Lithium changes the way light resets the brain’s internal clock in a mouse model of bipolar depression

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Key Words: Suprachiasmatic nucleus, circadian rhythm, lithium, bipolar disorder, light

Acknowledgements: A very big thank you to my mentor Dr. Rae Silver for her endless patience and guidance during each step of my project and to Dr. Joe LeSauter for all of his kind assistance and input. My thanks also goes to the rest of the wonderful Silver Lab, to Professor Peter Balsam, and my senior thesis class.
Abstract

Background: Lithium is the drug of choice for treating patients suffering from bipolar disorder (BP), a disorder characterized by mood swings between mania and depression. While in the manic state, individuals may be hyperactive, make poor decisions and experience difficulty sleeping. Symptoms of a depressive episode include a loss of interest in formerly engaging activities, persistent feelings of sadness, and fatigue. Though the exact pathway by which lithium acts to counteract the symptoms of BP is unknown, we do know that lithium helps animals entrain to a longer circadian rhythm. Studies have shown that patients with BP tend to have a shorter circadian rhythm than the near 24h period shown by a normal individual and experience difficulty synchronizing to the external light and dark (LD) cues of a 24h day. We hypothesize that because light is such a potent entraining cue, lithium may interact with LD signals to enable BP individuals entrain to the 24 hour day. One can test this hypothesis by examining locomotor activity and the levels of FOS expression following a light pulse during the animal’s internal night (as determined by onset of activity) or during the external night (as determined by the daily light-dark cycle).

Method: In order to determine the effects of lithium on brain and locomotor behavior, mice were placed in a 28 hour day consisting of 14:14 light:dark cycle. Animals were randomly divided into groups and fed either control chow or lithium supplemented chow. After receiving a one light pulse (LP) at 2 hrs after lights off (during the external night) or 2 hours after the start of their endogenous nightly activity bout (internal night), animals were sacrificed. Wheel running was measured and c-FOS expression in the SCN quantified. Under normal conditions, a nocturnal animal will exhibit increased levels of FOS expression if exposed to a light pulse of even five minutes during the nighttime.

Results: Measurements of wheel running activity indicated that ten of the fourteen mice housed in LD 14:14 had two peaks of activity at periods ~24h and ~28h both before and during lithium treatment. We assume that the 24h peak derives from the internal biological clock while the 28h peak is in response to the external cues of the 14:14 LD cycle. During the period of lithium administration, and the mean period of the highest activity peak increased significantly and two of fourteen animals had the period of their highest activity peak switched from ~24h to 28h. FOS expression in the SCN parallels analysis of the behavioral results. Mice on lithium had significantly more FOS in the entire SCN and specifically in the SCN core, regardless of whether they were sacrificed during their internal or external night. Lithium treatment decreased the amount of locomotor activity, a side effect with unknown import.

Conclusion: Analysis of wheel running behavior and levels of FOS expression in the SCN showed that animals on a lithium diet exhibited a greater response to the light pulse given in the night and were better able to entrain to the longer 28 hour day. We conclude that lithium acts upon an animal’s endogenous clock, thereby facilitating entrainment to a lengthened external cycle.
Introduction

Light is a powerful signal whose far-reaching effects we often take for granted. It regulates both how we structure and schedule our days as well as a myriad of internal processes of which we are largely unconscious. These internal circadian rhythms, ranging from sleeping and eating patterns to blood pressure levels and hormonal secretion, are tightly regulated by the synchrony of our body’s internal clock to light cues (Aronson et al., 1993). Without these two internal and external rhythms working in harmony, most of our normal bodily functions would be out of sync or even in jeopardy.

The problems that can occur when one fails to properly synchronize to external cues range from simple jet lag to elements of mental illnesses such as bipolar disorder. Jet lag is a circadian rhythm sleep disorder that occurs when the environmental light dark cycle is abruptly shifted leading to changes in sleep-wake timing (Avidan & Colwell, 2010). Bipolar disorder, on the other hand, is a not a temporary state but a mental illness characterized by periods of mania alternating with periods of depression. Normally, humans have a stable and precise 24 hour circadian period (Czeisler et al., 1999). However, the circadian clocks of bipolar patients have been shown to oscillate at faster frequencies than their environmental cues would suggest, prompting the possibility that BP may be a circadian rhythm disorder. (Kripke, Mullaney, Atkinson, & Wolf, 1978). BP individuals’ lack of entrainment to light cues may be a contributing factor to their symptoms. Patients often suffer from circadian deregulation during acute mood episodes and during depressive periods, contributing to disturbances in functions such as hormonal secretion and core temperature (Milhiet, Etain, Boudebesse, & Bellivier, 2011). Studies have shown that treatments that advance or delay circadian clocks via changes in sleep timing or scheduled light exposure can alleviate or aggravate abnormalities in mood (Kripke,
Lithium is frequently prescribed to combat a bipolar patient’s symptoms (Malhi, 2009). It is a proven mood stabilizer for BP, reducing the frequency and magnitude of manic episodes. Lithium is very effective in warding off suicidal actions (particularly common in BP patients) and is an excellent prophylactic agent for both mania and depression (Gershon, Chengappa, & Malhi, 2009; Jope, 1999). Though how lithium exerts its effects on the brain is not well known, previous studies have demonstrated that lithium can entrain an animal to circadian cycle longer than 24 hours (Li, Lu, Beesley, Loudon, & Meng, 2012).

The brain’s master clock, called the suprachiasmatic nucleus, (SCN), is, responsible for regulating the phase of circadian rhythms, including hormonal levels, body temperature, and locomotor behavior (Klein et al., 1991). The case for a master regulator has been substantiated by numerous experiments. Transplanted fetal SCN tissue into the third ventricle of SCN-lesioned hamsters was found to restore circadian locomotor activity (Lehman et al., 1987). SCN tissue from \textit{Tau} mutant hamsters (distinguished by their shortened circadian period) restored regular rhythms in SCN-lesioned wild-type hamsters that originally had the period length characteristic of their mutant donors (Ralph, Foster, Davis, & Menaker, 1990). The rhythms of genetically arrhythmic mice were restored following SCN grafts from wild type mice (Sujino & Masumoto, 2003). SCN neurons cultured in vitro displayed circadian electrical activity (Green & Gillette, 1982; Groos & Hendriks, 1982; Shibata, Oomura, Kita, & Hattori, 1982).

This clock operates independently of environmental cues but is capable of entraining to the external cycle via such cues (Aronson et al., 1993; C. H. Johnson, Elliott, & Foster, 2003). Light is its predominant zeitgeber (entraining cue) and so potent it is able to shift normal activity forwards or backwards if given during the dark phase of a cycle (Golombek & Rosenstein,
If an animal is administered a light pulse during the early night, it will delay the onset of activity; if the light pulse is administered during the late night, activity will be advanced (Rusak, Robertson, Wisden, & Hunt, 1990). In other words, light pulses can act as external cues that tell an animal it is daytime while their body’s endogenous clock indicates it is nighttime. There is evidence that lithium acts directly on the SCN. When the SCN is removed by lesioning then grafted back into the brain, lithium is just as effective as in non-SCN lesioned animals (LeSauter & Silver, 1993). If lithium acted primarily on other regions of the brain, one would expect SCN-grafted animals have more difficulty entraining to a longer circadian period. Furthermore, lithium increased the circadian period of firing rate rhythms in SCN neurons cultured in vitro (Abe, Herzog, & Block, 2000).

While no perfect animal model of bipolar disorder exists, we can alter an animal’s external environment to mimic the experience of a bipolar patient who is desynchronized to his external environment cues by placing the animal in an extended light dark cycle. Mice normally have an endogenous 24h cycle but when placed in 14 hours of light and 14 hours of darkness (i.e., a 28h day), they fail to synchronize properly to the external cues of light and dark and exhibit two activity bouts with 24h and 28h periods (Kripke & Wyborny, 1980; Schwartz & Zimmerman, 1990). Lithium is used to correct this internal-external rhythmic discrepancy by lengthening the circadian period, effectively delaying the internal or endogenous circadian rhythm (Kripke & Wyborny, 1980; Mceachron, Kripke, & Wyborny, 1981).

Light pulses have been shown to increase FOS expression, a neuronal marker of neural activity (Rusak et al., 1990). Arginine vasopressin (AVP) is a neuropeptide synthesized by SCN neurons that is localized to the outer shell of the SCN and aids in the regulation of SCN function (Jác, Kiss, Sumová, Illnerová, & Jezová, 2000). AVP levels of expression are elevated during
the dark phase and thus will serve to delineate the location of the SCN when a light pulse is administered regardless of the levels of FOS expression.

We hypothesize that lithium will augment responsiveness to light and better enable the endogenous clock to entrain correctly to external cues; relieving the symptoms of BP. In order to probe the effects of lithium on a molecular level, an LP will be given during either the internal 24 hour night or external 28 hour night. Lithium treated individuals should therefore exhibit greater neuronal activity after the LP is given than controls. If lithium promotes entrainment to a longer light-dark (external) circadian rhythm over the animal’s own internal rhythm, there should be an increase in FOS expression in the SCN of an animal sacrificed during the external night and an absence or diminished level of neuronal activity in animals sacrificed during its internal night. If lithium acts to promote entrainment to the animal’s endogenous rhythm, the opposite should occur.

Methods

Animal Housing

Twenty five male C57BL/6 mice (Jackson Laboratory, Sacramento, CA), 5 to 7 months old, were individually housed in clear polycarbonate cages (32 x 14 x 13 cm on pine shavings) in a ventilated, light-proof environmental chamber. Mice were placed on a 14:14h light-dark (LD) cycle with light at 10 lux. A red light less than 1 lux (multicoated red bulb for photographic use, Delta 1, Dallas, TX) was utilized for animal maintenance. Room temperature was maintained at 21 ± 2 °C and food and water were available ad libitum. Each cage was equipped with a running wheel (diameter 11 cm). All procedures were approved by the Columbia University Animal Care and Use Committee.

Experimental Groups
All animals (N=25) were fed regular chow (LabDiet 5001; PMI Nutrition, Brentwood, MO, USA) for the first 6-8 weeks. In the control group, mice (N=11) animals were sacrificed while the remaining 14 mice received wet powdered food mixed with 0.3% Lithium Carbonate and 2% KCl for 6-8 weeks prior to sacrifice.

**Locomotor Activity Measures**

Wheel running was monitored continuously using a computer based data acquisition system (Vitalview, Rand, OR) with counts collected in 10 minute bins and visualized using actograms (Minimitter, Bend, OR). Daily onset of activity bouts were calculated by ClockLab (Actimetrics, Wilmette, IL). Wheel-running activity was used to determine the animal’s behavioral phenotype and time of light pulse. After a period of several weeks on a lithium or non-lithium diet, animals were administered a one hour light pulse at 800 lux, two hours after the onset of activity during either the internal night (determined by analysis of the animal’s wheel running activity) or the external (28h) night and sacrificed the following half hour.

**Perfusion, Histology and Immunohistochemistry**

Mice were deeply anesthetized with sodium pentobarbital (200 mg/kg, i.p.) and perfused intracardially with 100 ml of 0.9% saline followed by 200 ml of 4% paraformaldehyde in 0.1 mol/L phosphate buffer, pH 7.3. Brains were post-fixed overnight, and then cryoprotected in 20% sucrose.

Immunohistochemistry for FOS and AVP was performed on free floating sections of the brain tissue. Coronal sections were cut at 50 µm in two series on a cryostat and collected in antifreeze [30% wt/vol sucrose, 1% wt/vol polyvinylpyrrolidone-40, 30% vol/vol ethylene glycol, in PB with 0.9% saline] and stored at −20 C until processing. For immunohistochemistry, sections were washed in PB containing 0.1% Triton X-100 (0.1 PBT) 3 times for 10 minutes
each, blocked in 2% Normal Donkey Serum for 1 hour and then incubated with primary antibodies: c-FOS antibody made in rabbit (1:5000, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and AVP antibody made in guinea pig (1:10,000, T-5048; Peninsula Laboratories, Inc., San Carlos, CA). After a 48 hour incubation in primary at 4°C, brain sections were washed in 0.1% PBT for 10 minutes (3 times) then incubated with secondary antibodies in 0.3 PBT for 2 h at room temperature (Cy2- and Cy3-conjugated, made in donkey, 1:200; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Sections were washed a final 3 times in 0.1M phosphate buffer (PB), wet mounted and left to dry overnight. Sections were dehydrated with a series of alcohol rinses, cleared with xylenes, and coverslipped with Krystalon (EMD Chemicals, Gibbstown, NJ).

**Microscopy**

Images of the SCN were captured using a Nikon Eclipse E800 microscope (Nikon, Tokyo, Japan) in order to determine FOS and AVP expression. Sections were excited and emission filtered using filter cubes for Cy2 (FITC-HYQ, excitation 460–500 nm, emission 510–560 nm; Nikon) and Cy3 (Y-2E/C Texas Red, excitation 540–580 nm, emission 600–660 nm; Nikon). The microscope was equipped with a CCD camera (RetigaExi; Q-Imaging, Surrey, Canada), with QCapture software (QImaging, Surrey, Canada) and images were stored for subsequent analysis. Images were captured in 8 bit grayscale and were loaded into Photoshop 7.0 (Adobe Systems, San Jose, CA). Images were optimized by adjusting the minimum and maximum levels to the ends of the image histogram.
Statistical Analysis

Behavioral activity was calculated over a period of 24 days before lithium administration and 24 days during lithium administration. The intervals of calculation began at least 2 weeks after animals had been placed in the 28 LD cycle and at least two weeks after the start of lithium treatment. The same 24 days was compared across all lithium fed animals. The period was calculated using chi-squared periodograms generated via ClockLab (Actimetrics, Wilmette, IL). Comparisons across four groups: control animals sacrificed during endogenous 24H cycle, controls sacrificed during exogenous 28H cycle, lithium-treated animals sacrificed during the 24H cycle, and lithium-treated animals sacrificed during the 28H cycle, were made using t-tests.

Results

Effects of Lithium on Circadian Period

Animals placed in an extended 28 hour cycle generally expressed rhythmic activity with power at both 24h and 28h (Figure 1). Seventeen out of twenty-five animals (ten out of fourteen experimental animals, and seven of the eleven controls) had peaks of activity at two periods. In the experimental group, four animals had only one peak at the ~24h period, while ten had peaks at both ~24h and ~28h periods both before and during lithium treatment (Figure 2). Of the peaks of rhythmic activity that occurred during the internal night before the start of lithium administration, periods ranged from 24.17h to 24.67h, and amplitudes from 254.71 to 1501.54. Peaks occurring during the external night had periods ranging from 27.83h to 28.67h, with amplitudes from 287.38 to 1204.34. During lithium administration, periods ranged from 24.5h to 26h and 27.83 h to 28.17 h, with amplitudes of 275.11 to 1245.81 and 216.83 to 867.53, respectively (Figure 2).
Comparisons of the period of the endogenous rhythm before and during lithium treatment reveal that lithium significantly lengthens the period of the endogenous rhythm, shifting the mean from 24.49±0.04 h to 25.10±0.09 h. (p<0.05, df=13, Student’s t-test; Figure 3A). Lithium did not significantly affect the period of the exogenous rhythm (p>0.05, df=13, Student’s t-test; Figure 3B).

Lithium significantly lengthens the circadian period of animals in a 14:14 LD cycle (p<0.01; Fig 4). During lithium administration, two animals had the peak of rhythmic activity with the greatest power (i.e., the highest) switched from 24h to 28h. The period of the highest peak of activity increased for every animal in the 24-25h range during lithium administration while animals whose period was at 28h before lithium remained the same (Figure 4A). All animals saw a significant elevation of the mean period. The mean period of the highest peak of activity shifted from 25.00±0.34 h to 25.85±0.37 h (p<0.01, df=13, Student’s t-test; Fig 4B). Lithium also had no significant effect on the amplitude of the peaks, regardless of the time of sacrifice (Table 1).
Effect of Lithium on Locomotor Activity

Comparisons of the amount of wheel running activity 24 days before and 24 days after lithium treatment revealed that lithium administration decreased the overall amount of wheel running activity (Figure 5). Twelve of the fourteen animals decreased their total number of wheel revolutions during the period of lithium administration while two had increased locomotor activity (Figure 5A). The mean number of wheel revolutions per day of animals during lithium administration was significantly less than the number before lithium treatment, decreasing from 11287.55±1758.58 wheel revolutions to 6318.36±1030.72 revolutions (p<0.05, df=26, Student’s t-test; Figure 5B).

Effect of Lithium on FOS Expression

Lithium administration amplified the suprachiasmatic nucleus’ response to light (Figure 7A). AVP neurons in the shell of the SCN delineated the outline of the SCN, within which FOS expression could be expected to occur (Figure 6). The lithium-fed group had significantly greater FOS expression in the suprachiasmatic nucleus than the control animals, regardless if they were sacrificed after being given a 2 hour light pulse (LP) two hours after activity onset (endogenous 24H clock) or 2 hours after lights off (exogenous 28H rhythm) (Figure 7). Controls sacrificed during internal night (n=5) exhibited significantly less FOS expression in the SCN than their lithium-fed counterparts (n=7). Mean relative optical density (ROD) of FOS in the SCN increased from 36.44±7.50 to 67.82±9.35 (p<0.05, df=10, Student’s t-test; Figure 7B). Likewise,
the mean ROD of FOS expression in the SCN core was significantly greater in the group treated with lithium, increasing from 50.44±9.37 to 94.91±9.57 (p<0.01, df=8, Student’s t-test; Figure 7C). Lithium-fed animals sacrificed during the external night also had greater FOS expression in the SCN and in the SCN core. In the SCN, the mean ROD of FOS of controls was 42.32±5.81 (n=6); for the lithium treated group (n=7), the mean was 62.55±12.01 (p < 0.05, df=10, Student’s t-test; Figure 7B). Mean ROD of FOS in the SCN core increased from 52.76±5.94 (n=6) to 97.38±14.59 (n=7) after the administration of lithium (p<0.05, df=8, Student’s t-test; Figure 7C).

Discussion

The present results indicate that light is a potent entraining cue for resetting the internal clock of lithium treated animals. Animals under lithium treatment that were given a light pulse during either the internal or the external night had both a lengthened circadian period and greater FOS expression in the SCN. Previous experiments with blinded rats demonstrated that lithium delayed free-running rhythms by 0.40h and activity rhythms in an extended 28h cycle by 2.49h, suggesting that lithium interacts with environmental cues of light and dark (Kripke & Wyborny, 1980). Lithium has been shown to lengthen the circadian period in humans as well as rodents (Klemfuss, 1992). In one study of bipolar disorder, lithium-treated patients that exhibited advanced circadian phase reported improved mood and exhibited delayed circadian phase. Patients that did not have an advanced circadian phase did not respond to lithium treatment (Kripke et al., 1978). And while lithium is not the most efficient antidepressant agent on its own,
it is considered to be one of the most effective drugs for cyclic bipolar disorder (Jefferson et al., 1978). Given this, it is likely that lithium’s efficacy in the treatment of mood disorders lies in its ability to lengthen the circadian rhythm.

Animals in an extended 28h LD schedule had two period peaks that remained even during the period of lithium administration. A previous experiment also found animals to have two period peaks before and during lithium treatment (Mceachron et al., 1981). Though the previous study reported that lithium-fed rats placed within a 28h cycle were able to entrain to the extended rhythm, the study also reported that older rats in another 27h cycle experiment exhibited slower rhythmicity and that not all were able to synchronize to the 27h LD cycle (Mceachron et al., 1981). The mice used in the present study resembled the older 27h experimental rats in that though they exhibited significantly longer periods during lithium administration, only four of fourteen animals had their highest activity peak at a ~28h period. The frequency of endogenous oscillators has been shown to become faster as rodents age, possibly indicating that lithium treatment was not fully able to synchronize that rhythms of older mice to the extended 14:14 LD cycle (Pittendrigh & Daan, 1976). The mice in this experiment were also advanced in age; during the start of the experiment, they were 5-7 months old. Given that lithium is a drug with side effects such as excessive drinking, urination, and diarrhea, all of which the mice exhibited, their age and the stressors of lithium may have been a contributing factor to their inability to completely entrain to the 28h LD cycle.

Lithium did not significantly increase or decrease the exogenous (28h) period, but it did significantly lengthen the period of endogenous (24h) rhythm, providing evidence that lithium acts on the period generated by the internal clock.

There was a slight decrease in amplitude of the period of the highest peak(s) and in the
amplitude of the internally and externally determined circadian periods, but the decrease was not enough to be significant. A previous study by Abe, Herzog, and et al. found that lithium had no effect on the amplitude of the rhythms of cultured SCN cells, while other studies have reported that lithium decreased the amplitude of circadian rhythms, leading to the hypothesis that lithium may act by stabilizing the mood swings of bipolar disorder (Abe et al., 2000; Klemfuss, 1992). Nonetheless, our present results indicate that lithium’s primary action is on the period of the internal clock and not the amplitude.

In parallel to the lengthened period, lithium treated animals also exhibited increased FOS expression in the SCN after receiving a light pulse. Light pulses given early during the dark phase of the LD cycle have been shown to cause rapid increases in the levels of c-FOS in the SCN, indicating increased levels of neuronal activity (Kornhauser, Nelson, Mayo, & Takahashi, 1990; Rea, 1989). Controls did exhibit FOS expression, which, concurrent with the splitting of rhythms, confirms the idea that placing animals in an extended light-dark cycle does not cause them to ignore external cues entirely, but to attempt to follow both the internal and external clocks. However, the increased levels of FOS found in the lithium-treated group strongly suggest that lithium augments response to light at the level of the SCN. Relative optical density of FOS was especially high in the SCN core, an area of the SCN with cells that are particularly sensitive to light cues (Lee, Billings, & Lehman, 2003; Travnickova et al., 2013).

FOS expression for controls sacrificed during the internal night did not differ from controls sacrificed during the external night; likewise for both lithium groups. This suggests that lithium treatment does not induce an animal to behave as if there is only one cycle (internal or external), an idea that is supported by the fact two activity peaks remain even after lithium administration, but that lithium acts to increase sensitivity to light in general. This increased
sensitivity to light may be the mechanism by which the mice are better able to respond to the external cues of light and dark and synchronize their behavior accordingly.

The specific mechanisms by which lithium acts to lengthen the circadian period remain unknown. So far, three mechanisms have been proposed: by slowing oscillators (or individual clocks), by affecting the strength of coupling between different internal circadian oscillators, or by altering sensitivity to photic stimuli (Hafen, 1994; Klemfuss, 1992). The discovery that lithium augments response to light in the SCN seems to lend strength to the third argument and seems to indicate an important interaction between lithium and light in counteracting the symptoms of bipolar disorder. Specifically, lithium is particularly effective in lowering the peaks of mania and reducing the risk of suicide (Gershon et al., 2009).

Though lithium treatment can clearly be advantageous, there are side effects to the drug. Lithium decreased the amount of wheel running activity in twelve out of fourteen animals. Others studies have also found lithium administration to suppress locomotor activity in rats and mice in measures such as wheel running and exploratory behavior (Berggren, 1988; Hafen & Wollnik, 1994; Johnson, 1976). Lithium has been documented to induce weight loss in rodents and chronic treatment causes nephrogenic diabetes insipidus in mammals (Klemfuss, 1992). The mice in our study also exhibited symptoms of lithium toxicity, such as excessive thirst and urination. Common side effects of lithium in humans also include excessive thirst and urination as well as gastrointestinal discomfort, diarrhea, nausea, tremors and fatigue (Mellerup & Plenge, 1990; Nikolić, Marinković, Zdravković, & Timotijević, 1997). It is possible that the manic episodes of patients suffering bipolar disorder may be alleviated in part due to lithium’s suppression of activity in humans.
In summary, this animal model of bipolar disorder confirmed that lithium lengthens circadian period and better enables animals to follow an abnormally extended 28h LD cycle. Additionally, lithium seems to increase sensitivity to light cues given during the night at the level of the brain’s master clock. Taken together, the behavioral and molecular analyses of this study suggest that lithium interacts with external photic cues to exert its influence on the SCN.
References


Figure 1: Comparison of behavioral activity before (Li-) and during (Li+) lithium treatment on animals in a 14:14 LD cycle. Figure depicts double plotted actograms and periodograms for 24 days before and 24 days during lithium treatment for representative animals (A) and (B). Actograms on the left are 48 (24:24) hour double plots of locomotor activity; actograms on the right are 56 (28:28) hour double plots of locomotor activity. Analysis of periodograms before and during lithium treatment demonstrates that lithium caused a shift in animal A’s endogenous (24h) period from 24.6h to 25h and an increase in the power of the 28h period. Animal B’s endogenous (24h) period shifted from 24.5h to 25.5h and the power of the 28h period increased. Actogram analysis corroborates the periodogram data.
Figure 2: Change in the period peak(s) before and during lithium treatment over 48 days. (24 days Li-, 24 days Li+). Periods fall within two clusters, around 24H and around 28H. (A) Analysis of fourteen individual animal’s highest (and second highest) period peak(s) before lithium treatment. (B) Individual animals’ period peak(s) during lithium administration. Periods of the internal rhythms are longer under lithium treatment (shifted to the right) while periods of external rhythms (28h) do not shift.
Figure 3: The effect of lithium of the mean period of the internal ~24H cycle and the external 28H cycle. The mean was calculated over 48 days (24 days before and 24 days during lithium treatment). (A) The mean period of the endogenous 24h rhythm of the animals on lithium supplemented chow is significantly greater than when the animals were on regular chow. (***p<0.0001, Student’s t-test). (B) No significant difference in the mean period of the external (28h) rhythm was found in animals pre and during lithium administration. Values shown are mean ± SEM. (p>0.05, Student’s t-test).
Figure 4: Change in the circadian period of the highest peak of activity before and during lithium treatment. The period was quantified over 24 days before lithium and 24 days during lithium treatment (n=14). (A) Period of individual animals before and during lithium treatment. (B) The mean period of the highest peak of rhythmic activity of all animals before lithium was significantly shorter than the mean period of animals while they were on lithium. Values shown are mean ± SEM. (**p<0.01, Student’s t-test).
Figure 5: The effects of lithium on locomotor activity. Activity of animals (n=14) over 24 days pre-lithium treatment was compared to activity over 24 days during lithium treatment. (A) The total locomotor activity for individual animals before and during lithium administration. (B) The mean number of wheel revolutions per day of animals pre-lithium and during lithium treatment. Values shown are mean ± SEM. (*p<0.05, Student’s t-test).
Figure 6: AVP and FOS expression in the SCN. SCN of a representative animal; double-labeled for AVP and FOS. FOS staining (top left) is in green, AVP staining (top right) in red. Bottom, FOS and AVP staining shown together.
Figure 7: FOS expression in the suprachiasmatic nucleus (SCN). Mice were given a 1h LP either 2h after activity onset (internal night) or 2h after lights off (external night), then sacrificed. Fourteen mice comprised the lithium group, eleven the control group. Values shown are mean ± SEM. (A) Representative SCN sections of mice fed either control chow (Li-) or lithium supplemented chow (Li+) and sacrificed during internal night (24h) or external night (28h). Bottom right, red circle, entire SCN; bottom left, red oval, SCN core. Inset, a single FOS cell. Scale bar, 100 mm. (B) The lithium treated group showed greater mean relative optical density (ROD) of FOS expression in both the SCN (*p<0.05, Student’s t-test) and the SCN core (**p<0.01, Student’s t-test). (C) Time of sacrifice (during internal 24H night or during external 28H night) did not affect the levels of FOS expression of controls or experimental groups. (p>0.05, Student’s t-test).
### Table 1: T-tests for Period Amplitude

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<th>Mean±SEM</th>
<th>df</th>
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<td><strong>Amplitude of Period of Highest Activity Peak</strong></td>
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<tr>
<td>Li-</td>
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<tr>
<td>Li-</td>
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<tr>
<td>Li+</td>
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<tr>
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<td>632.8±77.8</td>
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