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What is This?

Photic Sensitivity for Circadian Response to Light Varies with Photoperiod

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Abstract   The response of the circadian system to light varies markedly depending on photic history. Under short day lengths, hamsters exhibit larger maximal light-induced phase shifts as compared with those under longer photoperiods. However, effects of photoperiod length on *sensitivity* to subsaturating light remain unknown. Here, Syrian hamsters were entrained to long or short photoperiods and subsequently exposed to a 15-min light pulse across a range of irradiances (0-68.03 $\mu W/cm^2$) to phase shift activity rhythms. Phase advances exhibited a dose response, with increasing irradiances eliciting greater phase resetting in both conditions. Photic sensitivity, as measured by the half-saturation constant, was increased 40-fold in the short photoperiod condition. In addition, irradiances that generated similar phase advances under short and long days produced equivalent phase delays, and equal photon doses produced larger delays in the short photoperiod condition. Mechanistically, equivalent light exposure induced greater pERK, PER1, and cFOS immunoreactivity in the suprachiasmatic nuclei of animals under shorter days. Patterns of immunoreactivity in all 3 proteins were related to the size of the phase shift rather than the intensity of the photic stimulus, suggesting that photoperiod modulation of light sensitivity lies upstream of these events within the signal transduction cascade. This modulation of light sensitivity by photoperiod means that considerably less light is necessary to elicit a circadian response under the relatively shorter days of winter, extending upon the known seasonal changes in sensitivity of sensory systems. Further characterizing the mechanisms by which photoperiod alters photic response may provide a potent tool for optimizing light treatment for circadian and affective disorders in humans.

Key words   circadian, light, history, photoperiod, sensitivity, winter, phase shift

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Ocular light exposure is the primary signal by which mammalian circadian rhythms are entrained to the solar cycle. Acute light pulses delivered at various points throughout the subjective night differentially reset the phase of the central oscillator in the suprachiasmatic nucleus (SCN). These phase-dependent effects are described as phase-response curves (PRCs), with light early and late in the subjective night eliciting delays and advances of rhythms, respectively (Pittendrigh, 1981).

Photic input is conveyed to the SCN via melanopsin-containing ganglion cells (ipRGCs) along a neural pathway distinct from that of the classical visual system, the retinohypothalamic tract (RHT) (Güler et al., 2008).

Light rapidly triggers transcription in the SCN, including an induction of the molecular components of a transcription/translation feedback loop that generates circadian rhythmicity (Reppert and Weaver, 2001). Specifically, photic stimulation during the dark period induces the release of glutamate and neuropeptides from the RHT, leading to increased expression of *period* (*per*) *1* or *2* (Shigeyoshi et al., 1997; Field et al., 2000). The signal transduction pathways linking neurotransmitter binding to SCN gene expression involve a cascade of events, including phosphorylation of extracellular regulated kinase (pERK), which converge to activate the transcription factor CREB (Obrietan et al., 1998; Coogan and Piggins, 2003). Light during the dark period also induces the expression of *c-fos* in the SCN, although its transcription is not necessary for (Honrado et al., 1996), nor perfectly correlated with (Travnicková et al., 1996), light-induced shifts.

The circadian system is markedly influenced by light history. Seasonal changes in photoperiod alter the duration of responsiveness to light by a number of circadian-related parameters (Pittendrigh and Daan, 1976; Wehr et al., 1993; Sumová et al., 2003). Following exposure to bright-light pulses of identical intensity and duration, animals previously entrained to short day lengths of winter (e.g., 10 h of light and 14 h of dark, LD10:14) can show phase delays and advances that are approximately twice as great as those of animals under relatively longer photoperiods (e.g., LD14:10) (Goldman and Elliott, 1988; Evans et al., 2004). The maximum response to a light pulse and the system's sensitivity to light, however, are separable dimensions of the circadian response. Indeed, in the course of a single subjective night where direction and magnitude vary considerably as a function of circadian phase, light sensitivity (as measured by the amount of light required to produce half of the maximum response, i.e., ED_{50}) is unchanged (Nelson and Takahashi, 1991). We assess here whether the well-documented short photoperiod augmentation of phase shift size is accompanied by a heightened sensitivity to light.

To determine whether photoperiod alters sensitivity to light for phase resetting of the clock, we obtained complete fluence-response curves for light-induced phase advances in Syrian hamsters with short versus long photoperiod histories. A similar phase delay study confirmed our findings and established photoperiod influences were independent of circadian phase. Finally, we aimed to determine the level of regulation by which photoperiod modulates light sensitivity by examining photic induction of pERK, PER1, and cFOS proteins in the SCN of animals entrained to long versus short days.

MATERIALS AND METHODS

Male Syrian hamsters (Harlan Sprague Dawley, Inc., Indianapolis, IN), 3 to 4 weeks of age, were group housed in LD14:10 (long photoperiod; LP) or LD10:14 (short photoperiod; SP) for 3 weeks. The same photoperiod was maintained after transfer to individual housing in customized cages (27 cm × 20 cm × 15 cm) with 17-cm diameter running wheels. Food and water were available ad libitum. All procedures were conducted with approval of the UCSD Institutional Animal Care and Use Committee.

Light Sources

During entrainment, light phases (photophases) were illuminated by broad-spectrum white fluorescent bulbs (F4T5) (105 µW/cm²) while dark phases (scotophases) were dimly illuminated by narrowband light-emitting diodes (LEDs) affixed to the back wall of each chamber (560-nm, 23-nm half-peak bandwidth; 7.9×10^{-6} µW/cm²). This dim scotopic illumination, which compares in irradiance to the natural nighttime sky (~1/380 as bright as the lowest irradiance test pulse), was included because it facilitates photoperiodic entrainment relative to artificial complete darkness yet produces minimal phase resetting actions on its own (Evans et al., 2007). Seasonal changes in spectral sensitivity have been documented in invertebrate species and could confound the interpretation of results found with polychromatic light pulses (Cronly-Dillon and

Sharma, 1968; Takahiko and Yasuo, 1988). Therefore, for experimental light pulses, a 480-nm (23-nm half-peak bandwidth) 8-LED lamp source with diffuser was positioned atop the center of each cage lid. Irradiance levels of these lamps were manipulated with neutral density filters except for the highest intensity condition (68 µW/cm²), which was delivered with a 24-LED lamp with a spectral composition identical to the 8-LED lamp source. Spectral power distributions and half-peak bandwidth of the LED lamps were determined
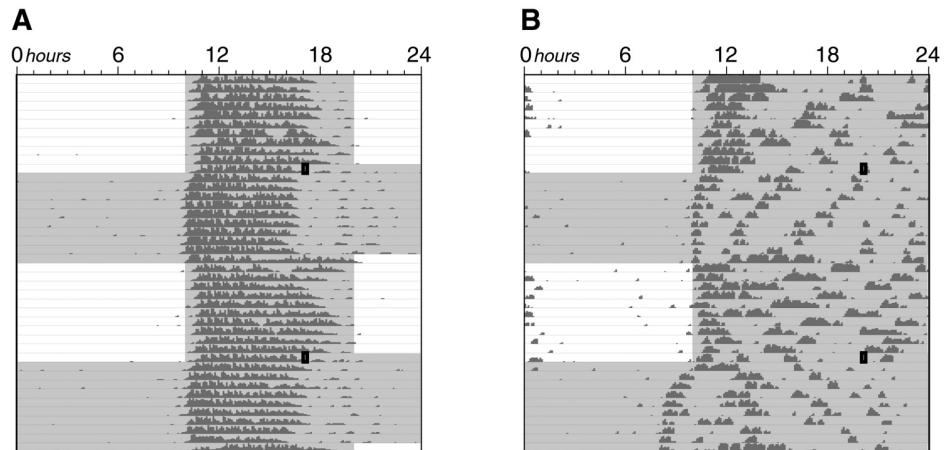

Figure 1. Representative actograms for animals under (A) long photoperiod (LP) and (B) short photoperiod (SP). Each line represents a 24-h day. Light gray areas represent dim light, white areas signify bright fluorescent white light, and the 2 black blocks each represent a 15-min short-wavelength light pulse at progressively increasing irradiances of 0.003 and 0.03 µW/cm². Dark gray patterns show wheel-running activity.

via an Ocean Optics spectral radiometer (model USB2000; Dunedin, FL). Reported irradiances were measured with an IL1700 radiometer (International Light, Inc., Newburyport, MA) with the sensor head positioned 5 cm from the center floor of the cage, approximating the hamster's eye level. Irradiance was measured in µW/cm² and converted to photon density (photons/cm²/sec) based on the energy per photon for 480 nm.

Phase Shifting of Activity Rhythms

Animals were initially entrained to LP or SP for at least 6 weeks in each phase shift study. An Aschoff type II design was employed to measure phase-shifting effects of a defined short-wavelength light pulse (see Figure 1). This paradigm was chosen instead of an Aschoff type I paradigm to ensure consistent prior entrainment status as well as amount of time in darkness prior to each light pulse within a group. For each light or sham pulse, animals were exposed to constant dim light conditions beginning at the normal time of lights-off. Animals remained under constant conditions for 10 days following the pulse and were then reentrained to their original photoperiod for an additional 10 days. Cages were changed on day 1 of reentrainment near the expected time of activity onset, using dim red illumination. This same protocol was repeated for each light pulse condition. Reproductive status was not assessed. Animals were presumed to have undergone gonadal regression and recrudescence over the course of the experiment.

Photoperiodic modulation of phase advances: Fluence-response curves. LP ($n = 13$) or SP ($n = 10$) animals were individually housed in running wheel cages located in ventilated, light-tight, matte white interior cabinets (43 cm × 36 cm × 46 cm). Each animal received six 15-min pulses of 480 nm light of progressively increasing irradiance: 0.003, 0.03, 0.25, 1.31, 4.86, and 68.03 µW/cm². Two sham (i.e., no light pulse) controls (one at the beginning and one at the end of the study) were also included. The light pulse was administered 7 h into the dark (ZT19) or 10 h into the dark (ZT22) for animals previously maintained in LP and SP, respectively. These times were chosen so that the light pulse was administered at phases representing comparable fractions of time into the subjective night.

Fluence-response curves for phase advances in each group were fit to a parametric model, as described by the following equation. The ED_{50} represents the half-saturation constant or the dose eliciting a response halfway between the minimum and maximum dose response, where p estimates the slope of the curve between the minimum and maximum response dose.

$$Y = \frac{\text{Minimum (0) response dose} + (\text{Maximal response dose} - \text{Minimum (0) response dose})}{1 + \left(\dfrac{\text{Photon dose}}{ED_{50}}\right)^{p}}$$

Photoperiodic modulation of phase delays. To test the generality of phase-advance results to a phase-delay
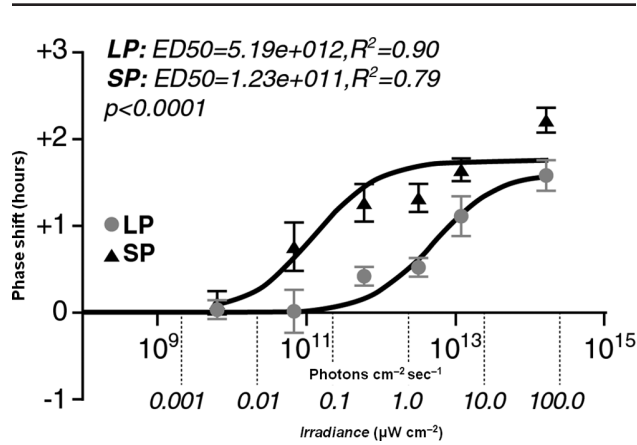
Figure 2. The fluence-response curves for phase advances in animals previously entrained to long photoperiod (LP; gray circles) or short photoperiod (SP; black triangles). SP animals are 40-fold more sensitive to light than LP, as indicated by the lower ED$_{50}$ ($p < 0.0001$).

Figure 3. Mean ± SE phase delay data for long photoperiod (LP; gray) versus short photoperiod (SP; black) ($n = 11$ per condition). Phase-shift scores were expressed relative to each animal's phase shift on the dark control night. Different letters illustrate statistically significant mean differences ($p < 0.05$).

condition, a separate sample of animals was entrained to LP ($n = 11$) or SP ($n = 11$) in 2 separate large-ventilated, light-tight, matte white interior chambers. Light pulses were administered 2 h into the dark (ZT14) for both LP and SP, with each cage being transferred to individual cabinets (as described in the phase advance section) for the duration of the 15-min pulse and returned to chambers afterwards. Each animal received a randomized order of narrowband short-wavelength light at the irradiances calculated to elicit a 1-h phase advance under SP and LP, as determined by the results of the phase-advance study (i.e., 0.14 μW/cm^2 or 3.38016×10^{11} photons cm^{-2} sec^{-1} and 3.5 μW/cm^2 or 8.4504×10^{12} photons cm^{-2} sec^{-1}, respectively; see Figures 2 and 3) as well as a dark control.

Phase-shift measurement. Each one-half wheel revolution generated a switch closure signal that was recorded via the DataQuest system (Mini-Mitter, Bend, OR). Actograms were analyzed via Clocklab software (Actimetrics, Evanston, IL). Activity onset was defined as the first point in time wherein the hamster ran ≥20 revolutions per min for 2 consecutive 6-min intervals. In quantifying activity responses, we excluded the first 3 days after a light pulse to avoid advancing transient effects. Phase shifts were calculated as the difference between the time of activity onset on the day of the light pulse and the time of the next activity onset as predicted by the post-pulse activity onset regression line. Phase-shift scores were expressed relative to each animal's phase shift on the sham control night. All group values are
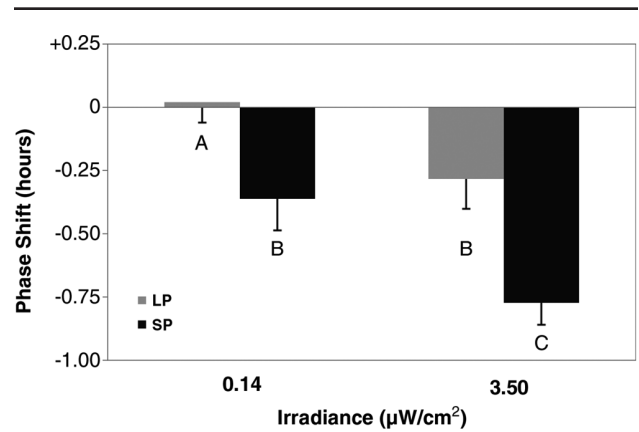
expressed as means ± SEM and analyzed with analysis of variance (ANOVA).

Photoperiodic modulation of SCN light-response. To assess the neural basis of short photoperiod enhancement of light sensitivity, a separate group of animals was entrained to LP ($n = 24$) or SP ($n = 36$) in the individual cabinets already described and administered a 5-min light pulse at ZT14 on the first cycle in constant conditions. This pulse consisted of short-wavelength light at 0.14 μW/cm^2 or 3.5 μW/cm^2, to match the irradiances of the behavioral phase-delay study. Brains were collected at 15 min post–light pulse for pERK immunostaining and 60 min post–light pulse for PER1 and cFOS immunostaining from animals with the following photoperiod history-irradiance conditions: (1) LP, 0.14 μW/cm^2 ($n = 6$ at 15 min; $n = 6$ at 60 min post-pulse); (2) SP, 0.14 μW/cm^2 ($n = 6$ at 15 min; $n = 6$ at 60 min post-pulse); (3) LP, 3.5 μW/cm^2 ($n = 6$ at 15 min; $n = 6$ at 60 min post-pulse); (4) SP, 3.5 μW/cm^2 ($n = 6$ at 15 min; $n = 6$ at 60 min post-pulse); and (5) a no-pulse dark control ($n = 6$ at 15 min; $n = 6$ at 60 min post-pulse) for LP and SP animals (see Figure 4). Essentially, those conditions served to test equal photic inputs versus light doses eliciting comparable phase shifts, as determined by the results of the described phase-shifting experiments.

Tissue Collection

At the assigned post-pulse time, animals were deeply anesthetized in complete darkness with
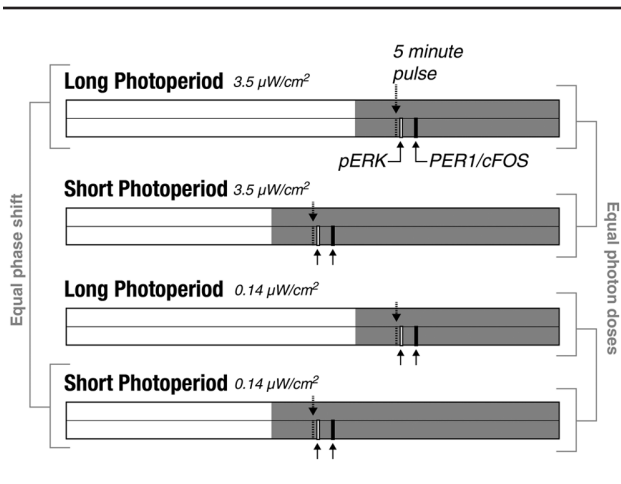
Figure 4. Schematic of the protocol for the immunocytochemistry experiment. The dotted lines represent the administration of a 5-min short-wavelength light pulse at ZT14, 2 h after lights-out. The white and black bars illustrate the timing of brain collections for study of pERK (15 min post-pulse) and PER1/cFOS (60 min post-pulse), respectively. Gray brackets along the outside help show the comparisons being made between the 4 different conditions (i.e., equal photon doses and light-eliciting comparable phase shifts). pERK = phosphorylation of extracellular regulated kinase.

sodium pentobarbital (intraperitoneal [i.p.]) and transcardially perfused with 100 mL 0.1 M phosphate-buffered saline (PBS) followed by 100 mL 4% paraformaldehyde in 0.1 M phosphate buffer (PB) with eyes covered, under dim red light. Brains were then removed, postfixed overnight, and cryoprotected in 30% sucrose with 0.01% sodium azide in 0.1 M PB prior to sectioning with a microtome. Four parallel series of 35-μm-thick coronal sections through the SCN were stored in cryoprotectant solution (Watson et al., 1986) at –20 °C until immunohistochemical processing.

Immunohistochemistry

A series of every fourth section was stained using an avidin-biotin-immunoperoxidase technique for pERK in tissue collected 15 min post-pulse and for PER1 or c-FOS in tissue collected 60 min post-pulse. All incubations were carried out at room temperature, and the tissue was washed with 0.1 M PBS between steps. Immunological reagents were diluted in PBS containing 0.4% Triton X-100 (Sigma-Aldrich, St. Louis, MO) and 0.1% bovine serum albumin (PBS+). Free-floating sections were quenched of endogenous peroxidase activity in 1% H_2O_2 in PBS for 10 min. The tissue was then permeabilized and blocked in PBS+ for 1 h prior to overnight incubation with a rabbit antibody specific for the phosphorylated forms of

ERK 1/2 (1:1000; Cell Signaling Technology, Inc., Danvers, MA; cat. 9101L), a rabbit c-Fos antibody (1:5000; Santa Cruz Biotechnology, Santa Cruz, CA; cat. sc-52), or a goat PER1 antibody (1:2000; custom made by Bethyl Laboratories, Inc., Montgomery, TX). The sections were subsequently incubated with a biotin-conjugated goat anti-rabbit IgG (1:500; Vector Laboratories [Burlingame, CA] for pERK and c-Fos) or a biotin-conjugated rabbit anti-goat IgG (1:500; Vector Laboratories for PER1) for 1 h and the signal amplified using the avidin-biotin-HRP method (1:1000 in PBS; Vector Laboratories). Staining was visualized using 0.02% diaminobenzidine (Sigma-Aldrich), 0.08% nickel sulfate, and 0.01% H_2O_2 in PB. The sections were mounted onto glass slides, dehydrated in a series of graded alcohols, cleared in CitriSolv (Fisher Scientific, Waltham, MA), and coverslipped with dibutyl phthalate xylene (DPX; Electron Microscopy Services, Hatfield, PA). Controls included omission of the primary antibodies as well as preadsorption of the diluted PER1 antibody for 24 h at 4 °C, with nanomolar concentrations of the purified antigen (see below) used to generate this antibody. Both omission and preadsorption controls resulted in a complete absence of specific staining.

The PER1 antibody was raised against a 15–amino acid synthetic peptide representing amino acid sequences 36 to 50 (CPGPSLADDTDANSN) near the N-terminus of hamster PER1 protein (Genbank accession number AAN38069). This antibody produces staining in the hamster (see Figure 5) and mouse SCN (I.C. Webb and M.N. Lehman, unpublished observations) that is exclusively nuclear, as expected, based on previous characterizations of PER1 immunoreactivity (Maywood et al., 1999; Field et al., 2000). In addition to the SCN, immunoreactive nuclei are also observed in other regions of the brain (e.g., the piriform cortex). Preadsorption of the diluted antibody with the immunizing peptide results in a complete loss of the nuclear staining in all areas, providing evidence that the staining observed is specific for PER1. In addition, pilot work in our (Lehman) laboratory and several published reports from other laboratories using this antibody (Yan et al., 2005; Yan and Silver, 2008) demonstrate that it detects rhythmic PER1 expression in the hamster SCN.

Densitometry

Two sections comprising the middle (~ –0.6 mm relative to bregma) and caudal (~ 0.9 mm) SCN of each animal were identified through use of a hamster

pERK

0 μW/cm² · 0.14 μW/cm² · 3.5 μW/cm²

LP

SP

pERK

% Area above threshold

LP
SP

0   0.14   3.5

Irradiance (μW/cm²)

cFOS

0 μW/cm² · 0.14 μW/cm² · 3.5 μW/cm²

LP   21 ± 7   275 ± 15   420 ± 28

SP   20 ± 5   359 ± 34   578 ± 105

cFOS

% Area above threshold

LP
SP

0   0.14   3.5

Irradiance (μW/cm²)

PER1

0 μW/cm² · 0.14 μW/cm² · 3.5 μW/cm²

LP   761 ± 100   699 ± 143   946 ± 122

SP   712 ± 65   1,174 ± 184   1,379 ± 41

PER1

% Area above threshold

LP
SP

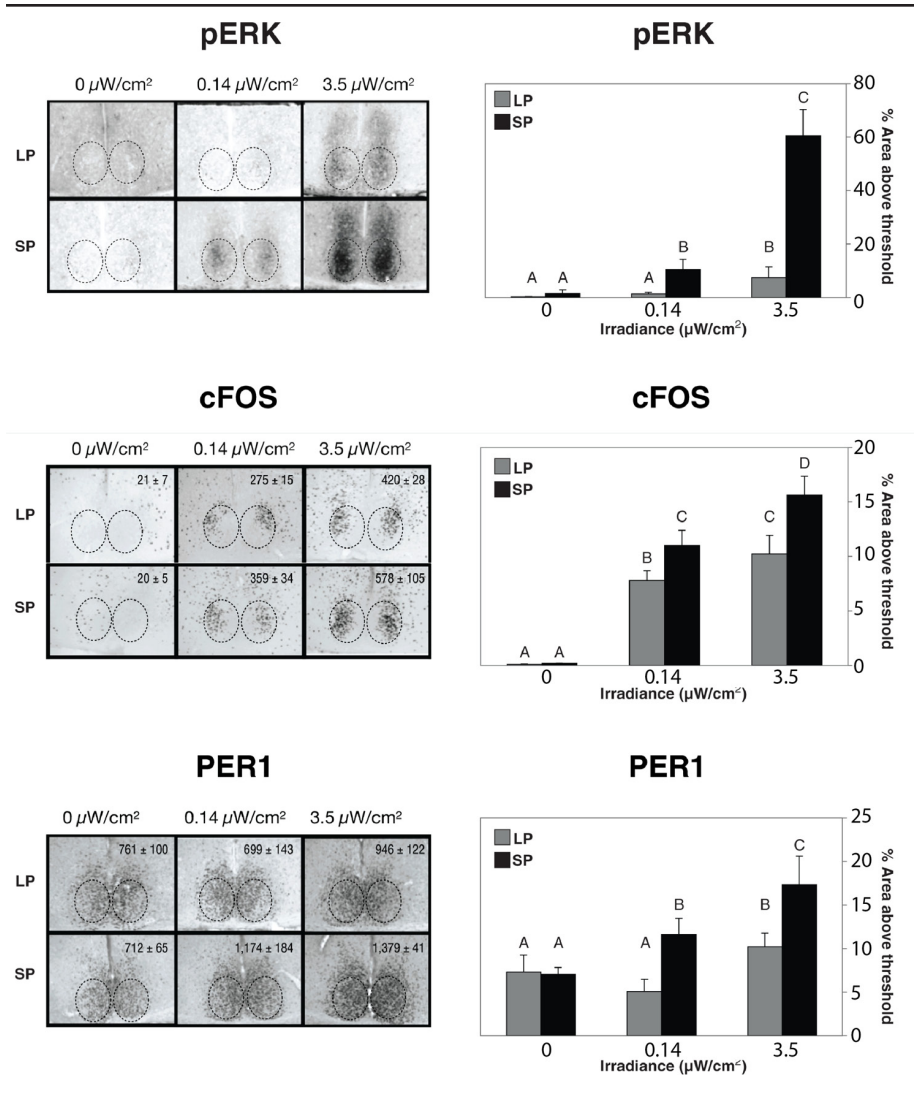0   0.14   3.5

Irradiance (μW/cm²)

Figure 5. Representative histology for pERK, cFOS, and PER1 in the SCN of animals previously maintained under long photoperiod (LP) or short photoperiod (SP). Dotted circles illustrate the area that was used for analyses. Data in the upper right corner of each section for cFOS and PER1 provide cell counts in mean ± SE for each condition. Bar graphs adjacent to SCN images show corresponding mean densitometry data (n = 4-6 per group). Bars identified with different letters are significantly different ($p < 0.05$).

molecules are analyzed and reported in detail. Cell counts are included for PER1 and cFOS. Using ImageJ software (National Institutes of Health, Bethesda, MD), boundaries encapsulating the SCN were consistently applied to each image (see Figure 5), and the percentage of the area that was above a fixed pixel intensity threshold (the average background from all sections) was then calculated. Measurements were taken bilaterally for each animal and averaged.

Statistical Analyses

SPSS was used to perform ANOVA and post hoc tests (version 13.0.0; SPSS, Inc., an IBM Company, Chicago, IL). GraphPad Prism software (version 5; GraphPad Software, San Diego, CA) was used for curve fitting of the fluence-response functions, constraining the minimum to 0 but allowing other parameters to remain unconstrained. Statistical differences between groups were considered significant if $p < 0.05$, with Bonferroni correction for post hoc comparisons.

atlas (Morin and Wood, 2001) and imaged via a digital camera (Microfire, Optronics, CA) attached to a microscope (DM500B; Leica Microsystems, Wetzlar, Germany). All images were taken using the same magnification and camera settings. Both PER1 and cFOS immunoreactivity were quantified via cell counts as well as densitometry, with similar results regardless of the analysis employed. Due to a high degree of pERK immunostaining in both neuronal cell bodies and dendritic processes, we were only able to examine pERK via densitometry. For comparison purposes, densitometry data for all 3

RESULTS

Fluence-Response Curves for Phase Advances

Within-subjects repeated-measures ANOVA indicated that phase-advance magnitude depends on light intensity ($F_{6, 126} = 52.4$, $p < 0.0001$) and that this dependence varies by photoperiod condition ($F_{6, 126} = 3.5$, $p < 0.005$). Representative actograms are provided to illustrate the phase-advance study protocol at the initial 2 progressively increasing irradiances of .003 and .03 μW/cm² (Figure 1). All irradiances

≥ 0.25 µW/cm^2 elicited phase advances that were significantly greater than those under 0.03 µW/cm^2 ($p < 0.01$), and 68 µW/cm^2 induced larger phase advances than all other irradiances ($p < 0.0001$). SP animals showed relatively larger light-induced phase shifts as compared with LP animals at all irradiances ≥ 0.03 µW/cm^2, although comparisons at individual irradiances showed a statistical difference in phase advances only at 0.25, 1.31, and 68 µW/cm^2 ($p < 0.05$). The phase advance data were well fit by 4-parameter sigmoid functions, with high coefficients of correlation (LP, $R^2 = 0.90$ and SP, $R^2 = 0.79$). Comparing LP versus SP, the ED_{50} was significantly lower for the short photoperiod animals ($p < 0.0001$), differing by 1.4 log units (Figure 2).

LP versus SP for Phase Delays

Phase delays also differed significantly as a function of light intensity ($F_{1, 20} = 22.6$, $p < 0.0001$), with the brighter pulse eliciting a greater phase shift. In addition, phase shifting varied by photoperiod condition ($F_{1, 20} = 10.4$, $p < 0.005$). More specifically, phase delays were greater in SP versus LP at both tested irradiances ($p < 0.001$; see Figure 3). The 2 irradiances that were calculated to elicit comparable 1-h advances in LP and SP (3.1 µW/cm^2 and 0.11 µW/cm^2, respectively) produced phase delays that did not differ significantly between conditions. Indeed, the values were very closely matched (mean shift ± SE for LP, 3.1 µW/cm^2 = –0.28 ± 0.11 and SP, 0.11 µW/cm^2 = –0.36 ± 0.11; see Figure 3).

Light-Induced Protein Expression in the SCN

A dose-dependent increase in expression levels was observed for all proteins (pERK: $F_{1, 15} = 15.8$, $p < 0.01$; PER1: $F_{1, 13} = 6.5$, $p < 0.05$; cFOS: $F_{1, 18} = 4.9$, $p < 0.05$). For the dark controls, pERK and cFOS expression were not statistically significant; however, PER1 baseline levels were elevated, consistent with reports of endogenous expression of this protein at the time of brain collection (Maywood et al., 1999; Nuesslein-Hildesheim et al., 2000). Lights pulses that elicited phase shifts in the behavioral studies (LP at 3.5 µW/cm^2 and SP at both irradiances) triggered increases in immunoreactivity for all proteins significantly above dark control levels ($p < 0.05$). Also, at both tested irradiances, immunoreactivity of all proteins was greater in the SP versus the LP condition (pERK: $F_{1, 15} = 19.5$, $p < 0.001$; PER1: $F_{1, 13} =$ 10.3, $p < 0.01$; cFOS: $F_{1, 18} = 7.3$, $p < 0.05$). Again, light pulses shown to elicit comparable phase shifts in SP and LP in the behavioral experiments failed to produce any differential levels of immunoreactivity in the SCN ($p \geq 0.398$) but instead were closely matched in numerical terms (see Figure 5).

DISCUSSION

This is the first study to demonstrate that the measured sensitivity to light is increased 1.4 log units under a short photoperiod. Analogously, light pulses of sufficient irradiance to generate robust phase delays and induction of pERK, PER1, and c-FOS in the SCN under short photoperiods were ineffective at eliciting a similar response under longer days. Furthermore, phase resetting and SCN activation equivalent to those in short photoperiods was achieved in the long photoperiod condition, but only with a much brighter light pulse. As detailed below, these studies suggest that seasonal changes in photoperiod modulate sensitivity to light upstream of or at the postsynaptic membrane of retinorecipient cells in the SCN.

Across photoperiods that alter the rhythm waveform, it is theoretically impossible to establish unambiguous phase equivalence. That is, alignment with respect to one phase marker (e.g., activity onset) necessarily introduces misalignment of other markers (e.g., activity offset). Although the phases for the light pulses in short and long photoperiods were selected based on roughly comparable proportions of the subjective night, they cannot be considered to represent precisely identical phases. Nonetheless, we consider it unlikely that circadian phase contributes substantially to the large differences in ED_{50} measured between photoperiods, as we are aware of no precedent for such an effect. To the contrary, Nelson and Takahashi (1991) found no evidence for variation in ED_{50} with circadian phase. Furthermore, the lower threshold for phase shifting and SCN responses early in the subjective night of short photoperiods points to a photoperiod-general enhancement of light sensitivity. Construction of full fluence-response curves at times throughout the circadian cycle, however, would be needed to establish definitive phase independence of light sensitivity.

It is important to note that these sensitivity effects are logically independent of the maximum phase shift obtainable with saturating light pulses. It has

long been appreciated that a bright light pulse produces phase shifts of systematically different magnitude and direction across the subjective night (i.e., a phase-response curve, PRC). In addition, the short photoperiod PRC of hamsters also has greater amplitude than does the long photoperiod PRC (Goldman and Elliott, 1988). Although not critical for our main conclusion, both phase advances and delays also yielded larger phase shifts in short photoperiods at the highest pulse irradiances, as expected based on prior work (Goldman and Elliott, 1988; Evans et al., 2004).

The fact that short photoperiod hamsters are 40 times more sensitive to light than those in a long photoperiod adds to the known influences of photoperiod on circadian rhythms. Seasonal changes in photoperiod influence the duration of circadian responsiveness to light as measured by behavioral, neuroendocrine, and cellular markers (Pittendrigh and Daan, 1976; Goldman and Elliott, 1988; Wehr et al., 1993; Sumová et al., 2003; Tournier et al., 2003). Even in constant darkness, the pacemaker maintains the influence of the previous photoperiod, as reflected in the overt rhythm period and waveform of the PRC for photic phase resetting. Indeed, hamsters previously entrained to short photoperiods have shown an approximately 2-fold greater mean maximal phase shift as compared with those under a longer photoperiod (Goldman and Elliott, 1988). In addition, a shift from a modest amplitude type 1 response to a much higher amplitude type 0 resetting has been found in short-day conditions in some animals (Pittendrigh et al., 1984). The fact that marked photoperiod differences in phase resetting persist after a number of days in constant darkness suggests an influence of the state of the SCN rather than an acute immediate effect of light history (Goldman and Elliott, 1988). Possibly related to this phenomenon, human studies have found enhanced light-induced circadian responsiveness under longer nights (Owen and Arendt, 1992) or after a relatively longer duration of sleep (Burgess and Eastman, 2006).

Increased photic phase shifts under short photoperiods could logically derive from either an enhancement of photic input or an altered entrainment state of the clock. Sensitivity of sensory systems in at least some nonmammalian vertebrates has been shown to vary seasonally. As an organ with intrinsic circadian oscillations and melatonin secretion, the vertebrate retina itself is potentially photoperiodic (Reme et al., 1986; Tosini and Menaker, 1996; Zawilska et al., 2007). Two reports specifically describe seasonal

changes in the spectral sensitivity of retinal photoreceptors in fish (Cronly-Dillon and Sharma, 1968) and crustacean species (Takahiko and Yasuo, 1988). In the present study, we employed a short-wavelength light pulse. A shift in spectral sensitivity and/or relative photoreceptor contribution could explain the photoperiod differences in light sensitivity reported here in hamsters, in the event that the peak spectral sensitivity to short-wavelength light were shifted to longer wavelengths in the summer. Yet, action spectra studies across a variety of species have mostly been conducted under longer photoperiods and consistently identify a peak in the short-wavelength region of the spectrum for various non-image-forming functions (Brainard et al., 2001; Hattar et al., 2002, 2003; Dacey et al., 2005). Therefore, seasonal changes in spectral sensitivity are unlikely to explain the increased sensitivity to short-wavelength light in the winter of mammals and, if anything, would act counter to the photoperiod differences reported here.

Alternatively, dark adaptation may potentially contribute to the enhanced short photoperiod response. Compared to rods and cones, which dark-adapt within minutes, melanopsin-containing ipRGCs appear to do so over several hours, although the precise time course has not been determined (Wong et al., 2005). Aggelopoulos and Meissl (2000) similarly comment on long dark-adaptation times for light-responsive neurons in the SCN. Although the period of reentrainment between each light pulse served to minimize photoperiod differences in duration of time in darkness, the chosen pulse times still resulted in 3 additional hours of scotopic exposure for short versus long photoperiod animals in the phase-advance study. Hence, more complete dark adaptation of ipRGCs could have occurred under relatively longer nights. However, our phase-delay experiment controlled for time in darkness prior to administration of the light pulse, and the increased resetting under a short photoperiod was maintained. Maximal phase shifts are also enhanced by extending the time in continuous darkness for many days (Shimomura and Menaker, 1994), well beyond any prior photoreceptor adaptation time frame. Those effects mirror that of prolonged short photoperiod exposure and further suggest the importance of the prior circadian entrainment state in altering response to light.

The regulation of this light sensitivity may originate in the SCN, as we demonstrate robust history-dependent differences in expression of light-induced pERK, PER1, and cFOS within the

pacemaker. Prior work has shown seasonal differences in patterns of gene expression and electrophysiological activity in the pacemaker, with longer periods of inducibility under shorter photoperiods (Travnicková et al., 1996; Sumová et al., 1995, 2003; Tournier et al., 2003; vanderLeest et al., 2007). Results from our immunohistochemical analyses complement these observations by demonstrating that the photic induction of pERK, PER1, and cFOS by the same light dose is each greater in animals previously maintained under a short photoperiod. Levels of immunoreactivity correspond to conditions that elicit a comparable behavioral phase-shift response and are not solely a function of irradiance input; instead, they depend on light history. We note that in other species, PER1 protein in the SCN is not responsive to light at night within 1 h of the light exposure (Field et al., 2000; von Gall et al., 2003; Yan and Silver, 2008); however, we do not know of any prior time course information for light induction of this protein in Syrian hamsters. The LP group shows only a modest 19% increase in PER1-expressing cells within 60 min of receiving the brightest light pulse. The much more robust response under short photoperiods, even at the very low irradiance, raises the possibility that the time course of induction may be altered by photoperiod history.

In view of the photoperiodic differences in light sensitivity observed very early in the signal transduction cascade at the level of the SCN (i.e., our pERK data), a modulatory mechanism upstream of the SCN is also possible. Enhanced sensitivity could derive from altered concentrations or distribution of melanopsin photopigment in ipRGCs and consequent increased photon capture under short days. A faster daytime rise in melanopsin under shorter days in rats may correspond to the increased sensitivity to light reported here (Mathes et al., 2007). Given functional connectivity between ipRGCs and classical photoreceptors (Sekaran et al., 2003; Dacey et al., 2005), the observed photoperiod modulation could also reflect altered retinal circuitry. Input to the ipRGCs from rods and cones and afferents from other neural structures (e.g., intergeniculate leaflet) innervated by these ipRGCs are potential pathways by which the SCN may be regulated by light (Morin and Blanchard, 1999; Moore et al., 2000; Gooley et al., 2003; Sekaran et al., 2003; Dacey et al., 2005). In addition, glutamatergic input from the RHT is modulated by serotonin (5-HT) receptors (Sollars et al., 2006). A role of 5-HT in the pathogenesis of seasonal mood disorders (Lam and Levitan, 2000)

and reduced photic sensitivity during the winter in the electroretinograms of such patients (Lavoie et al., 2009) further suggest various 5-HT receptor subtypes as candidate mechanisms. Another possible mechanism by which this photoperiod modulation of light sensitivity is occurring includes photoperiod changes in postsynaptic receptor density or sensitivity. The relationship of these various possible mechanisms to the known photoperiodic modulation of the network of coupled SCN oscillators (e.g., vanderLeest et al., 2009) remains to be explored. Given the capacity of the SCN to functionally reorganize itself under different lighting conditions (Watanabe et al., 2007; Yan et al., 2010), examination of regional differences in intra-SCN gene expression may elucidate the role of network changes in modulating photic sensitivity.

In conclusion, photic history potently alters subsequent circadian response to light. Specifically, prior exposure to short photoperiods substantially increases sensitivity to light for circadian phase resetting. Furthermore, this robust enhancement of sensitivity is reflected very early in the signal transduction cascade, at the level of the SCN. Ultimately, this collection of studies clearly establishes that seasonal changes in day length are an important and ecologically significant modulator of photic resetting, by which the clock is synchronized to the solar cycle.

CONFLICT OF INTEREST STATEMENT

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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