Research Article

The Effects of Pinealectomy and Melatonin Implants on Circadian Locomotor Activity Responses of the Mongolian Gerbils Exposed to Rapid Photoperiodic Transitions

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Summary

The jet-lag syndrome and the shift-work malaise take place by rapid changes in photoperiod and disturb people's life frequently. In the present study; we wanted to simulate these conditions. Control, pinealectomized and melatonin including silastic tube implanted Mongolian gerbils were respectively exposed into long (14L:10D), short (8L:16D), constant darkness (0L:24D) and constant light (24L:0D) photoperiods in order to investigate the effects of photoperiod, pineal gland and melatonin hormone in the photoperiodic reentrainment of this species. Gerbils showed an light/dark cycle entrained locomotor activity rhythm in long and short photoperiods, free-running in constant darkness and arrhythmic locomotor activity in constant light. The period lengths were similar to each other in the groups (p>0,05). Gerbils were reentrained by the changes in photoperiod nevertheless pinealectomy and melatonin implants did not make any significant influence on this reentrainment (p>0,05). Locomotor activity amounts varied in a group-photoperiod-dependent manner. These data suggest that photoperiod but not the pineal gland and constant release melatonin hormone is effective on the activity rhythm of the Mongolian gerbil.

Key words: Jet-lag, Shift-work malaise, Photoperiod, Pineal gland, Melatonin, Mongolian gerbil

Özet

Jet-lag sendromu veCARDIYALI çalışma rahatsızlığı fotoperiyodaki hızlı değişimlerden kaynaklanır ve sıkılkla insanların hayatını olumsuz etkilemektedirler. Bu çalışmada jet-lag veCARDIYALI çalışma simüle edilmek istendi. MoGOLİSTAN gerbillerinin fotoperiyod değişimlerine karşı gösterdiği yeni ritim düzenlenmesinde fotoperiyod, pineal bez ve melatonin hormonunun etkilerini araştırılmak üzere, kontrol, pinealetktomi ve melatonin implantları içeren gerbil grupları sırası ile uzun (14L:10D), kısa (8L:16D), sürekli karanlık (0L:24D) ve sürekli aydınlık (24L:0D) fotoperiyodlara yerleştirildi. Gerbiller uzun ve kısa fotoperiyodlarda ihashed/karanlık sıkılkusu ile düzenlenen bir aktivite ritmi gösterırken, sürekli karanlıkta serbest koшу ve sürekli aydınlıkta ise arıtmık bir aktivite vardı. Grupların periyod uzunlukları arasında anlamli bir farklılık yoktu (p>0,05). Gerbillerin aktivitesi fotoperiyod değişiklikleri ile yeniden düzenlenince, pinealetktomi ve melatonin implantları ise bu düzenleme üzerine anlamli bir etki yapmadı (p>0,05). Lokomotor aktivite miktarları fotoperiyod ve gruba bağlı olarak değişimler gösterdi. Bu çalışmanın sonuçları MoGOLİSTAN gerbillerinde lokomotor...
activite ritminin düzenlenmesinde fotoperiyodun etkili ancak pineal bez ve sürekli salınım yapan melatonin implantlarının ise etkili olmadığını gösterdi.

**Anahtar Kelimeler:** Jet-lag, Vardiyal çalışma rahatsızlığı, Fotoperiyod, Pineal bez, Melatonin, Moğolistan gerbil'i

**INTRODUCTION**

In mammals, endogeneous circadian rhythm of some physiological parameters (e.g., locomotor activity, body temperature, reproductive responses to photoperiod and release of some hormones) are generated and controlled by a circadian oscillator, located in the hypothalamic suprachiasmatic nuclei (SCN). These rhythms are synchronized by some zeitgebers such as light/dark cycle, temperature, food availability and hormones.\(^{(28,4,23)}\)

The jet-lag is a malaise often associated with transmeridian airplane travel. Some of the symptoms often reported are fatigue, irritability and inability to concentrate during the day, difficulty sleeping at night and gastrointestinal discomfort.\(^{(32,14)}\) The element of jet-lag that causes most distress, therefore, seems to be the rapid crossing of time zones. Shift work refers to work performed in shifts. Shift work often produces a malaise characterized by both sleeplessness and inability to concentrate in work hours.\(^{(25)}\) Complaints of gastrointestinal disturbances are also common.\(^{(1)}\)

Pineal gland is a small pine-shaped structure located in the epithalamus of the brain and melatonin is the main hormone of the pineal gland that informs the body about the enivromental light and darkness regimen.\(^{(15)}\) The production of melatonin is showing a diurnal fashion; with highest concentrations during the dark phase of the day and this rhythm is similar in all animals whether they are nocturnal or diurnal. The removal of the pineal gland (pinealectomy) abolishes the rhythmic endogeneous melatonin release and decreases plasma levels of melatonin.\(^{(8)}\) Pineal melatonin is important in the mammalian circadian organization. Although, pinealectomy did not change the rat locomotor activity rhythm in both free-running and light-dark cycle conditions\(^{(24)}\), several studies have shown the influence of melatonin on the regulation of the circadian rhythms in mammals.\(^{(16,18,19,31)}\)

Mongolian gerbil has been preferred as an animal model in the studies of reproduction\(^{(2)}\), pharmacology\(^{(30)}\) and brain research\(^{(29)}\) previously. Different types of activity patterns have been demonstrated in this species.\(^{(33,17,26,27,21,22,7,34,35,36,37)}\) Furthermore, we examined the photoperiod\(^{(6,10)}\), pineal gland\(^{(11)}\), food\(^{(13)}\) and environmental temperature\(^{(5)}\) dependent reproductive activity rhythm characteristics of the Mongolian gerbils. Food restriction causes phase advances in the locomotor activity of the Mongolian gerbils.\(^{(12)}\) Taken together that, pineal gland is an important component of the both reproductive and the locomotor activity cycle of the Mongolian gerbil.

The circadian rhythm of the locomotor activity is entrained mainly by the environmental photoperiod. In the present study, in order to understand the roles of photoperiod, pineal gland and melatonin hormone in the photoperiodic reentrainment, we examined the running-wheel activity of the Mongolian gerbils exposed to rapid changes in photoperiod in a short period of time.

**MATERIAL AND METHODS**

Adult male Mongolian gerbils (Meriones unguiculatus)\(^{(20)}\) (~80 g, 120 days of age) were obtained from our laboratory colony maintained at the Abant Izzet Baysal University. They were exposed from birth to 14L (14 hour of light, 10 hour of darkness, lights off at 2000 hr). Animals
were maintained in plastic cages (16x31x42 cm) with pine shavings used as bedding. Food pellets and tap water were accessible ad libitum. The procedures in this study were carried out in accordance with the Animal Scientific procedure and approved by the Institutional Animal care and Use Committee. All lighting was provided by cool-white fluorescent tubes controlled by automatic programmable timers. Ambient temperatures in the animal facilities were held constant at 22 ± 2 °C in air-ventilated rooms.

1 week after pinealectomy and melatonin implantation, we started to record the running-wheel activity in 14L:10D (lights off at 2000 hr) as a long photoperiod. Then, animals were respectively exposed to short (8L:16D) (lights off at 1400 hr), constant darkness (0L:24D) and constant light (24L:0D) photoperiods in 2 week intervals. The implants were renewed every 2 weeks.

Before surgery, gerbils were anesthetized subcutaneously with Ketamine (20 mg/kg BW, Sigma Chemical Company, MO, USA) and intraperitoneally with pentobarbital (32.5 mg/kg BW). Depth of anesthesia was monitored by frequent testing for the presence of leg flexion reflexes and active muscle tonus. After awaking from anesthesia, the animals were placed in the cages and two days later their locomotor activities were recorded.

Pinealectomy of gerbils was performed according to the method of Hoffmann and Reiter(8); aspiration was used to control the hemorraging. Anesthetized gerbils were placed in a stereotaxic apparatus to stabilize the head during surgery. After the head was shaved the surgical area was sterilized with 70% ethanol, an incision was made in the scalp. Muscle attachments were removed from the dorsal skull. After drying the skull, an incomplete circular cut was made with a dental drill burr at the λ (lambda) suture and a piece of cranium covering the pineal gland was folded forward anteriorly. Fine-tipped forceps was used to extend into the confluence of the sinuses to grasp and remove the pineal gland. After removal of the pineal gland, the bone flap was replaced and a small square of absorbable gelatin sponge (Gelfoam, Up John, Kalamazoo, MI) was applied to the skull surface to help promote clotting. The scalp was closed with stainless steel surgical clips. After surgery, the incision was treated with Newskin adhesive to prevent any contamination. At the end of the experiment, pinealectomized animals were decapitated and checked for the security of the pinealectomy.

Implants were prepared according to the methods of Horton et al.,(9). Crystaline melatonin (sigma) was dissolved in melted beeswax (1 mg melatonin/25 mg beeswax). Then the mixture was aspirated into 15 cm lengths of PE 320 tubing (2.69 mm i.d. x 3.5 mm o.d.; intramedic, clay Adams, Porsipony, NJ). When the beeswax had cooled to room temperature and hardened, the tubing was cut into 10 mm capsules. Control capsules were prepared in a similar way but filled with beeswax only. Implants were inserted s.c. in animals under ketamine and pentobarbital anesthesia through a small dorsal skin incision at a shaved area on the back. The wound was closed by a steel wound clip. The implants were changed with the new ones every two weeks.

Before starting to experiment, throughout a ten day period animals kept under lighting conditions of 14:10 LD cycle. Thirty gerbils which showed a distinct circadian rhythm of locomotor activity were selected for the experiment. Locomotor activity was measured for running-wheel activity under a constant temperature (22 ±2 °C) in three groups, control (sham operated) (n=10), pinx (n=10) and melatonin implanted (n=10). Animals respectively exposed to long (LD 14:10), short (LD 8:16), constant darkness (LD 0:24) and constant light (LD 24:0) photoperiods for two weeks in each. The number of wheel revolutions per ten minute interval was automatically recorded.
and stored on a hard disc by a computer. The stored results were analyzed by the program Vital View Data Acquisition Software (Mini Mitter Company, Inc. Bend, OR USA). The activity was shown by the double plotted actograms by the program Acti View Software (Mini Mitter Company, Inc. Bend, OR USA). The analysis of circadian locomotor activity rhythm was performed by the combination of visual inspection and computarization of actograms and frequency histograms. Circadian period was determined by chi-square periodogram analysis. Phase shifts were defined as the difference between the predicted and observed onset times on the day following the change in photoperiod. The predicted onset was determined from an eye-fit line drawn through the last 5 activity onsets prior to the change. This line was extended to the first post day of change to give the predicted onset for that day. The observed onset time was determined from an eye-fit line drawn through the first onsets following the change. Lines were drawn by experienced observers who were blind to the experimental groups.

Data (wheel revolutions which transformed to Microsoft Excel) were analyzed using SPSS (SPSS Statistical Software, SPSS Inc., Los Angeles, CA, USA, Ver. 11.0). Data were examined by one-way analysis of variance (ANOVA). Differences between groups within a treatment type were determined by t tests; values were considered statistically significant at p<0.05. Data are presented as MEAN ± SEM after back transforming from ANOVA results.

RESULTS
There was a significant difference among the average wheel turns of all groups in 14L (p<0.05). Animals started their activity at CT 12 hour when the lights turned off. The locomotor activity level of pinealectomized (9.29 ± 1.5 mean wheel turn (mwt)/day and melatonin implanted (36.50 ± 4.6 mwt/day) animals were significantly different from the controls (21.07 ± 3.1 mwt/day) (p<0.05) (Fig. 2). The period lengths were not statistically different among the groups under this photoperiod. (23.45 ± 0.12 h in control; 24.01 ± 0.2 h pinealectomized; 24.09 ± 0.10 h melatonin implanted) (Fig. 3).

In 8L, the activity onset was phase advanced about 6 hours in all groups. Wheel turns [controls (21.04 ± 3.0 mwt/day), pinealectomized (23.56 ± 3.3 mwt/day), and melatonin implanted (21.54 ± 3.2 mwt/day)] (Fig. 2) and the period lengths [controls (24.05 ± 0.11 h), pinealectomized (23.56 ± 0.20 h), and melatonin implanted (23.50 ± 0.14 h)] of the groups were similar to each other under this photoperiod (Fig. 3).

When the lighting regime was changed from 8L to 0L, a free running period of the circadian activity occurred in all groups. The locomotor activity level decreased in controls (16.82 ± 2.3 mwt/day), melatonin implanted (10.91 ± 1.6 mwt/day) and pinealectomized (3.55 ± 0.7 mwt/day) groups under 0L conditions (Fig. 2). The period lengths were similar in all groups under this photoperiod (23.50 ± 0.21 h in control; 24.10 ± 0.18 h pinealectomized; 23.55 ± 0.17 h melatonin implanted) (Fig. 3).

In 24L, circadian activity rhythm of the gerbils was in an arrhythmic manner. The locomotor activity levels were suppressed in 24L (p<0.05). Activity levels decreased to 10.56 ± 1.5 wt in controls, 3.65 ± 0.7 wt in pinealectomized and 4.23 ± 0.8 wt in melatonin implanted groups (Fig. 2).
Figure 1: Figure 1 represents the combined single plot actograms of the control, pinealectomized and melatonin implanted gerbils which were respectively exposed to long (14L), short (8L), constant darkness (0L) and constant light (24L) photoperiods for two weeks in each. Dark rectangle indicate the period of dark period and light rectangle indicate the light period. Dashed lines indicate the onset of the activity. Pnx is pinealectomy and mel-imp is melatonin implantation.

Figure 2: Figure 2 represents the daily mean locomotor activity amounts of the control, pinealectomized and melatonin implanted gerbils which were respectively exposed to long (14L), short (8L), constant darkness (0L) and constant light (24L) photoperiods for two weeks in each. Open bar represents the controls, right striated bar represents the pinealectomy and left striated bar represents the melatonin implantation. Different letters indicate the statistically significant difference among the groups.
DISCUSSION
The present study demonstrated for the first time that removal of the pineal gland or melatonin implantation did not disrupt the synchronization to the LD cycle of daily activity rhythms in Mongolian gerbils. The results show that there were significant differences in basal amount of daily locomotor activity among the groups under four different photoperiods. Melatonin-implanted gerbils were found to be more active than pinealectomized and control groups in 14L.

Our results is in good agreement with the fact that pinealectomy accelerated locomotor activity phase shift in response to photoperiodic change in hamster and rats.\(^{24,3}\) Moreover, the present study strongly supports the idea that the endogenous melatonin is actively involved in the regulation of circadian locomotor activity to the day light cycle in gerbils. Gerbils entrained to the LD cycles and showed the pattern of nocturnal animals as stated previously.\(^{35}\) Under constant darkness (0L), gerbils showed free-running rhythms.

Wheel-running activity was enhanced by the time of day. The light period corresponds to the time at which spontaneous locomotor activity is low, reflecting the circadian rhythm. A significantly higher response to the wheel was observed during the dark. Pinealectomy and melatonin implants decreased the wheel activity during the light and the dark periods in all gerbils. The tendency toward higher activity in gerbils in all photoperiods is the reflection of the intrinsic rhythmicity.

The present finding showing diurnal variations after melatonin implants suggest phase shifting of the activity rhythm of gerbils in constant darkness. On the other hand, from our results, we may not conclude whether entrainment to exogenous melatonin represent a physiological event. The possibility that the decrease in activity was induced by pinealectomy or melatonin implants is unlikely to be due to phase shifts.

Exogenous melatonin administration and implants did not have significant effects on
the circadian systems of gerbils. Melatonin caused either arrhythmicity or changed in the period of the free-running activity. Gerbils implanted with capsules containing melatonin show motor activity in 24L and biggest motor activity was in 14L. The levels of activity were similar in other photoperiods. These results show that continuous exposure to melatonin decreases the amplitude of the oscillator in long photoperiods comprising the circadian system rendering the oscillator more sensitive to the phase-shifting effects of light. Our recent experiment has shown that melatonin can also entrain the circadian system of gerbils. Daily injections of melatonin can entrain the activity rhythms of pinealectomized gerbils (unpublished data). The entraining effect of melatonin injections of pinealectomized gerbils may have due to direct inhibition of locomotor activity, rather than an effect on the circadian clock. However, it is still possible that the lack of entrainment of the free-running rhythm by the melatonin implants in intact gerbils may have been due to maintenance of the endogenous melatonin rhythm from the pineal gland. In rats, it was reported that the entraining effect was not distinguishable between intact and pinealectomized animals. However, it appears that melatonin does not entrain locomotor activity in the gerbils (Fig.1). These findings suggest that the effect of melatonin implants and pinealectomy differs among rodents.

However, when maintained under free-running conditions, produced by exposure to 24L, the circadian rhythm of the activity does not display considerable variability. On the other hand, wheel-turning numbers display considerable variability compared to other photoperiods. The alterations in the wheel turning numbers and phase of the activity rhythms may be related to the involvement of more than one circadian oscillator in the timing of the activity.

The cellular mechanism underlying the different wheel turning numbers of pinealectomy or melatonin implants in gerbils is unknown. Whether, for example, melatonin's effect on the SCN is mediated by activation of melatonin receptors remains unknown. In this species, one may not exclude a different and compensatory signal transduction mechanism for melatonin's circadian effects. For example, several non-photic cues (for example, leptin) modify clock phase by inducing locomotor activity. However, we did not observe that entrainment to melatonin.

The running-wheel cage is widely used to analyze the circadian activity rhythm. The wheel activity in relation to pinealectomy or melatonin implants in gerbils were not reported. Additional studies are warranted to determine the mechanism underlying these variations in running wheel activity by pinealectomy and/or melatonin implants. The underlying mechanisms presumably are located peripheral to the intrinsic timing system within the circadian pacemaker.

In conclusion, the results of the present study is giving important hints for understanding the whole physiological and behavioral activity properties of this species and support the hypothesis that the photoperiod is the most important component of the activity rhythm regulation of the Mongolian gerbils.

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