for drinking, there was water (H_2O) as the control and 10 mM lithium chloride (LiCl) as the experimental. For food, there were three different food types: High Fat (HF, 5.10 kcals per gram with kcal percentages 61.6% fat, 18.1% protein, and 20.3% carbohydrate, TestDiet 58Y1, St. Louis, MO), 10% Fat (TEN, 3.76 kcals per gram with kcal percentages 10.2% fat, 18.0% protein, and 71.8% carbohydrate, TestDiet 58Y2), and Regular Chow (RC, 3.36 kcals per gram with kcal percentages 13.4% fat, 29.8% protein, and 56.8% carbohydrate, LabDiet 5001, St. Louis, MO). The TEN food served as a second control when studying liquid consumption behaviors as it has been found previously that RC fed mice significantly drink more liquid than HF due to food consistency (Hicks et al. 2016). This experiment had 6 groups each with a n=6: 1) HF/H_2O, 2) HF/LiCl, 3) RC/H_2O, 4) RC/LiCl, 5) TEN/H_2O, 6) TEN/LiCl. Weekly measurements of body weight, and food and drink intake were recorded from aged-week 8-17 (10 weeks of study total). Food consumption was converted into kilocalories (kcals) per week consumed.

3.2.3 Behavior

See aforementioned behavioral tests in section 3.1.3 for protocols. The light-dark box was conducted during week 15, 8 weeks into the study. The sociability test, with the ten-minute acclimation open field test, was conducted during the last week of study, week 17.

3.2.4 BDNF and Vasopressin

Brains were collected following euthanasia and split into the frontal cortex and hypothalamus and then immediately stored in -80°C for later testing of BDNF and vasopressin using ELISA kits. A protease/lysis buffer cocktail was prepared for protection of the degradation of proteins while also allowing the lysing of the cells. The cocktail was prepared using 100 μl of protease inhibitor (Halt Protease Inhibitor Single-Use Cocktail EDTA-Free 100x, ThermoScientific) for every 10ml of Pierce IP Lysis buffer (ThermoScientific, Rockford, IL). For both the frontal lobe and hypothalamus, 520 μl of cocktail was added for homogenization. A low target concentration (working dilution 1:2) of sample and sample diluent buffer was created and then used in a BDNF ELISA (Mouse BDNF PicoKine ELISA Kit, Boster Biological Technology Co., Pleasanton, CA). ELISA kits were also used to test for the presence of Vasopressin (MyBioSource Inc., San Diego, CA) in respective brain regions.

3.2.5 Non-fasting Blood Glucose, Insulin, Triglycerides, and Cholesterol

During the last week of study, aged week 17, non-fasting blood glucose levels were measured. On the last day of study, trunk blood was collected following euthanasia to test for insulin levels using an ELISA (Ultra Sensitive Mouse Insulin ELISA Kit, Crystal Chem Inc., Downers Grove, IL). After clotting, samples were centrifuged at 4°C for 20 minutes at 2,000 x g to separate serum. Blood samples were also be used to determine concentrations of triglyceride

and cholesterol levels using the CardioChek system (Polymer Technology Systems Diagnostics, Indianapolis, IN).

3.2.6 Statistical Analysis

Two-way ANOVA tests using Systat 12 (Systat, Chicago, IL) statistical programming were done to analyze differences in weight, eating, and drinking amounts between the six experimental groups (based on food and liquid type). These were also done to determine differences in glucose tolerance, insulin, BDNF, and vasopressin, as well as all variables within all three behavioral tests (light-dark box, open field, and sociability). Expression of BDNF and vasopressin were normalized to the control group (RC, H2O) for comparison. Specifically for the sociability test, calculations in zone 1 and 4 were made to look at time spent in each respective zone (after-before). Zone 1 contained the control mouse and zone 4 was the furthest away from the control mouse. A repeated measures test was done to determine differences in rate of weight gain between and within the groups. Differences were considered significant at $p < 0.05$.

4. Results

4.1 Experiment 1: The Effects of a High Fat Diet and Lithium on Diabetes Symptoms, Anxiety, and Sociability in Balb/cJ Mice.

4.1.1 Behavior

There were no differences in any initial variable of the light-dark box test (Figure 1). No differences were present between food ($F_{2,21}$=1.549, p=0.236) or liquid ($F_{1,21}$=2.718, p=0.114) groups for dark latency (seconds). No differences were present between food ($F_{2,21}$=0.299, p=0.745) or liquid ($F_{1,21}$=2.162, p=0.156) groups for dark entries (number of entries). Also, there were no differences between food ($F_{2,21}$=1.176, p=0.328) or liquid ($F_{1,21}$=0.162, p=0.691) groups for dark occupancy (minutes). For further analysis, the light-dark box was analyzed for behavior in the dark and light zones respectively (Figure 2). Zone one, the dark zone, had no differences in distance for food ($F_{2,21}$= 0.291, p= 0.750) or liquid ($F_{1,21}$= 0.093, p= 0.764). Diet differences were present in velocity, however, with RC > HF ($F_{2,20}$= 3.742, p= 0.042) but not in liquid ($F_{1,20}$= 1.898, p= 0.184). Rear counts for zone 1 had no differences present in food ($F_{2,21}$= 0.099, p= 0.907) or liquid ($F_{1,21}$= 0.652, p= 0.429). Zone two, the light zone, had diet differences in distance traveled with RC = TEN > HF ($F_{2,29}$= 4.693, $p < 0.05$) but not liquid ($F_{1,29}$= 0.050, p= 0.824). No differences were present with velocity between food ($F_{2,30}$= 1.604, p= 0.218) or liquid ($F_{1,30}$= 0.306, p= 0.584). Diet differences were present in zone 2 with RC = TEN > HF ($F_{2,29}$= 8.656, p= 0.001) but not in liquid ($F_{1.29}$= 0.644, p= 0.429).

There were no differences in the open field variables of distance and velocity (Figure 3). For distance no differences between food ($F_{2,28}$= 0.150, p= 0.861) or liquid ($F_{1,28}$= 0.998, p= 0.326) were found. The same was true for velocity between both food ($F_{2,29}$= 0.053, p= 0.948) or liquid ($F_{1,29}$= 1.821, p= 0.188).

For the sociability test, occupancy of zone 1 after the addition of the companion mouse had diet differences of TEN > RC ($F_{2,27}$= 6.704, p = 0.003) and approaching significance of HF > RC ($F_{2,27}$= 6.704, p = 0.063) indicates a slight diet difference in sociability. Time spent in zone 4 before the addition of the companion mouse, H_2O > LiCl ($F_{1,29}$= 4.671, p=0.039). After the addition of the companion mouse, no differences were present between food ($F_{2,29}$= 1.544, p= 0.231) or liquid ($F_{1,29}$=0.207, p= 0.814) groups (Figure 4). There were differences in zone occupancy for the after-before calculations (Figure 5). Zone 1 difference, that which had the control mouse, had approaching significance with H_2O > LiCl ($F_{1,29}$=16.115 p = 0.052) indicating that the H_2O mice spent more time near the companion mouse. Zone 4 difference, the side in which was the furthest from the companion mouse had significant differences with LiCl > H_2O ($F_{1,29}$= 4.706, p = 0.038) confirming that the LiCl mice did not interact as much with the companion mouse. When looking at sniffing behavior by the experimental mouse, HF > RC ($F_{2,29}$= 6.213, p = 0.004). No differences in biting behavior between food ($F_{2,29}$= 2.110, p= 0.139) or liquid ($F_{1,29}$= 0.431, p= 0.517) groups (Figure 6).

No differences in BDNF levels in both the hypothalamus for food ($F_{2,29}$= 0.210, p= 0.812) or liquid ($F_{1,29}$= 0.254, p= 0.778) and in the frontal lobe for food ($F_{2,29}$= 2.014, p= 0.152) or liquid ($F_{1,29}$= 1.401, p= 0.682) (Figure 7).

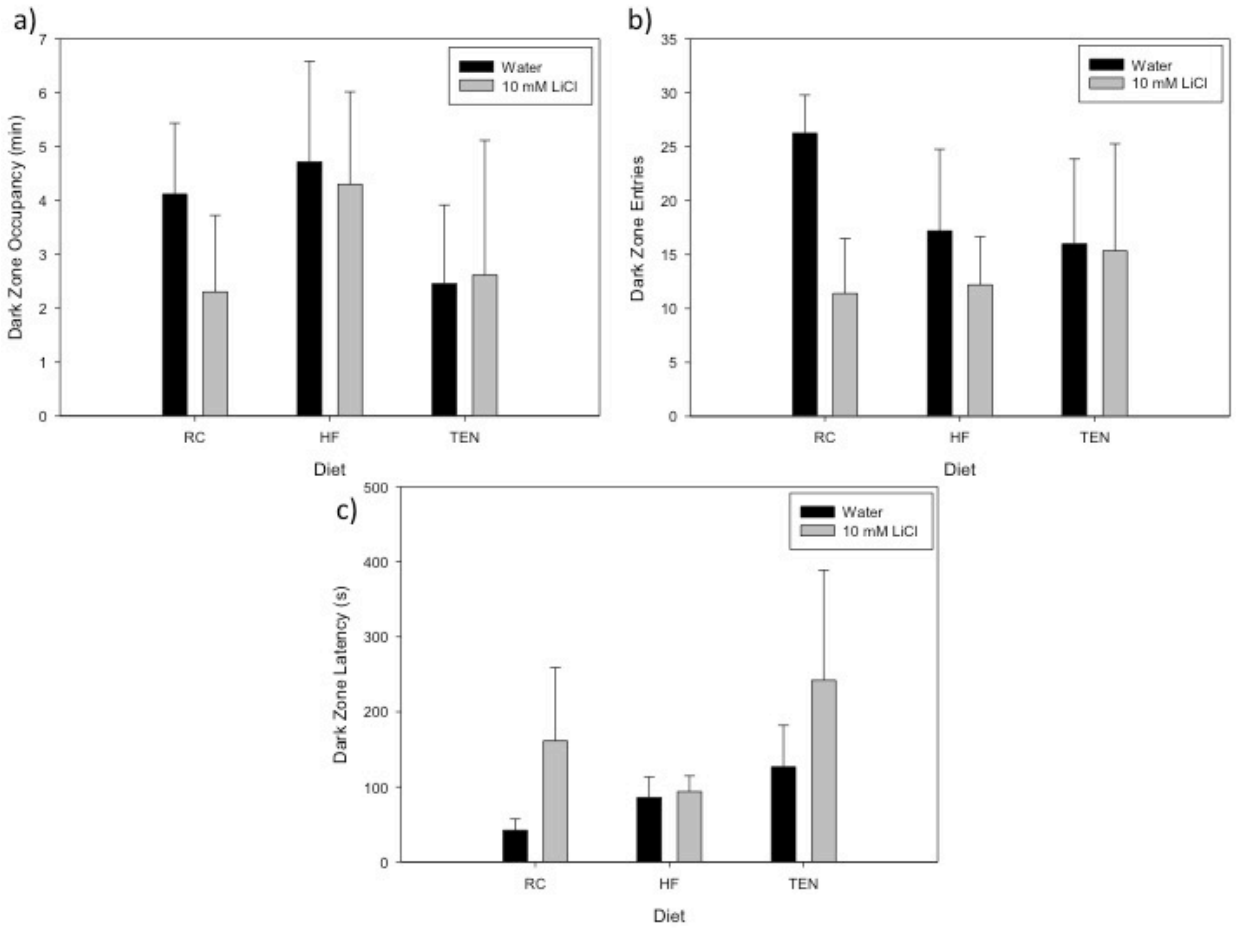

Figure 1. Effects of Diet and Lithium on the Light-Dark Box Test Variables. (a) The time occupied in minutes in the dark zone. No differences were present between food or liquid groups. **(b)** The number of times the mouse entered the dark zone. No differences were present between food or liquid groups. **(c)** The latency period in seconds for the mouse to enter the dark zone from the start of the test. No differences were present between food or liquid groups. Data are means +/- SEM.

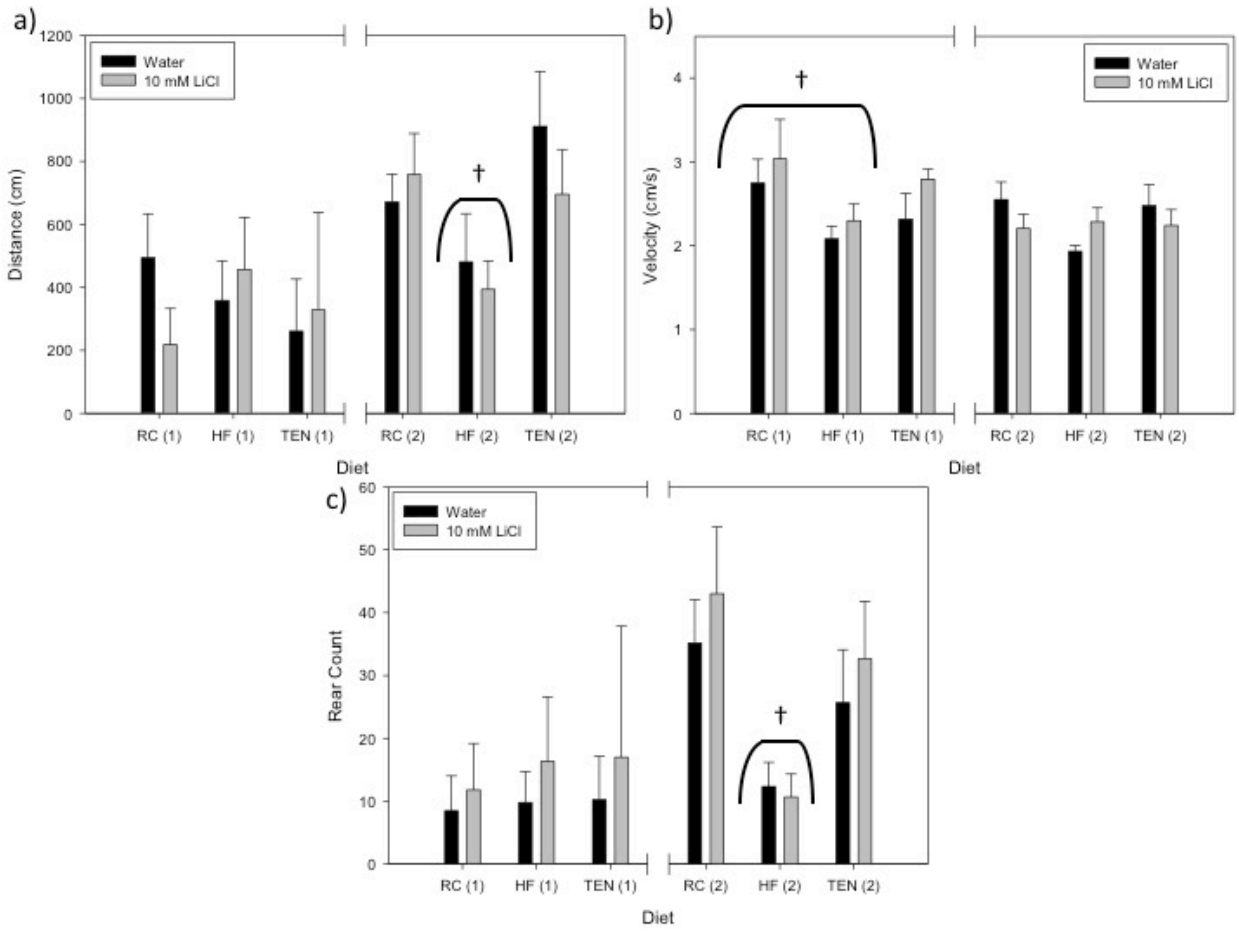

Figure 2. Effects of Diet and Lithium on Distance and Velocity in the Light-Dark Box Test. **(a)** Distance in centimeters for zone 1, the dark zone, and zone 2, the light zone, in the light-dark box test. For zone 1, no diet or liquid differences were present. For zone 2, diet differences were present with RC = TEN > HF, but no liquid differences were present. **(b)** Average velocity in cm/s for zone 1, the dark zone, and zone 2, the light zone, in the light-dark box test. Diet differences were present in velocity with RC > HF but not in liquid. No differences were present in zone 2 between food or liquid groups. **(c)** Rear counts for zone 1, the dark zone, and zone 2, the light zone, in the light-dark box test. No differences were present in zone 1. In zone 2, diet differences were present with RC = TEN > HF. (†) denotes diet differences, data are means +/- SEM ($p < 0.05$)

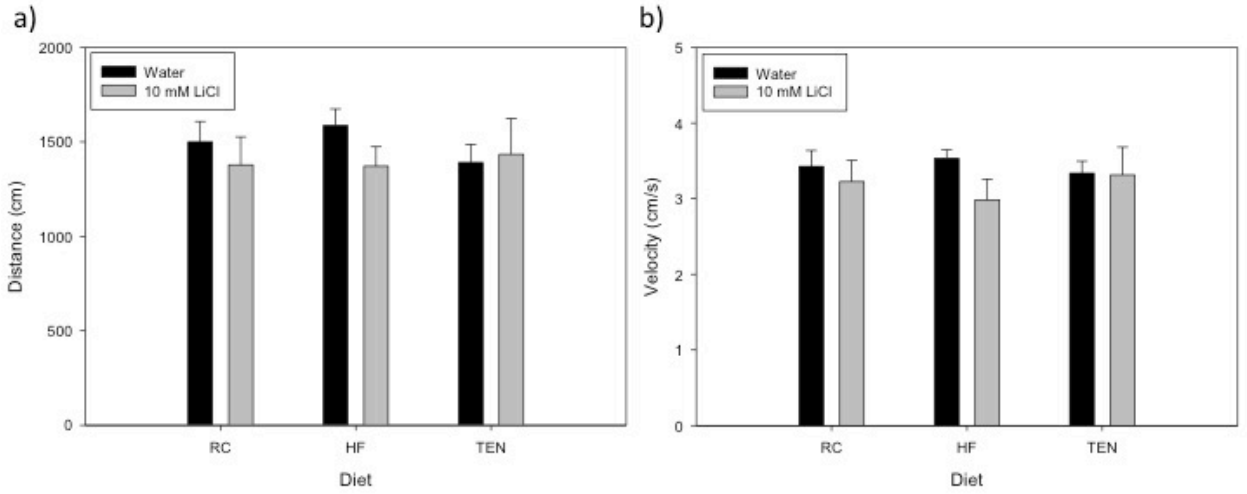

Figure 3. Effects of Diet and Lithium on Distance and Velocity in the Open Field Test. (a) Distance traveled in centimeters for the 10-minute acclimation period, open field test. No differences were present between food or liquid groups. **(b)** Average velocity in cm/s for the 10-minute acclimation period, open field test. No differences were present between food or liquid groups. Data are means +/- SEM.

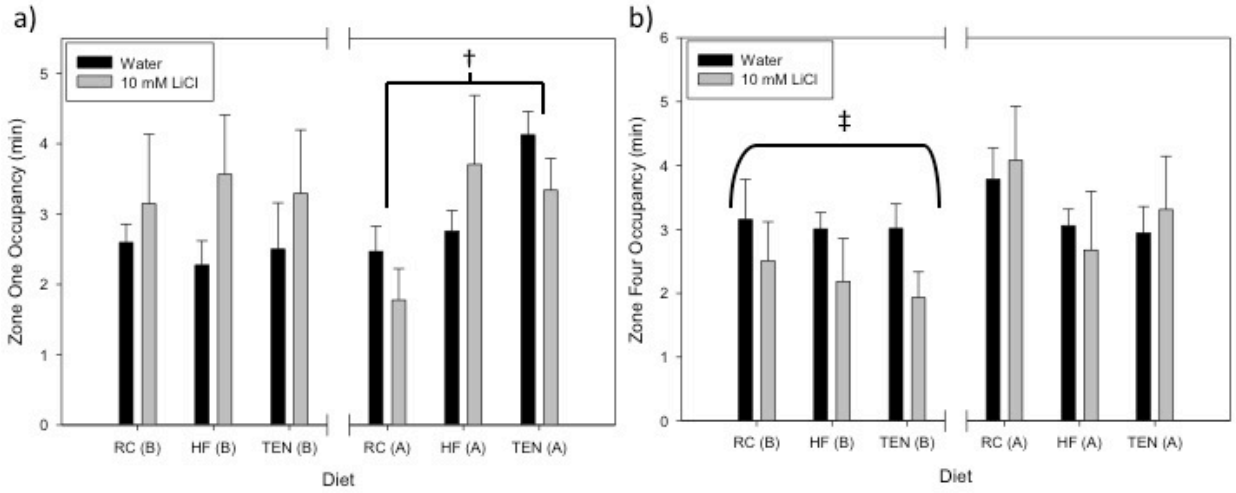

Figure 4. Effects of Diet and Lithium on the Social Test for Before and After the Addition of the Companion Mouse. (a) Zone 1 occupancy, the zone with the companion mouse, in minutes for before (B) and after (A) the addition of the companion mouse. No differences present between food or liquid groups for before the addition of the companion mouse. After the addition of the companion mouse, diet differences were present with TEN > RC and approaching significance of HF > RC. No liquid differences were present. **(b)** Zone 4 occupancy, the zone without the companion mouse, in minutes for before (B) and after (A) the addition of the companion mouse. Before the addition, liquid differences were present with H_2O > LiCl. No differences between food groups were present. (†) denotes diet differences, (‡) denotes liquid differences, data are means +/- SEM ($p < 0.05$).

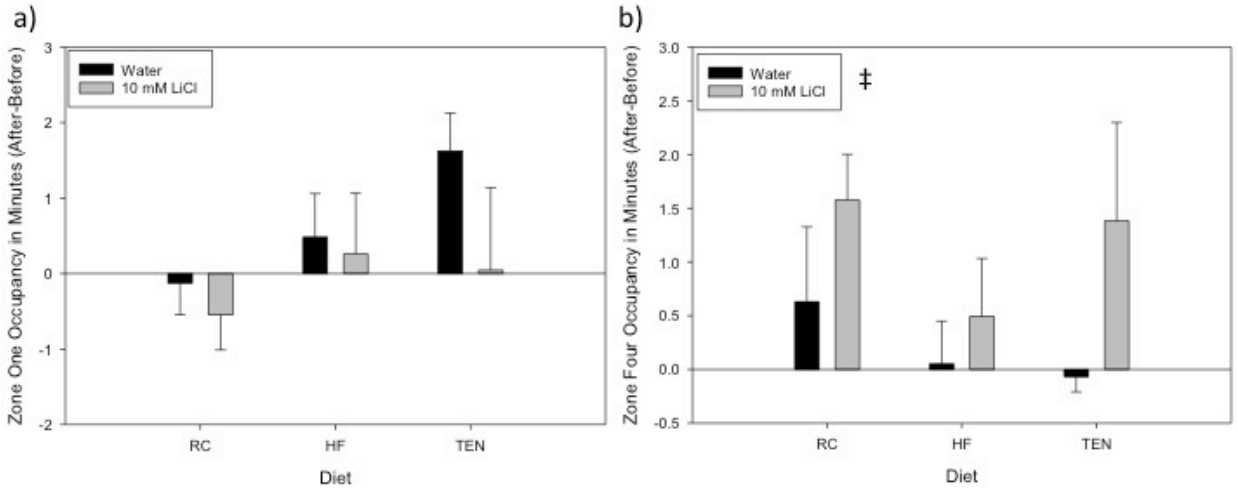

Figure 5. Calculations of After-Before Occupancy Time for Zones 1 and 4 of the Social Test. (a) Zone 1 occupancy calculation for after-before. No diet differences were present but had liquid differences with approaching significance with H_2O > LiCl suggesting that H_2O mouse spent more time near the companion. **(b)** Zone 4 occupancy calculation for after-before. No diet differences were present but liquid differences were, with LiCl > H_2O, confirming that the LiCl mice did not interact as much with the companion mouse. (‡) denotes liquid differences, data are means +/- SEM ($p < 0.05$).

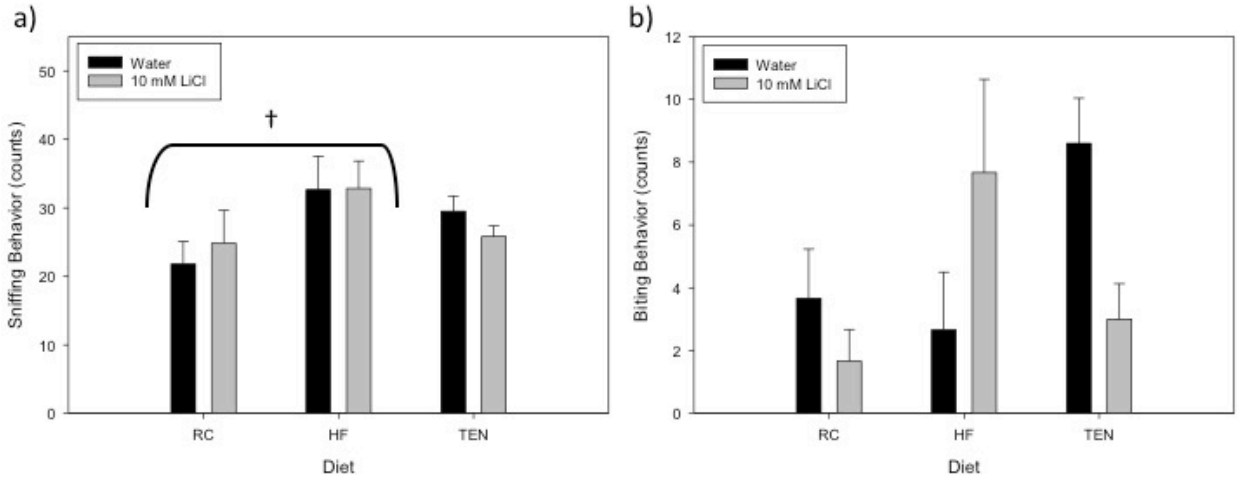

Figure 6. Effects of Diet and Lithium on Social and Aggressive Behavior in the Social Test. (a) Sniff counts towards the companion mouse after its addition. Diet differences were present with HF > RC. No liquid differences were present **(b)** Bite counts towards the companion mouse after its addition. No differences between food or liquid groups were present. (†) denotes diet difference, data are means +/- SEM ($p < 0.05$).

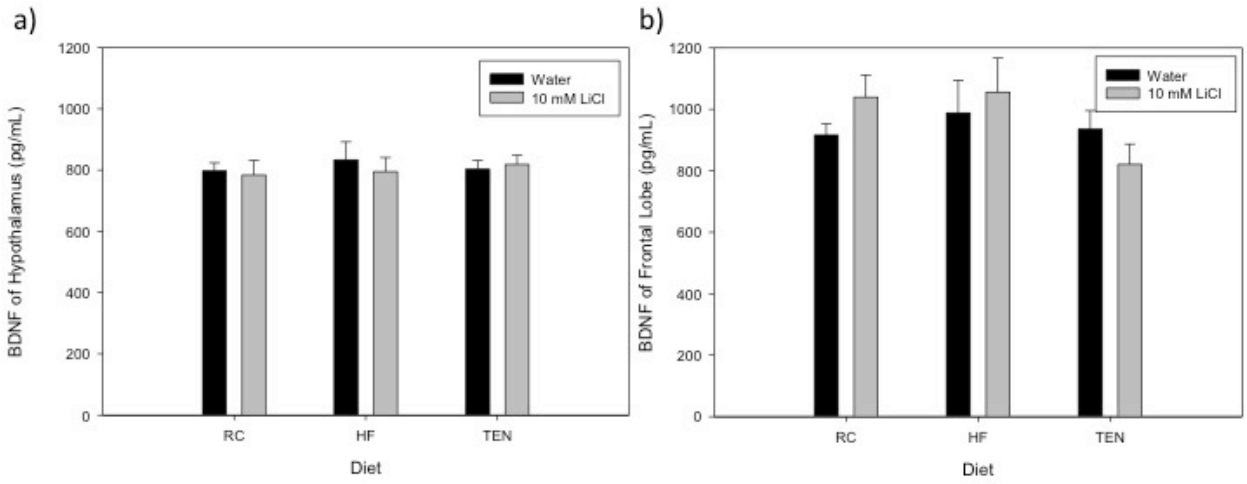

Figure 7. Effects of Diet and Lithium on BDNF Levels in the Frontal Lobe and Hypothalamus. (a) BDNF levels in pg/mL for the frontal lobe. No food or liquid differences were present. **(b)** BDNF levels in pg/mL for the hypothalamus. No differences were present for food or liquid groups. Data are means +/- SEM.

4.1.2 Physiology

In terms of grams of food consumption, throughout the duration of the study, diet differences were present with RC > TEN > HF ($p < 0.05$). There were no consistent differences in total kcals consumed per week, however, there were diet differences seen. Week 9 had differences with RC > TEN > HF. Week 10, the difference changed to RC > HF > TEN. For 11-13, the difference was consistently RC = HF > TEN, and then the experiment ended in week 14 with no diet differences (all $p < 0.05$). During weeks 8 and 13, there were differences in liquid, H_2O > LiCl ($F_{1,27}= 4.722$, $p = 0.039$), and LiCl > H_2O ($F_{1,30}= 4.096$, $p= 0.052$) respectively (Figure 8). Liquid consumption in milliliters differed between food groups with RC > TEN = HF ($p < 0.05$) throughout the duration of the study. During weeks 10, 11, and 12 liquid consumption differed between liquid groups with LiCl > H_2O ($F_{1,30}= 6.828$, $p= 0.014$), ($F_{1,30}= 4.468$, $p= 0.043$), and ($F_{1,30}= 5.671$, $p= 0.024$) respectively (Figure 9). When looking at weight gain, there was no difference among the groups in terms of repeated measures such that no group gained weight at a different rate than the other groups. The only differences present were in week 8 with HF > RC = TEN ($p < 0.05$) (Figure 10).

There were also no differences in glucose tolerance with all $p > 0.05$ for food and liquid at all time points. There were no differences in insulin levels between food ($F_{2,30}= 0.382$, $p= 0.686$) or liquid ($F_{1,30}= 1.877$, $p= 0.181$) (Figure 11).

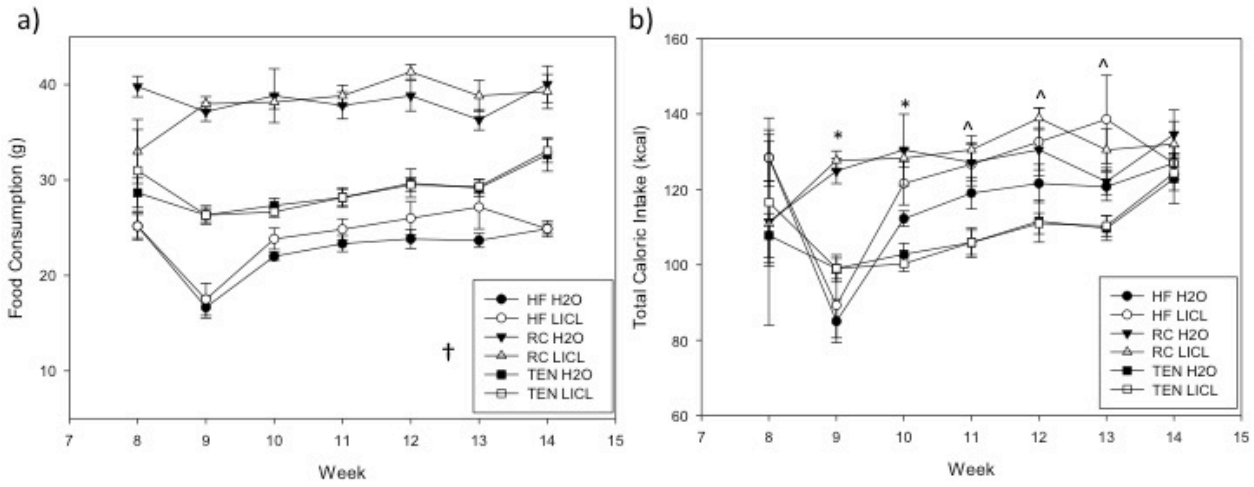

Figure 8. Effects of Diet and Lithium on Weekly Food and Kilocalorie Consumption. (a) Food consumption in grams per week. Diet differences were present throughout the duration of the study with RC > TEN > HF. No liquid differences were present. **(b)** Kilocalorie consumption per week. Week 9 had differences with RC > TEN > HF. Week 10, the difference changed to RC > HF > TEN. For 11-13, the difference was consistently RC = HF > TEN, and then the experiment ended in week 14 with no diet differences. No liquid differences were present. (†) denotes diet differences in all weeks, (*) denotes differences between all three food groups, (^) denotes differences between only two food groups, data are means +/- SEM ($p < 0.05$).

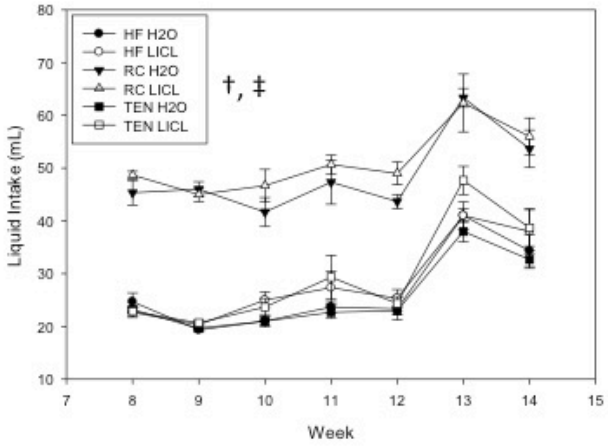

Figure 9. Effects of Diet and Lithium on Weekly Liquid Intake. Weekly measurements for liquid intake in milliliters. Diet differences were present with RC > TEN = HF throughout the duration of the study. During weeks 10, 11, and 12 liquid differences were present with LiCl > H_2O. (†) denotes diet differences between all weeks, (‡) denotes liquid differences data are means +/- SEM ($p < 0.05$).

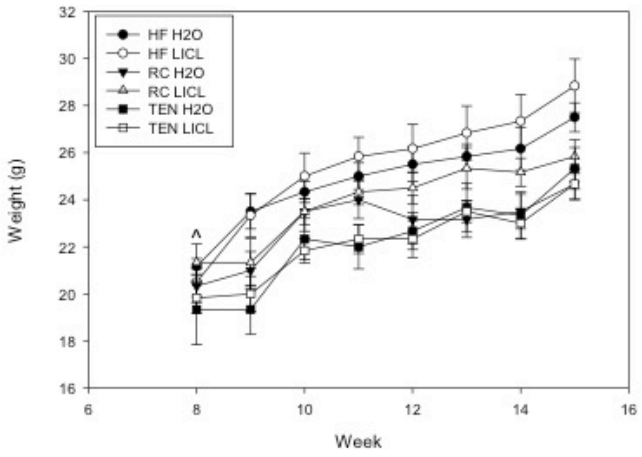

Figure 10. Effects of Diet and Lithium on Weekly Body Weight. Weekly measurements for body weight in grams. Weight differences were present during all weeks. There was no difference among the groups in terms of repeated measures such that no group gained weight at a different rate than the other groups. The only differences present were diet differences in week 8 with HF > RC = TEN. No liquid differences were present. (^) denotes differences between two groups, data are means +/- SEM ($p < 0.05$).

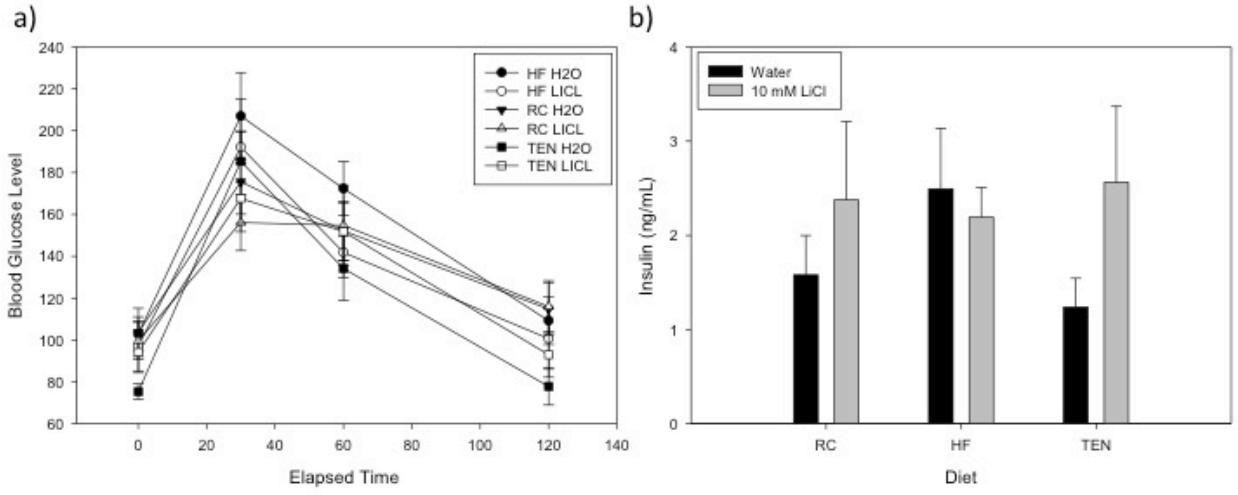

Figure 11. Effects of Diet and Lithium on Glucose Tolerance and Serum Insulin Levels. (a) Blood glucose levels in mg/dL for the baseline and multiple time points post-injection. No differences were present between food or liquid groups at any time point. **(b)** Serum insulin in ng/mL. No differences were present between food or liquid groups. Data are means +/- SEM.

4.2 Experiment 2: The Effects of High Fat Diet and Lithium on Diabetes Symptoms, Anxiety, and Sociability in Black Swiss Mice

4.2.1 Behavior

No differences were present between food ($F_{2,29}$= 2.494, p= 0.100) or liquid ($F_{1,29}$= 0.045, p= 0.834) for dark latency (seconds). For dark entries (number of entries) differences between liquid were present with LiCl > H_2O ($F_{1,29}$= 5.012, p= 0.033), but not for food ($F_{2,29}$= 1.167, p= 0.325). No differences were present for dark occupancy (minutes) for food ($F_{1,29}$= 0.054, p= 0.947) or liquid ($F_{1,29}$= 2.560, p= 0.120) (Figure 12). Again, split into dark and light zones, analysis of distance, velocity, and rears were done within each (Figure 13). For zone one, the dark zone, liquid differences in distance existed with LiCl > H_2O ($F_{1,29}$= 6.655, p= 0.015) but no diet difference ($F_{2,29}$= 1.225, p= 0.309). No differences were seen in velocity between food ($F_{2,30}$= 0.631, p= 0.539) or liquid ($F_{1,30}$= 0.007, p= 0.936). There were differences, however, for rear counts between liquid groups with LiCl > H_2O ($F_{1,30}$= 7.134, p= 0.012) but not in food ($F_{2,30}$= 1.117, p= 0.340). For zone two, the light zone, no diet ($F_{2,29}$= 1.109, p= 0.343) or liquid ($F_{1,29}$= 1.744, p= 0.197) differences were seen in distance traveled. The same for velocity for food ($F_{2,28}$= 0.279, p= 0.758) and liquid ($F_{1,28}$= 1.220, p= 0.279). No rear count differences occurred between food ($F_{2,29}$= 0.145, p= 0.866) or liquid ($F_{1,29}$= 0.002, p= 0.968).

Looking at open field variables during the 10-minute acclimation period, there were liquid differences in distance traveled with LiCl > H_2O ($F_{1,28}$= 7.782, p= 0.009) however the difference was not present when looking at only the first five minutes of the assay. No food differences were present ($F_{2,28}$= 1.536, p= 0.233). No differences were present in velocity for food ($F_{2,28}$= 2.228, p= 0.127) or liquid ($F_{1,28}$= 0.010, p= 0.921) (Figure 14).

There were no differences in any variable of the social test. Looking at zone 1 and 4, both before and after the additional of the companion mouse, no differences occurred between food or liquid groups ($p > 0.05$) (Figure 15). Differences did not occur when looking at the occupancy calculations of zone 1 after-before between food ($F_{2,28}= 2.087$, $p= 0.143$) or liquid ($F_{1,28}= 0.773$, $p= 0.387$). The same occurred for zone 4 after-before calculations for food ($F_{2,28}= 0.521$, $p= 0.600$) and liquid ($F_{1,28}= 0.693$, $p= 0.412$) (Figure 16). No difference in sniffing behavior for food ($F_{2,28}= 2.760$, $p= 0.081$) or liquid ($F_{1,28}= 0.642$, $p= 0.430$). Also, no differences in biting behavior for food ($F_{2,28}= 0.443$, $p= 0.647$) or liquid ($F_{1,28}= 0.787$, $p= 0.383$) (Figure 17).

Looking at both the frontal lobe and hypothalamus for differences in BDNF levels, only liquid differences were found in the frontal lobe with $LiCl > H_2O$ ($F_{1,25}= 7.441$, $p= 0.012$). No food differences were present ($F_{2,25}= 1.742$, $p= 0.196$). No differences were found in the hypothalamus for food ($F_{2,29}= 1.533$, $p= 0.233$) or liquid ($F_{1,29}= 2.129$, $p= 0.155$) (Figure 18).

Vasopressin levels were also measured in both the frontal lobe and hypothalamus. No differences occurred within the frontal lobe between food ($F_{2,30}= 2.114$, $p= 0.138$) or liquid ($F_{1,30}= 0.412$, $p= 0.666$). Differences were found, however, in the hypothalamus between food groups with RC > TEN ($F_{2,30}= 5.922$, $p= 0.007$) but not between liquid groups ($F_{1,30}= 0.114$, $p= 0.738$) (Figure 19).

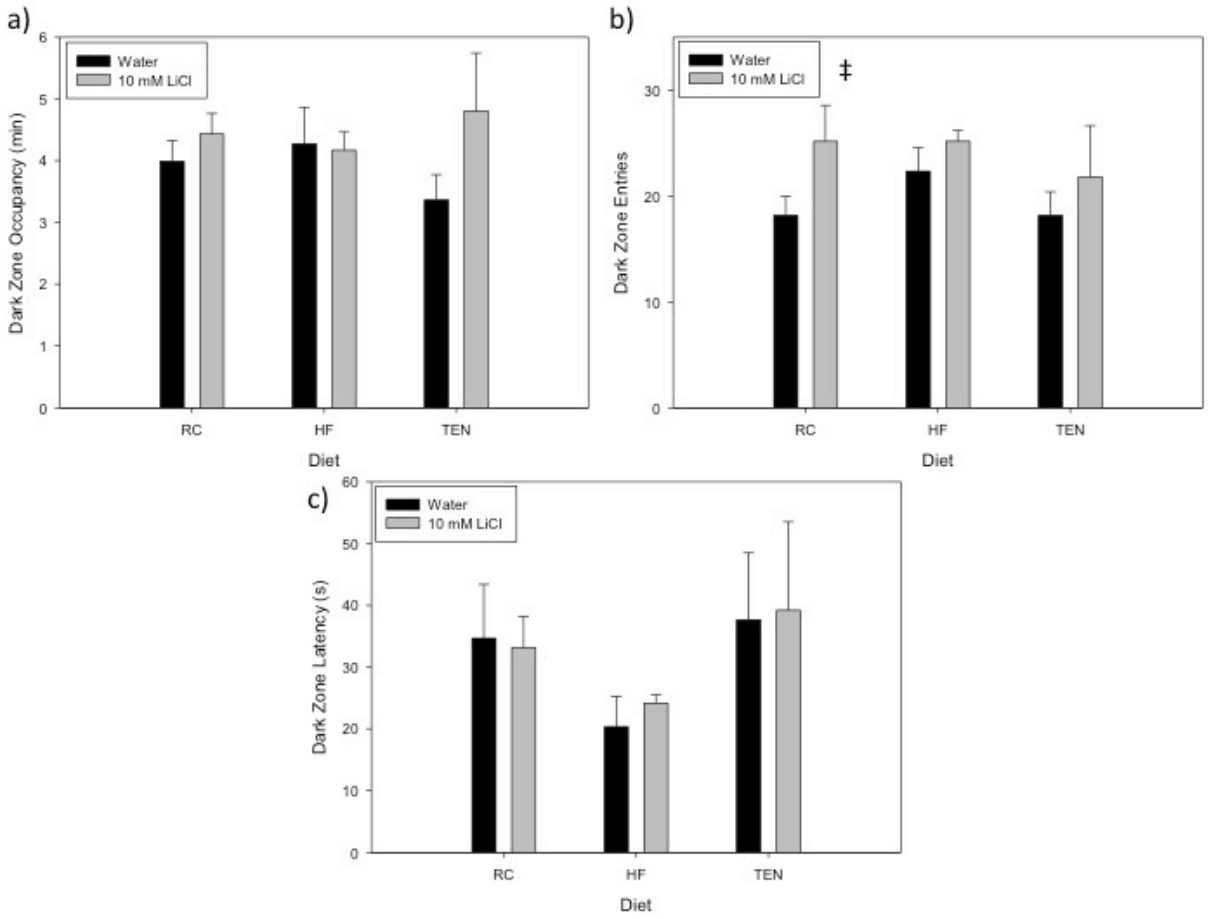

Figure 12. Effects of Diet and Lithium on the Light-Dark Box Test Variables. (a) The time occupied in minutes in the dark zone. No differences were present between food or liquid groups. **(b)** The number of times the mouse entered the dark zone. Differences between liquid groups were present with LiCl > H_2O, but none between food. **(c)** The latency period in seconds for the mouse to enter the dark zone from the start of the test. No differences were present between food or liquid groups. (‡) denotes liquid differences, data are means +/- SEM ($p < 0.05$).

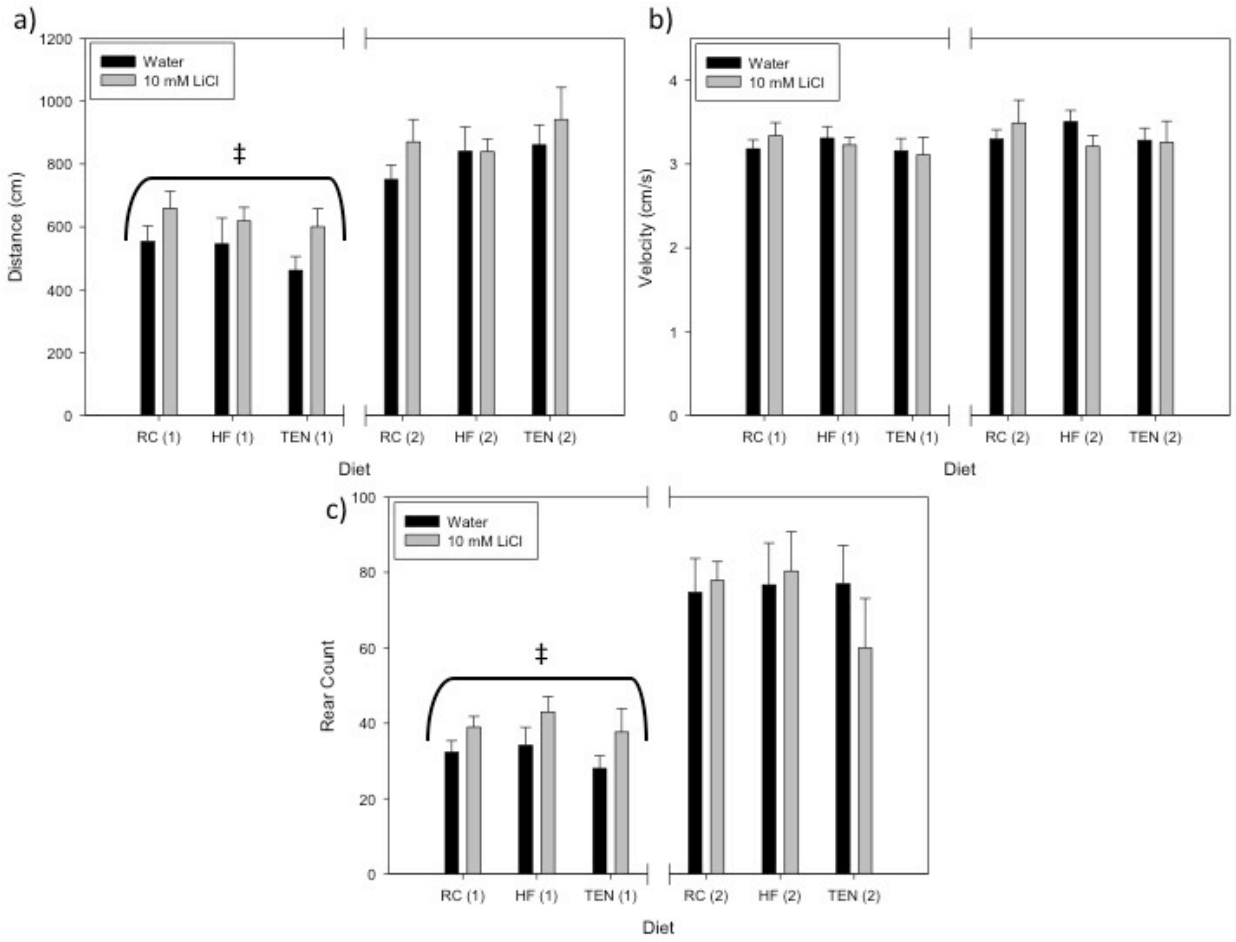

Figure 13. Effects of Diet and Lithium on Distance and Velocity in the Light-Dark Box Test. (a) Distance in centimeters for zone 1, the dark zone, and zone 2, the light zone, in the light-dark box test. For zone 1, liquid differences in distance were present with LiCl > H_2O, but no diet differences. For zone 2, no diet or liquid differences were seen. **(b)** Average velocity in cm/s for zone 1, the dark zone, and zone 2, the light zone, in the light-dark box test. No differences were seen in velocity for either zone between food or liquid groups. **(c)** Rear counts for zone 1, the dark zone, and zone 2, the light zone, in the light-dark box test. Liquid differences were present in zone 1 with LiCl > H_2O. (‡) denotes liquid differences, data are means +/- SEM ($p < 0.05$).

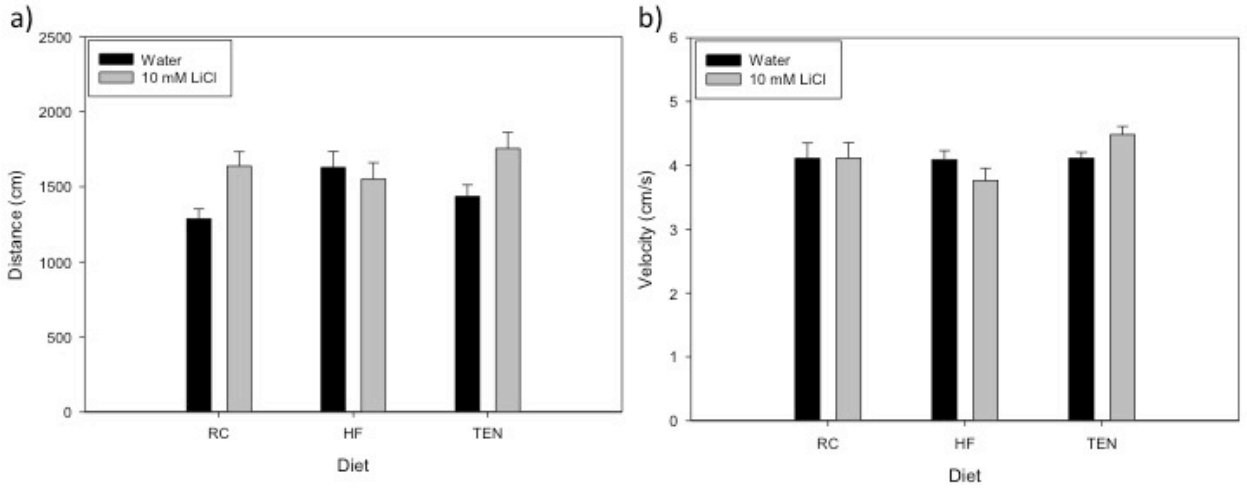

Figure 14. Effects of Diet and Lithium on Distance and Velocity in the Open Field Test. (a) Distance traveled in centimeters for the 10-minute acclimation period, open field test. No differences were present between food or liquid groups. **(b)** Average velocity in cm/s for the 10-minute acclimation period, open field test. No differences were present between food or liquid groups. Data are means +/- SEM.

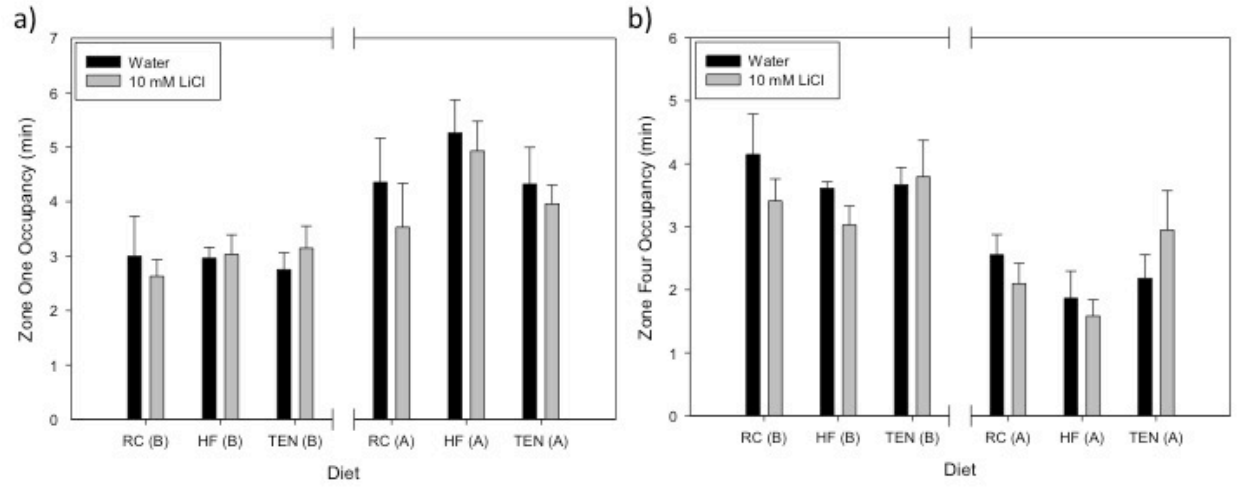

Figure 15. Effects of Diet and Lithium on the Social Test for Before and After the Addition of the Companion Mouse. (a) Zone 1 occupancy, the zone with the companion mouse, in minutes for before (B) and after (A) the addition of the companion mouse. No differences present between food or liquid groups for before and after addition of the companion mouse. **(b)** Zone 4 occupancy, the zone without the companion mouse, in minutes for before (B) and after (A) the addition of the companion mouse. No differences present between food or liquid groups for before and after. Data are means +/- SEM.

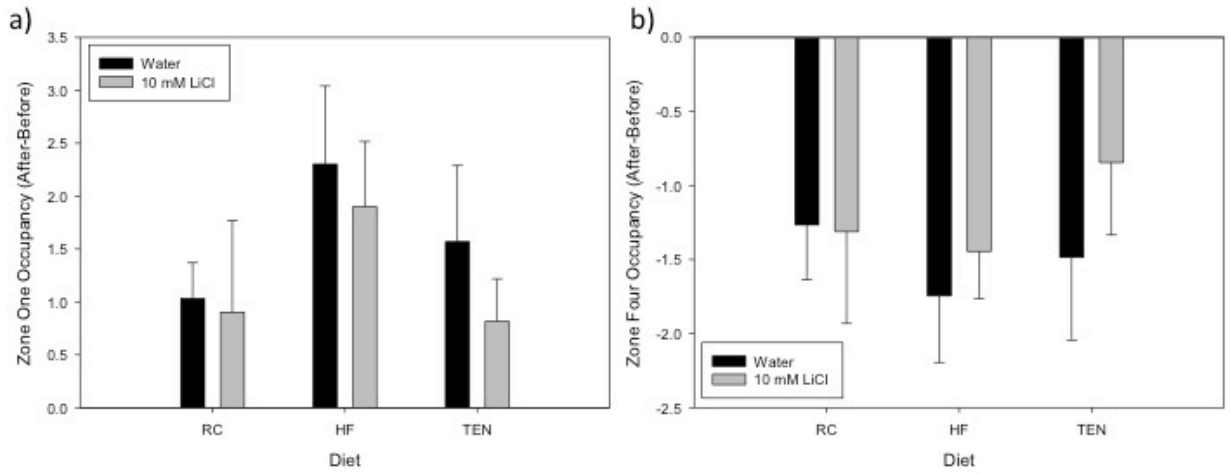

Figure 16. Calculations of After-Before Occupancy Time for Zones 1 and 4 of the Social Test. (a) Zone 1 occupancy calculation for after-before. No differences were present between food or liquid groups. **(b)** Zone 4 occupancy calculation for after-before. No differences were present between food or liquid groups. Data are means +/- SEM.

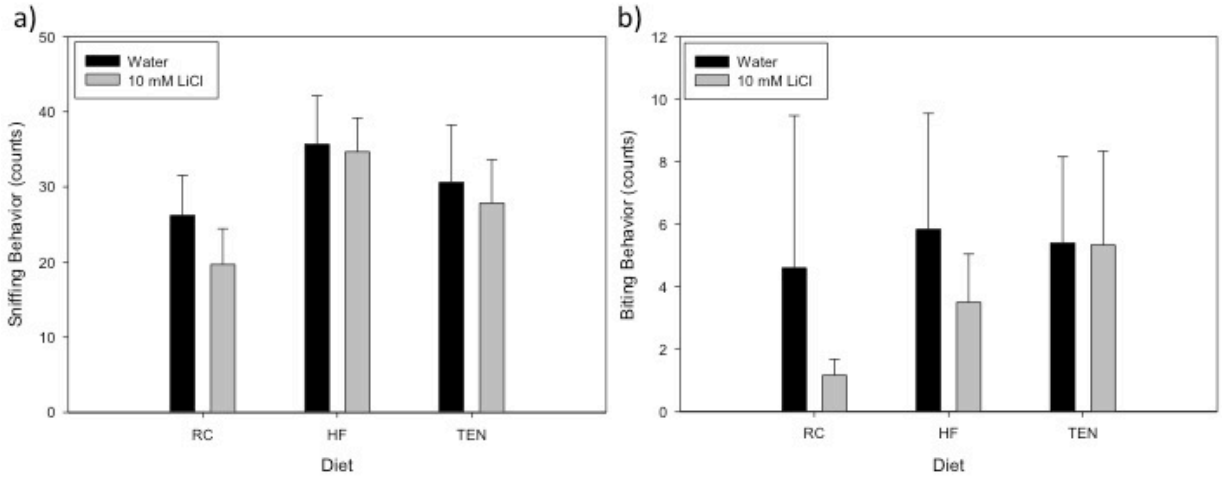

Figure 17. Effects of Diet and Lithium on Social and Aggressive Behavior in the Social Test. (a) Sniff counts towards the companion mouse after its addition. No differences between food or liquid groups were present. **(b)** Bite counts towards the companion mouse after its addition. No differences between food or liquid groups were present. Data are means +/- SEM.

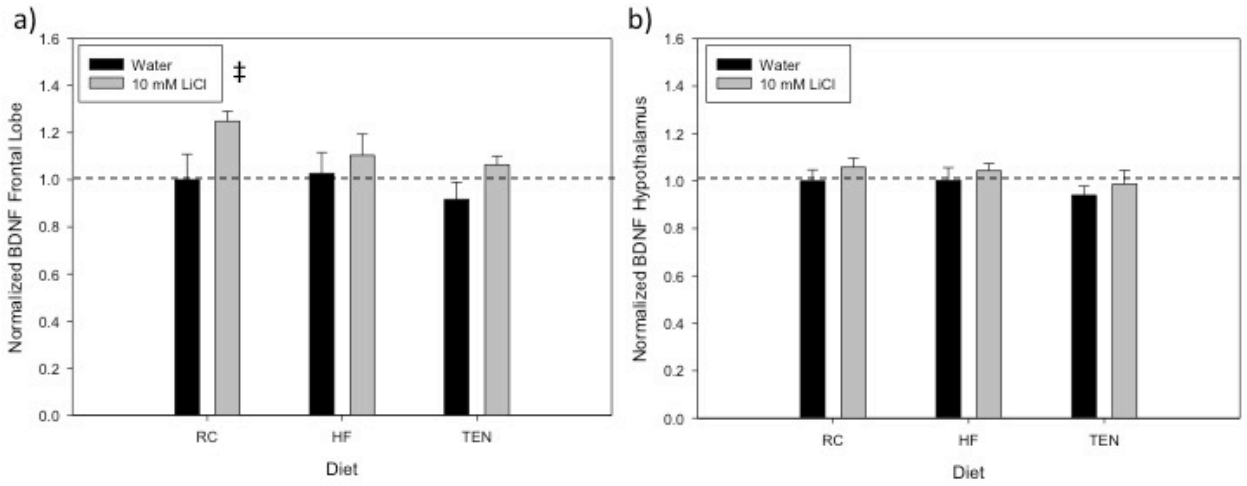

Figure 18. Effects of Diet and Lithium on BDNF Levels in the Frontal Lobe and Hypothalamus. (a) Normalized BDNF levels to the control group RC, H_2O for the frontal lobe. Liquid differences were found with LiCl > H_2O. No food differences were present. **(b)** Normalized BDNF levels to the control group RC, H_2O for the hypothalamus. No differences were present for food or liquid groups. (‡) denotes liquid differences, data are means +/- SEM ($p < 0.05$).

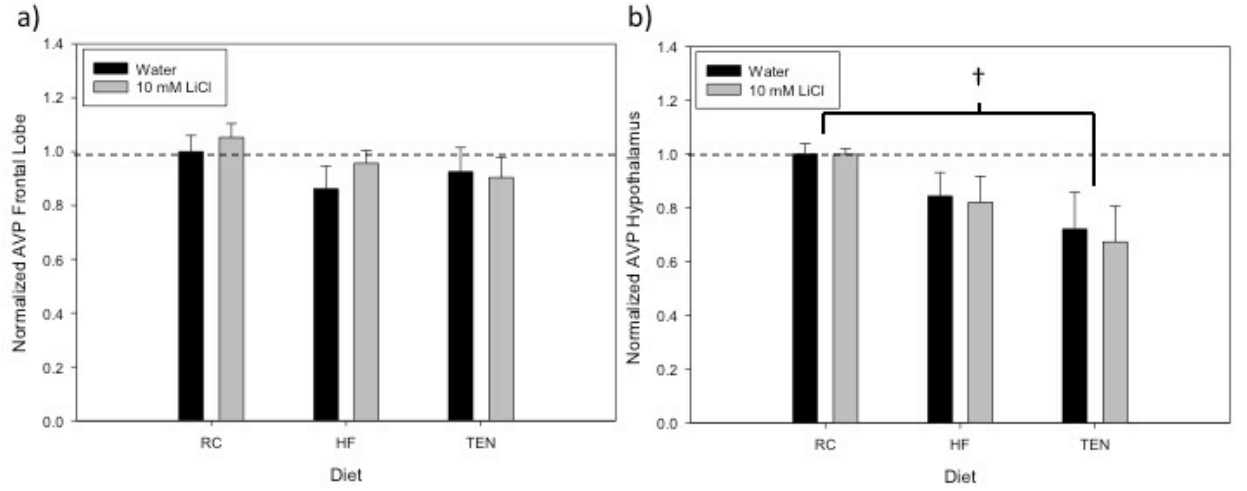

Figure 19. Effects of Diet and Lithium on Vasopressin Levels in the Frontal Lobe and Hypothalamus. (a) Normalized AVP levels to the control group RC, H_2O for the frontal lobe. No differences were present between food or liquid groups. **(b)** Normalized AVP levels to the control group RC, H_2O for the hypothalamus. Differences were present between food groups with RC > TEN but no differences were present between liquid. (†) denotes diet differences, data are means +/- SEM ($p < 0.05$).

4.2.2 Physiology

Differences in food consumption occurred between food groups during week 8 with RC > HF > TEN, and then in weeks 9-17 with RC > TEN > HF with all $p < 0.05$. Total kilocalories consumed varied every week between HF > RC > TEN and HF = RC > TEN ($p < 0.05$) with the exception of no differences being present during week 17. During week 15, differences of HF > RC only occurred in water drinking groups whereas HF > TEN and RC > TEN for only lithium drinking groups ($p < 0.05$) (Figure 20). Food differences occurred in liquid consumption in milliliters with RC > TEN = HF for all weeks ($p < 0.05$). Only in week 17 were there liquid differences with LiCl > H_2O ($F_{1,30}= 4.607$, $p= 0.040$) (Figure 21). Weight differences were present during all weeks with a semi-consistent pattern. During most weeks, HF = RC > TEN, only in week 12 and 17 did the difference change to HF > RC > TEN ($p < 0.05$). Liquid differences only occurred during the first week with LiCl > H_2O ($F_{1,29}= 5.505$, $p= 0.026$) (Figure 22).

There were no differences found between food ($F_{2,30}= 0.111$, $p= 0.895$) or liquid ($F_{1,30}= 1.156$, $p= 0.291$) for blood glucose levels. For insulin levels, differences were present between food with HF > RC ($F_{2,29}= 11.789$, $p= 0.047$), HF > TEN ($F_{2,29}= 11.789$, $p < 0.001$), and approaching significance of RC > TEN ($F_{2,29}= 11.789$, $p= 0.056$) (Figure 23). Differences in cholesterol levels were present between food with HF > TEN = RC ($F_{2,20}= 32.751$, $p < 0.001$) but not between liquid ($F_{1,20}= 1.984$, $p= 0.174$). No differences were found between food nor liquid for triglycerides ($F_{2,19}= 1.109$, $p= 0.350$) and ($F_{1.19}= 0.889$, $p= 0.358$) respectively (Figure 24).

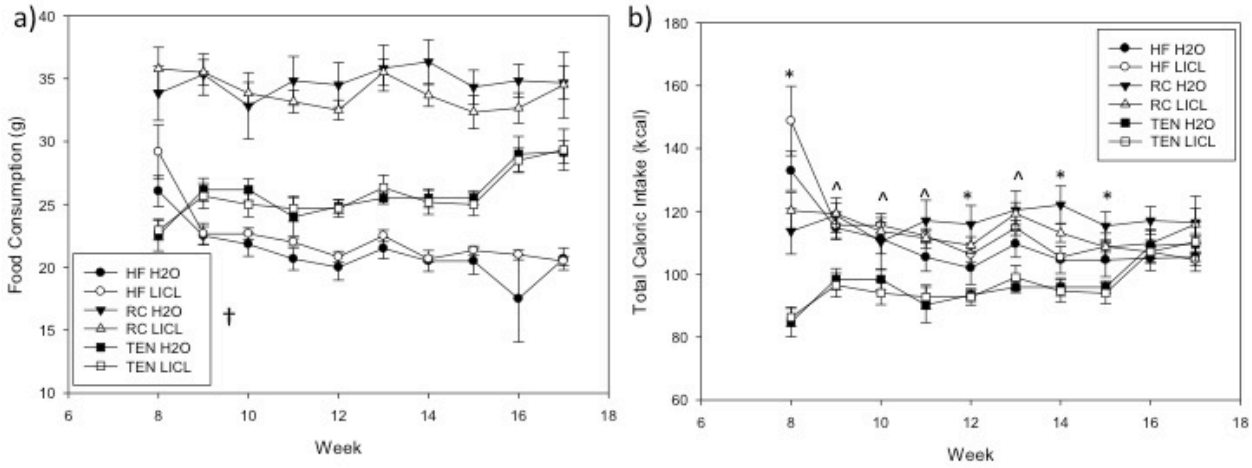

Figure 20. Effects of Diet and Lithium on Weekly Food and Kilocalorie Consumption. (a) Food consumption in grams per week. Differences in food consumption occurred between food groups during week 8 with RC > HF > TEN, and then in weeks 9-17 with RC > TEN > HF **(b)** Kilocalorie consumption per week. Total kcals consumed altered every week between HF > RC > TEN and HF = RC > TEN with no differences present during week 17. During week 15, differences of HF > RC only occurred in water drinking groups whereas HF > TEN and RC > TEN for only lithium drinking groups. (†) denotes diet differences in all weeks, (*) denotes differences between all three food groups, (^) denotes differences between only two food groups, data are means +/- SEM ($p < 0.05$).

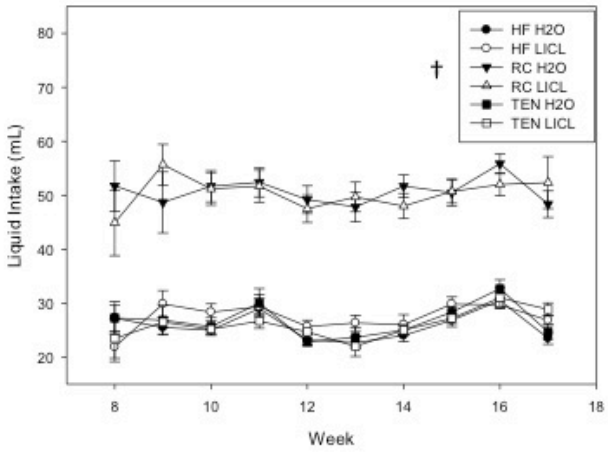

Figure 21. Effects of Diet and Lithium on Weekly Liquid Intake. Weekly measurements for liquid intake in milliliters. Food differences occurred in liquid consumption with RC > TEN = HF for all weeks. Only in week 17 were there liquid differences with LiCl > H_2O. (†) denotes diet differences between all weeks, data are means +/- SEM ($p < 0.05$).

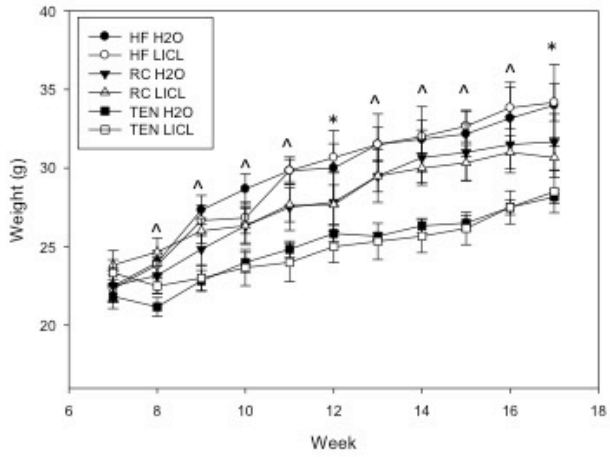

Figure 22. Effects of Diet and Lithium on Weekly Body Weight. Weekly measurements for body weight in grams. Weight differences were present during all weeks. During most weeks, HF = RC > TEN, only in week 12 and 17 did the difference change to HF > RC > TEN. Liquid differences only occurred during the first week with LiCl > H_2O. (*) denotes differences between all food groups, (^) denotes differences between two groups, data are means +/- SEM ($p < 0.05$).

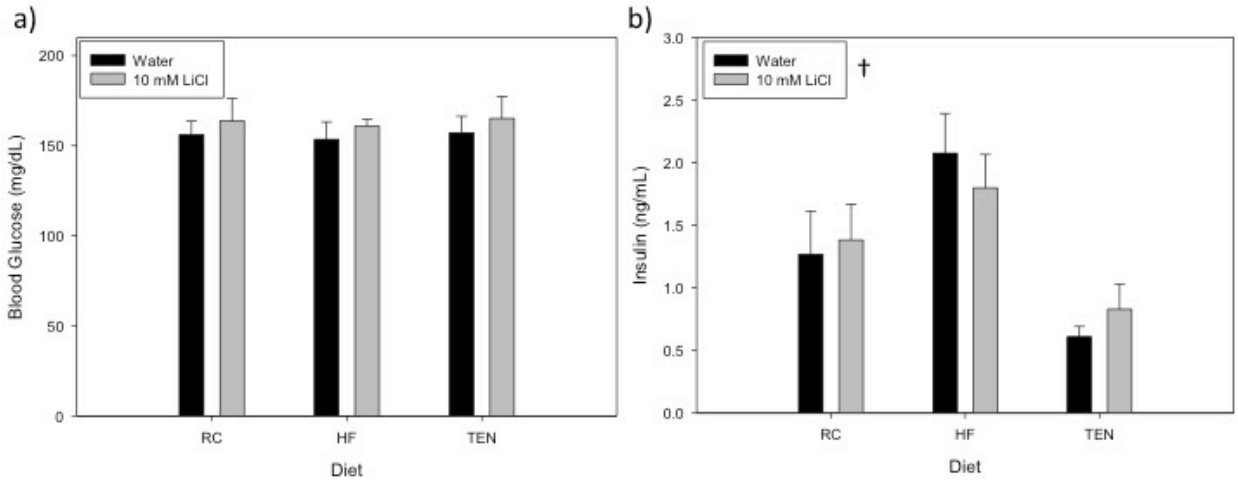

Figure 23. Effects of Diet and Lithium on Non-Fasting Blood Glucose and Serum Insulin Levels. (a) Serum glucose in mg/dL. No differences were present between food or liquid groups. **(b)** Serum insulin in ng/mL. Diet differences were present between food groups with HF > RC, HF > TEN, and approaching significance of RC > TEN. (†) denotes diet differences, data are means +/- SEM ($p < 0.05$).

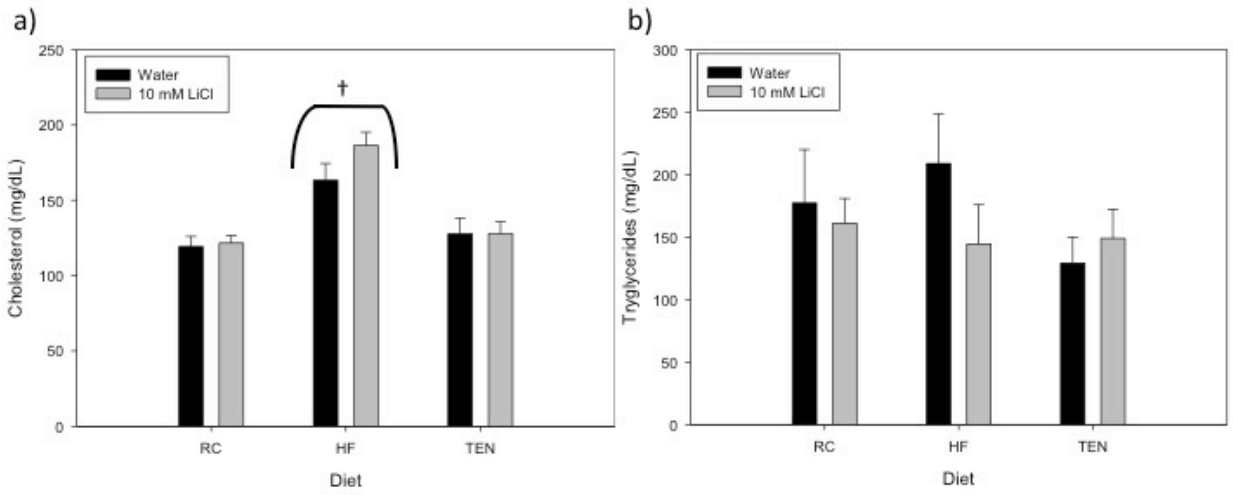

Figure 24. Effects of Diet and Lithium on Blood Cholesterol and Triglyceride Levels. (a) Cholesterol levels in mg/dL. Differences were present between food with HF > TEN = RC. No differences were present between liquid groups. **(b)** Triglyceride levels in mg/dL. No differences were present between food or liquid groups. (†) denotes diet differences, data are means +/- SEM ($p < 0.05$).

5. Discussion

There are many studies that have linked mood disorders with alterations in personality and diseases like Type 2 Diabetes Mellitus. Overall, individuals with mood disorders, such as Bipolar Disorder and Autism, have decreased cognitive functions that coincide with decreased sociability (Khanzada et al. 2017). The two diseases share 23 genes that lead to the distinctive phenotypes, relating them in terms of genetic factors. Further relationships can be made with environmental factors such as diet and medications. Although mouse strains cannot be diagnosed with such disorders, certain behavioral aspects are associated with specific strains that can then be used as models. The Balb/cJ and Black Swiss mice, although not comparable in terms of genetics, were both used in this study to elucidate effects of a high fat diet and lithium on mood disorders, and both responded differently in almost all factors studied.

Experiment 1:

The studying of Balb/cJ mice, the Autism Spectrum Disorder strain, showed behavioral rather than diabetic changes. The mice on regular chow moved faster when in the dark and further when in the light (Figure 2). This increase in locomotion is indicative of explorative-like behaviors and decreased anxiety, which was not seen in those mice with a HF or even TEN. In the light, those on HF had decreased distance traveled and rears. When a mouse rears, it is indicative of exploration and relates its increase in distance traveled to exploration rather than nervous darting. Those on the RC and TEN diets had increased and equal distance traveled and rear counts combining to create an explorative overall behavior. This leaves the HF diet mice to have increased anxious behavior with lack of travel and rearing, mirroring previous findings that HF increases anxiety in both humans and rodents (Bonnet et al. 2005; Collins et al. 2009; Zemdegs et al. 2015; Baker and Reichelt 2016). In mice that already naturally exhibit increased

anxiety (Brodkin 2007), the HF made it worse or the same as it is naturally, whereas the RC and TEN lessened the natural anxiety.

Specifically with sociability behavior, those on the lithium treatment were less sociable than those on water. Lithium treatment was ineffective in the autistic mouse model. As not everyone is responsive to lithium, it has been estimated that a range of responsive rates exists between 40-50%, however, these cases often involve lithium augmentation, and not sole treatment with lithium (Bauer et al. 2003). It has been shown previously however that lithium does reverse induced autistic features in rats (Wu et al. 2014). On the contrary, our mice on LiCl spent more time in the furthest zone from the companion mouse after its addition to the test, indicative of decreased social interaction and no reversal of autistic behaviors (Figure 5). Additionally, those on H_2O spent more time in the zone closest to the companion mouse after its addition. Looking strictly at the zone where the companion mouse was located, mouse on TEN were more sociable than the RC mice, and HF mice had approaching significance, being almost more sociable than RC (Figure 4, Figure 6). This can be an indication of fat differences, however the TEN food having less fat than the RC suggests that the high sugar may play a role in increased sociability. Although its been shown to increase anxiety, little research has been done to show how sugar plays a role on sociability (Baker and Reichelt 2016).

These mice exhibited no changes in BDNF levels in neither the frontal lobe nor the hypothalamus when on an altered diet or liquid (Figure 7). In previous studies, those on HF result in decreased BDNF accompanied with decreased overall cognitive function, learning, and memory (Liu et al. 2014; Molteni et al. 2002; Holloway et al. 2011; Sommerfield et al. 2004). However, such studies mainly looked into the hippocampus for BDNF differences, an area we did not examine. There has been a study however that found similar results as we, where a high-

fat diet, and even a high-sucrose diet, did not exhibit significant changes in BDNF (Jørgensen et al. 2014). Although not mentioned specifically about BDNF, Balb/cJ mice in general are known to have larger brain sizes (Brodkin 2007). Being linked specifically to ASD, it has also been found that alterations in BDNF levels is dependent on the severity of ASD. Milder phenotypes have been shown to have increased levels of BDNF whereas more severe have decreased levels (Kasarpalkar et al. 2014). Its hard to determine whether these mice show no differences in BDNF expression due to their severity of ASD, whether they display the same resistance they do in diabetic phenotypes, or whether we tested in the wrong brain regions to notice differences.

Weekly diet and liquid consumption were moderately altered depending on food and liquid groups. Differences in food and caloric consumption were present with RC mice eating more physical grams of food and kilocalories (Figure 8). With some weeks equaling caloric intake of the HF mice, RC kilocalorie intake was high. There were two weeks in which liquid type changed kilocalorie consumption, however the difference was not the same in the two weeks and thus were relatively random occurrences. There were a couple weeks in which liquid consumption was increased in LiCl mice (Figure 9), however the differences were not present during weeks of the glucose tolerance test, social test, or collection of final samples, but were present during the week of the light-dark box test. However, with liquid groups not differing in any light-dark box variables, it cannot be obviously correlated to have affected such results.

Physiologically, the Balb/cJ mice exhibited a resistance to diabetic symptoms when on HF. With increased weight gain often being the result of HF, and the Balb/cJ mice only presenting such in the second week of the study, it is indicative that the diet did not cause them to gain weight as seen in other strains (Figure 10). Also normally seen when on HF is an altered glucose tolerance or serum insulin levels. Neither was seen in any food group for this study

(Figure 11), adding to the idea that the Balb/cJ strain is not as susceptible to developing T2DM. It has been shown before that the Balb/cJ strain can exhibit a resistance to obesity (Fearnside et al. 2008; Olson et al. 2010; Kim et al. 2014) and that there have been genetic ties made to support this lack of phenotype (Marvelin et al. 2013). Such a finding is hard to justify the Balb/cJ strain as being the best model for ASD, as ASD has been correlated with an increased susceptibility to develop T2DM (Zuckerman et al. 2014). Perhaps other genes are influencing the development of T2DM, ones that we did not monitor.

Experiment 2:

The Black Swiss strain on the other hand seemed to have more changes with respects to diabetic symptoms but less in terms of sociability and behavior in general. With liquid differences seen in the light-dark box test, those on LiCl seemed to have decreased anxiety levels and increased explorative behaviors. In the light-dark box, lithium-drinking mice exhibited increased number of transitions between the light and dark zones, however did not spend more time in the dark zone, which is the true test of anxiety (Figure 12). Additionally, mice consuming lithium exhibited increased rears and distance traveled than the water-drinking counterparts regardless of diet (Figure 13). The combination of such behavior is indicative of exploration (Crawley 1985).

No alterations to social behavior were seen regardless of diet and drink (Figures 15-17). Being the result of a cross between Swiss Webster and C57BL6/JN mice, when compared to such, BLS mice are hyperactive, aggressive, have elevated sucrose preference, and exhibit reduced anxiety and depressive behavior (Logan and McClung 2016). A lack of effect on social behaviors may be indicative of lithium resistance, or the inability to respond to lithium. BLS

mice specifically were shown to exhibit such resistance, mimicking when BD individuals need increased doses of the drug in treatments (Hiscock et al. 2007).

There were no diet effects on BDNF levels, however, there were increased levels in the frontal lobe in mice on lithium (Figure 18). Lithium has been shown to increase BDNF levels, which is normally lessened overall in BD individuals (Knable et al. 2004; Rakofsky et al. 2012). With the frontal lobe being responsible for things such as motor function, memory, impulse control, and social behavior, an increase in BDNF in such region can correlate with an increase in such functions (Collins and Koechlin 2012). The increases in BDNF in the frontal lobe do not correlate with social behavior however, as there were no changes seen in sociability. This may have implications in the activity in the light-dark box test as LiCl mice moved more in distance traveled, possibly the acting of BDNF in the frontal lobe which controls motor functioning.

The only effect seen on vasopressin was in the hypothalamus and it was with RC having greater levels than TEN (Figure 19). This finding may be due to sugar and the TEN diet has higher amounts of sugar to supplement for its lower fat levels. Little research has been done to investigate how a high-sugar diet affects vasopressin in the body. One study done in which 2-buten-4-olide (2-B4O), an endogenous sugar acid, was administered to rats showed no changes to AVP expression (Kawasaki et al. 2006). As lithum treatments have been shown to increase AVP expression, it is unclear as to why LiCl drinking mice did not have any changes in levels (Watson et al. 2007).

Unlike Balb/cJ mice, the Black Swiss mice exhibited increased diabetic symptoms. The parental mouse strains of Black Swiss mice are C57BL/6J mice, and Swiss Webster mice. These two strains are both susceptible to obesity and T2DM when on a high-fat diet seen by increases in weight gain, decreased glucose tolerance, and increased insulin resistance (Surwit et al. 1988;

Lemke et al. 2008). Differing from their parental strains, the Black Swiss mice showed resistance to the obesogenic effects of a high-fat diet while still susceptible to other effects. Even though RC was consumed more in grams, the HF was consumed more in total kilocalories. This is reflective of similar eating habits, as the HF diet has increased kilocalories when compared to RC (Figure 20). With no significant liquid drinking patterns, the amount of LiCl drinking was not a factor in a lack of effects present in the study (Figure 21). The HF groups did indeed get fat, however, were only significantly different than RC groups in two weeks. All other weeks, HF was equal to RC and only different from TEN (Figure 22).

As increased insulin and altered glucose are seen in diabetic phenotypes, only one was seen in this study. The Black Swiss mice did not have altered glucose levels but did have increased insulin levels when on HF (Figure 23). This can be indicative the early stages of insulin resistance as there is increased insulin, but it is still maintaining proper glucose levels regardless of diet. Insulin resistance, which is seen in T2DM, often onset by an unhealthy and high fat diet, is when the body continues to produce insulin in response to increased glucose levels, however this insulin no longer works, thus leading to increased insulin and glucose. Those on HF also had increased cholesterol levels but no difference in triglyceride levels (Figure 24). Increased cholesterol is seen on a high fat diet but is usually accompanied by increased triglycerides (Howard 1987; Arca 2015). This does not match similar findings as increased triglyceride levels and lower levels of high-density lipoprotein cholesterol are seen with T2DM and insulin resistance (McLaughlin et al. 2003). However, since there is increased insulin levels but not glucose, insulin resistance may not be the answer, which would allign with the lack of triglycerides. A study looking to treat insulin resistance inhibited glucagon receptors and saw a

normalization of glucose levels (Okamoto et al. 2017). Such may be a similar mechanism present in the Black Swiss mice to explain why this also happened to them.

In conclusion, this study found differences in how each strain reacted to a change in diet and treatment with lithium. The Balb/cJ, an ASD mimicing strain, showed decreased sociability when treated with lithium and increased anxiety when on a high fat diet. They however did not show diabetic symtoms when on the high fat diet, indicative of a resistance to such. The Black Swiss, the BD mimicing strain, showed decreased anxiety and increased exploration when on lithium treatment but had no changes to social behavior. Increased BDNF levels in the frontal lobe may have played a role in behavior changes but more work should to be done to better connect this finding. Diet had no changes to behavior however did affect diabetic phenotype, but only partially. While they exhibited increased insulin and cholesterol levels, they did not exhibit much weight gain or alterations to glucose and triglyceride levels. Future studies will need to be done which will look at these two strains and the reasoning as to why the Balb/cJ strain was resistant to diabetic change but was affected socially by lithium, and why the Black Swiss strain had slight changes to BDNF and AVP but no changes to socaibility, and only partial changes to diabetic symptoms.

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BRIDGEWATER
STATE UNIVERSITY

Date: 12/15/15

To: Dr. Joseph Seggio
Biological Sciences
313 Science and Mathematics Center

From: Dr. Jonathan Roling, Chair
Institutional Animal Care and Use Committee

Re: IACUC Case#2015-05 - Modification Approval

The modifications to your IACUC Case#2015-05, *Behavioral Neuroscience Techniques in the Classroom,* are approved by the Institutional Animal Care and Use Committee (IACUC). The approval for this project is valid from the new animal usage dates of *9/1/15 – 8/31/17.* If you wish to continue the project, you will need to reapply at that time.

We wish you every success in this research.

[redacted]

JS/dfd

cc: Kenneth Adams

BRIDGEWATER
STATE UNIVERSITY

Date: 12/15/15

To: Dr. Joseph Seggio
Biological Sciences
313 Science and Mathematics Center

From: Dr. Jonathan Roling, Chair
Institutional Animal Care and Use Committee

Re: IACUC Case#2015-06 - Modification Approval

The modifications to your IACUC Case#2015-06, *The effects of chronobiological dysregulation, diet, alcohol consumption, and voluntary wheel-running on the symptoms of diabetes and performance on anxiety, learning, and social tasks,* are approved by the Institutional Animal Care and Use Committee (IACUC). The approval for this project is valid from the new animal usage dates of 9/1/15 – 8/31/17. If you wish to continue the project, you will need to reapply at that time.

We wish you every success in this research.

[redacted]

JS/dfd

cc: Kenneth Adams