Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter know, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Urania Dagalakis April 20, 2011

Analysis of Drug Treatments in a Potential Bipolar Rat Model

by

Urania Dagalakis

Dr. Jay Weiss
Adviser

Neuroscience and Behavioral Biology

Dr. Katherine Boss-Williams

Adviser

Dr. Kristen Frenzel

Committee Member

April 20, 2011

Analysis of Drug Treatments in a Potential Bipolar Rat Model

By

Urania Dagalakis

Dr. Jay Weiss

Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelors of Science with Honors

Neuroscience and Behavioral Biology

2011

Abstract

Analysis of Drug Treatments in a Potential Bipolar Rat Model
By Urania Dagalakis

One of the major problems in studying Bipolar Disorder (BD) is developing accurate models in which to test out new therapies and understand its prognosis. Through generations of selective breeding, Hyperactive (HYPER) rats exhibit periods of hyperactivity followed by periods of depressed activity which emulate the characteristics of BD. The first experiment tested if the administration of the BD medication, lithium citrate, was possible in this model using palatable treats (such as gelatin, fudge, cat food, etc) to mask its taste. While this investigation elucidated a possible drug administration method, the lithium was not able to reach significant levels to produce the predicted reduction in activity. The results showed that there was an increase in hyperactivity of dark as well as light ambulatory motor activity in the experimental HYPER rats compared to previously monitored non-experimental HYPER rats. The second experiment tested the effects of different BD medications on this model through the administration of three drug imbued rat chows (Lithium Citrate, Valproate, and Carbamazepine) along with a control chow group. The data from Experiment 2 reveals an increase in hyperactivity in dark and light ambulatory activity in the four rat groups, with the lithium citrate chow rats exhibiting some of the highest amount of motor activity. The lack of hyperactivity in the control chow group may have been due to the non-responsive rats placed into this group due to limitations in the quantity of HYPER rats available. The defensive withdrawal results did not yield any statistically significant effects between the four rat chow groups. Through these experiments it is clear that more testing and analysis needs to be done to assess the potential of the HYPER rats as a model for BD.

Analysis of Drug Treatments in a Potential Bipolar Rat Model

By

Urania Dagalakis

Dr. Jay Weiss

Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelors of Science with Honors

Neuroscience and Behavioral Biology

2011

Acknowledgements

I would like to offer immense thanks and gratitude to Dr. Boss-Williams and Dr. Weiss for allowing me to work with them in their lab as well as mentor and instruct me in conducting research. I would also like to thank Tiffany Drake and Rodney Parker in out lab for their help in the care and monitoring of the HYPER rats used in my experiments. As well as express my eternal gratitude to my family for their love and support throughout my learning both in life and science.

Table of Contents

List of Figures

Number *Page*

Abbreviations

Bipolar Disorder = BD
American Psychiatric Association = APA
Inositol Monophosphatase = IMPase
Ankyrin G = *ANK3*
Phosphatidylinositol = PI
Brain-Derived Neurotrophic Factor = BDNF
G protein-couples receptor kinase 3 = GRK3
D-box binding protein = DBP
Farnesyl-Diphosphate Farnesyltransferase 1 = FDFT1
Vertebrate LIN7 homolog 1 = MALS-1
Sulfotransferase 1A1 = SULT1A1
Insulin-like Growth Factor 1 = IGF-I
Hyperactive rats = HYPER
Cherry Gelatin = CG
Lamb & Rice Dog Food = LRDF
Beef & Liver Cat Food = BFCF
Mint Fudge = MF
Liver & Chicken Cat Food = LCCF
Ocean Whitefish Cat Food = OWCF
Orange Gelatin = OG
Bacon Dough = BD
Peanut Butter Fudge = PBF
Chicken & Rice Dog Food = CRDF
Mixed Grill Cat Food = MGCF
Repeated Measures One Way Analysis of Variance = RM-ANOVA
one-way analysis of variance = one way-ANOVA

Introduction

Bipolar disorder (BD) is a very severe mental illness characterized by periods of prolonged depression alternating with periods of mania. According to the American Psychiatric Association's (APA) Diagnostic and Statistical Manual of Mental Disorders IV (2000) there are two classifications of BD, a patient can either have BD-I or BD-II. A patient with BD-I must experience a manic episode, for at least a week, which is characterized by persistently elevated or irritable mood as well as three of seven symptoms, which include an increase in goal-directed activity, increased speaking, flight of ideas, inflated self-esteem, decreased need for sleep, distractibility and over involvement in high risk activities all of which must be detrimental to the patient's everyday functioning. A patient with BD-II must experience a hypomanic episode, which is characterized by inflated self-esteem, decreased need for sleep, flight of ideas, increased goal directed activity excessive activities; it is distinguished from mania based on its absence of psychotic symptoms and a lower degree of impairment to functioning. These manic or hypomanic periods must last at least 7 days and be followed by periods of prolonged depression in mood and activity to register as either BD-I or BD-II. These episodes of depression must last at least 2 weeks and include symptoms of appetite disturbance, sleep disturbance, psychomotor retardation or agitation, suicidality, decreased interest in life, and guilt. The APA recommended treatment for this disorder is the prescription of mood stabilizers, such as lithium, valproate and carbamazepine. According to the National Institute of Mental Health those afflicted with BD have a lifetime prevalence of 3.9%. Despite current treatment and medication, 15% of BD patients will eventually die by suicide showing its risk of mortality (Craddock & Jones,

1999). Studies have shown that the predominant age of onset is in the mid-20's, with women and African Americans as higher risk groups (Angst, 1978).

Lithium was approved for BD treatment in 1970 and has become a standard treatment since then (Price & Heninger, 1994). Some possible mechanisms are its stabilizing effects through the inhibition of the enzyme inositol monophosphatase (IMPase) which when active in the phosphatidylinositol (PI) pathway has been linked to the hyperactivity seen in BD (Odonnell & Gould, 2007). Despite some experimental evidence of this, lithium also affects other aspects of the PI signal transduction, so it is not evident if modulation of the PI responses by lithium can be solely linked to IMPase inhibition (Atack, Broughton, & Pollack, 1995). Lithium has also been proven to work jointly with the brain-derived neurotrophic factor (BDNF)/TrKB signaling pathway to mediate its neuroprotective effect (Frey et al., 2006). Despite the benefits of the use of lithium as treatment, not all patients respond positively.

Valporate, also known as valproic acid, is an anti-convulsant and was approved for BD treatment in 1995 (Bowden & Singh, 2005). Despite it only being recently used as a BD treatment, valproate has increased in prescription by 26.9% since 1996 (Blanco, Laje, Olfson, Marcus, & Pincus, 2002). Research has shown that it plays a role in the inhibition of histone deacetylases (HDAC) which when inhibited arrests growth and induces cell differentiation (Gurvich, Tsygankova, Meinkoth, & Klein, 2004). Despite several hypotheses, the direct mechanism of this drug pathway is still unknown, yet valproate is a standard drug treatment used for BD patients. Despite the benefits of the use of valproate as a BD treatment, not all patients respond positively.

Carbamazepine is another anti-convulsant which has shown prophylaxis in many cases against mania and it is especially beneficial in patients with rapid-cycling BD. Studies comparing carbamzepine to lithium have not shown any statistically significant differences between the two drug treatments. Except that patients taking carbamazepine and exhibiting rapid cycling of BD have had fewer occurrences of hospitalization (Atack, et al., 1995). Despite the evidence of the beneficial effects of carbamazepine, little is known about its mechanisms or its biological effects on BD (Belmaker, 2004). The development of a BD animal model could help further clarify the mechanisms of these therapies.

Several different BD animal models have been developed using different experimental techniques, even though none have been able to completely characterize the full spectrum of BD. A widely used model is the psychostimulant model in rats which administers drugs such as amphetamine. Researchers have been able to produce increased psychomotor activity emulating the mania seen in BD (Machado-Vieira, 2004). The limitations to this model are that it requires continual drug administration for maintenance of manic behavior, it only illustrates the manic portion of the disorder, and the manic phase is only maintained for a short duration. Another experimental model is based on nutrition, which administers homocysteine to rats for prolonged periods to increase their serum level due to the fact that increased serum levels have been observed in BD patients (Kato, Kubota, & Kasahara, 2007). The limitations of this model are that it did not exhibit any abnormalities when tested in several behavioral tests (Kato, et al., 2007). Another nutritional model is based on omega-3 fatty acid administration in rats to mimic human data of a reduced prevalence of BD in populations with a diet high in

omega-3 fatty acids (Kato, et al., 2007). The limitation to this model is that it does not produce mania and only illustrates the depression portion of BD (Kato, et al., 2007). Yet another experimental model is based on modulating environmental factors, such as sleep deprivation, in rats to induce anxiety and mimic the mania seen in BD (Machado-Vieira, 2004). The limitations to this model are that it only produces mania, it does not produce depression as seen in BD patients (Machado-Vieira, 2004), and the hierarchical interactions of the rats may produce a confound (Gessa, Pani, Fadda, & Fratta, 1995). A relatively new experimental model is based on transgenic rodents in an attempt to produce characteristics of BD. The chromosomal dislocation of *DISCI* in transgenic mice has exhibited some hyperactivity as well as sensorimotor gating and impaired sociality (Kato, et al., 2007). Another genetic abnormality associated with BD is the deletion of the 22q11 locus. The limitations to both of these genetic models are that the mice show schizophrenia-like behaviors (Kato, et al., 2007).

All these experimental discrepancies in the development of a BD model demonstrate the difficulties in being able to exhibit the full manic and depression spectrum of BD. This investigation utilized selectively breed hyperactive (HYPER) rats, raised in the lab for the past 20 years, as a prospective BD model. This Sprague Dawley strain of rats in each generation is monitored and exhibits fluctuations of abnormal hyperactivity with subsequent hypoactive low periods, atypical of normal rats. These high and low periods of activity seem to follow the same behavioral spectrum of mania and depression in BD. They also exhibit an elevated degree of sensitivity to any stressor, such as a shock, that will cause them to show an exaggerated ambulatory motor activity for several days. To test the validity of this HYPER rat as a possible animal model of BD

it is necessary to investigate their reaction to BD medications. The dual purpose of this investigation is to verify if the HYPER rats will ingest lithium orally as well as the administration of several different BD medications to HYPER rats to see if these drugs will mimic the effects seen in humans. These effects will be assessed by monitoring ambulatory motor activity during drug administration.

Methods

Experiment 1: Lithium Administration

Due to the importance of lithium in BD treatment of humans it is a natural choice to test voluntary ingestion of lithium in HYPER rats. The exaggerated hyperactive response in these rats to stressful events, such as surgery or injections, necessitates a more subtle drug administration vehicle that will mask it. Previous attempts at oral administration of lithium in HYPER rats have failed due to their dislike and rejection of lithium. Therefore masking the lithium in palatable treats and maintaining a random treat schedule are viable options to overcoming the HYPER rats taste aversion and rejection of the lithium. It is predicted that at a high enough dose of lithium in the HYPER rats should lead to a reduction in their hyperactivity.

Animals. One and a half month old male HYPER rats weighing an average of 258 grams (n=8) were housed individually in standard polycarbonate translucent cages and ambulatory motor activity was monitored. The ambient temperature in the room was maintained between 20-22° Celsius with a 12:12 light:dark period (lights on from 0700-1900). The 5001 Purina rat chow and tap water were provided *ad libitum*. Additionally, they received five daily treats one of which contained lithium. Food and water intake were measured daily and body weights were measured once a week.

Drug and Drug-Treats. The Lithium Citrate was ordered from Sigma Aldrich (catalogue # 62484) and was incorporated into a palatable treat. The concentration of lithium changed based on the tolerance of the drug ingested by the HYPER rats as

measured by their food and water intake. The concentration of lithium ranged from 70-80 mg of drug/per kg of body weight and administered in one of five palatable treat.

There were 18 total treats in addition to their food chow, five of which were given each day and only one of those contained the lithium. Each treat flavor was given without any drug for two days prior and two days after it contained lithium to ensure that the rats could not identify it (see Figure 1). The treat schedule was organized so that treats were given with at least one week between re-administration periods. Each drug treat was created specifically for each rat based on their individual body weight. Of these treats, three were gelatin flavors (cherry, orange, and lime), four fudge flavors (chocolate, peanut butter, butterscotch and mint), and intermixed between these sweet treats were seven different kinds of cat foods (beef and liver, liver and chicken, seafood feast, turkey and giblets, whitefish, mixed grill and cod sole with shrimp), two different dog food treats (lamb and rice as well as chicken and rice), and two different flavors of dough (bacon and butter). HYPER rats have previously shown palatability towards all of the treats listed above.

Monitoring Ambulatory Activity. Each HYPER rat was individually housed in standard translucent cages surrounded by eight infrared beams (see Figure 2). Ambulatory motor activity was recorded by a computer which represents horizontal activity produced by the animal. Repetitive movements of the animal in the same location are not counted as ambulatory motor activity. Motor activity is monitored by two software programs; a DOS version of Crosstalk Script (programmed by Bob Bonsall of Circular Solutions using Windows 98 as an operating system) and a BASIC program that connected each cage to the computer-controlled system measured via the Labview Version 5.1 programmed by

Bob Bonsall that connects each cage to the computers controlled system. The eight infrared beams are monitored by the software and any change in at least one beam will be recorded as a "sentence". A "sentence" represents the state of all eight infrared beams; changes are calculated by the computer and expressed as ambulatory counts which are representative of ambulatory motor activity. An ambulatory count is defined as a change in an infrared beam that remained unchanged in the previous four "sentences". This enabled the continual monitoring (24 hours a day, 7 days a week) of their daily activity and allowed for the examination of the drug effects. Activity data were expressed by averaging 12 hours or 2 hours during the dark phase to assess the lithium effects on the HYPER rats. The 12 hour dark ambulatory motor activity represents the whole dark time frame, while the 2 hour dark ambulatory motor activity represents the last two hours of the dark period and is marked by its increased activity compared to the whole 12 hour dark period. The 12 hour light ambulatory motor activity was also monitored graphically to assess any lithium effects, since typically HYPER rats exhibit decreased ambulatory activity during this period when compared to normal rats.

Procedures. The HYPER rats (n=8) were placed in individual cages where their activity was monitored for a baseline period, of seven days, to allow them to become acclimated to their new environment as well as the treat schedule regime (see Figure 1). During the baseline period, rats were orally given five different non-drug treats a day. Their food and water intake were measured daily as well as weekly body weight intake. After the baseline period, rats were given the Lithium within one of their daily administered five treats. The original dose of 80 mg/kg of lithium was administered for seven days, followed by sixteen days of 70 mg/kg. A lack of behavioral effects lead to an increase in

their dose to 75 mg/kg for seven days. Soon thereafter they began to reject the lithium and the experiment was terminated.

Statistical analyses were conducted to assess whether lithium affected the ambulatory motor activity of the HYPER rats. For the analysis of the data, an additional group of non-experimental animals was added to provide a relative comparison of the differences in activity between the lithium HYPER rats and the non-experimental HYPER rats. The data from these non-experimental animals were obtained two years previous to the current experiment. For each measure a repeated measures one way analysis of variance (RM-ANOVA) was performed using SPSS version 18 (SPSS, Inc. Chicago IL). The baseline period was averaged and a mean was obtained to create the covariate for the RM-ANOVA. The analysis compared rat groups (non-experimental HYPERs vs. lithium HYPERs) with repeated measure across time and incorporated the covariate.

Experiment 2: Lithium, Valproate & Carbamazepine Administration

The common use of Lithium Citrate, Valproate and Carbamazepine in treatment of BD patients makes them significant tools in the assessment of the HYPER rat as a potential BD animal model. Through a comparison of these different drugs a clearer picture can be drawn as to the validity of this model. This drug comparison strove to investigate what kind of reaction the HYPER rats would have to these drugs. By infusing rat chow with the drugs this eliminates the problem of voluntary ingestion by eliminating their choice. The prediction is that the buildup of these BD drugs in the HYPER rats

should lead to a reduction in hyperactivity, but it does raise the risk of possibly heightening their hyperactivity due to the stressor of the drug chow.

Animals. Two and a half month old male HYPER rats weighing an average of 450 grams (n=36) were housed in pairs in the colony during the first portion of the experiment. Then they were housed individually in standard polycarbonate translucent cages and ambulatory motor activity was monitored. The ambient temperature in the room was maintained between 20-22° Celsius with a 12:12 light:dark period (lights on from 0700-1900). The rats were provided with either 5001 Purina rat chow or drug-chow (Lithium Citrate-chow, Valproate-chow, or Carbamazepine-chow) and tap water *ad libitum.*

Drug and Drug Chow. The drugs used to create the drug chows were ordered from Sigma-Aldrich using Lithium Citrate (catalogue # 62484), Valproic acid (catalogue # P4543), and Carbamazepine (catalogue # C4024). The drug-chows were created by the Custom Animal Diets Company (in Bangor, PA) after specification of the drug dosage necessary for each rat chow Valproate-14 g of drug/per kilo of chow (Gilmor, Skelton, Nemeroff, & Owens, 2003; Gould, Chen, & Manji, 2004; Marx et al., 2008), Carbamazepine-3.5 g drug/per kilo of chow (Gould, et al., 2004), Lithium Citrate-3.0 g of drug/kilo of food (Fukumoto, Morinobu, Okamoto, Kagaya, & Yamawaki, 2001; Gilmor, et al., 2003; Gould, et al., 2004; Gould & Einat, 2007; Hammonds & Shim, 2009; Marx, et al., 2008; Yuan, Chen, & Manji, 1999).

Monitoring Ambulatory Activity. Each HYPER rat was individually housed in a standard translucent cage surrounded by eight infrared beams (see Figure 2). Ambulatory motor activity is recorded by the Labview Version 5.1 programs described in Experiment

1. This enabled the continual monitoring (24 hours a day, 7 days a week) of their daily activity and allowed for the examination of the drug effects.

Defensive Withdrawal Test. The defensive withdrawal test was used to investigate the anxiety of the HYPER rats. This test contrasts the anxiety levels of the control group of HYPER rats receiving 5001 Purina rat chow to the HYPER rats receiving the Valproate, Lithium and Carbamazepine drug chows. Anxious rats will usually spend more time on the 5 x 5 border squares of the open field as oppose to the inner squares (Ferreira et al., 2008). The defensive withdrawal field is 101.6 x 101.6 x 45.7 cm in size with each individual square being 19.1 x 19.1cm. Each individual HYPER rat is placed into a dark polyvinyl cylindrical tube measuring 25.4 cm in length and 11.4 cm in diameter. The cylinder is placed into a specified square diagonally to the corner of the defensive square so that its opening faces into the open field (see Figure 3). The rat is left within the open field for 10 minutes during which a timer is used to record its emergence time as well as any re-entry into the tube. The parameter key to indicating the anxiety level of the rat is the total distance it moved within the open field especially a comparison with how long it spent within the inner 3 x 3 squares compared to the surrounding outer 5x5 squares of the open field.

Procedure. Four different chows were administered to four different groups of HYPER rats within the rat colony (2 rats per cage). The group of HYPER rats given control food (n=12) received the usual 5001 rat chow. The second group of HYPER rats (n=8) had chow embedded with 14 g of valproic acid per kilo of rat chow. The third group of HYPER rats (n=8) had chow embedded with 3.5 g of carbamazepine per kilo of rat chow. The fourth group of HYPER rats (n=8) had chow embedded with 3 g of lithium citrate

per kilo of rat chow. Throughout the time in the colony the food intake was taken daily, while the body weight was taken once a week. After spending nine days within the colony during their initial exposure to the drug food they were placed into individual cages in the monitor room (see Figure 2). The HYPER rats spent fourteen days in the monitor room with continued administration of the drug chows to the specific rats in each group. In the monitor room due to the individual water dispensers (not possible in the colony) the food and water intake was taken daily as well as a weekly body weight intake. Immediately after this fourteen day period in the monitor room each rat was put through the 10 minute defensive withdrawal test to assess their motor ability and anxiety.

Statistical analyses were conducted to assess whether lithium, valproate or carbamazepine affected the ambulatory motor activity of the HYPER rats. For each measure a repeated measures one way analysis of variance (RM-ANOVA) was performed using SPSS version 18 (SPSS, Inc. Chicago IL). The analysis compared rat groups (controls, lithium, valproate and carbamazepine) with repeated measure across time. A one-way analysis of variance (one way-ANOVA) was conducted on the data collected from the defensive withdrawal test comparing the data across rat groups. A one way-ANOVA was conducted comparing daily activity of the three drug chow groups to the control group to assess any significant interactions between them.

Results

Experiment 1: Lithium Administration

This investigation was successful in its purpose to administer lithium through palatable treats. Even though the HYPER rats did eat the lithium treats, due to their reaction to the lithium it was not possible to administer drug treats with a dosage high enough to result in the desired reduction of hyperactivity. The 80 mg/kg dose was an attempt to start off at an increased lithium dosage in the HYPER rats but due to their rejection of drug treats, after seven days it had to be lowered to 70 mg/kg. After sixteen days at this dosage without any reduction in activity as well as no rejection of treats, the dosage was raised to 75 mg/kg to try and get more lithium into the HYPER rats. But their rejection of treats soon after this dosage increase meant that it would not be possible to administer more lithium in this study.

While the HYPER rat drug treat intake was the primary purpose of this investigation the measure of their dark and light ambulatory motor activity was used to monitor any effects the lithium treats might have had on their behavior. Figure 4 depicts the dark ambulatory motor activity of the lithium experimental group compared to the non-experimental group. A RM-ANOVA analysis of the 12 hour dark ambulatory data between the experimental and the non-experimental HYPER rats across ambulatory days incorporating a covariate of baseline days, generated a significant interaction [$F(29, 493) = 3.523$, $p < 0.001$] of group by days. The main effects of either day or group did not reach statistical significance. A RM-ANOVA analysis of the 2 hour dark ambulatory data, comparing the same two groups, also incorporating a covariate generated a significant interaction [$F(29, 493) = 4.252$, $p < 0.001$] of group by days and a significant

main effect [$F(1, 17) = 10.063$, $p = 0.006$] of group type. The main effect of day did not reach statistical significance. An inspection of the data on the graph (Figure 4) reveals that the experimental group during measurement of both the 12 and 2 hour dark ambulatory motor activity exhibited elevated activity on certain days.

Figure 5 depicts the light ambulatory motor activity of the lithium experimental group compared to the non-experimental group. A RM-ANOVA analysis of the 12 hour light ambulatory data, comparing the experimental and non-experimental HYPER rats across the ambulatory days incorporating a covariate of baseline days generated a significant interaction [$F(29, 493) = 11.623$, $p < 0.001$] of group by days and a significant main effect [$F(29, 493) = 1.934$, $p = 0.003$] of activity days. The main effect of group type did not reach statistical significance. An inspection of the data on the graph (Figure 7) reveals that the experimental group during the measurement of the 12 hour light ambulatory motor activity exhibited elevated activity on certain days.

Experiment 2: Lithium, Valproate & Carbamazepine Administration

Figure 6 depicts the dark ambulatory motor activity of the four different HYPER rat groups (Control, Valproate, Carbamazepine, Lithium) used. A RM-ANOVA analysis of the 12 hour dark ambulatory data comparing the four HYPER rat chow groups across the ambulatory days generated a significant interaction [$F(39, 416) = 3.018$, $p < 0.001$] of group by days and a significant main effect [$F(13, 416) = 5.958$, $p < 0.001$] of days as well as a significant main effect [$F(3, 32) = 9.991$, $p < 0.001$] of group type. Additionally, each day was separately analyzed with a one way-ANOVA analysis comparing daily 12 hour dark activity of the four HYPER rat chow groups. Where appropriate, Dunnett's post hoc tests were conducted and generated a significant difference between the lithium

group and the control group for days 12 through 23 excluding days 17 and 19. A RM-ANOVA analysis of the 2 hour dark ambulatory data comparing the four HYPER rat groups across the ambulatory days generated a significant interaction [$F(39, 416) = 2.849$, $P < 0.001$] of group by days and a significant main effect [$F(13, 416) = 9.812$, $P < 0.001$] of days as well as a significant main effect [$F(3, 32) = 15.768$, $P < 0.001$] of group type. Additionally, each day was separately analyzed with a one way-ANOVA analysis comparing the four HYPER rat chow groups of their daily 2 hour dark activity. Where appropriate, Dunnett's post hoc tests were conducted and generated a significant difference between the lithium group and the control group for days 12 through 23.

Figure 7 depicts the light ambulatory motor activity of the four different HYPER rat groups (Control, Valproate, Carbamazepine, Lithium) used in this investigation based on the type of chow they received. A RM-ANOVA analysis of the 12 hour light ambulatory data comparing the four HYPER rat chow groups across the ambulatory days generated a significant interaction [$F(36, 384) = 2.029$, $P < 0.001$] of group by days and a significant main effect [$F(12, 384) = 5.958$, $P < 0.001$] of days as well as a significant main effect [$F(3, 32) = 9.662$, $P < 0.001$] of group type. Additionally, each day was separately analyzed with a one way-ANOVA analysis comparing the four HYPER rat chow groups of their daily 12 hour light activity. Where appropriate, Dunnett's post hoc tests were conducted and generated a significant difference between the lithium group and the control group for days 12 through 23 except for day 18.

Figures 8-13 show the data for the four different HYPER rat groups obtained from the defensive withdrawal test. The data measures were broken down into separate components to assess their degree of anxiety as well as motor ability. These measures

were expressed in several different graphic ways presenting the different components of the open field. Figure 8 displays the total number of squares (inner + outer square) traveled as well as the same data but excluding the HYPER rats that did not enter the open field. Figure 9 displays the total number of inner squares as well as the same data but excluding the HYPER rats that did not enter the open field. Figure 10 displays the total number of squares traveled per second as well as the same data but excluding the HYPER rats that did not enter the open field. Figure 11 displays the time (seconds) of emergence from the tube as well as the same data but excluding the HYPER rats that did not enter the open field. Figure 12 displays the total time spent in the tube summating the emergence time and the re-entries into the tube. Figure 13 displays the total boluses left by the HYPER rats within the open field. Separate one way- ANOVA analyses generated non-significant effects for all of the variables shown in Figures 8-13.

Discussion

The purpose of experiment 1 was an investigation into the oral administration of the widely used BD medication lithium. Despite HYPER rat ingestion of lithium drug treats they did not ingest high enough lithium to produce clinically relevant blood levels (based on previous results) to decrease hyperactivity as predicted. In most BD patients lithium leads to a reduction in their incidence of manic activity, leading to the supposition that it could also reduce the hyperactivity of HYPER rats (Cipriani, Pretty, Hawton, & Geddes, 2005). Contrary to this assumption, the experimental HYPER rats in this investigation exhibited hyperactivity during certain experimental days. This hyperactivity may have been due to an inability to achieve high enough blood levels of lithium (based on previous results) to cause the predicted reduction in activity. Lithium takes approximately two to four weeks to produce therapeutic effects (Cipriani, et al., 2005). This slow onset of lithium effects in BD patients raises questions regarding the time of onset for the desired reduction of hyperactivity in the HYPER rat model. Lithium administration is complicated in the HYPER rat model due to a difficulty in getting them to ingest treats with a high enough dosage, as was seen during the 75 mg/kg dosage, to lead to the predicted reduction in activity. The measure of ambulatory motor activity during this experiment served as a way to monitor any lithium effects on the HYPER rats.

The significant interaction of the dark and light ambulatory motor activity data confirms a variation in ambulation of the experimental group compared to the ambulation of the non-experimental group during the activity days. The variation in ambulation of the experimental group is not uniform, but exhibits positive and negative fluctuations in

activity throughout the experiment (see Figure 4). Their initial hyperactivity in both dark and light ambulatory motor activity (see Figure 4 and 5) during the 80 mg/kg dosage could have been due to their already exacerbated hyperactivity from the stress of being single housed as was seen during the baseline period of the lithium. The significant interaction in both light and dark ambulatory activity raises questions regarding how the stress of the lithium treats might have reversed any possible drug effects and further aggravated the HYPER rats into a continual hyperactive state. Despite the sensitivity of the HYPER rat model, to any external stressors, their intake of the drug treats as well as their elevated ambulatory motor activity indicates that the lithium was unable to block or at least attenuate their hyperactivity. The continued induction of hyperactivity in the experimental HYPER group throughout the 30 days of the investigation could also be attributed to the inability of lithium to reverse their hyperactivity once it had begun.

The purpose of experiment 2 was to examine the intake of three frequently used BD medications (Valproate, Carbamazepine, and Lithium Citrate) on the HYPER rats as well as evaluate any effects on their activity. The lithium group showed the highest ambulatory motor activity in both the dark and light ambulatory measures (see Figure 6 and 7). This increased activity in the lithium chow group is contrary to the predicted reduction of activity. The hyperactivity of the lithium group may have been due to the elevated stress from the drug chow. It also may have been due to the inability of lithium to block or reverse the hyperactivity of the HYPER rat. The unforeseen low activity observed in the ambulatory activity of the control chow group may have been due to the limited availability of the HYPER rat during the time of experimentation. The allotment of the HYPER rat to each group attempted to equally distribute the HYPER rat based on

its responsive activity status, with the HYPER rat that exhibits more responsive hyperactivity as the best example of this behavioral model, and the non-responsive ones being unable to illustrate its characteristic hyperactivity. This activity status was determined based on HYPER rat responses in activity monitoring conducted previous to the investigation. The limitation in HYPER rat availability meant that the control group, as well as the other three groups, had some with a non-responsive status. The use of this non-responsive HYPER rat reduces the ability to properly assess the full effects that the administration of drug or non-drug imbued chows had on the HYPER rat ambulation. They also diminish the ability of the control group to properly demonstrate the normal hyperactivity of the HYPER model, and act as a good comparison for the three drug chow groups. All these factors make an assessment of the effects of the BD drugs on the HYPER rat very complicated. The monitoring period of 14 days may have also been too short to accumulate enough ambulatory data for a more complete analysis of the differences between the groups.

Despite the hyperactivity observed during the monitoring period, the defensive withdrawal test yielded no significant effects in all the measures taken across rat chow groups. This behavioral test assesses the degree of motor ability and the anxiety levels of the rats, through a comparison of the number of inner versus outer squares the rat enters, as well as measures such as emergence time from the tube and squares per second. A non-anxious rat would be expected to show less exploration of the open field. While anxious rats are more reluctant to enter into the inner portion of the open field preferring to stay closer to the outer edges, as well as showing the highest ambulation around the edges of the field (Navarro et al., 1997). There was no effect found in any of the

measures taken from the results of the defensive withdrawal test between the four chow groups, even with the exclusion of the rats that did not emerge from the tube and therefore never entering the open field (Figures 8-13). This lack of a significant difference between the four rat chow groups could be due to a similar amount of anxiety across all four groups during this test. While the lithium group showed the highest ambulatory activity its non-significant performance in the defensive withdrawal test, compared to the other three groups, points to a similarity in its degree of anxiety. This behavioral test may not have been able to completely probe into the detailed characteristics of the interactions between the four rat chow groups.

The purpose of this study was to investigate whether it was possible to administer BD medications orally as well as assess the reaction of the HYPER rat to them to evaluate it as a potential BD model. Its fluctuations between manic and depressed periods in ambulatory motor activity present it as a strong possible model, since it exhibits the full behavioral spectrum of BD. But its sensitivity to stressors in its environment makes any type of drug assessment intricate. Further work is required to gain a more complete understanding of the HYPER rat as a potential BD model, both experiments provided further insight into the type of inquiry needed to better asses this model. The first experiment was able to establish that lithium could be administered orally by being disguised in palatable treats, but due to low lithium levels it did not reduce activity as predicted. This exposes the need for less stressful administration vehicles for BD medications, such as lithium, for the HYPER rat. While the second experiment did yield significant effects between the drug chow groups it did not establish the kind of effect expected from these BD medications. The lithium group exhibited the highest dark and

light ambulatory motor activity. This could be due to a number of experimental factors and will need to be further analyzed and replicated to substantiate the results further. Certain limitations, such as a small monitoring time frame and the use of non-responsive rats, demonstrate the need for further testing and analysis to better evaluate the reaction of this HYPER rat model to BD medications. Even though the investigation utilized many commonly used BD medications, there has been evidence that medications such as lithium have effects that can actually induce increased rapid activity cycling in rats instead of reducing activity as seen in humans (Antelman et al., 1998). A possible solution to this would be the use of other effective BD medications, such as Risperidone, to provide a wider array of testing on the HYPER rat model. The growing prevalence of BD in our world makes the search for a better animal model of BD critical in uncovering the mystery behind this disorder. The difficulty in creating a model with the full spectrum of symptoms seen in BD is an ongoing process that could provide neurological and biochemical insights into this disorder later on in the future. This animal model is also critical in the testing and development of more effective treatments to improve the quality of life of patients. Since BD is a lifelong disorder the development of better medications and therapies provides hope that patients can live a better life.

Figure 1. Weekly Drug Treat Schedule

	CG	LRDF	BLCF	MF	LCCF	OWCF	OG	BD	PBF	CRDF	MGCF
Mon	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g						
Tues		0.5 g	0.5 g	0.5 g	0.5 g	0.5g					
Wed			0.5 g	0.5 g	0.5 g	0.5g	0.5g				
Thur				0.5 g	0.5 g	0.5g	0.5g	0.5 g			
Fri					0.5 g	0.5g	0.5g	0.5 g	0.5g		
Sat						0.5g	0.5g	0.5 g	0.5g	0.5 g	
Sun							0.5g	0.5 g	0.5g	0.5 g	0.5 g

Figure 1. Shows a weekly treat schedule for HYPER rats, vertical axis contains abbreviations for various food treats used (Cherry Gelatin = CG, Lamb & Rice Dog Food = LRDF, Beef & Liver, Cat Food = BFCF, Mint Fudge = MF, Liver & Chicken Cat Food = LCCF, Ocean Whitefish Cat Food = OWCF, Orange Gelatin = OG, Bacon Dough = BD, Peanut Butter Fudge = PBF, Chicken & Rice Dog Food = CRDF, Mixed Grill Cat Food = MGCF) and horizontal axis represents days of the week. The measure of 0.5 g is the size of each treat given to the HYPER rat. The red shading indicates the treats containing Lithium Citrate for that day, normal preceded and followed by two days without drug within same treat.

Figure 2. Monitor Room Cage

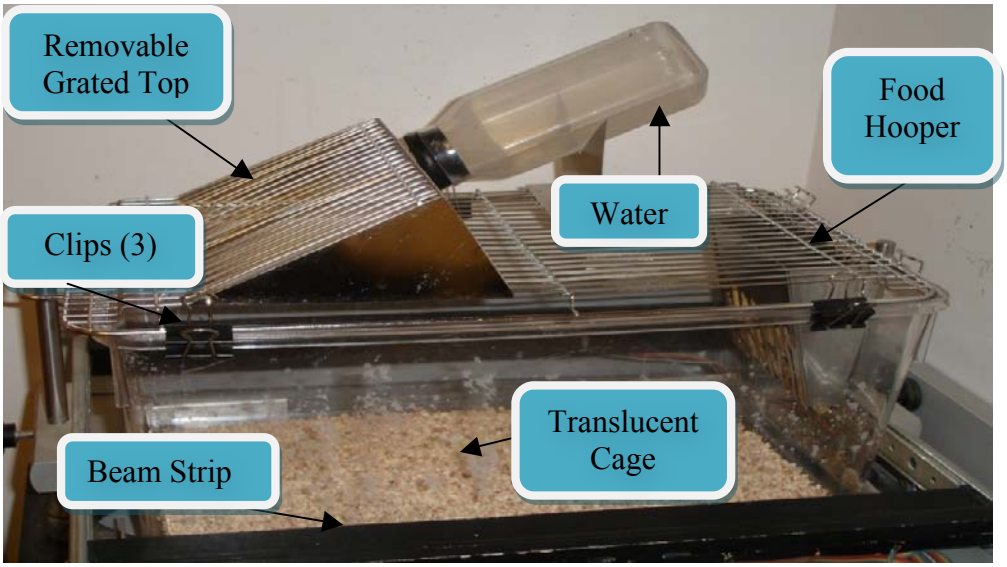

Figure 2. A picture of a translucent cage within the monitor room which holds a single HYPER rat. The translucent cage is surrounded by infared beams that utilize the computer SMA software to monitor ambulatory activity of a HYPER rat.

Figure 3. Diagram of Open Field used in Defensive Withdrawal Test

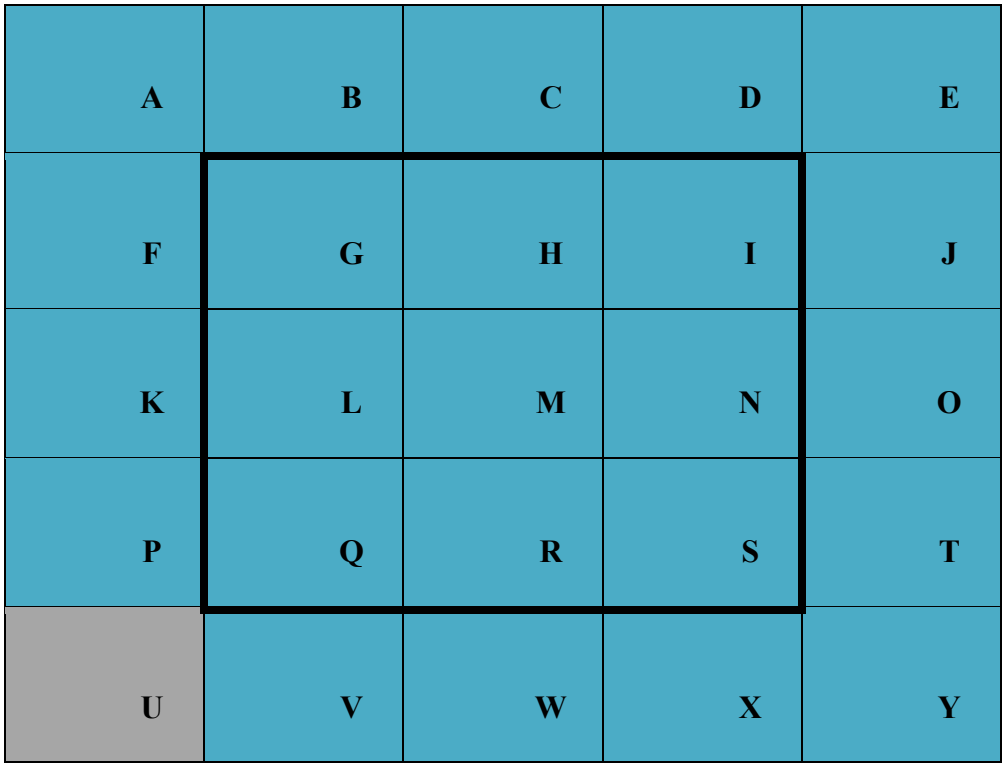

Figure 3. The 5 X 5 open field diagram represents the open field testing box used to test motor and anxiety of HYPER rats, during the defensive withdrawal test. The gray U square represents where the one sided tube which contains HYPER rat is placed at the onset of the test. Darker outlined portion of the open field differentiates the 3 x 3 inner squares from the 5 x 5 outer squares which are used as part of the assessment of the HYPER rat anxiety.

Figure 4. Dark Ambulatory Motor Activity from Experiment 1

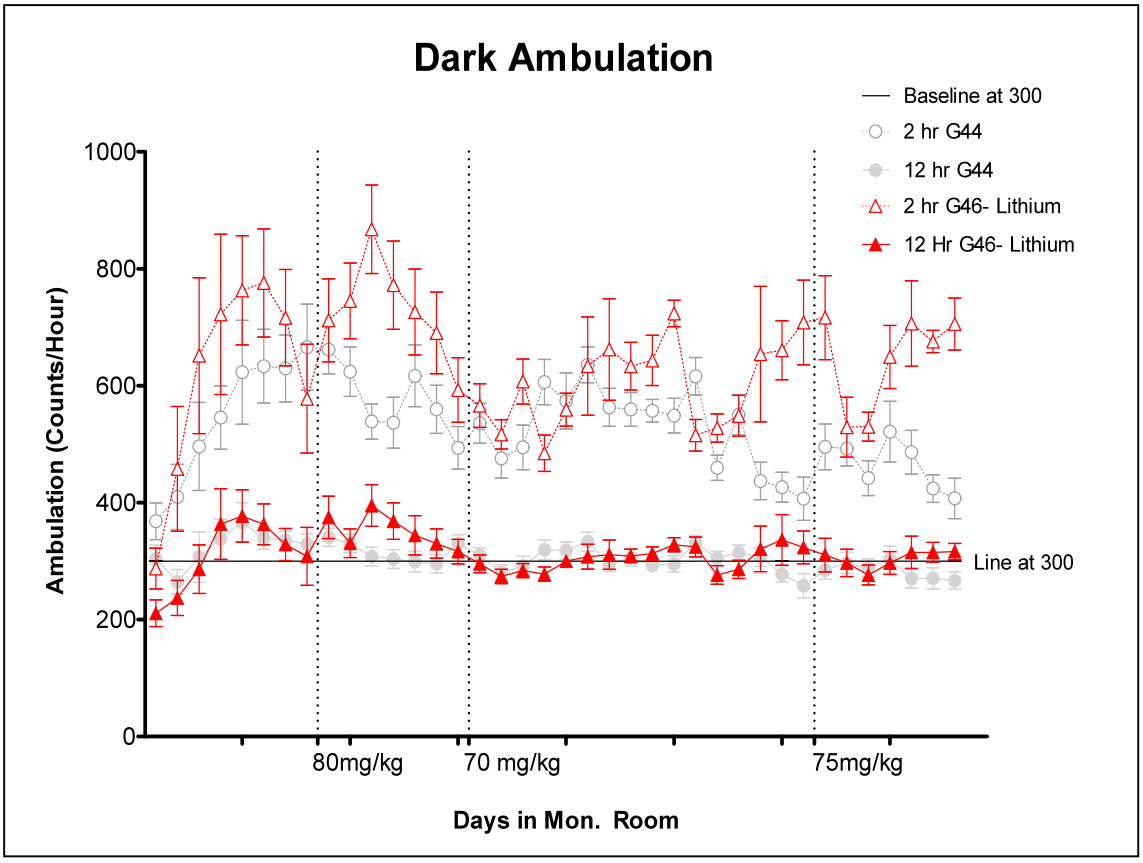

*Figure 4.*This graph depicts the days of dark ambulatory activity against the ambulation counts. The data in red is of the experimental HYPER rats that received the lithium treat administration (G46) while the data in grey is of the previous non-experimental HYPER rats (G44). Along the x axis the days are classified according to each specific drug dose period throughout the lithium administration to the experimental HYPER rats. The graph contains both the 12 hour and 2 hour dark activity data to give the full spectrum of dark ambulatory activity within the experimental period.

Figure 5. 12 Hour Light Ambulation from Experiment 1

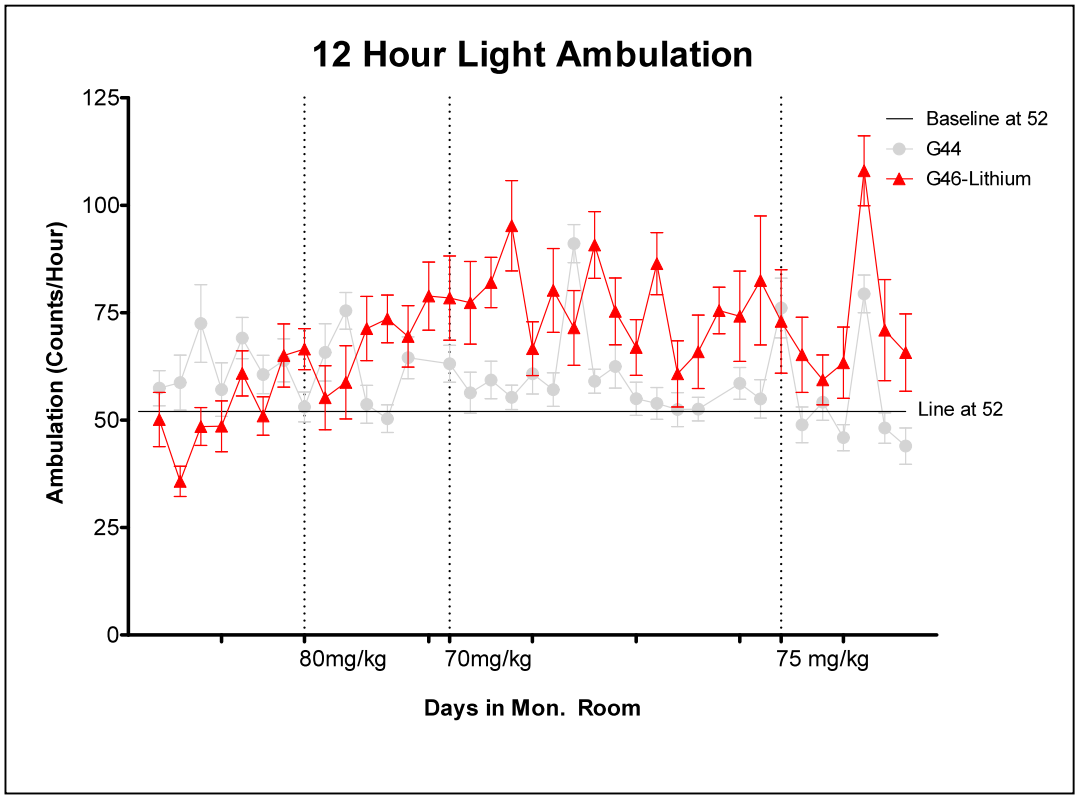

*Figure 5.*This graph depicts the days of light ambulatory activity against the ambulation counts. The data in red is of the experimental HYPER rats that received the lithium treat administration (G46) while the data in grey is of the previous non-experimental HYPER rats (G44). Along the x axis the days are classified according to each specific drug dose period throughout the lithium administration to the experimental HYPER rats. The graph contains the 12 hour light activity data to give the spectrum of light ambulatory activity within the experimental period.

Figure 6. Dark Ambulatory Motor Activity from Experiment 2

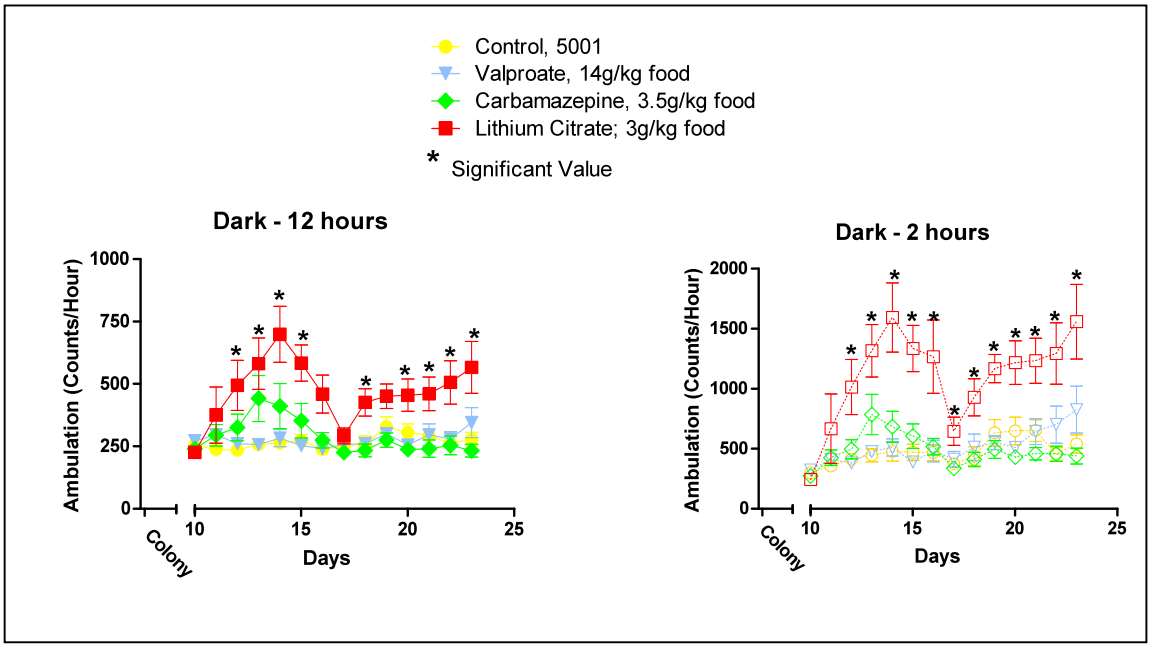

Figure 6. These graphs depict the 12 hour and 2 hour dark ambulatory motor activity of each group of HYPER rats. The x axis is divided in two portions the first one relating to the initial period in which the HYPER rat groups received their specific chows and were in group housing within the colony. The second potion of the x-axis illustrates their individual monitoring within the monitor room for the duration of the experiment. The y-axis represents their ambulatory motor activity. The legend denotes the color divisions between each HYPER rat group according to the type of chow they received (Control- yellow, Valproate- blue, Carbamazepine- green, Lithium- red). The asterisk denotes the days with a significant effect in the activity data between the lithium and control group.

Figure 7. 12 Hour Light Ambulatory Motor Activity from Experiment 2

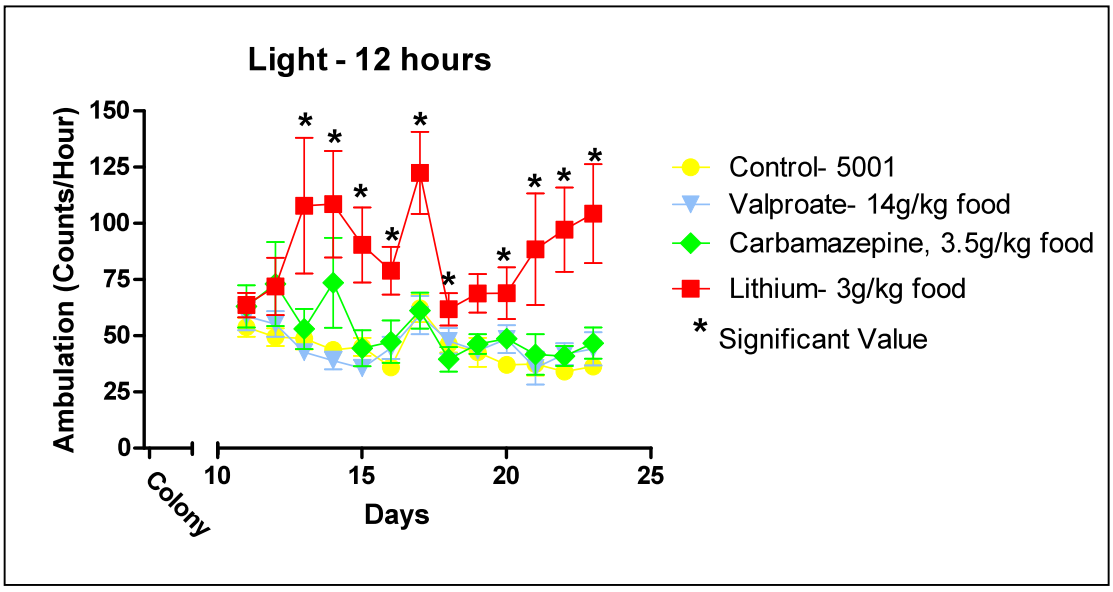

*Figure 7.*This graph depicts the 12 hour light ambulatory motor activity of the four different HYPER rat groups. The x axis is divided in two portions the first one relating to the initial period in which the HYPER rat groups received their specific chows and were in group housing within the colony. The second potion of the x-axis illustrates their individual monitoring within the monitor room for the duration of the experiment. The y-axis represents their ambulatory motor activity. The legend denotes the color divisions between each HYPER rat group according to the type of chow they received (Control- yellow, Valproate- blue, Carbamazepine- green, Lithium- red). The asterisk denotes the days with a significant effect in the activity data between the lithium and control group.

Figure 8. Total Number of Squares for Experiment 2

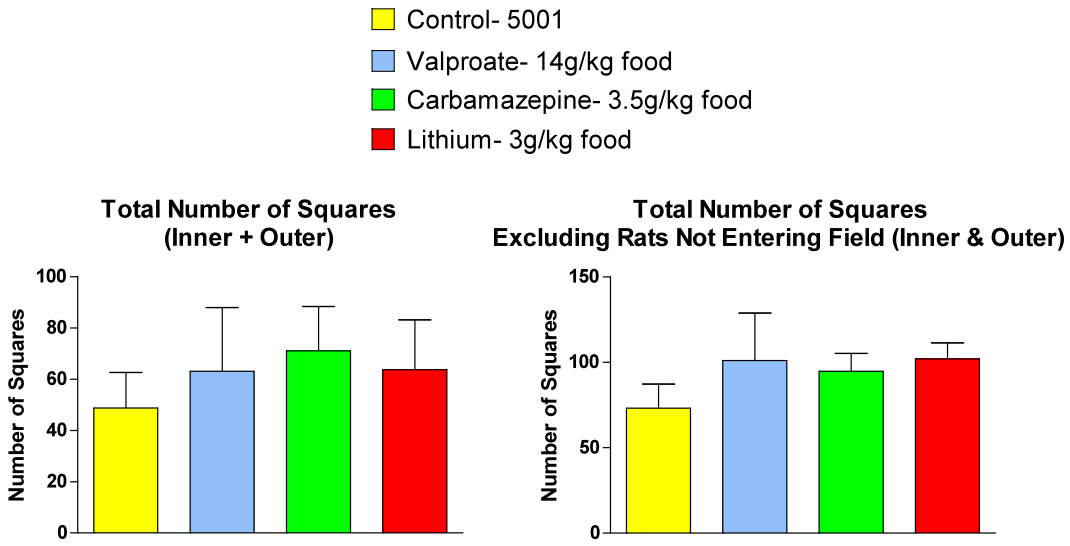

Figure 8. Both graphs represent the total number of squares (both inner and outer) entered by the HYPER rats tested in a defensive withdrawal test. The graph on the right has excluded the rats that did not enter the open field out of the analysis to assess the magnitude of the interaction between the four groups. The legend denotes the color divisions between each HYPER rat group according to the type of chow they received (Control- yellow, Valproate- blue, Carbamazepine- green, Lithium- red).

Figure 9. Total Number of Inner Squares for Experiment 2

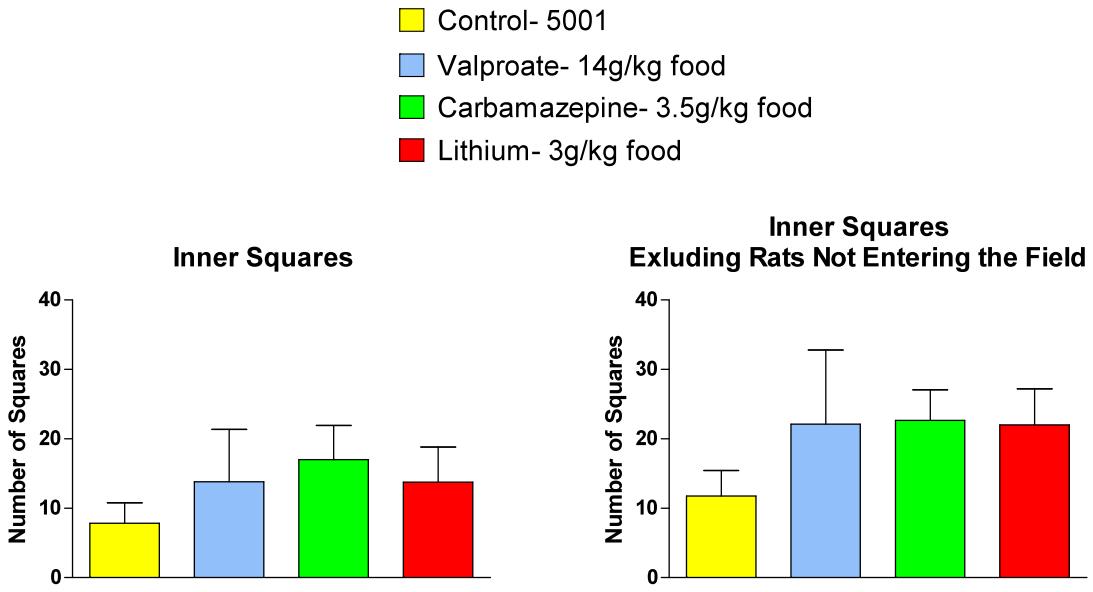

Figure 9. Both graphs represent the total number of inner squares entered by the HYPER rats within the open field across group. The graph on the right depicts the total number of inner squares entered by the HYPER rats excluding the rats that did not enter the open field. The legend denotes the color divisions between each HYPER rat group according to the type of chow they received (Control- yellow, Valproate- blue, Carbamazepine- green, Lithium- red).

Figure 10. Total Number of Squares per Second for Experiment 2

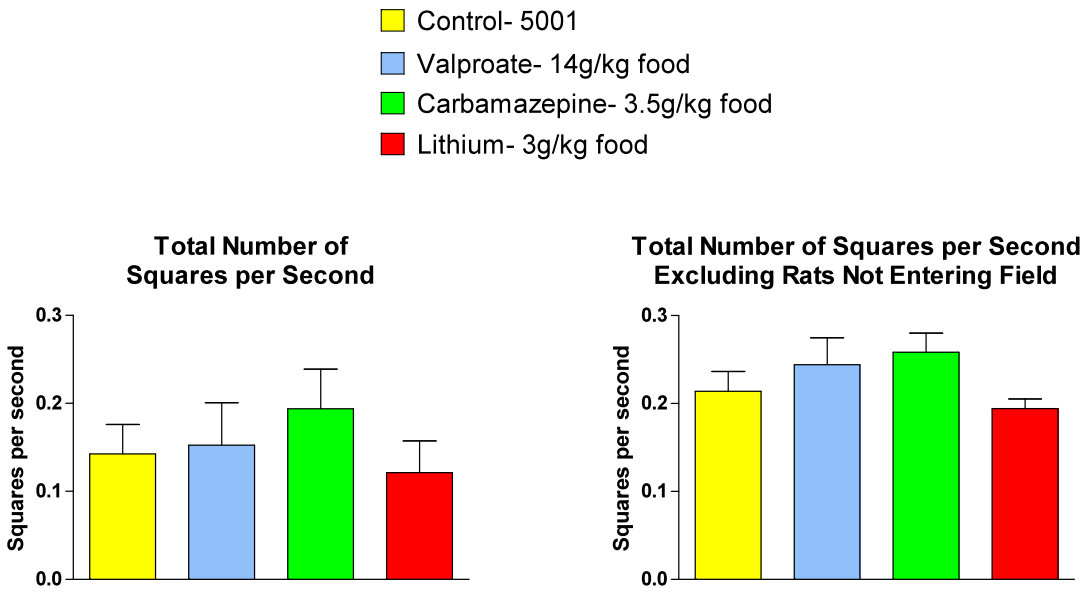

Figure 10. Both graphs represent the total number of squares per second entered by the HYPER rats tested in a defensive withdrawal test. The graph on the right has excluded the rats that did not enter the open field out of the analysis to assess the magnitude of the interaction between the four groups. The legend denotes the color divisions between each HYPER rat group according to the type of chow they received (Control- yellow, Valproate- blue, Carbamazepine- green, Lithium- red).

Figure 11. Total Time of Emergence from Tube for Experiment 2

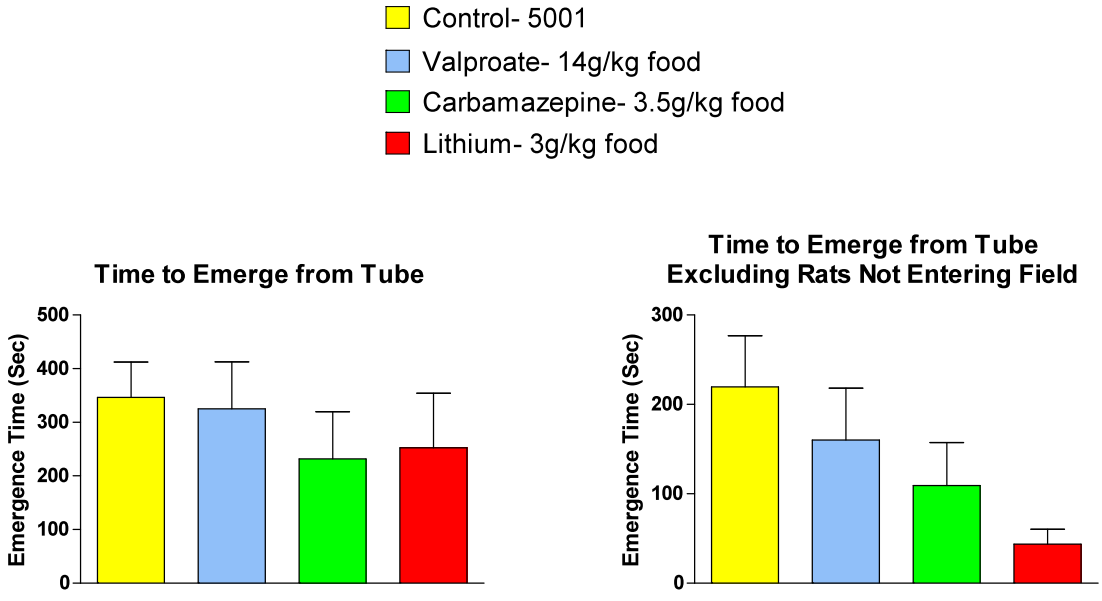

Figure 11. Both graphs represent the time of emergence from the tube by the HYPER rats tested in a defensive withdrawal test. The graph on the right has excluded the rats that did not enter the open field out of the analysis to assess the magnitude of the interaction between the four groups. The legend denotes the color divisions between each HYPER rat group according to the type of chow they received (Control- yellow, Valproate- blue, Carbamazepine- green, Lithium- red).

Figure 12. Total Time Spent in Tube for Experiment 2

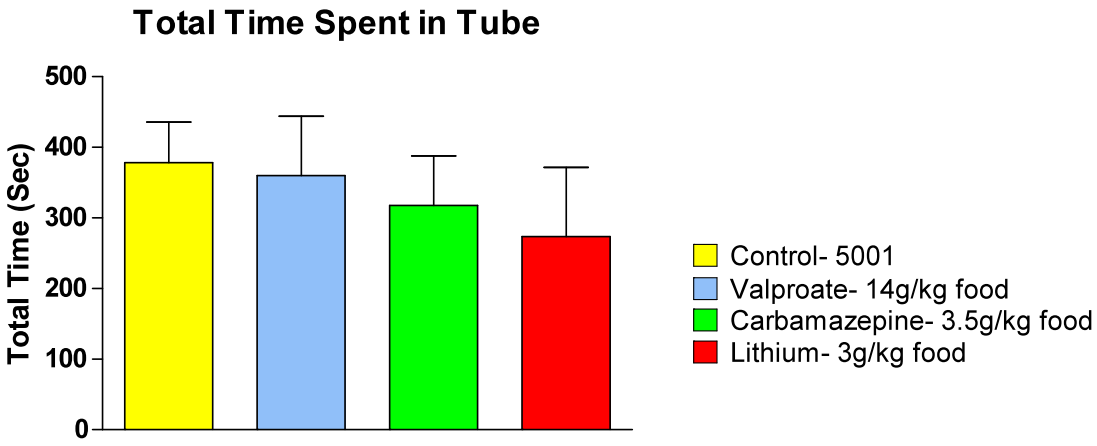

Figure 12. This graph represents the total time spent in the tube by the HYPER rats tested in a defensive withdrawal test. The legend denotes the color divisions between each HYPER rat group according to the type of chow they received (Control- yellow, Valproate- blue, Carbamazepine- green, Lithium- red).

Figure 13. Total Number of Boluses for Experiment 2

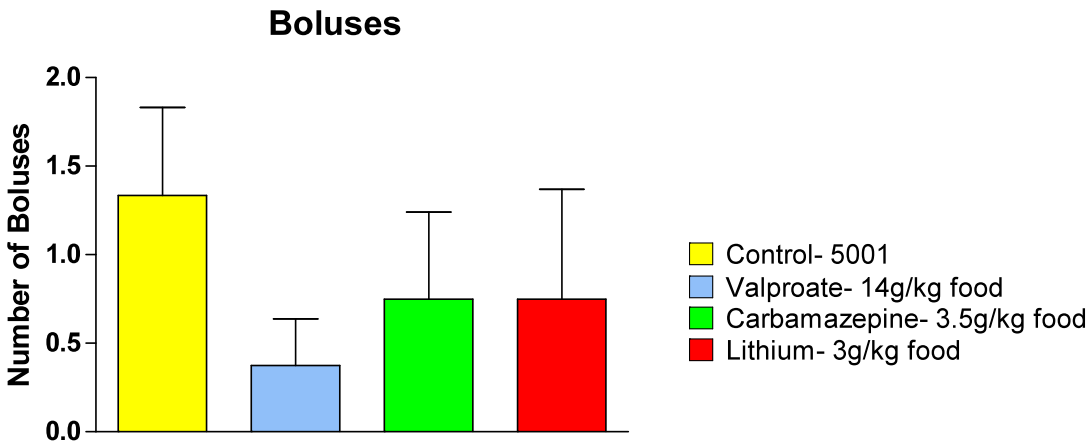

Figure 13. The graph represents the total boluses left by the HYPER rats in a defensive withdrawal test across rat chow groups. The legend denotes the color divisions between each HYPER rat group according to the type of chow they received (Control- yellow, Valproate- blue, Carbamazepine- green, Lithium- red).

References

American Psychiatric Association (2000). *Diagnostic and statistical manual of mental disorders* (4th ed., Text Revision). Washington, DC: Author.

Angst, J. (1978). The course of affective disorders. II. Typology of bipolar manic-depressive illness. *Arch Psychiatr Nervenkr, 226*(1), 65-73.

Antelman, S. M., Caggiula, A. R., Kucinski, B. J., Fowler, H., Gershon, S., Edwards, D. J., . . . Kocan, D. (1998). The effects of lithium on a potential cycling model of bipolar disorder. [Research Support, U.S. Gov't, P.H.S.]. *Progress in neuro-psychopharmacology & biological psychiatry, 22*(3), 495-510.

Atack, J. R., Broughton, H. B., & Pollack, S. J. (1995). Inositol monophosphatase--a putative target for Li+ in the treatment of bipolar disorder. [Review]. *Trends in neurosciences, 18*(8), 343-349.

Belmaker, R. H. (2004). Bipolar disorder. [Review]. *The New England journal of medicine, 351*(5), 476-486. doi: 10.1056/NEJMra035354

Blanco, C., Laje, G., Olfson, M., Marcus, S. C., & Pincus, H. A. (2002). Trends in the treatment of bipolar disorder by outpatient psychiatrists. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *The American journal of psychiatry, 159*(6), 1005-1010.

Bowden, C. L., & Singh, V. (2005). Valproate in bipolar disorder: 2000 onwards. [Research Support, Non-U.S. Gov't Review]. *Acta psychiatrica Scandinavica. Supplementum*(426), 13-20. doi: 10.1111/j.1600-0447.2005.00522.x

Cipriani, A., Pretty, H., Hawton, K., & Geddes, J. R. (2005). Lithium in the prevention of suicidal behavior and all-cause mortality in patients with mood disorders: a systematic review of randomized trials. [Meta-Analysis Research Support, Non-U.S. Gov't Review]. *The American journal of psychiatry, 162*(10), 1805-1819. doi: 10.1176/appi.ajp.162.10.1805

Craddock, N., & Jones, I. (1999). Genetics of bipolar disorder. *J Med Genet, 36*(8), 585-594.

Ferreira, M. A., O'Donovan, M. C., Meng, Y. A., Jones, I. R., Ruderfer, D. M., Jones, L., . . . Craddock, N. (2008). Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Nature genetics, 40*(9), 1056-1058. doi: 10.1038/ng.209

Frey, B., Andreazza, A., Cereser, K., Martins, M., Valvassori, S., Reus, G., . . . Kapczinski, F. (2006). Effects of mood stabilizers on hippocampus BDNF levels in an animal model of mania. *Life Sciences, 79*(3), 281-286. doi: 10.1016/j.lfs.2006.01.002

Fukumoto, T., Morinobu, S., Okamoto, Y., Kagaya, A., & Yamawaki, S. (2001). Chronic lithium treatment increases the expression of brain-derived neurotrophic factor in the rat brain. *Psychopharmacology (Berl), 158*(1), 100-106. doi: 10.1007/s002130100871

Gessa, G. L., Pani, L., Fadda, P., & Fratta, W. (1995). Sleep deprivation in the rat: an animal model of mania. *Eur Neuropsychopharmacol, 5 Suppl*, 89-93. doi: 0924977X9500023I [pii]

Gilmor, M. L., Skelton, K. H., Nemeroff, C. B., & Owens, M. J. (2003). The effects of chronic treatment with the mood stabilizers valproic acid and lithium on corticotropin-releasing

factor neuronal systems. *J Pharmacol Exp Ther, 305*(2), 434-439. doi: 10.1124/jpet.102.045419jpet.102.045419 [pii]

Gould, T. D., Chen, G., & Manji, H. K. (2004). In vivo evidence in the brain for lithium inhibition of glycogen synthase kinase-3. *Neuropsychopharmacology, 29*(1), 32-38. doi: 10.1038/sj.npp.13002831300283 [pii]

Gould, T. D., & Einat, H. (2007). Animal models of bipolar disorder and mood stabilizer efficacy: a critical need for improvement. *Neurosci Biobehav Rev, 31*(6), 825-831. doi: S0149-7634(07)00068-1 [pii]10.1016/j.neubiorev.2007.05.007

Gurvich, N., Tsygankova, O. M., Meinkoth, J. L., & Klein, P. S. (2004). Histone deacetylase is a target of valproic acid-mediated cellular differentiation. [Research Support, U.S. Gov't, P.H.S.]. *Cancer research, 64*(3), 1079-1086.

Hammonds, M. D., & Shim, S. S. (2009). Effects of 4-week treatment with lithium and olanzapine on levels of brain-derived neurotrophic factor, B-cell CLL/lymphoma 2 and phosphorylated cyclic adenosine monophosphate response element-binding protein in the sub-regions of the hippocampus. *Basic Clin Pharmacol Toxicol, 105*(2), 113-119. doi: PTO416 [pii]10.1111/j.1742-7843.2009.00416.x

Kato, T., Kubota, M., & Kasahara, T. (2007). Animal models of bipolar disorder. *Neurosci Biobehav Rev, 31*(6), 832-842. doi: S0149-7634(07)00025-5 [pii]10.1016/j.neubiorev.2007.03.003

Machado-Vieira, R. (2004). Perspectives for the development of animal models of bipolar disorder. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 28*(2), 209-224. doi: 10.1016/j.pnpbp.2003.10.015

Marx, C. E., Yuan, P., Kilts, J. D., Madison, R. D., Shampine, L. J., & Manji, H. K. (2008). Neuroactive steroids, mood stabilizers, and neuroplasticity: alterations following lithium and changes in Bcl-2 knockout mice. *Int J Neuropsychopharmacol, 11*(4), 547-552. doi: S1461145708008444 [pii]10.1017/S1461145708008444

Navarro, M., Hernandez, E., Munoz, R. M., del Arco, I., Villanua, M. A., Carrera, M. R., & Rodriguez de Fonseca, F. (1997). Acute administration of the CB1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat. [Research Support, Non-U.S. Gov't]. *Neuroreport, 8*(2), 491-496.

Odonnell, K., & Gould, T. (2007). The behavioral actions of lithium in rodent models: Leads to develop novel therapeutics. *Neuroscience & Biobehavioral Reviews, 31*(6), 932-962. doi: 10.1016/j.neubiorev.2007.04.002

Price, L. H., & Heninger, G. R. (1994). Lithium in the treatment of mood disorders. *N Engl J Med, 331*(9), 591-598. doi: 10.1056/NEJM199409013310907

SPSS (PASW) for Windows, Rel. 18.0.0. [Computer software]. (2009). Chicago, IL: SPSS, Inc. http://www.spss.com .

Weiss, J. M., West, C. H., Emery, M. S., Bonsall, R. W., Moore, J. P., & Boss-Williams, K. A. (2008). Rats selectively-bred for behavior related to affective disorders: proclivity for intake of alcohol and drugs of abuse, and measures of brain monoamines. [Review]. *Biochemical pharmacology, 75*(1), 134-159. doi: 10.1016/j.bcp.2007.09.027

Yuan, P., Chen, G., & Manji, H. K. (1999). Lithium activates the c-Jun NH2-terminal kinases in vitro and in the CNS in vivo. *J Neurochem, 73*(6), 2299-2309.