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Lithium Effects on Ethanol Intake in Impulsive Mice

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LITHIUM EFFECTS ON ETHANOL INTAKE

IN IMPULSIVE MICE

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ABSTRACT

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The present study sought to identify the effects of chronic lithium administration on ethanol intakes in high alcohol-preferring (HAP) mice. Lithium is a well-established treatment for bipolar disorder and has demonstrated efficacy in reducing impulsivity, an endophenotype of the disease. Impulsivity is also a prominent trait of alcoholism. HAP mice display a preference for consuming substantial amounts of ethanol and exhibit abnormally high levels of impulsivity. Previous work has determined that chronic lithium exposure in HAP mice reduces their levels of impulsivity. The present study analyzed fluctuations in established intake patterns after lithium exposure and how pre-exposure to lithium would affect ethanol intake acquisition. The results showed an increase in ethanol intake and no change in preference for ethanol over water in lithium treated mice. There was an increase in overall total fluid consumption in these mice, likely resulting from polydipsic effects. There also appeared to be a potentiated lithium toxicity effect found in those mice pre-exposed to lithium. The conclusion was that lithium therapy does not decrease ethanol consumption in HAP mice.

INTRODUCTION

For over a century, lithium has been a standard treatment for patients suffering from a wide range of illnesses. It was first prescribed to treat mania in 1871 at Bellevue Hospital in New York (Shorter, 2008). In the time since, it has fallen in and out of favor as medical trends evolved and other medications became available. Lithium, however, has been studied for treatment in bipolar and unipolar disorder numerous times and continues to have proven efficacy. In 1973, Prein and colleagues demonstrated that patients who were prescribed lithium when released from the hospital after a manic episode had only a 31% relapse rate compared to 67% in placebo at one year. A 5-year prospective study to determine the long-term effects of lithium revealed that patients who had maintained their medication scored better on diagnostic scales and had fewer hospitalizations than patients who had discontinued use (Maj, 1998). A review done in 2004 evaluated data from 5 separate randomized control trials and concluded that lithium is effective at reducing the risk of manic relapse and also has a clear preventative effect against manic episodes (Geddes, 2004).

Bipolar disorder is also highly correlated with lifetime diagnoses of alcohol or drug addictions or abuse (Cassidy, 2001). Patients with bipolar disorder are 4.6 times more likely to have comorbid alcoholism than subjects without bipolar disorder (Winokur, 1998). Winokur (1994) showed that the average age for patients with comorbid bipolar disorder and alcoholism was 20, while the age of onset for patients with primary bipolar disorder only was 25. Further research revealed that a family history of mania was correlated with elevated prevalence rates

of bipolar disorder with comorbid alcoholism (Winokur, 1995). There is a correlation between age of onset of alcoholism and the course of bipolar disorder itself. Earlier onset of bipolar disorder is more likely to be associated with later substance abuse than later onset bipolar disorder (Feinman & Dunner, 1998).

There is also evidence that patients with comorbid alcoholism are more likely to have repeating manic episodes. It was found that patients with comorbid alcoholism had a 100% relapse rate, while patients without any substance abuse problem had a relapse rate of only 33% at a four year follow-up (Tohen, 1990).

More recent studies have begun to evaluate possible underlying connections between bipolar disorder and alcoholism. One of the main facets of bipolar disorder is impulsivity. Holmes (2009) found that subjects with bipolar disorder comorbid with alcoholism were more impulsive than both healthy controls and subjects with bipolar disorder without comorbid alcoholism. In this study, bipolar patients and controls were given both the Barratt Impulsiveness Scale (BIS) and the Balloon Analogue Risk Task (BART). These tasks are commonly utilized in studies to assess impulsivity in humans. The scores of the bipolar patients were significantly elevated compared to controls on both measures. Even in euthymic states (neither manic nor depressed), bipolar patients that are alcoholics are more impulsive than bipolar individuals that are not alcoholics (Swann, 2004).

Moeller (2001) found that there is an association between impulsivity and several psychiatric disorders, including bipolar disorder. Heightened levels of impulsivity are evident across all states of the disorder, indicating that it is a trait dependent quality, rather than a state dependent aspect (Swann, 2001; Peluso, 2007; Swann, 2003). Bipolar patients consistently have higher scores on the Barratt Impulsiveness Scale (BIS) compared to healthy controls, regardless

of whether they are in a manic or depressive episode. These studies lead to the conclusion that impulsivity is an endophenotype of bipolar disorder. It is a stable, measureable, heritable aspect of the disease which can be used to study and develop treatments for bipolar disorder.

Impulsivity is present across all subtypes of bipolar disorder and is consistently decreased by lithium treatment as measured by psychiatrists in hospitalized patients (Swann, 2002). Lithium has, in fact, been shown to reduce impulsivity in individuals with bipolar disorder and other behavioral disorders. It reduces impulsivity levels in patients with Attention-Deficit/Hyperactive Disorder (Dorrego, 2001). Sustained-release lithium is also effective in reducing impulsivity in gamblers with bipolar disorder, diminishing the severity of gambling when impulsivity of gambling is being studied (Hollander, 2005).

One possible effect of lithium is a reduction of impulsivity levels. Suicidal behavior is a significant risk associated with bipolar disorder and, in treatment, patients treated with lithium are less likely than patients being treated with other medications, such as Valproate, to attempt suicide (Kovacsics, 2009). It has also been shown to be more effective than atypical antipsychotics at reducing suicidal behaviors (Ahearn, 2012). Since impulsivity is highly associated with suicide attempts, it can be inferred that lithium is reducing these attempts by decreasing impulsivity in the patients (Kovacsics, 2009).

On a neurobiological level, it has been determined that low levels of CSF 5 – HIAA (a metabolic product of serotonin) lead to higher levels of impulsivity in mice (Nelson, 2001; Ramobz, 1996). These effects are seen primarily in areas of the brain associated with emotional regulation such as the limbic regions. Studies using animal models have demonstrated that lithium acts to increase levels of serotonin and its release in these brain regions after long-term treatment (Carli, 1997), suggesting a possible mechanism for lithium's therapeutic effects on

impulsivity. There is also evidence that a polymorphism in GSK-3, which codes for an enzyme that is a target for mood stabilizers, has effects on sensitivity to lithium treatment, further associating lithium treatment with the serotonin system and impulsivity (Numajiri, 2012).

There have been few experiments using animal models to investigate lithium effects on impulsivity. However, a recently completed study in our lab showed that daily consumption of lithium chow led to a reduction in impulsivity as measured by the delay discounting task in the selectively bred High Alcohol Preferring (HAP2) mouse line (Halcomb, Gould & Grahame, in press). Delay discounting is a measure of cognitive impulsivity. Mice receiving lithium chow showed significantly lower levels of impulsivity compared to mice in the control group, which received regular chow.

Impulsivity also has a well-known association with alcoholism and substance abuse. Both human studies and animal models have demonstrated this link (Vuchinich, 1998; Mitchell, 2005; Wilhelm, 2007). There is evidence that a family history of alcoholism may predict higher rates of impulsivity. Subjects whose family histories contain alcoholism have higher false alarm rates in a GoNoGo task, a measure of impulsivity (Saunders, 2008).

It has also been shown that prospective studies of impulsivity may be used to predict alcohol abuse. Murphy and Garavan (2011) evaluated 89 college students in a delay-discounting task and found that high impulsivity scorers also scored high on the Alcohol Use Disorders Identification Test (AUDIT). Individuals who have histories of alcohol abuse or are currently abusing alcohol are more likely to steeply discount rewards, displaying more cognitive impulsivity (Bjork, *et al.,* 2004; Vuchinich & Simpson, 1998). Another study compared cognitive impulsivity and motor impulsivity using abstinent alcoholics and healthy controls. They found that the alcoholics did not differ from controls on motor impulsivity, but there were significant

differences in cognitive impulsivity, which contradicts findings in animal literature (Mitchell, 2005; Ohmura, 2011).

Studies evaluating substance abuse and impulsivity in human subjects generate information on correlating factors, but fail to provide causality. For example, all the previously cited studies could indicate either that those who are predisposed towards impulsive behavior are more likely to have drug abuse problems or it could indicate that a history of drug or alcohol use causes impulsivity. Using animal models allows for causal inferences about impulsivity and drug taking.

There has been a great deal of research using animal models to characterize impulsivity. Alcohol-preferring inbred strains of mice also show increased impulsiveness (Wilhelm, 2007). Oberlin and Grahame (2009) demonstrated that High Alcohol-Preferring, alcohol-naïve (HAP) 1 and HAP 2 mice are also more impulsive than Low Alcohol-Preferring (LAP) 1 and HS/Ibg (progenitor line) mice. They showed a steeper discounting curve in alcohol-naïve HAP1 and HAP2 mice. These studies lend credence to the concept that impulsivity is an endophenotype of alcoholics and that there may be a genetic link between impulsivity and alcoholism. Since this endophenotype of impulsivity exists in both bipolar disorder and alcoholism and lithium reduces this effect in both populations, I hypothesize that lithium treatment should also have an effect on alcohol consumption.

Research into lithium effects on alcohol intake have produced mixed results in both human and animal model studies. While some clinical trials have established that lithium therapy decreases drinking episodes, others have found no significant difference in intake (Wren, 1974; Fawcett, 1987; Lejoyeux, 1993). All of these studies were done with depressed subjects, which may have influenced the data. Relatively few studies have evaluated lithium

effects on intake of other substances, but it has been shown to have no effect on cocaine craving and administration or rewarding effects of opioids (Gawin, 1984; Bolanos, 1996).

The results from animal models have been no more definitive. Alexander and Alexander (1977) Found that Wistar-NTRU rats injected with lithium carbonate and given free choice between tap water and a 10% ethanol solution increased their total fluid intake, but drank less alcohol than controls (Alexander, 1977). Other studies have not demonstrated this effect (Hines 1986; Hines 1988).

The following study was comprised of two separate experiments. Given the findings that lithium is effective at reducing levels of impulsivity in HAP mice, the fact that heightened impulsivity is a prominent trait in both bipolar disorder and alcoholism and the high co-morbidity rate between the two disorders, Experiment One was employed to evaluate the effect of lithium on ethanol consumption in HAP mice which have acquired an established drinking pattern. Mice in this experiment were exposed to ethanol for three weeks to acquire a consistent drinking pattern then lithium chow was added to their cages for two weeks. It was hypothesized that lithium treatment would reduce ethanol intake and preference for ethanol over water.

There is evidence that separate neural circuits are involved in maintenance of ethanol consumption versus drinking acquisition (Ikemoto, 1997). It is possible that lithium may act differently in these two pathways. In addition, HAP mice are selectively bred to consume significant amounts of ethanol and to prefer ethanol over water. These traits may blunt the ability for lithium to decrease ethanol intake once those drinking patterns have been established. For these reasons, Experiment Two was devised to assess the efficacy of lithium in the prevention of acquisition of ethanol intake. In this experiment, mice received lithium

treatment for two weeks prior to the onset of ethanol exposure. It was hypothesized that lithium treatment would hinder the initiation of ethanol consumption.

METHODS

Experiment 1

Subjects

Subjects were 24 female and 24 male HAP II mice. They were individually housed on a reverse light/dark cycle with lights out at 8:30 am and lights on at 8:30 pm.

Experimental Design

Mice were initially divided into two groups, balanced across sex, age and family, an ethanol group (E), and a water group (W). During Phase I, the ethanol group was given a two bottle choice, free access to tap water and 10% ethanol in tap water. The ethanol solution was available in a 50ml tube and water was placed in a 25ml tube. Tubes were side-switched at every intake reading to control for side preference. Intakes were measured three times a week. The water group was given water in 50ml and 25ml tubes. These were also side-switched at each reading. In addition, each animal was given a bottle of 9g NaCl/L tap water in glass bottles placed on the opposite side of the cage to help maintain isotonic balance.

During Phase II, after 3 weeks of continuous access to alcohol, g/kg/day ethanol intakes were calculated for the last three days of ethanol exposure and mice were separated into four groups: lithium chow/ethanol (LE), lithium chow/water (LW), control chow/ethanol (CE) and

control chow/water (CW), with each group receiving the same liquid as Phase I. The regular chow was removed from the cages and was replaced with either a 4% lithium chloride or control chow (Custom Animal Diets, Bangor, PA). All mice were weighed three times per week to ensure they were not losing an unhealthy amount of weight on the lithium diet. Intakes were taken three times per week. Phase II lasted for 2 weeks (Fig 1).

Experiment 2

Subjects

Subjects were 18 female and 18 male HAP I mice. They were individually housed on a reverse light cycle with lights out at 8:30 am and lights on at 8:30 pm.

Experimental Design

In this study, mice were separated into two groups, balanced across sex, age and family. For the first 2 weeks of the experiment, the lithium group was given chow containing 4% lithium chloride while control group were given control chow. They also had *ad libitum* access to water. Water was available in both 50ml and 25ml tubes. The sides the tubes were available on were switched at every intake reading. They were weighed twice weekly as lithium may cause unhealthy weight loss. Each cage was also given a 9g NaCl/L tap water solution in glass bottles to help maintain isotonic balance during lithium exposure.

After 2 weeks of access, when lithium had built up to therapeutic levels, the lithium and control chow groups were each divided into ethanol or water groups. The lithium/ethanol (LE) and control chow/ethanol (CE) groups had a 10% ethanol in tap water solution replaced the tap

water in the 50mL tubes. The lithium/water (LW) and control chow/water (CW) groups continued with tap water on their cages. Intakes were measured three times weekly. They were to remain on their lithium diets for 3 weeks with ethanol access. Hereafter these groups will be identified as LE, CE, LW and CW (Fig 1).

Statistics

In Experiment 1, a two factor ANOVA was used to analyze total fluid consumption differences between the four groups in Experiment 1 both before and after lithium exposure using both fluid and group as the independent variables. Follow-up *t*-tests were used to identify differences in total fluid consumption between each group. In addition, a two factor ANOVA was also run on water intakes using both fluid and group as independent variables, with associated follow-up *t*-tests used to uncover differences between specific groups.

Further repeated measures ANOVAs were used to evaluate differences between LE and CE groups in g/Kg/day, preference for ethanol over water, total fluid consumption, ethanol intakes in mL and water intakes in mL, with chow type as the independent variable. A separate repeated measures ANOVA was run to determine if sex differences existed in g/kg/day intake or total fluid consumption. Independent *t*-tests were run to identify any differences in weight between the LE, LW, CE and CW groups.

In Experiment 2, independent *t*-tests were run to analyze weight differences between the lithium and control chow groups during Phase I and a two factor ANOVA was used to evaluate weight differences between all groups in Phase II using both fluid and chow as independent variables. An independent samples *t*-test was also used to identify differences in g/kg/day intake of ethanol between the LE and CE groups.

RESULTS

Experiment 1

The two factor ANOVA analyzing total fluid consumption found no effect of chow or fluid during Phase I of the experiment, with all groups consuming similar amounts of total fluid. There was a main effect of day, $F(9,378) = 12.86$, $p < 0.001$, with all groups increasing total fluid consumption over the course of Phase I (Fig 2).

During Phase II, a two factor ANOVA found a main effect of chow, with LE and LW groups both consuming significantly more total fluid than both CE and CW groups, $F(1,42) = 11.40$, $p < 0.001$. There was also a main effect of day in the LE and LW groups for total fluid intake, $F(7,45) = 6.99$, $p < 0.001$, with mice in both groups significantly increasing total fluid intake over the course of Phase II (Fig 2). There was no interaction of day and fluid type on total fluid consumption. This indicates that increases seen in total fluid consumption in the LE group were the result of increases in both ethanol and water intakes. There was no significant difference between the LE and LW groups on total fluid consumption. Although an ANOVA analyzing water intakes in the LW and CW mice found no main effect of chow during Phase II, there was an interaction of chow by day, $F(7,154) = 2.134$, $p = 0.043$. A follow-up independent samples *t*-test found that by 32 of the experiment, mice in the LW condition were consuming significantly more water than mice in the CW group, $t(22) = -2.304$, $p = 0.031$ (Fig 3). One interpretation of these results would be that the higher intakes seen in the LE and LW groups were driven by thirst, since lithium is known to create polydipsia.

Prior to lithium exposure (Phase I), there was no significant difference between the lithium and control chow groups on g/kg/day intake of ethanol. A repeated measures ANOVA found a main effect of day for both chow groups during this pre-exposure phase (Phase I), $F(9,180) = 13.12$, $p < 0.001$, with intakes increasing over the three weeks, with mice in both LE and CE groups showing over 94% preference for ethanol over water (Fig 4). In addition, there was a main effect of day on preference for ethanol over water, $F(9,180) = 22.97$, $p < 0.001$, with an escalation of preference as Phase I progressed . Over the course of Phase I, mice acquired a preference for ethanol over water and increased their consumption significantly (Fig 5). At the end of Phase I, mice were separated into lithium and control chow groups such that there were no significant differences between the LE and CE groups on any of these measures.

Although it was hypothesized that there would be a decrease in g/kg/day ethanol intakes for mice in the LE group after lithium exposure (Phase II), a repeated measures ANOVA found a significant main effect of day for this group, $F(7,140) = 7.482$, $p < 0.001$ (Fig 6), but it was not in the direction it was hypothesized to be. Contrary to the prediction, mice in the LE group elevated their ethanol intake rather than reducing it. In addition, the g/kg/day intakes for the lithium chow mice were significantly higher than the g/kg/day of the control chow mice, $F(1,20) = 1357.71$, $p < 0.001$ (Fig 6).

The preference for ethanol over water observed in Phase I did not weaken during Phase II. A paired samples *t*-test revealed no significant difference in preference for ethanol over water in either the LE or CE group, nor was there a significant difference between the two groups (Fig 7).

Although subjects in the LE and LW groups initially lost weight at the initiation of lithium exposure, their weights quickly rebounded and by the end of the experiment there were no significant weight differences between the LE, LW, CE and CW groups (Fig 8).

There were also no main effects of sex seen on total fluid consumption, ethanol intakes or preference for ethanol over water.

Experiment 2

Mice in the LE group in this study suffered from extreme weight loss and ill health after initiation of ethanol exposure. This study was terminated one week early and only weight data and ethanol intakes for two days were analyzed. Mice in the LW condition also weighed less, they did not drop below 80% of baseline weight, which have required removal from the experiment.

A *t*-test analyzing the ethanol intakes in g/kg/day for the two days of measurement available found a significant difference in the LE and CE groups, $t(16) = -3.342$, $p < 0.001$ (Fig 9). Subjects in the LE group consumed significantly higher amounts of ethanol during these two days. There was also a significant difference in water consumption between the CE and LE groups, $t(16) = -2.237$, $p = 0.04$.

A repeated measures ANOVA found a main effect of day on weight, $F(4,136) = 10.04$, $p < 0.001$ (Fig 10). Although there was no main effect of group on weight, there was a group X day interaction, $F(4,136) = 7.64$, $p < 0.001$. Follow-up t tests found a significant difference in weights between the two groups beginning on day 4 of lithium exposure (Phase I), $t(34) = -2.16$, $p = 0.04$. Within one week, mice in the LE condition had severely deteriorated in health and the experiment was terminated.

DISCUSSION

In Experiment 1 in this study, mice were separated into water and ethanol groups then exposed to ethanol for three weeks to establish a consistent intake pattern (Phase I). They were separated into control and lithium chow groups based on mean ethanol intakes in grams per kilogram for the final three days of Phase I. At lithium induction (Phase II), both groups in the ethanol condition were consuming approximately 20 g/Kg of 10% ethanol solution and 7 ml of tap water per day. Over the course of the lithium treatment, mice in both lithium groups steadily increased consumption of both ethanol and water. Intakes increased to a mean of 26 g/Kg/ day of ethanol and 9 ml/day of tap water. These consumption levels were significantly higher than their pre-exposure intakes and the intakes of mice in control chow groups. Although the first hypothesis of this study posited that lithium treatment would reduce ethanol intake, the results do not support that conclusion.

Consistent with previous findings, this study suggests that treatment with lithium creates polydipsia, resulting in an increase of total fluid consumption (Alexander, 1978). It is possible that the mice in the LE group increased their intake of ethanol due to its rewarding effects; however three lines of evidence counter this argument. First, there was no significant group by fluid interaction. Increases in ethanol intake were accompanied by increases in water intake. Within one week of lithium treatment (Day 32 of the experiment), mice had significantly increased both water and ethanol consumption. Second, mice in the LW group increased their

water intake compared to Phase I such that their total fluid consumed matched total fluid consumed in the LE group during Phase II. This indicates that the nature of the fluid was not a motivating factor in the escalation of consumption. In addition, there was no significant increase in ethanol or water intake in either of the control chow groups. The subjects in the CW group did not significantly increase their water intakes, while subjects in the CE group maintained stable consumption levels throughout Phase II, rather than increasing consumption due to rewarding effects. These results indicate that increases in ethanol intake were consistent with non-specific polydipsic effects.

Subjects also displayed a statistically significant preference for ethanol over water in Phase I which was not decreased by lithium treatment. Since the preference level was so high during Phase I, there was likely a ceiling effect limiting analysis of whether or not lithium treatment could actually increase preference for ethanol over water. Prior to lithium exposure, 95% of the total fluid consumed by mice in the LE condition was ethanol. This preference was not significantly reduced by lithium. It was also not significantly different from the preference exhibited in the CE condition. The mice in this study are selectively bred to prefer and consume high amounts of ethanol and the lithium treatment did not alter this behavior. The conditioned taste aversion often seen with lithium exposure due to its gastrointestinal effects was not seen here, presumably because the mice had already formed positive associations during the ethanol pre-exposure in Phase I, thereby preventing a negative association from developing between ethanol intake and nausea.

This preference for ethanol over water is contradictory to previous findings in Wistar rats exposed to lithium carbonate which showed increased total fluid consumption but decreased consumption of ethanol (Alexander, 1978). Rats in that study were given

intraperitoneal injections of lithium carbonate rather than exposure through chow. Other findings of ethanol preference reduction also involved injection of lithium carbonate or chloride (Zakusov, 1978; Ho, 1975; Sinclair, 1974) In contrast, lithium exposure through graduated methods, such as drinking water or chow, resulted in no decrease in ethanol intake or preference (Hines, 1986; Hines, 1988). This suggests that the decrease in preference observed in injection studies may be a result of a negative association between the injections and ethanol exposure or a conditioned taste aversion rather than lithium action.

The results from Experiment one do not support the hypothesis that lithium treatment reduces ethanol intake. It is possible that we were unable to detect effects due to the polydipsia-induced increases in total fluid consumption; however, this seems unlikely since the preference for ethanol after lithium exposure was not significantly altered. Future studies could evaluate the preference for ethanol at titrated doses to determine if higher or lower concentrations would affect preference. Higher doses of ethanol may be aversive to mice and although HAP mice are selectively bred to consume large quantities of ethanol, increasing the dose may override this predisposition when combined with lithium treatment.

An unforeseen unavailability of HAP II mice precluded them from being used in Experiment 2. Instead of 48 HAP II mice, 36 HAP I mice were used. These mice have comparable rates of ethanol intake and levels of impulsivity. Subjects were separated into control chow and lithium chow groups and given two weeks of lithium exposure. They were then further divided into water and ethanol groups. At the beginning of Experiment 2, there was no significant difference in weights between the two groups; however mice in both lithium chow groups showed steady weight loss over the course of the three weeks culminating in a significant

difference in weights between the lithium con control chow groups at the onset of ethanol exposure.

Data collection for this experiment was sparse due to the loss of subjects; however after two days of ethanol exposure mice in the LE group had consumed significantly more ethanol then mice in the CE group. Although the LE group also consumed significantly more water than the CE group, it is possible that the excessive amount of ethanol intakes observed in the LE group may have contributed to their ill health.

Within one week of ethanol exposure, three of the mice in the LE group had expired and four more were removed due to excessive weight loss. The weights of all of the mice in the LE and LW groups were significantly lower than mice in the CE and CW groups, although subject weights in the LW group were not such that they needed to be removed from the experiment. There is a possibility that the combination of lithium and then ethanol exposure produced a toxic effect in the mice. There are two possible mechanisms driving the extreme weight loss and ill health seen in the mice in this experiment.

Lithium has never been administered to HAP I mice. Although the HAP I line is similar to the HAP II line in many dimensions, there may have been some unknown effect that was revealed through this study. Previous work with these lines has shown that they attain comparable weight levels and respond similarly to ethanol consumption and measures of impulsivity. Subjects in Experiment one of this study (HAP II mice) did not suffer from extreme weight loss. The fact that the mice in the LW condition also weighed less lends plausibility to this line of thought. Although the addition of ethanol to the cages led to death in almost all of the mice in the LE condition, it was not the factor contributing to the ill health of subjects in the LW

group. There is little information describing the exact mechanisms of action of lithium, therefore it is feasible that one of these mechanisms affects HAP I mice differently than HAP II mice.

The other possibility is that the combination and sequence of administration of lithium and ethanol in this experiment produced a toxic effect. Lithium has a very narrow therapeutic index and is toxic at moderate doses. Ho and Ho (1978) analyzed the increases in lithium toxicity after ethanol exposure in rats and mice. Both acute and chronic exposure to ethanol after acute lithium carbonate treatment led to a reduction of excretion of lithium, allowing toxic build-up in the system. Lithium is excreted through the renal system and subjects given lithium or ethanol treatment increased urine excretion; however exposure to lithium then ethanol created a significant decrease in urinary excretion. Many of the mice in the lithium treatment condition in the present experiment were exhibiting signs of lithium toxicity. Although no gastrointestinal effects were observed, the mice displayed poor muscle control and weakness. These effects were noted but not measured. Since this was not observed in Experiment one with HAP II mice, the negative effects seen were possibly the result of a combination of the alternate line and the potentiated lithium toxicity levels. This may have serious implications for treatment of bipolar disorder patients with comorbid alcoholism. Literature examining this phenomenon in humans is scarce. If the resulting toxicity is a consequence of the sequence of administration, lithium exposure preceding ethanol exposure, it could prove a dangerous combination for patients who initiate lithium treatment at an early age.

Bipolar disorder and alcoholism are highly comorbid diseases, sharing the key endophenotype of heightened impulsivity (Cassidy, 2001; Holmes, 2009). Lithium is effective in reducing impulsivity levels in both disorders and in general symptom treatment in bipolar disorder (Maj, 1998; Kovsciscs, 2009; Halcomb, Gould & Grahame, in press). Although lithium is

effective at reducing impulsivity levels in HAP II mice and it was anticipated to reduce ethanol intake, the findings described here do not support the hypothesis. There are several possible explanations for these findings.

First, impulsivity as a construct may be dissected into separate classifications; primarily cognitive impulsivity and motor impulsivity. Ohmura (2011) saw a reduction of motor impulsivity in mice treated with lithium and tested in the 3 choice serial reaction time task (3CSRTT). The 3CSRTT is a method of determining motor impulsivity levels, while the delay discounting task used in our lab is a measure of cognitive impulsivity (Rachlin & Green, 1972). As stated above, our lab has found that chronic lithium treatment is effective in reducing impulsivity in the delay discounting task (Halcomb, Gould & Grahame, submitted, 2012). These tasks recruit alternate neural pathways, some of which may overlap pathways associated with ethanol intake (Hinshaw, 2003).

The exact mechanisms through which lithium acts are not yet completely discerned. It has been associated with the inhibition of GSK-3beta, a serine/threonine kinase with varied functions on glycogen synthesis and neural precursor growth (Qu, 2011). Recent work in our lab to replicate lithium effects on impulsivity with a GSK-3beta inhibitor, have thus far yielded no results (Halcomb, Gould and Grahame, personal communication, 2012). Lithium effects on this system are still unclear.

Lithium treatment is also correlated with more grey matter in limbic and paralimbic structures in bipolar patients, compared to patients receiving anticonvulsant treatment (Germana, 2011). There is evidence that abnormalities in these systems, primarily the amygdala, are involved in many of the key aspects of bipolar disorder, including impulsivity (Blond, 2012).

Long-term treatment with lithium may lead to increased serotonin levels in these regions, decreasing impulsivity (Carli, 1997).

Also unknown are the exact mechanisms contributing to the development of alcoholism. There is evidence suggesting frontal cortex involvement in addiction, including the orbitofrontal cortex and the anterior cingulate gyrus (Goldstein, 2002). These areas show connectivity to limbic areas which are likely affected in patients with bipolar disorder. These regions may be influencing impulsivity levels in patients; however although impulsivity is a prominent aspect of the disorder and lithium is effective at attenuating it, the mechanism through which it is acting may not overlap with pathways regulating ethanol consumption. This is consistent with the findings of this study.

Second, established ethanol intake patterns in HAP mice may be highly resistant to reduction. Although some compounds have been shown in our lab to reduce ethanol intake prior to the acquisition of a consistent intake pattern, long term exposure may prevent drug effects. Experiment 2 was devised to determine if being treated with lithium preceding the establishment of a consistent drinking pattern would reduce acquisition and/or intake. Since the experiment was terminated, this continues to be unclear. As stated previously, HAP mice are selectively bred to consume high quantities of ethanol and it is possible that once they have acquired a high ethanol intake pattern, it is problematic to decrease it.

Third, we did not evaluate lithium brain serum levels in this study. If lithium levels were not elevated enough to produce effects then that may underlie these results; however we believe levels were adequate. Ohmura (2011) also administered lithium through chow with similar means and previous work in our lab administering lithium through chow has found that brain serum levels were within the human therapeutic range (Halcomb, Gould & Grahame, in

press). Since that same chow was used in this study, it follows that comparable lithium levels would be achieved.

Lastly, the polydipsia observed here may have interfered with the ability to detect decreases in ethanol consumption, but this seems unlikely. Although the increased thirst may account for the increase in ethanol intake, lithium did not affect preference. Subjects continued to show a significant preference for ethanol over water during Phase II lithium treatment in Experiment 1. A follow-up study could examine how titrating the ethanol concentration during lithium treatment would affect preference. Higher doses may decrease intake levels and preference.

Evaluation of lithium treatment for alcoholism in human studies is often confounded by other concomitant disorders and may therefore be unreliable. The literature from animal studies indicates that while lithium chow or solution is affecting impulsivity levels in high alcohol-preferring subjects, there is not an effect on ethanol intake or preference. The results from the current study support this conclusion. The most probable conclusion is that the mechanism of action through which lithium is reducing impulsivity is not the driving force in ethanol consumption.

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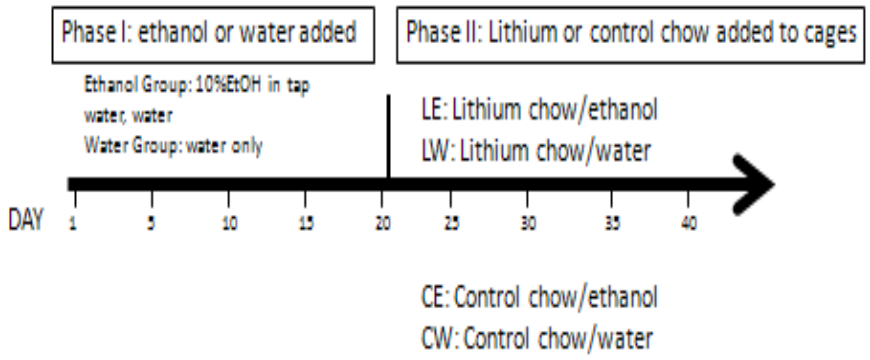
FIGURES

A.



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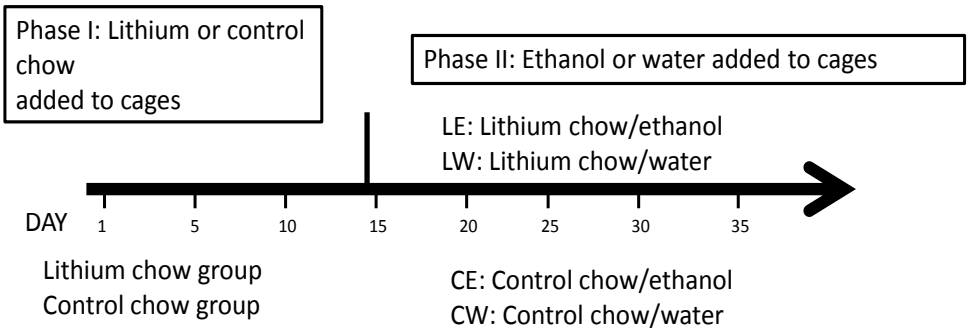

Fig 1. Timeline of Experiments. A. Procedure for Experiment one: ethanol exposure for three weeks followed by two weeks of lithium and ethanol exposure. B. Procedure for Experiment two: lithium exposure for two weeks followed by two weeks of ethanol exposure.

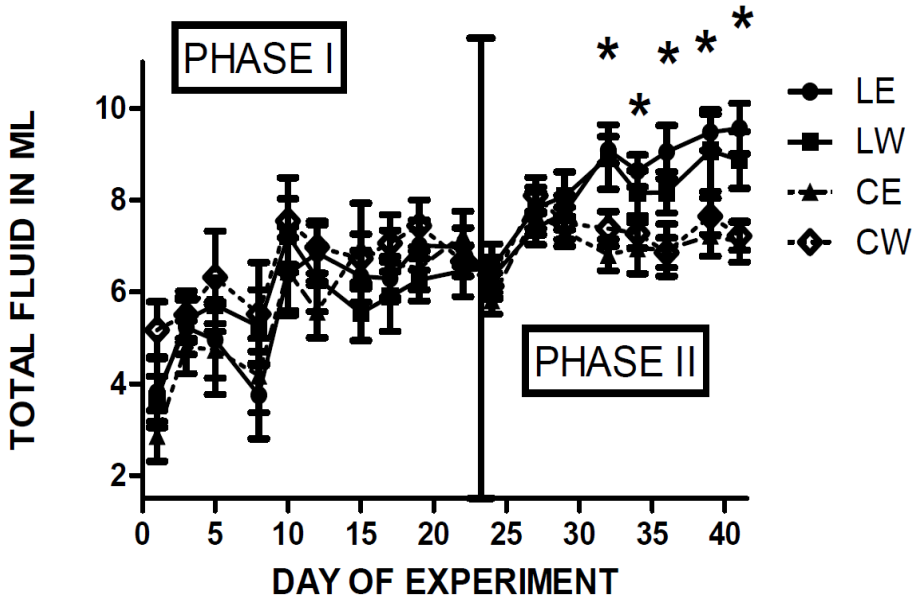

Fig 2. Total Fluid Consumption All Groups. A. During Phase I, there was no effect of group since all groups were consuming similar amounts. There was a main effect of day with all groups increasing total consumption. During Phase II, there was a significant difference between groups on lithium chow (LE and LW) and groups on control chow (CE and CW), $F(1,42) = 11.40$, $p < 0.001$. There was also a main effect of day in the LE and LW groups with intakes significantly higher in Phase II than in Phase I for those groups, $F(7,45) = 6.99$, $p < 0.001$. B. Total fluid consumption in just the LE and CE groups. C. Total fluid consumption in just the LW and CW groups.

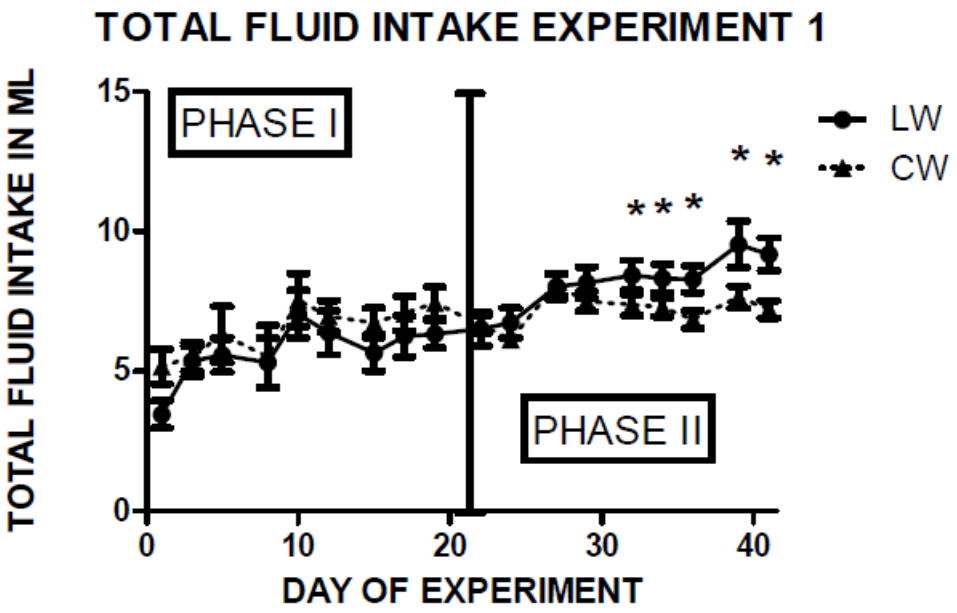

Fig 3. Total Fluid Intake Water Groups. A repeated measures ANOVA found a significant day X chow interaction on total fluid intakes in the LW and CW groups, F (7,154) = 2.134, p = 0.043. A follow-up t-test ascertained that by day 32 of the experiment, mice in the LW group were consuming significantly higher amounts of fluid than the CW group, t(22) = -2.304, p = 0.031.

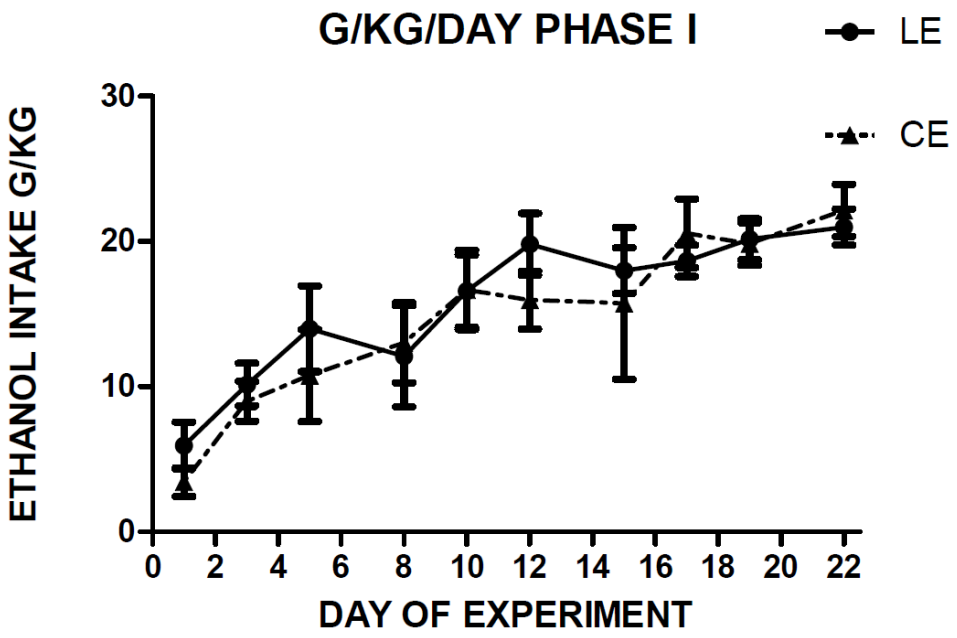

Fig 4. Ethanol Intake Phase I. Ethanol intakes measured by g/kg/day during Phase I. There was no significant difference between LE and CE groups, but there was a main effect of day, with intakes in both groups significantly increasing during Phase I, F (9,180) = 13.12, $p < 0.001$.

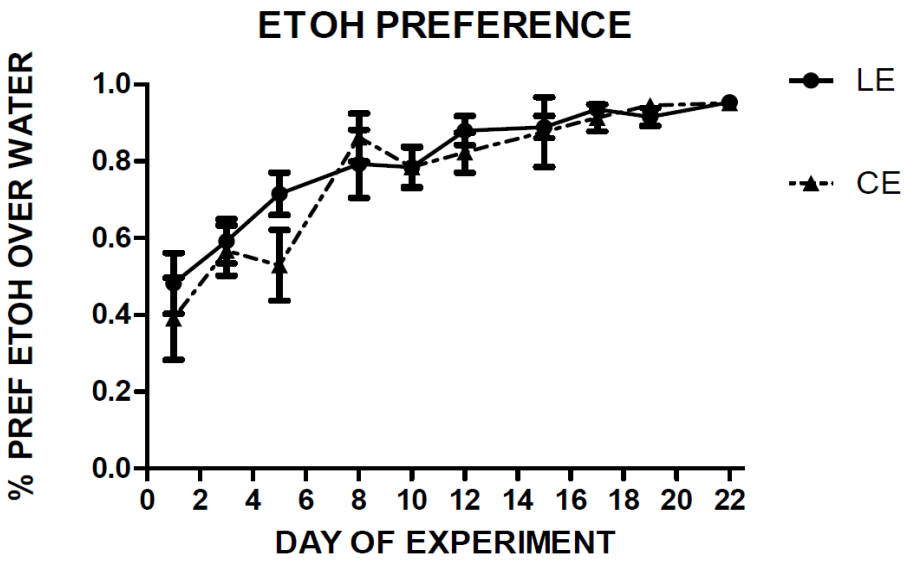

Fig 5. Ethanol Preference Phase I. Both LE and CE groups significantly increased their preference for ethanol over water over the course of Phase I, $F(9,180) = 22.97$, $p < 0.001$. There was no significant difference between the two groups in preference for ethanol over water.

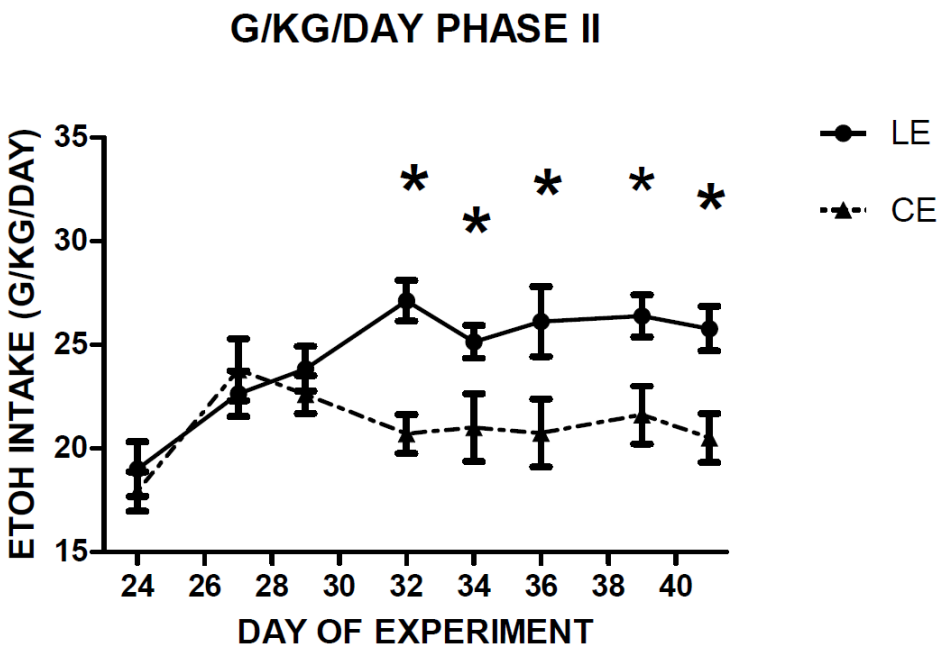

Fig 6. Ethanol Intakes Phase II. Ethanol intakes measured in g/kg/day during Phase II. There was a main effect of day for the LE group on ethanol intake, $F(7,140) = 7.482$, $p < 0.001$. Subjects in the LE group significantly increased ethanol intakes during Phase II. There was also a main effect of chow, with mice in the LE group consuming significantly more ethanol than subjects in the CE group, (1,20) = 1357.71, $p < 0.001$.

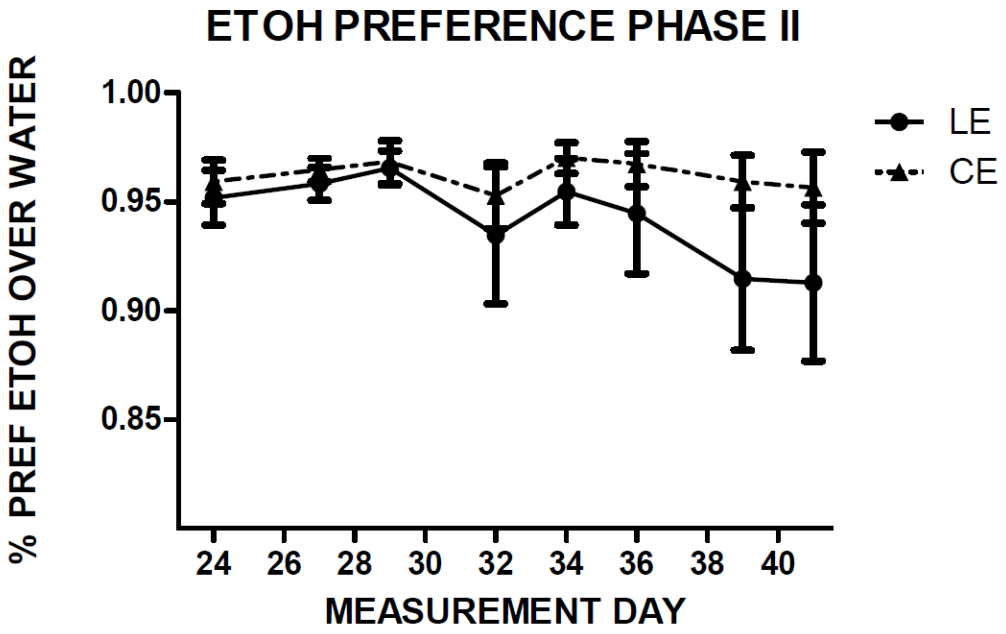

Fig 7. Ethanol Preference Phase II. Preference for ethanol over water during Phase II. There were no statistically significant differences between the LE and CE groups in preference for ethanol over water in Phase II, although there is a trend in the LE group.

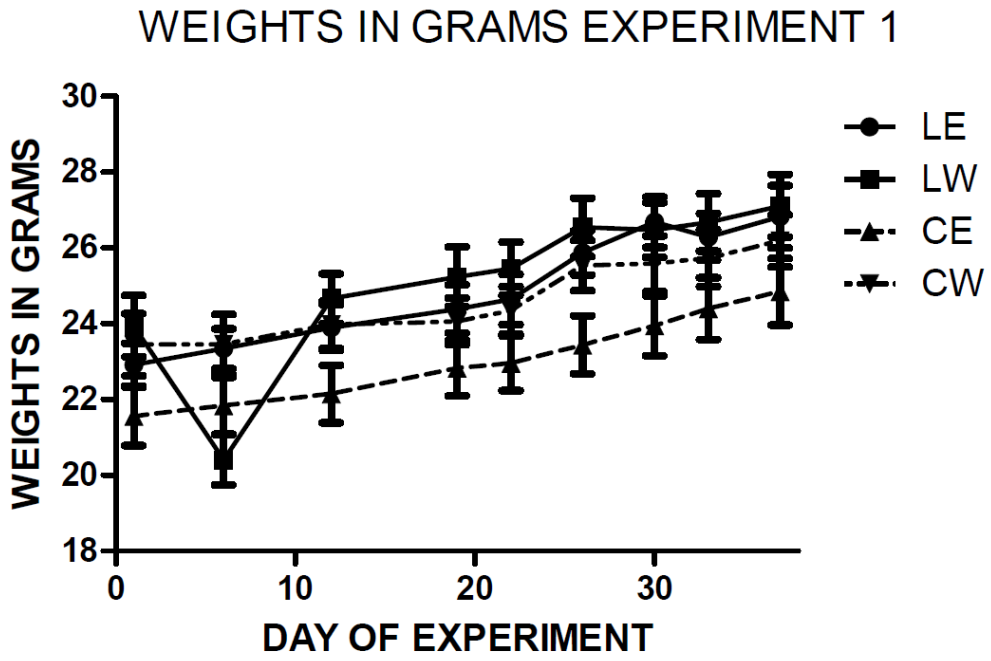

Fig 8. Weights Experiment 1. There were no statistically significant differences between the groups in weight.

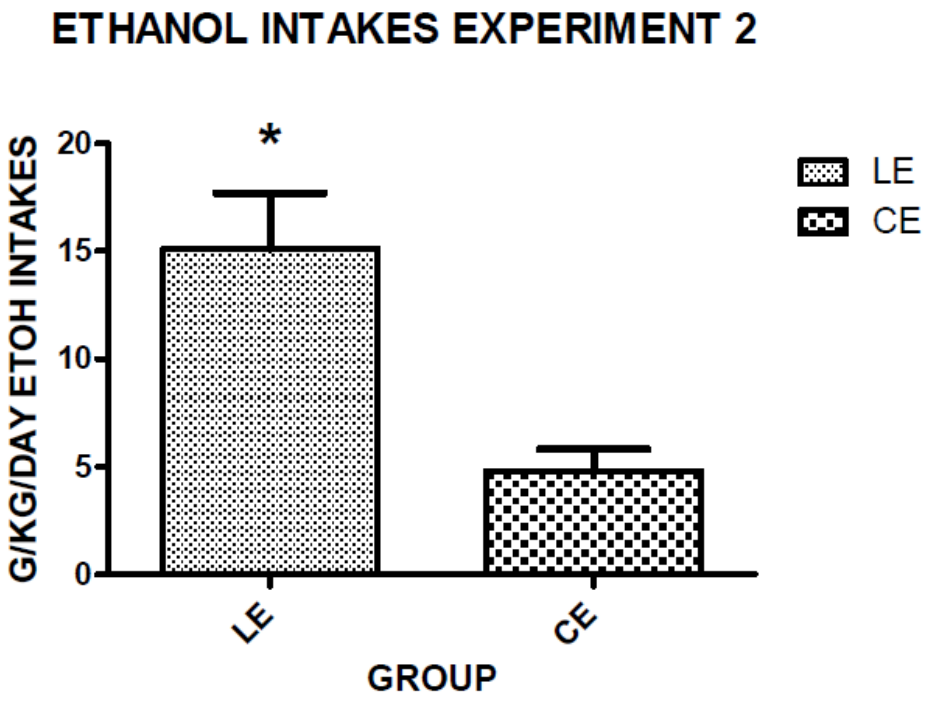

Fig 9. Ethanol Intake Experiment 2. Subjects in the LE group had significantly higher ethanol intakes than subjects in the CE group, $t(16) = -3.342$, $p < 0.001$.

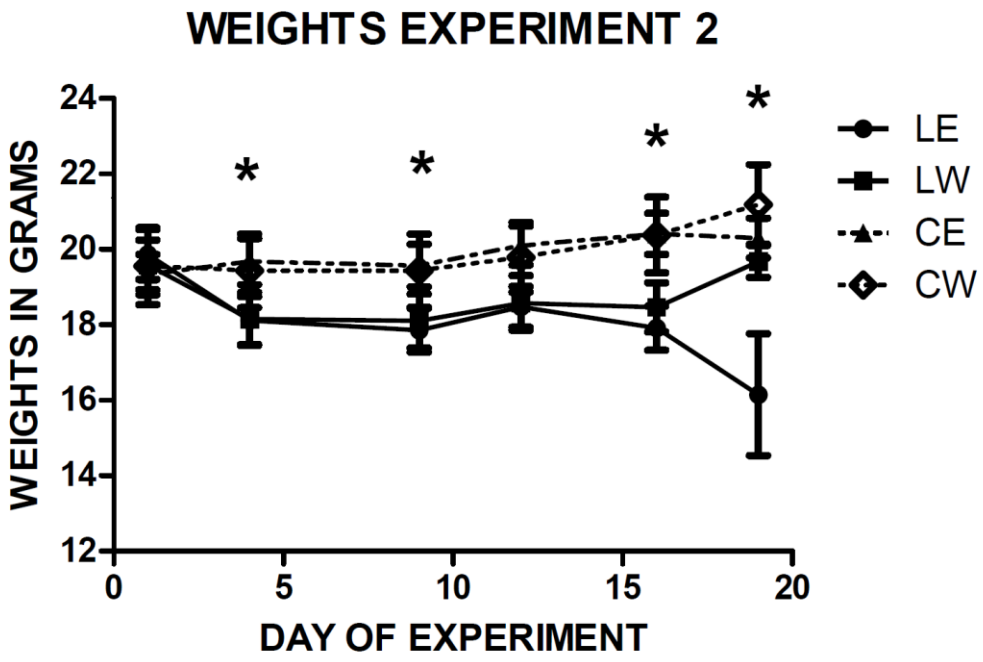

Fig 10. Weights Experiment 2. There was a main effect of day on weight, with mice in the LE and LW groups decreasing in weight, $F(4,136) = 10.04$, $p < 0.001$. There was also an interaction of chow X day, $F(4,136) = 7.64$, $p < 0.001$ and a follow-up t-test found a significant difference between the lithium groups (LE and LW) and the control groups (CE and CW) by day 4 of the experiment, $t(34) = -2.16$, $p = 0.04$.