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Food Restriction Affects Locomotor Activity in Mongolian Gerbils (*Meriones unguiculatus*)

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Abstract: The circadian rhythm of the locomotor activity is regulated by at least 2 external pacemakers, environmental photoperiod and food availability. In the present study, the effects of short-term (2 weeks) food restriction on locomotor activity rhythm in adult male and female Mongolian gerbils were investigated. Animals were fed ad libitum with no restriction during the first week of the experiment and their daily ad libitum food consumptions were determined. Then the food was restricted 40% of ad libitum for the next 2 weeks. At constant temperature, locomotor activity was monitored by running wheel in animals exposed to a long photoperiod (14L) for 3 weeks. A significant phase advance was recorded in the locomotor activity rhythm in both male and female animals. The shift was bigger in males (12 ± 1.1 h) than in females (6 ± 0.5 h) ($P < 0.05$). Activity levels and body weights decreased in males ($P < 0.01$) and females ($P < 0.012$) compared with the first week values. The gerbils' locomotor activity was significantly affected by the food restriction. These results indicate that the rhythm and the level of the locomotor activity in the Mongolian gerbil can be entrained by food restriction.

Key Words: Rhythm, Food restriction, Locomotor activity, Mongolian gerbil

Moğolistan Gerbillerinde (*Meriones unguiculatus*) Besin Kısıtlaması Lokomotor Aktiviteyi Etkilemektedir

Özet: Lokomotor aktivitenin sirkadiyen ritmi en az iki çevresel parametre ile düzenlenebilir, bunlar fotoperiyot ve besin mevcudiyetidir. Bu çalışmada, kısa dönem (iki hafta) besin kısıtlamasının yetişkin erkek ve dişi Moğolistan gerbillerinin lokomotor aktivite ritmi üzerine olan etkileri araştırıldı. Deneyde ilk bir hafta kontrol amaçlı normal besin tüketimi uygulandı ve gerbillerin günlük besin tüketimleri tespit edildi. Daha sonra ki iki hafta süresince tüketim % 40 kısıtlandı. Lokomotor aktivite ölçümleri koşu tekerlekli veri toplama sistemi ile üç hafta süresince uzun fotoperiyotta (14L), sabit sıcaklıkta kaydedildi. Erkek ve dişi gerbillerde besin kısıtlaması ile beraber bir safha kayması yaşandığı ve aktivitenin daha erken saatlerde başladığı tespit edildi. Erkeklerdeki safha kayması ($12 \pm 1,1$ saat) dişilerdekenden ($6 \pm 0,5$ saat) büyüktü ($P < 0,05$). Aktivite miktarları ve vücut ağırlıkları erkek ($P < 0,01$) ve dişilerde ($P < 0,012$) ilk hafta sonuçları ile karşılaştırıldığında azalma gösterdi. Gerbillerin lokomotor aktivitesi besin kısıtlamasından anlamlı şekilde etkilendi. Bu sonuçlar Moğolistan gerbillerinde toplam aktivite ritmi ve miktarının besin kısıtlaması ile düzenlenebileceğini göstermektedir.

Anahtar Sözcükler: Ritim, Besin kısıtlaması, Lokomotor aktivite, Moğolistan gerbil'i

Introduction

The daily light-dark cycle is the main environmental cue that entrains the suprachiasmatic nuclei (SCN) to 24 h (1). Besides light, the phase of the SCN can be shifted by a variety of non-photoc factors such as food availability (2), temperature (3) and social factors (4).

There is evidence that food restriction is capable of entraining a circadian clock (1,2). Food restriction decreases the level and phase advances the rhythm of the locomotor activity in rats (*Rattus rattus*) (5,6) and field

mouse (*Mus booduga*) (7). Restricted feeding during the naturally inactive phase (daytime for nocturnal species) induces a decrease in food intake. Animals try to adapt to underfeeding by increasing feed efficiency and decreasing locomotor activity (8).

Food restriction reduces fat reserves and serum leptin concentrations in the Siberian hamster (9), and suppresses serum gonadotropins, and induces increased anabolic and decreased catabolic gene expression in the arcuate nucleus in rats (10).

Own previous studies examined the dependency of the reproductive activity rhythm characteristics of the Mongolian gerbils on photoperiod, food and temperature. Short photoperiods ($\leq 8L$) inhibited but long photoperiods ($\geq 10L$) stimulated the gonadal weight of the male Mongolian gerbils. However, body weight was independent of changes in ambient photoperiods (11,12). In our latest work, on the other hand, 12-week food restriction inhibited the gonadal development and the maintenance of the gerbils significantly (13). According to our unpublished data, gerbils show an entrained motor activity rhythm under light/dark cycles, free-running circadian periods during constant darkness, and arrhythmic motor activity rhythm during constant light.

In the present study, locomotor activity rhythm responses of Mongolian gerbils to short-term restricted feeding was investigated in a LD 14:10 photoperiod.

Materials and Methods

Mongolian gerbils (*Meriones unguiculatus*) (14) were obtained from our colony maintained at the Abant İzzet Baysal University. The procedures used in this study were carried out in accordance with the Animal Scientific procedure, Act of 1986, and approved by the Institutional Animal Care and Use Committee. All animals were kept in LD 14:10 throughout the experiment with the light off at 8 pm. The animals were maintained in plastic cages (16 x 31 x 42 cm) with pine shavings used as bedding. Cool-white fluorescent tubes controlled by automatic programmable timers, and light intensities at the animals' eye level exceeded 200 lux. Food (Purina Rodent Chow, Formula # 5001) was provided ad libitum for the first week of the experiment and restricted for the next 2 weeks. Ambient temperature was held constant at 22 ± 2 °C in air-conditioned rooms. Adult male ($n = 10$) and female ($n = 10$) gerbils were housed individually. Food intake in gerbils was determined prior to performing the food-restricted portion of the experiment (8 g for males, 7 g for females). Food amounts equal to 60% of ad lib (based on our previous study, (13)) were provided at 6 am on a daily basis (4.8 g males, 4.2 g females). Body weights were recorded every week throughout the experiment.

Locomotor activity

Locomotor activity was measured for running-wheel activity under a constant temperature (22 ± 2 °C) in

males and females. The animals were exposed to running wheels for 3 weeks (1 week for control and 2 weeks for food restriction). The number of revolutions per 10-min interval was automatically recorded and stored on a computer hard disk. The stored results were analyzed by Vital View Software (Mini Mitter Company, Inc., Bend, OR, USA). The activity was shown by the double plotted actograms by Acti View Software (Mini Mitter Company, Inc.). The mean period of the circadian running wheel activity rhythm was calculated for each group of animals.

Statistics

Wheel revolutions and body weights were analyzed using a repeated measures 2-way analysis of variance (ANOVA: SPSS for Windows, ver 10.0). Differences between means within or between groups were determined by t-tests. Values were considered statistically significant at $P \leq 0.05$. Data are presented as mean \pm SEM.

Results

During the first week of the experiment, the animals were fed ad libitum. They were entrained by the light/dark cycle and they started to their activity about at CT 12 when the lights were turned off. On the fourth day of the experiment, there was a little phase advance in males that cannot be explained by the experimental design. During the second week of the experiment, locomotor activity was phase advanced in both males and females by the food restriction. There was a significant difference between the phase advances of the males (12 ± 1.1 h) (Figure 1) and the females (6 ± 0.5 h) ($P < 0.05$) (Figure 2). Locomotor activity levels (running-wheel turns) decreased in both males (650 ± 23 wt (wheel turn)) and females (480 ± 15 wt) in comparison with the first week values of the males (4827 ± 150 wt) and the females (4449 ± 135 wt) ($P < 0.001$) (Figure 3). A rapid decrease was observed in body weights. Body weight of the males decreased from 85 ± 4.5 g to 75 ± 3.2 g ($P < 0.05$) and that of the females decreased from 76 ± 3.1 g to 68 ± 2.8 g ($P < 0.05$) in 1 week (Figure 4). During the third week of the experiment, the animals' running wheel activity was very low. Their activity levels were 57 ± 5 wt in males and 43 ± 4.3 wt in females. The rhythm of activity disappeared in the gerbils and arrhythmic locomotor activity occurred. Body weights

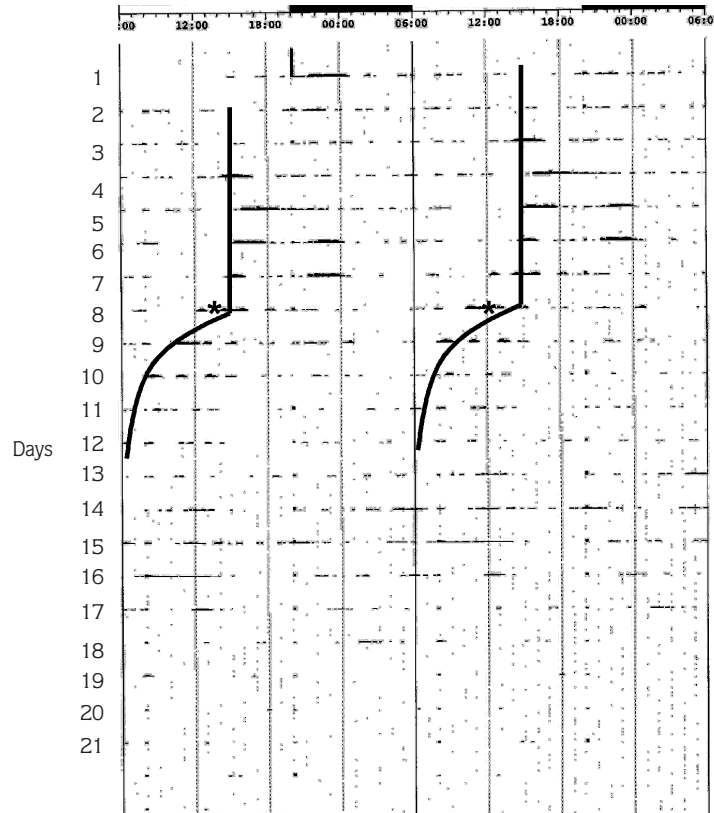


Figure 1. A representative actogram of one male Mongolian gerbil in LD 14:10. The running wheel activity was recorded for 3 weeks. * shows the beginning of food restriction. Line indicates the phase shift.

decreased to 63 ± 2.5 g ($P < 0.01$) in males and 55 ± 2.4 g ($P < 0.02$) in females (Figure 4).

Discussion

The results reported here show that the Mongolian gerbil is a good model for physiological studies of entrainment by feeding schedules in rodents. Food restriction phase advanced the activity rhythm of the Mongolian gerbils. This effect occurred by the phase advance of the internal clock, SCN. However, it could not be clarified yet how the food restriction phase-shifts the activity rhythm of the SCN. One possible mechanism is via melatonin hormone, because melatonin is released by the pineal gland, which is entrained by photoperiod. It is well established that the daily rhythm of pineal melatonin phase advances in food restriction (6,15). The phase advance in melatonin rhythm phase advances the SCN

clock via special melatonin neurons and receptors in SCN (16).

Another possible mechanism is via leptin hormone. Leptin is a fat-derived hormone that acts to monitor daily metabolism. It is suggested that the rhythmic pattern of endogenous leptin production may play a role in modulating circadian clock phase. The rhythm of plasma leptin is driven by the animal's feeding rhythm, so that when food availability is restricted to the daytime, leptin levels peak in the day rather than at night (17). An early peak in leptin levels due to an advance in feeding could have a phase-advancing effect on the SCN clock. However, there are also studies that suggest that the SCN clock is not phase shifted by food restricted schedules (18). In our latest study, we have shown that 40% food restriction decreased leptin values in adult male and female Mongolian gerbils (13). In that study, we found a

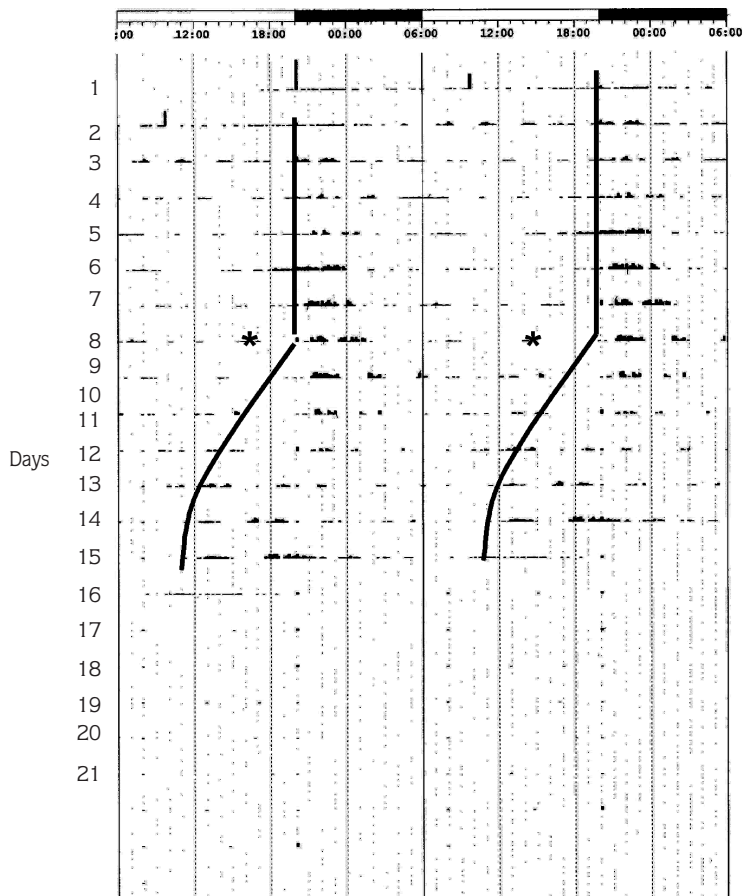


Figure 2. A representative actogram of one female Mongolian gerbil in LD 14:10. The running wheel activity was recorded for 3 weeks. * shows the beginning of food restriction. Line indicates the phase shift.

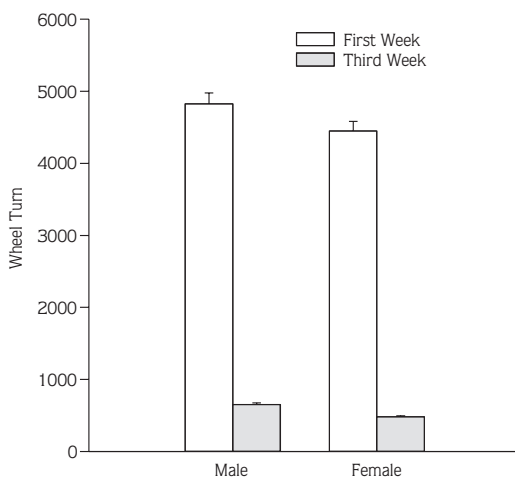


Figure 3. Mean \pm S.E.M running wheel turns of the gerbils. $P < 0.05$ indicates a significant difference.

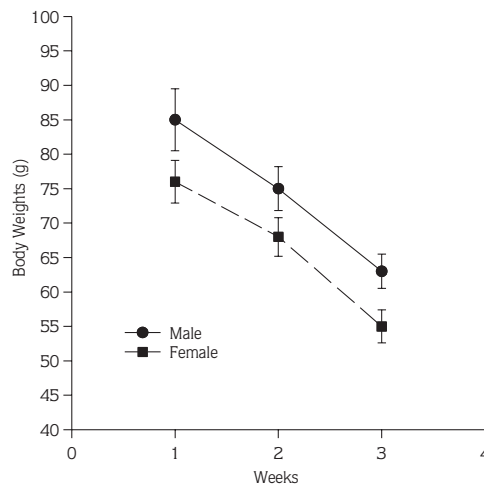


Figure 4. Mean \pm S.E.M. body weights of male and female Mongolian gerbils. $P < 0.05$ indicates a significant difference.

sex-related difference in leptin concentrations. The serum leptin concentration was lower than that of females in *ad libitum* groups. The high level of circulating leptin in the female could be attributed to a gonadal activity.

It has been shown that the rhythm of the leptin hormone release is regulated by the SCN (19). Leptin also phase advances the suprachiasmatic nuclei in vitro (20). Our observations support these data, because leptin also phase advances the locomotor activity rhythm when administered in vivo in Syrian hamsters (Karakas et al., unpublished data).

The phase advance of the activity rhythm was bigger in males than in females. The SCN of the male individuals may be more sensitive to food availability than that of females. Wheel turns were reduced in both males and females significantly by the food restrictions. It is suggested that the gerbils tried to tolerate the inhibitory effects of food restriction by decreasing locomotor activity (8). Food deprivation has been reported to increase NPY release and synthesis (21). NPY cells in the arcuate nucleus (ARC) express leptin receptors and are thought to play an important role in the negative feedback control of appetite by responding to leptin derived from adipocytes.

It is an expected seasonal adaptation for some animals living at higher latitudes to increase their energy stores before winter, like ground squirrels (*Spermophilus beldingi*), Syrian hamsters (*Mesocricetus auratus*), and prairie voles (*Microtus ochrogaster*) (22-24). Conversely, some species, like meadow voles (*Microtus pennsylvanicus*), deer mice (*Peromyscus maniculatus*) and Siberian hamsters (*Phodopus sungorus*), decrease their body weight when exposed to short winter-like days (25,26). In the present study, a fast reduction occurred in the body weights of male and female gerbils with the food restriction. The combination of the food restriction and the running wheel activity may be responsible for the fast body weight reduction. This suggests that to decrease the locomotor activity may be another

adaptation used by Mongolian gerbils to reduce energy consumption under restricted conditions as in Siberian hamsters (27).

The locomotor activity responses of the Mongolian gerbils to photoperiod is well known. The rhythm of the activity is regulated by the photoperiod. In addition, the reproductive activity responses of the Mongolian gerbils to photoperiod indicate the influence of the pineal gland. The reproductive organ weight responses to photoperiod change according to the presence or absence of the pineal gland (28). The rhythm of the reproductive responses to photoperiod is also produced and controlled by the SCN. It is reported that the rhythms in rats, for example, are influenced by restricted access to food, which serves as a more potent entrainer than photic cues in controlling running activity (29), and that anticipatory wheel-running activity is generated by photoperiodic cues from the time of food presentation. We investigated whether Mongolian gerbils have the ability to be entrained by restricted food presentation. When restricted access to food was offered during the illuminated portion of the LD cycle, gerbils developed a new rhythmic pattern of locomotor activity, being more advanced. However, more detailed investigations are needed to illuminate the interaction among the SCN, photoperiod and pineal gland in the Mongolian gerbil.

Under constant light/dark cycle (LD 14:10) food restriction phase advanced the locomotor activity rhythm. According to these results we hypothesize that food restriction has a large effect on the regulation of locomotor activity rhythm in Mongolian gerbils.

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