Mammalian Photoperiodic System: Formal Properties and Neuroendocrine Mechanisms of Photoperiodic Time Measurement

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OVERVIEW

Mammalian Photoperiodic System: Formal Properties and Neuroendocrine Mechanisms of Photoperiodic Time Measurement

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Abstract Photoperiodism is a process whereby organisms are able to use both absolute measures of day length and the direction of day length change as a basis for regulating seasonal changes in physiology and behavior. The use of day length cues allows organisms to essentially track time-of-year and to "anticipate" relatively predictable annual variations in important environmental parameters. Thus, adaptive types of seasonal biological changes can be molded through evolution to fit annual environmental cycles. Studies of the formal properties of photoperiodic mechanisms have revealed that most organisms use circadian oscillators to measure day length. Two types of paradigms, designated as the external and internal coincidence models, have been proposed to account for photoperiodic time measurement by a circadian mechanism. Both models postulate that the timing of light exposure, rather than the total amount of light, is critical to the organism's perception of day length. In mammals, a circadian oscillator(s) in the suprachiasmatic nucleus of the hypothalamus receives photic stimuli via the retinohypothalamic tract. The circadian system regulates the rhythmic secretion of the pineal hormone, melatonin. Melatonin is secreted at night, and the duration of secretion varies in inverse relation to day length; thus, photoperiod information is "encoded" in the melatonin signal. The melatonin signal is presumably "decoded" in melatonin target tissues that are involved in the regulation of a variety of seasonal responses. Variations in photoperiodic response are seen not only between species but also between breeding populations within a species and between individuals within single breeding populations. Sometimes these variations appear to be the result of differences in responsiveness to melatonin; in other cases, variations in photoperiod responsiveness may depend on differences in patterns of melatonin secretion related to circadian variation. Sites of action for melatonin in mammals are not yet well characterized, but potential targets of particular interest include the pars tuberalis of the pituitary gland and the suprachiasmatic nuclei. Both these sites exhibit uptake of radiolabeled melatonin in various species, and there is some evidence for direct action of melatonin at these sites. However, it appears that there are species differences with respect to the importance and specific functions of various melatonin target sites.

Key words photoperiodism, melatonin, pineal, mammal, circadian

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This article reviews major aspects of the formal properties of the mammalian photoperiodic system and discusses the neuroendocrine substrate for photoperiodic timekeeping. Mammals are the only group for which a particular "photoperiodic hormone" melatonin—has been identified, and this has allowed for progress in understanding the neuroendocrine basis for photoperiodic responses in this group. Therefore, we can discuss what is known of the mammalian photoperiodic system and pose questions regarding possible similarities or differences that have yet to be explored for other photoperiodic animals.

EARLY DISCOVERIES IN PHOTOPERIODISM RESEARCH

The first clear demonstration of a photoperiodic response was reported in studies of a mutant tobacco plant called Maryland Mammoth that flowered later than other tobacco. It was determined that the difference in flowering time could be explained by different photoperiodic requirements; Maryland Mammoth flowers later in the summer, as compared with other varieties, because it requires a reduction of day length to 14 h to induce flowering (Garner and Allard, 1920, 1923). Earlier studies had yielded some evidence for the importance of day length in flowering of *Humulus* and *Cannabis* (Tournois, 1912) and of *Sempervivum* (Klebs, 1913), but it was Garner and Allard who first clearly recognized that flowering and other responses in plants could be accelerated by either long or short days, depending on the species. The term *photoperiodism* was concocted by United States Department of Agriculture (USDA) scientist O. F. Cook and was introduced in the literature by Garner. (Allard preferred his own terminology—haemeronyctotropism, or day and night response.) Garner and Allard worked for the USDA, which estimated that their studies leading to the discovery of photoperiodism cost about $10,000 over a 10-year period. This investment in basic research has brought billions of dollars in benefits to farmers, horticulturists, and plant breeders (Sage, 1992). Garner and Allard believed that photoperiodism might also account for the seasonal reproduction of some algae and for the timing of bird migration. The first report of photoperiodic response in an animal was the documentation that appearance of sexual forms of the strawberry louse in late fall is regulated by day length (Marcovitch, 1923, 1924). Shortly thereafter followed the first observations of photoperiodic responses in birds (Rowan, 1925) and mammals (Baker and Ranson, 1932; Bissonnette, 1932).

PHOTOPERIODISM AND CHRONOBIOLOGY

Photoperiodism entails the use of day length cues to time seasonal changes in physiology and behavior. It is important to distinguish between photoperiodic responses and circadian responses, even though these two phenomena usually share a common mechanism; that is, the same circadian oscillator(s) that is used to regulate overt daily rhythms is also used to measure day length (photoperiodic time measurement, or PTM). Circadian rhythms are oscillations with approximately 24-h-period lengths that entrain to the daily light:dark cycle. Photoperiodism, in contrast, employs the annual cycle of day length to time seasonal adaptations. It is not correct to label an organism as photoperiodic merely because it is capable of entraining circadian rhythms to light cues.

Circadian rhythms themselves may exhibit seasonal variations. For example, ground squirrels show circannual variations in the phase angle of entrainment to a constant 14L:10D photocycle (Lee et al., 1986; Freeman and Zucker, 2000). Also, the duration of human sleep increases as a function of shortened day length (Wehr, 1991, 1992; Wehr et al., 1993). However, photoperiodism can be most unequivocally demonstrated if an organism exhibits noncircadian changes in response to changes in day length. For example, many species switch from periods of reproductive activity to periods of reproductive quiescence as a function of changes in photoperiod. Indeed, since reproductive seasonality has been the most commonly studied feature of photoperiodism in mammals, it has sometimes been suggested that a particular mammal (i.e., laboratory rat or laboratory mouse) is relatively nonphotoperiodic if day length does not affect reproductive status. This is clearly a premature conclusion; some species fail to show a photoperiod influence on reproduction yet exhibit other responses (such as seasonal pelage change) that are under photoperiodic regulation (Smale et al., 1988).

Biological seasonality presumably evolved as a consequence of the increased fitness that accrues to individuals that alter their physiological and behav-

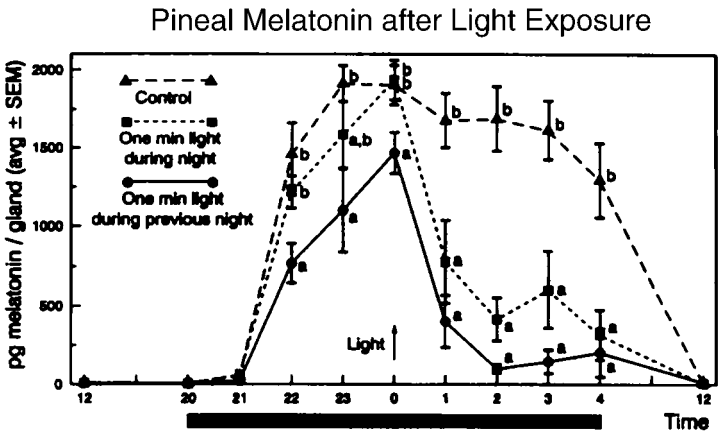

Figure 1. Night break paradigm. A single 1-min light pulse presented at the middle of the dark period in Siberian hamsters resulted in a rapid decrease in pineal melatonin content that persisted for the remainder of the night. The duration of elevated pineal melatonin was also decreased on the day following the brief night interruption pulse, suggesting a lasting effect on the circadian oscillator that regulates pineal melatonin (from Lerchl, 1995). For animals housed under short day lengths, light pulses of this type can evoke long-day responses—for example, reproductive activation in long-day breeders.

ioral states to meet the demands of relatively predictable environmental events that recur in the form of annual cycles. Environmental parameters that provide the selective basis for biological seasonality include annual variations in food and water availability, temperature, and exposure to predation or disease. These have been termed *ultimate* factors. In contrast, the environmental cues that are directly employed to help time an organism's seasonal cycles have been designated as *proximate* factors (Baker, 1938). These are often not the same as the ultimate factors that shape the evolution of biological seasonality. Photoperiodism provides a prime example of response to a proximate factor (day length) that is not itself of major importance to reproductive fitness. Nevertheless, the annual progression of day length change is a readily accessible and accurate indicator of time of year. Therefore, photoperiod, a time-of-year signal that allows organisms to anticipate and prepare for important changes in ultimate factors, is widely employed as a major cue for the timing of biological seasonality.

The study of photoperiodism includes three general areas of interest for chronobiologists: (a) Changes in photoperiod provide perhaps the most widely used cue for the timing of seasonal (annual) biological rhythms. (b) The measurement of day length, an essential component of the photoperiodic response, usually is accomplished by a mechanism involving a circadian oscillator. (c) Frequently, photoperiodism is intimately linked to either an endogenous interval timing mechanism or an endogenous circannual oscillation, to produce annual biological rhythms.

PHOTOPERIODIC TIME MEASUREMENT

Several methodologies have been used to establish that a circadian mechanism is used for PTM.

In *night break experiments*, very brief light pulses interrupting long nights induce long-day type responses despite exposure to an otherwise short-day photoperiod (Fig. 1). This result suggests that the total duration of light (or dark) exposure in each 24-h cycle is not the critical parameter in PTM. Rather, the results are more consistent with the concept that the circadian timing of photic stimulation is of fundamental importance (Hoffmann, 1979; Lerchl, 1995).

Resonance light cycles, also designated as the Nanda-Hamner paradigm, have been used to further probe the involvement of the circadian system in PTM. Resonance cycles involve repeated exposure of animals to 4- to 6-h light pulses presented at various intervals. Of particular interest in this type of study is a comparison of results obtained using intervals that result in photocycle lengths that are exact multiples of 24 h (i.e., 6L:18D and 6L:42D) and intervals that result in cycles that are not multiples of 24 h (i.e., 6L:30D and 6L:54D), respectively. In these experiments, animals exposed to cycles of 24- or 48-h-period lengths generally exhibit short-day responses, as would be expected since the light pulses are of only 4 to 6 h duration. However, animals exposed to non-24-h cycles (i.e., 36 h, 60 h) exhibit long-day responses. This result has been attributed to the fact that with non-24-h cycles, successive light pulses fall at different phases of the animal's circadian cycle (Fig. 2). Thus, as with the night break paradigm, some of the pulses fall during a

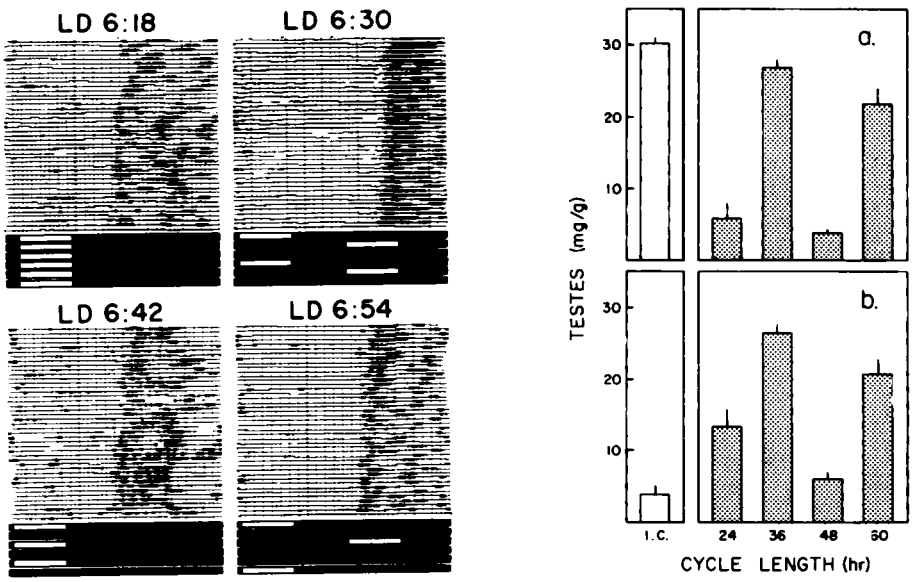

Figure 2. Resonance light cycles. Left panel: Wheel-running records of Syrian hamsters that were exposed to one of four resonance light cycles (light:dark cycles indicated above each actogram and depicted graphically below each actogram, with open bars indicating 6-h light pulses). For animals on photoschedules that are multiples of 24 h (LD 6:18 and LD 6:42), each successive 6-h light pulse falls at the same phase of the (entrained) hamster's circadian cycle; for hamsters exposed to the other resonance cycles (LD 6:30 and LD 6:54), light falls at different times of the animal's circadian cycle on different days. Right panel: Testis weights of hamsters exposed to each of the resonance cycles depicted in the left panel. Animals in section a were in LD 14:10 prior to the study, and controls (I.C.) remained in LD 14:10 for the 89 days of the experiment. Hamsters in section b were in LD 6:18 for 10 weeks prior to the beginning of the study (to induce testicular regression) and were sacrificed after 63 days in either resonance cycles or after further exposure to LD 6:18 (I.C.). In both paradigms, testicular maintenance (Section A) or testicular recrudescence (Section B) resulted only with resonance cycle lengths that were not multiples of 24 h, presumably because some of the light pulses in these cycles fell during the photoinductive phase (during subjective night) of the circadian cycle (from Elliott, 1976).

circadian phase during which they will evoke a long-day response (Elliott, 1976; Nanda and Hamner, 1959).

Note that resonance cycle experiments are expected to yield conclusive results only in organisms that do not require long-day stimulation on every day to evoke long-day responses. This is because with the longer resonance cycles, the test subjects will experience some periods of more than 24 h in which no light at all is present. Gonadal regression was prevented either partially or completely in Syrian hamsters that were maintained in otherwise continuous darkness by presenting 24 h of continuous light at 4-, 8-, or 12-day intervals (Stetson et al., 1975). This result suggests that long-day information does tend to drive the response when animals are presented alternatively with both long-day and short-day information. In a later experiment with Siberian hamsters, alternating long- and short-day signals were provided by experimentally manipulating the durations of nocturnal melatonin peaks (see below). The results of this study also supported the notion that long-day information is "favored" when long- and short-day signals are provided in alternating sequence (Prendergast et al., 1998).

T-cycle paradigms usually employ very short light pulses, sometimes of only a few minutes duration, repeated at either 24-h or non-24-h intervals against a background of darkness. When presented at 24-h intervals, short light pulses do not evoke long-day responses; in contrast, long-day responses are often observed in association with non-24-h T cycles. For T-cycle studies to be readily interpretable, the period length of the non-24-h cycles must be close enough to 24 h to allow for stable entrainment of circadian rhythms (i.e., within the limits of entrainment). Overt circadian rhythms (such as locomotor activity in rodents) are generally monitored to establish that entrainment has occurred and to determine the phase angle of entrainment relative to the light pulses (Fig. 3). The typical result of T-cycle studies has been attributed to properties of circadian oscillators that result in different phase angles of entrainment to light cycles of different period lengths. Thus, light falls at different phases of the circadian cycle in non-24-h T cycles as compared with 24-h T cycles (Elliott, 1976). Note that evidence for circadian involvement in PTM as obtained from night-break experiments, resonance cycles, and T cycles depends on the ability of appro-

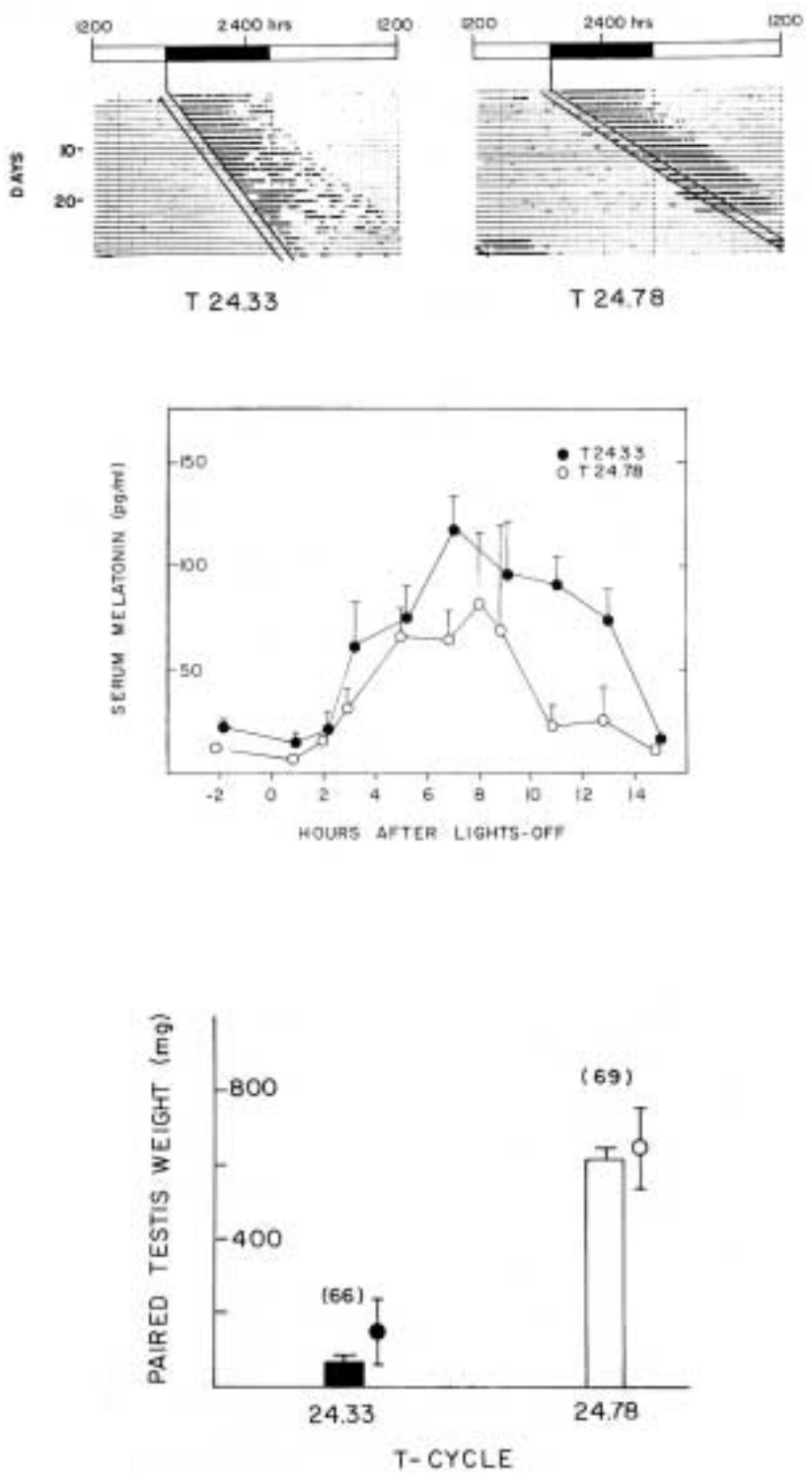

Figure 3. T cycles. Top panel: Wheel-running activity rhythms are shown for individual Siberian hamsters exposed to 1-h light pulses administered at T = 24.33 or T = 24.78. Time of the light pulse is indicated by the parallel lines in each record. The bars above the actograms depict the 16L:8D photocycle that was in effect prior to the initiation of T cycles. Note the different phase angles of entrainment, with light falling during the time of activity onset in T 24.78 but not in T 24.33. Middle panel: Circadian variations in serum melatonin concentrations in hamsters entrained to T 24.33 or T 24.78, with time plotted relative to lights-off on the day of sampling. Note the shorter duration of elevated melatonin concentrations in the hamsters exposed to T 24.78. Bottom panel: Paired testis weights of hamsters exposed to T 24.33 or T 24.78. Height of each bar indicates mean, and SEM is also indicated. Numbers in parentheses indicate group sizes. The circles indicate testis weights of animals that had access to activity wheels during exposure to the T cycles; the other hamsters were group housed without wheels. Only the animals on the T 24.78 cycle maintained large testes, a typical long-day response in this species. This reproductive response was associated with a short-duration melatonin signal and a phase angle of entrainment that resulted in illumination of the first hour of the active phase (from Darrow and Goldman, 1986).

priately timed, short light pulses to evoke long-day-type responses.

Disruption of the circadian system leads to disruption of photoperiodic responses. Most notably, following destruction of the suprachiasmatic nuclei (SCN), mammals fail to exhibit different seasonal responses to long versus short days. SCN-lesioned rodents behave as if they are unable to discriminate between different photoperiods; they do not respond appropriately to either long or short days (Rusak and Morin, 1976; Stetson and Watson-Whitmyre, 1976).

HYPOTHETICAL MODELS FOR MECHANISM OF PTM

Two major models have been proposed to describe possible mechanisms for PTM. The external coincidence model was first proposed by Bunning (Bunning, 1960; Pittendrigh and Minis, 1964). In this model, light has two distinct and separate roles. First, light is the major zeitgeber for entrainment of the circadian system. Second, light will result in long-day responses (photoinductive effect of light) only if it falls during a particular photoinductive phase of the circadian cycle. In the absence of illumination during the photoinductive phase, the organism will display short-day responses. When the model was first proposed, chronobiologists tended to classify photoperiods as either "long day" or "short day." It is now clear that whereas such a classification may be useful for simplifying descriptions of photoperiodic responses, it is only a rough approximation of reality. In fact, organisms are capable of responding to gradations of day length—that is, there is no clear dividing line between long and short days. The external coincidence model thus needs modification to account for this ability to discriminate day lengths on a continuum. It seems that the simplest modification would be to postulate that the photoinductive phase of the circadian cycle is itself subdivided in a relatively continuous fashion. Light falling at one point during the photoinductive phase might be "interpreted" as representative of a 15-h photophase, and light falling at a different point might be interpreted as a 16-h photophase, and so forth. It is difficult to obtain a complete and precise plot of the photoinductive phase, but it is clear that it is largely included within the portion of the circadian cycle where light is able to evoke phase shifts (i.e., subjective night).

For an external coincidence system to unerringly discriminate between various day lengths, it is crucial that the organism be appropriately entrained to the light:dark cycle. Thus, a change in the phase angle between the circadian oscillator and the light:dark cycle might be expected to result in an alteration in the interpretation of day length. In particular, a short day would likely be interpreted as a long day if the phase angle of entrainment resulted in illumination of some part of the photoinductive phase. (This is what is presumed to happen in T-cycle experiments, where long-day responses are evoked following entrainment to brief light pulses administered at recurring non-24-h intervals.) Since the phase angle of entrainment is clearly important in organisms that rely on their circadian system for PTM, one must wonder about the possible implications of entrainment to nonphotic zeitgebers. Does entrainment to zeitgebers such as temperature cycles, intense locomotor activity, or timed feedings interfere with the "accuracy" of PTM?

Note the parallel between the external coincidence model for PTM and the classical model for entrainment of circadian rhythms. In external coincidence, light will evoke a photoperiodic (long-day) effect only if it falls during a specific phase of the circadian cycle. In the model for circadian entrainment, the magnitude and direction of light-evoked phase shifts depend on the circadian time of light exposure, as illustrated in a phase-response curve.

A second model for PTM is the internal coincidence model. Here, the phase relation between two circadian oscillators varies as a function of day length, and it is the degree of the phase difference between these oscillators that determines the type of photoperiodic response (i.e., long-day or short-day responses). Light is the zeitgeber for entrainment of both oscillators. However, the two oscillators may be differentially phase shifted by a given light pulse, so that the phase relation between them can vary depending on the parameters of the photic stimuli. The phase of each oscillator is also influenced via a mutual coupling between the two oscillators, and the strength of the coupling relation may vary in response to a number of factors, perhaps including the day length (Boulos and Rusak, 1982; Daan et al., 2001).

The internal coincidence model is less amenable to experimental test as compared with the external coincidence model. This is largely because to critically test for internal coincidence, it would probably be necessary to identify anatomically the two relevant oscillators and then to systematically alter their mutual phase relations, preferably by use of nonphotic stimuli, to demonstrate that it is the phase relation between

the oscillators and not a "direct" effect of light that is critical. This type of experiment has not been accomplished in any organism. Nevertheless, the internal coincidence model has taken on added appeal with the increasing evidence in support of a dual-oscillator model for the circadian system (Boulos and Rusak, 1982; Illnerova, 1991).

The external and internal coincidence models have frequently been treated as if they were distinct alternatives. They are clearly different in that internal coincidence requires the interaction of two oscillators, whereas external coincidence requires only one. However, an internal coincidence system could behave in a manner fully consistent with the external coincidence model. Thus, it could be that light falling during the photoinductive phase (external coincidence) evokes long-day responses because that is the phase at which light will produce differential phase shifts of two oscillators (internal coincidence), causing them to assume a "long-day" phase relation to each other.

The external coincidence model is appealing because of its testability and its apparent simplicity. The internal coincidence model may seem more complex than external coincidence in that it proposes involvement of two oscillators rather than one. However, with internal coincidence the phase-shifting action of light, already firmly established as the basis for entrainment of circadian oscillators, accounts for PTM as well (but see p. 292). Indeed, with internal coincidence, it is not necessary to assume that light has any effects on circadian oscillators beyond its phase-resetting action. In contrast, with external coincidence, the photoinductive effect of light must be an action (either on the oscillator or on some target that is under oscillator control) different from phase resetting.

OTHER TYPES OF INTERNAL TIMING ASSOCIATED WITH PHOTOPERIODISM: CIRCANNUAL AND NON-CIRCANNUAL SEASONALITY

In addition to the involvement of circadian oscillators in PTM, there are other types of biological timing mechanisms that are generally associated with photoperiodism. Some photoperiodic species exhibit endogenous circannual rhythms when held for long periods of time under seasonally constant conditions—that is, constant photoperiod and temperature. Two mammalian examples of circannual species are ground squirrels and sheep (Fig. 4). Both show circannual rhythms of reproductive activity that can be "entrained" to yield a strictly annual rhythm if the animals are exposed to naturally changing day length (Lee and Zucker, 1991; Woodfill et al., 1994). For circannual rhythms, changes in day length may be viewed as a zeitgeber for entrainment of the putative circannual oscillator. However, the anatomical substrate for a circannual rhythm has not been unequivocally identified in any species (Zucker and Prendergast, 1999). Therefore, the concept of a circannual oscillator remains a hypothetical construct used to "explain" the expression of circannual rhythms. It remains possible that overt circannual rhythms result from interactions among multiple anatomical structures (perhaps including both endocrine and neural substrates) rather than being a product of one discrete structure as for circadian oscillations (Mrosovsky, 1970).

Many photoperiodic species do not exhibit repeated annual (or circannual) cycles when held under seasonally constant conditions. For example, a number of photoperiodic rodents remain reproductively active for most of their lives when held continuously under a long-day photoperiod, but will exhibit gonadal regression within a few weeks following transfer from long to short days. If short-day exposure is continued, a complete recrudescence of the reproductive system occurs about 5 months after the initial exposure to short days, with the exact time varying somewhat among species (Goldman and Nelson, 1993). This "spontaneous" reversal essentially returns the animal to a long-day (summer) condition even in the face of continued short-day exposure, and the reversal is proposed to occur as a consequence of the organism's switch to a photorefractory (or, in this particular example, a short-day refractory) state. Once the state of photorefractoriness has been achieved, exposure to long days is required to "break" photorefractoriness and return the animal to the photosensitive state (Fig. 5; Stetson et al., 1977). Thus, organisms continue to discriminate day lengths when in the photorefractory state, but the nature of the day length response changes to breaking of refractoriness (by long days) rather than to changes in overt responses (such as reproduction).

ROLE OF PINEAL GLAND IN MAMMALIAN PHOTOPERIODISM

Two early discoveries focused interest on the pineal hormone, melatonin, as a potentially crucial component of the mammalian photoperiodic mechanism: (a)

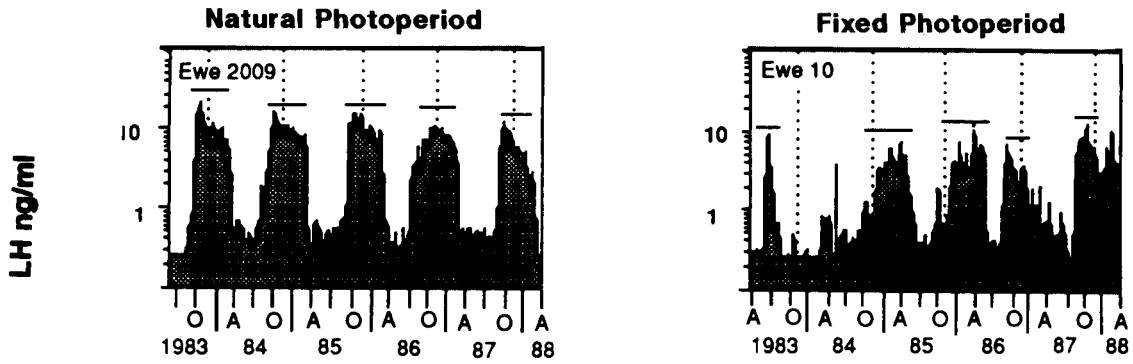

Figure 4. Seasonal changes in luteinizing hormone (LH) secretion in a circannual, photoperiodic mammal. Ewes were housed outdoors with natural photoperiod (left panel) or indoors under a fixed short (8L:16D) photoperiod (right panel). The animal under natural photoperiod showed five peaks of serum LH concentrations over the 5 years of the study, and these peaks occurred at about the same time each year, as indicated by the dotted vertical lines which indicate the average midpoints of the high stage of the LH cycle for the entire study (November 20). Ewes held under a fixed photoperiod exhibited circannual variations in serum LH; peaks did not occur at the same times in successive years (adapted from Karsch et al., 1989).

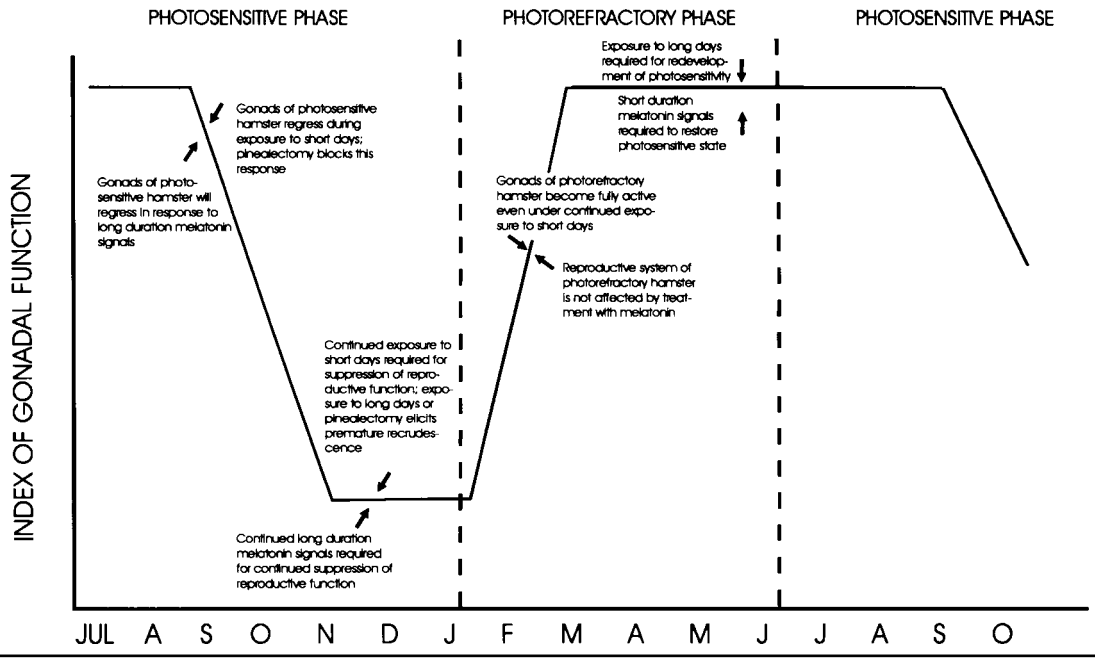

Figure 5. Effects of day length and melatonin in a noncircannual, photoperiodic mammal. The diagram was constructed based on data from Syrian hamsters but could serve as a prototype for a wide variety of noncircannual mammals. Gonadal regression occurs in late summer and is triggered by decreased day length and the associated increased duration of the nocturnal melatonin signal. "Spontaneous" recrudescence of the gonads occurs in early spring and does not require increased day length. Rather, recrudescence appears to be timed by an endogenous interval timer that was set in motion by the previous exposure to short days. As the animal achieves the photorefractory state, the reproductive system is no longer inhibited by exposure to short days and long-duration melatonin signals. The hamster is returned to a photosensitive state (breaking of photorefractoriness) by exposure to long summer days and the associated short duration melatonin signals (adapted from Goldman et al., 1982).

Pinealectomy abruptly leads to the loss of discrimination between long and short days as indicated by overt photoperiodic responses, such as seasonal changes in reproductive state, body fat stores, and pelage cycles (Czyba et al., 1964; Hoffman and Reiter, 1965; Vitale et al., 1985). In contrast, pinealectomy does not compromise entrainment of circadian rhythms to light:dark cycles (Finkelstein et al., 1978). (b) The nocturnal rhythm of pineal melatonin synthesis/secretion is regulated by a circadian oscillator(s) in the SCN that stimulates the pineal via a multisynaptic neural pathway (Moore et al., 1967; Moore, 1995). These early observations were followed by a number of studies that provided evidence for a pivotal role of

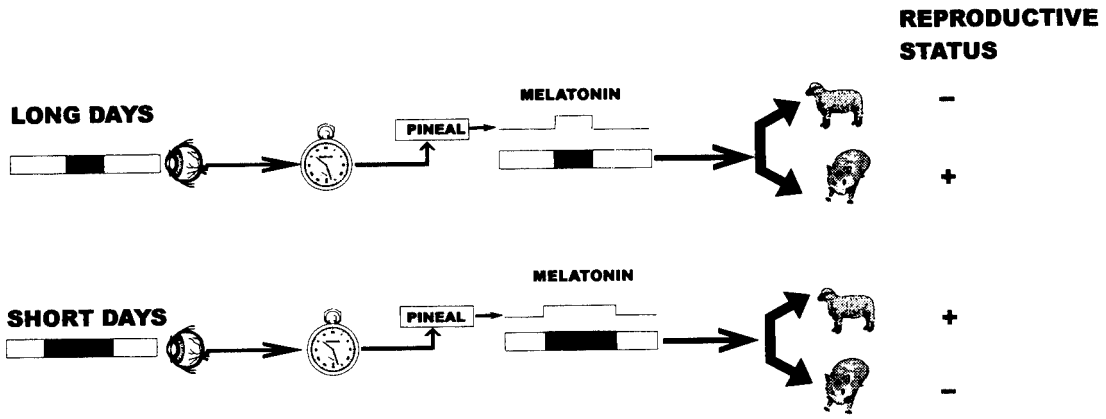

Figure 6. Pineal melatonin rhythm encodes day length information. Photoperiod information is transmitted from the eye to a circadian oscillator(s) in the SCN. Signals from the SCN drive the rhythmic synthesis and secretion of pineal melatonin. The duration of the nocturnal elevation of pineal melatonin increases as day length decreases. In short-day breeders (e.g., sheep), short-duration melatonin peaks (associated with long days) inhibit reproduction, whereas long-duration peaks (associated with short days) are stimulatory. In long-day breeders (e.g., hamster), short-duration melatonin peaks are stimulatory to reproduction, and long-duration peaks are inhibitory (adapted from Goldman, 1999).

melatonin; administration of the hormone to photoperiodic rodents could evoke responses like those observed in response to photoperiod manipulations (Goldman and Nelson, 1993).

This line of research has yielded further insight into the involvement of the mammalian circadian system in PTM. Namely, the circadian system regulates the pattern of melatonin secretion, and the melatonin pattern serves as a humoral signal that conveys day length information (see Schwartz et al., 2001 [this issue], and also Stehle et al., 2001 [this issue]). This statement is further supported by observations suggesting that the results obtained with various experimental interventions used to provide evidence for circadian involvement in PTM (i.e., night-break experiments, use of non-24-h T cycles) can be explained by the effects of these interventions on the pineal melatonin rhythm (Figs. 1 and 3; Darrow and Goldman, 1986; Lerchl, 1995).

Several studies in two long-day breeding mammals, namely, Siberian hamsters (Carter and Goldman, 1983a, 1983b; Goldman, 1991) and Syrian hamsters (Maywood et al., 1990; Karp et al., 1991), employed a paradigm of timed daily infusions of melatonin in pinealectomized subjects. Infusions of various durations and amounts of hormone were used in different treatment groups, and infusions were administered at various times of the circadian cycle. The results revealed that the duration of each daily infusion was by far the most important factor for determining the nature of the overt responses to melatonin. Daily infusions of relatively short duration (4 to 6 h in Siberian hamsters) evoked responses that are normally associated with long days; these responses include testis growth, as well as high serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin. This was true regardless of whether the pinealectomized, melatonin-infused subjects were housed in long or short photoperiods and regardless of the time of day of the infusions. In contrast, infusions of longer duration (8 to 12 h) resulted in testis regression and low serum levels of LH, FSH, and prolactin; these responses are typical of short-day hamsters (Fig. 6).

Similar melatonin infusion experiments were performed in female sheep. In contrast to hamsters, sheep are reproductively stimulated by exposure to short days. Short-duration daily melatonin infusions decreased the frequency of LH pulses in ewes and inhibited ovulatory cycles; these responses are normally associated with long days in sheep. Longer duration infusions were stimulatory to these reproductive parameters, similar to the effects of short-day exposure (Bittman and Karsch, 1984; Wayne et al., 1988). Thus, in both short-day breeding sheep and long-day breeding hamsters, short-duration melatonin infusions led to the long-day responses typical for the given species, whereas long-duration infusions evoked responses that are associated with short days (Fig. 6; for review, see Bartness et al., 1993). These results corresponded to the effects of day length on the pattern of pineal melatonin secretion. In a wide variety of mammals, the duration of nocturnal melatonin secretion is inversely related to day length (Bartness

and Goldman, 1989; Reiter, 1993). The types of observations cited above have been interpreted to suggest that day length signals are encoded in the duration of nocturnal melatonin secretion. These signals are presumably decoded in melatonin target cells to produce responses that are associated with the day length represented by the melatonin pattern (but see p. 294).

As mentioned earlier, the internal coincidence model for PTM requires only one type of action of light on the circadian system—namely, its phase resetting effect. However, the model leaves open the question of how the phase relation between two oscillators could be translated into a measure of day length. In an intriguing application of the internal coincidence model for PTM to melatonin physiology, it has been suggested that two light-entrainable circadian oscillators are responsible for regulating the duration of nocturnal melatonin secretion. Thus, a dusk oscillator may trigger the onset of melatonin secretion and a dawn oscillator might determine the termination of pineal activity (Illnerova, 1991). This idea closely parallels the popular notion that a dual-oscillator model of the circadian system could help to account for systematic changes in the duration (alpha) of the locomotor activity rhythm (Boulos and Rusak, 1982). If the duration of melatonin secretion does indeed depend on the phase relation between dawn and dusk oscillators, then there is a direct link between this mutual phase relation and the endocrine coding of day length via the duration of nocturnal melatonin secretion.

Does the circadian system play a significant role in the "decoding" of melatonin signals, and is the "interpretation" of the melatonin message strictly a function of its duration? In addition to the characteristic rhythmic pattern of melatonin biosynthesis and secretion, there is also evidence for circadian variation in responsiveness to melatonin. When melatonin was administered by daily injections in pineal-intact, long-day-housed Syrian hamsters, reproductive inhibition was observed in animals that were treated during the late afternoon or at the very end of the dark phase; no effect was seen for injections given during the early part of the light phase or during most of the dark phase (Stetson and Tay, 1983). However, different results were obtained when melatonin injections were given in pinealectomized hamsters, suggesting that exogenous melatonin might in some way interact with the pineal to inhibit reproduction (Tamarkin et al., 1977). Results in further studies suggested that some of the effects of melatonin injections in pineal-intact hamsters might be explained by additive effects of the exogenous hormone and the nocturnal peak of circulating melatonin resulting from pineal secretion; thus, exogenous and endogenous melatonin might summate to produce a long-duration melatonin signal (Goldman et al., 1984). Therefore, the results obtained with daily injections of melatonin in intact hamsters do not provide evidence for an intrinsic circadian rhythm of responsiveness in melatonin target tissues, but may only reflect the circadian pattern of endogenous melatonin.

There is some evidence for a circadian variation in responsiveness to melatonin even in pinealectomized hamsters, where additive effects of exogenous and endogenous melatonin cannot be invoked as an explanation (Stetson and Watson-Whitmyre, 1986). Very short duration (1 or 4 h) melatonin infusions resulted in partial testicular regression in pinealectomized Siberian hamsters, provided that the infusions were administered during the first hour of darkness in animals housed under 16L:8D. This response was not seen when melatonin was infused at other times, and it occurred for animals receiving 50 ng melatonin/day but not for hamsters infused with 10 or 25 ng melatonin/day (Gunduz and Stetson, 2001a). It is difficult to know whether this experimental result has implications for the normal function of the pineal melatonin rhythm for the following reasons: (a) When melatonin was infused for 8 h/day rather than 1 h, complete testis regression was observed in animals that received a total of only 10 ng melatonin/day, and this response occurred even with infusions at times that did not overlap into the first hour of darkness (Carter and Goldman, 1983a; Gunduz and Stetson, 2001b). (b) The regression seen in the hamsters receiving 1-h or 4-h melatonin infusions that provided hormone during the first hour of the night was not as pronounced as that observed in pineal-intact, short-day hamsters. Thus, these infusions did not completely mimic the action of short photoperiod, whereas the 8-h melatonin infusions did evoke regression equivalent to that observed in short days (Gunduz and Stetson, 2001a, 2001b). (c) Finally, although pinealectomized hamsters were responsive to 1-h melatonin infusions administered during the first hour of darkness, pineal melatonin secretion does not begin until 2 to 3 h after onset of darkness in both long- and short-day photoperiods (Goldman et al., 1984). It is of interest to note that the single circadian phase when 1-h melatonin infusions were inhibitory to reproduction corresponds closely to the only phase when exogenous melatonin can evoke phase advances and

thereby entrain the circadian rhythm of locomotor activity in rats (Cassone, 1998). It is not known whether there is any relation between the ability of melatonin to act as an entraining agent and its involvement in photoperiodic responses. Could it be that not only light but also melatonin itself might have phase-shifting actions on oscillators whose mutual phase relations contribute to PTM?

A different sort of circadian involvement in the response to melatonin signals may be suggested by the results of experiments that explored the frequency at which melatonin infusions must be delivered to evoke short-day-type responses in wild-type and *tau* mutant Syrian hamsters, respectively. Wild-type Syrian hamsters housed in continuous darkness exhibit free-running circadian period lengths that are very close to 24 h (Elliott, 1976). When 10-h melatonin infusions were administered to pinealectomized wild-type hamsters at various frequencies, short-day responses (reproductive inhibition) were evoked with signals presented at intervals of 20, 23, 24, or 25 h, but not when the infusions were given at 28-h intervals (Maywood et al., 1990). *Tau* mutant Syrian hamsters have free-running periods of approximately 20 h, considerably shorter than those exhibited by their wild-type counterparts. When 10-h melatonin signals were given in pinealectomized *tau* mutant hamsters, reproductive inhibition was obtained when infusions were presented at 16- or 20-h intervals, but not when intervals were 24 or 28 h (Stirland et al., 1996). The results in wild-type and *tau* mutant Syrian hamsters suggest that a series of 10-h melatonin signals may be effective only when presented at frequencies that are close to the animal's endogenous circadian period length. This apparent match between circadian period and the required melatonin signal frequency may be a mere coincidence (no pun intended), or it may reflect an as yet unknown role of the circadian system in the decoding of a series of melatonin signals. The results do not, however, provide direct evidence for importance of the circadian phase of exposure.

PHOTOPERIOD HISTORY EFFECTS: DOES THE PINEAL MELATONIN RHYTHM FAITHFULLY ENCODE DAY LENGTH?

The specific role of the pineal melatonin rhythm as a code for day length can be perhaps most critically tested by an examination of so-called photoperiod history effects. Seasonal physiological state is determined not only by the photoperiod that is currently in effect but also by the animal's photoperiod history. The state of photorefractoriness may be considered as one type of photoperiod history effect. After 4 to 5 months of exposure to short (winter) days, photoperiodic mammals revert to the spring/summer physiologic condition, as they become refractory to the effects of short days. In this situation, the photoperiodic mechanism appears to be altered by long-term exposure to short days. Several reports indicate that the pineal melatonin rhythm is not significantly altered in association with the switch to the photorefractory state in this paradigm; that is, the melatonin rhythm during photorefractoriness matches the ambient day length similarly to the way that it correlates with day length during the photosensitive phase of the annual cycle (Malpaux et al., 1987; Malpaux et al., 1988). Other reports have suggested changes in melatonin patterns associated with the onset of photorefractoriness (Almeida and Lincoln, 1984; Lerchl and Nieschlag, 1992). In any event, it is clear that responses to melatonin change dramatically with the onset of photorefractoriness. For example, photorefractory hamsters fail to exhibit inhibition of reproduction when given exogenous melatonin in paradigms that reliably inhibit reproductive activity in photosensitive hamsters (Bittman, 1978; Fig. 5). Similarly, photorefractoriness in sheep is associated with the loss of responsiveness to a melatonin signal (Karsch et al., 1986). Thus, it appears that photorefractoriness is primarily, if not exclusively, the result of a change in postpineal processing of information.

There is a parallel between the respective roles of melatonin and day length in photorefractory mammals: Short melatonin signals are required to break refractoriness to long-duration melatonin (Bittman and Zucker, 1981), just as long days are required to break refractoriness to short days. Thus, the day length information that is encoded in the melatonin rhythm is used both to drive overt photoperiodic responses and to break photorefractoriness. All photoperiodic mammals become photorefractory after long-term exposure to short days. Some species, including sheep (Malpaux et al., 1988), become photorefractory after prolonged exposure to either long or short days. The ability of a given species to become photorefractory after several weeks exposure to either long or short photoperiods may be associated with circannual rhythms.

Another paradigm in which photoperiod history has a major role in determining photoperiodic

response is seen when comparing the effects of increasing versus decreasing day lengths. In Siberian hamsters, exposure to 14L can be either stimulatory or inhibitory for the reproductive system, depending on photoperiod history. Male hamsters that were transferred from 16L to 14L exhibited testicular regression, whereas males that had already undergone regression in an 8L photoperiod showed testicular growth when the day length was increased to 14L. Since 14L day lengths occur both in early and late summer in nature, the ability to respond differently to this day length in the two experimental paradigms may represent a mechanism by which these animals improve their ability to respond in an adaptive fashion to the annual photoperiod cycle, by unambiguously identifying the 14L day length as indicative of an early summer (increasing day lengths) or a late summer (decreasing day lengths) day, respectively (Hoffmann et al., 1986).

A particularly intriguing type of photoperiod history effect is the case whereby a mother rodent is able to differentially alter the photoperiodic responses of her offspring depending on the photoperiod that is in effect during gestation (Horton, 1984; Lee and Zucker, 1988; Lee, 1993). Male Siberian hamster pups raised from birth in 14L (an "intermediate" day length) showed rapid testicular maturation if their mother had experienced a shorter photoperiod (10L or 12L) during gestation, whereas pups born to mothers that had been exposed to 16L throughout pregnancy showed slower testicular development during postnatal rearing in 14L (Stetson et al., 1986; Elliott and Goldman, 1989). There is evidence that melatonin passes from the maternal circulation to the fetus, establishing a circadian rhythm of melatonin in the fetal circulation similar to that of the mother (Yellon and Longo, 1987, 1988). This rhythm of melatonin may then act as a day length signal in the fetus as it does in the adult, in this case to establish a photoperiodic history for the developing rodent (Weaver and Reppert, 1989). Communication of day length information from mother to fetus might serve to give the newborns a head start in assessing time of year and thereby adopting an appropriate developmental trajectory. This could be particularly important for a species where reproductive competence potentially can be achieved within a few weeks after birth, but in which puberty may be delayed through the winter months for individuals born near the end of the breeding season (Hoffmann, 1978).

Male hamster pups raised from birth in 14L exhibited patterns of pineal melatonin (at 18 days of age) that depended, in part, on the photoperiod of gestation. Thus, pups with a gestational photoperiod history of 10L exhibited a shorter duration of nocturnal melatonin production as compared with pups whose mothers had been housed under 16L during gestation. This difference in the postnatal pineal melatonin rhythm under 14L could explain the different rates of reproductive development in the male pups with different prenatal photoperiod histories. In this case, it appears that gestational photoperiod history exerts its effect by altering the melatonin rhythm generating system (Shaw and Goldman, 1995a, 1995b). Therefore, it seems that developing hamsters may experience an action of photoperiod history to alter the photoperiodic mechanism at the prepineal level, as compared with the effect of photoperiod history on postpineal processes that appears to be instrumental in the case of photorefractoriness. In view of these results, it may be appropriate to reexamine the concept that the melatonin rhythm provides a faithful representation of the current day length. Rather, photoperiod history may influence not only melatonin target sites but also the melatonin rhythm generating system.

CRITICAL PHOTOPERIODS

In general, photoperiod history effects force us to reconsider the popular notion of "critical photoperiods." The concept of critical photoperiod serves as a clear descriptor for data such as those obtained in a study of effects of day length on reproductive parameters in Syrian hamsters. When hamsters were raised in 14L and then transferred to various shorter day lengths, gonadal regression was observed for animals moved to 12L or shorter photoperiods, whereas reproductive activity was maintained in photoperiods of 12.5L or longer. A second set of hamsters was exposed to 6L to induce testicular regression; animals were then transferred to various longer day lengths. Testicular recrudescence was seen only for hamsters transferred to 12.5L or longer photoperiods. Thus, 12.5L was said to be the critical day length for maintenance of reproductive activity in this species (Elliott, 1976). Critical photoperiods vary among species; Siberian hamsters raised in 16L undergo gonadal regres-

sion when the day length is decreased to 14L (Duncan et al., 1984) and therefore exhibit a longer critical photoperiod than Syrian hamsters.

The critical photoperiod may also be different for different responses in a single species. Thus, male Siberian hamsters undergo testicular regression when day length is decreased to 14L, but another winter-type response in this species, the molt to winter pelage, does not occur unless the day length is reduced even further (Duncan et al., 1984). Finally, results cited earlier indicate that 14L can be either stimulatory or inhibitory to reproduction in Siberian hamsters, depending on whether the animals had previously experienced a longer or shorter day length (Hoffmann et al., 1986). Thus, the term *critical photoperiod* may be useful in some cases, but one must be aware of the important caveats described above.

GENETIC BASIS FOR DIFFERENCES IN PHOTOPERIODIC RESPONSES BETWEEN INDIVIDUALS, BREEDING POPULATIONS, AND SPECIES

There are many differences among mammals in responses to photoperiod cues. For example, there are substantial species differences with respect to the critical day lengths required for maintaining reproductive activity. Also, in some species photoperiod influences several traits, whereas in other species the effects of photoperiod may be more restricted or altogether absent (Goldman and Nelson, 1993). In some cases, striking variations in response to day length are also seen within a single species. Differences in photoperiodic responsiveness between northern and southern populations of white-footed mice were shown to be based on genetic differences between the two breeding populations (Lynch et al., 1981). Individual differences in photoperiodic responsiveness have also been observed within breeding populations of rodents, and a genetic (heritable) basis for these differences has been demonstrated in Siberian hamsters (Kliman and Lynch, 1992), deer mice (Desjardins et al., 1986), and white-footed mice (Heideman and Bronson, 1991). Heritable differences in photoperiod responsiveness among members of a breeding population presumably could provide the raw material for the evolution of species differences in photoperiod responses.

The physiological bases for differences in responsiveness to photoperiod are of at least two types in the rodents that have been examined to date. Short-day nonresponsive morphs taken from a population of deer mice proved to be unresponsive to treatment with melatonin; the photoperiod nonresponsive morphs failed to undergo gonadal regression when given a regimen of the hormone that evokes reproductive inhibition in photoperiod-responsive mice. Hence, individual differences in *postpineal* processing of melatonin signals appear to account for variations in photoresponsiveness among deer mice (Blank and Freeman, 1991).

Significant proportions of the individuals in laboratory populations of Siberian hamsters fail to exhibit the species-typical winter season responses (reproductive inhibition, molt to winter pelage, reduction of body fat stores) when exposed to short days. The failure of these animals to respond appears to be explained by their different phase angle of entrainment to short days as compared to the short-day responsive morphs (Puchalski and Lynch, 1988). Presumably, the different pattern of entrainment in the nonresponder hamsters results in a stimulation of the photoinductive phase of their circadian cycle by light. Short-day nonresponsive Siberian hamsters are apparently not incapable of producing, and responding to, an expanded nocturnal melatonin peak, because these animals uniformly exhibit gonadal regression when exposed to continuous darkness (Freeman and Goldman, 1997). Therefore, in contrast to the situation in deer mice, individual differences in short-day nonresponsiveness in Siberian hamsters appear to be related to *prepineal* processing of photoperiod information.

The implications of genetic variability in prepineal versus postpineal processing of information may be considerable. If the melatonin signal itself is altered, as in the case of Siberian hamsters, then all photoperiodic responses that depend on melatonin signaling would be expected to be affected in a similar fashion. In contrast, if genetic variability occurs at the postpineal level, it would seem likely that several of the overt photoperiodic responses could be differentially affected. For example, in the latter case, reproduction might be resistant to melatonin (day length) effects, whereas certain other types of responses might remain under photoperiodic regulation.

In Siberian hamsters, variable responsiveness to short days is a function not only of genetics but also of photoperiod history. Hamsters that have been exposed to very long day lengths (16L or 18L) are far more likely to be unresponsive to subsequent short

days as compared with hamsters raised exclusively under an intermediate (14L) photoperiod. Based on this finding, it has been proposed that in this relatively short-lived rodent, animals born early in the spring/summer and subsequently exposed to the longest summer days would be more likely to remain reproductively active during winter as compared with (younger) individuals that were born later during the breeding season (Gorman and Zucker, 1997). It may make sense with respect to an adaptive program that the older individuals in the population, and thus those presumably least likely to survive until the following primary breeding season, would be the animals most likely to "gamble" on winter breeding. In Siberian hamsters, an age-related distribution of winter breeders may be achieved via a mechanism based on photoperiod history rather than relying on age per se as a determinant of resistance to the reproductive inhibitory effects of short winter days.

This photoperiod history effect "interacts" with genetic variation in Siberian hamsters. One strain of hamsters, designated PNR, was obtained by artificial selection for short-day nonresponsiveness. Virtually all individuals in this strain as well as all animals from the original strain (UNS strain, not selected for photoperiod response) were fully responsive to short days when exposed to 10L from the time of birth. However, when both strains of hamsters were raised from birth in 16L or 14L prior to testing in short days, the PNR animals showed a much greater incidence of short-day nonresponsiveness than did the UNS hamsters. Thus, it may be that PNR hamsters become predominantly unresponsive to short days under standard rearing conditions because they are particularly sensitive to the photoperiod history effects that can result from long-day exposure (Goldman et al., 2000). Curiously, when newly weaned PNR hamsters were given access to running wheels, the animals became uniformly responsive to short days (Freeman and Goldman, 1997). Thus, in Siberian hamsters the short-day nonresponsive phenotype, though having a heritable basis, is quite labile in response to environmental conditions.

SITES OF ACTION OF MELATONIN FOR MEDIATION OF PHOTOPERIODIC RESPONSES

In mammals, the pineal melatonin rhythm is crucially involved in mediation of a wide variety of photoperiodic responses. Therefore, one is compelled to ask whether melatonin acts at a corresponding variety of target sites to regulate these overt responses, or whether one site of melatonin action is involved in regulating several types of responses. The second hypothesis could have some appeal in that it would be somewhat analogous to the operation of the circadian system, where a single oscillator (or pair of oscillators) regulates many rhythms. On the other hand, melatonin is a hormone, and most (but by no means all) hormones have multiple target sites. (Note that this statement might require some modification if melatonin is transported to its targets via the cerebrospinal fluid rather than the blood, as discussed by Malpaux et al. [2001 (this issue)].)

Uptake of radiolabeled melatonin has been observed in the pars tuberalis of the pituitary in virtually all mammals that have been examined, and in mustelids (ferret, mink, spotted skunk) this is reported to be the only uptake site in structures associated with the brain. In addition to binding in the pars tuberalis, many species show binding in various brain sites, including most notably the SCN (Bittman, 1993). There is remarkably little direct evidence for specific actions of melatonin at these various sites, however. Therefore, it remains possible that some target sites are associated with actions of the hormone that are unrelated to photoperiodism—for example, actions related to the regulation of circadian rhythms (Cassone, 1998). Several studies involving pituitary stalk sections and the use of localized microimplants of melatonin have suggested that the sheep pars tuberalis is an important site of melatonin action for regulation of seasonal variations in prolactin secretion but that melatonin may act in the mediobasal hypothalamus to regulate the secretion of gonadotropins (Lincoln, 1994; Malpaux et al., 1993; Malpaux et al., 1995). A study performed in Syrian hamsters also suggested separate sites of action for melatonin in regulating prolactin and gonadotropins, respectively. In this species, lesions in the mediobasal hypothalamus prevented the action of melatonin to inhibit LH secretion but did not interfere with inhibition of prolactin by melatonin (Maywood and Hastings, 1995). In Siberian hamsters, inhibition of testis growth was observed following localized infusions of very small amounts of melatonin into the SCN, thalamic reuniens nucleus, or thalamic paraventricular nucleus (Badura and Goldman, 1992); these three sites all exhibit uptake of radiolabeled melatonin in this species (Weaver et al.,

1989). Following melatonin infusions into the SCN, serum prolactin was decreased. Gonadotropins were not measured, but since testis growth was completely blocked by these infusions, it is likely that secretion of gonadotropins was decreased as well (Badura and Goldman, 1992). These results suggest a possible redundancy among these sites with respect to melatonin actions on the reproductive axis.

Overlap among the functions of the three central nervous system melatonin uptake sites in Siberian hamsters is further suggested by the results of a recent study that investigated both testicular regression and the development of refractoriness during chronic treatment with melatonin. Microimplants of melatonin were placed in SCN, nucleus reuniens, or the thalamic paraventricular nucleus in male Siberian hamsters. Testicular regression ensued in animals receiving any one of these treatments, and in each case gonad recrudescence was initiated after about 16 to 19 weeks. This is similar to the timing of regression and recrudescence in pineal-intact animals transferred to short days or in hamsters receiving chronic systemic treatment with melatonin, suggesting that each of the three brain sites can become refractory to the reproductive inhibitory actions of melatonin. When the melatonin implants were removed and the animals received systemic treatment with melatonin, a second period of testis regression was observed. This result suggests that whereas the site that initially received a melatonin implant may have become refractory to reproductive effects of the hormone, the non-implanted sites retained responsiveness to melatonin (Freeman and Zucker, in press).

There appears to be an interesting species difference between Siberian (*Phodopus sungorus*) and Syrian (*Mesocricetus auratus*) hamsters with respect to the SCN as a possible target for the photoperiodic actions of melatonin. SCN lesions prevented long-duration melatonin infusions from inhibiting the reproductive system in Siberian hamsters (Bartness et al., 1991), but comparable lesions did not prevent a similar action of exogenous melatonin in Syrian hamsters (Bittman et al., 1989; Maywood et al., 1990). This correlates with the observation that uptake of radiolabeled melatonin is evident in the SCN of Siberian hamsters but is weak or absent in the Syrian hamster SCN (Weaver et al., 1989).

Recent studies suggest that melatonin may act directly on cells of the immune system, resulting in seasonal variations in immune response. This observation raises the possibility that a relatively wide variety of cells might be capable of responding directly to the photoperiodic signal conveyed by the melatonin rhythm (Drazen et al., 2000; Prendergast et al., 2001).

MOLECULAR BIOLOGY OF THE PHOTOPERIODIC SYSTEM

In most organisms, PTM is accomplished by a circadian mechanism. For this reason, we might anticipate that the spectacular advances that have been made toward elucidating the molecular biology of circadian clocks might lead to comparable progress in uncovering the molecular basis for photoperiodic timekeeping. Some intriguing discoveries have already appeared, and these are discussed in the manu- script of Hazlerigg et al. (2001 [this issue]). The finding that the clock gene, *Per1*, is rhythmically expressed in the rodent pars tuberalis—a melatonin target site—is especially interesting. It will be important to determine whether this gene is involved as part of a timing mechanism in melatonin target cells of the pars, or whether it serves a different sort of function there.

SUMMARY AND QUESTIONS

This review has focused on characterizing the photoperiodic system of mammals, and we probably know more about the neuroendocrine substrate for photoperiodism in this group than in any other. For mammals, it appears safe to conclude, at least to first approximation, that day length information is processed in some fashion in the SCN and is transcribed to an endocrine signal, the pineal melatonin rhythm. Yet, we know little about how this endocrine signal is "decoded" in melatonin target tissues or why small differences in the duration of the nocturnal melatonin signal sometimes can lead to opposite overt responses, such as inhibition versus stimulation of the secretion of pituitary gonadotropic hormones.

Even less is known of the neuroendocrine basis for photoperiod history effects. Is information related to photoperiod experience stored in some discrete location for future reference? Since the SCN is a major component of the melatonin rhythm generating system, it seems a likely candidate for storage of photoperiod history that might influence the photoperiodic mechanism at the prepineal level; there is evidence to support a role of the SCN in this respect

(Sumova et al., 1995; Schwartz et al., 2001). What about photoperiod history effects on responsiveness to melatonin (a postpineal phenomenon)?

As with mammals, most photoperiodic organisms employ a circadian mechanism for PTM. In most cases, this has been established solely by experimental approaches like those described on pp. 285-288 of this review. Only for mammals do we know an important step in PTM that lies downstream from the circadian oscillator—the pineal melatonin rhythm and its role in coding day length information. Yet, for birds and reptiles, neither melatonin nor the pineal gland appears to be a crucial component of the photoperiodic mechanism, although the pineal melatonin rhythm is important for other circadian functions in a number of reptilian and avian species (Underwood and Goldman, 1987). Does another endocrine tissue serve a function like that of the mammalian pineal in these vertebrate classes? Or is PTM strictly a function of neural (nonendocrine) circuitry in nonmammalian vertebrates? It will be important to clearly establish the roles of various melatonin target sites in mammals. Once this has been accomplished, it should be possible to determine whether these sites are similarly involved in photoperiodic responsiveness in other vertebrates. If they are, and if melatonin is not the photoperiod "messenger" in these species, then how does the circadian system communicate photoperiod information to these sites?

For invertebrate animals as well, it will be interesting to determine how circadian (time-of-day) information is transcribed into a photoperiod (day length) signal. A recent article in this journal develops hypotheses based on current knowledge of mammalian clock genes, proposing ways in which these genes might form the basis for a dual-oscillator circadian system. Although the paper is largely conjectural and is primarily aimed at formulating a molecular basis to match formal models of circadian systems, the ideas that are put forward have implications for a possible parallel (molecular) explanation for PTM. Furthermore, though the hypothesis is developed on the basis of data for mammalian clock genes, it would seem appropriate to look for similar explanations in other types of animals where dual-oscillator circadian systems may exist (Daan et al., 2001).

The articles that follow in this issue deal with a variety of specific issues in photoperiodism research and cover a wide range of organisms.

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