BIOLOGY OF REPRODUCTION 32, 947–956 (1985)

Photoperiodic Time Measurement in the Male Deer Mouse, *Peromyscus maniculatus*[1]

HERBERT UNDERWOOD,[2] J. MAL WHITSETT,
and TIMOTHY G. O'BRIEN

Department of Zoology
North Carolina State University
Raleigh, North Carolina 27695

ABSTRACT

Weanling male deer mice, *Peromyscus maniculatus*, were exposed for three weeks either to light-dark (LD) cycles with periods (T=L+D) ranging from T=23 (1L:22D) to T=25.16 (1L:24.16D) or to 24-h LD cycles with photoperiods ranging from 1 (1L:23D) to 19 (19L:5D) h. Both the circadian locomotor activity rhythms and the response of the reproductive system to these LD cycles were assessed. The results demonstrate that the photoperiodic effectiveness of light depends on the phase of the light relative to the animal's circadian system, as marked by the circadian activity rhythm. Light falling during the animal's subjective night, from activity onset to at least 11.8 h after activity onset, stimulates growth and maturation of the reproductive system, whereas light falling during the rest of the circadian cycle is nonstimulatory.

INTRODUCTION

The annual change in day length offers a precise, noise-free cue that is used by many temperate-zone animals to time such important events as fattening, migration, molting, and reproduction. Animals can anticipate, and prepare for, conditions that are most conducive to their survival as well as the survival of their offspring. Within the past decade rapid strides have been made, especially in photoperiodic mammals, in elucidating the mechanisms by which animals decipher the photoperiodic cue and translate this into a neuroendocrine response.

One central theme in this research has been an investigation of the nature of photoperiodic time measurement itself; that is, how organisms discriminate long from short days. Historically, the underpinnings of our current concepts concerning photoperiodic time measurement began with a hypothesis first formulated by Bünning in 1936. According to the Bünning hypothesis, photoperiodic time measurement depends on a daily (circadian) rhythm of sensitivity to light; the organism is insensitive to light during one-half of a circadian cycle but becomes sensitive to the inductive effects of light during the remainder of the cycle. A particular light-dark (LD) cycle will be inductive (i.e., stimulate a photoperiodic response) or not depending on whether or not light falls on the light-sensitive or light-insensitive portion of the rhythm. Although originally proposed to explain photoperiodic time measurement in plants, Bünning's concept has been extended to explain photoperiodic time measurement in vertebrates as well (Follett, 1973; Elliott and Goldman, 1981). Refinements to Bünning's model have included the concept that light must have a dual role in photoperiodic time measurement. First, light must entrain the circadian oscillator responsible for driving the photoperiodic photosensitivity rhythm and, second, light is inductive, or not, depending on whether light illuminates the sensitive portion of the circadian rhythm of photoperiodic photosensitivity (CRPP) (Pittendrigh and Minis, 1964). In general, this kind of model is termed an "external coincidence" model since it demands the coincidence of an external stimulus (light) and the photosensitive part of an internal circadian rhythm; light has a dual role (entrainment and induction) (Pittendrigh, 1972). Another "variation" of a circadian-based model for photoperiodic time measurement postulates that the phase relationship between two internal circadian oscillators (or

Accepted December 10, 1984.
Received November 19, 1984.
[1] This investigation was supported in part by a National Science Foundation Grant (PCM 8116880) and a U.S. Environmental Protection Agency Grant (CR809428010). H.U. is the recipient of Research Career Development Award 5 K03 HD00276–02 from the National Institute of Child Health and Human Development.
[2] Correspondence.

groups of oscillators) determines whether or not induction will occur (Pittendrigh, 1972, 1981). For example, the phase of one oscillator may be set by dawn and the other by dusk so that the mutual phase relationship between the two oscillators changes as the photoperiod shortens or lengthens. In this "internal coincidence" model light has only one role – entrainment of the constituent oscillators.

To date, investigations in several species of mammals, including hamsters, voles, and rats, indicate that the circadian system is involved in photoperiodic time measurement (Grocock and Clarke, 1974; Elliott and Goldman, 1981; Nelson et al., 1982). Several kinds of experimental protocols have been developed to test for a role of the circadian system in photoperiodic time measurement, including night breaks, resonance cycles, and "T" experiments. These protocols exploit the entraining actions of light on the circadian system and assess whether the observed photoperiodic response (e.g., gonadal development) depends on the phase at which light falls relative to the animal's circadian system. Typically, because of its ease of measurement, the circadian rhythm of locomotor activity is used as an assay for the phase of the animal's circadian clock. The present studies were conducted to assess the nature of photoperiodic time measurement in the prairie deer mouse (*P. maniculatus*) by examining both gonadal development and locomotor activity patterns in individuals exposed to various 24-h and non-24-h LD cycles.

MATERIALS AND METHODS

Animals and Housing

Prairie deer mice (*Peromyscus maniculatus bairdii*) were born in the laboratory from a stock originally obtained from Dr. John A. King of Michigan State University. The mice were maintained in 0.29 × 0.19 × 0.12-m polypropylene cages on a bedding of shredded aspen. They were provided with tap water and Wayne F-6 Blox and Wayne Breeder Blox (during pregnancy and lactation) ad lib. Adult males and females, housed previously on a lighting regimen of 15L:9D, were paired for breeding in a room having a cycle of 6L:18D. Males were removed from the breeding cages about a week later. Mothers and offspring remained on the 6L:18D schedule until weaning. Male offspring were weaned at 21 days of age and introduced into experimental treatments at that time in Experiments 1 and 2. After 3 wk of exposure to the experimental photoregimens, the animals were killed and the body weights, testis weights, and seminal vesicle weights were measured.

During the experiments the deer mice were individually housed in cages in one of three types of light-tight wooden chambers. Type A chambers measured 0.76 × 0.41 × 0.36 m and held a single 8-watt fluorescent bulb (Westinghouse F8T5/cw) giving an average intensity of 100 lux at the floor of the animal's cage. The cages (2 per chamber) contained a running wheel connected to a 20-channel Esterline-Angus event recorder. Type B chambers measured 0.64 × 0.44 × 0.36 m and held a single 4-watt fluorescent bulb (Sylvania F4T5/cw) giving an average intensity of 50 lux at the floor of the animal's cage. Each B chamber held 2 polypropylene cages without a running wheel. Type C chambers measured 1.21 × 0.45 × 0.61 m and each held a single 30-watt fluorescent bulb (Sylvania F30T12-CW-RS) giving an average intensity of 600 lux at the floor of the animal's cage. Each C chamber held 9 polypropylene cages without running wheels.

Experiment 1

Upon weaning, male deer mice were assigned on a random basis of one of the following "T" light cycles: 1L:21D, 1L:22D, 1L:23D (control), 1L:24D, or 1L:25D. The light cycles were controlled by Chrontrol programmable electronic timers (Lindburg Enterprises, Inc., San Diego, CA). Eight individuals in each treatment were housed in Type A chambers and had access to running wheels, thus enabling us to record locomotor activity. The remaining mice were housed in Type C chambers and yielded no information on activity patterns. For cycles in which the period (T=L+D) of the LD cycle was <24 h the initial light pulse was programmed to fall at the end of the animal's activity time (late subjective night); for T cycles ≥24 h the initial light pulse fell at the beginning of the animal's activity time (early subjective night). This lighting protocol minimized transients into reentrainment to the various T cycles from the 6L:18D cycle under which the deer mice had been reared.

Experiment 2

Male deer mice were transferred at weaning to either Type A or Type B chambers. Those in Type A chambers were assigned randomly to LD cycles of 1L:21.67D or 1L:24.16D. The light cycles were controlled by Flexopulse timers (Eagle Signal Co., Davenport, IA). All individuals in Type B chambers were exposed to 1L:23D. Thus activity data were recorded from individuals on ahemeral light cycles but not from those on 1L:23D. For T=22.67, the initial pulse was placed at the end of the subjective night and for cycles in which T=24 or T=25.16 the initial pulse fell at the beginning of the subjective night.

Experiment 3

Adult male deer mice were exposed to the following standard (T=24) LD cycles inside Type A chambers: 1L:23D, 6L:18D, 8L:16D, 10L:14D, 12L:12D, 14L:10D, 16L:8D, and 18L:6D. The light cycles were controlled by Tork timers (Tork, Mount Vernon, NY). Activity patterns were recorded from 10 individuals on each of the LD cycles for a minimum of 14 days on each cycle.

Analyses

The circadian phase of the light pulse during entrainment to different T cycles is expressed by the

phase angle difference (ψ) in hours measured between activity onsets and the beginning of the 1-h light pulse. Phase angles were measured only during steady-state entrainment. A positive phase angle denotes that activity onset preceded light onset, a zero phase angle indicates that light onset and activity onset coincided, and a negative phase angle shows that activity onset occurred after light onset. For standard (T=24 h) photoperiods the phase angle reference points were activity onsets and light *offsets*. "Circadian time" (CT) refers to a time scale measured in hours (CT0-CT24) covering one full circadian cycle. For deer mice CT12 marks activity onset and indicates the beginning of the half-cycle (CT12-CT24) termed the "subjective night." The "subjective day" encompasses the remainder of the cycle (CT0-CT12). Non-24-h LD cycles were normalized to 24 h when presented on the "circadian time" scale.

The design of Experiments 1 and 2 was a single classification analysis of variance. Seminal vesicle weights were subjected to a logarithmic (base 10) transformation because of their highly skewed distributions (Whitsett et al., 1984a). The *F* tests for organ weights were calculated using the General Linear Models procedure of the Statistical Analysis System computer program (SAS Institute Inc., 1982). Multiple comparisons were made using the Duncan-Waller test (SAS Institute Inc., 1982).

RESULTS

The interpretation of the results necessitates a brief discussion of the mechanism of entrainment of circadian rhythms by LD cycles. Under constant conditions circadian rhythms exhibit a "free-running" period (τ) that is approximately, but rarely exactly, 24 h in length. One of the most consistent and universal features of circadian rhythms is their response to single light pulses applied at different phases of an otherwise free-running rhythm. Light pulses can elicit phase shifts in the rhythm whose magnitude and duration are functions of the phase at which the light pulse was administered (Daan and Pittendrigh, 1976). A graph of these phase shifts yields the "phase response curve" (PRC). During entrainment the period (τ) of the circadian rhythm is forced to assume the period (T=L+D) of the entraining LD cycle. Entrainment of a circadian rhythm typically is restricted to LD cycles with periods between $\sim$18 and $\sim$30 h; outside of this range entrainment does not occur. A model of entrainment developed by Pittendrigh is especially germaine (Pittendrigh, 1965). According to this model a phase shift ($\Delta\phi$) occurs during each steady-state entrainment cycle that is equal to the difference between the free-running period (τ) of the rhythm and the period (T) of the light cycle: $\Delta\phi = \tau - T$. The phase shift required is obtained only when the light falls at a particular phase of the circadian rhythm. This phase can be predicted, if a PRC is available, since the phase corresponds to the point in the cycle at which the single light pulse elicited the requisite $\Delta\phi$. During entrainment, therefore, the LD cycle controls not only the *period* of the circadian rhythm but also its *phase*. If, for example, the period (T) of an entraining light cycle is varied, then the phase relationship between the light and the entrained circadian rhythm will also vary.

Fig. 1 shows representative activity patterns from Experiments 1 and 2 of deer mice entrained to LD cycles with periods of T=23 (1L:22D), T=24 (1L:23D), and T=25.16 (1L:24.16D). Light cycles of 1L:21D and 1L:25D fell outside the range of entrainment and the animals in these groups were excluded from analysis. The phase of the activity pattern relative to the LD cycle varies with the period of the cycle. When T=24 h (1L:23D), the 1-h light pulses occurred in the subjective day, with cycles of T<24 h the light pulses occurred in the late subjective night, and with light cycles of T>24 h the light pulses fell in the early subjective night (Fig. 1).

We cannot directly measure the circadian rhythm of photoperiodic photosensitivity as we can locomotor activity. However, if the CRPP and the rhythm of activity show a fixed phase relationship to each other, then the entraining light pulses in the different T cycles must be illuminating different phases of the CRPP just as they are illuminating different phases of the activity rhythm. Tables 1 and 2 show the response of the reproductive organs to the various T cycles. Overall *F* tests were significant for all variables measured. Reproductive organs of individuals in 1L:22D and 1L:24D (Table 1) and 1L:21.67D and 1L:24.16D (Table 2) were significantly larger than those of males in the 1L:23D control groups. Fig. 2 shows the reproductive organ responses of the deer mice entrained to the various T cycles as a function of the phase of the light relative to their circadian activity rhythm. Light pulses falling during the subjective day (1L:23D) were nonstimulatory to the reproductive system, whereas light falling during either the early (1L:24D, 1L:24.16D) or late (1L:22D, 1L:21.67D) subjective night were inductive. These data demonstrate that the photoinducible phase (ϕ_i) of the CRPP in deer mice extends from 0.1 h after activity onset to at least 11.8 h after activity onset.

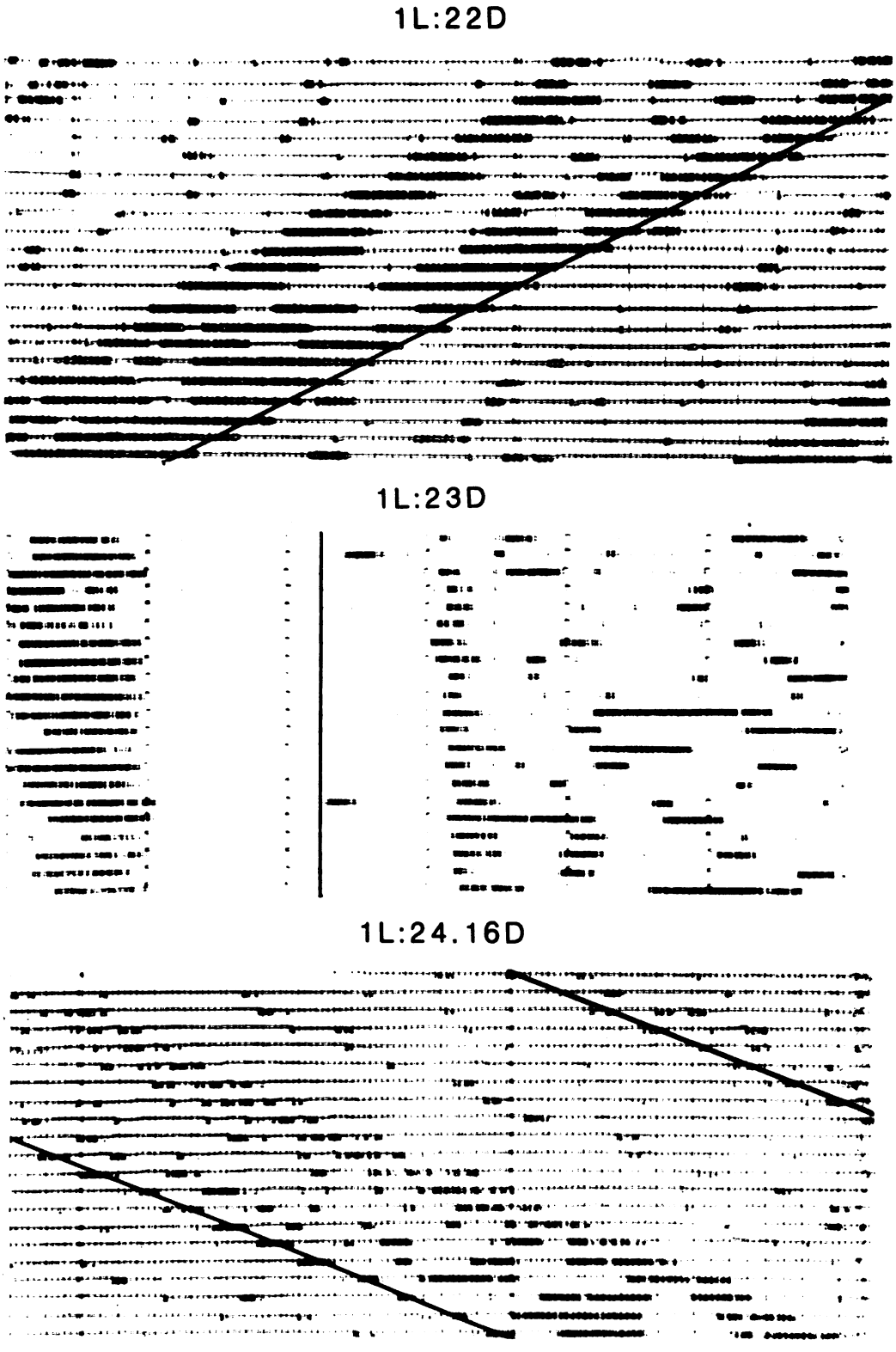

FIG. 1. Activity records of deer mice entrained to several different light-dark cycles. The diagonal or vertical lines indicate the onset of the daily 1-h light pulse.

Fig. 3 shows representative activity records of individual deer mice exposed to 24-h LD cycles with photoperiods of 6, 10, 12, 16, and 18 h (Expt. 3). Fig. 4 presents the average phase relationships between activity onsets and light offsets under 24 LD cycles of groups of deer mice exposed to photoperiods ranging from 1 (1L:23D) to 18 (18L:6D) h (Expt. 3). Under photoperiods of less than 10 h, light offsets occur before activity onsets, although the negative ψ decreases as photoperiod duration lengthens. In photoperiods durations of between 10 and 12 h the activity onsets begin occurring at, or before, light offsets, and ψ becomes increasingly positive as photoperiod duration lengthens up to the 18-h photoperiod.

Fig. 5 shows the gonadal responses of deer mice, drawn from a previous study (Whitsett et al., 1984b), to 24-h LD cycles with photoperiods ranging from 3 (3L:21D) to 19 (19L:5D) h. Photoperiods <11 h are noninductive, whereas in mice receiving from 11.5 to 19 h of light per day there is a quantitative increase in testis weight as a function of photoperiod. Therefore, rather than a "critical" photoperiod, there is a gradual increase in the effectiveness of photoperiod as photoperiod duration lengthens.

By combining the results obtained from the activity patterns on various LD cycles (Figs. 1, 3, 4) with the gonadal responses to these LD cycles (Figs. 2 and 5) we can determine the duration and phase of ϕ_i in deer mice. For example, the T protocol indicates that ϕ_i extends from 0.1 h to 11.8 (CT) h after activity onset (CT12.1-CT23.8) (Fig. 2). Examination of Figs. 3 and 4 shows that, for standard (T=24 h) photoperiods greater than 10–11 h, light begins falling during the early subjective night and for photoperiods greater than 12 h light falls on the late subjective night as well. The gonadal response to various duration standard photoperiods (Fig. 5) shows a gradual increase in the inductive effects of photoperiods beginning around 11.5 h. Since 10L:14D (and shorter duration) photoperiods are noninductive we conclude that illumination from CT1.8 (light onset of 10L:14D) to CT11.8 (light offset, 10L:14D) falls during the insensitive phase of the CRPP. Therefore, ϕ_i onset must occur between CT11.8 and CT12.1, that is, between 12 min before and 6 min after activity onset. Similarly, the offset of ϕ_i must occur between CT23.8 (where light is inductive; Fig. 2) and CT1.8 (where it is not; Figs. 4 and 5). Fig. 6 shows a graphic representation of ϕ_i in deer mice relative to the animal's circadian cycle.

DISCUSSION

Our experimental methodology assumes that the photoinducible phase of the CRPP and the circadian rhythm of activity bear a fixed phase relationship one to another. Since we cannot directly assess the phase of the CRPP on a daily basis, we must assume that it bears some constant phase relationship to a rhythm that we

TABLE 1. Influence of light-dark cycles on body weight and reproductive organ weight in male deer mice at 6 wk of age in Experiment 1 (data expressed as mean ± SEM).

	LD cycle				
	1:22	1:23	1:24	$F_{2,53}$	P<
Body weight (g)	15.9 (0.4)	15.1 (0.4)	16.0 (0.3)	1.33	NS
Testis weight (mg)	68.1[a] (6.5)	41.4 (6.8)	77.8[a] (5.0)	8.03	0.0009
Log seminal vesicle weight (mg)	1.21[a] (0.11)	0.58 (0.09)	1.18[a] (0.08)	12.55	0.0001
Sample size	22	17	19		

[a]Significantly different from 1L:23D group (α = 0.05).

TABLE 2. Influence of light-dark cycles on body weight and reproductive organ weight in male deer mice at 6 wk of age in Experiment 2 (data expressed as mean ± SEM).

	LD cycle				
	1:21.67	1:23	1:24.16	$F_{2,50}$	P<
Body weight (g)	1.65[a] (0.6)	15.0 (0.3)	17.0[a] (0.5)	4.29	0.02
Testis weight (mg)	60.3[a] (6.6)	39.1 (4.4)	68.4[a] (7.7)	6.09	0.005
Log seminal vesicle weight (mg)	0.83[a] (0.12)	0.44 (0.07)	0.97[a] (0.12)	7.22	0.002
Sample size	19	20	14		

[a]Significantly different from 1L:23D group ($\alpha = 0.05$).

can measure (i.e., locomotor activity). It is now universally accepted that vertebrates are "multi-oscillator" in structure; that is, individuals possess more than one circadian clock (Pittendrigh, 1981). Accordingly, our assumption will hold either 1) if one clock drives both the rhythm of activity and the CRPP or 2) if two clocks are involved but they are tightly coupled and/or show similar responses to light. The validity of our assumption is strengthened a posteriori by the close relationship we observed between light, the phase of the activity rhythm, and the gonadal response.

Previously we demonstrated that photoperiodic time measurement in *P. maniculatus* relies on a circadian responsiveness to light by exposing both male and female deer mice to "resonance" LD cycles (6L:18D, 6L:30D,

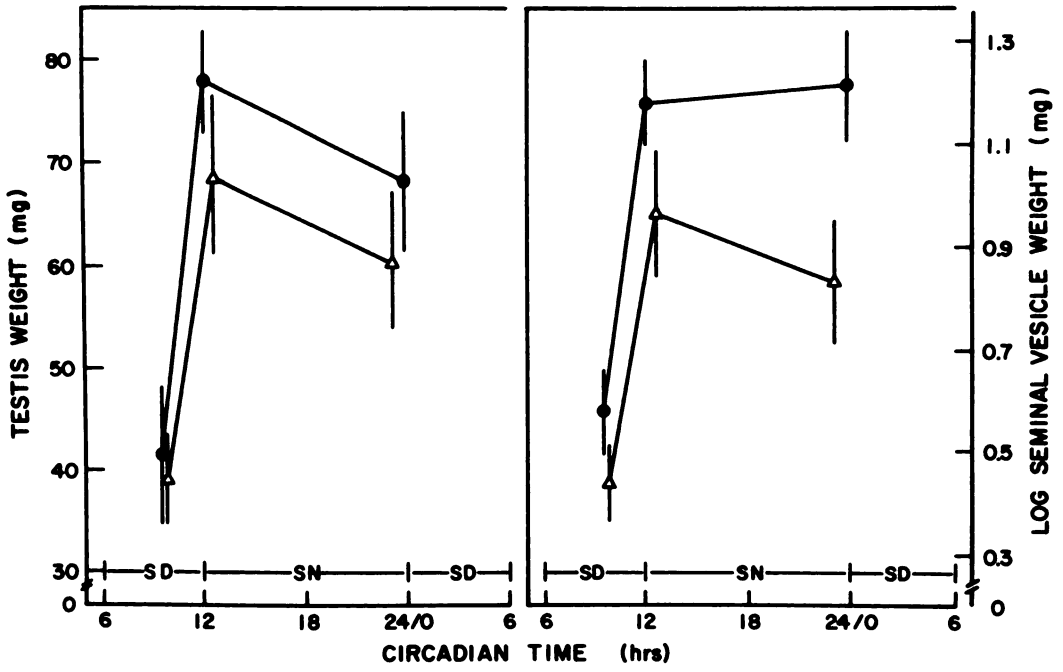

FIG. 2. Reproductive organ weights of deer mice subjected to various T lighting protocols as a function of the phase (light onset) of the 1-h light pulse relative to the circadian activity rhythm. *Solid circles* (Expt. 1) or *open triangles* (Expt. 2) denote means ± SE. *SD* and *SN* indicate the subjective day and subjective night, respectively. Activity onset marks the beginning of the "subjective night" (CT12). The phase relationships between the light onset and the activity onset on the non-24-h LD cycles were normalized to 24 h.

6L:42D, and 6L:54D) (Whitsett et al., 1983, 1984c). The circadian system of animals exposed to any of these LD cycles was assumed to be entrained with a period of 24 h. The 6-h photoperiod in the T=24 and T=48 cycles occurred at the same time every day or every other day (during the subjective day) and was not inductive. However, the T=36 and T=60 h cycles were inductive, since there was an alteration of light in the morning (subjective day) with light in the evening (subjective night) the following day (6L:30D) or 2 days later (6L:54D). The day or two of darkness between light was assumed to have little effect on the pattern of entrainment. The pulses falling during the subjective night (6L:30D, 6L:54D) impinged on the photoinducible phase and stimulated gonadal development, but pulses falling during the subjective day (6L:18D, 6L:42D) were noninductive. Therefore, the "resonance" paradigm was sufficient to show the existence of a CRPP in deer mice, but gave little information on the exact phase or duration of ϕ_i other than to demonstrate that it was positioned in the subjective night.

Combining information on the effects of different LD cycles on both the activity rhythm and the gonadal response of deer mice, we conclude that the onset of the photoinducible phase occurs at, or about, activity onset and extends for at least 11.8 h after activity onset. The termination of ϕ_i was not precisely determined, but it must occur by 13.8 h after activity onset. The middle of the subjective night (between 0.6 and 11.1 h after activity onset) was not tested. Part of this portion of the circadian cycles corresponds to the transition between delays and advances in the PRC (Daan and Pittendrigh, 1976), and stable entrainment is not possible in this portion of the PRC. The photoinducible phase therefore could either encompass the entire subjective night or exhibit a bimodal sensitivity pattern with photoinducible phases early and late in the subjective night and an insensitive phase in the middle. The former possibility seems more likely, however, in view of the findings of Elliott (1981) in the golden hamster (*Mesocricetus auratus*). By presenting single light pulses every 10 days to the middle of the subjective night of hamsters otherwise held in continuous darkness, Elliott (1981) demonstrated that this phase was also photoperiodically inductive. To our knowledge, Elliott's extensive studies on photoperiodic time measurement in golden hamsters are the only studies, other than our present study in deer mice, that empirically demonstrate the position and duration of a photoinducible phase in a mammal. The hamster studies made extensive use of resonance and "T" protocols and showed that ϕ_i in the hamster begins about 0.5 h after activity onset and ends between 11 and 11.5 h after activity onset (Elliott, 1976, 1981; Elliott et al., 1972). The photoinducible phase in the nocturnal deer mouse and hamster therefore seem to be quite similar. Both begin near activity onset and both extend at least until 11–11.5 h after activity onset.

The hamster exhibits a sharp "critical" photoperiod of about 12.5 h; photoperiods less than 12.5 h are not inductive, whereas in longer photoperiods the reproductive system is maximally stimulated (Elliott, 1981). However, deer mice exhibit increasing gonadal responsiveness as photoperiod duration increases from 11.5 to 19 h. The potential reasons for the different responses of hamsters and deer mice are several. For example, in hamsters a 1-h light pulse, if presented during ϕ_i, is as effective as a long photoperiod in stimulating the reproductive system (Elliott, 1981). It is possible that, in deer mice, increased duration of illumination of ϕ_i is associated with increased stimulation of the reproductive system. In certain birds, for example, night breaks of longer duration are more inductive than shorter duration breaks (Follett and Milette, 1982). Alternatively, the differences between hamsters and deer mice might reside in their respective entrainment characteristics. The precision of entrainment may be less in deer mice than in hamsters. An increased variability in ψ, for example, would be associated with a less precise photoperiodic time measuring ability. This idea is supported by studies of Pittendrigh and Daan (1976), who showed that the variation in τ is greater in deer mice than in golden hamsters and that, based on the species' average PRCs, the variation in ψ would also be greater in deer mice. One cannot exclude the possibility of differences at other neuroendocrine levels, including the hypothalamo-pituitary-gonadal axis and/or pineal physiology. Perhaps there are selective advantages to deer mice in not exhibiting a "critical" photoperiod per se.

The physiologic substrate underlying photoperiodic time measurement in mammals involves both the suprachiasmatic nuclei (SCN) of the hypothalamus and the pineal organ. A neural circadian clock(s) resides in the SCN and drives

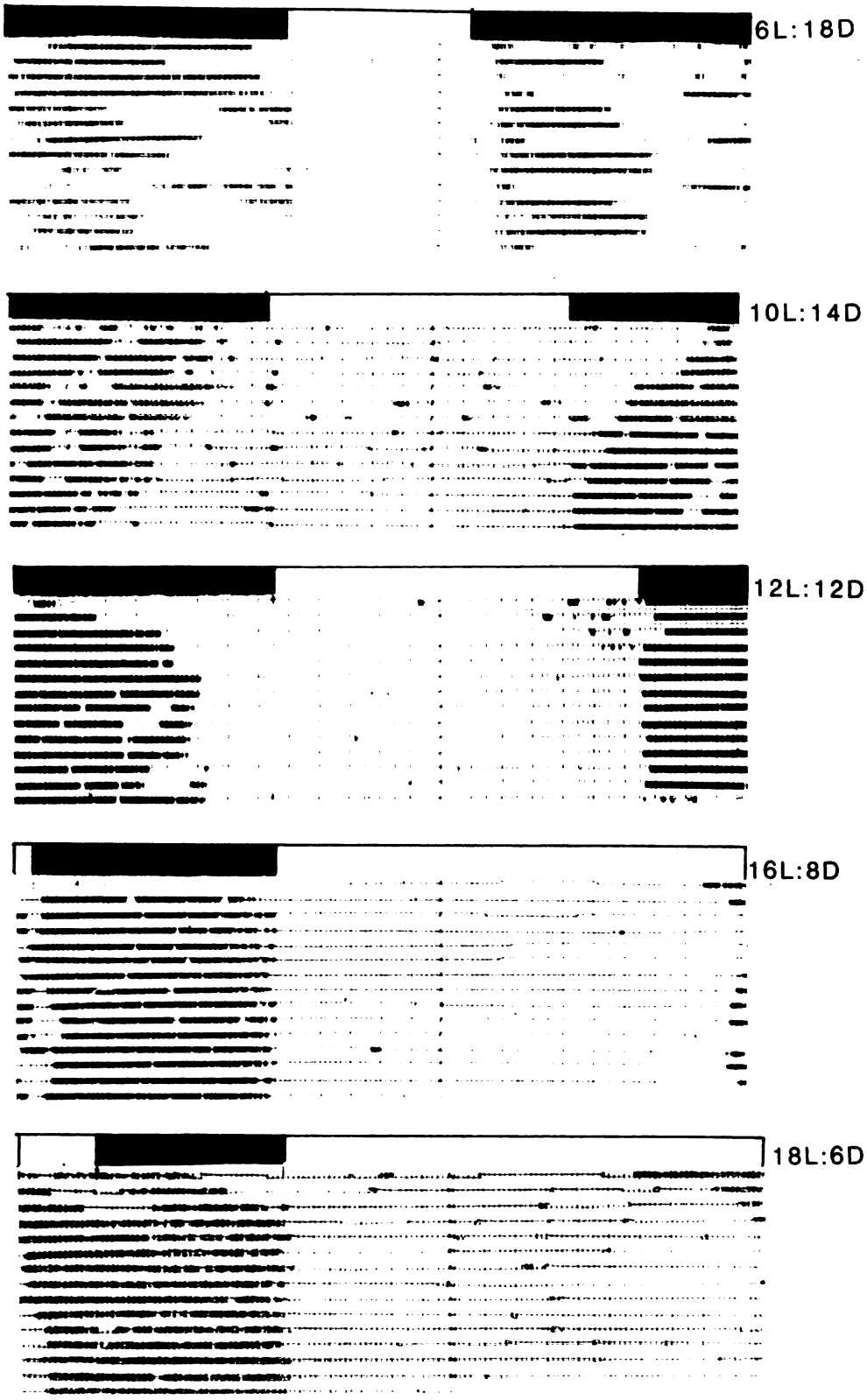

FIG. 3. Activity records of individual deer mice exposed to various 24-h LD cycles (Expt. 3). The light cycles are diagrammed at the top of each record.

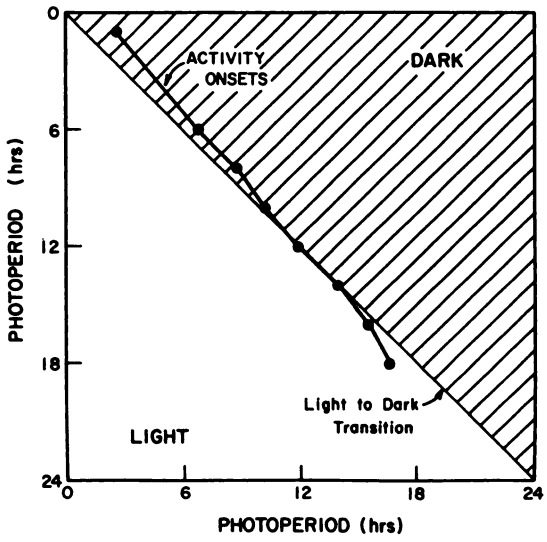

FIG. 4. Phase relationships between activity onsets and LD cycles during entrainment of deer mice to 24-h LD cycles with photoperiods of 1–18 h in duration.

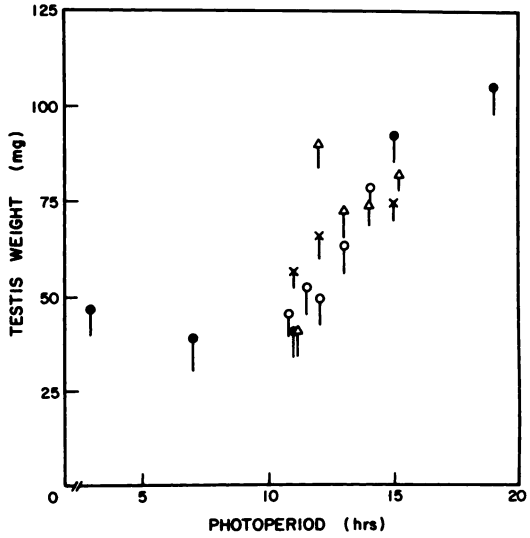

FIG. 5. The testicular response, measured at 6 wk of age, of deer mice exposed to various 24-h LD cycles. The symbols denote the means minus 1 SEM. Similar symbols indicate animals in the same experiment (of a series of 4). (Data drawn from Whitsett et al., 1984b.)

the circadian metabolism of the pineal, including the biosynthesis of the indoleamine melatonin. Pineal melatonin levels in all animals examined to date are highest at night regardless of the habits (nocturnal or diurnal) of the animal. Pineal melatonin is rapidly secreted into the blood. Recent studies in sheep and Djungarian hamsters offer convincing evidence that the duration of the nightly melatonin "pulse" is the cue by which these animals discriminate between photoperiodically inductive and noninductive day lengths (Bittman et al., 1983; Goldman et al., 1984). Our studies in *P. maniculatus* have shown that melatonin has antigonadal effects (Whitsett et al., 1984d) and that the shape (duration and amplitude) of the nocturnal melatonin pulse is profoundly affected by the length of the photoperiod (Noden and Underwood, unpublished). However, our studies on deer mice do not allow us to discriminate between the "internal" versus "external" coincidence models for the role of the circadian system in photoperiodic time measurement. If the pineal is involved in photoperiodic time measurement in deer mice via its nightly melatonin pulse, one could envisage an external coincidence mechanism whereby the onset and/or offset of the circadian melatonin rhythm is modified by the (external) coincidence of

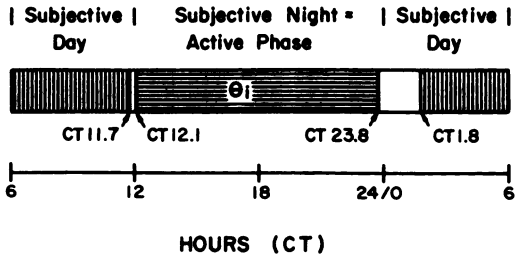

FIG. 6. The photoinducible phase of the CRPP in deer mice. The area with horizontal lines denotes the photoinducible phase (ϕ_i) (CT12.1 to CT23.8) of the circadian rhythm of photoperiodic photosensitivity relative to the circadian rhythm of activity. Activity onset marks the beginning of the subjective night (CT12-CT24). The *unmarked* areas (CT11.7 to CT12.1 and CT23.8 to CT1.8) denote untested phases and the areas with *vertical lines* (CT1.8 to CT11.7) denote the phase of the CRPP that is insensitive to the inductive effects of light.

light with the melatonin rhythm. Alternatively, an internal coincidence mechanism could be operating whereby light (internally) phases circadian oscillators that separately drive the onset and offset of the nightly melatonin rhythm. The duration of the melatonin pulse would be a function of the phase relationship between these two oscillators (Illnerová and Vaněček, 1982).

REFERENCES

Bittman, E. L., Dempsey, R. J. and Karsch, F. J. (1983). Pineal melatonin secretion drives the reproductive response to daylength in the ewe. Endocrinology 113:2276–2283.

Bünning, E. (1936). Die endogene tagesrhythmik als Grundlage der photoperiodischen Reaktion. Ber. Deutsh. Bot. Ges. 54:590–607.

Daan, S. and Pittendrigh, C. S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents II. The variability of phase response curves. J. Comp. Physiol. 106:253–266.

Elliott, J. A. (1976). Circadian rhythms and photoperiodic time measurement in mammals. Fed. Proc. 35:2339–2346.

Elliott, J. A. (1981). Circadian rhythms, entrainment and photoperiodism in the Syrian hamster. In: Biological Clocks in Seasonal Reproductive Cycles (B. K. Follett and D. E. Follett, eds.). John Wright and Sons Ltd., Bristol, pp. 203–217.

Elliott, J. A. and Goldman, B. D. (1981). Seasonal reproduction: photoperiodism and biological clocks. In: Neuroendocrinology of Reproduction (N. T. Adler, ed.). Plenum Publ., New York, pp. 377–423.

Elliott, J. A., Stetson, M. H. and Menaker, M. (1972). Regulation of testis function in golden hamsters: a circadian clock measures photoperiodic time. Science 178:771–773.

Follett, B. K. (1973). Circadian rhythms and photoperiodic time measurement in birds. J. Reprod. Fertil. Suppl. 19:5–18.

Follett, B. K. and Milette, J. J. (1982). Photoperiodism in quail: testicular growth and maintenance under skeleton photoperiods. J. Endocrinol. 93: 83–90.

Goldman, B. D., Darrow, J. M. and Yogev, L. (1984). Effects of timed melatonin infusions on reproductive development in the Djungarian hamster (*Phodopus sungorus*). Endocrinology 114:2074–2083.

Grocock, C. A. and Clarke, J. R. (1974). Photoperiodic control of testis activity in the vole, *Microtus agrestis*. J. Reprod. Fertil. 39:337–347.

Illnerová, H. and Vaněček, J. (1982). Two-oscillator structure of the pacemaker controlling the circadian rhythm of *N*-acetyltransferase in the rat pineal gland. J. Comp. Physiol. 145:539–548.

Nelson, R. J., Bamat, M. K. and Zucker, I. (1982). Photoperiodic regulation of testis function in rats: mediation by a circadian mechanism. Biol. Reprod. 26:329–335.

Pittendrigh, C. S. (1965). On the mechanism of entrainment of a circadian rhythm by light cycles. In: Circadian Clocks (J. Aschoff, ed.). North-Holland, Amsterdam, pp. 277–297.

Pittendrigh, C. S. (1972). Circadian surfaces and the diversity of possible roles of circadian organization in photoperiodic induction. Proc. Natl. Acad. Sci. USA 69:2734–2737.

Pittendrigh, C. S. (1981). Circadian organization and the photoperiodic phenomena. In: Biological Clocks in Seasonal Reproductive Cycles (B. K. Follett and D. E. Follett, eds.). John Wright and Sons Ltd., Bristol, pp. 1–35.

Pittendrigh, C. S. and Daan, S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents IV. Entrainment: pacemaker as clocks. J. Comp. Physiol. 106:291–331.

Pittendrigh, C. S. and Minis, D. H. (1964). The entrainment of circadian oscillations by light and their role as photoperiodic clocks. Am. Nat. 98:261–294.

Whitsett, J. M., Underwood, H. and Cherry, J. (1983). Photoperiodic stimulation of pubertal development in male deer mice: involvement of the circadian system. Biol. Reprod. 28:652–656.

Whitsett, J. M., Lawton; A. D. and Miller, L. L. (1984a). Photosensitive stages in pubertal development of male deer mice (*Peromyscus maniculatus*). J. Reprod. Fertil. 72:269–276.

Whitsett, J. M., Noden, P. F., Cherry, J. and Lawton, A. D. (1984b). Effect of transitional photoperiods on testicular development and puberty in male deer mice (*Peromyscus maniculatus*). J. Reprod. Fertil. 72:277–286.

Whitsett, J. M., Cherry, J. and Underwood, H. (1984c). Involvement of the circadian system in photoperiodic control of pubertal development in female deer mice, *Peromyscus maniculatus*. Experientia 40:887–888.

Whitsett, J. M., Underwood, H. and Cherry, J. (1984d). Influence of melatonin on pubertal development in male deer mice (*Peromyscus maniculatus*). J. Reprod. Fertil. 72:287–293.