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# Maternal transfer of photoperiodic information in Siberian hamsters. vi. effects of time-dependent 1-hr melatonin infusions in the mother on photoperiod-induced testicular development of her offspring

Abstract: We tested in Siberian hamsters the nature of the maternal signal that relays photoperiodic information to the developing fetuses. As previous investigations have identified maternal hormonal and circadian components in this process, the specific goal of this presentation is to determine quality of the signal that connotes daylength when it is imparted to the fetus. Does the function of the signal received by the fetus best support the coincidence or duration hypotheses of photoperiodic induction? Pregnant hamsters received 1 or 8 hr melatonin or vehicle infusions everyday. Juveniles of intact mothers gestated on 16 hr of light per day (16L) experienced maximal suppression of testicular development when reared on 14L. However, when intact mothers gestated on 10L received a 1-hr melatonin infusion daily at 20:00–21:00 hr, their young responded to 14L with greatly accelerated testicular development. In the absence of the maternal pineal gland (and, therefore, the maternal melatonin signal), the effects of maternal melatonin infusions were reversed. Here, only the juveniles of 16L-gestated females infused at 20:00–21:00 hr daily responded to 14L with enhanced testicular development. All other groups showed the same extent of gonadal development, independent of the time or type of infusion their mothers received. Testicular development on 14L of all juveniles from pinealectomized mothers gestated on 10L was of the same magnitude, regardless of the type and time of infusion their mothers received during pregnancy. The results suggest that the maternal signal transferred to the fetuses during gestation consists not only of the daily melatonin signal, but also some circadian-based component that greatly affects the effect of the former. The timing, and not the duration, of the maternal melatonin signal with respect to the animals' (mother and fetus) circadian day is of crucial importance in the transfer of photoperiodic information from mother to fetus.

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## Introduction

In several species reproductive responses to daylength during juvenile life are influenced by photoperiodic information received in utero from the mother during pregnancy. Maternal transfer of photoperiodic information to the fetus was first reported by Horton [1] in the montane vole (*Microtus montanus*), then in the Siberian hamster (*Phodopus sungorus*) [2] and in the meadow vole (*Microtus pennsylvania*) [3, 4]. Horton [1] showed that the reproductive development of montane voles was altered by changing the prenatal photoperiod and by exposing pups to an intermediate daylength (14 hr of light per day = 14L). Males which were transferred on the day of birth from 16L to 14L had a significantly lower rate of gonadal growth, while rapid genodal growth occurred in males gestated in light/darkness (LD) 12:12 (=12L) and transferred to 14L at birth. Cross-fostering experiments were utilized to determine whether the young received photoperiodic information in utero, during lactation, or both. In these experiments, litters from long or short photoperiod mothers were divided, on the day of parturition, between foster mothers who had experienced the opposite photoperiod during pregnancy – all litters were raised in 14L from birth. The results indicated that information transferred from mother to fetus during gestation determines the reproductive response of the prepubertal animal to photoperiods of intermediate duration [5]. Similar findings were obtained in Siberian hamsters [6, 7]. Specifically, exposure to 14L during juvenile life promotes rapid testicular development in young gestated on 12L or 10L but inhibits testicular development in those gestated on 16L. This effect of

gestation photoperiod on testicular development is mediated through information transferred from mother to the young. Removal of the mother's pineal gland [7] or inhibition of pineal function by gestation on constant light [8] prevents this transfer of photoperiodic information. Other studies have demonstrated that the photoperiod during the first 2 wk after birth does not affect the rate of reproductive development – only photoperiods experienced during gestation and after 2 wk of age are effective [6]. To determine whether pinealectomy of the mother resulted in the transfer of a long day signal to the young or represented the absence of photoperiodic information being transferred, young were raised in constant light after birth [8]. As constant light functionally inhibits pineal secretory activity, testicular development is dependent on the photoperiodic information received prior to exposure to constant light. These experiments proved that the maternal pineal gland is necessary for the transfer of long and short day signals to the fetuses. In the absence of the maternal pineal gland no coherent photoperiodic information is transferred to the young [8].

In contrast to the Siberian hamster and montane vole, gonadal development of prepubertal golden hamsters (*Mesocricetus auratus*) is independent of maternal influence [9]. Similarly, the photoperiod received before birth in Turkish hamsters (*Mesocricetus brandti*) is not important; instead, gonadal maturation is governed by the length of the postweaning photoperiod [10].

There is further evidence that the rhythm of maternal pineal melatonin secretion is involved in providing the fetus with information about daylength [11]. Infusion of melatonin in pregnant pinealectomized mothers for eight or more hours per day mimics the effects of a short photoperiod; the pups exposed to 14L develop large testes. This response to melatonin is dependent on the number of infusions and the timing of infusions relative to birth [11, 12]. Manipulations of maternal melatonin also influence the rate of testicular development of young reared in constant light. Daily injections of melatonin given at selected times of the day to pregnant pineal-intact females housed on 16L also yields young with inhibited testicular development when reared in constant light [13]. This observation clearly indicates that melatonin is important in the transfer of photoperiodic information from mother to fetus.

We previously demonstrated a sensitive time point (20:00–21:00 hr) in transferring photoperiodic information (short day) in either intact or pinealectomized juvenile Siberian hamsters [14] with 1 hr melatonin (50 ng/hr) infusion. The present experiments deal with maternal transfer of photoperiodic information; the means by which short duration melatonin infusions during gestation modified the reproductive mechanism of the young, i.e. the study was designed to investigate whether prenatal (maternal) melatonin affected the responsiveness of developing male hamsters to various photoperiods experienced during the postweaning periods. Thus, pregnant hamsters exposed to melatonin infusions of 1–8 hr during gestation were pinealectomized at the beginning of the experiment and at parturition, pups were kept in the same photoperiod or transferred to different photoperiods. Reproductive responses of the animals receiving these treatments were examined.

## Materials and methods

All protocols in this study were approved by the Lab Animal Care and Use Committee and were conducted in strict accordance with the NIH Guidelines for the Care and Use of Laboratory Animals.

### Animals and housing

All hamsters were born in our colony either under long photoperiod (LD16:8 = 16L; lights 04:00–20:00 hr Eastern Day Time (EDT)) or short photoperiod (LD10:14 = 10L; lights 10:00–20:00 hr EDT). Breeding pairs were housed on pine shaving beddings in plastic cages measuring $45 \times 21 \times 15$ cm. The breeding cages were checked daily for litters, usually after 16:00 hr. Hamsters were designated 1 day of age on their day of birth. 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 200lx.

Before experimental use, all adult male and female hamsters (3–6 months of age) were maintained in 16L. In short photoperiod experiments, adult females were transferred from 16L to 10L 2 wk prior to mating to allow them to entrain to the new photoperiod before breeding. Females were paired with an unoperated male that had been housed in 16L.

### Photoperiodic treatments

Several photoperiods were used in the experiments with treated/untreated adult Siberian hamsters. In all cases, the onset of the dark period was kept at 20:00 hr to optimize the probability of and reduce the time of transition of entrainment following a phase shift. Long day treatment was provided by LD16:8 = 16L. Short photoperiod was provided by LD10:14 = 10L. LD14:10 = 14L was used as an intermediate photoschedule.

### Pinealectomy and cannulation

For surgery, hamsters were anesthetized with pentobarbitol (32.5 mg/kg BW, i.p.) and with ketamine (20 mg/kg BW, 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.

Pinealectomy of adult female hamsters was performed according to the method of Hoffman and Reiter [15]. Cannulation of the animals for infusion was performed following the technique reported by Carter and Goldman [16] using a 60-cm length of polyethylene tubing (Clay Adams, PE-20, Parsippany, NJ, USA). Newskin adhesive was applied to the incision area to prevent any contamination. The entire cannula assembly was sterilized with 70% ethanol before use.

### Infusions

Infused animals were housed singly in plastic cages. All animals received food and tap water ad libitum. Animals

were fitted with a subcutaneous catheter for infusions of melatonin, as described [14]. The infusion flow rate was 0.12 mL/hr. Syringes were refilled everyday just before the infusion started. The infusion apparatus, including the syringes pump and polyethylene tubes up to flow-through swivels was covered with aluminium foil as a precautionary measure against light exposure and subsequent degradation of melatonin.

### Melatonin solutions

Melatonin solutions were made by dissolving crystalline melatonin in a small volume of 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/hr. Melatonin concentration used was 50 ng per hour (see below).

### Statistical analysis

Testes weights were analyzed using two-way analysis of variance (ANOVA; SAS, Version 6.07) for the effect of dose, time and all interactions. 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 homogeneous. Differences between groups within a treatment type were determined with a least-squares mean test – mean values were considered significantly different if $P < 0.05$. Data are presented as mean ± SEM after back-transforming from ANOVA results.

### Experimental protocols

Our aim in this study was to determine the temporal and durational dimensions of the effective long day and short day melatonin signals provided to the mother and from her to the fetus.

*Experiment 1*. Adult hamsters were paired for breeding and assigned to either 16L or 10L. Adult male hamsters were housed with either intact or pinealectomized females for 8 days to ensure that successful mating occurred. In the case of pinealectomized groups, females were pinealectomized before the start of the experiment and housed singly. After a 2-day recovery period adult pinealectomized females and unoperated males were paired. Periodic vaginal lavage indicated the presence or absence of spermatozoa, and when sperm was found in the vagina, this day was indicated as day 1 of pregnancy. After 8 days, males were removed from the cages. On the day of parturition, the mothers and litters were transferred to 14L for the remainder of the experiment. On day 30 of postnatal life, juvenile male hamsters were killed and paired testes weights were determined.

*Experiment 2*. In this experiment adult hamsters were paired for breeding in 16L or in 10L. Adult male hamsters were left with females until day 8 of gestation to ensure that mating had occurred. Intact pregnant animals were implanted with subcutaneous cannulas for infusion of melatonin or vehicle at day 8 of gestation. Pregnant hamsters received a daily 1-hr melatonin (50 ng/hr) infusion at one of the four different time points (19:00–20:00, 20:00–21:00, 24:00–01:00 or 03:00–04:00 hr), or 8 hr melatonin (50 ng/hr) infusions at one of the two time points (20:00–04:00 or 09:00–17:00 hr). Control animals received either a 1-hr or an 8-hr infusion of vehicle on a daily basis. Infusions of melatonin or vehicle were administered from day 8 of gestation to the day of birth. After birth the mothers were carefully removed from the infusion apparatus and transferred to 14L with their litters. Animals remained in this photoperiod for the remainder of the experiment. On day 30 of postnatal life, juvenile male hamsters were killed and paired testes weights were determined.

*Experiment 3*. In this experiment adult female hamsters were pinealectomized before the start of the infusions; thus the only source of melatonin for fetuses was the melatonin infused into the mothers. Adult females were pinealectomized and housed singly. After a 2-day recovery period adult pinealectomized females and unoperated males were paired for breeding and assigned to either 16L or 10L. Adult males were kept with females until day 8 of gestation and were then removed from the cages. On the same day (day 8 of gestation) pinealectomized mothers were implanted with subcutaneous cannulas for infusion of melatonin or vehicle. They received 1-hr melatonin (50 ng/hr) at one of the four different time points (19:00–20:00, 20:00–21:00, 24:00–01:00 or 03:00–04:00 hr) or 8 hr Mel (50 ng/hr) at one of the two time points (20:00–04:00 or 09:00–17:00 hr) or vehicle infusions throughout the day. Infusions of melatonin or vehicle were performed from day 8 of gestation to the day of birth. After birth the mothers were gently disconnected from the infusion apparatus and transferred to 14L with their litters. Animals remained in this photoperiod for the remainder of the experiment. On day 30 of postnatal life, juvenile male hamsters were killed and paired testes weights were determined.

## Results

Animals gestated and born to intact unoperated mothers exposed to 16L during pregnancy responded to 14L as a short day; at 30 days of age they had significantly ($P < 0.0001$) smaller testes than animals born of pinealectomized mothers treated similarly (Fig. 1). Animals born to pinealectomized mothers in 16L and transferred to 14L after birth developed larger testes than their controls, i.e. animals born to intact mothers in 16L, but smaller testes compared with animals born to intact mothers in 10L. Testis development on 14L occurred at the same rate in animals born to pinealectomized mothers irrespective of the gestational photoperiod (Fig. 1). Animals born to intact unoperated mothers exposed to 10L during pregnancy responded to 14L as a long day; at 30 days of age they had significantly ($P < 0.0001$) larger testes than animals from pinealectomized mothers treated similarly (Fig. 1). These results support the hypothesis that the maternal pineal gland is a critical component of the mechanism by which information about ambient photoperiod is transferred from mother to fetus in the Siberian hamster.

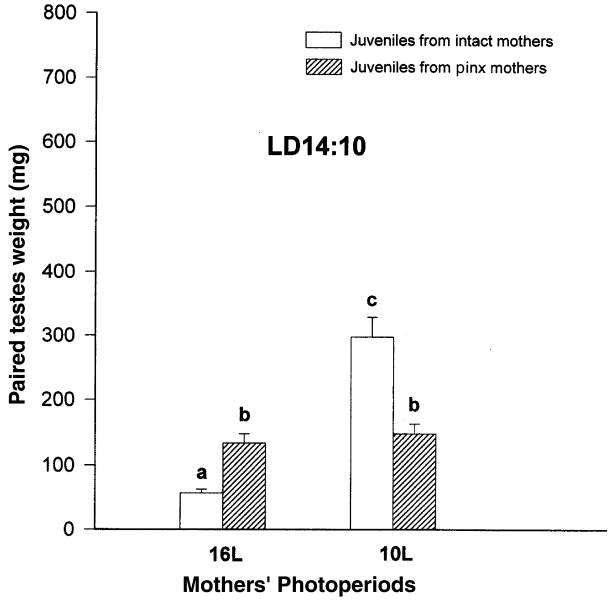


*Fig. 1.* Paired testes weights of juvenile Siberian hamsters born to intact or pinealectomized mothers exposed to long (16L) or short (10L) photoperiod during gestation and transferred to an intermediate photoperiod (14L) at birth. All values are shown as mean ± S.E.M. Similar letters indicate statistical similarity. (n = 8 animals per group).

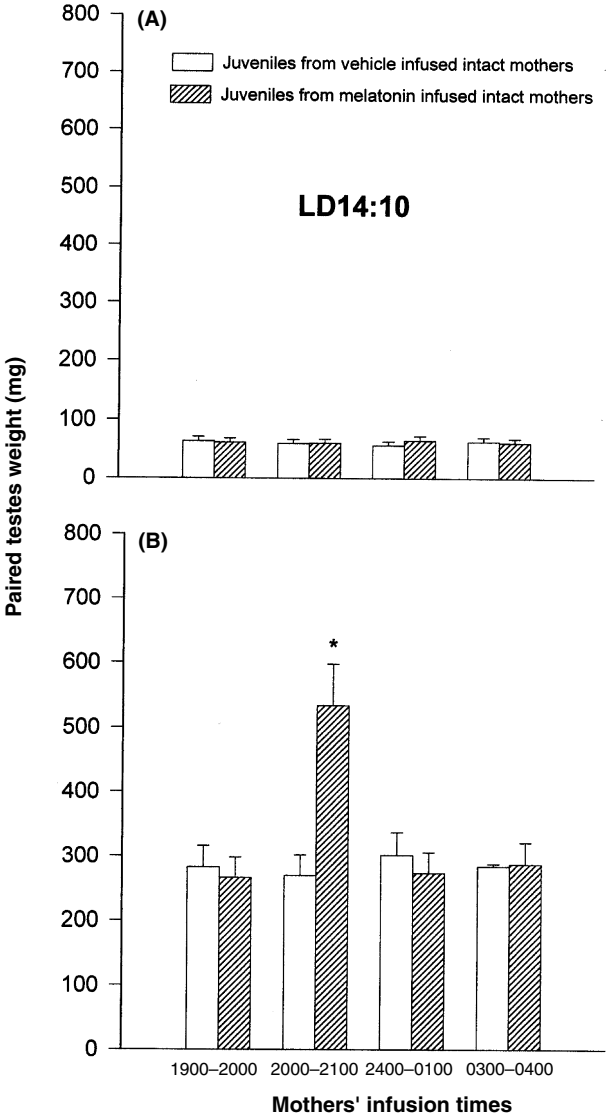


*Fig. 2.* (A) Paired testes weights of juvenile Siberian hamsters born to intact, 1-hr melatonin or vehicle-infused mothers in 16L and transferred to 14L at birth. (B) Paired testes weights of juvenile Siberian hamsters born to intact 1-hr melatonin or vehicle-infused mothers in 10L and transferred to 14L at birth. Intact pregnant mothers received melatonin (50 ng/hr) or vehicle infusions at four different time points (19:00–20:00, 20:00–21:00, 24:00–01:00 or 03:00–04:00 hr). All values are shown as mean ± S.E.M. *Significant difference ($P < 0.05$) (n = 8 animals per group).

All animals from 1-hr melatonin-infused mothers on 16L and transferred to 14L at birth, responded to the latter as a short photoperiod, i.e. testicular development was significantly delayed (Fig. 2A). This response is independent of melatonin and vehicle infusions given to the mothers during pregnancy, and suggests that in intact gestating animals the pineal gland is a major factor in generating the signal through which photoperiodic information is transferred from the mother to the fetus. In the presence of the daily pineal signal from the mother, short (1 hr) melatonin infusions are ineffective in altering the photoperiodic signal received by the fetus. Testicular development of the young of intact 8-hr 50 ng/hr melatonin-infused mothers on 16L and transferred to 14L at birth was greatly enhanced, indicating that the maternal signal received during gestation connoted 'short day' (Fig. 3A). On the other hand, testicular development in control animals (of intact vehicle-infused mother gestated on long days and transferred at birth to 14L) was significantly retarded (Fig. 3A).

The young of intact mothers exposed to 10L during pregnancy and receiving 50 ng/hr melatonin infused at 20:00–21:00 hr, developed significantly larger testes ($P < 0.0001$) when transferred to 14L at birth (Fig. 2B). Testicular development was similar in all other groups in this experiment; animals born to intact vehicle-infused mothers and animals born to intact melatonin (50 ng/hr) infused mothers at 19:00–20:00, 24:00–01:00 and 03:00–04:00 hr had similar testicular weights (260–300 mg), smaller ($P < 0.0001$) than in animals born to intact 50 ng/hr melatonin-infused mothers at 20:00–21:00 hr (Fig. 2B). The results of this infusion group clearly indicate that melatonin administered right after lights-off (at 20:00–21:00 hr) provided an inhibitory signal (i.e. short day) above and beyond the maternal pineal signal and that these combined signals were readily transferred to the young as 'short day'. This sensitive time point (20:00–21:00 hr) appears to remain phase locked to 'lights out' of the ambient photoperiod. Indeed, the experimental protocol was designed to allow rapid re-entrainment to novel photoperiods with a minimum of transient cycles. Testicular development in the young gestated on 10L was not affected by the time or type of infusions when they extended for 8 hr (Fig. 3B).

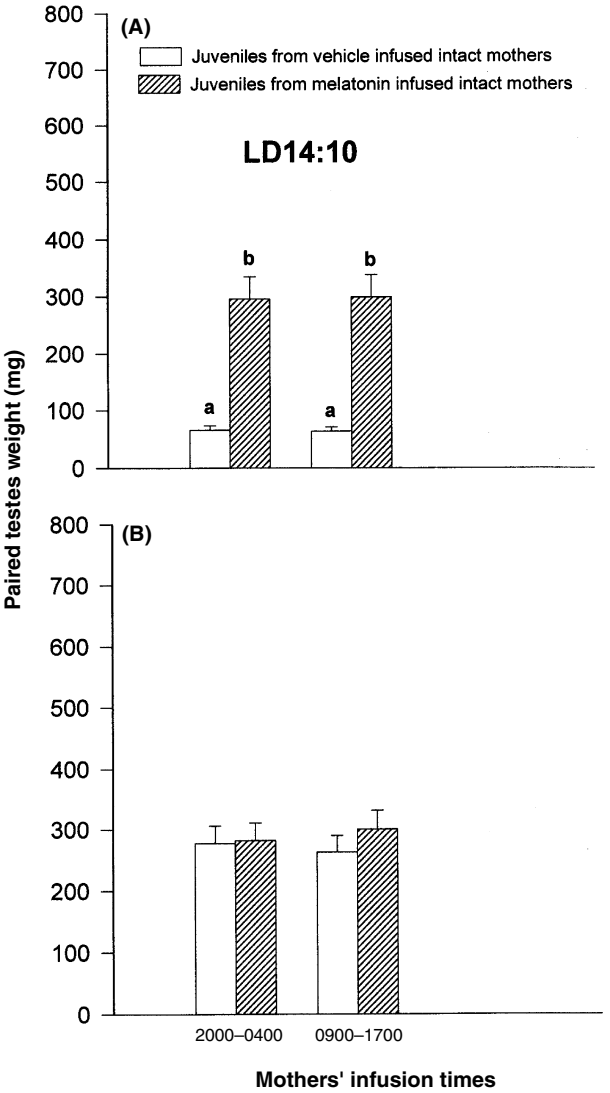


*Fig. 3.* (A) Paired testes weights of juvenile Siberian hamsters born to intact, 8-hr melatonin or vehicle-infused mothers in 16L and transferred to 14L at birth. (B) Paired testes weights of juvenile Siberian hamsters born to intact, 8-hr melatonin or vehicle-infused mothers in 10L and transferred to 14L at birth. Intact pregnant mothers received melatonin (50 ng/hr) or vehicle infusions at 20:00–04:00 or 09:00–17:00 hr. All values are given as mean ± S.E.M. Similar letters indicate statistical similarities. (n = 8 animals per group).

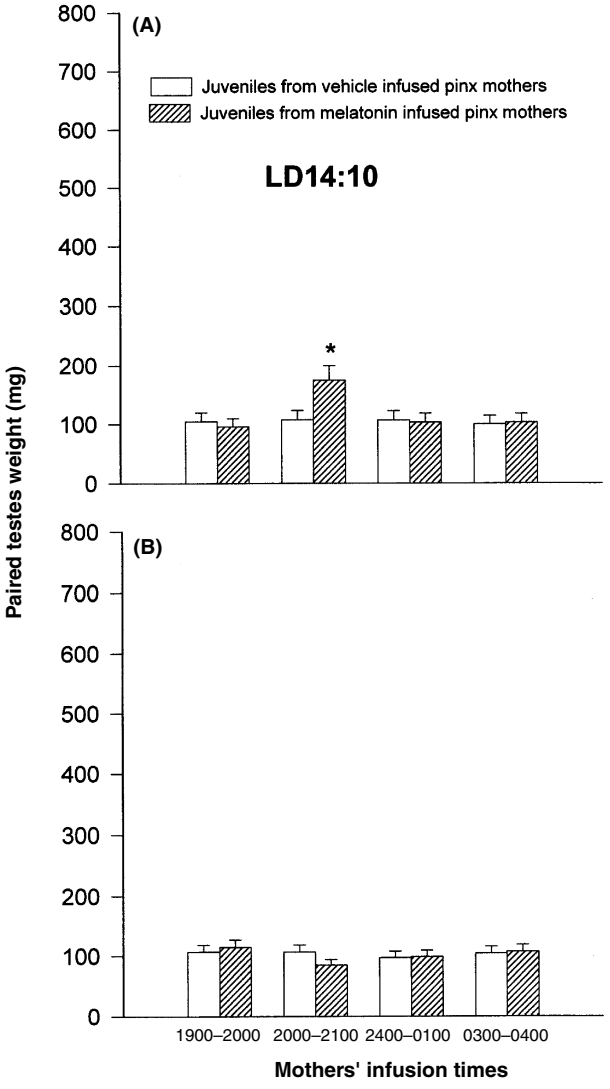


*Fig. 4.* (A) Paired testes weights of juvenile Siberian hamsters born to pinealectomized, 1-hr melatonin or vehicle-infused mothers in 16L and transferred to 14L at birth. (B) Paired testes weights of juvenile Siberian hamsters born to pinealectomized mothers receiving 1-hr melatonin or vehicle infusions daily from day 8 of pregnancy to birth. Animals were housed on 10L and transferred to 14L at birth. Pinealectomized pregnant mothers received melatonin (50 ng/hr) or vehicle infusions at four different time points (19:00–20:00, 20:00–21:00, 24:00–01:00 or 03:00–04:00 hr). All values are given as mean ± S.E.M. *Significant difference ($P < 0.05$). (n = 8 animals per group).

In animals gestated in 16L and transferred to 14L at birth, testicular development in young of pinealectomized mothers receiving 1-hr melatonin (50 ng/hr) infusion at 20:00–21:00 hr in 16L photoperiod was clearly enhanced ($P < 0.0001$) over that in young from pinealectomized mothers receiving vehicle at the same time and for the same duration (20:00–21:00 hr) each day (Fig. 4A). There were no differences in testicular weights among juveniles of melatonin or vehicle-infused mothers at 19:00–20:00, 20:00–21:00 (vehicle), 24:00–01:00, and 03:00–04:00 hr (Fig. 4A).

Testicular development was enhanced in all young born to pinealectomized, 8-hr melatonin (50 ng/hr) infused mothers, irrespective of the times of infusion (20:00–04:00 or 09:00–17:00 hr) (Fig. 5A). However, testicular development in animals born to pinealectomized mothers receiving 8-hr vehicle infusions at the same time points was significantly slower ($P < 0.0001$) compared with animals born to melatonin-infused mothers (Fig. 5A).

Testicular development occurred at a similar rate in juveniles of pinealectomized mothers receiving 1-hr melatonin (50 ng/hr) or vehicle infusions at each time point tested (Fig. 4B). Animals born to pinealectomized mothers receiving melatonin infused at a rate of 50 ng/hr for 8-hr daily developed large testes when exposed to 14L at birth; while

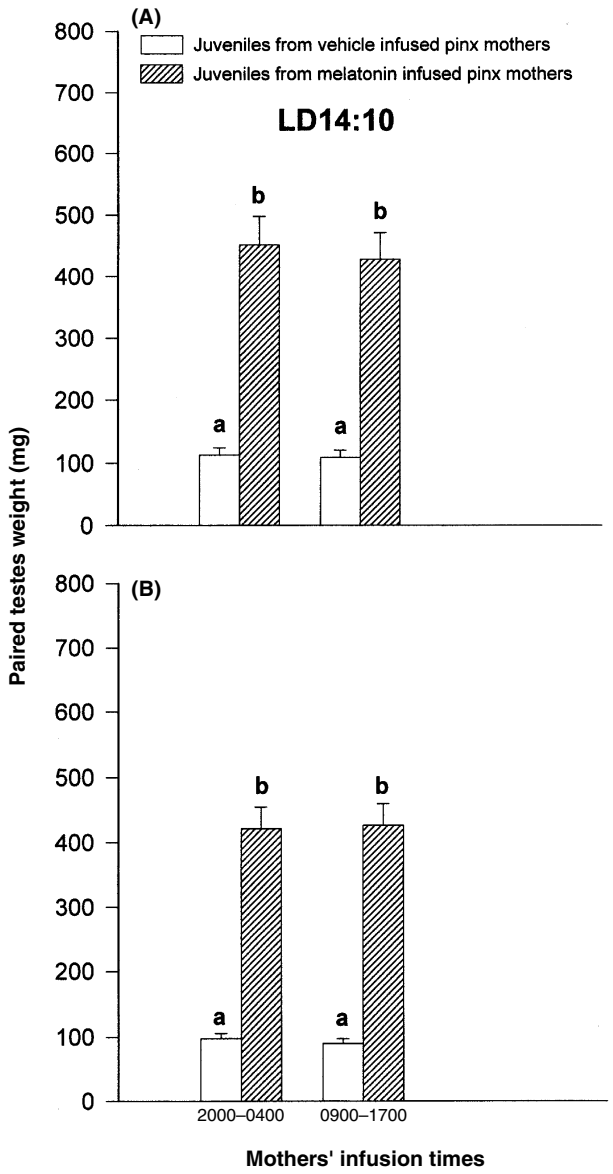


*Fig. 5.* (A) Paired testes weights of juvenile Siberian hamsters born to pinealectomized, 8-hr melatonin or vehicle-infused mothers in 16L and transferred to 14L at birth. (B) Paired testes weights of juvenile Siberian hamsters born to pinealectomized mothers on 10L receiving melatonin or vehicle infusions for 8 hr daily between day 8 of pregnancy and birth. They were then transferred to 14L at birth. Pinealectomized pregnant mothers received melatonin (50 ng/hr) or vehicle infusions at 20:00–04:00 or 09:00–17:00 hr. All values are given as mean ± S.E.M. Similar letters indicate statistical similarity. (n = 8 animals per group).

animals born to pinealectomized 8-hr vehicle-infused mothers developed small testes ($P < 0.0001$) in 14L (Fig. 5B).

## Discussion

In a previous study [14], we reported the inhibitory effect of 1 hr melatonin infusion on testicular growth in either intact or pinealectomized juvenile Siberian hamsters. Infusion immediately after lights off (20:00–21:00 hr) produced a short day effect, significantly retarding testicular growth. The present study was carried out to determine if the maternal organism communicated with her developing fetuses in a similar fashion. A number of investigations have demonstrated that the maternal pineal gland, through its hormone melatonin, communicates photoperiodic information to the fetus [7, 8, 17]. Maternal photoperiodic cues or melatonin given externally by injection or infusion effectively program the fetal brain only if administered 2–6 days before birth [7, 17]. This information is stored and utilized by juvenile hamsters long after birth in an integrated response to ambient photoperiod. In the absence of the maternal pineal gland, there is no transfer of information of the prenatal photoperiod [7, 13]. We investigated the testicular response of pups that were born to melatonin-infused mothers exposed to different photoperiods. When pups that are born to intact mothers in either 16L or 10L and transferred at birth to 14L, 16L born pups have smaller testes than 10L born pups (Fig. 1). These results are in good agreement with other studies and show the maternal effect on the pup's testicular development; 14L is an intermediate photoperiod for Siberian hamsters and testes development in this photoperiod is dependent on the information received during gestation. That is, animals gestated on 10L (e.g. typical of the spring of the year) respond to 14L as a long day while animals gestated on 16L (e.g. typical of the summer of the year) respond to 14L as a short day (Fig. 1). When we compare these results (Fig. 1) with the data from a previous study [13], animals gestated and raised in 16L (see 13, vehicle groups) develop much larger testes (controls: 600 mg), than the animals gestated in 16L and raised in 14L (~50 mg) (Fig. 1, intact). If animals are gestated in 10L and raised in 14L testis development is not as intensive as in animals gestated and raised on 16L (Fig. 1; see 13). When pups gestated on either 16L or 10L are born to pinealectomized mothers and raised in 14L, testicular development is the same in both groups (Fig. 1) indicating that removal of the maternal pineal gland abolishes the long or short day signal transmitted to the fetus.

Interesting observation is that mothers transfer to their fetuses a 1-hr melatonin infusion signal as indicating exposure to short photoperiod. This result is only observed when melatonin is infused to pinealectomized mothers at 20:00–21:00 hr (compare Fig. 2A and Fig. 4A); 1-hr melatonin infusions are without effect in intact mothers (Fig. 2A). When we consider that testicular development is dependent on the gestational photoperiod and the availability of melatonin in maternal circulation during gestation, the presence or absence of the mother's pineal gland modulates the effect of a 1-hr melatonin infusion at 20:00–21:00 hr, and thereby affects testicular development of her pups. On the other hand, considering the effect of infusions of 50 ng melatonin at 20:00–21:00 hr on juvenile hamsters in previous experiments [14], it appears that the maternal pineal gland (endogenous melatonin secretion) emits a more effective signal than infused (exogenous) melatonin in delivering a photoperiodic signal to the fetuses. Pups born to pinealectomized, melatonin-infused mothers in 16L and raised in 14L, undergo rapid testicular development, i.e. received a short photoperiod signal from

their mothers, only when pinealectomized mothers receive melatonin infusion at 20:00–21:00 hr, but not at other time points (Fig. 4A). The situation observed in pups of intact mothers exposed to 10L during gestated is different. These pups, raised in 14L, undergo rapid testicular development ($P < 0.0001$) when gestating mothers received melatonin at 20:00–21:00 hr (Fig. 2B). It appears that intact pregnant hamsters in short days (10L) are more sensitive to infused melatonin at 20:00–21:00 hr than are intact pregnant hamsters on long days (16L) (compare Fig. 2B and Fig. 2A). The effects of short photoperiods (10L) and the short day signal resulting from melatonin infusion at 20:00–21:00 hr appear to the additive, resulting in a much stronger 'short day' signal being imparted to the fetus. Therefore, two possibilities arise: melatonin infused in pinealectomized animals exposed to short photoperiod is either without effect or higher doses of melatonin may be required for an effect to be observed. The results show that the effects of 1-hr melatonin (50 ng/hr) infusion at 20:00–21:00 hr had a significantly greater effect only in pinealectomized mothers infused in 16L (Fig. 4A). The same effect is observed in melatonin-infused intact mothers in 10L (Fig. 2B). Thus, not only the time of melatonin infusion but also gestational photoperiod and the presence or absence of the maternal pineal gland governs postnatal reproductive development of juveniles. The data indicate that there is a restricted time for reception of the prenatal signal, with maximal sensitivity to melatonin occurring after 'lights off'. The appearance of sensitivity to infused melatonin after lights off could coincide with the time that responsive maternal and/or fetal tissues are most sensitive to melatonin. There results provide confirmation of the role of the pineal gland and its hormone melatonin in transducing photoperiodic information in the pregnant Siberian hamsters.

Our 8-hr melatonin infusion results agree with the findings reported by Carter and Goldman [16] in Siberian hamsters. The 8-hr melatonin-infused animals had large testes regardless of whether the animals were intact or pinealectomized or animals were housed in long or short photoperiods and regardless of the time of day of the infusion. In fact, the day time 8-hr melatonin infusions did not overlap the putative critical phase during the first hour of the dark phase, yet they produced the same effect as 1-hr infusions given during that phase. However, the mechanism by which the light–dark transition interferes with 8-hr infusions is still unknown and remains to be determined. Apparently, the daily infusion of melatonin for 1-hr did not mimic the pattern of endogenous melatonin associated with exposure to a 14L (intermediate) photoperiod close enough to induce a gonadal response. Nevertheless, this duration was extremely important that a short exposure to melatonin during the first hour of darkness is capable of producing a short day effect. The dose, procedure and timing of delivering melatonin may be an important feature of its action on the reproductive axis of developing fetuses.

As melatonin is known to pass the placenta freely from mother to fetus [18], the results from our experiments suggest that it is possible that fetal target system(s) for the melatonin signal(s) are functional by late fetal life and that the fetus is capable of responding to the mother's melatonin rhythm. Although Weaver et al. [11, 12] suggested that the response to infused melatonin is dependent on the number of infusions, the timing of the infusions relative to birth and the duration of each infusion, our results provide evidence that infusions of less than 4 hr duration in pregnant mothers during the last 6–8 days of pregnancy affect testicular development of her pups during postnatal juvenile life. Our data further demonstrate that this effect is photoperiod and circadian-dependent. The absence of the maternal pineal gland may contribute to a slightly increased sensitivity to exogenous melatonin in long photoperiod, while the presence of the maternal pineal in gestating females on short photoperiods greatly enhances the effect of infused melatonin. Pinealectomized golden hamsters have been shown to be more sensitive than their sham controls in reproductive responses to exogenous melatonin [19].

In summary, our results clarify the way in which melatonin has been delivered to juvenile Siberian hamsters [13, 20] or pregnant adult Siberian hamsters by daily infusion for a period of 1-hr and in some cases 4-hr add considerable strength to the hypothesis that coincidence of melatonin and a period of melatonin sensitivity is the primary feature of the melatonin signal that conveys photoperiodic information to the neuroendocrine system, but the fundamental mechanisms of this process remain elusive.

The results of melatonin infusions in pregnant Siberian hamsters provide evidence that can be interpreted as that the fetal neuroendocrine system is functional prior to birth. The identity of the melatonin sensitive structures in the fetal brain has not been determined. These structures function during the gestational period, especially during the last 6 days of gestation and are probable components of the system that stores photoperiodic information provided by the mother, to be used weeks later in integrating a gonadal development response to ambient photoperiod by the juvenile hamster.

The suprachiasmatic nucleus (SCN) of the Siberian hamster appears to be a target for the regulation of reproduction by melatonin [21, 22]. Melatonin-specific binding sites are found in the SCN and their density may be regulated by melatonin [23], but studies in other photoperiodic species including Syrian hamsters, fail to support such a role [24]. In the Syrian hamster, it has been shown that destruction of two areas of the brain in which melatonin receptors are in very high density, the SCN and paraventricular nucleus (PVN), does not prevent the suppressive effect of melatonin on luteinizing hormone levels [24]. In the Siberian hamster, however, lesions of the SCN prevent testicular regression normally induced by systemic infusions of melatonin, which mimic the effect of short days, suggesting a central site that mediates reproductive responses [21, 22]. Acute melatonin treatment in rats enhances SCN glucose utilization and protein synthesis [25]; phase advances the peak in SCN electrical activity in vitro [26] and induces SCN expression of immediate early genes [27]. Yellon [28] also suggests that the biological clock mechanism that generates the circadian melatonin rhythm is responsive to the influence of daily melatonin treatments and presumably to the feedback action of endogenous melatonin on its own rhythm in the Siberian hamsters. Furthermore, expression of melatonin receptors in the SCN

is not dependent on neuronal input from surrounding hypothalamic areas, because excitotoxic hypothalamic lesions that spare the SCN do not eliminate the melatonin binding sites in the SCN [29]. Hastings et al. [30] proposed a model of non-photic (melatonin) resetting of the circadian clock. Melatonin can reduce the activity at specific times of the day. Exogenous melatonin will decrease cellular activity and cause phase delay when administrated in the early subjective day. Accordingly, melatonin administrated in the late subjective day will cause phase advance. The model provides an explanation as to why the clock is sensitive for melatonin stimulation at different phases of the circadian cycle and is consistent with our results because the models explain why we obtained an entrainment to 1-hr melatonin infusion. Thus, the biological clock that generates a variety of circadian signals may use the daily melatonin signal to distribute the circadian message, which is then translated into changes in reproductive status.

Although the neuroendocrine mechanism is not clear for the effect of melatonin in transferring the short day signal to the reproductive systems, it seems possible that melatonin may function differently in several aspects of the developmental process. As for the ability of the data from these experiments to determine whether the duration and/or the coincidence models constitute null hypotheses, we conclude that the application of a short signal of melatonin at the beginning of the dark period, that is when the production of melatonin started, and that is when membrane-bound receptors on melatonin target structures are active [31, 32], is sufficient by itself to entrain a gonadal response. Therefore, the presence of a period of sensitivity to melatonin can explain the observed physiological response and this response is similar to the one observed with a long duration infusion (8 hr) (Fig. 4A and Fig. 5A). Therefore, the effectiveness of 8-hr melatonin infusions in pinealectomized animals does not rule out the existence of duration hypothesis. Further investigations will be required to understand the basis for what appear to be infusion differences in sites of melatonin action.

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