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Photoperiodic regulation of the hamster testis: Dependence on circadian rhythms

(seasonal reproduction/photosensitivity/deuterium oxide/period homeostasis)

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ABSTRACT **The testes of hamsters exposed to short days (10 hr of light per day) regress within 13 weeks. Administration of 7.5% deuterium oxide to hamsters lengthens the period of free running circadian activity rhythms by 2.2% and prevents testicular regression during short-day exposure. This is consistent with predictions derived from an external coincidence model for photoperiodic time measurement: Deuterium oxide changes phase relationships between the light-dark cycle and the circadian system, the hamster's daily photosensitive phase is stimulated with light during short days, and the testes remain large. Conservation of the period of circadian rhythms within narrow limits has adaptive significance for hamster photoperiodism and for the occurrence and phasing of the annual reproductive cycle.**

During the period beginning in late pregnancy and extending through birth and lactation, the mammalian mother and the newborn are most susceptible to detrimental effects from the external environment. The seasonal breeding cycles characteristic of most mammals in temperate zones ensure that the young are born at times optimal for survival (1). Golden hamsters (*Mesocricetus auratus*) exposed to natural photoperiods undergo annual gonadal cycles that are determined in part by seasonal changes in the day length (2, 3). In the laboratory, hamsters exposed daily to 12.5 hr or more of light (long days) maintain functionally mature gonads (4). In the long-day photostimulated male, full spermatogenesis is maintained (5) and androgen secretion is normally adequate to ensure copulatory behavior (6, 7) and fertilization of ova. When day length decreases below the critical value of 12.5 hr (short days), or if the animal is blinded, the gonads regress, spermatogenesis ceases, androgen levels decline to low values within 4–6 weeks, and copulation ceases (4–6, 8). If short days are maintained, the testes will spontaneously recrudesce and completely regrow in 25–30 weeks. Testicular recrudescence can be induced by long photoperiods in advance of its spontaneous occurrence; during the regressed phase of the cycle the animals remain photosensitive, but the short days are inadequate to maintain the reproductive system (8).

Bünning (9) first proposed that measurement of day length [photoperiodic time measurement (PTM)] could be based on an endogenous circadian rhythm of sensitivity to light; the photoperiodic effect of a particular light-dark cycle (LD) depends on the position of the light relative to the circadian rhythm of photosensitivity. Photostimulation or photoinduction occurs only when light extends into the sensitive portion of the rhythm. This can be accomplished by long days; short days fail to photostimulate because light falls only in the insensitive phase. This external coincidence model is one of two specific

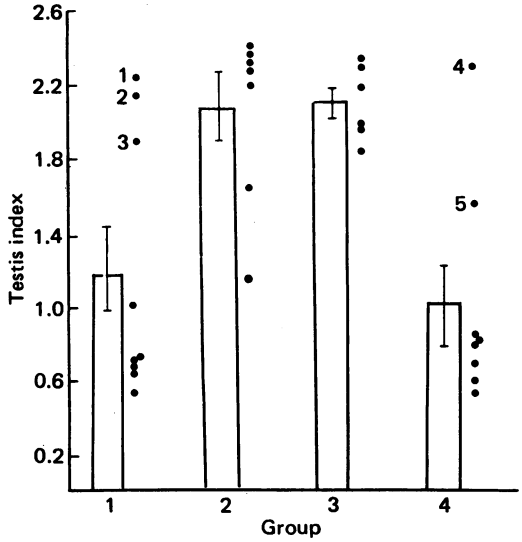

FIG. 1. TI of individual animals after 13 weeks of exposure to the LD 10:14 photoperiod. The bars and thin vertical lines indicate the mean ± SEM. The numbered points (1–5) identify individual animals.

hypotheses that have been advanced to explain how circadian clocks may be involved in PTM. Pittendrigh and Minis (10) noted that light has a dual role in such a model for PTM: It entrains the many circadian rhythms of the organism, including the rhythm of photoperiodic sensitivity; and it produces the photoperiodic response when it coincides with the light-sensitive phase of the circadian photosensitivity rhythm.

Multicellular organisms comprise a population of circadian oscillations whose mutual phase relationships may have significant effects on physiological functions, in particular PTM. The internal coincidence model (11) assumes that some oscillations in the system are coupled to dawn (lights-on) and others to dusk (lights-off) and that their phase relationships change as a function of day length. The single role of light in this scheme is to entrain circadian oscillations whose mutual phase relationships determine the photoperiodic response. The external and internal coincidence models are not mutually exclusive and it is often difficult to generate distinct predictions based on the separate models (12); this is especially true in hamsters because light is the only established *Zeitgeber* for entraining circadian rhythms in this species. The experiments described herein are not contingent upon which model is adopted; the results have been described in terms of the external

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Abbreviations: PTM, photoperiodic time measurement; LD, light-dark cycle; PSP, photosensitive phase; TI, testis index; T, period of entraining LD cycle; τ, endogenous period of circadian rhythm; DD, constant darkness.

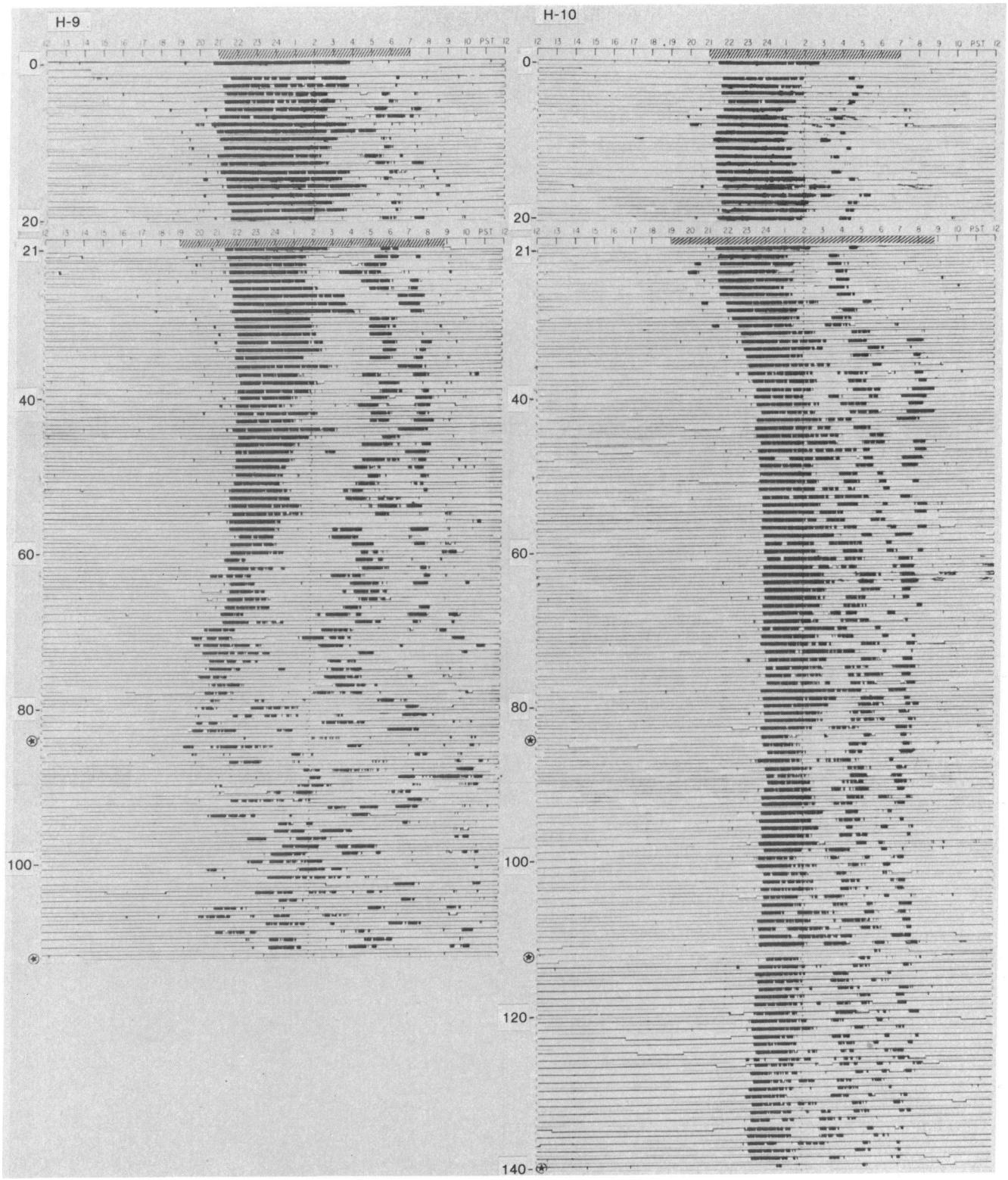

FIG. 2. Continuous records of wheel running activity of two hamsters: H-9 in (group 1) and H-10 in group 2 (7.5% ^2H_2O from day 26 to day 140). The LD cycle is indicated on a 24-hr time scale (Pacific Standard Time) with the hatched horizontal bar signifying darkness. During days 0–20, both animals were exposed to 14 hr of light per day; beginning on day 21 they received 10 hr of light per day. Numbers at the left indicate number of days. Laparotomies (designated by circled stars) were performed after 9, 13, and 17 weeks of exposure to the LD 10:14 cycle (days 84, 112, and 140). The testes of H-9 were regressed at 9 and 13 weeks (TIs of 0.86 and 0.68, respectively); those of H-10 were not regressed at weeks 9, 13, or 17 (TIs of 2.34, 2.34, and 2.21, respectively).

coincidence model but do not exclude the involvement of an internal coincidence scheme.

The hamster's measurement of day length is thought to be mediated by a circadian photosensitivity rhythm that bears a constant phase relationship to the overtly recorded circadian locomotor activity cycle (13, 14). The daily photosensitive phase (PSP) begins approximately 0.5 hr prior to the onset of wheel-running activity and ends between 11 and 11.5 hr after onset

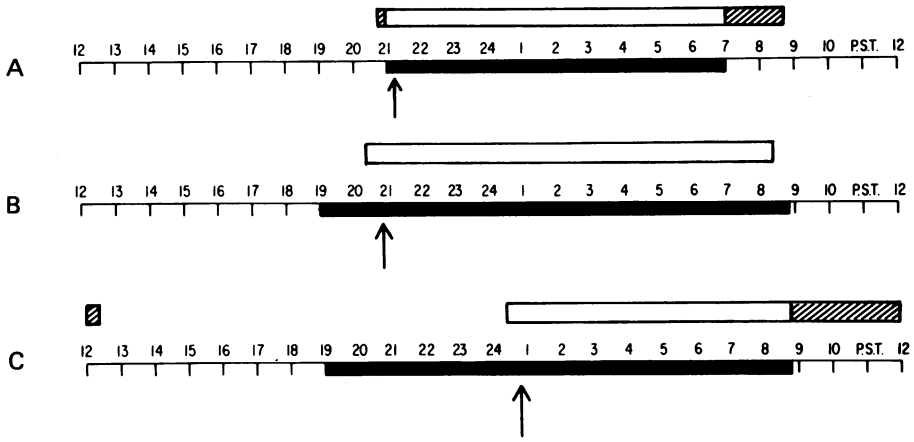

FIG. 3. Schematic representation of activity onsets (arrows) and PSPs (horizontal bars above numbers) for hamsters drinking H_2O (*A* and *B*) or 7.5% ^2H_2O (*C*). The LD cycle is shown on a 24-hr time scale with the solid black bar signifying darkness. In *A* the animals ($n = 26$) were exposed to 14 hr of light per day and in *B* ($n = 6$), to 10 hr of light. The intervals during which the daily photosensitive and light phases coincided (hatched horizontal bars) were 2.0, 0, and 3.5 hr long in *A*, *B*, and *C*, respectively. The estimated duration and position of the PSP are derived from experiments on populations of animals (13, 14); the extent to which the PSP varies among individuals is not known. Nor has it been established what minimum duration of light stimulation of the PSP is adequate to maintain the gonads. Based on values in refs. 13 and 14, H-9 (Fig. 2) received 0.3 hr of light stimulation of his PSP while entrained to the LD 10:14 photoperiod. Either his PSP was of shorter than average duration or 0.3 hr of photostimulation was below the threshold value necessary for gonadal maintenance. All other values were empirically derived from records such as those illustrated in Fig. 2. Only records from hamsters with stable entrainment of wheel running to the LD cycles were used in these analyses. Days 6–15 were analyzed in deriving values in *A* and days 53–62 for most values in *B* and *C*. The testes of group 1 hamsters regressed in LD 10:14 (*B*); those of group 2 animals did not (*C*).

of activity. Long-day responses occur when as little as 1 hr of light per day coincides with the PSP (13, 14).

Under entrained conditions, the phase angle of activity relative to the 24-hr photoperiod depends upon the period (τ) of the underlying circadian pacemaker (15); the range of τs adopted by the circadian system affects the phase angle and thus the positioning of the daily PSP. We speculated that the effectiveness of the circadian system for accurately measuring day length is contingent upon conservation of τ within the narrow range normally observed in hamsters. The main goal of the present study was to test the prediction that a slight change in the τ of the hamster's circadian rhythms would have functional consequences for PTM and the annual reproductive cycle. A second aim was to confirm and extend the findings of Elliott (13, 14) concerning circadian mediation of PTM in hamsters.

MATERIALS AND METHODS

Male hamsters, derived from stock (LVG-LAK) purchased from the Lakeview Hamster Colony (Newfield, NJ) and born in our laboratory, were maintained from birth on a photoperiod of 14 hr of light per 24 hr (LD 14:10). The light phase began at 0700 hr and ended at 2100 hr, Pacific Standard Time.

Sexually mature hamsters were placed in individual activity cages on 17 June 1976 and were provided with continuous access to food (Simonsen rat pellets, maintenance diet), tap water, and an activity wheel. The average light intensity at the cage level was 130 lumen/m^2 of cool white fluorescent light.

Wheel revolutions were continuously monitored for each animal on Esterline–Angus event recorders. After the wheel running of hamsters had entrained to the LD 14:10 cycle, the daily photoperiod was shortened to 10 hr of light (LD 10:14). Photoperiod length was monitored by means of a photocell; the L phase of the LD 10:14 cycle was actually 10 min longer than intended—i.e., lights were on from 0850 hr to 1900 hr daily.

Before the transition to LD 10:14, the mature size of the testes was confirmed via external palpation of the gonads. Actual testis measurements (16, 17) indicate that the gonads of animals exposed to LD 14:10 photoperiods are large and functional.

Four to five days after the transition to the LD 10:14 photoperiod, the 30 hamsters were divided into four groups. Group 1 ($n = 9$) was offered tap water in their bottles. For group 2 ($n = 7$), the tap water was replaced with 7.5% ^2H_2O (deuterium oxide). Group 3 ($n = 6$) was offered 7.5% ^2H_2O for the first 2 weeks in LD 10:14 and 10% ^2H_2O thereafter. Two concentrations of ^2H_2O were used to provide a broad range of phase angle differences between light-onset and activity-onset (18). Group 4 ($n = 8$) was blinded by bilateral orbital enucleation, performed under ether anesthesia, and the tap water was replaced with 7.5% ^2H_2O.

Testicular condition was assessed during laparotomies performed under pentobarbital sodium anesthesia (80 mg/kg of body weight) 9 and 13 weeks after transfer to the LD 10:14 photoperiod. Some hamsters also were laparotomized after 17 weeks, because regression is not always complete after 13 weeks of short-day exposure in hamsters housed in activity wheels (13). The length and width of each animals's right testis were measured to the nearest 0.1 mm and the testis index (TI) computed as follows: (length × width)/bodyweight. A TI of approximately 2.0 indicates a large functional gonad; TI $\leq$ 1.0 signifies a regressed hypofunctional testis (16).

RESULTS

After 13 weeks of exposure to the LD 10:14 photoperiod, six of the nine hamsters drinking water (group 1) and six of the eight blind hamsters drinking 7.5% ^2H_2O (group 4) had regressed testes. None of the 13 sighted hamsters drinking ^2H_2O (groups 2 and 3) had completely regressed testes ($P = 0.001$, Fisher exact probabilities test, group 1 *vs* groups 2 and 3; $P = 0.0005$, group 4 *vs* groups 2 and 3) (Fig. 1). The TIs of animals in groups 2 and 3 were greater than those of animals in group 1 ($P < 0.02$; $P < 0.008$, respectively, *t* tests) and were also greater than those of animals in group 4 ($P < 0.003$; $P < 0.002$, respectively). The TIs of groups 2 and 3 did not differ significantly. Animals drinking ^2H_2O did not exhibit testicular regression during prolonged exposure to the LD 10:14 photoperiod. ^2H_2O did not prevent regression of the gonads of blind hamsters; progonadal

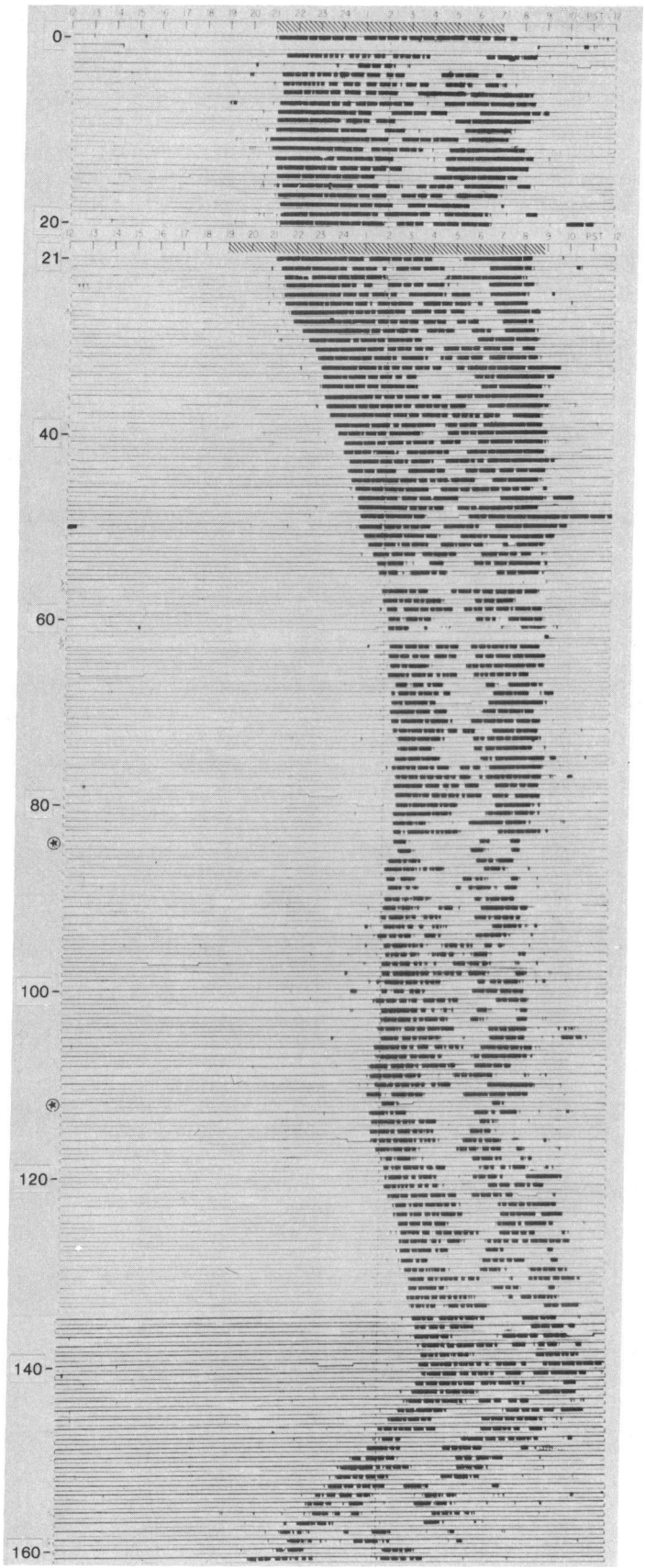

FIG. 4. Continuous record of wheel running activity for H-15 (group 3); 7.5% ^2H_2O was offered on days 25–38 and 10% ^2H_2O on days 39–141; thereafter, the animal was given H_2O. From day 114 until the end of the record the animal was maintained in constant darkness. TIs at weeks 9 and 13 were 1.92 and 1.85, respectively. Other treatments and symbols as in Fig. 2.

effects of ^2H_2O are seemingly continent on its effect on endogenous rhythms and their interaction with the LD cycle (see below).

In LD 14:10, activity onset for all stably entrained animals began at 2114 hr ± 3 min (mean ± SEM). Records in Fig. 2 show representative entrainment to this photoperiod during days 0–20. The PSP in LD 14:10 was estimated to extend from 2044 hr until 0844 hr each day (Fig. 3*A*). The light phase and PSP coincided from 2044 hr to 2100 hr and from 0700 hr to 0844 hr daily (2 hr). This duration of photostimulation is adequate to maintain large functional testes (13, 14).

In LD 10:14, activity for group 1 hamsters drinking H_2O and undergoing testicular regression began at 2053 hr ± 24 min; their PSP extended from 2023 hr to 0823 hr daily and was not stimulated with light (Fig. 3*B*).

An increased lability in the activity pattern and the loss of a stable phase reference point coincided with testicular regression (Fig. 2, H-9, days 70–112). Activity fragmentation was recorded for the majority of sighted and blind hamsters undergoing testicular regression and may reflect effects of gonadal hormones on the hamster circadian system (see ref. 19).

The testes of three animals in group 1 showed little or no regression by week 13. For a large portion of the 13-week interval in LD 10:14, their entrainment to the photoperiod was such that light coincided with the PSP. For example, H-1, whose activity onset began at 2223 hr, received approximately 1 hr of light stimulation of the PSP. This duration of PSP photostimulation is adequate to maintain the testes (13, 14). Others (e.g., ref. 20; Fig. 1) have observed that the testes of some hamsters maintained in short days fail to regress. This may be understood in terms of atypical entrainment to the LD cycle. Animals 2 and 3 (Fig. 1) eventually adopted nonstimulatory phase angles of entrainment and their testes began to regress (TIs of 1.53 and 1.26 at week 17). The TIs of two blind hamsters (animals 4 and 5 in Fig. 1, group 4) declined to 1.41 and 1.08, respectively, by week 17, suggesting that their testes eventually also would have regressed completely.

Activity onset for hamsters drinking 7.5% ^2H_2O (group 2) occurred at 2452 hr ± 19 min in the LD 10:14 photoperiod. The average delay of 4 hr in activity onset induced by ^2H_2O changed the phase relationship between the LD cycle and the postulated circadian rhythm of photoperiodic sensitivity so that light coincided with 3.5 hr of the PSP (Fig. 3*C*). The corresponding time of activity onset for hamsters ingesting 10% ^2H_2O (group 3) was 0138 ± 30 min, which provided 4.3 hr of light stimulation of the PSP. Representative activity records for group 2 and group 3 animals are shown in Fig. 2 (H-10) and Fig. 4. Five of the deuterated hamsters from groups 2 and 3 were tested through week 17 and their testes still had not regressed (e.g., H-10 in Fig. 2).

Blind animals ingesting 7.5% ^2H_2O manifested free-running activity rhythms whose period was 24.63 ± 0.06 hr. The τ for activity in nondeuterated hamsters free-running in constant darkness (DD) is 24.11 hr, computed as the average value reported in three recent studies (13, 21, 22). The change in τ induced by 7.5% ^2H_2O thus was approximately 0.52 hr or a 2.2% increase over the average τ recorded in DD.

Changes in phase angle induced by 10% ^2H_2O were large (Fig. 4). Wheel running was stably entrained during days 58–85 and the active phase (α) was compressed. A gradual drift toward earlier activity onsets began on day 86. At the end of week 13 (day 112), this animal was placed in DD while continuing to drink ^2H_2O. The free running τ of 24.1 hr is far shorter than one would predict, given the phase angle observed during entrainment to the LD 10:14 photoperiod and the hamster phase

response curve (23). An unusually short τ in DD (23.61 hr) was also seen when ^2H_2O was replaced with H_2O (day 142). These data suggest a substantial after-effect on τ related to ^2H_2O ingestion. Comparable results were observed in the majority of similarly treated hamsters.

DISCUSSION

During entrainment, τ, the endogenous period of circadian rhythms, is matched exactly to the period (T) of the entraining LD cycle. Because the τ of circadian rhythms is not exactly 24 hr, entrainment by the LD 10:14 photoperiod (T = 24 hr) requires a phase shift each cycle to eliminate the discrepancy between τ and T (13, 14). ^2H_2O lengthens the τ of free-running hamster circadian rhythms; if ^2H_2O exerts a similar lengthening effect on circadian rhythms entrained to LD cycles, it will increase the phase advance necessary to adjust τ to T. Particular phase shifts can only be obtained when light falls at a particular phase of the circadian cycle; ingestion of ^2H_2O therefore imposes a changed phase relationship between the LD cycle and the entrained circadian system (18). The aspect of ^2H_2O action most likely responsible for this effect is the lengthening in τ; there is no evidence that ^2H_2O has an effect on the shape of the phase response curve (21).

When hamsters are exposed to short day lengths, ingestion of ^2H_2O produces a coincidence of light with the hypothesized circadian rhythm of photosensitivity. The hamster's neuroendocrine system then responds as though short days were long days and the testes inappropriately fail to regress. Three nondeuterated animals spontaneously exhibited similar atypical entrainment to the LD 10:14 cycle; the testes of these animals also failed to regress or regressed more slowly, depending on whether and when a shift to a nonstimulatory phase angle occurred. These findings are consistent with an external coincidence model of PTM; accurate measurement of day length depends on specific phase relationships with the external LD cycle.

An increase approximating 2% in the hamster's underlying circadian τ establishes phase relationships that cause hamsters to misinterpret short days as long days. The normal conservation of the hamster's τ within narrow limits, which is a remarkable property of hamster circadian oscillations (24), has functional significance: In the absence of such τ homeostasis, the circadian system loses its utility for PTM and for phasing of the annual reproductive cycle. It remains to be established whether and how other photoperiodic mammals with a greater range of circadian periods than hamsters are able to use their circadian rhythms for accurate measurement of day length.

Large aftereffects in the τ of the free-running activity rhythm were observed in deuterated hamsters released into DD after entrainment to the LD 10:14 photoperiod. Entrainment to various 24-hr photoperiods *per se* has no clear after effect on the hamster's τ (24); nor does ^2H_2O produce after effects on τ when deuteration occurs under constant conditions (DD or LL) (21, 22). It is possible that the large discrepancy between τ and T during entrainment in the deuterated state is responsible for the aftereffects. The large daily advancing phase shifts necessitated by deuteration may shorten the τ of the underlying circadian oscillator and could account for the gradual drift toward earlier activity onsets in LD 10:14.

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