Establishment and persistence of photoperiodic memory in hamsters

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Long summer days unequivocally stimulate, and short winter days inhibit reproduction in Siberian hamsters. By contrast, intermediate-duration day lengths (12.5–14 h long) either accelerate reproductive development or initiate regression of the reproductive apparatus. Which of these outcomes transpires depends on an animal's photoperiodic history, suggesting that hamsters must encode a representation of prior photoperiods. The duration of nocturnal melatonin secretion is the endocrine representation of day length, but nothing is known about how long it takes to establish photoperiodic histories or how long they endure. Hamsters exposed for 2 or more weeks to long summer day lengths acquired a long-day photoperiodic history that determined subsequent reproductive responses to intermediate-duration day lengths and melatonin signals. The memory for long-day lengths persisted in pinealectomized hamsters for 6.5 weeks, faded significantly after 13 weeks, and was functionally absent after 20 weeks. These findings indicate that hamsters are influenced only by relatively recent day lengths and melatonin signals and ignore earlier ones that might cause them to misinterpret the salience of current day lengths.

Seasonal breeding is timed so that lactation and subsequent weaning of young coincide with annual peaks in food availability, thereby increasing fitness (1, 2). Variation in day length (DL) is a major environmental cue for phasing reproduction (3); the light-dark cycle entrains an endogenous circadian rhythm of pineal melatonin (Mel) secretion; elevated nightly Mel production is directly proportional to the length of the dark phase and is much longer under short days (SDs) than long days (LDs; typically $\geq$10 h vs. 5 h per night, respectively; ref. 4). Summer DLs ($\geq$14 h light per day; 14L) stimulate, and winter days ($\leq$10L) inhibit reproduction in long-day breeding photoperiodic rodents. Nightly infusions of Mel that match endogenous durations mimic the effects of corresponding DLs on reproductive physiology in pinealectomized rodents deprived of endogenous Mel (5, 6).

In temperate regions, each DL occurs twice per year. Identical intermediate-duration DLs (12L–14L) can be separated by as many as 6 months (e.g., at the vernal and autumnal equinoxes); thus, a given intermediate DL can be a harbinger of radically different environmental conditions. In the Siberian hamster (*Phodopus sungorus*), as in other photoperiodic LD breeding rodents (e.g., *Microtus* and *Peromyscus*), divergent reproductive responses occur in early spring and late summer in response to identical intermediate DLs. Individuals with a recent history of longer DLs (shorter Mel signals) interpret intermediate DLs and their associated Mel signals as SDs, and initiate gonadal regression, whereas animals with a recent history of shorter DLs (longer Mel signals) initiate gonadal and somatic growth; thus, the reproductive effects of a given intermediate DL or Mel signal depend largely on the DL and Mel signals that preceded it (7–11). Such history-dependent responses imply that a ''memory'' for prior Mel signals dictates gonadotrophic responses to current intermediate DLs, where memory is shorthand for prior DL information that influences how animals respond to current photoperiod signals. A hamster in late summer exposed to decreasing intermediate DLs has a photoperiodic history consisting of both long and short DLs, from the preceding summer and winter, respectively. Whether and how such animals are influenced by more recent DLs and ignore earlier ones is unknown. The amount of experience with a particular range of DLs an animal must have before it encodes them as a photoperiodic history also remains to be specified. Herein, we determine how long it takes to establish a long-day photoperiodic history and describe the persistence and eventual decay of this representation of DL over a seasonal time scale.

Materials and Methods

Animals and Procedures. Siberian hamsters (*P. sungorus*) were obtained from our breeding colony, which was maintained in a 15L:9-h dark (15L) photoperiod. Onset of darkness was 1800 h Pacific Standard Time for all light-dark cycles. Under light methoxyflurane vapor anesthesia, testis dimensions were obtained by measuring the length and width of the left testis ($\pm$0.1 mm) with analogue calipers. The product (testis width)^2 $\times$ (testis length) was calculated to indicate estimated testis volume (ETV), which is highly correlated ($R > 0.95$) with testis weight (12).

The first experiment assessed the history-dependence of responses to intermediate DLs and Mel infusion regimes. Hamsters were maintained from birth until 8–10 weeks of age in either 15L (LD) or 12L (SD), then transferred to 12L, 13.5L (intermediate DL), or 15L for 8 weeks, at which time ETV was determined. Additional groups of hamsters were maintained in 15L (LD) or 10L (SD) from birth and at 8–10 weeks of age were pinealectomized (according to procedures described in ref. 13), fitted with s.c. polyethylene cannulae (14), and provided with timed daily 7-h infusions of either Mel (5-methoxytryptamine; 100 ng per infusion) or saline (0.9% NaCl) for 28 consecutive days, after which ETVs were determined.

The next experiment determined the duration of exposure to a long DL necessary and sufficient to encode a long-day photoperiodic history. Hamsters were derived from a breeding colony maintained in a 13.5L photoperiod; male offspring were housed in 13.5L until 12 weeks of age, at which time those with large testes (ETV $>$ 400) were transferred to 15L for 0, 1, 2, 4, 8, or 12 weeks. All males were returned to 13.5L after their prescribed duration of 15L treatment. ETVs were determined biweekly for $>$30 weeks after return to 13.5L. Individuals manifesting a reduction in ETV $\geq$48% sustained for two consecutive measurements within the first 12 weeks in 13.5L were considered to have undergone gonadal regression (12). Hamsters that sustained gonadal regression in 13.5L eventually

Abbreviations: Mel, melatonin; DL, day length; *n*L, number of hours of light; ETV, estimated testis volume; LD, long day; SD, short day; PTW, paired testis weights.

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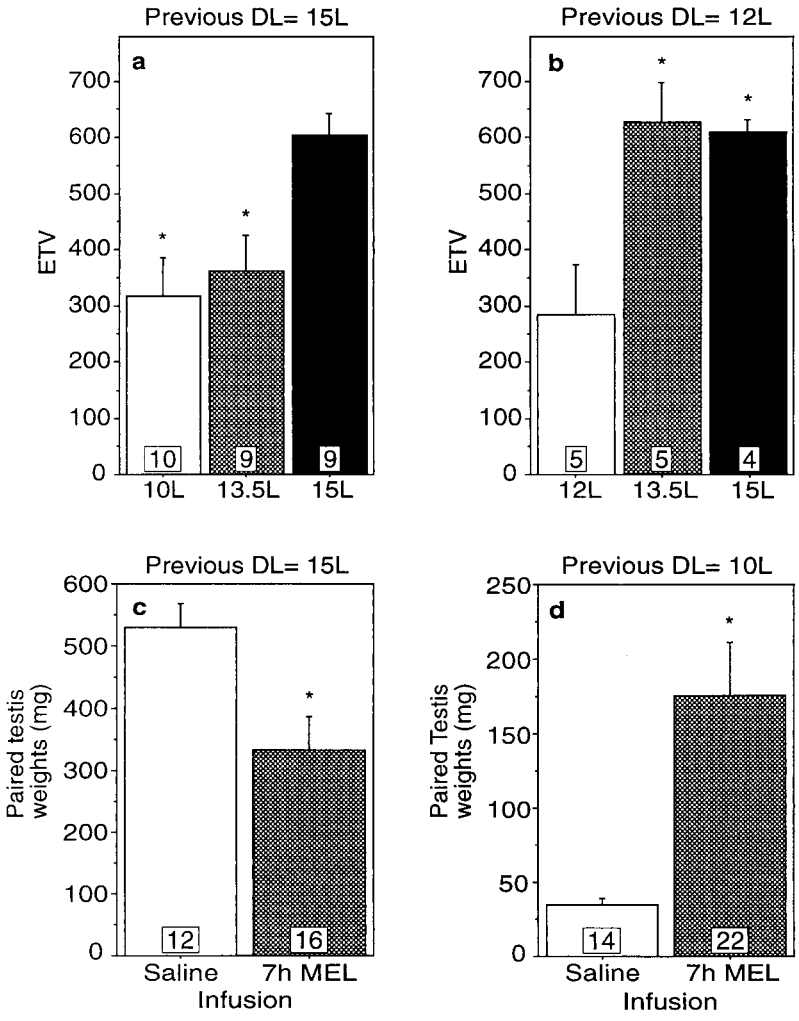

Fig. 1. Effects of DL and intermediate-duration Mel infusions on testis size depend on prior photoperiod history. (*a*) Mean (± SEM) ETV of hamsters from a 15L photoperiod 8 weeks after they were transferred to a short (10L) or intermediate (13.5L) DL or kept in the original long (15L) DL (ANOVA, $P < 0.005$). (*b*) ETV of hamsters from a 12L photoperiod 8 weeks after they were either kept in the original short DL (12L) or transferred to an intermediate (13.5) or long (15L) DL (ANOVA, $P < 0.01$). *, $P < 0.01$ vs. 15L group (*a*) or 12L group (*b*). (*c*) PTWs of hamsters from a 15L photoperiod after 4 weeks of daily 7-h Mel or saline infusions (ANOVA, $P < 0.01$). *, $P < 0.01$ vs. saline-infused controls. (*d*) PTW of hamsters from a 10L photoperiod after 4 weeks of 7-h Mel infusions (ANOVA, $P < 0.005$). Sample sizes appear within bars. *, $P < 0.01$ vs. saline-infused controls.

experience spontaneous testicular recrudescence (12). In these individuals, the duration of responsiveness to 13.5L was calculated as the interval between the week the animal was returned to 13.5L and the week of onset of gonadal recrudescence (defined as the week ETV > 400 and remained >400 for two consecutive measurements). Hamsters that did not experience gonadal regression after 12 weeks in 13.5L were transferred to 10L for 6 additional weeks, after which ETVs were determined.

The final experiment assessed the persistence of a long-day photoperiodic history. Male hamsters gestated and kept thereafter in 15L were pinealectomized at 8–9 weeks of age (week 0). Beginning at week 0, 6.5, 13, or 20, hamsters were fitted with infusion cannulae and provided daily with 7-h or 10-h Mel infusions (100 ng per infusion, both groups) or saline infusions. Treatments endured for 28 consecutive days, after which animals were autopsied, and paired testis weights (PTW) were determined to ±0.1 mg. All procedures were approved by the Animal Care and Use Committee of the University of California at Berkeley.

Statistical Analyses. Standard parametric and nonparametric statistical analyses (ANOVA, simple regression, paired *t* test, and χ^2) were conducted with STATVIEW 4.1 software for the Macintosh (Abacus Concepts, Berkeley, CA). Where significant *F* ratios were obtained, pairwise comparisons were conducted with Fisher's protected least significant difference test or paired *t* tests, as appropriate. ω^2 values, an index of the magnitude of the treatment effect, were calculated according to equation 4-2 in ref. 15. ω^2 is a relative measure that reflects the proportional amount of the total variance attributable to the variation among the infusion treatments; $\omega^2 = 0$ in the absence of treatment effects and ranges from 0 to 1 when effects are present.

Results

Gonadal Responses to Intermediate DLs and 7-h Mel Infusions Depend on Photoperiod History. Hamsters with a recent photoperiodic history of long DLs (15L) underwent gonadal regression when exposed to an intermediate DL (13.5L) for 8 weeks; a similar outcome obtained when LD hamsters were infused daily with Mel for 7 h for 4 weeks (Fig. 1 *a* and *c*). Identical intermediate DLs and Mel signals provoked gonadal growth in hamsters with a recent photoperiodic history of shorter DLs (Fig. 1 *b* and *d*). Unambiguously long (15L) and short (12L or 10L) DLs were

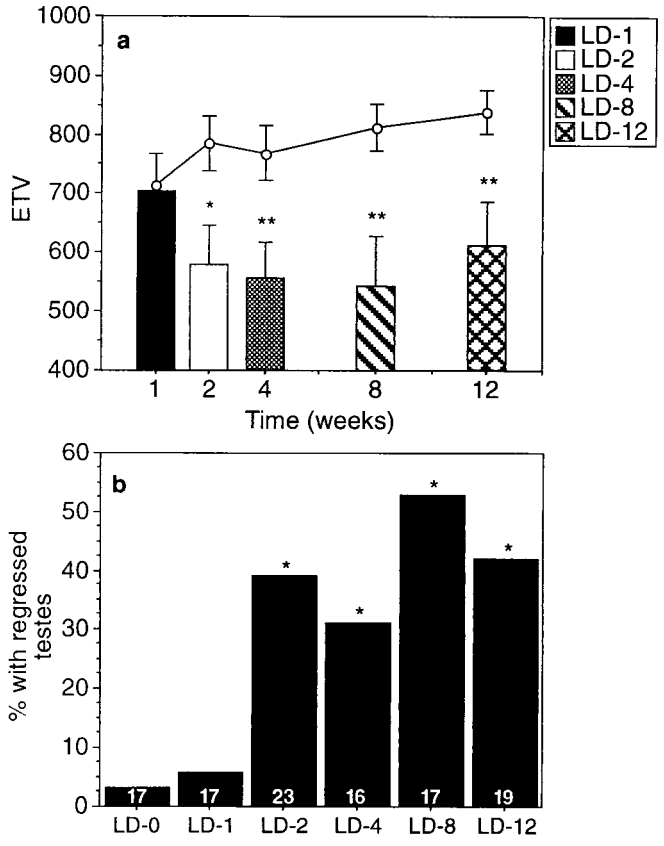

Fig. 2. Encoding of a long-day photoperiodic history. (*a*) Mean (± SEM) ETV of hamsters kept from birth in 13.5L, then exposed to 15L for 1, 2, 4, 8, or 12 weeks (designated as LD-1, LD-2, LD-4, LD-8, and LD-12, respectively), and then returned to 13.5L. Histograms indicate ETV of hamsters 10 weeks after return to 13.5L; ETV of age-appropriate control (LD-0) animals are indicated by open circles. LD-1 hamsters did not undergo testicular regression; their ETVs were similar to those of hamsters kept in 13.5L throughout (LD-0; $P > 0.90$). All other 15L exposure durations induced substantial gonadal regression in animals returned to 13.5L ($P < 0.05$, all comparisons). *, $P < 0.05$ vs. LD-0 controls; **, $P \leq 0.01$. (*b*) Percentage of hamsters with testicular regression (>48% decrease in ETV) over 10 weeks in 13.5L, after durations of exposure to 15L as indicated on the abscissa ($\chi^2 = 22.7$; df = 5; $P < 0.0005$). Sample sizes appear within bars. *, $P < 0.05$ vs. LD-0.

compatible with maintenance of developed and regressed testes, respectively, independent of photoperiodic history.

Exposure to a Long DL for 2 or More Weeks, but Not 1 Week, Renders an Intermediate DL Inhibitory. Hamsters maintained from birth in an intermediate photoperiod (13.5L) underwent rapid gonadal growth. At 3 months of age, 13.5L hamsters with fully developed testes were exposed for 0, 1, 2, 4, 8, or 12 weeks to 15L and then returned to 13.5L (see *Materials and Methods*). Hamsters kept for 1 week in 15L did not experience testicular regression on return to 13.5L (Fig. 2*a*). In contrast, exposure for 2 or more weeks to 15L induced testicular regression in 35–50% of hamsters that were returned to 13.5L (Fig. 2 *a* and *b*). The proportion of individuals that manifested testicular regression did not differ significantly among groups treated with 15L for 2 or more weeks (Fig. 2*b*).

Hamsters that experienced gonadal regression on return to 13.5L ($n = 32$) eventually manifested spontaneous gonadal recrudescence. Among these responsive individuals, the duration of responsiveness to 13.5L did not vary as a function of the duration of prior 15L treatment (Fig. 3 *a* and *b*). Of the 15L-treated hamsters that did not undergo gonadal regression after 12 weeks in 13.5L, 54% were likewise nonresponsive to 10L.

Responsiveness to 7-h Mel Infusions Declines over Time in the Absence of Endogenous Long-Day Mel Signals. Testicular regression in response to 7-h Mel infusions depended on a recent long-day photoperiodic history (Fig. 1 *c* and *d*). In hamsters maintained from birth in 15L and pinealectomized at 9 weeks of age, we determined how long the memory for 15L persisted; hamsters were infused for 7 h or 10 h with Mel or saline beginning 0, 6.5, 13, or 20 weeks after pinealectomy (see *Materials and Methods*). Mel infusions of 7-h duration initiated 0 or 6.5 weeks after pinealectomy induced significant testicular regression (Fig. 4 *a* and *b*). After a delay of 13 weeks, 7-h Mel signals caused slight, nonsignificant decreases in testis weights (Fig. 4*c*). Finally, 7-h Mel infusions initiated 20 weeks after 15L exposure did not induce any gonadal regression (Fig. 4*d*). In contrast, gonadal regression was elicited by 10-h Mel infusions at all time points tested. The magnitude of regression elicited by 7-h Mel signals decreased in a linear manner with time since last exposure to long-day Mel signals (15L), whereas responses to 10-h Mel signals were undiminished with the passage of time (Fig. 4*e*).

Discussion

These experiments describe and quantify the manner in which prior exposure to long DLs influences reproductive responses to intermediate DL stimuli and thus constitute an information processing mechanism (memory) in the reproductive neuroendocrine system. This mechanism mediates acquisition, retention, and decay of photoperiodic history, presumably by modifying responses to endogenous Mel signals. The concept of memory in this context is shorthand for "stored DL information;" there is no implication that memories for photoperiod and Mel are comparable to those involved in cognitive tasks.

Reproductive responses to intermediate DLs and Mel signals depend on a history of recent DL signals, and as few as 2 weeks of exposure to long DLs established an effective LD photoperiodic history; 1 week of long DLs was completely ineffective in this regard, whereas longer intervals (>2 weeks) of 15L exposure were no more effective than 2 weeks in influencing subsequent reproductive responses to 13.5L. The significance of 1 vs. 2 weeks in encoding the long-day photoperiod is not readily apparent. Whereas the decrease in duration of nocturnal Mel secretion is accomplished after the first night in 15L (16), entrainment of the circadian system to a 1.5-h phase shift requires approximately 2 weeks (17). Either a critical number of short Mel signals is required to establish an LD history (i.e., between 7 and 14 signals), or short-duration Mel signals must coincide with some feature(s) of the entrained circadian system to encode a functional photoperiodic history.

The present data afford insights into the nature of the memory encoded by 2 or more weeks of 15L. The proportion of individuals acquiring a long-day photoperiodic history did not increase with longer intervals of 15L treatment, although it is likely that photoperiod nonresponsiveness (to 10L, a history-independent, short DL) masked our ability to resolve the encoding of 15L in some individuals—the high incidence of photoperiod nonresponsiveness in the present study would have prevented the expression of short-day responses after transfer to 13.5L and could account for the observation that, at asymptote, only 50% of individuals were photo-inhibited. Nevertheless, 2 weeks of 15L were no more effective than 12 weeks in imparting a long-day history. Because gonadal involution in 13.5L depends on reference to a long-day photoperiodic history, the duration of gonadal involution in 13.5L served as one measure of the persistence of the 15L history. The duration of gonadal responsiveness to 13.5L in hamsters that acquired a long-day photoperiodic history did not vary as a function of the duration of 15L

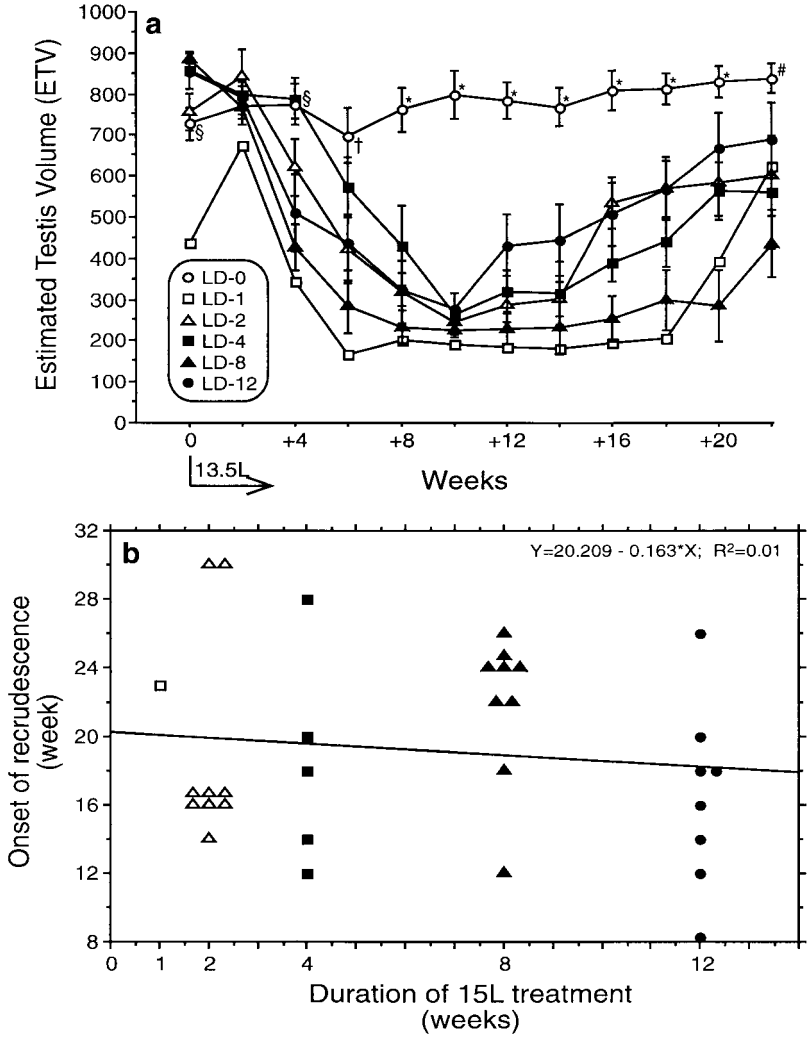

Fig. 3. Persistence of photoperiod history effect did not vary as a function of the duration of 15L treatment in individuals that acquired a photoperiodic history. (*a*) Mean (± SEM) ETV of hamsters that manifested testicular regression in 13.5L (data are replotted relative to the week animals in each group were returned to 13.5L). Groups did not differ in the timing of the onset of recrudescence (ANOVA, $P > 0.30$). Symbols indicate that the LD-0 mean differs significantly from that of other groups: *, $P < 0.05$ vs. all other groups; #, $P < 0.05$ vs. LD-2, LD-4, and LD-8; §, $P < 0.05$ vs. LD-1, LD-8, and LD-12; †, $P < 0.05$ vs. LD-1, LD-2, LD-8, and LD-12. (*b*) Simple regression reveals essentially no correlation ($R^2 = 0.01$) between the duration of 15L exposure and the week of onset of testicular recrudescence in 13.5L; photoperiodic histories encoded by longer intervals of 15L exposure did not differ from those encoded by shorter 15L treatments.

treatment. Once established, be it after few or many weeks, photoperiodic histories seem to endure equally long.

To investigate retention of photoperiodic history, hamsters maintained from birth in 15L (a long DL) were pinealectomized at 9 weeks of age, eliminating circulating Mel and presumably the physiological means to update Mel-mediated photoperiod information. Hamsters were reproductively responsive to 7-h Mel infusions initiated immediately after pinealectomy (week 0). After a delay of 6.5 weeks, during which the hamsters were not exposed to any Mel, they were still able to compare the current 7-h Mel signal to the shorter Mel signal (≈5 h) previously encoded by 15L. The magnitude of gonadal regression elicited by 7-h Mel signals decreased, however, in a linear manner with time since last exposure to long-day Mel signals (15L), whereas responses to 10-h signals that represent unambiguously short DLs were undiminished with the passage of time. The inability to compare current intermediate Mel signals with prior short Mel signals implies that the hamsters could not access prior long-day information; thus, the previously available 15L information had faded or decayed over this interval. Alternatively, the observed decline in testicular responses to 7-h Mel signals with time since pinealectomy may reflect deterioration (caused by aging or long-term absence of Mel) of physiological systems responsible for Mel-mediated gonadal regression. However, 10-h Mel infusions were equally effective in inducing gonadal regression after delays of either 0 or 20 weeks, casting doubt on this conjecture. A progressive decay in photoperiodic history information is evident in the diminution of responses to intermediate but not long Mel signals.

In the absence of subsequent Mel signals, the memory for previous LDs fades and is functionally unavailable. Either it is expunged, or it can no longer be retrieved. In nature, the utility of any prior photoperiod episode as a reference DL depends on the strength with which it was encoded and its dissipation over time. The encoding of a long-day photoperiod history information occurs in an all-or-none manner, i.e., once encoded, be it after a few or many weeks, photoperiodic histories seem to endure equally. With longer intervals of exposure to a range of

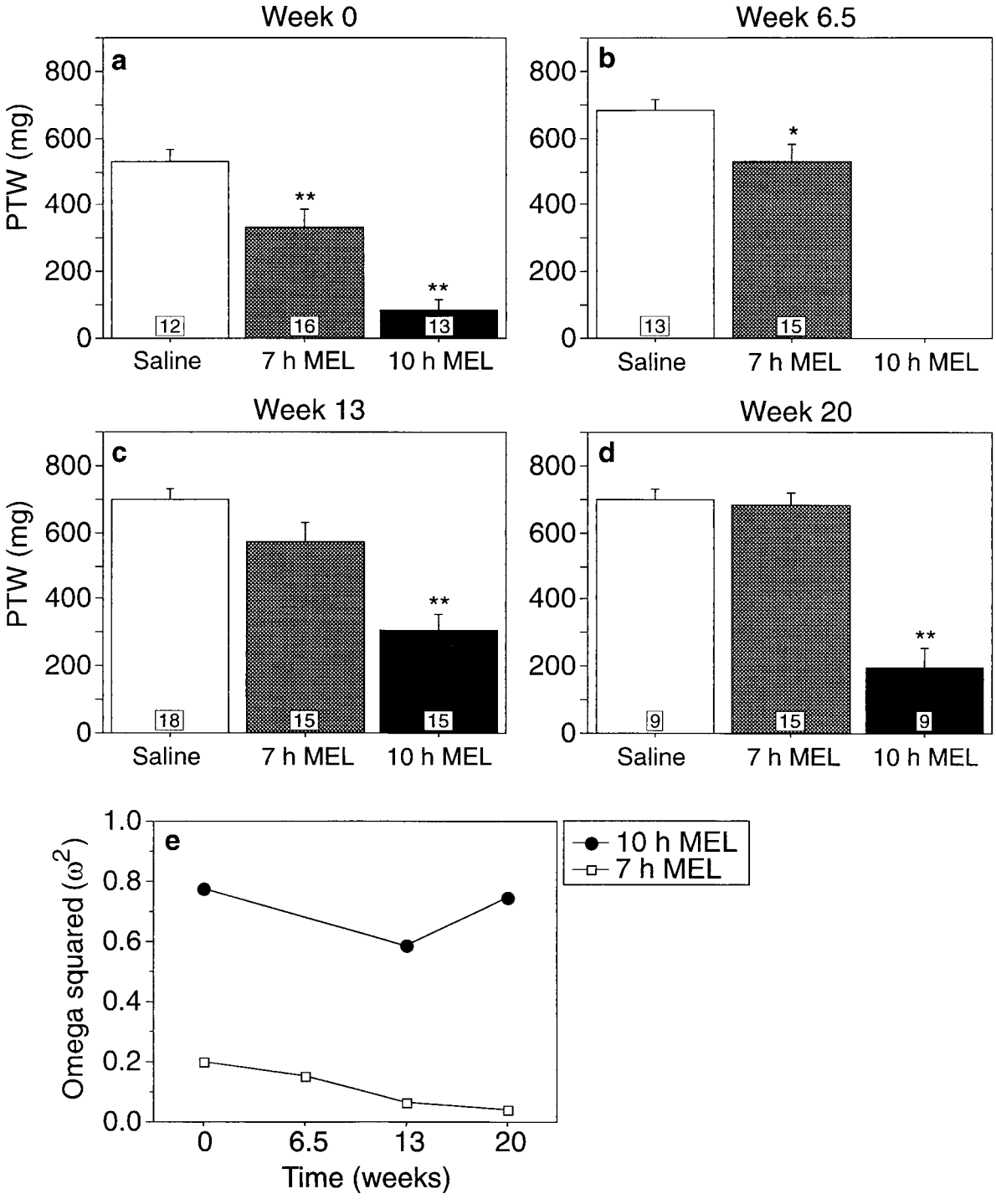

Fig. 4. Decline in PTWs in response to 4 consecutive weeks of daily Mel or saline infusions in pinealectomized hamsters. (*a*) Infusions initiated coincident with pinealectomy at week 0 (ANOVA, $P < 0.0001$). (*b–d*) Infusions initiated 6.5 weeks (*b*; ANOVA, $P < 0.05$), 13 weeks (*c*; ANOVA, $P < 0.0001$), and 20 weeks (*d*; ANOVA, $P < 0.0001$) after pinealectomy. Sample sizes appear within bars. (*e*) ω^2 values for the 7-h and 10-h Mel infusion treatments as a function of time since pinealectomy (performed at week 0). Intermediate-duration (7 h/day) Mel infusions provoked significant testicular regression when initiated 0 or 6.5 weeks after pinealectomy ($P < 0.05$, both comparisons). Intermediate-duration infusions did not result in significant regression after 13 ($P = 0.07$) or 20 weeks ($P > 0.90$). Long duration (10-h/day) Mel infusions provoked significant testicular regression at week 0, week 13, and week 20 ($P < 0.001$, each comparison). A linear decline was observed in the magnitude of the 7-h Mel treatment effect but not that of the 10-h Mel treatment. *, $P < 0.05$; **, $P < 0.01$ vs. saline-infused controls.

DLs, the encoded history may be "stronger" and allow animals to evaluate subtle differences between past and present DLs. The discrepancy in duration between a prior and the current DL presumably affects whether they are recognized as similar or different DLs. Progressive decay of the 15L information after 20 weeks is irrelevant for purposes of evaluating a 10-h Mel signal but compromises the hamster's ability to judge a 7-h Mel signal. Thus, a decay in photoperiodic history over time selectively

impairs evaluation of intermediate but not unequivocally short DLs.

It is not clear how the establishment and retention of photoperiodic history information in laboratory studies of pinealectomized hamsters held in fixed DLs translates into the richer and more complex situation in nature, where hamsters are never without daily DL and Mel signals. A simple model based on these data suggests that in late summer, DLs from as long ago as the summer solstice (<13 weeks earlier) may cause intermediate DLs to be read as relatively *shorter* days (DL disparity is maximized). Were hamsters to reference episodes of less recent DLs (e.g., 12.5L from the previous spring), they might interpret late-summer intermediate DLs as relatively *longer* days and inappropriately initiate gonadal growth. An information buffer for Mel signals that contains only relatively recent DL information would effectively constrain comparisons to recent, more relevant DLs. Whether DL episodes from only certain phases of the year and not others (e.g., summer and winter solstices) are encoded or whether hamsters continuously update and forget DLs remains to be determined. Furthermore, given the rich amount of seasonal information hamsters extract from naturalistic changes in DL (i.e., sinusoidally changing photoperiods), such photic information may well reduce the stimulus requirements for encoding of a photoperiodic history or ameliorate its decay. In nature, hamsters may acquire and discard photoperiodic information continuously. These issues await further experimentation.

Temporal changes in reproductive responses to intermediate Mel signals parallel some as-yet unidentified changes in responsiveness to Mel upstream of reproductive status *per se*. Unlike more conventional mammalian memory mechanisms that involve relatively complex input/output systems, the pathways by which light alters Mel secretion (18) and thereby controls gonadotrophic activity are well characterized (6) and should provide clues to localization and characterization of the neural basis of photoperiodic history. The DL-comparison mechanism may reside solely within these photic-neural-endocrine effector pathways or may necessitate input from brain structures implicated in classical memory paradigms (e.g., hippocampus or bed nucleus of the stria terminalis) that have a high density of Mel binding sites and have been implicated in seasonal photoperiodic responses of hamsters (19, 20).

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