

Temporal integration of melatonin infusion duration: signal averaging versus frequency dependence

Abstract: Day length affects somatic and reproductive physiology of Siberian hamsters via regulation of the duration of nocturnal pineal melatonin secretion. Nightly 'long' (e.g. 12 hr) or 'short' (e.g. 6 hr) melatonin signals inhibit or stimulate gonadal growth, respectively. When long and short signals are presented in combination, however, neuroendocrine mechanisms exhibit a frequency-dependent response, stimulating gonadal growth only if short signals are presented every second night or more frequently. The present experiments further assessed formal models for the temporal integration of melatonin signals changing abruptly in duration from night to night. Photo-inhibited Siberian hamsters were housed in constant light and infused subcutaneously with various combinations of nightly short or long melatonin signals according to one of the several regimes that varied the frequency of short melatonin signal occurrence, average duration of the nightly melatonin signal, or both. Six weeks of nightly alternating short and long signals yielded different gonadal responses depending on the average melatonin signal duration. Moreover, when average melatonin signal duration was held constant between groups, gonadal stimulation was independent of the frequency of the constituent melatonin signals except when the duration of the short signal was reduced to 3 hr. Thus, neuroendocrine mechanisms do not solely categorize melatonin signals as either long or short but attend also to the duration of each component signal. In the majority (six of seven) of infusion regimes, reproductive responses to chimeric patterns of long and short melatonin signals were compatible with a simple signal-averaging mechanism.

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Introduction

Many mammalian species, including humans, exhibit marked seasonal changes in physiology and behavior in response to the annual cycle of ambient day length [1, 2]. As the number of hours of light per day decreases in autumn, Siberian hamsters, *Phodopus sungorus*, suppress reproductive activity, reduce body weight, molt to a more insulative fur and initiate various thermoregulatory behaviors, presumably as adaptations to increased energy expenditures and reduced caloric resources associated with winter [3, 4]. Summer and winter phenotypes are readily induced in the laboratory by exposing animals for several weeks to typical long (e.g. 15 hr light/day) or short (10 hr light/day) photoperiods, respectively [5]. More proximally, these responses are mediated by the pattern of nocturnal pineal melatonin secretion: long patterns of elevated melatonin secretion (e.g. > 10 hr/night), as generated in winter, induce the winter phenotype, whereas short durations of elevated melatonin secretion (e.g. < 6 hr/night), typical of summer photoperiods, stimulate the reproductive phenotype [6–10]. Studies have generally emphasized a categorical interpretation of melatonin signal duration, noting a functional equivalence of day length or melatonin signals above a

critical duration (i.e., 'long' signals) or below that critical value (i.e., 'short' signals), respectively (but c.f. [11–13]).

Near the so-called critical day length, however, a given photoperiod can induce either gonadal growth or inhibition depending on whether the prior day length was shorter or longer, and this history dependence likely holds for melatonin durations as well [14–17]. These effects of photoperiod history establish that neuroendocrine mechanisms functionally integrate environmental signals over time. Although several studies have investigated the integration of photoperiodic signals changing from one several week period to another [18, 19], few have addressed the processing of photoperiod signals that change markedly in duration from day to day. In one study short day gonadal regression was attenuated in hamsters exposed to a brief night-time light pulse every 2, 3 or 7 days [20]. Such studies are difficult to interpret mechanistically, however, because night-time light exposures not only suppress melatonin acutely, but also affect circadian entrainment. Even infrequent light exposures, therefore, may alter melatonin secretion patterns throughout the study. The entraining actions of light, moreover, render it unlikely that animals would ever produce, under natural conditions, melatonin durations that changed in duration by several hours from

day to day. Such a pattern may be produced artifactually, however, among humans through the inconsistent timing or intermittent administration of exogenous melatonin or of drugs that affect melatonin secretion (e.g., beta adrenergic antagonists, such as propranolol, that acutely lower blood melatonin concentrations [21]).

Using more direct manipulations of melatonin, two studies recently assessed the temporal integration of daily changing melatonin signals in hamsters. In both [22, 23], systemic propranolol treatments were given during the dark phase in order to pharmacologically shorten the duration of the endogenous melatonin signal. Melatonin secretion on subsequent nights was unaffected. Against a background of long (12–13 hr) endogenous melatonin signals, shortened (4 hr) melatonin signals presented every third night or less frequently exerted no effect on testis size. Truncation of the signal every second night, however, provoked gonadal growth. Because these two prior studies used only one long and one short melatonin duration, it is not possible to ascertain whether the observed frequency dependence generalizes to all short and long melatonin durations as would be expected if these signals were processed categorically. Such a ‘categorical frequency’ model may be contrasted with an ‘analog’ model of melatonin signal processing whereby the exact length of each signal influences the gonadal response. As an example, the mechanism integrating melatonin signals over several days might determine the arithmetic mean of signal duration: with more frequent short duration signals, the average duration is correspondingly shortened and might be expected to induce gonadal development below a critical value.

The present study, therefore, evaluated two competing models for the temporal integration of melatonin signal length by the reproductive neuroendocrine system using subcutaneous hormone infusions to exert precise control over signal duration. The signal averaging (analog) model predicts that varying the duration of the respective ‘long’ and ‘short’ melatonin signals will alter the apparent frequency dependence of the gonadal response. Melatonin patterns comprised of different component signals but with the same average signal duration, moreover, should yield identical outcomes. The categorical model, by contrast, predicts that the frequency dependence of the gonadal response will be independent of the length of the component ‘long’ and ‘short’ signals.

Materials and methods

Male Siberian hamsters (*Phodopus sungorus*) were gestated and raised in a reproductively stimulatory photoperiod [15 hr light, 9 hr dark; lights on 05:00 hr Pacific Standard Time (PST); light intensity is 100–400 lux]. Prior to melatonin infusions all hamsters were housed in polypropylene cages (27 × 16 × 13 cm) on corncob bedding at 22 ± 2°C with food (Purina Mouse Chow no. 5015, St Louis, MO, USA) and water available ad libitum. At 18–25 days, animals were transferred to a reproductively inhibitory photoregime (10 hr light daily; lights on 10:00 hr PST).

After 6 weeks, hamsters for both experiments were anesthetized, examined for reproductive inhibition, and implanted with infusion catheters as described below. Upon recovery from surgery, hamsters were transferred to round polypropylene cages (20 cm diameter × 25 cm) equipped for melatonin infusion. From this point, the lights remained on continuously to suppress endogenous melatonin production [24] until the end of the study 6 weeks later.

Experiment 1

Hamsters with regressed gonads were randomly assigned to one of seven melatonin infusion regimens that were initiated the night following surgery. Three groups received melatonin infusions that were 7, 8 or 9 hr in duration nightly (Groups Static7, n = 9; Static8, n = 11; and Static9, n = 8, respectively). Two additional groups received melatonin signals that alternated daily between a short signal known independently to stimulate reproductive development when delivered daily and a long signal known to be reproductively inhibitory. In one group (Group 6/12, n = 17), 6-hr melatonin infusions were alternated with 12-hr infusions. An additional treatment group (Group 5/9; n = 18) received 5- and 9-hr melatonin signals on alternate nights. The final two groups received a repeating 3-day pattern of one short infusion followed by two longer signals: Group 5/8/8 (n = 17) presented subjects with a 5-hr signal on every third night, and 8-hr signals on the two nights following. Group 5/11/11 (n = 16) presented a 5-hr infusion followed by two 11-hr signals on subsequent nights. All infusions were timed such that their midpoint occurred at 03:00 hr PST. As summarized in Table 1, these stimuli systematically vary average melatonin duration and frequency of short melatonin signals.

Experiment 2

Because 6 weeks of 8-hr nightly melatonin infusions were not inhibitory in Experiment 1, Group 5/8/8 did not receive stimulatory signals at a frequency of 0.33 as intended. A second study was therefore conducted to include a melatonin pattern with an average duration of 7 hr and a short signal presented every third night (Table 1). We took this opportunity to verify the stimulatory effect of 8-hr

Table 1. Stimulus characteristics of daily-changing melatonin regimens

Group	Average duration (hr)	Short signal frequency
Expt 1		
5/9	7	0.50
6/12	9	0.50
5/8/8	7	*
5/11/11	9	0.33
Expt 2		
3/9/9	7	0.33
5/8/8	7	1.00
11/8/8	9	0.67

*Intended value of 0.33 is not indicated because daily 8-hr infusions failed to be reproductively inhibitory (see text).

melatonin durations and to assess whether it depended on a contrast effect with interpolated melatonin signals of longer or shorter duration. Separate groups of hamsters were treated as described in Experiment 1 but randomly assigned to one of the following infusion schedules: groups Static5 ($n = 14$) and Static8 ($n = 13$) received invariant daily melatonin infusions of 5- or 8-hr duration, respectively. Three other groups received 3-day melatonin patterns. Group 3/9/9 ($n = 13$) received a 3-hr infusion followed by two 9-hr signals. In Group 5/8/8 ($n = 14$), a 5-hr signal was followed by two 8-hr signals, and in Group 11/8/8 ($n = 14$), an 11-hr melatonin signal was preceded by two 8-hr infusions. As in Experiment 1, these infusion patterns generated average durations of 7 or 9 hr, but represented a broader range of frequency of stimulatory signals than was used in that experiment (Table 1).

Infusions

Polyethylene infusion catheters were implanted subcutaneously under methoxyflurane anesthesia vapors (Metofane; Pittman Moore, St Louis, MO, USA) as described in detail elsewhere [25]. Catheters were connected to a swivel device mounted from the cage lid that allowed free movement of hamsters around the container, and programmed infusions of melatonin were delivered by timer-controlled pumps (Razel Scientific Instruments, Stamford, CT, USA) at a flow rate of 0.017 mL/hr. The infusate was prepared by dissolving 5.0 mg melatonin (Sigma, St Louis, MO, USA) in 1.0 mL 70% ethanol and diluting with 79 mL of sterile 0.9% NaCl solution to yield a stock solution, which was frozen and stored for later use. Aliquots (1.00 mL) were thawed and further diluted with 99 mL NaCl solution. Because animals received different duration melatonin on subsequent nights, it was not feasible to administer a fixed total melatonin dosage nightly. Thus, we elected to infuse melatonin at a constant rate of 10 ng/hr.

Measures

At the time of catheter implantation, the left testis was measured externally to verify that the gonads were regressed [26], and body weight was recorded. After 6 weeks of melatonin infusions, hamsters in both experiments were weighed, killed with a lethal intraperitoneal sodium pentobarbital injection, and paired testis weight was determined. All procedures were previously approved by the Institutional Animal Care and Use Committee (University of California, San Diego) where the work was undertaken.

Statistics

Because of significant heterogeneity of variance in dependent measures, paired testes weight and change in body weight were analyzed with the non-parametric Kruskal–Wallis test for the equality of the populations (Statview 5.0; SAS Institute, Cary, NC, USA). Following a significant omnibus result, planned comparisons were conducted with the non-parametric Mann–Whitney U -test. Results were considered significant at $P < 0.05$. Additional analyses

were conducted to assess group differences in the proportion of animals that were reproductively inhibited by melatonin treatment. Animals with paired testis weights below 80 mg were considered fully regressed, and the resulting proportions were examined pairwise with χ^2 statistics.

Results

In Experiment 1, both testis weight and change in body weight showed significant between-group variation (Fig. 1; Kruskal–Wallis test: $H = 25.4$, $P < 0.001$ for paired testis weight; $H = 22.0$; $P < 0.01$ for change in body weight). With melatonin infusions of invariant duration, gonadal growth was suppressed by 9-hr infusions compared with shorter infusions (7- or 8-hr infusions, $P < 0.05$ for both comparisons), but no difference was observed between 7- and 8-hr infusions ($P > 0.8$). Somatic growth followed the same pattern with smaller increases in body weight achieved during 9-hr infusions than with 7-hr ($P < 0.05$) or 8-hr signals ($P < 0.01$). Again, the latter two signals did not differentially affect somatic growth ($P > 0.7$).

The two groups receiving different schedules of daily alternating long and short signals (5/9 versus 6/12) differed from one another in testis weight ($P < 0.05$). A corresponding group difference in somatic growth, however, was not apparent ($P > 0.5$). The two versions of the 3-day melatonin infusion regimen likewise generated significantly different paired testis weights ($P < 0.01$) and somatic growth ($P < 0.01$).

An average 9-hr signal delivered at different frequencies of longer and shorter melatonin infusions (5/11/11 and 6/12) did not generate significant differences in testis weight ($P > 0.2$) nor did either group differ significantly from static 9-hr infusions ($P > 0.4$ for both comparisons with 9 hr). Somatic growth, in contrast, was significantly greater among hamsters exposed to alternating 6- and 12-hr infusions than in the group receiving the same 9-hr average duration delivered over 3 days (Group 5/11/11; $P < 0.01$). Neither group, however, had significantly different body weight changes from that obtained with static 9-hr infusions ($P > 0.2$ for both comparisons).

The two groups receiving an average melatonin duration of 7 hr delivered at different frequencies of long and short signals likewise failed to differ significantly in terms of gonad size ($P > 0.2$) or body weight gain ($P > 0.9$). Neither group differed significantly from static 7-hr-infused hamsters in either testis weight ($P > 0.05$ for both comparisons) or somatic growth ($P > 0.5$).

Comparisons of the proportion of animals with fully regressed gonads led to identical conclusions as those described above based on group averages (Fig. 1B): significantly more animals exhibited complete gonadal inhibition when average signal duration was 9 versus 7 hr regardless of whether the short melatonin signals was presented every second or every third night ($P < 0.05$ for both comparisons). Signal frequency had no significant effect on the proportion of animals exhibiting threshold stimulation.

In Experiment 2, the five melatonin treatments led to significant between-group variation in paired testis weight

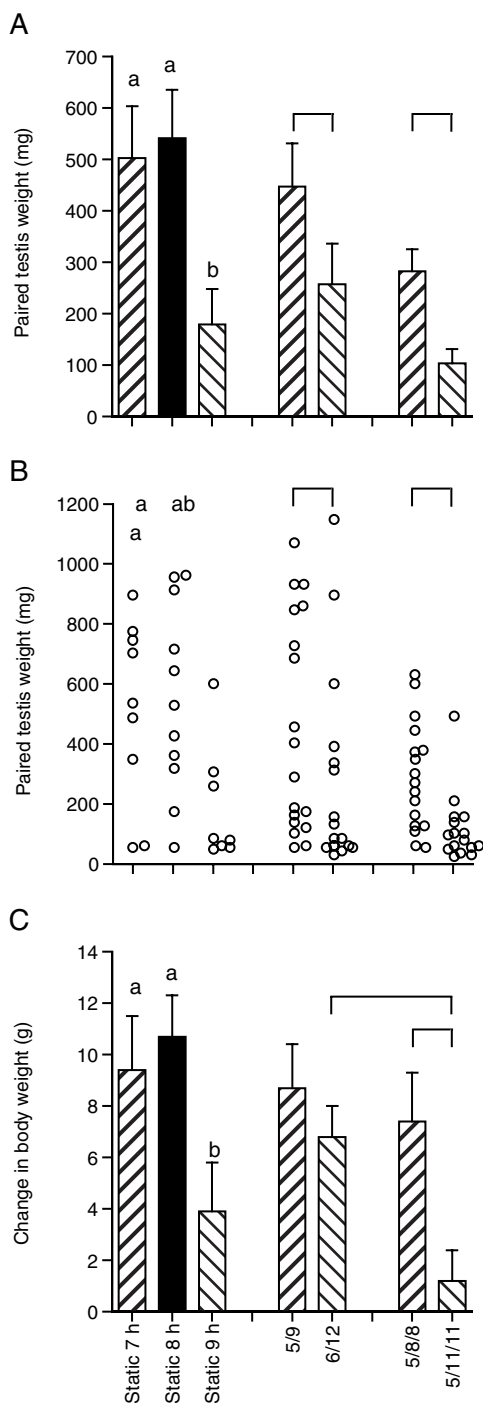


Fig. 1. Mean + S.E.M. (A) and individual (B) paired testis weights and mean change in body weight (C) after 6 weeks of melatonin infusions. Groups receiving the same average duration of melatonin are denoted with bars of the same cross-hatching. Brackets spanning two experimental groups indicate significant differences between groups of similar average durations or frequency of short melatonin signals ($P < 0.05$, Mann-Whitney U -test). Static duration groups that differ from one another are denoted with different letters. Group differences (χ^2) in the proportion of animals exceeding a threshold level of gonadal stimulation are indicated on scatterplots of individual data.

(Fig. 2A; Kruskal-Wallis test: $H = 9.9$; d.f. = 4; $P < 0.05$) but not in somatic growth (Fig. 2C; $H = 6.1$; d.f. = 4; $P > 0.1$). Static infusions of 5 or 8 hr induced comparable degrees of gonadal growth ($P > 0.6$). The two treatment regimens that delivered an average melatonin duration of 7 hr, however, induced different degrees of gonadal growth ($P < 0.05$): compared with a 3-day pattern of 5- and 8-hr signals, the same 7-hr average generated from 3- and 9-hr signals was reproductively inhibitory. Against a background of stimulatory 8-hr melatonin signals, one long duration (11 hr) signal every third day was sufficient to inhibit gonadal growth ($P < 0.01$ compared with 5/8/8; $P < 0.05$ compared with 8/8/8). Analysis of the proportion of animals with fully regressed gonads yielded generally similar results (Fig. 2B).

Discussion

The frequency-dependent effects of intermittent short melatonin signals on gonadal growth reported in other experiments [22, 23] are shown here to be context specific. Prior work illustrated that a shortened melatonin signal provoked gonadal growth in photo-inhibited hamsters if delivered every second night, but not if the short melatonin signal was presented less frequently. Consistent with these earlier findings, gonadal developmental was arrested with melatonin infusion patterns containing a short signal every third night (Group 5/11/11) but stimulated with more frequent presentation (Group 5/9). Gonadal growth could be inhibited, however, with certain infusion patterns that presented short melatonin signals at the same frequency (Group 6/12) or even when the short signals predominated (Group 11/8/8). The frequency of 'short' signals necessary to induce gonadal growth thus depends on the duration of each component signal. These findings argue against a categorical interpretation of daily melatonin signals as either simply 'long' or 'short.' Instead, various durations of 'long' and 'short' signals are processed differently depending upon the context within which they are delivered.

This interpretation requires an independent validation that each of the components of the 2- or 3-day melatonin patterns is indeed stimulatory or inhibitory, respectively, as presumed. The literature provides ample documentation in hamsters that very long melatonin infusions (e.g. 10–12 hr) are reproductively inhibitory and very short signals (e.g. 4–6 hr) are reproductively stimulatory [7, 8, 18, 27, 28]. Effects of intermediate duration signals (e.g. 8, 9 hr), however, are less consistent between studies [7, 29]. With the demonstration in Experiment 1 that daily 9-hr signals inhibited gonadal growth, there can be little question that 9-, 11-, and 12-hr components of the melatonin regimens should be considered to be reproductively inhibitory. Similarly, daily 7- and 8-hr signals were shown to stimulate testis growth in Experiment 1, and in Experiment 2, daily 5- and 8-hr signals both induced gonadal growth, albeit not to the same extent as in Experiment 1. We have no explanation for this between-experiment variation, although we note that these experiments were conducted months apart and in different laboratory rooms. As a further assurance that 5–8-hr components of the chimeric patterns may be safely considered to be reproductively

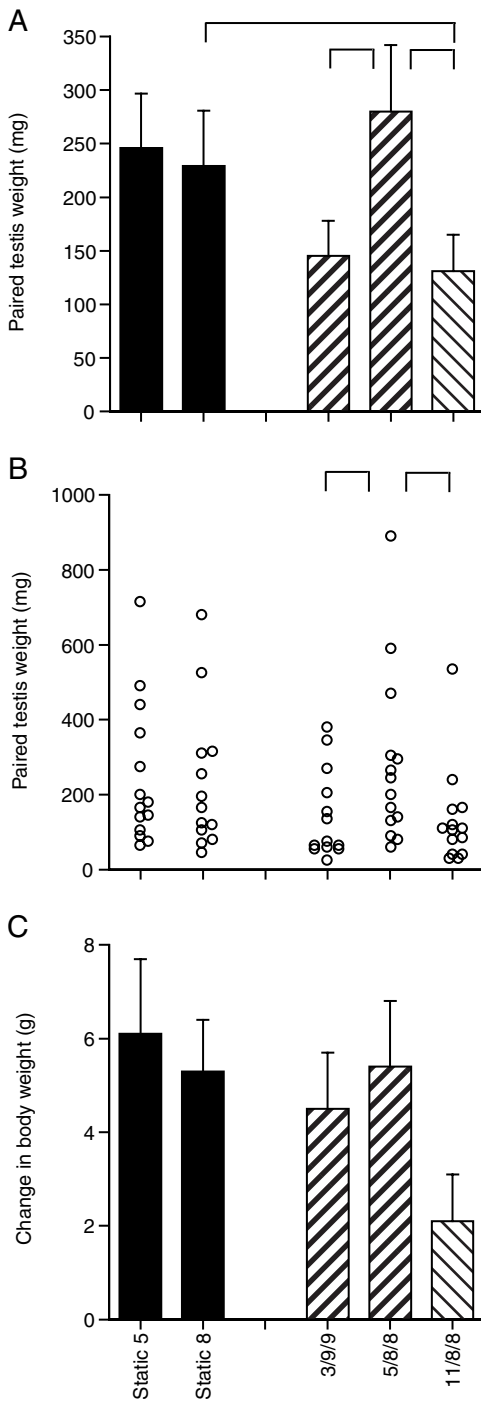


Fig. 2. Mean + S.E.M. (A) and individual (B) paired testis weights and mean change in body weight (C) after 6 weeks of melatonin infusions described in the text. Conventions as in Fig. 1.

stimulatory, 5-hr and even 4-hr signals have been repeatedly demonstrated to stimulate gonadal development in similar experimental paradigms [23, 29, 30]. No validation is available, however, for the stimulatory effects of 3-hr infusions. To our knowledge only one published study in Siberian hamsters has assessed the effects of melatonin durations shorter than 4 hr. In that report, infusions of 1-hr

duration failed to induce reproductive growth in hamsters pinealectomized at 15 days of age and infused for 2 weeks thereafter [12]. These short melatonin infusions were functionally equivalent to the complete absence of melatonin, which in Siberian hamsters, is neither intrinsically inhibitory nor stimulatory to the gonadal axis [7, 8, 31].

Contrary to expectations when Experiment 1 was designed, daily 8-hr signals stimulated gonadal growth. Group 5/8/8, therefore, received stimulatory melatonin components daily, and this group fails to provide a critical test of whether gonadal growth could be provoked with a short signal only every third night. Moreover, an unplanned post hoc comparison paradoxically revealed this group to be reproductively inhibited compared with static 8-hr infusions ($P < 0.05$), a result predicted neither by a signal averaging nor a signal frequency model of melatonin integration. Because much evidence suggests that interpretation of intermediate duration melatonin and day length signals may depend on photoperiodic context [17, 32], we hypothesized post hoc that static 8-hr infusions might only be stimulatory by comparison with the longer endogenously produced signals prior to the infusions. Intermittent exposure to 5-hr infusions might therefore disambiguate the interpretation of 8-hr signals, causing them to be perceived as inhibitory. As they occurred only every third night, the 5-hr signals may have been infrequent enough to provoke gonadal growth but sufficient to provide a photoperiodic context. Experiment 2 provided no support for this conjecture, however, as Group 5/8/8 failed to differ from either daily 5- or 8-hr infusions. Because an interpretation of 8-hr infusions as stimulatory would not be expected to be reversed by the intermittent inclusion of very long 11-hr signals (i.e. in Group 11/8/8), the gonadal inhibition evident in that group establishes that even a high frequency of stimulatory signals (two of three) is insufficient to induce gonadal growth under some conditions. Thus, the present study contains numerous findings inconsistent with a fixed frequency dependent response to categorically interpreted 'long' and 'short' melatonin signals.

If the gonadal response cannot be predicted on the basis of the frequency of short duration melatonin signals, can it be adequately explained by a mechanism that calculates the arithmetic mean of the various infusion durations? Prior to this report, the few relevant infusion studies were generally consistent with such an averaging model: among hamsters with large testes, 10-hr melatonin infusions presented every 48 hr failed to induce gonadal regression and were functionally equivalent to daily 5-hr infusions in this regard [33, 34]. In the present Experiment 1, the testis data of the chimeric hamsters in no case differed significantly from those of hamsters infused with the same average duration. The body weight response, however, differed between hamsters in Group 6/12 and Group 5/11/11 despite the fact that the average melatonin duration was 9 hr in both cases. This discrepancy between dependent measures is not unprecedented, however, as the photoperiodic regulation of body weight is mediated by changes in prolactin as well as in the gonadal axis [35–37]. These two systems, moreover, appear to be differentially regulated by photoperiod [11, 38, 39]. Indeed, pelage coloration, also mediated principally through prolactin secretion, and gonad size exhibited

different patterns of frequency dependence on night-time propranolol injections [22].

With respect to the reproductive axis, the averaging model is not supported by the differential gonadal response in Experiment 2 of hamsters in Groups 3/9/9 and 5/8/8, which both received average melatonin durations of 7 hr. Whereas gonad size in Group 5/8/8 was consistent with a static short duration melatonin signal, hamsters in Group 3/9/9 were reproductively inhibited. Thus, the only group response not explicable in terms of an averaging hypothesis is that which received the shortest (i.e. 3 hr) melatonin durations. As noted above, somewhere between 4 and 1 hr in duration is a response threshold below which daily melatonin infusions no longer stimulate gonadal development. An averaging model could be sustained if there is a comparable threshold for the mechanism that integrates melatonin signals over successive days.

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References

- GORMAN MR, GOLDMAN BD, ZUCKER I. Mammalian photoperiodism. In: Circadian Clocks. Takahashi JS, Turek FW, Moore RY, eds, Kluwer, New York, 2001; pp. 481–510.
- WEHR TA. Seasonal photoperiodic responses of the human circadian system. In: Circadian Clocks. Takahashi JS, Turek FW, Moore RY, eds, Kluwer, New York, 2001; pp. 715–744.
- WEINER J. Limits of energy budget and tactics in energy investments during reproduction in the Djungarian hamster (*Phodopus sungorus sungorus* Pallas 1770). Symposia of the Zoological Society of London 1987; **57**:167–187.
- GOLDMAN BD. The Siberian hamster as a model for study of the mammalian photoperiodic mechanism. *Adv Exp Med Biol* 1999; **460**:155–164.
- HOFFMANN K. The critical photoperiod in the Djungarian hamster *Phodopus sungorus*. In: Vertebrate Circadian Systems: Structure and Physiology. Aschoff J, Daan S, Gross G, eds, Springer, New York, 1982; pp. 297–304.
- DARROW JM, GOLDMAN BD. Circadian regulation of pineal melatonin and reproduction in the Djungarian hamster. *J Biol Rhythms* 1985; **1**:39–54.
- CARTER DS, GOLDMAN BD. Antigonadal effects of timed melatonin infusion in pinealectomized male Djungarian hamsters (*Phodopus sungorus sungorus*): duration is the critical parameter. *Endocrinology* 1983; **113**:1261–1267.
- CARTER DS, GOLDMAN BD. Progonadal role of the pineal in the Djungarian hamster (*Phodopus sungorus sungorus*): mediation by melatonin. *Endocrinology* 1983; **113**:1268–1273.
- BARTNESS TJ, POWERS JB, HASTINGS MH et al. The timed infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses? *J Pineal Res* 1993; **15**:161–190.
- GOLDMAN BD. Parameters of the circadian rhythm of pineal melatonin secretion affecting reproductive responses in Siberian hamsters. *Steroids* 1991; **56**:218–225.
- DUNCAN MJ, GOLDMAN BD, DiPINTO MN et al. Testicular function and pelage color have different critical daylengths in the Djungarian hamster, *Phodopus sungorus sungorus*. *Endocrinology* 1985; **116**:424–430.
- GUNDUZ B, STETSON MH. A test of the coincidence and duration models of melatonin action in Siberian hamsters: the effects of 1-hr melatonin infusions on testicular development in intact and pinealectomized prepubertal *Phodopus sungorus*. *J Pineal Res* 2001; **30**:97–107.
- GUNDUZ B, STETSON MH. A test of the coincidence and duration models of melatonin action in Siberian hamsters. II. The effects of 4- and 8-hr melatonin infusions on testicular development of pinealectomized juvenile Siberian hamsters (*Phodopus sungorus*). *J Pineal Res* 2001; **30**:56–64.
- HORTON TH. Growth and maturation in *Microtus montanus*: effects of photoperiods before and after weaning. *Can J Zool* 1984; **62**:1741–1746.
- NIKLOWITZ P, LERCHL A, NIESCHLAG E. Photoperiodic responses in Djungarian hamsters (*Phodopus sungorus*): importance of light history for pineal and serum melatonin profiles. *Biol Reprod* 1994; **51**:714–724.
- STETSON MH, RAY SL, CREYAUFMILLER N et al. Maternal transfer of photoperiodic information in Siberian hamsters. II. The nature of the maternal signal, time of signal transfer, and the effect of the maternal signal on peripubertal reproductive development in the absence of photoperiodic inputs. *Biol Reprod* 1989; **40**:458–465.
- HOFFMANN K, ILLNEROVA H, VANECEK J. Change in duration of the nighttime melatonin peak may be a signal driving photoperiodic response in the Djungarian hamster (*Phodopus sungorus*). *Neurosci Lett* 1986; **67**:68–72.
- SHAW D, GOLDMAN BD. Influence of prenatal photoperiods on postnatal reproductive responses to daily infusions of melatonin in the Siberian hamster (*Phodopus sungorus*). *Endocrinology* 1995; **136**:4231–4236.
- GORMAN MR, ZUCKER I. Pattern of change in melatonin duration determines testicular responses in Siberian hamsters, *Phodopus sungorus*. *Biol Reprod* 1997; **56**:668–673.
- EARNEST DJ, TUREK FW. Periodic exposure to a brief light signal stimulates neuroendocrine-gonadal activity in golden hamsters. *J Androl* 1984; **5**:64–69.
- CHAMPNEY TH. β -Adrenergic blockers prevent short photoperiod-induced gonadal regression, but not melatonin-induced regression in male Syrian hamsters. *J Exp Zool* 1989; **249**:221–228.
- PRENDERGAST BJ, ZUCKER I, YELLON SM et al. Melatonin chimeras alter reproductive development and photorefractoriness in Siberian hamsters. *J Biol Rhythms* 1998; **13**:518–531.
- FLYNN AK, FREEMAN DA, ZUCKER I et al. Testicular development in Siberian hamsters depends on frequency and pattern of melatonin signals. *Am J Physiol* 2000; **279**:R1182–R1189.
- STIEGLITZ A, GWINNER K, SPIEGELHALTER F et al. Urinary 6-sulphatoxymelatonin as an index of pineal function in the Djungarian hamster: influence of photoperiod and ambient temperature. In: Advances in Pineal Research. Møller M, Pévet P, eds, John Libbey, New York, 1994; pp. 285–291.
- PRENDERGAST BJ, KELLY KK, ZUCKER I et al. Enhanced reproductive responses to melatonin in juvenile Siberian hamsters. *Am J Physiol* 1996; **271**:R1041–R1046.

26. GORMAN MR. Seasonal adaptations of Siberian hamsters: I. Accelerated gonadal and somatic development in increasing versus static long day lengths. *Biol Reprod* 1995; **53**:110–115.
27. BARTNESS TJ, GOLDMAN BD. Effects of melatonin on long-day responses in short-day housed adult Siberian hamsters. *Am J Physiol* 1988; **255**:R823–R830.
28. BARTNESS TJ, GOLDMAN BD. Peak duration of serum melatonin and short-day responses in adult Siberian hamsters. *Am J Physiol* 1988; **255**:R812–R822.
29. FREEMAN DA, LARKIN JE, SELIBY L. Testicular and somatic growth in Siberian hamsters depend on the melatonin-free interval between twice daily melatonin signals. *J Neuroendocrinol* 2002; **14**:228–233.
30. PRENDERGAST BJ, HUGENBERGER JL. Frequency coding of melatonin signals sufficient to induce testicular growth in photoregressed Siberian hamsters. *J Neuroendocrinol* 1999; **11**:237–241.
31. KELLY KK, GOLDMAN BD, ZUCKER I. Gonadal growth and hormone concentrations in photoregressed Siberian hamsters: pinealectomy versus photostimulation. *Biol Reprod* 1994; **51**:1046–1050.
32. GORMAN MR, ZUCKER I. Seasonal adaptations of Siberian hamsters. II. Pattern of change in day length controls annual testicular and body weight rhythms. *Biol Reprod* 1995; **53**:116–125.
33. ELLIOTT JA, BARTNESS TJ, GOLDMAN BD. Effect of melatonin infusion duration and frequency on gonad, lipid, and body mass in pinealectomized male Siberian hamsters. *J Biol Rhythms* 1989; **4**:439–455.
34. MAYWOOD ES, BUTTERY RC, VANCE GHS et al. Gonadal responses of the male Syrian hamster to programmed infusions of melatonin are sensitive to signal duration and frequency but not to signal phase nor to lesions of the suprachiasmatic nuclei. *Biol Reprod* 1990; **43**:174–182.
35. WADE GN, BARTNESS TJ. Effects of photoperiod and gonadectomy on food intake, body weight, and body composition in Siberian hamsters. *Am J Physiol* 1984; **246**:R26–R30.
36. VITALE PM, DARROW JM, DUNCAN MJ et al. Effects of photoperiod, pinealectomy and castration on body weight and daily torpor in Djungarian hamsters (*Phodopus sungorus*). *J Endocrinology* 1985; **106**:367–375.
37. NIKLOWITZ P, HOFFMANN K. Pineal and pituitary involvement in the photoperiodic regulation of body weight, coat color and testicular size of the Djungarian hamster, *Phodopus sungorus*. *Biol Reprod* 1988; **39**:489–498.
38. SHAW D, GOLDMAN BD. Influence of prenatal and postnatal photoperiods on postnatal testis development in the Siberian hamster (*Phodopus sungorus*). *Biol Reprod* 1995; **52**:833–838.
39. DONHAM RS, PALACIO E, STETSON MH. Dissociation of the reproductive and prolactin photoperiodic responses in male golden hamsters. *Biol Reprod* 1994; **51**:366–372.