Testicular development in Siberian hamsters depends on frequency and pattern of melatonin signals

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Flynn, Alison K., David A. Freeman, Irving Zucker, and Brian J. Prendergast. Testicular development in Siberian hamsters depends on frequency and pattern of melatonin signals. Am J Physiol Regulatory Integrative Comp Physiol 279: R1182–R1189, 2000.—We investigated the impact of frequency and pattern of melatonin signals on reproductive development in Siberian hamsters. Juvenile males gestated in short day lengths and housed in constant illumination to suppress melatonin secretion were infused with melatonin for 5 h either once or twice per day for 20 days. Melatonin infusions at either frequency produced equivalent increases in testes and body weights that exceeded those of animals infused with saline but were indistinguishable from those of hamsters transferred to long day lengths. The reproductive system appears to be maximally stimulated by a single short melatonin signal per day. Other animals kept from birth in a short photoperiod were treated 6 h after onset of darkness with the β-adrenergic receptor antagonist dL-propranolol to shorten melatonin secretion on the night of injection but not on subsequent nights. This permitted interpolation of short nightly melatonin signals of 4–5 h duration against a background of long melatonin signals of 10–12 h duration on other nights. Treatment regimes that maintained a 1:1 ratio of short to long melatonin signals for 8 wk stimulated reproductive development; a 1:2 signal ratio, in each of three different patterns, was uniformly ineffective. The number of successive short melatonin signals had little influence on the interval across which successive melatonin signals were summated to influence photoperiodic traits. The neuroendocrine axis appears more responsive to short melatonin signal frequency than pattern for development of the reproductive system. These experiments employed lighting regimes that addressed, albeit indirectly, in Syrian hamsters provided with intermittent light exposure. Males transferred from a stimulatory photoperiod to constant darkness and given 24-h light pulses every 4, 8, or 12 days for 73 days had heavier testes than those left in uninterrupted darkness (10). This and another experiment (8) revealed that infrequent light exposure achieves at least partial maintenance of the reproductive apparatus. These experiments employed lighting regimes that alter melatonin secretion for an indeterminate number of nights (21) and thus do not permit conclusions regarding melatonin signal frequencies sufficient to sustain reproduction.

Day length mediates seasonal effects on the neuroendocrine axis by controlling the duration of nocturnal pineal melatonin secretion, which is proportional to the length of the scotophase. Melatonin duration is thus an accurate endocrine representation of day length. In the Siberian hamster nightly melatonin secretion endures for 5 h under long days and for 10 h under short days (5). Pinealectomized hamsters infused nightly for 5 h or 10 h with melatonin adopt summer and winter reproductive phenotypes, respectively (1). Duration of the nocturnal melatonin signal is widely accepted as the principal means for transducing seasonal effects of day length on reproductive physiology. An alternative hypothesis, that a circadian rhythm of sensitivity to melatonin determines whether melatonin exerts pro- or anti-gonadal effects (27), has been criticized (18, 23). The present approach is predicated on the assumption that the duration of the nightly melatonin signal is the preeminent determinant of seasonal effects of day length.

Although melatonin signals normally are generated every night, it is unclear whether nightly signals are needed to promote gonadal growth or whether less frequent signals will suffice. Thus the minimum signal frequencies necessary and sufficient to maintain gonadal stimulation are not well specified, nor are the formal rules governing the manner in which a series of successive melatonin signals are translated into altered gonadotrophic output. This issue was first addressed, albeit indirectly, in Syrian hamsters provided with intermittent light exposure. Males transferred from a stimulatory photoperiod to constant darkness and given 24-h light pulses every 4, 8, or 12 days for 73 days had heavier testes than those left in uninterrupted darkness (10). This and another experiment (8) revealed that infrequent light exposure achieves at least partial maintenance of the reproductive apparatus. These experiments employed lighting regimes that alter melatonin secretion for an indeterminate number of nights (21) and thus do not permit conclusions regarding melatonin signal frequencies sufficient to sustain reproduction.

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Short-duration nightly melatonin infusions of 4–6 h duration stimulate gonadal growth in Siberian hamsters if provided on a daily basis but are ineffective if administered less frequently (28). Likewise, long-duration melatonin infusions of 10- to 12-h duration induce gonadal regression if provided at 24- but not 36- or 48-h intervals (9). In Syrian hamsters, long-duration melatonin infusions given at 24- or 20-h intervals provoke gonadal regression, but frequencies that deviate substantially from 24 h are less effective (25). Neuroendocrine responsiveness to melatonin may be frequency tuned such that signals that recur at the endogenous frequency of once every day most effectively produce appropriate physiological responses. Complicating this interpretation is the contention that hamsters measure day length “not solely by the presence of melatonin... but also by the length of its absence...” (25); i.e., the melatonin-free interval between successivesignals may be a major determinant of substrate responsiveness to melatonin. Elliott and colleagues (9) also raised this issue in speculating that there may be a refractory period after each melatonin signal, during which the system is temporarily unresponsive to the hormone while it resets in preparation for the next measurement of melatonin duration. This conjecture is incompatible with the demonstration that several weeks of daily treatment with 9-h (long) and 3-h (short) melatonin infusions, separated by a 3-h melatonin-free interval, yielded testis weights intermediate between those maintained by either signal alone (11). Summation appears possible even when the second daily melatonin signal falls during the postulated refractory period induced by the first signal.

The present study sought to specify the respective contributions of melatonin signal frequency and pattern for reproductive development in prepubertal hamsters. It is commonly assumed that gonadal growth evoked by long day lengths represents the maximal degree of reproductive stimulation that can be elicited by melatonin; however, this conjecture has never been adequately tested, as photoperiodic treatments provide only one melatonin signal per 24-h interval. Experiment 1 tested whether the endogenous frequency of a single short-duration melatonin pulse once per day provokes maximal testicular growth or whether more frequent short-duration melatonin signals stimulate greater or more rapid gonadal and somatic development. Spacing of the two daily signals as far apart as possible maximized the melatonin-free interval at 7 h and provided a strong test of the hypothesis that the gonadal axis can respond to multiple daily stimulatory signals.

Paradoxically, inductive effects on reproductive development are evident when short melatonin signals are presented every other day against a background of long melatonin signals; i.e., melatonin-responsive neuroendocrine targets bridge a gap of 48 h between short melatonin signals when long-duration melatonin signals are interspersed (30). These data suggested that the presence of any melatonin (even long-duration signals) at approximately daily intervals may serve to maintain reproductive responsiveness to short-melatonin signals; this experiment did not, however, assess whether the pattern of melatonin signals contributes to melatonin signal processing. A sequence of 2 or 3 consecutive days of short melatonin signals might permit bridging of longer intervals, not possible after only a single short melatonin signal. In support of this hypothesis, two consecutive melatonin signals of maternal origin communicate photoperiodic information to the hamster fetus, whereas one signal is ineffective (34), and two long-duration signals are far more effective than a single signal in inhibiting reproductive development in juvenile hamsters (12). Therefore, Experiment 2 evaluated the relative contribution of the melatonin pattern (number of consecutive short- or long-duration signals) and frequency (number of short signals/unit time) on gonadal development in photoregressed male hamsters.

GENERAL METHODS

Siberian hamsters (Phodopus sungorus) were from our local colony which was established in 1985 and originally derived from wild-caught stock trapped near Omsk, Soviet Union, and outbred once. Hamsters were housed in polypolyethylene cages (1–4 hamsters/cage) from the time of weaning. Food (mouse chow no. 5015; Purina Mills, St. Louis, MO) and tap water were available ad libitum, and ambient temperature was held at 21 ± 2°C.

Experiment 1: High-Frequency Short Melatonin Signals

Animals. Adult male-female pairs were formed from colony hamsters housed in a 16-h light photoperiod (16L; lights on at 0200 h, Pacific Standard Time) and transferred to 10-h light photoperiod (10L; lights on at 0800 h) 4 days later. Cages were inspected daily for presence of litters beginning 17 days after pairing. Male offspring were weaned at 14 days of age (day 14) and transferred during the light phase to a constantly illuminated chamber (LL, 300–700 lux at cage level); LL suppresses endogenous melatonin secretion in Siberian hamsters (32).

Infusions. On day 18, each male hamster was anesthetized with methoxyflurane vapors (Metofane; Pitman-Moore, St. Louis, MO) and implanted with a subcutaneous polyethylene infusion catheter, subsequently connected to a programmable timed-infusion pump (29). Hamsters in LL were infused with melatonin (Mel; 100 ng/infusion, in saline vehicle) for 5 consecutive hours either once (1030–1530 h; n = 17) or twice (1030–1530 h and 2230–0330 h; n = 17) per 24-h interval. One group of control hamsters kept in LL (2 × Sal; n = 16) and a second transferred to 16L on day 18 (2 × Sal; n = 17) were infused twice daily with saline vehicle at times corresponding to those of hamsters that received twice daily melatonin infusions. An initial control group (n = 17) killed on day 18 provided baseline (preinfusion) somatic and reproductive data. Infusions were administered from days 18 to 38.

Measurements. Body weights (BW; ± 0.1 mg) and paired testis weights (PTW; ± 0.1 mg) were determined at necropsy.

Experiment 2: Melatonin Signal Number vs. Pattern of Melatonin Signals

Animals. Adult male and female hamsters housed in a 16L photoperiod (lights on 0900–0100 h) were paired and monitored daily for the presence of litters. On the day of birth,
dam and pups were transferred to 10L (lights on 1500–0100 h). Male offspring were weaned at 18 days of age, randomly assigned to treatment groups, and housed in 10L with age-matched conspecifics (1–4 hamsters/cage). Cages were kept in environmental chambers fitted with dim red lights that remained on continuously to facilitate treatment during the dark phase. Beginning at 29–31 days of age (week 0), animals were injected subcutaneously with 0.1 ml of the β-adrenergic antagonist β1-propranolol (PR; 0.5 mg in 0.1 ml of 0.9% saline; Fig. 1A); this drug inhibits melatonin secretion beginning shortly after injection and eliminates circulating melatonin for the remainder of the dark phase without altering secretion on the subsequent night (30; Fig. 1B). Propranolol or 0.1 ml saline (Sal) was injected daily 6 h after onset of darkness (0700 h) for 8 consecutive weeks according to the treatment schedules summarized in Fig. 1C. Hamsters (n = 12–16/group) received propranolol injections every other day (PR2), for 2 consecutive days followed by 2 days of no treatment (PR22), every third day (PR3), for 2 consecutive days followed by 2 days of no treatment (PR32), or for 3 consecutive days followed by 6 days of no treatment (PR33). Sal injections were provided for 3 consecutive days followed by 6 consecutive days of no treatment (i.e., on the PR33 schedule). Treatments were discontinued after 8 wk for hamsters in the PR2 and PR22 treatment groups (a total of 28 propranolol injections) and for a subset (n = 12–13) of hamsters in the PR3, PR32, and PR33 groups (a total of 19 propranolol injections). So as to provide PR3, PR32, and PR33 hamsters with the same number of propranolol injections (=28) as the two PR2 groups, PR3, PR32, and PR33 hamsters (n = 6–8/group) received an additional 4 wk of nightly injections. Subsets of Sal-treated hamsters were likewise injected for either 8 or 12 wk.

**Measurements.** BW (±0.1 g) and PTW (±0.1 mg) were determined at necropsy on week 8 or week 12. Pelage (fur) color was assessed on a nominal scale that ranged from 1 (dark, “summer” fur) to 4 (white, “winter” fur), without knowledge of the animals' treatment condition (7).

**Nonresponder exclusions.** Some Siberian hamsters fail to undergo gonadal regression in short day lengths (19). These “nonresponders” exhibit atypical durations of nightly melatonin secretion and locomotor activity and maintain their reproductive apparatus irrespective of ambient short photo-periods (31). Prior to the initiation of propranolol treatments at week 0, estimated testis volumes (ETV) were determined for all hamsters by externally measuring the left testis; volume calculation was based on a formula for an elongated spheroid (14). Two of 108 individuals were classified as non-responders and excluded from all subsequent analyses because their ETV exceeded twice the mean week 0 ETV among all animals.

**Statistical Analyses**

BWs and PTWs were analyzed separately by repeated-measures analyses of variance. Where significant F ratios were obtained, pairwise comparisons between treatment groups were conducted using the Fisher protected least significant difference test. Pelage scores were compared with the nonparametric Kruskal-Wallis and Mann-Whitney U tests for pairwise comparisons (Statview 4.01; Abacus Concepts, Berkeley, CA). Observed differences were considered significant if P < 0.05.

**RESULTS**

**Experiment 1:** High-Frequency Short Melatonin Signals

**Paired testis weights.** Significant differences in PTW were detected after 20 days of infusions (F = 7.49; df = 3.63; P < 0.001; Fig. 2A). Over the course of treatment, testis weights increased in all groups relative to initial control values (P < 0.01, all comparisons). Hamsters housed in LL and infused with saline twice per day (2 × SalLL) had lighter testes (201 ± 44 mg) than those housed in 16L and treated similarly (2 × SalLD; 458 ± 29 mg; P < 0.001). Five-hour melatonin infusions once per day (1 × Mel) elevated PTW of hamsters relative to values of 2 × SalLL animals (P < 0.001). Testes of hamsters infused for 5 h with melatonin twice per day (2 × Mel) were also significantly heavier than those of 2 × SalLL animals (P < 0.001); testis weights of 2 × Mel-treated hamsters did not, however, differ from those of 1 × Mel-treated hamsters (392.7 ± 39 vs. 378.9 ± 45 mg, respectively). Animals in LL infused with melatonin either once or twice per day had PTW that did not differ from those of hamsters housed in 16L and infused with saline (P > 0.20 in each case).

**Body weights.** BW increased in all groups over the course of treatment (P < 0.001 vs. initial control values, all comparisons). On day 38, BW differed across
treatment groups \((F = 3.66; \text{df} = 3.63; \ P < 0.05; \text{Fig. 2B})\) in a pattern identical to that obtained for testis weights. Sal hamsters in LL were lighter than those in all other groups \((P < 0.05, \text{all comparisons})\). BW among hamsters transferred to long days and infused with saline were indistinguishable from those in LL that were infused with melatonin once or twice per day \((P > 0.40, \text{all comparisons})\).

**Experiment 2: Melatonin Signal Number vs. Pattern of Melatonin Signals**

**Week 8.** **Paired Testis Weights.** Significant differences in PTW were observed after 8 wk of treatment \((F = 7.2; \text{df} = 5.74; \ P < 0.001; \text{Fig. 3A})\). Hamsters injected with Sal sustained regressed testes throughout the first 8 wk of treatment. Animals in the PR3, PR32, and PR33 groups also failed to undergo testicular growth; week 8 PTW values for these groups did not differ from those of Sal-treated hamsters \((P > 0.10, \text{all comparisons})\), nor were any of the PR3 treatments statistically distinguishable from one another \((P > 0.20, \text{all comparisons})\). In contrast, substantial testicular growth was evident in PR2- and PR22-treated hamsters. Each of the PR2 treatments resulted in testis weights that significantly exceeded those observed in PR3-, PR32-,
PR3\textsuperscript{3}, or Sal-treated hamsters (\(P < 0.01\), all comparisons). PTW of PR2 and PR2\textsuperscript{2} hamsters did not differ (\(P > 0.40\)) from each other.

**Body Weights.** BW did not differ significantly among treatment groups after 8 wk of injections (\(F = 1.5; \text{df} = 5, 74; P > 0.10\); Fig. 3B).

**Pelage.** Propranolol injections significantly affected pelage molt among treatment groups (\(H = 43; \text{df} = 5; P < 0.001\); Fig. 3C). All 13 PR2-treated hamsters, and 14 of 16 PR2\textsuperscript{2}-treated hamsters exhibited the dusky *stage 1* (summer) pelage after 8 wk of injections; in contrast, 11 of 13 Sal-treated hamsters manifested the white *stage 4* (winter) pelage (\(P < 0.001\)) at week 8. The intermediate pelage scores of PR3, PR3\textsuperscript{2} and PR3\textsuperscript{3} hamsters (median scores of 2, 3, and 2, respectively) each differed significantly from those of both PR2 and PR2\textsuperscript{2} hamsters (\(P < 0.01\), all comparisons) and from Sal controls (\(P < 0.005\), all comparisons) but did not differ from each other (\(P > 0.10\), all comparisons).

**Week 12. Paired Testis Weights.** After an additional 4 wk of saline and propranolol injections, PTW among groups treated with propranolol once every third day in each of three different patterns did not differ from those of Sal controls (\(F = 1.2; \text{df} = 3, 23; P > 0.30\); Fig. 4A). Week 12 PTW values of PR3-, PR3\textsuperscript{2}-, and PR3\textsuperscript{3}-treated hamsters did not differ significantly from one another (\(P > 0.10\), all comparisons), nor did they differ from those of Sal-treated hamsters (\(P > 0.30\), all comparisons). Testis weights of PR3-treated hamsters were significantly lower than those of PR2-treated hamsters at week 8 (\(t = 2.69, \text{df} = 17, P < 0.05\)).

**Body Weights.** No differences were observed between any of the PR3 and Sal groups (\(F = 0.7; \text{df} = 3, 23; P > 0.50\); Fig. 4B).

**Pelage.** The overall patterns of fur color did not differ statistically among groups in which injection treatments were extended until week 12 (median pelage scores = 3 or 4 in all groups; \(H = 4.8; \text{df} = 3; P > 0.10\); Fig. 4C). The pelage of PR3 hamsters was significantly darker than that of PR3\textsuperscript{3} hamsters (\(P < 0.05\)), although this group was indistinguishable from Sal- or PR3\textsuperscript{2}-treated hamsters.

**Discussion**

Gonadal and somatic development were accelerated in photoregressed Siberian hamsters administered one or two 5-h melatonin signals/day for 20 consecutive days. Both melatonin infusion frequencies induced equivalent somatic and testicular growth, indistinguishable from that recorded in hamsters transferred from short to long day lengths. The single daily 5-h melatonin signal, which matches duration and frequency characteristics of melatonin secretion in Siberian hamsters housed in spring day lengths (5), may be optimal for reversing inhibitory effects of short days on the neuroendocrine axis. Nightly changes in the duration of melatonin secretion during the spring accelerate reproductive development in young born at the beginning of the breeding season and break refractoriness to short day lengths in overwintering animals (reviewed in Ref. 13).

Why does the second nightly melatonin signal fail to increase gonadal growth? One possibility is that sub-
strate refractoriness to melatonin may begin shortly after the end of the first 5-h melatonin signal, thereby negating effectiveness of the successor signal. The importance of a melatonin-free interval, emphasized previously in relation to long melatonin signals (25) presumably also applies in the present context. If it exists, a discrete episode of daily refractoriness to melatonin (not to be confused with seasonal refractoriness to melatonin after ~20 wk of exposure to short day lengths) is not simply a matter of insensitivity after 5 h of elevated circulating melatonin. This conclusion follows from the observation that a 10-h melatonin signal produces effects on the reproductive system opposite to those of a 5-h signal (gonadal inhibition vs. gonadal growth; Ref. 4). Perhaps daily refractoriness only begins several hours after the end of each nightly episode of melatonin secretion, regardless of its duration; melatonin target tissues thus may remain responsive to uninterrupted nightly melatonin signals of 4–12 h duration, but once secretion terminates, responsiveness to melatonin is diminished for the remainder of the night.

In maximizing the melatonin-free interval between twice daily 5-h melatonin infusions to 7 h, we reduced the likelihood of “false-negative” conclusions regarding responsiveness to multiple melatonin signals in a single day. Earlier studies that provided multiple short-duration melatonin infusions per 24 h, conducted with different goals in mind, did not combine photostimulatory melatonin signals with long melatonin-free intervals (12, 27). The single exception alternated 6-h melatonin infusions with 12-h melatonin-free intervals for several weeks (T = 18-h melatonin cycle); deployment of adult male hamsters that had large gonads when melatonin treatment began, however, minimized the possibility of detecting photostimulatory effects of increased melatonin signal frequencies (9). In the present study, melatonin treatments which lasted for 20 days, yielded gonadal weights at necropsy well below the maximal possible testis weights in adults (19). It is unlikely, therefore, that the second melatonin signal produced more rapid growth during the early phases of treatment or that “ceiling” effects on testicular dimensions limited the response to twice daily melatonin infusions.

We cannot discount the possibility that some components of the neuroendocrine system are responsive to the second of the two daily short melatonin signals. Each short melatonin signal may provoke increased gonadotrophin secretion without, however, accelerating reproductive development. This would transpire if testis growth rates were asymptotically high at the lower gonadotrophin concentrations associated with the single daily melatonin signal. The manner in which any additional stimulatory information accruing from the second melatonin signal is lost remains unknown. Neuroendocrine cells with melatonin receptors may be incapable of initiating duration-specific gonadotrophin-releasing hormone (GnRH) gene transcription or translation more than once in a 24-h period. The present data, although compelling rejection of the hypothesis that the gonads are responsive to more than one short melatonin signal per day, do not delineate the mechanisms underlying such nonresponsiveness; these data leave open the possibility that some components of the system track higher frequency signals. This issue could be resolved by measurement of GnRH protein in the brain or circulating concentrations of gonadotrophins or prolactin in hamsters that receive one vs. two 5-h melatonin infusions per day.

Whether reproductive inhibition can be accelerated by multiple daily long-duration melatonin signals is more difficult to answer because two 10-h signals per 24 h allow for only a brief melatonin-free interval. The time required to clear melatonin from the circulation at the end of the first infusion (27, 33), and possible receptor occupancy by melatonin for some time thereafter, may vary as a function of the duration of the particular long-duration melatonin signal (17). Consequently, with multiple daily long-duration infusions, the melatonin-free interval may be short or nonexistent. This could be problematic, because two melatonin signals separated by 1 h are read as a single longer-duration signal (12). The data for long-duration melatonin signals presented in a T-cycle paradigm (9, 25) nevertheless suggest a frequency-response curve with maximum reproductive inhibition to signals at circadian intervals. Maximal gonadal responsiveness to short- and long-duration melatonin signals at a frequency of one per day may be yet another instance in which physiological systems have co-opted circadian organization.

When long- and short-duration melatonin signals were alternated on successive days, the gonads underwent development; gonadal growth failed, however, when two long-duration signals intervened between successive short signals. We concluded previously that in hamsters with regressed or undeveloped gonads short melatonin signals stimulate gonadal development only if spaced no more than 48 h apart, i.e., that the number of successive long-duration melatonin signals determines reproductive responses to intervening short melatonin signals (30). The present data contradict this conjecture. Animals that received a repeating sequence of two consecutive long melatonin signals (i.e., an interval >48 h without a short-duration melatonin signal) sustained gonadal growth provided two consecutive short melatonin signals followed the two long signals. The pattern of presentation evidently affected the interval across which successive short melatonin signals were summated to establish the long-day phenotype. The number of successive short signals, however, does not by itself determine reproductive status. Two successive short signals were stimulatory when followed by two successive long signals, but not when followed by four successive long signals. Clearly, a given pattern of either short or long melatonin signals alone that discounts signal frequency is not sufficient to ensure gonadal development. The variable that best predicted reproductive responses at the end of the 8-wk treatment interval was the overall frequency of short melatonin signals. Animals that received a ratio
of 1:1 and 1:2 short to long signals responded with gonadal growth and inhibition, respectively, regardless of which of the several melatonin signal patterns were delivered.

An alternative hypothesis is that hamsters were responding to the total number of short melatonin signals received. One way to test for a frequency- vs. number-based mechanism is to provide groups with different short-duration melatonin frequencies for an extended interval, such that the number of short signals approaches equality but signal frequency differs for the two groups. We approximated such a test by extending injection treatments in some PR3, PR3², and PR3³ hamsters to 12 wk; these individuals received the same number of short melatonin signals (28 signals) that the PR2 and PR2² had received by week 8. Nevertheless, testis weights of PR3 hamsters at week 12 were still significantly below those of PR2-treated hamsters at week 8. Reproductive stimulation from 28 short melatonin signals is frequency sensitive.

The balanced ratio of short to long signals provided by the PR2 regimen may be equivalent to an intermediate, history-dependent signal. The response to intermediate day lengths and melatonin signals is dictated by the hamster’s prior photoperiodic history (7, 15, 20). The PR2 regimen would under such circumstances promote gonadal growth because antecedent day lengths from birth to initiation of treatment had provided a longer melatonin signal. This conjecture would require that PR3 represent an unambiguously short (i.e., history-independent) day length and that the neuroendocrine system averages the durations of melatonin signals over a longer than daily interval. In the present study hamsters were gestated in long day lengths and maintained from birth in short photoperiods. The neuroendocrine system may regard the PR2 dividend as an intermediate-duration melatonin signal (shorter in absolute duration than the photoperiodic history) and therefore initiate gonadal growth.

As in previous experiments, melatonin signal integration over the course of several weeks was trait specific (7, 30). In contrast to the categorical effects of the various propranolol treatments on the gonadal axis (i.e., either fully stimulatory or inhibitory), pelage color exhibited a graded dependence on short melatonin signal frequency: pelage color of PR3-treated hamsters was intermediate between that of PR2- and Sal-treated hamsters. Testicular size is influenced mainly by circulating concentrations of gonadotrophins, whereas pelage color is mainly dependent on prolactin secretion (6). That a short melatonin signal every third day does not affect gonadotrophic mechanisms, but is attended to by mechanisms responsible for pelage coloration, agrees with prior functional and neuroanatomical dissociations of photoperiodic gonadotrophic and lactotrophic outputs in other photoperiodic rodents (24, 26). Melatonin-mediated day length information appears to be processed by a system of parallel distributed effector pathways.

In summary, Siberian hamsters do not accelerate gonadal development when the frequency of daily short melatonin signals is increased beyond the normal value of one per day. When challenged with alternating short and long melatonin signals in several patterns, a balanced ratio favors gonadal development. Signal frequency is more critical than pattern for stimulation of gonadal growth.

**Perspectives**

The melatonin signals generated in the present study probed latent frequency responsiveness in the gonadal system. In nature, hamsters would never experience two 5-h melatonin signals separated by a melatonin-free interval of 7 h in a single day; the circadian control of melatonin secretion ensures that under naturally occurring short day lengths (or even in continuous darkness) nightly melatonin secretion occurs in a single episode that does not exceed 12 h in duration (5, 21, 35). The duration of nightly melatonin secretion does not remain static over the course of several weeks, but instead changes as day length increases or decreases. The predictive information contained in such patterns of melatonin secretion (15, 16) was not incorporated in the present study and might affect testicular development. Whether frequency responsiveness of substrates that decode and integrate melatonin signal frequencies are changed by the use of static vs. changing melatonin signals awaits further investigation. The probing of physiological systems with unnatural hormone frequencies deployed in the present and many previous studies is, nevertheless, useful in establishing the optimality of natural signal frequencies and patterns; for example, frequencies that deviated by ±30 min from the endogenous circalunar pattern of GnRH secretion failed to sustain normal gonadotrophin secretion in rhesus monkeys (reviewed in Ref. 22). In the present study, hamsters failed to increase gonadal size when the frequency of melatonin signals was doubled from the normal value. The mechanism by which the second daily signal is disregarded awaits a better understanding of the molecular mechanisms by which a single short melatonin signal is translated into gonadotrophic sequelae: changes in melatonin binding, melatonin-neurotransmitter coupling, GnRH pulsatility, or pituitary responsiveness to GnRH are all possible rate-limiting stages for photic regulation of gonadal status. Hamsters are capable of integrating a series of discordant melatonin signals over the course of many days for the purposes of directing gonadal growth. Their testes do not respond to more than one short-duration melatonin signal per day, but are simulated by short-duration melatonin signals appearing every other day, provided a long-duration melatonin signal occurs on the intervening day. In responding to an integrated series of signals, rather than on a signal-by-signal basis, the hamsters gain “photoperiodic inertia” that may buffer the reproductive axis from the occasional errors in melatonin secretion.
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