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Melatonin Implants Have No Effect on the Testis Volume and Body Weight in Mongolian Gerbils

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Abstract: Photoperiod is an important environmental cue in the regulation of reproductive activity in Mongolian gerbils. Photoperiodic information is ultimately transduced into a hormonal signal by the pineal gland. We examined the effect of constant-release melatonin capsules on the testicular size and body weights of male Mongolian gerbils exposed to a long (14L: 10D) photoperiod. Three groups were formed: control (n = 10), intact + melatonin implant (n = 10) and pinealectomy + melatonin implant (n = 10). The testes and body weights of the gerbils were recorded every week for 8 weeks. Body weights (76 ± 2.0 g intact + melatonin implant; 79 ± 3.0 g pinealectomy + melatonin implant) and testes weights (1.54 ± 0.21 g intact + melatonin implant; 1.47 ± 0.19 g pinealectomy + melatonin implant) of intact + melatonin implanted and pinealectomy + melatonin implanted groups were not statistically different from those of the control group (80 ± 3.1 g body weight; 1.55 ± 0.20 g testes weight) ($P > 0.05$) at the end of the study. Regardless of treatment, the animals fully reflected the characteristics of a long photoperiod. These results indicate that masking the endogenous melatonin rhythm via constant-release melatonin implants has no effect on the testis volume or body weight of Mongolian gerbils when the animals are kept in a long photoperiod.

Key Words: Implantation, Melatonin, Pinealectomy, Gerbil

Mongolian Gerbillerde Melatonin İmplantasyonlarının Testis Hacmi ve Vücut Ağırlıkları Üzerine Bir Etkisi Yoktur

Özet: Mongolian gerbillerin üreme aktivitelerinin düzenlenmesinde fotoperiyot önemli bir faktördür. Fotoperiyodik bilgi en son pineal bez tarafından hormonal sinyale çevrilir. Bu çalışmada uzun fotoperiyotta (14L: 10D) tutulan Mongolian gerbillerin testis boyutlarına ve vücut ağırlıklarına, sürekli salınım gösteren melatonin hormonunun etkisini inceledik. Üç grup oluşturuldu; kontrol (n = 10), intakt + melatonin implantasyonu (n = 10) ve pinealektomi + melatonin implantasyonu (n = 10). Sekiz hafta süresince gerbillerin testis ve vücut ağırlık değişimleri haftada bir kaydedildi. Deney sonunda intakt + melatonin implantasyonu ve pinealektomi + melatonin implantasyonu gruplarının vücut ağırlığı ($76 \pm 2,0$ g İntakt + melatonin implantasyonu; $79 \pm 3,0$ g pinealektomi + melatonin implantasyonu) ve testis ağırlıkları ($1,54 \pm 0,21$ g İntakt + melatonin implantasyonu; $1,47 \pm 0,19$ g pinealektomi + melatonin implantasyonu) ile kontrol grubu ($80 \pm 3,1$ g vücut ağırlığı; $1,55 \pm 0,20$ g testis ağırlığı; $P > 0,05$) arasında anlamlı bir farklılık tespit edilmedi. Uygulamalara bakmaksızın, hayvanlar uzun fotoperiyodun özelliklerini gösterdiler. Bu sonuçlar göstermektedir ki sürekli salınan melatonin implantasyonları ile maskelenen endojen melatonin ritmi, hayvanlar uzun fotoperiyotta tutuldukları zaman testis hacmi ve vücut ağırlığı üzerinde bir etki göstermemektedir.

Anahtar Sözcükler: İmplantasyon, Melatonin, Pinealektomi, Gerbil

Introduction

Animals exposed to long or short photoperiods show a number of physiological and morphological changes that presumably increase the animal's probability of overwintering, such as a molt to a winter pelage, a change in body mass and the ability to enter torpor. Many animals use daylength to anticipate seasonal transitions. Daylength information is transduced into a hormonal signal by the pineal gland. The pineal hormone melatonin is synthesized and released rhythmically and the rhythmic

changes in melatonin regulate the reproductive activity of these animals. The reproductive activity of Mongolian gerbils (*Meriones unguiculatus*) (1) (lat 44° N, long 116° E, East Mongolia) is also under control of ambient photoperiod (2-5). Long days (>8L) stimulate but short days (<8L) inhibit the reproduction of gerbils (6,7). In different gerbil species (*Tetera indica cuvieri*, *Meriones hurriane*) constant light (24L) has an inhibitory effect but constant darkness (0L) has a stimulatory effect on the reproductive activities of these animals (8,9).

The response of animals to melatonin presented in a nonrhythmic, chronic fashion is variable and depends on the species tested and the dose used. Several species have been examined for their response to treatment with constant-release, subcutaneous implants of melatonin. This treatment produces relatively high concentrations of circulating hormone sustained at a constant level throughout the day, and presumably interferes with the perception of the endogenous melatonin rhythm (10,11).

A number of studies have examined the effect of melatonin implants on animals. Melatonin implants have no effect on physiological parameters in Siberian hamsters maintained in constant conditions (12). The gonadal response of long-day-housed Syrian hamsters to Silastic melatonin implants is variable; whereas one study reported a dose-dependent inhibition (13), another observed no effect (14). In sheep maintained in long photoperiod, melatonin implants result in a short-day-like response (15,16).

The effects of melatonin implant treatment have also been studied in animals subjected to a change in photoperiod. In both Siberian hamsters and sheep, constant-release implants block the response to photoperiod transitions (12,17,18). Although melatonin-implanted Syrian hamsters fail to respond to a decrease in photoperiod, they do respond to an increase in photoperiod (19). In one experiment in Mongolian gerbils, melatonin implants caused a regression in the accessory sex organs but not in the testes (20). Taken together, these data suggest that constant-release melatonin implants interfere with the animal's ability to accurately transduce daylength information into physiological responses. The species-specific nature of the response to melatonin implants may reflect inherent differences in the neuroendocrine condition in the absence of a perceived daily melatonin rhythm.

Responses to melatonin implantations can change after pinealectomy. In pinealectomized golden hamsters, smaller beeswax implants generally do not affect gonadal size on long days (14). In pinealectomized Siberian hamsters, the effect of melatonin implantation depends on the preoperative photoperiod (21). The effects of melatonin implants have not been reported in pinealectomized Mongolian gerbils.

Some species show a significant decrease in body mass in winter or the dry season, whereas others demonstrate the opposite trend (22,23). These changes

in body mass can be explained directly by the activity time length or their adaptive strategy to changes in environmental conditions. In Mongolian gerbils body mass is independent of ambient photoperiod (7).

To determine the importance of a daily melatonin rhythm in conveying photoperiod information, we masked the endogenous melatonin rhythm with constant-release melatonin implants and monitored the animals' ability to respond to a long photoperiod.

Materials and Methods

Animal care: Mongolian gerbils (*Meriones unguiculatus*) were obtained from our laboratory colony maintained at Abant İzzet Baysal University. They were exposed from birth to 14L (14 h of light, 10 h of darkness, lights off at 2000 h). Animals were maintained in plastic cages (16 x 31 x 42 cm) with pine shavings as bedding. Food pellets (Purina rodent chow) and tap water were accessible ad libitum. Principles of laboratory animal care and specific national laws were followed. All lighting was provided by cool-white fluorescent tubes controlled by automatic programmable timers. Ambient temperatures in the animal facilities were held constant at 20 ± 1 °C in air-ventilated rooms.

Experimental Design: Male Mongolian gerbils were housed 2 per cage. The gerbils were divided into 3 groups. A control group was left intact. The second group received melatonin implants (1 mg in 24 mg of beeswax) every 2 weeks for 8 weeks. The last group was pinealectomized and received melatonin implants. Each group contained 10 animals. Body weights and testes weights were recorded every week throughout the experiment.

Anesthesia: Before surgery, the 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.

Surgical Procedures

Pinealectomy: Pinealectomy of adult gerbils was performed according to the method of Hoffmann and Reiter (24); aspiration was used to control the hemorrhaging (25).

Preparation of the melatonin implants: Implants were prepared according to the methods of Horton et al. (11). Crystalline 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, Parsipony, NJ, USA). 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.

Implantation of the melatonin implants: 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.

Testes Measurements: The length and width were measured with calipers to the nearest 0.1 mm by external palpation of the left testis in the scrotum. In most cases, only one of the testes was measured, alternating sites on successive animals. However, if the testes were suspected of being disparate in size (as assessed by gentle palpation) both were measured. Paired testicular volume was calculated from this measurement using the formula for a prolate spheroid (26):

$$\text{Volume} = 0.5236 (\text{length}) \times (\text{width})$$

This single testis volume (STV) was then converted to paired testes weight (PTW) using a pre-determined linear regression formula:

$$\text{PTW} = 1.846 (\text{STV}) - 0.015$$

All data from in situ testicular measurements are reported in the form of paired testes weights derived by this method.

Statistics: Data were analyzed using SPSS (SPSS Statistical Software, SPSS Inc., Los Angeles, CA, USA, Ver. 10.0). Data were examined by one-way or two-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 \pm SEM after back transforming from ANOVA results.

Results

Melatonin implants did not significantly affect ($P > 0.05$) body weights of any group during the course of the

experiment (Figure 1). The initial and final body weights of these 3 groups were similar to each other (Table 1).

Melatonin implants did not affect the testes weights of Mongolian gerbils in any group ($P > 0.05$) (Figure 2). Testes weights were not different when compared after 1 and the 8 weeks of the experiment in the control, intact + melatonin implanted and pinealectomy + melatonin implanted groups (Table 2).

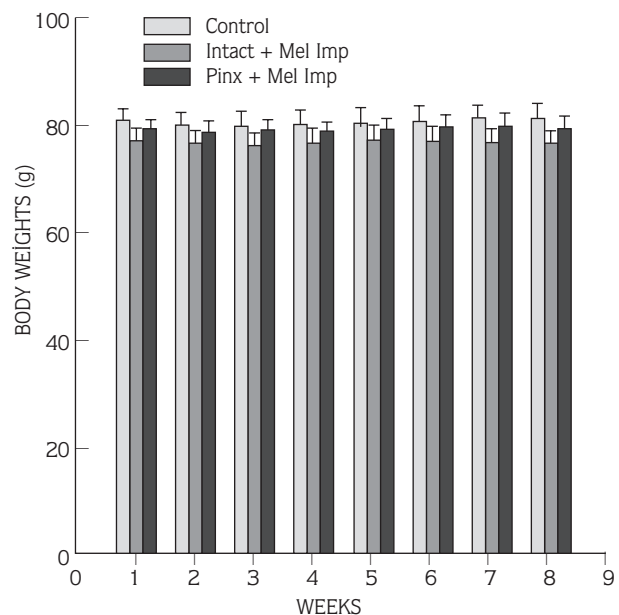


Figure 1. Body weights of Mongolian gerbils in a 14L photoperiod for 8 weeks. Open bar represents controls, right striated bar represents intact + melatonin implants and left striated bar represents pinealectomized + melatonin implants. Data are given as mean \pm SEM. n: 10 animal/group Pinx: Pinealectomy, Mel Imp: Melatonin implants

Table 1. Body weights of control, intact + melatonin implants and pinealectomy + melatonin implants groups of Mongolian gerbils in 14L for 8 weeks. Data are given as mean \pm SEM. Pinx: Pinealectomy, Melatonin Imp: Melatonin implants

Weeks	Control Body Weights (g) Mean \pm SEM	Melatonin Imp Body Weights (g) Mean \pm SEM	Pinx + Melatonin Imp Body Weights (g) Mean \pm SEM
1-	80.700 \pm 2.500	77.300 \pm 2.400	79.500 \pm 2.000
2-	80.200 \pm 2.500	76.800 \pm 2.500	78.900 \pm 2.300
3-	79.800 \pm 2.800	76.500 \pm 2.400	79.300 \pm 2.300
4-	80.300 \pm 2.800	76.800 \pm 3.100	79.000 \pm 2.200
5-	80.600 \pm 3.000	77.400 \pm 3.000	79.400 \pm 2.200
6-	80.900 \pm 3.200	77.200 \pm 3.000	79.800 \pm 2.500
7-	81.400 \pm 2.800	77.100 \pm 2.600	80.000 \pm 2.500
8-	81.500 \pm 3.000	77.000 \pm 2.600	79.900 \pm 2.300

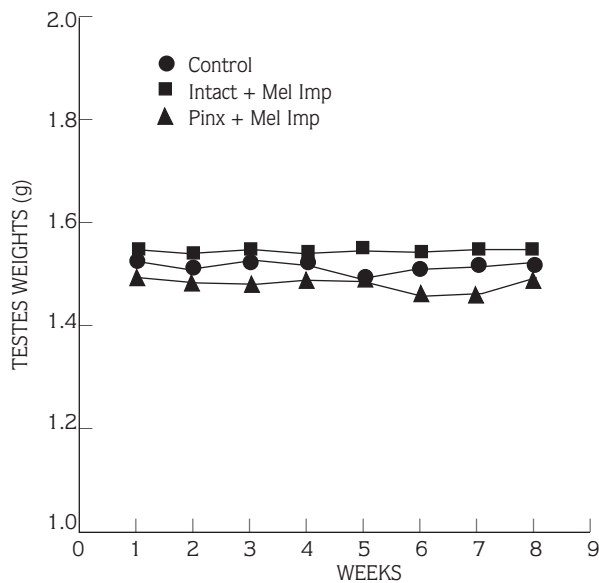


Figure 2. Testes weights of Mongolian gerbils in a 14L photoperiod for 8 weeks. Circle represents controls, square represents intact + melatonin Implants and triangle represents pinealectomized + melatonin Implants groups. Data are given as mean \pm SEM. n: 10 animal/group Pinx: Pinealectomy, Mel Imp: Melatonin implants

Table 2. Testes weights of control, intact + melatonin implants and pinealectomy + melatonin implants groups of Mongolian gerbils in 14L for 8 weeks. Data are given as mean \pm SEM. Pinx: Pinealectomy, Melatonin Imp: Melatonin implants

Weeks	Control Body Weights (g) Mean \pm SEM	Melatonin Imp Body Weights (g) Mean \pm SEM	Pinx + Melatonin Imp Body Weights (g) Mean \pm SEM
1-	1.524 \pm 0.010	1.545 \pm 0.012	1.500 \pm 0.015
2-	1.509 \pm 0.010	1.539 \pm 0.012	1.493 \pm 0.013
3-	1.530 \pm 0.011	1.550 \pm 0.014	1.486 \pm 0.013
4-	1.523 \pm 0.012	1.543 \pm 0.015	1.495 \pm 0.013
5-	1.499 \pm 0.010	1.551 \pm 0.011	1.490 \pm 0.013
6-	1.513 \pm 0.010	1.542 \pm 0.014	1.465 \pm 0.014
7-	1.517 \pm 0.011	1.550 \pm 0.015	1.469 \pm 0.014
8-	1.523 \pm 0.010	1.552 \pm 0.015	1.495 \pm 0.015

Discussion

The present experiment showed that melatonin implants have no effect on the testis volume and body weight of Mongolian gerbils maintained in a long photoperiod. Gerbils given constant-release melatonin implants did not differ from animals given empty implants over the course of the 8-week treatment period.

This suggests that the perception of the endogenous melatonin rhythm may not be required for the animals to respond to a photoperiod. When the endogenous melatonin rhythm was removed by pinealectomy or masked by the sustained release of exogenous melatonin from the implants, the animals remained in the physiological condition characteristic of the photoperiod to which they were exposed prior to implantation.

Conversely, body weight response to photoperiod was not prevented by melatonin implants. In fact, for animals born in long days, melatonin treatment had no statistically significant effect on body weight. These data indicate that the body weight response to photoperiod and melatonin implants is somewhat independent of the perception of an endogenous rhythm.

Several explanations can be offered for the melatonin-independent body weight response. Photic information is transmitted to the suprachiasmatic nuclei (SCN) and then impulses travel to the paraventricular nuclei and the superior cervical ganglia before reaching the pineal gland. Implantation of a melatonin capsule does not interfere with the neural transmission of photoperiodic information as evidenced in melatonin-implanted female Siberian hamsters (11). Consequently, information regarding the ambient photoperiod could be available via mechanisms other than the melatonin rhythm.

Melatonin-independent transmission of photoperiodic information is likely to involve the SCN. Circadian rhythms of a variety of physiological processes including motor activity, body temperature and hormone concentrations are governed by an endogenous clock in the SCN (27). These endogenous rhythms are entrained by the daily light-dark cycle, and this entrainment may not be influenced by the presence of a melatonin implant.

Motor activity is one process that could easily influence body weight. If, for example, total daily activity is higher under a long as opposed to a short photoperiod, a relatively high rate of energy expenditure may contribute to the lower body weight observed in long-day animals. An effect of photoperiod on total daily activity may occur in melatonin-implanted animals as well as in controls (27).

If the regulation of body weight changes is not controlled by photoperiod or melatonin implants in Mongolian gerbils, it should be investigated what type of mechanism(s) controls this process. Food quantity and

quality may be other factors. When we consider the natural distribution area of Mongolian gerbils (steppes of Mongolia), the food factor is a strong possibility. Animals can find enough food in the wet season (short days, winter) and this causes a body weight increase but they cannot find enough food in the dry season (long days, summer) and body weight decreases. Experiments to test this possibility are ongoing in our laboratory.

Mongolian gerbils are a nocturnal species and the extensive geographical distribution of this genus suggests that the gerbil may be a valuable wild species for studying the environmental regulation of seasonal reproductive cyclicity. In the wild, Mongolian gerbils reproduce from February to September and their testes, like those of other seasonal breeders, undergo regression during autumn, the period of sexual inactivity (2). In our recent studies, we have shown the involvement of the pineal gland in the regulation of reproduction activity in Mongolian gerbils (6,7,28). However, we were unable to determine if the observed effects occur via melatonin or any other factors. It has been shown that the continuous release of melatonin by subcutaneous implants, without daily rhythmicity, induces gonadal regression or stimulation, depending on the species. The use of melatonin implants is controversial since the most striking characteristic of melatonin secretion is its rhythmicity. Syrian hamsters need a daily interval free of melatonin to allow exogenous melatonin signals to be measured (29). However, the continuous release of melatonin by subcutaneous capsules, which eliminates the daily rhythmic changes in serum melatonin, replicates the effect of short days on the pituitary-gonadal system in the mink (30,31) as it does in the fox (32). Similarly, in the ram, continuous melatonin administration replicates short day length effects (33). In adult golden hamsters, implants do not alter testis size in animals housed in long

days (13,34). In adult Siberian hamsters, the effect of melatonin implants is dependent on the photoperiod prior to implants. In adult Turkish hamsters, implants induce testicular regression in long days (35). Our observations on testis size in adult gerbils are similar to those in golden hamster studies. Regardless of the presence or absence of the pineal gland, subcutaneous melatonin implants did not affect testis size in adult gerbils. These observations suggest that the effect of pinealectomy and of exogenous melatonin are synergistic in animals exposed to a stimulatory photoperiod (16L).

Although the role of melatonin in reproductive physiology is reasonably established, its potential function via implants in sexual behavior has received only poor attention in Mongolian species. This is the first evidence that a constant release of melatonin via subcutaneous capsules failed to affect the sexual performance of the gerbils. It will be of interest to determine the extent to which the present conclusions apply when the actions of melatonin implants are tested in other photoperiodic conditions or when implants are given at different times of the annual reproductive cycle.

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