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# Reproductive Response of Adult Siberian Hamsters to One-Hour Melatonin Infusions

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**Abstract:** The pineal hormone melatonin is known to play an important role in mediating photoperiodic messages to the reproductive system in seasonal breeding animals. In this study, we investigated whether a 1 h subcutaneous infusion of 50 ng of melatonin at 2000-2100 hours, effective in causing inhibition of testicular development in the juvenile Siberian hamsters (*Phodopus sungorus*), would cause testicular regression in adults of this species housed on a long photoperiod (LD16:8). Pinealectomized adult hamsters received programmed subcutaneous infusions of 50 or 500 ng of melatonin or vehicle (0.12 ml) at 2000-2100 hours each day for 8 weeks. We found that the testicular weights of infused animals receiving melatonin or vehicle were not different from each other; all animals retained large testes. These results show that a 50 ng melatonin infusion at a particular time point (2000-2100 hours) is not effective in altering the functional status (size) of the testes of adults.

Key Words: Melatonin, Siberian hamster, Photoperiod, Testes, Infusion

## Bir-Saat Melatonin İnfüzyonlarına Yetişkin Sibirya Hamsterlerinin Üreme Yanıtı

**Özet:** Pineal bezi hormonu olan melatonin mevsimsel üreyen hayvanlarda fotoperiyodik mesajları üreme sistemine iletmede önemli bir role sahiptir. Bu çalışmada, yavru Sibirya hamsterlarında (*Phodopus sungorus*) etkili olduğunu bulduğumuz 50 ng melatoninin 2000-2100 saatleri arasında 1 saat infüze edildiğinde uzun fotoperiyotta (LD16:8) tutulan yetişkin erkek Sibirya hamsterlarında etkili olup olmadığı araştırıldı. Pineal bezleri alınmış (pinealektomi) yetişkin hamsterler 8 hafta süresince hergün saat 2000-2100 arası 50 ng ya da 500 ng dozlarında melatonin ya da kontrol solüsyonu (0.12 ml) ile infüze edildiler. Sonuçta melatonin ya da kontrol solüsyonu ile infüze edilen tüm grupların testis ağırlıklarında bir farklılık bulunmadı; tüm hayvanlar testis ağırlıklarını muhafaza ettiler. Bu sonuçlar infüze edilen 50 ng melatonin dozunun 2000-2100 saatleri arasında yetişkin hayvanların testislerinin fonksiyonel durumunu (boyutlarını) değiştirmede etkili olmadığını göstermektedir.

Anahtar Sözcükler: Melatonin, Sibirya hamsteri, Fotoperiyot, Testis, İnfüzyon

## Introduction

Like many temperate zone rodent species, Siberian hamsters (*Phodopus sungorus*) display marked physiological and behavioral adaptations in response to annual changes in the ambient photoperiod. While Siberian hamsters are reproductively active in long photoperiods, they are inactive in short photoperiods (less than 13-14 h of light per day) (1-3).

The pineal gland is primarily concerned with conveying the photoperiodic message to components of the neuroendocrine axis, irrespective of whether this message is stimulatory or inhibitory. The pineal gland functions as a neuroendocrine transducer, receiving photoperiodic input ultimately from the retina, and responding with melatonin output during the hours of darkness each day. Thus, long days (short nights) are represented by melatonin release over fewer hours each night than are short days (long nights) (1,4-7). The daily rhythm of melatonin production and release has very stable features in a light-dark cycle and has the same characteristics in nocturnal and diurnal mammals;

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melatonin levels in the pineal are low during the day, begin to increase shortly after lights off, reach peak levels at mid-darkness and then decrease in the late night to reach daytime levels shortly before light onset (8-10).

The nature of the endocrine message communicated via the melatonin rhythm has been the subject of much investigation. Two hypotheses have been proposed to explain how melatonin functions in the regulation of seasonal breeding (11). The duration hypothesis proposes that animals measure the length of the nightly melatonin message and that uninterrupted exposure to melatonin for a particular duration elicits a long-day response, while a longer duration of exposure elicits a short-day response (11,12). Evidence in support of this hypothesis has come from melatonin infusions in pinealectomized Siberian and Syrian hamsters (5,6,13,14).

The coincidence hypothesis proposes that induction (stimulation or inhibition of reproductive function, depending on the species) occurs when melatonin released from the pineal coincides in time with melatonin target organ(s) sensitivity. This hypothesis supposes that the time of presence of melatonin is important. Support for this hypothesis has come from experiments in intact adult Syrian hamsters (15), Turkish hamsters (16) and Siberian hamsters (17), pinealectomized adult Syrian hamsters (18-20) and intact and pinealectomized juvenile Siberian hamsters (21,22).

Pinealectomy (removal of the pineal gland and, hence, the source of melatonin) prevents an animal from responding to a change in photoperiod with the appropriate reproductive response. Results obtained from pinealectomized animals suggest that the pineal is involved in the transduction of photoperiodic effects, not only upon the neuroendocrine-gonadal axis, but also upon other or all functions regulated by photoperiod. Pinealectomy blocks short photoperiod induced gonadal regression of hamsters previously housed on long photoperiod (4) and prevents long photoperiod induced gonadal growth of hamsters previously housed on short photoperiods (23-25). Thus, the general effect of pinealectomy in the adult Siberian hamster is either to prevent the perception of a change in daylength or to maintain the daylength measurement system in the preoperative mode, i.e., the gonads are maintained in a functional state appropriate to the previous photoperiod (25,26).

When pinealectomized, long-day-housed adult Siberian hamsters receiving as few as 5 weeks of short-day melatonin infusions (12 h) exhibited short-day-type testicular regression and decreased body weight (27). To test the importance of the dose of melatonin for short-day-type responses, long-day-housed adult Siberian hamsters were pinealectomized and given short-day-type, long duration melatonin signals (10 h) at 1.56, 6.25, 25 or 100 ng melatonin infusions daily for 5 weeks. Hamsters receiving saline-vehicle only or 1.56 ng melatonin daily did not show reproductive inhibition (27).

The purpose of the research reported here was to attempt to test both the duration and coincidence hypotheses in adult male Siberian hamsters by evoking short-day-like responses to long duration melatonin infusions in one group of pinealectomized adult males, and by administering to a second set of groups a short (1 h) infusion at a time (2000-2100 hours) demonstrated in juveniles to actively retard testicular development and times demonstrated to be ineffective (i.e., times when juveniles were insensitive to melatonin).

## Materials and Methods

Adult male Siberian hamsters (*Phodopus sungorus*) approximately 3-6 months old and weighing 40-50 g were obtained from our breeding colony. 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. Hamsters were born and maintained on a 16:8 h light:dark cycle (lights on at 0400 hours). Hamsters received tap water and food ad libitum. Room temperature was maintained at 22 ± 1 °C and all lighting was provided by cool-white fluorescent tubes; light intensities at the animal's eye level exceeded 200 lux.

### Surgery

For surgery, hamsters were anesthetized with pentobarbitol (32.5 mg/per animal, i.p.) and with ketamine (20 mg/per animal, i.m.; Sigma Chemical Company, St. Louis, MO, USA). Depth of anesthesia was monitored by frequent testing for the presence of leg flexion reflexes and active muscle tonus.

### Melatonin Infusions

The method of cannulation and infusion was based on that described by Carter and Goldman (5,6). A simple

flow-thru swivel for infusion into unrestrained animals was designed according to the method of Brown et al. (28). In brief, adult animals were pinealectomized, with minor modifications, according to the methods of Hoffman and Reiter (29) and fitted with a subcutaneous catheter under the dorsal skin of the back for the infusion of melatonin. The infusion flow rate was 0.12 ml/h. Syringes were refilled every day just before the infusion started. The infusion apparatuses, including the syringes, pump and polyethylene tubes up to flow-through swivels, were covered with aluminum foil as a precautionary measure against light exposure and subsequent degradation of melatonin. Cannulas were checked at least twice daily and replaced when necessary.

### Melatonin Solutions

Melatonin solutions were made by dissolving crystalline melatonin in 100% ethanol and diluting this mixture in sterile saline (0.9% NaCl) to the desired concentrations. Stock solutions were kept at 4 °C prior to use. Fresh working melatonin solutions were made at room temperature by diluting the stock with sterile saline to the desired concentrations. Vehicle solutions were made in the ratio of one part absolute ethanol to 1000 parts sterile saline. Each animal was infused with vehicle or melatonin in vehicle at a volume of 0.12 ml/h. Melatonin concentrations used were 50 and 500 ng per hour.

### Testis Measurement

Testicular volume was determined from measurement of the length and width of one testis through the scrotum with calipers to the nearest 0.1 mm under light ketamine and pentobarbital anesthesia. Single testicular volume was calculated from this measurement using the formula for a prolate spheroid (30)

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

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

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

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

### Experiment

Hamsters were divided into five groups. The first group served as an intact control (n = 10). Hamsters in the second group (n = 10) were pinealectomized at the beginning of the experiment and housed individually. Hamsters in the third group (n = 80) were pinealectomized and implanted with subcutaneous cannulas for the infusion of melatonin or vehicle solutions at the beginning of the experiment (time = 0). Hamsters were housed individually during the experimental period. Each animal received a daily 1 h (50 ng/h) melatonin or vehicle infusion at one of four different time points (1900-2000, 2000-2100, 2400-0100 or 0300-0400 hours). Infusions continued for 8 weeks. Animals in the fourth group (n = 20) were pinealectomized and received 8 h (50 ng/h) melatonin or vehicle infusions at 2000-0400 hours. The last (fifth) group of animals (n = 40) was pinealectomized and received 1 h 500 ng melatonin or vehicle infusions at 2000-2100 hours or 0300-0400 hours. At the end of the experiment the animals were sacrificed by decapitation and the last measurement of testes was taken by weighing the testes.

### Statistics

Data were analyzed using SAS (Statistical Inst., version, 6.07). Because the data were not always normally distributed in each group of the experiment, they were log-transformed prior to statistical analysis to make the sample variances homogenous. Data were examined by one-way or two-way analysis of variance (ANOVA) for the effect of dose, time and all interactions. Differences between groups within a treatment type were determined with a least-squares means test; means were considered significantly different if $p < 0.05$. Data are presented as MEAN ± SEM after back-transforming from ANOVA results.

## Results

The testicular response of intact and pinealectomized adult Siberian hamsters to long photoperiods is well known and has been described in many studies. The animals in this study demonstrated a similar response; hamsters with large testes remained reproductively active during the course of the 8 week experiment regardless of the presence or absence of the pineal gland (Fig. A).

The testicular weights of animals receiving either 50 ng/h melatonin or vehicle solutions at all time points were not different from each other. All animals retained large testes (Fig. B). In contrast, animals receiving a daily infusion of melatonin (50 ng/h) for 8 h during the

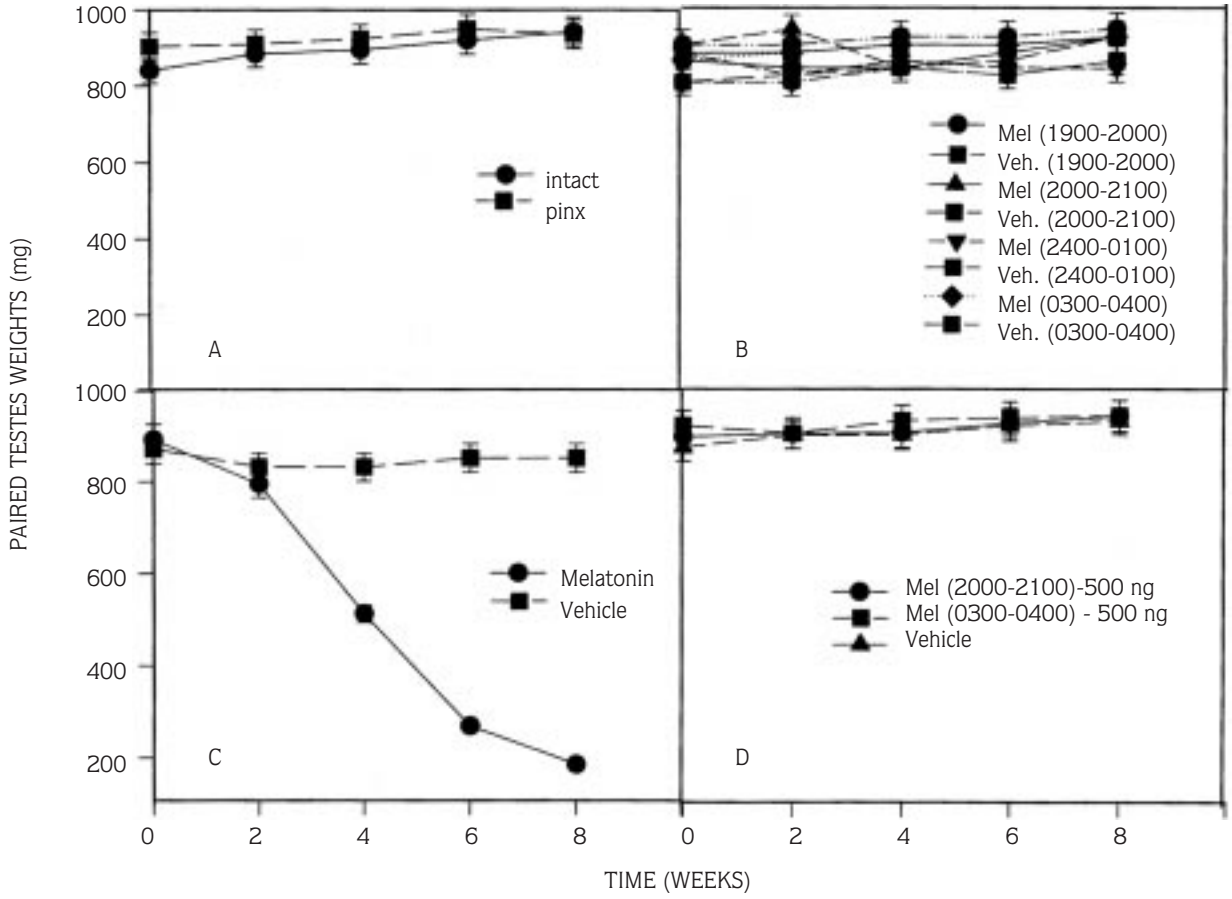


Figure. **A)** represents paired testes weights (mg) of intact and pinealectomized (pinx) hamsters housed on 16 h of light. **B)** represents paired testes weights (mg) of pinealectomized hamsters housed on 16 h of light and infused with either 50 ng/h melatonin (Mel) or vehicle (Veh.) solutions at four different time points (1900-2000, 2000-2100, 2400-0100 or 0300-0400 hours). **C)** represents paired testes weights (mg) of pinealectomized hamsters housed on 16 h of light and infused 8 h with either 50 ng/hr melatonin or vehicle solutions at 2000-0400 hours. **D)** represents paired testes weights (mg) of pinealectomized hamsters housed on 16 h of light and infused 1 h with 500 ng/hr melatonin or vehicle solutions at 2000-2100 hours or 0300-0400 hours. Control groups combined into one group. Each symbol represents the mean ± SEM of 10 animals.

experimental period had significantly smaller testes than those receiving vehicle infusions. This difference was significant ($p < 0.0001$) from week 4 to the end of the experiment (Fig. C). The testicular weights of animals receiving a daily infusion of 500 ng melatonin at 2000-2100 hours were not different from those of the 500 ng groups at 0300-0400 hours and those of the control groups at each corresponding time point (Fig. D).

## Discussion

The most significant result obtained from this study is the demonstrated different effect of a short duration (1 h) of melatonin infusion on the reproductive status of adult Siberian hamsters. The effect of melatonin in juvenile Siberian hamsters was dose and time dependent; i.e., only 50 ng of melatonin infused between 2000 and 2100 hours was effective (21). Studies in which melatonin injections effectively alter testicular size and function suggest that the timing of hormone administration relative to the ambient light/dark cycle; i.e. relative to the animal's circadian day, is a significant factor in the resulting reproductive response (18). Injection studies have been criticized on the basis that the results may be explained either as an additive effect of exogenous and endogenous melatonin or as a result of the use of a pharmacological dose of melatonin (12,14,31). Critical examination of the published data

from intact animals and the demonstration that single daily injections of melatonin induce gonadal regression in pinealectomized male Syrian hamsters housed in LD12:12 only if the injection is timed to occur within a 6 h window of sensitivity negate the criticism that the effect of the injected exogenous melatonin results from adding this hormone to the endogenously released hormone, thereby providing melatonin to the animal for a long duration (19,20). Studies in which melatonin was infused in Siberian hamsters revealed that in adult or juvenile pineal intact and/or pinealectomized hamsters a 4-6 h daily infusion of melatonin in doses ranging from 10 ng to 5000 ng always stimulated gonadal development of the juvenile or maintained gonadal function of the adult reproductive system. On the other hand, daily 8+ h infusions of melatonin always inhibited the gonadal development of juveniles or caused gonadal atrophy of long-day-housed adult animals (5,6,32). We found that the reported stimulatory effect of daily melatonin infusions of any duration less than 6 h on reproductive development in pinealectomized Siberian hamsters was not supported by the data in our previous experiments and, therefore, suggested that the duration hypothesis was a null hypothesis (21,22). We demonstrated that a daily 1 h melatonin infusion, only if administered right after lights off (2000-2100 hours), had an inhibitory, not stimulatory, effect on testicular development in pinealectomized juvenile Siberian hamsters.

The fact that daily 1 h melatonin infusions over a period of 8 weeks in adult Siberian hamsters on LD16:8 did not cause testicular atrophy (Fig. B) suggests that the photoperiodic response system in adult males may be far less sensitive to the effective (in juvenile males) dose of melatonin infused over a 1 h time period. However, we note that a similar amount of melatonin infused at the same time (2000-2100 hours) into pregnant female Siberian hamsters held on the same photoperiod (LD16:8) was sufficient to program the fetuses (i.e., presented to the fetuses a signal indicating gestation occurred under short, not long, days), thereby affecting their neonatal response to ambient photoperiod (Gündüz and Stetson, unpublished). Thus, in adult females at least, 50ng of melatonin produces a "short day" effect only when administered between 2000 and 2100 hours.

Our 8 h melatonin infusion results agree with findings reported by Carter and Goldman (5) in Siberian hamsters. Eight-hour melatonin infused animals had large testes. In fact, the day time 8 h melatonin infusion did not overlap the putative critical phase during the first hour of the dark phase, so the animals did not produce a similar effect as 1 h infusions given during that phase. However, the mechanism by which the light-dark transition interfere with 8 h infusions is still unknown and remains to be determined.

It appears that in males a 50 ng melatonin infusion at 2000-2100 hours is effective in altering the testicular development of juveniles, but it is not effective in adults of this species. The reasons for this difference between juvenile and adult responses to 1 h melatonin infusions are not readily apparent, but may be attributable to the differential sensitivity of the system of adults and juveniles to the infused hormone or, perhaps, the melatonin-sensitive period in the long day adult may not be at 2000-2100 hours. It is also possible, for example, that the infusion of melatonin at highly sensitive time points (after lights off, 2000-2100 hours) to juvenile hamsters inhibits already maturing GnRH pulse generator activity that is in turn sufficient to inhibit LH/FSH secretion. Although we used a 10 x higher dose of melatonin (500 ng) in one group of adult males (Fig. D), it did not cause gonadal atrophy during the course of the experiment. Therefore, the facts that a single hour exposure at 2000-2100 hours to melatonin for a period of 15 days (juvenile males) (21) effectively alters normal physiological processes in these animals while the same infusion administered daily for a period of 8 weeks had no apparent effect on testicular function in adults exposed to long days (Fig. B) may be due to (a) differential sensitivity of the affected systems responding to the infused hormone, (b) different minimal periods of exposure (in days) of the affected systems to the infused hormone, (c) a phase shift in the sensitive time period (from 2000-2100 hours to another time point) in adult males or (d) adult hamsters respond to durational exposures to melatonin while juvenile hamsters respond to phase exposures to melatonin (not supported by data from injection studies in adult males).

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