Original Article

Effect of Lithium Upon Circadian Rhythm of Locomotor Activity in Gerbil Meriones ungiculatus: Gender Differences

Efeito do Litio sobre o Ritmo Circadiano na Atividade Locomotora do Gerbil *Meriones unguiculatus*: Diferenças Sexuais

*Juárez-Tapia Cinthia Rocío, Miranda-Anaya Manuel, Moreno-Sáenz Enrique, Durán Pilar**

ABSTRACT

The effect of lithium on circadian rhythms has been studied to understand whether affective and bipolar disorders are related to disturbances on the circadian regulation. Some species of Gerbils (sand rat, Mongolian gerbil) have been proposed as a model to study mood disorders. The aim of the present work was to study the effects of chronically ingested lithium upon the structure of locomotor activity in male and female gerbils exposed to constant conditions as well as light/dark cycles; in order to understand if the free running period and activity levels and phase relationship with a light cycle is affected by chronic lithium chloride administration in water. Locomotor activity was recorded from individual gerbils, by means of running wheels coupled to infrared light beams in environmental controlled chambers. Results obtained indicate a lengthening of the free running period and activity phase in males, but an opposite effect in females until 20 days after beginning the ingest of LiCl, therefore its effects upon circadian free running period was different regarding the gender.

Key words: circadian, locomotor activity, Mongolian gerbil, lithium, gender.

Departamento de Biología Celular, Facultad de Ciencias, Universidad Nacional Autónoma de México. México D.F. 04510.
*To whom correspondence should be send, e mail: pilis@unam.mx
Departamento de Biología Celular, Facultad de Ciencias, UNAM, México D.F. 04510, Tel +52-55-56224833, Fax +52-55-56224828

RESUMO

O efeito do lítio sobre os ritmos circadianos foram estudados para entender se as doenças afetivas e bipolares estão relacionadas com as alterações da regulação circadiana. Algumas espécies do gerbil (rata do deserto, gerbil mongoliano) propuseram-se como modelos para o estudo das doenças anímicas. O propósito do presente trabalho foi estudar os efeitos do lítio ingerido de modo crônico sobre a estrutura da atividade locomotora em gerbil macho e fêmea expostos a condições constantes bem como ciclos claro/escuro, para determinar se o período do ritmo em livre locomoção, os níveis de atividade e sua relação de fase foram afetados pela administração crônica de cloreto de lítio (LiCl) em água. A atividade locomotora foi registrada em câmaras controladas individualmente para a cada gerbil, usando rodas de exercício acopladas a sensores de luz infravermelha. Os resultados obtidos indicaram um alargamento do período do ritmo em livre locomoção e a fase de atividade nos machos, com um efeito oposto nas fêmeas após 20 dias de iniciado o tratamento de LiCl. O efeito sobre o período do ritmo em livre locomoção foi diferente entre machos e fêmeas.

Palavras chave: Circadiano, atividade locomotora, gerbil, lítio, fêmeas, machos, gênero.

INTRODUCTION

Many depressed and bipolar patients suffer from alterations in their sleep-wake cycle and its circadian rhythmicity; therefore mood disorders are of particular interest to be studied from the perspective of the circadian clock function [16, 31, 33, 34]. A connection between the molecular basis of the circadian clock and mood disorders have been recently established, and behavior of the circadian Clock mutant mice has been proposed to be studied as model for mania, since its treatment with lithium is similar to the observed in humans[28].

The effect of lithium on circadian rhythms has been studied to understand whether affective and bipolar disorders are related with disturbances of the circadian regulation. Lithium lengthens the free running period of the circadian locomotor activity in different animals such as flies[22], rats[12]; hamsters[20], mice[13] and even primates[32] including humans[14]. Lithium also can lengthen the free running circadian period of electrical firing rate from mouse's SCN isolated neurons[1]. It has been speculated that the action of lithium upon free running period seems to be through the Glycogen Synthase Kinase3-β (GSK3 β) and lithium has a direct inhibition of it[8,13,21,22,35]. The inhibition of GSK3 β by lithium thus leads to suppression of Rev-erb β, activation of Bmal1 and a slowing of the molecular circadian feedback loop lengthening the period of the circadian cycle[35]. The fat sand rat (a species of gerbil) has been proposed as a model to study mood disorders produced by changes in photoperiod[5,10], meanwhile Mongolian gerbil has been proposed as a model to study anxiety and novel antidepressant agents since their neurokitin NK1 receptors maintain a closer homology to human NK1 receptors than mice and rats[6,30].

Mongolian gerbil *Meriones unguiculatus* is well adapted species to extreme environments, where seasonal change in photoperiod is followed by changes in availability of other natural Resources[18]. The gerbil has a social organization and does not hibernate[2]. In laboratory conditions, gerbil displays an unstable circadian activity profile in such way that activity is wide spread in short bouts present along the light-dark cycle. There are controversial results whether its circadian activity is diurnal, nocturnal, or crepuscular[17,24,25,26]. Irregular activity-rest cycles and instability of phase regarding light conditions are features that arises the possibility of studying this species.

There are no studies on gender differences in the effect of Li and the circadian system in rodents, however there are studies in which gender differences and therapeutics on treatment for bipolar disorders exist[7].

The aim of the present work is to study the effects of chronically ingested lithium upon the structure of locomotor activity in males and females gerbils exposed to constant conditions as well as light/dark cycles; in order to observe if the instability of phase of activity as well as the circadian distribution of activity is affected by chronically lithium administration.

MATERIAL AND METHODS

Animals and housing

Eight male and eight female juvenile *Meriones unguiculatus* (57.3 ± 2.4g SEM) about three month-old, were obtained from the vivarium at the Facultad de Ciencias UNAM. Animals were kept in individual polycarbonate cages containing woodchips, with *ad libitum* food (Rodent Lab Chow 5001, Purina Inc.) and tap water. Environmental temperature was maintained at 23 ± 3°C, and lighting cycle (LD) set at 12:12 (0600-1800, 280 lx). Animals were individually transferred to cages for long term recordings. Cleaning of cages occurred once a week. When in constant conditions, animals were continuously exposed to a dim red light (1-2 lx) provided by a small red bulb lamp which allow to give maintenance without changing light conditions. Animals were maintained and treated according with the official Mexican regulation for experimentation in animals (NOM-062-ZOO-1999).

Locomotor activity recordings

Locomotor activity was recorded from individual gerbils, by means of running wheels coupled to infrared light beams; data were obtained by acquisition data board in a PC. A light beam was located at the top of a glass cage (18x30x20 cm) and every half revolution of the wheel was recorded as one event. Data were summarized every ten minutes (10 min. bin cell) and stored in a PC until further analysis. Each cage was placed in a light-tight wooden box (70x40x43 cm), equipped with a fluorescent lamp (280 Lx) controlled by a timer. Ventilation was kept constant using a small fan and temperature was maintained at 24 ± 2°C.

Experimental protocol

Animals were exposed initially to Light-Dark conditions (LD 12:12, 06:00-18:00) during at least 15 days, then, constant Dim red Light (DrL, 1-2 lx) was used as constant condition to observe free running rhythms during 50 days, then exposed in LD during 15 more days. Cages cleaning was performed once a week during all the experiment using the same dim light as a background. By the day 25th in DrL to the end of the experiment, a solution of 1 mM LiCl (sigma) in water was provided in substitution of regular water. LiCl solution was refreshed every other day. Volume of consumed water was quantified every change by weighing bottles. In order to reduce the sodium loss by the effect of consuming lithium (*diabetes insipidus*), bulk grains of sea-salt were available at the bottom of the cage. After 50-60 days of consuming LiCl-water, locomotor activity was recorded on LD cycles.

Data analysis

Locomotor activity data were organized into double plotted actograms; a minimum of ten days of recording were used to calculate X^2 periodogram, and waveforms were performed by means of DISPAC software[3]. For evaluate activity levels during constant conditions, waveforms were fit to the dominant period and analyzed in free running rhythms. Segments of each recording used for analysis consisted in blocks of ten consecutive days: initial LD; dim red-light with regular water (DrL), and three segments of ten days each during LiCl-water, as well as the final ten days in LD.

Data of activity were compared among segments. For each separate segment variables as

period, waveform, activity levels and in case of LD, phase relationship with the zeitgeber was analyzed. Data were compared for every group using Student´s t-test by means of the software Statistica, where $p<0.05$ was considered significant. Data are expressed as the mean value with corresponding standard error.

Results

Figure 1 Show two representative actograms of two female gerbils with activity in LD mainly diurnal, and some spread during the dark phase. When exposed to constant DrL, irregular patterns during the first 6 days in A or a consolidated activity bout in B were present, disperse activity persisted in all the recording, however main bout of activity showed free running. When LiCl was given in water, some animals started to consolidate clear activity bouts as in the example of A, being more conspicuous by day 50. Entrained locomotor activity in final LD conditions from day 57, showed mainly nocturnal activity bouts in both cases showed.

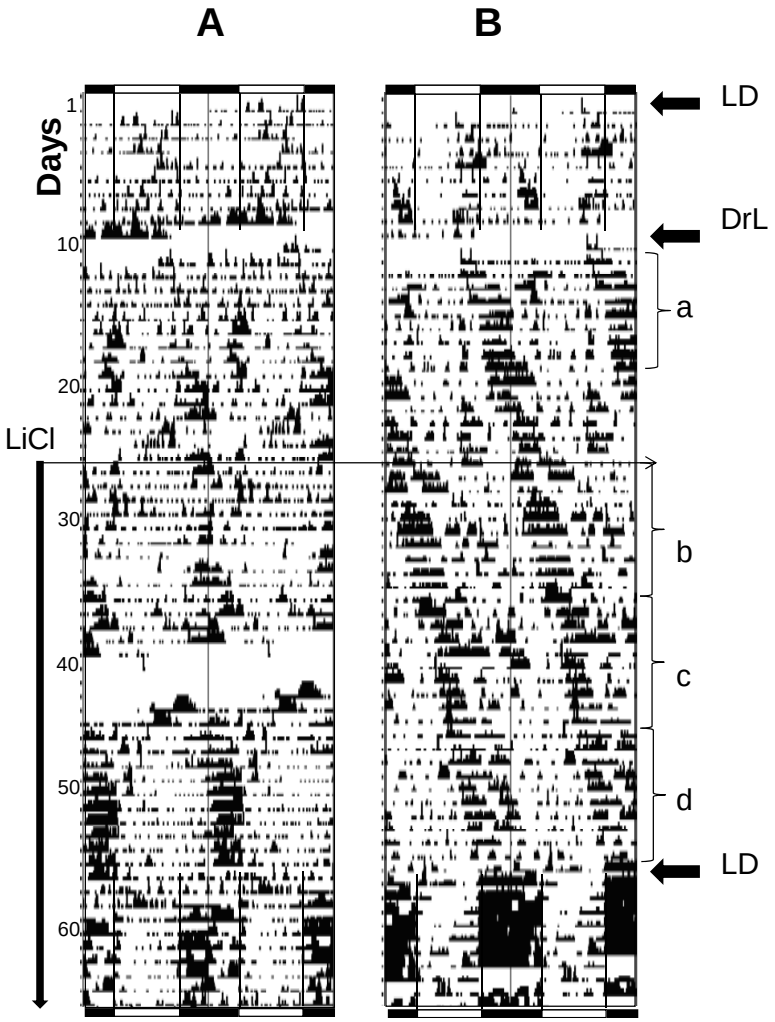

Figure 1 Actograms of two female gerbils before and during consumption of LiCl in water (left arrow), In A, after day 50, activity bout becomes less disperse and when in LD shows nocturnal instead of diurnal as in initial entrainment is observed. Brackets indicate segments analyzed by periodogram and average waveform. LD conditions are indicated at the top and bottom of each actogram. DrL= constant Dim red Light.

Figure 2, shows two representative actograms of male gerbils. During the initial LD conditions, diurnal activity with some bouts dispersed in darkness can be observed. When in free running conditions (DrL), it is common to observe that activity is spread all over each circadian cycle; however a main bout of activity can be followed indicating a circadian profile. When LiCl in drinking water was given (indicated by an arrow on the left side of actogram), no immediate effect on period was observed. In A, activity phase seems to be more consolidated and change in free running period was observed from day 45. In B, splitting in activity was observed from day 33 and these two components free run until next LD. Empty days in actograms are due to technical failure. When exposed to LD cycles, both animals showed strong nocturnal profiles by day 75; however, unstable phase relationship can be seen in B.

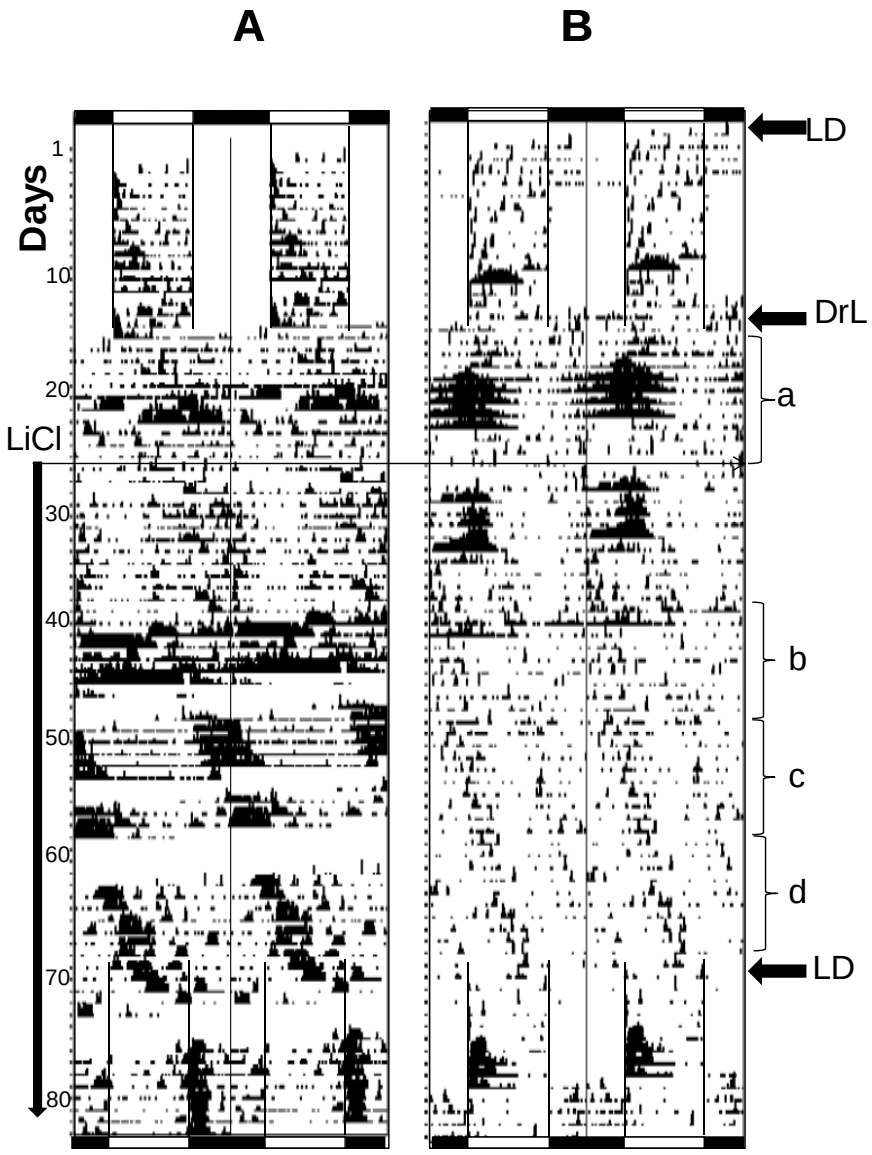

Figure 2 Actograms of two male gerbils. In A, after day 50, activity bout becomes less disperse and when in LD shows nocturnal instead of diurnal as in initial entrainment. Activity in B splits during chronic administration of LiCl.

Period and activity changes

Figure 3 shows the average of the free running period (FRP) without LiCl administration (indicated with *a*) and three consecutive segments of 10 days (b, c and d) after using lithium in water. Statistical differences were observed in males (dark bars) since the first 10 days after beginning drinking LiCl solution. By other hand, period average in females displayed at the beginning a shortening in the period, then until after 20 days of ingest a lengthening was observed in the population. Average activity length was also affected during the first days of consuming LiCl (fig 4). Both males and females increased total hours of activity spread all over the circadian cycle; however this tendency persisted in males during all the time they were drinking LiCl, while females decreased activity by the 10th day.

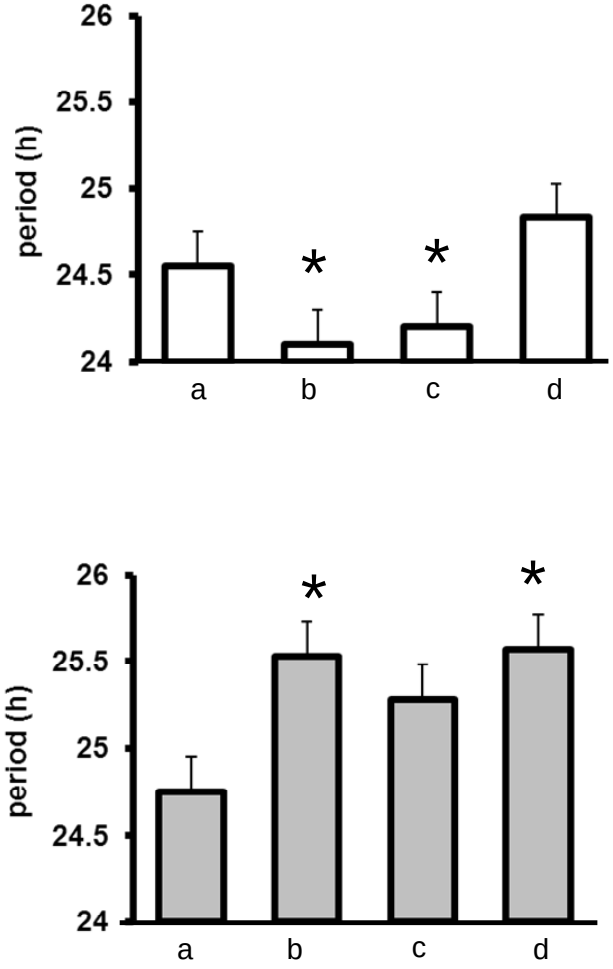

Figure 3 Free running period in controls and after LiCl administration in drinking water. Stars denote significant differences regarding controls (a) and blocks of 10 consecutive days while drinking LiCl (b, c and d). A lengthening is observed in males (dark bars) while at the beginning a shortening was seen in females (clear bars).

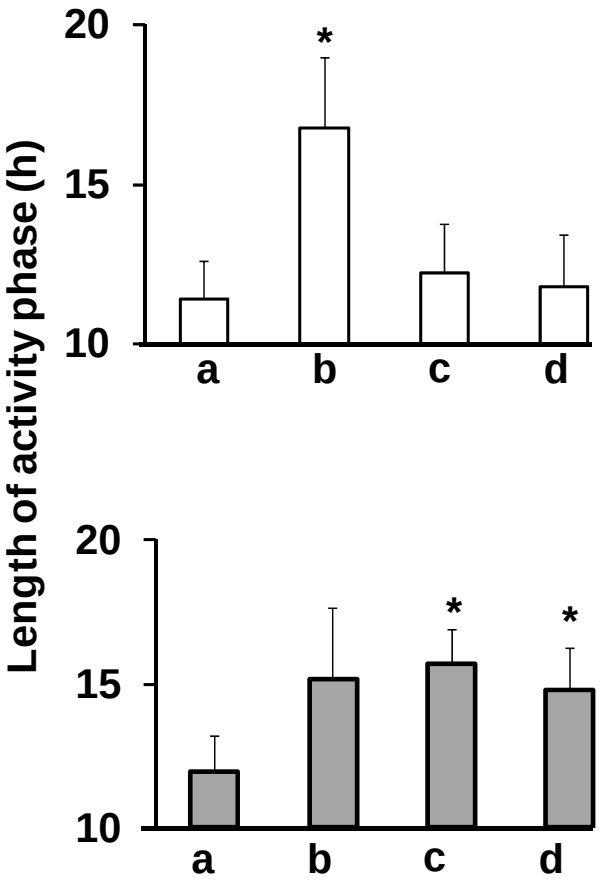

Figure 4 Average activities displayed between control condition and days after chronically drinking LiCl in water. An effect on increasing was observed in males (dark bars) while in females (clear bars) only during the first 10 days. Stars denote differences regarding control (a).

Discussion

Activity wheel running in long term recordings seem to be a reliable method for analyzing FRP of circadian rhythms in Mongolian gerbils. Although a disperse pattern of activity is consistent in all organisms tested, which mean that this species does not show a consistent rest phase during the circadian cycle. There are no differences in FRP between genders before consuming LiCl, and both display average periods larger than 24 hr, indicating a diurnal rhythms regarding Aschoff´s rule[4] which is consistent with other studies[26].

Irregular locomotor activity patterns arises the question about the way in which therapeutics for bipolarity may have an impact on the activity structure in a species such as the gerbil. It is known that bipolar patients have less stable and more variable circadian activity patterns than controls. Irregular circadian activity in bipolar patients is present even when not acutely ill[15].

At the present study we observed that chronically drinking a solution of LiCl water produce changes differentially in some circadian rhythm parameters regarding the gender. Lithium lengthens free running circadian period in most of the species studied. Its action seems to be mainly through the activity of the enzyme GSK3 β[35]. In the present study, such effects were confirmed in males; however females displayed in average a lengthening in FRP after 20 days of drinking LiCl in regular water. Overall quantification of drink solution of LiCl during three consecutive weeks, indicated that males drink larger volumes (541.2±15.21 ml) than females (413.7±26.43 ml) which may indicate that the lengthening effect lately observed in females might be due to a delayed reaching of the needed

concentrations to provoke the length in FRP. Gender differences have been reported regarding drinking behavior where males usually drink more volume than females[24]. Also gender differences in activity were not noted in controls as observed elsewhere[27]. Nevertheless is not clear why there is at the beginning a shortening in FRP.

It is likely that endocrine differences may interfere on the effect of circadian regulation in different species of mammals[11]. The recent association of circadian rhythms physiology and mood disorders suggest that also sex differences observed in bipolar affections may have some relationship in the existence of sex differences in the sensitivity to lithium therapeutics affects patients regarding gender[23]. Together with the aforementioned, there is a possibility that in animal models used for studying pharmaceutical effects of drugs requires more attention regarding gender[9,19].

Disturbances of circadian rhythms are being more frequent in the industrialized world (jet lag, shift work). Most of these disturbances are accompanied with some other clinical illness condition such as obesity, sleep and psychiatric disorders including affective disorders. It is of particular attention that affective disorders are related with decreased circadian amplitude of several rhythms as well as a phase advance or delay[29]. Treatment with lithium changes FRP and phase relationship with zeitgebers, indicating a close association of the mood disorders symptoms and regulation of circadian rhythms.

Acknowledgements

The present work is dedicated to the memory of Prof. León Cintra, an outstanding Neuroscientist and mentor. We thanks to Agustin Carmona, Dora Salazar, Isabel Antunez, and Mario Soriano for their invaluable technical support in the vivarium facilities.

REFERENCES

1. Abe M, Herzog ED, Block GD. Lithium lengthens the circadian period of individual suprachiasmatic nucleus neurons. Neuroreport 2000 Sep 28; 11(14): 3261-4.

2. Agren G, Zhou Q, Zhong W. Ecology and social behaviour of Mongolian gerbils *Meriones unguiculatus*, at Xilinhot, Inner Mongolia, China. Anim. Behav. 1989; 37:11-27.

3. Aguilar-Roblero R, Salazar-Juarez A, Rojas-Castañeda J, Escobar C, Cintra L. Organization of circadian rhythmicity and suprachiasmatic nuclei in malnourished rats. Am J Physiol. 1997 Oct; 273(4 Pt 2):R1321-31.

4. Aschoff J. Free running and entrained rhythms. In: Aschoff J, editor. Handbook of behavioral neurobiology; Vol 4. Biological Rhythms., Press NY and London: Ed. Plennum: 1981, p. 81-92.

5. Ashkenazy T, Einat H, Kronfeld-Schor N. Effects of bright light treatment on depression- and anxiety-like behaviors of diurnal rodents maintained on a short daylight schedule. Behav Brain Res 2009 Aug 12; 201(2): 343–6.

6. Bradley BF, Bridges NJ, Starkey NJ, Brown SL, Lea RW. Anxiolytic and anxiogenic drug effects on male and female gerbils in the black-white box. Behav Brain Res 2010; doi:10.1016/j.bbr.2010.08.006

7. Burt VK, Rasgon N. Special considerations in treating bipolar disorder in women. Bipolar Disord. 2004 Feb; 6(1):2-13.

8. Dokucu ME, Yu L, Taghert PH. Lithium- and valproate-induced alterations in circadian locomotor behavior in Drosophila. Neuropsychopharmacology 2005 Dec; 30(12):2216-24.

9. Eichling PS, Sahni J. Menopause related sleep disorders. J Clin Sleep Med. 2005 Jul 15; 1(3): 291-300.

10. Einat H, Kronfeld-Schor N, Eilam D. Sands rats see the light: short photoperiod induces a depression-like response in diurnal rodent. Behav Brain Res 2006 Oct 2; 173(1):153-7.

11. Gorman MR, Lee TM. Hormons and Biological Rhythms. In: Becker S, Breedlove M, Crews D, McCarthy MM Editors. Behavioral Endocrinology (2nd ed). London: The MIT Press: 2002, p 451-494.

12. Hafen T, Wollnik F. Effect of lithium carbonate on activity level and circadian period in different strains of rats. Pharmacol Biochem Behav 1994 Dec; 49(4): 975-83.

13. Iwahana E, Akiyama M, Miyakawa K, Uchida A, Kasahara J, Fukunaga K, Hamada T, Shibata S. Effect of lithium on the circadian rhythms of locomotor activity and glycogen synthase kinase-3 protein expression in the mouse suprachiasmatic nuclei. Eur J Neurosci 2004 Apr; 19(8): 2281-7.

14. Johnsson A, Engelmann W, Pflug B, Klemke W. Period lengthening of human circadian rhythms by lithium carbonate, a prophylactic for depressive disorders. Int J Chronobiol 1983; 8(3) 129-47.

15. Jones SH, Hare DJ, Evershed K. Actigraphic assessment of circadian activity and sleep patterns in bipolar disorder. Bipolar Disord. 2005 Apr; 7(2):176-86.

16. Katz G, Durst R, Zislin Y, Barel Y and Knobler HY. Psychiatric aspects of jet lag: review and hypothesis. Med Hypotheses 2001 Jan; 56(1): 20-3.

17. Klaus, U., Weinandy, R. y Gattermann, R. Circadian activity and sensitivity to noise in the mongolian gerbil *Meriones unguiculatus*. Chronobiology Int 2000 Mar; 17(2): 137-45.

18. Klir, J, Heath, JE. Bennani N. An infrared thermographic study of surface temperature in relation to external thermal strees in the Mongolian gerbil, *Meriones unguiculatus*. Comp Biochem Physiol. 1990; 96 (1): 141-6.

19. Leibenluft E. Women with bipolar illness: clinical and research issues. Am J Psychiatry; 1996 Feb; 153(2):163-73.

20. LeSauter J and Silver R. Lithium lengthens the period of circadian rhythms in lesioned hamsters bearing SCN grafts. Biol Psychiatry 1993 Jul 1-15; 34(5):75-83.

21. O'Brien WT, Harper AD, Jove F, Woodgett JR, Maretto S, Piccolo S and Klein PS. Glycogen synthase kinase-3beta haploinsufficiency mimics the behavioral and molecular effects of lithium. J Neurosci 2004 Jul; 24(30) 6791-8.

22. Padiath QS, Paranjpe D, Jain S, Sharma VK. Glycogen synthase kinase 3beta as a likely target for the action of lithium on circadian clocks. Chronobiol Int. 2004 Jan; 21(1): 43-55.

23. Peternel S, Pilipović K, Zupan G. Seizure susceptibility and the brain regional sensitivity to oxidative stress in male and female rats in the lithium-pilocarpine model of temporal lobe epilepsy. Prog Neuropsychopharmacol Biol Psychiatry 2009 Apr 30;33(3):456-62. Epub 2009 Jan 21.

24. Pietrewicz AT, Hoff MP. Hoggins SA. Activity rhythms in the Mongolian gerbil under natural light conditions. Physiol Behavior 1982 Aug; 29(2): 377-80.

25. Probst B, Eisermann K. Stöhr W. Diurnal patterns of scent-marking, serum testosterone concentration and heart rate in male Mongolian gerbils. Physiol Behav 1987; 41(6): 543-7.

26. Refinetti R. Variability of diurnality in laboratory rodents. J Comp Physiology A Neuroethol Sens Neural Behav Phisiol 2006 JuL; 192 (7): 701-14. Epub 2006 Jan 19.

27. Roper TJ. Sex differences in circadian wheel running rhythms in the Mongolian gerbil. Physiol Behav 1976 Sep;17(3):549-51.

28. Roybal K, Theobold D, Graham A, DiNieri JA, Russo SJ, Krishnan V, Chakravarty S, Peevey J, Oehrlein N, Birnbaum S, Vitaterna MH, Orsulak P, Takahashi JS, Nestler EJ, Carlezon WA, Jr. McClung CA. Mania-like behavior induced by disruption of CLOCK. Proc Natl Acad Sci U S A. 2007 Apr 10; 104(15): 6406-11.

29. Schulz P, Steimer T. Neurobiology of circadian systems. CNS Drugs. 2009; 23 Suppl 2:3-13. doi: 10.2165/11318620-000000000-00000.

30. Varty GB, Cohen-Williams ME, Morgan CA, Pylak U, Duffy RA, Lachowicz JE, Carey GJ and Coffin VL. The gerbil elevated plus-maze II:

anxiolytic-like effects of selective neurokinin NK1 receptor antagonists. Neuropsychopharmacology 2002 Sep; 27(3): 371-9.

31. Wehr TA and Wirz-Justice A Circadian rhythm mechanisms in affective illness and in antidepressant drug action. Pharmacopsychiatria 1982 Jan; 15(1):31-9.

32. Welsh DK, Moore-Ede MC. Lithium lengthens circadian period in a diurnal primate, Saimiri sciureus. Biol Psychiatry 1990 Jul 15; 28(2):117-26.

33. Winget CM, DeRoshia CW, Markley CL, Holley DC. A review of human physiological and performance changes associated with desynchronosis of biological rhythms. Aviat Space Environ Med 1984 Dec; 55(12):1085-96.

34. Wirz-Justice A, Benedetti F, Berger M, Lam RW, Martiny K, Terman M, Wu JC. Chronotherapeutics (light and wake therapy) in affective disorders. Psychol Med. 2005 Jul; 35(7): 939-44.

35. Yin L, Wang J, Klein PS and Lazar MA. Nuclear receptor Rev-erbalpha is a critical lithium-sensitive component of the circadian clock. Science. 2006 Feb 17. 311(5763):1002-5.