COVER SHEET

TITLE: Lithium and its effects on a novel (non drug-induced) hyperactive mouse model

AUTHOR'S NAME: Alexandra Ostromecki

MAJOR: Biology

DEPARTMENT: Biology in CALS

MENTOR: Stephen Gammie

DEPARTMENT: Zoology

MENTOR(2): Melissa Scotti

DEPARTMENT(2): Zoology

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ABSTRACT

Bipolar disorder (BPD) is a devastating long-term disease for which a significant symptom is mania. Researchers have long relied on the use of rodent models to study psychological disorders (like BPD), as well as to develop pharmaceuticals to treat these diseases. Thus far, however, the most common behavioral proxies of manic symptoms are induced in rodents via treatment with psycho-stimulants. Recently, our lab isolated a line of organically hyperactive mice (L6 mice) that exhibit behavioral characteristics that may be analogous to symptoms of mania (i.e., reduced sleep, increased boldness, increased aggression, and hyperactivity). To test the potential that these animals could be used to model mania, we treated our animals with either standard rodent chow or chow supplemented with the anti-mania drug lithium. We applied various tests commonly used to gauge hyperactivity and other "mania-like" symptoms in rodents. Tests were run both prior to and post drug treatment to assess lithium's efficacy in attenuating the "mania-like" symptoms expressed in the L6 mice, and lithium was in fact found to have an effect in decreasing in-cage hyperactivity. Collectively, this work represents the first step in investigating the possibility that this line of mice could serve as a novel model for mania.

Alexandra Ostromecki / Biology
Author Name/Major

Alexandra Ostromecki
Author Signature

Stephen Gammie
Mentor Name/Department

Mentor Signature

04/29/2010
Date

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Alexandra Ostromecki

Mentors: Dr.'s Stephen Gammie and Melissa Scotti
215 Zoology Research Building
Madison, WI, 53703

Abstract

Bipolar disorder (BPD) is a devastating long-term disease for which a significant symptom is mania. Researchers have long relied on the use of rodent models to study psychological disorders (like BPD), as well as to develop pharmaceuticals to treat these diseases. Thus far, however, the most common behavioral proxies of manic symptoms are induced in rodents via treatment with psycho-stimulants. Recently, our lab isolated a line of organically hyperactive mice (L6 mice) that exhibit behavioral characteristics that may be analogous to symptoms of mania (i.e., reduced sleep, increased boldness, increased aggression, and hyperactivity). To test the potential that these animals could be used to model mania, we treated our animals with either standard rodent chow or chow supplemented with the anti-mania drug lithium. We applied various tests commonly used to gauge hyperactivity and other "mania-like" symptoms in rodents. Tests were run both prior to and post drug treatment to assess lithium's efficacy in attenuating the "mania-like" symptoms expressed in the L6 mice, and lithium was in fact found to have an effect in decreasing in-cage hyperactivity. Collectively, this work represents the first step in investigating the possibility that this line of mice could serve as a novel model for mania.

Introduction

Bipolar disorder, or manic-depression, is a devastating long-term disease affecting approximately 5.7 million Americans each year (NIMH, 2008). Although many components exist to the illness, a significant and often destructive one is mania. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), mania is defined as a distinct period of abnormally and persistently elevated, expansive, or irritable mood lasting at least one week. Characteristics consist of elevated distraction, accelerated speech, restlessness, insufficient sleep,

long periods of "highs," agitation, and engaging in impulsive and high-risk behaviors (NIMH, 2008). Currently, various treatments exist for the disorder, but difficulties include poor treatment outcomes with considerable side effects, high relapse rates, and ongoing symptoms (Flaisher-Grinberg et al., 2009). Treatments for mania include lithium, valproic acid, lamotrigine, gabapentin, topiramate, and oxcarbazepine (NIMH, 2008); however, improvements in care are constantly being made.

Researchers have long relied on the use of rodent models to study psychological disorders such as mania, as well as to help develop pharmaceuticals to treat these diseases (Sankoorikal et al., 2006). For example, one paradigm utilizes a sweet solution (saccharin) preference test to determine whether a rodent model could exist for increased reward-seeking/hedonistic behavior, a common attribute of mania (Flaisher-Grinberg et al., 2009). Another common test used to demonstrate locomotor activity and exploratory behavior (both attributes of mania) is placement of the mouse in a novel open field box, where boxes are drawn on the surface of the floor of the apparatus, and the number of boxes entered are an indication of one unit of exploratory activity (Crawley, 1985). A light/dark box, in which one portion of the box is open and one portion is closed, is also a commonly utilized measure of locomotive, risk-taking behavior (Crawley, 1985). A fourth test, used to measure irritability in mice, is the elevated plus maze, in which there are two open arms and two enclosed. Anxiety is then measured by more frequent entry into the closed portion than the open portion of the maze (Hilakivi et al., 1989). Typically the behaviors researchers choose to use as proxies for mania symptoms, or other psychological disorders, are considered to be analogous to components of the disorder's overall symptomology (i.e., endophenotypes) (Harrison-Read, 2008; Einat, 2006). The endophenotype concept establishes subclinical elements of, and is used to examine, the genetic

foundation for complex psychiatric conditions (Harrison-Read, 2008; Einat, 2006). An animal model may be created if an endophenotype and the relevant clinical disorder share genetic features, and similar neurobiological variables may be found or induced in experimental animals without necessarily imitating the clinical features of the psychiatric ailment itself (Harrison-Read, 2008). An example of this is "prepulse inhibition of the startle response, showing deficits in sensory-motor gating that can be measured in schizophrenic and bipolar patients (Franks, et al., 1983) and in rodents in which genetic and pharmacological influences have been reported (Geyer, et al., 2002; Ong, et al., 2005)" (Harrison-Read, 2008).

However, in many instances, the behavioral proxies of disorder syptoms are drug-induced. For example, previous research tested the effects of anti-mania medication on hyper-locomotion in mice. The hyper-locomotion (a proxy for hyperactivity) is usually achieved through the administration of drugs such as *d*-amphetamine (Gould et al., 2007). These methods ultimately diminish their own usefulness since induced behavioral changes are likely to depend on neurobiological mechanisms isolated from those causing mania, and it is recommended that environmental manipulations be used in place of drug treatments (Harrison-Read, 2008).

Regardless of the behavioral model of mania being tested, however, studies involving rodent mania models have often found that lithium is effective in reducing "mania-like" and other behaviors. According to Bersudsky et al. (2007), lithium is the drug of choice in bipolar disorder treatment, and successfully produces a strong antidepressant effect in the forced swim test in mice. Not limited to bipolar disorder, lithium is also used in the treatment of schizophrenia, aggressive disorders, and other symptoms associated with a variety of psychiatric ailments. The disadvantages of these previous mouse models, however, include the inability to measure more than one aspect of mania at once, as well as the fact that the mice have been

administered drugs in order to achieve such a state and the behaviors associated with it (which does not parallel how the disorder occurs in humans).

Our lab has developed a unique line of mice that express behaviors such as hyperactivity, sleeplessness, elevated aggression, and increased risk-taking, all of which are considered endophenotypes for mania. Thus, these mice may be appropriate candidates for mania research. This mouse strain allows us the ability to test more than just one endophenotype of the disorder and, more importantly, does not require drugs to achieve any of these behaviors. To determine if this unique line could be a good model for mania, we fed a subset of these mice chow supplemented with lithium chloride (LiCl). We then used various known tests for its associated behaviors. In addition to in-cage observations conducted multiple times on a weekly basis, tests used to study manic behaviors included the light/ dark box, open-field, and forced swim tests, which were performed both pre and post treatment. In order to determine which mice displayed specific hyperactive tendencies, in-cage observations were used, and open-field and forced swim tests were performed to measure overall hyperactivity. The light/ dark box was used as a measure of risk-taking behaviors. We hypothesized that mice treated with a lithium-supplemented diet would display less "manic-like" behaviors than animals fed a control diet.

This research is unique because, until now, no other study has tested the effects of lithium in a *naturally* hyperactive mouse model in order to study mania. The lithium treatment was used because of its known history for controlling manic symptoms and inhibiting the return of manic and depressive episodes (NIMH, 2008; Gould et al., 2007). Furthermore, some in the scientific community believe that unless testing of already approved existing medications occurs, such as antipsychotics and anticonvulsants, it is unlikely that advances in treatments for mood disorders can be made (O'Donnell et al., 2007).

Methods

Forty adult male mice, (> 60 days old) from our unique mania line were separately housed in standard size cages, each measuring 27.8 x 17.5 x 13.0 cm. We created an extended light cycle of fourteen hours light and ten hours dark, with lights on at 7AM CST, in the room where the mice were housed. We then allowed them to adjust to this schedule for a period of one week before any observations or testing occurred. Temperature and humidity were stable throughout the study at 20 ± 2°C and 24.0 ± 5%, respectively. Food and water were available *ad libitum*.

One week after housing and allowing for the mice to adjust to the new light schedule, preliminary in-cage observations were made over the course of three consecutive days. Specific focus was on measures of mania-like hyperactivity, including behaviors such as climbing, spinning, etc. in the cage. A total of thirty observations per mouse were made during each observation period with the mice remaining in their home cage. Other baseline tests included five-minute light/ dark box trials and seven-minute forced swim tests, both of which were also used to measure mania, and described below. Twenty of the animals were then randomly assigned to a control food treatment, and twenty to a lithium food treatment. Animals began receiving this diet two days after the final baseline test, for 35 days. Light/ dark box, open field and forced swim tests (post LiCl treatment) were performed on days 33 and 34, and 35 respectively.

In total, two trials of light/ dark box, open field tests, and forced swim tests were performed. Multiple trials performed on separate days on the same mouse were later scored by the same observer to ensure accuracy in scoring for each test. All animals were cared for according to the National Institute of Health's Guide for the Care and Use of Laboratory

Animals, as well as the University of Wisconsin's Institutional Animal Care and Use Committee (IACUC).

Food Preparation

40 mice were randomly assigned to either LiCl treatment (n=20) or control (n=20). Two days after the final baseline test, animals received regular breeder chow (Harlan Teklad, Madison, WI diet 7004) or breeder chow supplemented with 2% LiCl (Harlan Teklad, Madison WI). After one week, LiCl-treated mice were switched to chow containing 4% LiCl (Harlan Teklad, Madison WI). This protocol was chosen because it has been found to produce serum and brain levels of lithium in rodents that are within the therapeutic range seen in humans (Gould et al., 2008; 2004). An additional bottle filled with a 450 mM solution of saline was also placed inside in the cages in order to regulate salt balance due to lithium treatment.

In-cage Observations

Three times a week, 30 observations of in-cage activity were made for each animal over a 60-90 minute time course. Observations occurred at 9AM, 4PM, or 11PM CST, over the course of three consecutive days each week. Cages were changed once every seven days, and mice were given one day to adjust before observations began. Behaviors were then categorized as asleep (both eyes closed and motionless), awake but not hyperactive (both eyes open but not displaying any hyperactive tendencies), or hyperactive (performing behaviors such as running, spinning, climbing, or doing back flips).

Light/ Dark Box

The light/ dark box is a plastic box (30 x 24.5 x 25.5 cm) in which one half is enclosed by an opaque insert, and one half is left open. In our study, each mouse was placed inside the opaque insert to start, and time to open (time it takes the mouse to move into the open portion of

the box), number of transitions from dark to light, total time spent in the light, and number of head pokes (times the mouse did not fully enter the light), were noted by an observer. An animal was only considered to be in the open if all four paws were in the light portion; otherwise, any fewer was considered a head poke. Each trial lasted five minutes, was videotaped, and later scored by an observer.

Open field test

The open field test was a five-minute test performed in a large plastic box (39 x 39 x 39cm) with lines drawn on the floor surface forming 16 total squares. Each time the mouse moved from one square to the next, it was considered as one unit of movement. The test was videotaped and later scored. Total crosses from one unit to the next, total crosses to the center squares of the box, and total crosses to the outer squares were all counted in the scoring.

Forced Swim Test

This was a seven-minute test in which mice were placed in a clear, cylindrical glass (3620.81 cm^3) filled half way with room temperature tap water (~24°C) and videotaped. Among the behaviors focused on were latency to first float, total floats, and total time spent floating, with an animal considered to be floating when all four legs had stopped moving.

Weight Measurement

Mice were weighed at the start of the study, and then once every week at the time that cages were changed throughout the study. Measurements were in grams.

Statistical Analysis

Statistical Analysis was done using Statistical Analysis Software. Repeated measure ANOVA's (analyses of variance) were used, and if the assumptions of normality and equal variance were violated, natural logarithm transformations were then performed.

Results

Hyperactivity: For hyperactivity, the 11PM time point yielded the most significant data. Within individuals across time, there was no change. However, there was a trend towards interaction between time point and treatment ($F_{3,114} = 2.160$, $p = 0.097$). There was a significant effect of treatment ($F_{1,38} = 6.940$, $p = 0.012$). Animals that received lithium were less active over time than control animals, demonstrated by higher hyperactivity averages for baseline LiCl-treated groups than all three post-treatment LiCl-treated groups. All three post-treatment groups were deemed significant ($p<0.05$) (Figure 1). Pre-treatment control groups maintained comparable means for hyperactivity as compared to all three post-treatment groups.

Although 9AM and 4PM tests were also performed, there was no effect of treatment for the 9AM observations ($p>0.05$). Across all time points for 4PM hyperactivity testing, there was no significance ($p>0.05$). There was, however, an overall change over time in behavior, and treatment was found to provide a trend towards interaction between time point and treatment here. No treatment effects were found on sleep in either group for hyperactivity.

Open field: For total open field, within individuals, regardless of treatment, there was an increase toward crossing (demonstrating locomotion), for which $F_{1,38} = 3.601$, $p = 0.065$, thus, overall, individuals, regardless of treatment, increased in their locomotor activity. For the percent in the center boxes of the open field test, there was no change over time, no interaction, and no effect of treatment or time point on the number of center squares crossed ($p>0.05$) (Figure 2).

Forced Swim Test and Light/ Dark Box Test: Swim tests and light/ dark box tests all showed no significance ($p>0.05$).

Weight: Mean weights decreased in groups treated with LiCl, and control groups remained the same.

Discussion

In order to study mania in our novel line of mice, we hypothesized that mice treated with a lithium-supplemented diet would display less "manic-like" behaviors than animals fed a control diet, and our research does in fact suggest that LiCl does decrease such behaviors. This is demonstrated by the significant effect of treatment from the 11PM hyperactivity observations, for which the p-values were all less than 0.05 for observations 1, 2, and 3 (post-treatment) (Figure 1). As figure 1 illustrates, LiCl-treated mice had lower overall averages of hyperactive behavior than both control and pre-treatment groups, showing that LiCl did in fact attenuate some of the mania. Although 9AM and 4PM observations of hyperactivity showed $p>0.05$ (no significance), the 4PM observations did show an overall trend towards a change over time in behavior. This shows that an increase in dosage of LiCl may have had its effects here. Another issue may be that 9AM and 4PM observation times were inherent periods of inactivity for these mice anyway, thereby leaving the 11PM observation time (during lights off) prone to the hyperactive behaviors observed. This may provide support for additional observations during the lights out portion of the cycle in the future.

As is shown in Figure 2, the LiCl treatment had no significant effect on total crosses in the open field test. One possibility for future testing may be to use a higher dosage of LiCl in order to achieve statistically significant effects. The trend for an increase in means for total crosses in the open field in both control and LiCl, and pre vs. post-treatment groups demonstrates an increase in locomotor activity, and therefore, an increase in hyperactivity, showing effects somewhat conflicting with the in-cage hyperactivity observation results. Because the mice may have become habituated to the given dose, future testing may include week-by-week increases in the dosage. Another possible explanation for these somewhat conflicting results is the inherent

difference in the nature of in-cage observations vs. the open-field test. It is also possible that an increase occurred due to acclimation of the mice (i.e. natural adaptation to the home cage vs. novelty of the open-field) to the test, or that these animals become more manic as they age. One solution for this may include performing multiple tests and collecting averages of the data in order to really focus on locomotor activity in the open-field tests, and ensure accuracy. Again, further increases in LiCl dosage, as well as timing of testing, may have provided the desired effect of decreased locomotion.

The LiCl-treated mice decreased in weight throughout the study, which was expected as previous research notes that LiCl-treated mice *do* typically experience weight loss. This decrease then suggests evidence of ingestion of the drug by the mice.

The implications of our research are numerous. Foundations of future drug development and other treatment options for manic patients could potentially come from our findings, and also provide new options for both young and old populations struggling with the disease. The ramifications of our research may also provide better insight into how LiCl affects the behaviors of mania, as well as the necessary duration of prescription of the drug in order to achieve such effects. This also extends to a variety of other ailments such as schizophrenia and numerous psychotic disorders, not only mania, providing broad implications in both the medical field and the research of such disorders. In addition, all those treated with any sort of psychotropic medication may be affected.

In conclusion, LiCl did appear to reduce mania-like behaviors in our novel line of organically hyperactive mice. We believe this study has provided us with the foundation for future research, and hope to pursue further testing, both with this drug and other psychotropic medications, in the study of mania in our novel line of mice.

Appendix

Figure1

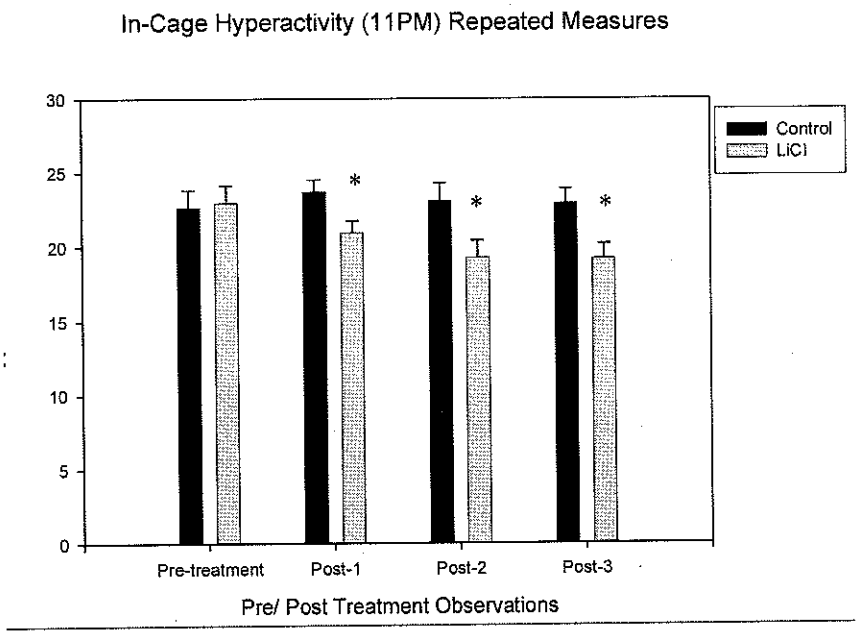
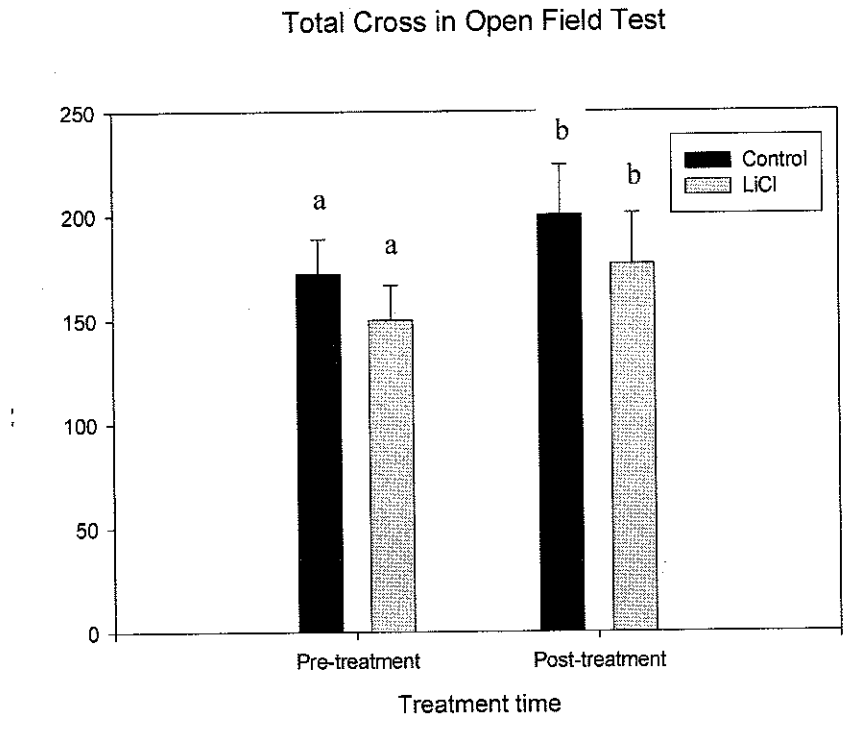


Figure 2



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