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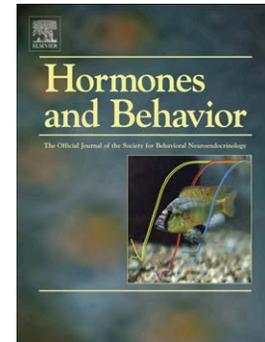
Increased photic sensitivity for phase resetting but not melatonin suppression
in Siberian hamsters under short photoperiods

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Title: Increased photic sensitivity for phase resetting but not melatonin suppression in Siberian hamsters under short photoperiods

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Abstract: Light regulates a variety of behavioral and physiological processes, including activity rhythms and hormone secretory patterns. Seasonal changes in the proportion of light in a day (photoperiod) further modulate those functions. Recently, short (SP) versus long days (LP) were found to markedly increase light sensitivity for phase shifting in Syrian hamsters. To our knowledge, photoperiod effects on light sensitivity have not been studied in other rodents nor is it known if they generalize to other circadian responses. We tested whether photic phase shifting and melatonin suppression vary in Siberian hamsters maintained under LP or SP. Select irradiances of light were administered, and shifts in activity were determined. Photic sensitivity for melatonin suppression was examined in a separate group of animals via pulses of light across a 4 log-unit photon density range, with post-pulse plasma melatonin levels determined via RIA. Phase shifting and melatonin suppression were greater at higher irradiances for both LP and SP. The lower irradiance condition was below threshold for phase shifts in LP but not SP. Melatonin suppression did not vary by photoperiod, and the half saturation constant for fitted sigmoid curves was similar under LP and SP. Thus, photoperiodic modulation of light sensitivity for phase shifting is conserved across two hamster genera. The dissociation of photoperiod effects on photic phase shifting and melatonin suppression suggests modulation of sensitivity occurs downstream of the common retinal input pathway. Understanding the mechanistic basis for this plasticity may yield therapeutic targets for optimizing light therapy practices.

Highlights:

- Photic thresholds for circadian responses were assessed in Siberian hamsters
- Light sensitivity for phase shifting is enhanced in short versus long daylengths
- Light sensitivity for melatonin suppression is identical in long versus short days
- Modulation of light sensitivity occurs downstream of common retinal input pathway
- Photoperiod effects on circadian resetting are conserved across hamster species

Keywords: light, photoperiod, melatonin, circadian, sensitivity, phase shift, hamster

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Introduction: Circadian rhythms include a host of physiological and behavioral processes that maintain an endogenous period of approximately 24 hours in the absence of any environmental cues. Common examples of circadian rhythms in mammals include sleep-wake cycles, hormone fluctuations and core body temperature patterns. Under natural conditions, light resets the phase of the pacemaker in the suprachiasmatic nuclei (SCN) of the hypothalamus in order to maintain a stable relationship between circadian functions and the daily photocycle via a process called entrainment (Pittendrigh and Dann 1976; Pittendrigh 1981; Elliott and Tamarkin 1994). Such shifts reflect the differential effects of light on the SCN at different circadian phases, which are described experimentally in phase response curves (PRCs) (Johnson et al., 2003).

Acute exposure to light during the night resets the phase of circadian rhythms and suppresses nocturnal elevations of the pineal hormone melatonin. Both responses require ocular photoreception in mammals (Czeisler et al. 1995; Lockley et al., 1997, 1998; Wright et al., 2001), and similar spectral sensitivity further suggests a shared photoreceptor system (Provencio et al., 1995; Yoshimura et al., 1996; Hattar et al., 2003; Brainard et al., 2001; Thapan et al., 2001). Anatomical studies also support the existence of a common neural circuit (Klein et al., 1991; Moore, 1995; Panda et al., 2002). A well-established neural pathway conveys photic input for phase resetting and melatonin suppression, including phototransduction by melanopsin-containing ganglion cells (ipRGCs) that project directly to the SCN via the retinohypothalamic tract (RHT) (Klein et al., 1991; Panda et al., 2002). From the SCN, light information follows a multisynaptic pathway to the pineal gland for melatonin regulation, with intermediate connections in the paraventricular hypothalamus, the upper thoracic intermediolateral cell column, the superior cervical ganglion and post-ganglionic sympathetic fibers (reviewed in Moore, 1995). The precise point of divergence between the pathways for phase resetting and the acute suppressive effects of light on plasma melatonin remains unknown.

Because melatonin suppression by light shares similar properties to phase resetting, it has been a useful method for characterizing the physiology underlying the circadian system (Klein et al., 1991; Arendt, 1998; Brainard et al., 2001). Both demonstrate a characteristic dose-dependent function wherein a threshold amount of light is required to elicit a response; a steep rise in response occurs with increasing light intensities; and finally, a maximal saturating response is achieved, which cannot be surpassed with brighter light (Brainard et al., 1982, 2001; Neslon and Takahashi, 1991a; Zeitzer et al., 2000). Sensitivity is commonly indexed as the ED_{50} , defined as the quanta of light required to produce half of the maximum response. Melatonin suppression has been shown to be relatively more sensitive to light than phase resetting, as indicated by the leftward displacement of the melatonin fluence response curve to lower irradiances relative to that for phase shifts (Nelson and Takahashi, 1991b; Zeitzer et al., 2000).

Not only is light the primary environmental cue for entraining rhythms to a 24 hour day, but the duration of light within a given 24-hour cycle (i.e. photoperiod) regulates the seasonality of myriad functions including reproduction, body weight, pelage, immuno-competence and behavior, to name a few (Goldman, 2001). Photoperiod also alters the 24 h waveform, or shape, of circadian rhythms. In both nocturnal and diurnal animals, the high nighttime levels of pineal melatonin are sustained for longer intervals in winter than in summer (Binkley et al., 1977; Rollag et al., 1980; Goldman and Elliott, 1988; Bartness et al., 1993; Lerchl and Schlatt, 1993; Wehr et al., 1993). Parallel lengthening of nocturnal activity under short days (Elliott and Tamarkin, 1994) along with alterations in waveform of SCN neuronal activity (vanderLeest et al., 2007) indicate that these photoperiod influences reflect changes in pacemaker network organization. With regard to phase resetting, under winter photoperiods, light-induced phase shifts occur across a broader fraction of the circadian cycle and the magnitude of the shifts is markedly greater (Pittendrigh et al., 1984; Millette and Turek, 1986; Puchalski and Lynch, 1988; Goldman and Elliott, 1988). These seasonal differences in circadian organization reflect entrainment effects and not sequela of immediate light history since they persist for many days after release into constant conditions (Pittendrigh and Daan, 1976).

In the Syrian hamster, the known effects of photoperiod on circadian function have recently been extended to include the modulation of photic sensitivity. The ED_{50} is approximately 40-fold lower in hamsters entrained to a short photoperiod than in a long photoperiod (Glickman et al., 2012). Furthermore, those behavioral differences are preceded by differential expression of SCN activation, with a much greater amount of light being required to induce similar levels of expression of pERK, PER1 and cFOS in animals previously maintained under long versus short days (Glickman et al., 2012). Importantly, the increased sensitivity of the short day pacemaker appears to be specific to light inputs since enhanced responsiveness is not found with non-photic resetting cues (Evans et al.,

2004). Thus, the enhanced short day sensitivity may be specific to the light input pathway.

In view of the potential utility of enhancing sensitivity to light for treatment of circadian and affective disorders, it is important to identify whether photoperiod effects generalize to melatonin suppression, a response that is often used as a proxy for studies of circadian regulation by light in humans. Limited previous works suggests that photic sensitivity for melatonin suppression by light may indeed be altered by seasonal changes in photoperiod (Thompson et al., 1990; Owen and Arendt, 1992). However, the influence of photoperiod on light sensitivity for melatonin suppression has not previously been studied in an animal model wherein light history can be rigorously controlled and monitored. Siberian hamsters are a particularly good model for studies of circadian function as they demonstrate rhythms in locomotor activity and melatonin secretion, both of which also show robust and predictable changes in waveform as a function of photoperiod (Milette and Turek, 1986; Puchalski and Lynch, 1986; Goldman and Elliott, 1988). In order to determine whether photoperiod history alters sensitivity to light for melatonin suppression, we constructed complete fluence-response curves for light-induced melatonin suppression in Siberian hamsters previously maintained under short versus long days. We also tested the generality of photoperiod influences on photic sensitivity to phase shifts in this species. Increased sensitivity to light for both responses under shorter days would suggest a common mechanism of action is serving to alter light sensitivity as a function of photoperiod. Alternatively, a photoperiod difference in light-induced phase shifts, but not melatonin suppression, would indicate seasonal modulation of photic response is targeting mechanisms unique to phase resetting.

Methods: Male Siberian hamsters (*Phodopus sungorus*), 4-6 weeks old, were selected from a breeding colony established in 1994. In both studies, animals for each photoperiod condition were maintained in separate large ventilated, light-tight, matte white interior chambers. On test nights, cages were temporarily transferred to a separate matte white interior pulsing cabinet (43 cm x 36 cm x 46 cm) for the 15-min light exposure regime. For the phase shift experiment, animals were individually housed in polypropylene cages (27 cm x 20 cm x 15 cm) equipped with 12 cm diameter wheels from when they first entered their randomly assigned photoperiod entrainment condition until the completion of the study. Animals in the melatonin experiment were group housed in polypropylene cages (48 cm x 27 cm x 20 cm) until the test night, when each animal was transferred to the smaller polypropylene cage (27 cm x 20 cm x 15 cm) immediately before placement in the pulsing cabinet. Food and water were available ad libitum. All procedures were approved by UCSD Institutional Animal Care and Use Committee.

Phase shifting experiment:

General protocol: An Aschoff type II randomized within-subjects design was employed in a group of Siberian hamsters that were entrained to LD14:10 (LP, 14 h light, 10 h dark; n =10) or LD10:14 (SP, n = 10) for 6 weeks. To examine photosensitivity associated with phase delay shifts, light pulses were administered 2 h into the dark (ZT14; with ZT12 representing the time of lights out) for both LP and SP. Each animal received three 15-min short wavelength light pulse at 0 (dark control), 0.11 and 3.14 $\mu\text{W}/\text{cm}^2$, with each test night separated by 21 days and each photoperiod group counterbalanced for the order of irradiance condition. Animals were exposed to constant darkness beginning at the normal time of lights-off on the night of each test pulse, remained under constant conditions for 10 days following the pulse, and were then re-entrained to their original photoperiod for 11 days. Cages were changed on day 1 of re-entrainment near the expected time of activity onset, under dim red illumination.

Assessment of wheel-running activity: Each one-half wheel revolution generated a switch closure signal that was recorded via VitalView software (Mini-Mitter, Bend, OR). Actograms were analyzed via Clocklab software (Actimetrics, Evanston, IL). **Activity onsets and offsets were determined in Clocklab, with adjustments to automated selections being made via eye-fit by raters who were blind to pulsing condition.** As a check of proper entrainment to photoperiod condition, activity duration (α) was determined as the mean difference between regression lines fitted to the onsets and offsets for the two weeks preceding the first test pulse. **Due to significantly greater variance in offsets versus onsets in baseline entrainment data, the more reliable activity onset was the phase marker used for calculation of phase shifts.** Phase shifts were calculated as the difference between the time of activity onset on the day of the light pulse (as determined by a regression line fitted to the onsets of the 7 days prior to the pulse) and the time of the activity onset on the day after the pulse (as predicted by the post-pulse regression line, calculated through activity onsets for days 4-10 following the light pulse). Two animals were eliminated from our analysis due to recording issues on days of constant conditions, making an accurate assessment

of phase shift difficult. Phase-shift scores were expressed relative to each animal's phase shift in response to the sham control condition. All group values are expressed as means \pm SEM and analyzed with analysis of variance (ANOVA).

Melatonin suppression experiment:

General protocol: A separate cohort of hamsters (n=106 total) was maintained on LP or SP for 6-7 weeks prior to melatonin suppression tests. Each animal was pulsed individually for 15-min with short wavelength light at ZT18 (LP) or ZT20 (SP) at one of the following prescribed intensities: 0, 0.003, 0.03, 0.3, 1.31, 4.8 or 68 $\mu\text{W}/\text{cm}^2$ (n=6-8 per irradiance for each photoperiod condition). The timing of the light pulse was chosen to occur approximately 60% of the way through the scotophase (60% for LP, 57% for SP) at a time when circulating melatonin is at its peak under each photoperiod in Siberian hamsters (Darrow and Goldman, 1986; Lerchl and Schlatt, 1993). Precisely one hour following the start time of each light pulse, animals were anesthetized with isoflurane and blood was collected via the retro-orbital sinus using heparinized caraway micro collecting tubes (Fisherbrand). Animals were then euthanized. Blood was transferred to tubes containing 40 μL of heparin, centrifuged, and plasma was stored at -70°C until assay.

Melatonin assay: Melatonin was extracted from samples, and concentrations were determined via an adaptation of the Buhlmann melatonin radioimmunoassay (RIA) kit (01-RK-MDI) from ALPCO (Salem, NH). Extraction included the following, with centrifuging of columns in between each step: conditioning with methanol and water, loading with sample, washing with 10% methanol and hexane, eluting melatonin with methanol, and evaporating to dryness. Extracted material was then reconstituted and diluted using the kit's zero calibrator, resulting in a functional sensitivity of 2.6 pg/ml. Standard curve calibrators (1-81 pg/ml), experimental and control samples were incubated with the Kenway anti-melatonin antibody and ^{125}I -melatonin for 18-24 hours at $2-8^\circ\text{C}$. Second antibody was then added, and samples were incubated for 15 minutes at $2-8^\circ\text{C}$. After the subsequent addition of water, samples were centrifuged, unbound supernatant was aspirated, and antibody-bound precipitate was counted with a Gamma-counter (Titertek Instruments, Inc., Huntsville, AL). The gamma counter software utilizes the standard curve to determine relative potency estimates of melatonin levels in pg/ml. Intra- and inter-assay coefficients of variation of this RIA were estimated to be 6.7% and 10.4%, respectively.

Analyses: Melatonin data were analyzed as concentrations (pg/ml) and percent control-adjusted melatonin suppression ($(\text{mean melatonin for control} - \text{mean melatonin at each irradiance})/\text{mean melatonin for control} \times 100$; %CA melatonin suppression). Adjustment for dark control values is commonly employed for studies of melatonin suppression to account for baseline differences in melatonin concentrations as would be expected of no pulse controls groups maintained under different baseline conditions (Brainard et al., 1984, 2001; Nelson and Takahashi, 1991; Owen and Arendt, 1992). SPSS (version 13.0.0) was used to perform ANOVA and post hoc tests. **Effect size estimates were determined via eta-squared (η^2) for ANOVA and Cohen's d for pairwise comparisons. Eta-squared was calculated as the between group sum of squares/total sum of squares. Cohen's d was calculated as the difference between two group means divided by the average of the standard deviations for those means. Effect size for within-subjects data was also corrected in order to make direct comparisons to estimates for between-subjects studies (Morris and DeShon, 2002).** 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.

Light Sources: From the time of gestation until entry into the study, environmental lighting conditions consisted of 24 hour periods of cycled light and darkness, with 14 hours of light and 10 hours of darkness (i.e. LD14:10). The LP groups remained on the same room photocycle, and the photoperiod for SP animals was achieved by extending the time of lights off by 4 hours. During the entrainment phase of both experiments, photophases were illuminated with broad-spectrum white fluorescent bulbs (F4T5) ($100-150 \mu\text{W}/\text{cm}^2$). Dark phases (scotophases) were dimly illuminated by narrowband light-emitting diodes (LEDs) affixed to the shelves of each chamber (560 nm, 23 nm half-peak bandwidth; $7.9 \times 10^{-6} \mu\text{W}/\text{cm}^2$, ~ 0.01 lux) during both entrainment and post-pulse free-run periods. This dim scotopic illumination is comparable in irradiance to natural ambient light at night and represents a very small fraction ($\sim 1/380$) of our lowest irradiance test pulse. Dim light scotophases have been shown to facilitate rapid and full entrainment to SP and reduce the incidence of short day non-responder Siberian hamsters (i.e. animals who fail to adopt the more typical winter phenotype, including expansion of activity duration, gonadal regression and weight

loss) (Gorman and Elliott, 2004). Experimental light pulses were administered in separate pulsing cabinets that were optimized for a homogenous, highly controlled exposure. Test pulses consisted of a 480 nm (23 nm half-peak bandwidth) 8-LED lamp source with diffuser, positioned in a standardized location at the center top of each cage lid. Irradiance levels of these lamps were obtained using neutral density filters, except for the highest intensity condition ($68 \mu\text{W}/\text{cm}^2$), which was administered via a 24-LED lamp with the same spectral composition as the 8-LED lamp source. Spectral power distributions and half-peak bandwidth of the LED lamps were characterized with an Ocean Optics spectral radiometer (model USB2000; Dunedin, FL). Irradiance measures were determined 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 $\mu\text{W}/\text{cm}^2$ and converted to photon density (photons/ cm^2/sec) based on the energy per photon for 480 nm.

Results:

Phase shifting study: Light pulses of increasing irradiance elicited greater phase delays (main effect repeated-measures ANOVA, $F_{1,18} = 25.50$, $p < 0.001$, $\eta^2 = 0.28$). Phase shifting also varied by photoperiod condition ($F_{1,18} = 6.91$, $p < 0.02$, $\eta^2 = 0.19$), with greater delays in SP versus LP at both tested irradiances ($p < 0.001$; **Figures 1 and 2**). In addition, LP response to the lower irradiance condition was not different from 0, whereas all other conditions produced significant phase delays. The mean phase shift induced by the sham dark control was not statistically different from 0 for either photoperiod condition (95% CI for LP and SP is [-0.280, 0.248] and [-0.528, 0.272], respectively), confirming that the practice of administering the pulse (independent of the light) was minimally disruptive to the animals. The duration of wheel-running activity (α) differed between long and short day conditions, verifying that animals were appropriately entrained to their respective photoperiods (mean \pm SE α for LP: 9.03 ± 0.22 hours and SP: 13.07 ± 0.23 hours).

Melatonin suppression study: There was a significant effect of both irradiance ($F_{7,92} = 7.49$, $p < 0.001$, $\eta^2 = 0.33$) and photoperiod (two-way between subjects ANOVA, $F_{1,92} = 10.51$, $p < 0.005$, $\eta^2 = 0.16$) on melatonin concentrations. **Figure 3A** shows the mean \pm SEM melatonin for the two photoperiod conditions (mean range, 4.9 to 277.7 pg/ml). Melatonin concentrations did not differ between the dark control and $0.003 \mu\text{W}/\text{cm}^2$ exposure conditions ($p = 1.00$, $d = 0.09$) but levels in both groups were higher compared to irradiances $> 0.03 \mu\text{W}/\text{cm}^2$ ($p \leq 0.05$, $d \geq 0.61$). Mean melatonin at $0.03 \mu\text{W}/\text{cm}^2$ was also greater than the remaining higher irradiance conditions ($p < 0.005$, $d \geq 0.81$). Finally, melatonin levels at irradiances $\geq 1.31 \mu\text{W}/\text{cm}^2$ were not statistically different, reflecting saturating responses in both LP and SP. Post-hoc tests showed higher melatonin in SP versus LP animals at all but the highest irradiance condition.

Melatonin suppression scores varied with irradiance ($F_{6,84} = 4.96$, $p < 0.001$, $\eta^2 = 0.26$) but not by photoperiod ($F_{1,84} = 0.003$, $p = 0.95$, $\eta^2 < 0.00$, **Figure 3B**). All intensities above $0.03 \mu\text{W}/\text{cm}^2$ suppressed melatonin more than $0.003 \mu\text{W}/\text{cm}^2$ ($p < 0.05$, $d \geq 1.26$). In addition, irradiances $\geq 1.31 \mu\text{W}/\text{cm}^2$ reduced circulating melatonin to a greater extent than the two lowest irradiances ($p < 0.05$, $d \geq 1.39$). Percent control adjusted data were converted to a best-fit sigmoidal fluence-response curve, with melatonin suppression plotted as a function of photon density on a log linear plot (**Figure 3C**). The formula for the curve is: $Y = \text{Minimum} + (\text{Maximum} - \text{Minimum}) / (1 + 10^{-(\text{LogED}_{50} - X) * p})$ (where p estimates the slope of the curve between the minimum and maximal response dose). The data for both photoperiod conditions were well fit to this sigmoid function, as demonstrated by high coefficients of correlation (R^2 for SP = 0.97 and LP = 0.95). However, there were no photoperiod differences in ED_{50} ($p = 0.74$) or maximum response ($p = 0.99$).

Discussion:

Here we demonstrate increased photic sensitivity for phase resetting but not melatonin suppression under short photoperiods in Siberian hamsters, **suggesting a functional divergence in photoperiod modulation of these two light responses**. In addition, our combined study findings are consistent with reports in other species, where lower levels of light are required for melatonin suppression as compared to phase shifting (Nelson and Takahashi, 1991b). Indeed, the lower irradiance in the phase shifting study, which surpassed threshold for SP but not LP, falls on the saturating portion of the melatonin suppression response curves for both photoperiod conditions.

The lower threshold for photic phase delays under SP versus LP in both Siberian and Syrian hamsters suggests a general enhancement of light sensitivity for phase shifting that is common to both species. Because quantitative data on circadian light sensitivity of Siberian hamsters were lacking, irradiances for these studies were selected on the basis of similar experiments in Syrian hamsters, where SP animals show a 40-fold increase in photic sensitivity (Glickman et al., 2012). **The range of irradiances employed for the melatonin suppression experiment corresponded to that used in constructing fluence-response for phase advances in Syrian hamsters. The two select irradiances of the phase shifting experiment were calculated from those same fluence-response curves, representing the doses required to elicit an equivalent one hour phase shift under long and short days (which were also used in our Syrian hamster phase delay and SCN experiments).** In the present phase shifting study of Siberian hamsters, the lower of the two selected irradiances elicited a delay under short but not under long daylengths whereas a higher irradiance induced shifts under both photoperiods, closely mirroring patterns of behavioral and molecular data from previous Syrian hamster studies (Glickman et al., 2012).

In contrast to results for phase resetting, melatonin suppression does not demonstrate analogous photoperiod modulation. Instead, response curves for the two photoperiod conditions are virtually indistinguishable and do not vary in terms of threshold, sensitivity, or maximum response. The absolute change in melatonin may give the illusion of increased photic sensitivity under shorter days; however, **statistical equivalence of control-adjusted scores suggests that this apparent photoperiod effect is being driven by baseline differences** (see Figure 3). Photoperiod-dependent disparities in raw melatonin values confirm several but not all previous reports in this species (Hoffman et al., 1981, 1985; Illnerova et al., 1984; Darrow and Goldman, 1986; Hoffman and Illnerova, 1986; Lerchl and Schlatt, 1993; Niklowitz et al., 1994). The fluence response curves for suppression in LP and SP show statistically similar ED_{50} s of 4.78×10^{10} and 4.98×10^{10} photons/cm²/sec, respectively. In terms of threshold, both LP and SP animals require at least $0.03 \mu\text{W}/\text{cm}^2$ in order to significantly reduce circulating melatonin levels. Maximum melatonin suppression is also statistically similar regardless of photoperiod history. This is to be expected since there is an obvious ceiling effect that renders it a less robust comparison measure than the threshold sensitivity or ED_{50} . Ultimately, no matter how the two conditions are compared, photoperiod history does not appear to modulate melatonin suppression by light in the Siberian hamster.

To our knowledge, these are the first published fluence response curves for photic responses in this species. Melatonin suppression by light in Siberian hamsters is well fit to the sigmoid function obtained in other model systems. Dose response curves for melatonin suppression have been described in Syrian hamsters under LD14:10-but not shorter photoperiods- and yield comparable ED_{50} s to that reported here for Siberian hamsters entrained to the same photoperiod (Brainard et al., 1984; Nelson and Takahashi, 1991b). We also do not know of published studies examining photoperiod effects on light-induced melatonin suppression in other mammalian models. In pilot work with Syrian hamsters, we found a sharp and short-lived melatonin peak, particularly under LP, that limited the utility of that model system (personal observations).

In humans, dimensions of photic history other than entrainment photoperiod have been shown to alter melatonin suppression by light (Thompson et al., 1990; Owen and Arendt, 1992; Hebert et al., 2002; Jasser et al., 2006). Specifically, prior exposure to higher daytime light intensities over a one-week period decreases the melatonin suppressing effects of test pulses at night (Hebert et al., 2002). Even just two hours of 18 lux light versus complete dark pre-adaptation results in an attenuated melatonin suppression response (Jasser et al., 2006). **Under more naturalistic seasonal conditions, humans demonstrate increased light-induced melatonin suppression during the winter versus summer** (Thompson et al., 1990; Owen and Arendt, 1992); however, daylight exposure patterns of the seasonal conditions in those studies were not explicitly monitored or controlled, so it is difficult to know if photoperiod can account for the results. Separate reports have not consistently found a seasonal variation in the distribution of daily light exposure in humans (Cole et al., 1995; Hebert et al., 1998).

In any study administering single light pulses under different photoperiods, two potential confounds to be acknowledged are the amount of time in darkness prior to a test pulse and the circadian timing of the stimulus. With regard to possible dark adaptation effects, both melanopsin-containing ipRGCs and SCN neurons demonstrate hours-long dark-adaptation kinetics (Aggelopoulos and Meissl, 2000; Wong et al., 2005), so it is possible that animals were not completely dark-adapted prior to the administration of light pulses. Because the timing of the light pulse for the melatonin study sampled time points representing similar proportions of the subjective night, animals under short days were exposed to darkness for 2 hours more than those in long days. Notably, in that experiment there was no discernible photoperiodic effect on light sensitivity. In contrast, photic sensitivity was influenced by

photoperiod in the phase-resetting experiment in which the immediate duration of darkness was equivalent for the two groups. Therefore, dark adaptation effects cannot account for the increased response to light for phase resetting under shorter days.

With regard to equivalence of circadian phase, it is not possible to match the phase of two different waveforms with single time points. For example, if two different photoperiods are aligned based on one phase indicator (e.g. activity onset), they are rendered misaligned with respect to another (e.g. activity offset). Therefore, within each reported experiment, we cannot claim that our selected pulse times represent identical circadian phases for the SP and LP conditions. In addition, the melatonin suppression data were derived using light stimuli in the late subjective night (a time that reliably elicits phase advancing), whereas the phase shifting study employed phase delaying stimuli administered in the early subjective night. We cannot, therefore, rule out the possibility that photoperiod modulation of sensitivity to light may be partly due to circadian phase in Siberian hamsters. However, there is no evidence indicating the timing of a light pulse contributes significantly to threshold response or absolute sensitivity to light in hamsters. On the contrary, full dose response curves to light for phase shifting of activity rhythms at two selected phases do not reveal differences in sensitivity (i.e. ED₅₀) (Nelson and Takahashi, 1991). Furthermore, photoperiod effects have not been limited to a particular circadian phase in Syrian hamsters, where the greater photic phase shifts under SP versus LP are maintained at different times in the subjective night (Evans et al., 2004; Glickman et al., 2012).

Constant dim illumination throughout all scotophases was employed to be consistent with our previous work, wherein dimly lit nights serve to enhance photoperiod entrainment but do not induce robust shifts in rhythms on their own (Evans et al., 2007). Dim light of similar irradiance has also been relatively ineffective at suppressing melatonin in Syrian hamsters (Brainard et al., 1982, 1984; Nelson and Takahashi, 1991b). Consistent with those findings, our melatonin study does not show levels of the hormone to differ between the lowest irradiance pulse and the sham condition for either photoperiod group, suggesting that the dim light alone is not potent enough to elicit a neuroendocrine response in Siberian hamsters.

In summary, these studies suggest that photic responses of phase resetting and melatonin suppression are modulated differently by photoperiod history. In short daylengths, the increased photic sensitivity for the phase-resetting function of the SCN without comparable changes to melatonin suppression suggests a mechanism downstream of their common retinal input. **This divergence may take place at the level of the SCN and/or involve alternate paths to the pineal (e.g. intergeniculate leaflet (Mikkelsen and Møller, 1990)).** It has been proposed that light induces *per1* mRNA expression and suppresses melatonin via separate synaptic mechanisms as NMDA receptor activation in the SCN is required for the former but not the latter (Paul et al., 2003). Since photoperiod modulation of photic sensitivity is evident very early in the signal transduction cascade of SCN neurons (Glickman et al., 2012) but not for melatonin suppression (as reported here), we suggest that only the pathway involving NMDA receptor activation is photoperiod sensitive. **Better understanding the mechanistic basis for the profound impact of photoperiod on light response may ultimately lead to new strategies for optimizing light therapy for circadian sleep disturbances and affective disorders.**

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Figure Legends

Figure 1. Representative wheel-running actograms of Siberian hamsters tested for phase-shifting responses following entrainment to A) long photoperiod (LP) and B) short photoperiod (SP). Animals were repeatedly entrained to LP or SP, exposed to a brief light pulse of three different irradiances- 0, 0.11 and 3.14 $\mu\text{W}/\text{cm}^2$ (as indicated along the left of each actogram), and allowed to free-run in constant conditions for assessment of phase-shifting. Each line represents a 24-h day. Unshaded and shaded areas represent times of bright light and relative dark, respectively. The 15-min short wavelength light pulses after release into constant conditions are indicated with white circles.

Figure 2. Dependence of phase-delay magnitude on photoperiod entrainment and irradiance. Mean \pm SE phase delays (h) for long photoperiod (LP; gray) versus short photoperiod (SP; black) ($n = 10$ per condition). Phase-shift scores are expressed relative to each animal's phase shift on the dark control night. Phase delays differed by both irradiance ($p < 0.001$) and photoperiod ($p < 0.02$).

Figure 3. Melatonin data are illustrated in the following figures: A) Raw mean melatonin concentrations (pg/mL) for long photoperiod (LP; gray) versus short photoperiod (SP; black) ($n=6-8$ per irradiance for each photoperiod condition). Two-way between-subjects ANOVA demonstrates a significant effect of both irradiance ($p < 0.001$) and photoperiod ($p < 0.005$) on melatonin concentrations. Note, however, the significant photoperiod differences for the dark control condition. B) In order to account for the photoperiod differences in baseline levels, these means reflect the percent change in melatonin from the dark control for the photoperiod-matched condition under long (LP; grey) and short (SP; black) days. Suppression scores varied with irradiance ($p < 0.001$) but not by photoperiod ($p = .954$). C) Fluence-response curves for the same suppression scores are illustrated for animals previously entrained to LP (grey circles) or SP (black squares). Data for both conditions were well fit to this sigmoid function, as demonstrated by high coefficients of correlation (R^2 for SP= 0.97 and LP= 0.95). However, there are no photoperiod differences in ED_{50} ($p=0.74$) or maximum response ($p=0.99$).

Figure 1.

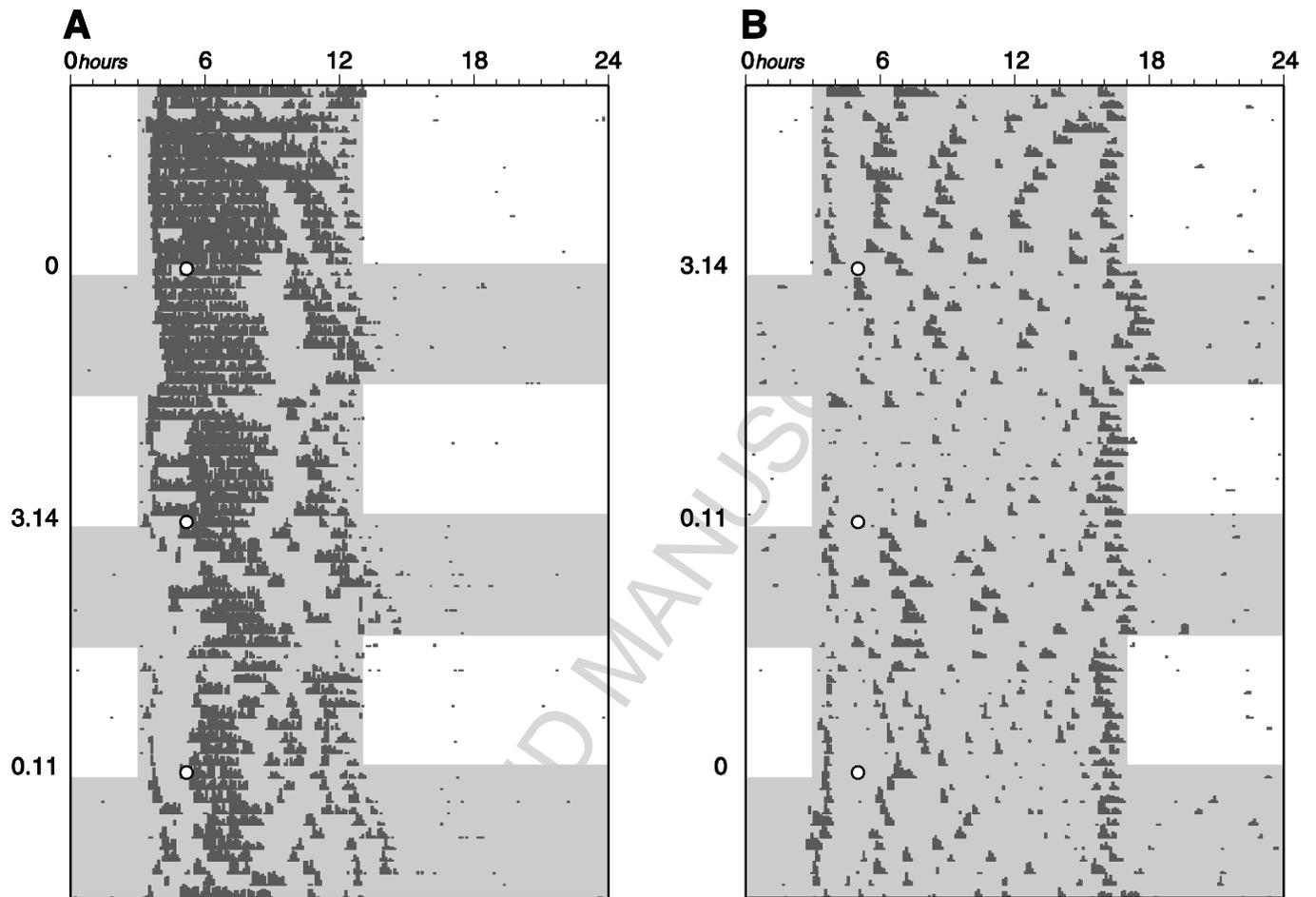


Figure 2.

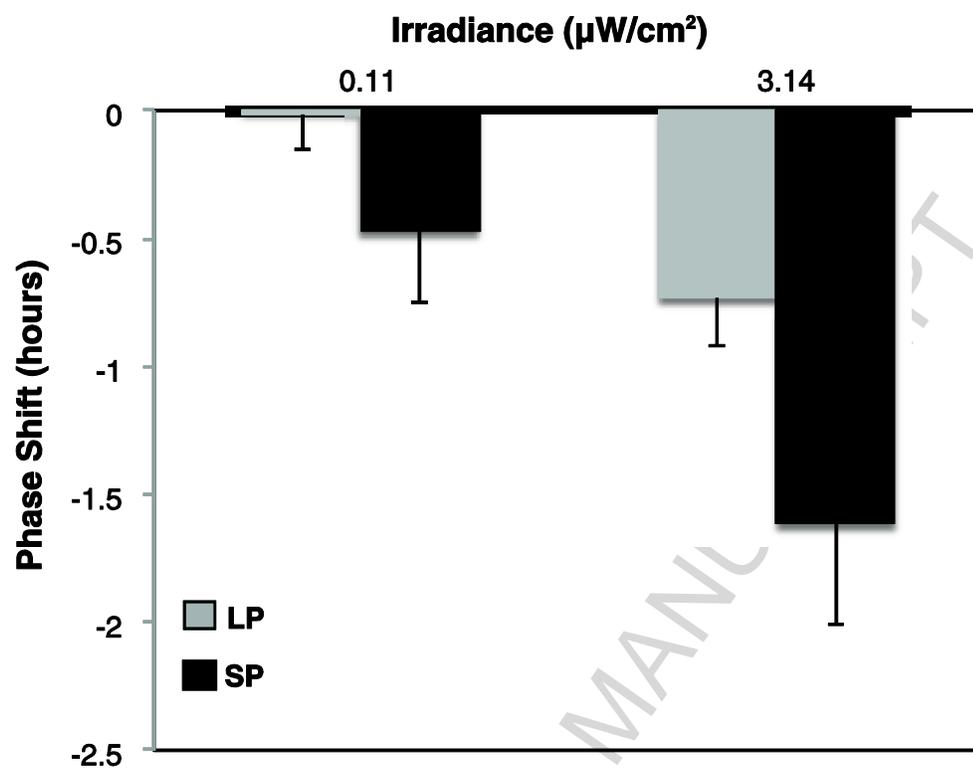


Figure 3A.

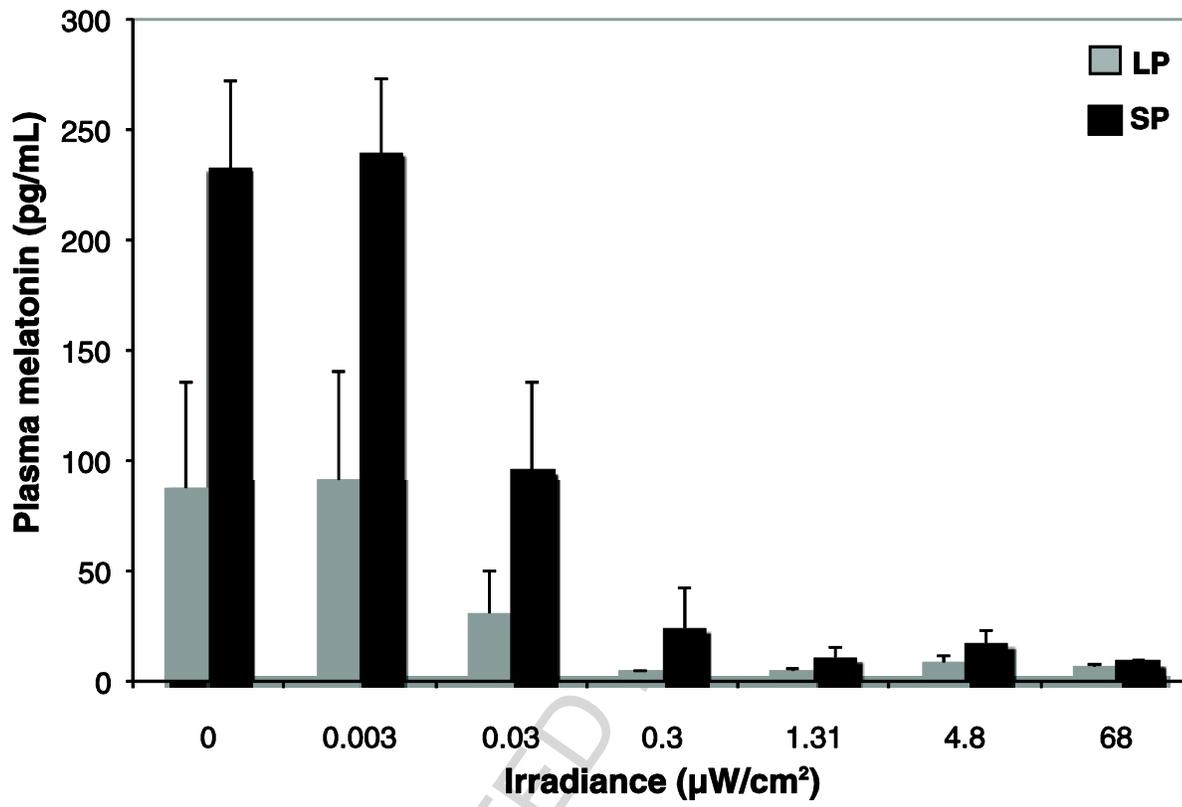


Figure 3B.

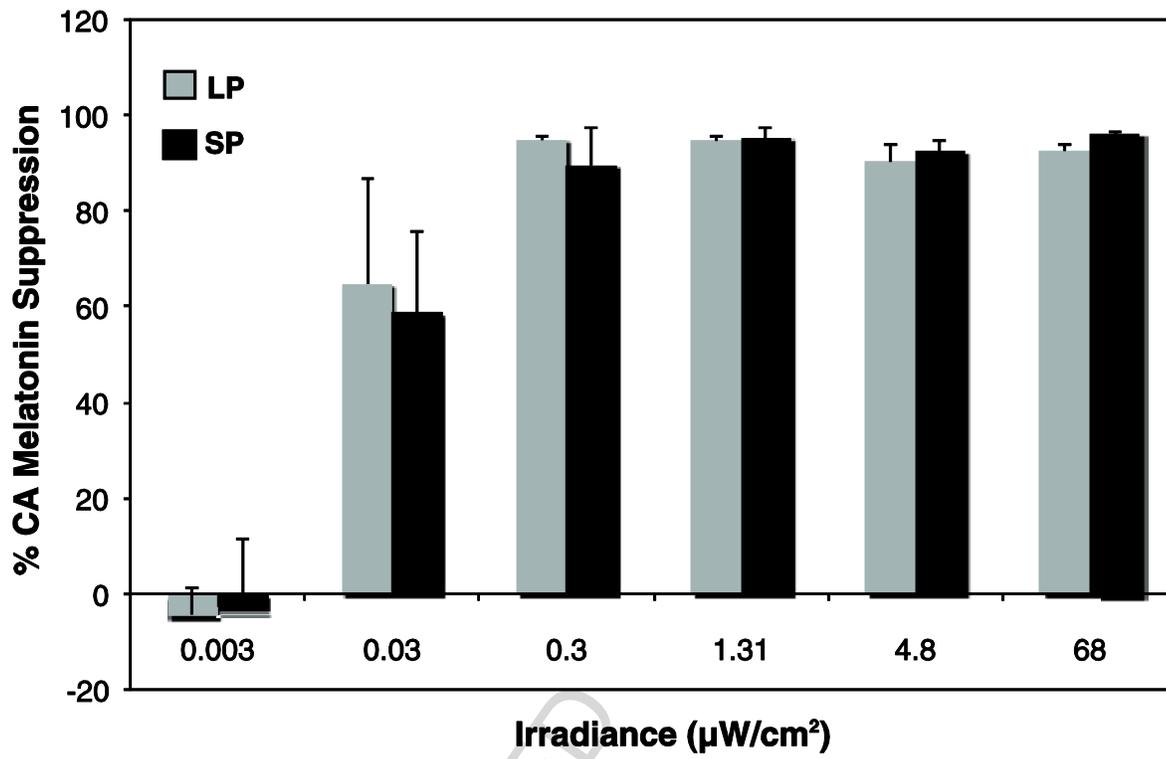
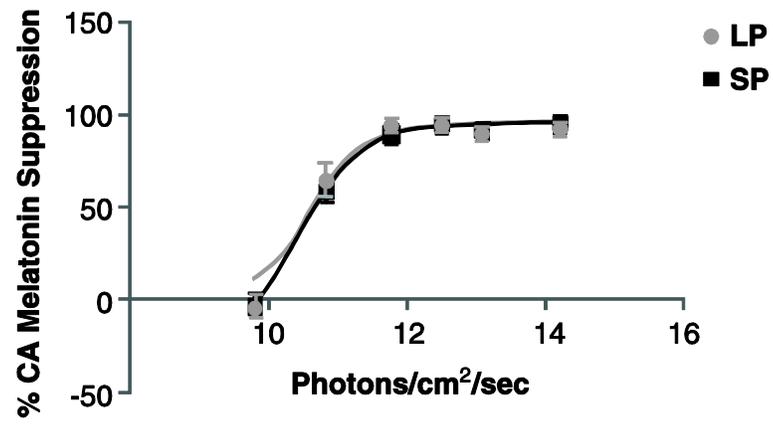


Figure 3C.



AC

Highlights:

- Photic thresholds for circadian responses were assessed in Siberian hamsters
- Light sensitivity for phase shifting is enhanced in short versus long daylengths
- Light sensitivity for melatonin suppression is identical in long versus short days
- Modulation of light sensitivity occurs downstream of common retinal input pathway
- Photoperiod effects on circadian resetting are conserved across hamster species