UC San Diego
UC San Diego Electronic Theses and Dissertations

Title
The Influence of Photoperiod History on Circadian Response to Light

Permalink
https://escholarship.org/uc/item/7xf6k46c

Author
Glickman, Gena Lynne

Publication Date
2013

Peer reviewed|Thesis/dissertation

eScholarship.org Powered by the California Digital Library
University of California

UNIVERSITY OF CALIFORNIA, SAN DIEGO

The Influence of Photoperiod History on Circadian Response to Light

A dissertation submitted in partial satisfaction of the requirements for the degree of

Doctor of Philosophy

in

Psychology

by

Gena Lynne Glickman

Committee in charge:

Professor Michael Gorman, Chair
Professor Stephan Anagnostaras
Professor Sonia Ancoli-Israel
Professor Alexander Kaufman
Professor Don MacLeod

2013

©

Gena Lynne Glickman, 2013

All rights reserved.

The Dissertation of Gena Lynne Glickman is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

____

____

____

____

____

Chair

University of California, San Diego

2013

DEDICATION

This is dedicated to my family, my most staunch supporters.

Especially, to my grandmother, Angelina DeSalvo, who has always believed I could accomplish whatever I set out to do.

Also, to my parents, Sheila and Gerry Glickman, for cheering me on as I muddled through graduate school.

And to the most important person in my life, my partner in crime, Aaron Steckelberg. You have helped me personally and professionally, without hesitation, each step of the way.

Last but not least, to our little one, Cora Lynne Steckelberg. Though non-existent for the greater part of my dissertation, you have been the heart that allowed me to finally complete what I started.

EPIGRAPH

A sensible man will remember that the eyes may be confused in two ways - by a change from light to darkness or from darkness to light; and he will recognize that the same thing happens to the soul.

- *Plato*

Master Plato once said that Lux est umbra Dei; light is the shadow of God. I say this way: Light is the god of shadow!

- *Mehmet Murat ildan*

In the right light, at the right time, everything is extraordinary.

- *Aaron Rose*

TABLE OF CONTENTS

Signature Page……………………………………………………………...............iii

Dedication…………………………………………………………………………..iv

Epigraph…………………………………………………………………………..v

Table of Contents…………………………………………………………...........vi

List of Figures……………………………………………………………………..viii

Acknowledgements……………………………………………………………….x

Vita…………………………………………………………………………………xii

Abstract……………………………………………………………………………..xviii

Chapter 1. General Introduction…………………………………………………..1

Chapter 2. Increased photic sensitivity for circadian phase resetting under shorter days

…………………………………………………………………………...........23

2.1. Introduction…………………………………………………….......23

2.2. Methods…………………………………………………….............25

2.3. Results……………………………………………………………........30

2.4. Discussion…………………………………………………………..31

Chapter 3. Light sampling: Impact of photoperiod history, irradiance and circadian phase……………………………………………………………………………........40

3.1. Introduction……………………………………………………...40

3.2. Methods……………………………………………………..............45

3.3. Results…………………………………………………………………47

3.4. Discussion…………………………………………………………..48

Chapter 4. Photoperiod modulates sensitivity to light in the SCN…………………...60

4.1. Introduction……………………………………………………………...60

4.2. Methods………………………………………………………..............61

4.3. Results……………………………………………………………………66

4.4. Discussion…………………………………………………………….....67

Chapter 5. Sensitivity to light for melatonin suppression does not vary by photoperiod…………………………………………………………………………...72

5.1. Introduction……………………………………………………………...72

5.2. Methods………………………………………………………..............75

5.3 Results……………………………………………………………………80

5.4. Discussion…………………………………………………………….....82

Chapter 6. Conclusions…………………………………………………………96

References………………………………………………………………………...105

LIST OF FIGURES

Figure 1.1. Free running period (τ) in A) nocturnal and B) diurnal animals…...........17

Figure 1.2. Entrainment of a nocturnal rodent to LD14:10…………………………..18

Figure 1.3. Light-induced phase advance of activity rhythms in a nocturnal rodent…………………………………………………………………….…..19

Figure 1.4. Phase response curve……………………………………………………..20

Figure 1.5. Diagram of the neural pathway by which light regulates circadian rhythms……………………………………………………………………………..21

Figure 1.6. Dose response function…………………………………………...........22

Figure 2.1. Possible outcomes for phase advance study..…………………………….36

Figure 2.2. Representative actograms for phase advance study.....…………………...37

Figure 2.3. Fluence-response curves for phase advances…………………………….38

Figure 2.4. Photoperiod effects on phase delays……………………………………...39

Figure 3.1. Experimental set-up for light sampling study……………………………54

Figure 3.2. Proportion of a sub-saturating light pulse received as a function of photoperiod…………………..……………………………………………………..55

Figure 3.3. Proportion of a sub-saturating light pulse received as a function of circadian phase………………………………………………………………………..56

Figure 3.4. Proportion of a saturating light pulse received as a function of photoperiod ……………………………………………………………………………………...57

Figure 3.5. Proportion of a saturating light pulse received as a function of circadian phase…………………………………………………………………………………..58

Figure 3.6. Proportion of total photons received as a function of irradiance…………59

Figure 4.1. Schematic of the protocol for the immunocytochemistry experiment……70

Figure 4.2. Representative histology in the SCN for long (LP) or short photoperiod (SP) animals…………………………………………………………………………..71

Figure 5.1. Short day versus long day: mean melatonin scores……………….........90

Figure 5.2. Short day versus long day: melatonin change scores……………………91

Figure 5.3. Short day versus long day: percent control-adjusted melatonin………….92

Figure 5.4. Fluence-response curves for melatonin suppression ……….………........93

Figure 5.5. Representative actograms for phase shifting in Siberian hamsters….........94

Figure 5.6. Photoperiod effects on phase delays in Siberian hamsters……………….95

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Michael Gorman for his support as the chair of my committee. His guidance in developing, designing and presenting all of my disseration experiments has been invaluable, and I am very lucky that our lives have intersected. The vast knowdledge and effort that came from Jeff Elliott over the years is also greatly appreciated. He started the investigation into photoperiod history long before I entered the scene and was particularly integral to the melatonin experiment. I would be remiss if I failed to include Norman Anderson among those who have supported me during this work. He has been incredibly generous in sharing his wisdom from the very early stages of my studies, ensuring my statistical analyses were sound and that my enthusiasm for my work never ceased. In addition, I am grateful to the amazing collective of scientists who have served as my dissertation committee members: Stephan Anagnostaras, Sonia Ancoli-Israel, Sasha Kaufman and Don MacLeod.

Many thanks goes to our Animal Care staff, particularly Antonio Mora, who was the primary technician responsible for the animals used in all the reported projects. Never hesitating to lend a helping hand and with whom it is always a pleasure to work, his involvement is very much appreciated. I would also like to acknowledge the important role of Divya Ahuja in obtaining results for the light sampling study, spending many hours of her time assisting me in diligently scoring Syrian hamster behaviors. Also, my deepest gratitude to Aaron Steckelberg who

helped with the formatting of videos for the light sampling study and enahnced many of the figures with his tremendous artistic talent.

Additional thanks to Liz Harrison whose work helped make the melatonin study more meaningful. Chapter 5, in part, is currently being prepared for submission for publication of the material. Glickman Gena; Harrison Elizabeth; Elliott Jeff; Gorman Michael. The dissertation author was the primary investigator and author of this material.

I would also like to acknowledge the lab of Mike Lehman, without whom my immunohistochemistry exeperiment would have no doubt taken three times as long. Chapter 4, in part, is a reprint of the material as it appears in Photic sensitivity for circadian response to light varies with photoperiod in Journal of Biological Rhythms 2012. Glickman, Gena; Webb, Ian; Elliott, Jeff; Baltazar, Ricardo; Rheale, Meghan; Lehman, Michael; Gorman, Michael. The dissertation author was the primary investigator and author of this paper.

A very special thanks to my first scientific mentor, George (Bud) Brainard, who started this fellow clown on an illuminating journey and has been unwavering in his support and encouragement. Finally, I will be eternally grateful to the community of chronobiologists who treated me like family from the very start, promoting my engagement and learning with rigorous scientific papers, accessible presentations, and personal insights.

You all make me want to be a better scientist.

VITA

Education

University of California, San Diego- La Jolla, California
Doctor of Philosophy in Experimental Psychology, May 2013
Master of Arts in Experimental Psychology, May 2007

Thomas Jefferson University- Philadelphia, Pennsylvania
Bachelor of Science in Occupational Therapy, December 2001

Villanova University- Villanova, Pennsylvania
Bachelor of Arts in Psychology, May 2001

Publications

Peer-Reviewed Articles

Glickman G, Webb I, Elliott J, Rheale M, Baltazar R, Lehman M, Gorman M (2012) Photic sensitivity for circadian response to light varies with photoperiod. Journal of Biological Rhythms 27(4):308-318

Raiewski E, Elliot J, Evans J, Glickman G, Gorman M (2012) Twice daily melatonin peaks in Siberian but not Syrian hamsters under 24 hour Light:Dark:Light:Dark Cycles. Chronobiology International 29(9):1206-1215

Glickman G (2010) Circadian Rhythms and Sleep in Children with Autism. Neuroscience and Biobehavioral Reviews 34(5):755-768

Brainard G, Sliney D, Hanifin J, Glickman G, Byrne B, Greeson J, Jasser S, Gerner E, Rollag M (2008) Sensitivity of the human circadian system to short wavelength (420 nm) light Journal of Biological Rhythms 23(5):379-386

Glickman G, Byrne B, Pineda C, Hauck W, Brainard G (2006) Light therapy for Seasonal Affective Disorder with Blue Narrow-Band Light-Emitting Diodes (LEDs). Biological Psychiatry 59:502-507

Glickman G, Hanifin J, Rollag M, Wang J, Cooper H, Brainard G (2003) Inferior retinal light exposure is more effective than superior retinal exposure in suppressing melatonin in humans. Journal of Biological Rhythms 18(1):71-79

Glickman G, Levin R, Brainard G (2002) Ocular Input for Human Melatonin Regulation: Relevance to Breast Cancer. Neuroendocrinology Letters 23(2):17-22

Brainard G, Hanifin J, Rollag M, Greeson J, Byrne B, Glickman G, Gerner E (2001) Action spectrum for melatonin regulation in humans. Photodermatology, Photoimmunology and Photomedicine 17:139

Brainard G, Hanifin J, Greeson J, Byrne B, Glickman G, Gerner E, Rollag M (2000) Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. Journal of Neuroscience 21(16):6405-6412

Brainard G, Hanifin J, Rollag M, Greeson J, Byrne B, Glickman G, Gerner E and Sanford B (2000) Human melatonin regulation is not mediated by the three cone photopic visual system. Journal of Clinical Endocrinology & Metabolism 86(1):433-436

Book Chapters and Symposium Papers

Glickman G, Byrne B, Rollag M, Tavener S, Hanifin J, Brainard G, Light-induced melatonin suppression in healthy humans with narrow-band light-emitting diodes. 15th Annual Meeting 2003 Society for Light Treatment and Biological Rhythms, Virtual Meeting (published in Chronobiology International)

Brainard G and Glickman G The biological potency of light in humans: significance to health and behavior 2003 25th Session of the CIE, June 25- July 2, 2003, sponsored by International Commission on Illumination (CIE)

ABSTRACT OF THE DISSERTATION

The Influence of Photoperiod History on Circadian Response to Light

by

Gena Lynne Glickman

Doctor of Philosophy in Psychology

University of California, San Diego, 2013

Professor Michael Gorman, Chair

Light entering the mammalian eye activates a neural pathway independent of the classical visual system, serving to regulate circadian rhythms via input to the central pacemaker in the suprachiasmatic nuclei (SCN) of the hypothalamus. Various factors affect light regulation of the circadian system, including the physical properties of the light stimulus and the position of the light stimulus relative to the eyes. Photic response is also markedly altered by prior light exposure and thus, the non-image forming visual system represents an attractive model of neural plasticity.

Seasonal changes in the duration of daylight (i.e. photoperiod) provide a rich example of how photic history can influence circadian regulation by light. Specifically, previous entrainment to a short winter-like photoperiod results in an approximately two-fold greater maximal phase shift as compared to relatively longer summer days (Goldman and Elliott, 1988; Evans et al., 2004). The work presented here is the first to characterize *sensitivity* to light as a function of photoperiod history. Indeed, there is a 40-fold increase in photic sensitivity for phase advancing of activity rhythms in Syrian hamsters previously entrained to shorter days, refining our understanding of how seasonal changes in photoperiod modulate circadian response to light. Additional experiments test the effects of photoperiod on various measures associated with non-visual, light-mediated responses, including: circadian phase delays, light sampling behavior, light-induced melatonin suppression and photic-induction of proteins in the SCN. Collectively, these projects further identify the level of regulation by which photoperiod modulates photic sensitivity of the circadian system.

CHAPTER 1. GENERAL INTRODUCTION

Circadian Rhythms are Regulated by Environmental Light-Dark Cycles:

Circadian rhythms are physiological and behavioral processes that have an endogenously generated cycle with a period (τ) of approximately 24 hours. Various phenomena demonstrate such a rhythm, including the sleep-wake cycle, hormone secretory patterns and core body temperature fluctuations. In mammals, these rhythms are maintained via a central pacemaker in the suprachiasmatic nuclei (SCN) of the hypothalamus (Klein et al., 1991; Moore, 1995). In order to determine the τ of a particular circadian rhythm, it should be examined under free running conditions, in the absence of any environmental cues.

Locomotor activity is the most thoroughly studied behavioral rhythm in rodents. The activity rhythms of both *Mesocricetus auratus* (Syrian hamster) and *Phodupos sungorus* (Siberian hamsters) are particularly robust and have been well characterized in the literature. Figure 1.1.A is an actogram that illustrates the activity rhythm of an animal with a τ of slightly less than 24 hours. Each line represents a 24-hour day, with this figure showing a total of 5 days. The grey shading indicates the days are spent under constant dark conditions. The black patterns represent wheel-running activity. In this example, each day the animal begins running slightly earlier relative to the 24 hour day and so, for heuristic purposes, the animal will be said to have a τ of approximately 23 hours. In nocturnal animals, such as hamsters, it is common to demonstrate a τ of less than 24 hours whereas diurnal animals (e.g.

humans) typically have a τ slightly longer than 24 hours (see Figure 1.1.B).

In the absence of temporal cues, even a seemingly minor deviation from a 24-hour period of just 0.2 hours (12 minutes) each day (which is not uncommon) results in a gradual divergence from the environmental day, until phase differences become detrimental to an animal's survival. In this example, within just 5 days, there will be a one-hour phase difference relative to the natural 24-hour cycle. In two months time, the rhythm will have become completely inverted, with the nocturnal animal behaving as if diurnal and vice-versa.

The endogenous rhythms of both nocturnal and diurnal animals are reset daily to a precise 24-hour period via environmental signals. The most significant cue for synchronization of rhythms (entrainment) is the natural light-dark cycle, with the light and dark period of the cycle known as the photophase and scotophase, respectively. Figure 1.2. depicts entrainment of a nocturnal animal to a light-dark cycle that has 14 hours of light and 10 hours of darkness (i.e. LD14:10). The white areas represent the period of light within the 24-hour day, a time when the animal is at rest. Once the lights go out, the animal begins running and activity is largely confined to the scotophase (grey shaded regions).

Photic entrainment of circadian rhythms occurs via daily light-induced shifts that adjust for the difference between the endogenous period of the rhythm and the period of the entraining cycle. Even just a single brief pulse of light is enough to shift the clock. Figure 1.3 shows how a light pulse late in the subjective night may cause the activity rhythm to shift to an earlier time. Such shifts reflect the differential effects

of light on the SCN at different phases of the clock in mammals, which are often described via phase response curves (PRCs) (Klein et al., 1991; Moore, 1995). Under normal conditions, there is no response to a light pulse administered during the subjective day, which is consequently referred to as the "dead zone." Light early in the subjective night elicits a delay in rhythms whereas light later in the subjective night will cause an advancing shift to an earlier time (see Figure 1.4 for a graphical depiction of a sample PRC).

Phase resetting by light is primarily mediated by the monosynaptic retinohypothalamic (RHT) projection to the SCN (Moore and Lenn, 1972; Johnson et al., 1988). Evidence suggests the RHT uses glutamate to transmit photic information to the pacemaker (Liou et al. 1986). Glutamate elicits phase-dependent shifts in SCN neuronal firing and activity rhythms that parallel those induced by light (Meijer et al. 1988; Ding et al. 1994; Shirakawa and Moore 1994). The greatest density of retinal fibers in the SCN is in the ventrolateral (vl) core region, although projections to other sites provide additional parallel pathways by which the SCN may be regulated (Gooley et al., 2003). For example, there are retinal projections to both the subparaventricular zone (SPZ) of the hypothalamus and the intergeniculate leaflet (IGL) of the thalamus, which each also innervate the retinorecipient core region of the SCN (Moga and Moore, 1997; Moore et al., 2000; Krout et al., 2002). In its role as pacemaker, the SCN integrates these various photic signals (see Figure 1.5).

Light Regulates Melatonin Rhythms:

Of the neural pathways from the SCN to distal targets, the one regulating pineal melatonin synthesis and secretion is perhaps the best understood. After light information is detected by the eyes and transmitted to the SCN, it is subsequently sent to the pineal gland via a well-defined multi-synaptic pathway. This includes sequential synapses in the paraventricular nucleus of the hypothalamus, the upper thoracic intermediolateral cell column, and the superior cervical ganglion (Klein et al., 1991; Moore, 1995) (Figure 1.5). Via this pathway, light serves to regulate pineal melatonin, with high levels at night and low levels during the day (Arendt, 1995). Ocular exposure to light at night can also acutely suppress production of the hormone (Cardinali et al., 1972; Klein and Weller, 1972; Minneman et al., 1974; Lewy et al., 1980). Because of the shared retinal input pathway, this acute response has been a useful tool for studying the physiology underlying the circadian system (Klein et al., 1991; Arendt, 1995).

Characteristics of the Light Source Influence Circadian Response:

Myriad factors influence photic regulation of the circadian system, including the position of the light source relative to the eyes and the physical properties of the light stimulus. Melatonin suppression by light as well as phase resetting have been shown to depend on the region of the retina exposed in humans, as significantly greater photic responses are obtained with inferior and nasal retinal illumination (Lasko et al., 1999; Visser et al., 1999; Glickman et al., 2003; Rueger et al., 2005). Identifying the physiology underlying these differences is difficult in humans;

however, photoreceptor distribution may be responsible as receptors are not homogenously distributed within the retina of other mammalian models (Cooper et al., 1991; Provencio et al., 2000; Berson et al., 2002; Hattar et al., 2002; Hannibal et al., 2002).

Circadian responses are also affected by intensity and wavelength of the light source. Various circadian measures demonstrate a characteristic intensity-dependent response, including both phase shifting and melatonin suppression (Brainard et al., 1982, 2001; Takahashi et al., 1984; Nelson and Takahashi, 1991a; Shigeyoshi et al., 1997; Zeitzer et al., 2000; Muscat et al., 2005; Hut et al., 2008). Fluence-response curves may be fit to a parametric model as described by the equation below and illustrated in Figure 1.6. The dynamic range of a response includes irradiances within the boundaries of threshold and maximum response. The threshold is the amount of light required to elicit a detectable difference from 0, and the maximum response dose represents irradiances at which saturation is achieved (i.e. additional light will not increase the response further). The half-saturation constant (ED_{50}) is the dose eliciting a half maximum response and is often used as a point of comparison for determining relative sensitivity. Finally, *p* estimates the slope of the curve between the minimum and maximum response dose:

$$Y = \text{Minimum (0) Response} + \frac{\text{(Maximum response-Minimum (0) response)}}{1+10\hat{}(\text{Log}ED_{50}-X)^{p}}$$

Spectral characteristics of the light source further influence the intensity needed to elicit a response. Comparable circadian responses are obtained with lower intensities of short wavelength light relative to all other tested longer wavelengths. Indeed, short wavelength light is the most potent for stimulating circadian and non-image forming systems across species (Brainard et al., 2001; Thapan et al., 2001; Hattar et al., 2002, 2003; Dacey et al., 2005; Gamlin et al., 2007).

Intrinsically Photosensitive Melanopsin-Positive Retinal Ganglion Cells (ipRGCs):

At least five different action spectra identify a common reasonably narrow 459-484 nm region of peak sensitivity for various non-image forming functions mediated through the eye. Action spectra studies describe wavelength sensitivities for responses such as: melatonin suppression in humans (Brainard et al., 2001; Thapan et al., 2001), electroretinogram B-waves in humans (Hankins et al., 2002), pupillary constriction in mice lacking rod photoreceptors (Lucas et al., 2001), and direct retinal ganglion cell response to light stimuli in rats (Berson et al., 2002). This spectral signature is distinct from the classical photoreceptors, suggesting a non-rod, non-cone photoreceptor is mediating light for these functions. Separate work led to the discovery of the photopigment melanopsin in the retinas of both rodents and humans (Provencio et al., 2000, 2002; Morin et al., 2003) and more specifically, within a subset of intrinsically photosensitive retinal ganglion cells (ipRGCs) (Berson et al., 2002; Hattar et al., 2002). These melanopsin-containing ipRGCs project to the SCN and respond to light in a manner akin to photic resetting (Gooley et al., 2001;

Provencio et al., 2002; Morin et al., 2003). Together, these findings reveal that the melanopsin containing ipRGCs are the primary photoreceptors involved in circadian and neuroendocrine phototransduction. More recent work demonstrating that the ipRGCs mediate light input from both classical photoreceptors as well as melanopsin-positive cells indicates a potentially complicated interplay between the different photoreceptor systems (Güler et al., 2008).

Evidence for Classical Photoreceptor Contribution:

Despite strong evidence indicating melanopsin-containing ipRGCs are the *primary* photoreceptors for a variety of non-image forming functions, it appears that the rod and cone photoreceptors also play a role. For example, melanopsin-knockout mouse studies show that the classical visual photoreceptors are sufficient for mediating non-visual responses to light, as both pupillary reflex and phase resetting are maintained, albeit altered, in these animals (Panda et al., 2002; Lucas et al., 2003). In addition, the different threshold responses of SCN neurons to 505 nm light after dark versus light adaptation suggests that there are separate rod and cone inputs to the SCN, with most neurons receiving input from both (Aggelopoulos and Meissel, 2000). Cellular recordings in primate retina further demonstrate that rod and cone cells can, in fact, directly activate ipRGCs (Dacey et al., 2005). Although tremendous progress has been made in understanding ipRGC anatomy and physiology over the past decade, the role of these newly discovered photoreceptors and the classical visual photoreceptors is seemingly quite complex and has yet to be fully clarified.

Regulation of SCN-Mediated Photobiological Effects at the Cellular Level:

Regulation of the clock by light is accomplished via the rapid triggering of transcriptional activation in the SCN. Those transcribed gene products subsequently reset a transcription/translation feedback loop that generates the rhythm (Dunlap, 1999; Reppert and Weaver, 2001). The sequence of biochemical events and the circadian outputs that follow are thought to be regulated via the post-translational process of phosphorylation, which plays an important role in the control of core clock proteins (Lee et al., 2001). Three molecules, phosphorylated extracellular signal-regulated kinase (pERK), PER1 and cFOS, share a common anatomical co-localization within retinorecipient regions of the SCN, exhibit rapid induction by light during the subjective night, and potentially contribute to the coupling of photic input to behavioral rhythms.

pERK Represents Early Signal Transduction:

Extracellular signal-regulated kinase (ERK) appears to play an important role early in the signaling cascade mediating light-induced expression of the clock protein product, PER. The phosphorylated form of ERK, pERK, exhibits endogenous circadian oscillations in the SCN, rising during the day and declining at night in the shell-like outer region in both hamsters and mice (Obrietan et al., 1998; Coogan and Piggins, 2003; Lee et al., 2003; Agostino et al., 2004). Bright light at night also activates phosphorylation of ERK in the SCN of rodents (Obrietan et al., 1998; Coogan and Piggins, 2003), peaking 15 min after light onset (Butcher et al., 2003).

Moreover, the anatomical distribution of light-induced pERK activation within the SCN largely corresponds to light-induced *Per1* gene expression and consequently, it was speculated that this very proximal level of regulation could be responsible for the phase-dependent effects of light (Albrecht et al., 1997; Obrietan et al., 1998). In addition, the attenuation of light-induced phase shifts when ERK phosphorylation is disrupted (Butcher et al., 2002) as well as the absence of pERK expression in the shell of hamster SCNs after enucleation (Lee et al., 2003) both further support the hypothesis that pERK plays a role in the coupling of photic input to circadian phase resetting. Photic induction of pERK has not been examined across a range of irradiances, so it is not known whether the induction of this marker shows a dose dependent response. However, together, these studies suggest that the ERK signaling cascade serves to mediate biochemical events critical to the generation and photic regulation of circadian rhythms.

Changes in Per Allow for Resetting of the Translational-Transcriptional Feedback Loop:

PER1 is a core clock protein product thought to participate both in the generation of endogenous rhythms as well as photic resetting. *Per1* peaks in the SCN during the subjective day and nadirs during the subjective night (Tei et al., 1997; Takumi et al., 1998). In addition, light administered early in the subjective night (but not during the day) induces rapid increases in *mPer1* expression in the SCN (Shigeyoshi et al., 1997; Albrecht et al., 2001). Along with localization of *Per1* in

retinorecipient regions of the SCN, phase shifting kinetics of *mPer1* further implicate this molecule in the coupling of photic input to entrainment of behavioral rhythms. Specifically, the dose response and sensitivity for *mPer1* induction by light is quantitatively similar to photic resetting of activity rhythms (Shigeyoshi et al., 1997). Although tested under a narrow set of conditions, temporal description of *mPer1* reveals a peak induction 60 minutes after onset of the light pulse in mice (Shigeyoshi et al., 1997), which is relatively longer than that of pERK (Butcher et al., 2003). This is consistent with the notion that PER1 induction is occurring downstream of the phosphorylation of ERK. Finally, a study employing a luciferase reporter system indicates that ERK markedly increases *mPer1* gene promoter activity (Nomura et al., 2003). In sum, *Per1* is a part of the core clock machinery that also reflects the influence of light on the circadian system.

Photic Induction of cFOS Reflects the Light Sensitive Photophase:

The *cFos* gene is an immediate early gene that is activated by light during the subjective night, making it an excellent molecular marker for times at which light can phase shift the clock (Kornhauser et al., 1990). Similar to *Per1*, *cFos* is induced in areas of the SCN that receive light input, demonstrates an endogenous rhythm of expression (low at night, high during the day), and parallels behavioral phase shifting with its rapid induction by light (Kornhauser et al., 1990; Guido et al., 1999). Photic induction of *cFos* mRNA in Syrian hamsters peaks 30 minutes after initiation of the light stimulus (Kornhauser et al., 1990). Despite the aforementioned work, the

interpretation that *cFos* is, in fact, responsible for coupling photic input to behavioral rhythms is complicated: *cFos* induction fails to saturate at similar light intensities as phase resetting (Kornhauser et al., 1990), and *cFos* knockout mice are still able to entrain to light (Honrado et al., 1996). Data suggesting a more complex relationship were obtained in mice lacking the PACAP type 1 receptor, wherein behavioral phase shifts and *cFos* induction were disassociated from one another (Hannibal et al., 2001). Therefore, though its precise relationship to phase shifting by light remains unclear, *cFos* is a well-studied molecular marker of circadian function, and its induction by light is indicative of phases of photic responsiveness.

Effects of Photic History:

The circadian effects of light have been studied rather extensively at the behavioral, neural and molecular level; however, far less attention has been directed toward the influence of photic history. Several experiments have begun to elucidate the effects of previous lighting conditions on various circadian responses. Animal studies examining phase resetting by light have found that the magnitude of a phase shift is reduced after pre-exposure to a non-saturating stimulus (Nelson and Takahashi, 1999). Stability of entrainment in Syrian hamsters to light-dark (LD) cycles with a 22 h period also appears to depend on prior lighting conditions (Chiesa et al., 2006). Some of these results could be explained by strong early effects of light on the circadian system leading to consequent dampening due to light adaptation, as proposed by Comas et al (2006) in their methodical examination of phase sensitivity to

various duration light pulses. In contrast to experiments that control for previous light exposure, studies that instead extend duration of darkness prior to administration of a light pulse demonstrate increased light sensitivity of neurons in the SCN (Aggelopelous and Meissel, 2000). Similarly, phase-shifts in rodents are enhanced after many days in continuous darkness (Shimomura and Menaker, 1994; Refinetti, 2003). This extended period is well beyond any known photoreceptor adaptation time frame and thus, suggests prior state of entrainment can also alter photic response.

Photoperiod Influences Various Biological Functions:

An ecologically relevant example of the effects of light history on the circadian system includes the marked influence of prior photoperiod. Photoperiod is the duration of light within a 24-hour cycle, and it provides an important signal for the seasonal timing of a variety of physiological and behavioral processes. In photoperiodic species such as hamsters, reproductive functions, body weight and pelage are all regulated by seasonal changes in day length. Laboratories simulate summer days via photoperiods of ≥14 hours, and virtual winter is achieved with ≤ 10 hours of light.

Seasonal changes in photoperiod lead to changing durations of light responsiveness as reflected in the waveform (i.e. shape) of various circadian rhythms. For example, relatively greater durations of nocturnal activity and melatonin secretion have been well documented under the longer nights of winter as compared with summer-like photoperiods across a variety of species (Pittendrigh and Daan, 1976; Binkley et al., 1977; Rollag et al., 1980; Goldman and Elliott, 1988; Bartness et al.,

1993; Lerchl and Schlatt, 1993; Wehr et al., 1993; Elliott and Tamarkin, 1994). Light-induced phase shifts of rhythms also occur across a broader range of the circadian cycle and with increased amplitude in hamsters previously entrained to a short photoperiod (Milette and Turek, 1986; Puchalski and Lynch, 1988; Goldman and Elliott, 1988). Specifically, hamsters with a short photoperiod history show an approximately two-fold greater maximal phase shift by bright light as compared to animals under longer days (Goldman and Elliott, 1988; Evans et al., 2004). Importantly, photoperiod changes in the rhythm's τ and waveform persist for many days after release into constant dark conditions, indicating alterations in the state of entrainment rather than an effect of immediate light history (Pittendrigh and Daan, 1976; Goldman and Elliott, 1988).

Limited work suggests that prior photoperiod may also exert an influence on the circadian system of humans, albeit the photocycle was not explicitly controlled or monitored. One of the earliest experiments to examine the influence of photic history in humans found increased melatonin suppression by light in winter versus summer in a small population of men in Antarctica (Owen and Arendt, 1992). In addition, altering sleep duration between 6 or 9 hours elicits variable light-induced phase shifts, with 9-hour sleep times resulting in a significantly greater response (Burgess and Eastman, 2006).

The Interval in Which Light Can Induce PER1 and cFOS is Altered by Photoperiod:

In both hamsters and rats, the interval of photic induction of *Per1* is lengthened under short versus long photoperiods (Tournier et al., 2003; Sumova et al., 2003). While this pattern is similar to that of increased duration of melatonin secretion with longer nights, *Per1* does not appear to be regulated by acute or chronic melatonin, and pinealectomy does not affect the *Per1* rhythm in the SCN of Syrian hamsters (Messager et al., 1999, 2001). Additional evidence that the circadian clock is able to incorporate and respond to variations in photoperiod comes from studies employing light induction of *cFos* in the SCN wherein the duration of the photosensitive phase is also dependent on photoperiodic history (Sumova et al., 1995; Tournier et al., 2003; Sumova et al., 2003). To date, studies of photoperiodic regulation of circadian rhythms at the cellular level have focused primarily on the interval of induction of *Per* and *cFos* by bright light; however, comparisons of photic *sensitivity* via examination of induction by a range of sub-saturating light pulses have not yet been done.

Effects of Dim Light:

The influence of dim light provides another robust example of the significant role of photic history on circadian responses. The term ‘dim light,’ has been used to describe light stimuli that have been historically ineffective in eliciting a circadian response or even less appropriately, based on visual perceptual quality. Early studies did not show direct effects of dim light on many tested circadian responses (Brainard et al, 1982, 1984; Nelson and Takahashi, 1991a, 1991b); however, growing evidence suggests that dim light of similar intensity does indeed exert an influence on the

circadian system. Circadian entrainment in hamsters shows sensitivity to dim green light scotophases (<0.01 lux) under a variety of tests, with the following findings: elevated levels of wheel-running activity (Gorman et al, 2003), facilitated split patterns of entrainment (Gorman et al., 2003; Evans et al., 2005), enhanced entrainment to lengthening T cycles (i.e. periods >24 hours) (Gorman et al, 2005), accelerated re-entrainment in a simulated jet lag paradigm (Evans et al., 2009; Frank et al., 2010) and more rapid re-entrainment to short photoperiods (Gorman and Elliot, 2004). It has been proposed that these myriad examples of dim light influences are a consequence of altered coupling of circadian oscillators in the SCN (Evans et al., 2005, 2009).

Summary:

Light regulates a variety of circadian responses via mechanisms that have been fairly well described. However, the modulation of sensitivity to light via prior photic exposure remains an important but less understood phenomenon. Seasonal changes in photoperiod represent an ecologically relevant light history that has a significant impact on the circadian system. This collection of projects provides the first rigorous characterization of photic sensitivity for circadian responses in hamsters previously maintained under long versus short days. In addition, multiple levels of regulation are examined, including behavioral, neuroendocrine and molecular, in order to elucidate the mechanisms by which photoperiod history influences circadian response to light.

Specifically, these studies aim to answer the following questions:

1) Does photoperiod alter sensitivity to light for circadian phase resetting?

- And if so, does photoperiod modulation of light sensitivity depend of circadian phase?

2) Does light sampling behavior vary as a function of photoperiod, irradiance and/or circadian phase?

3) Does photoperiod modulation of light sensitivity occur at the level of the SCN?

- And if so, where specifically along the signal transduction cascade does it take place?

4) Does photoperiod history alter photic sensitivity for light-induced melatonin suppression?

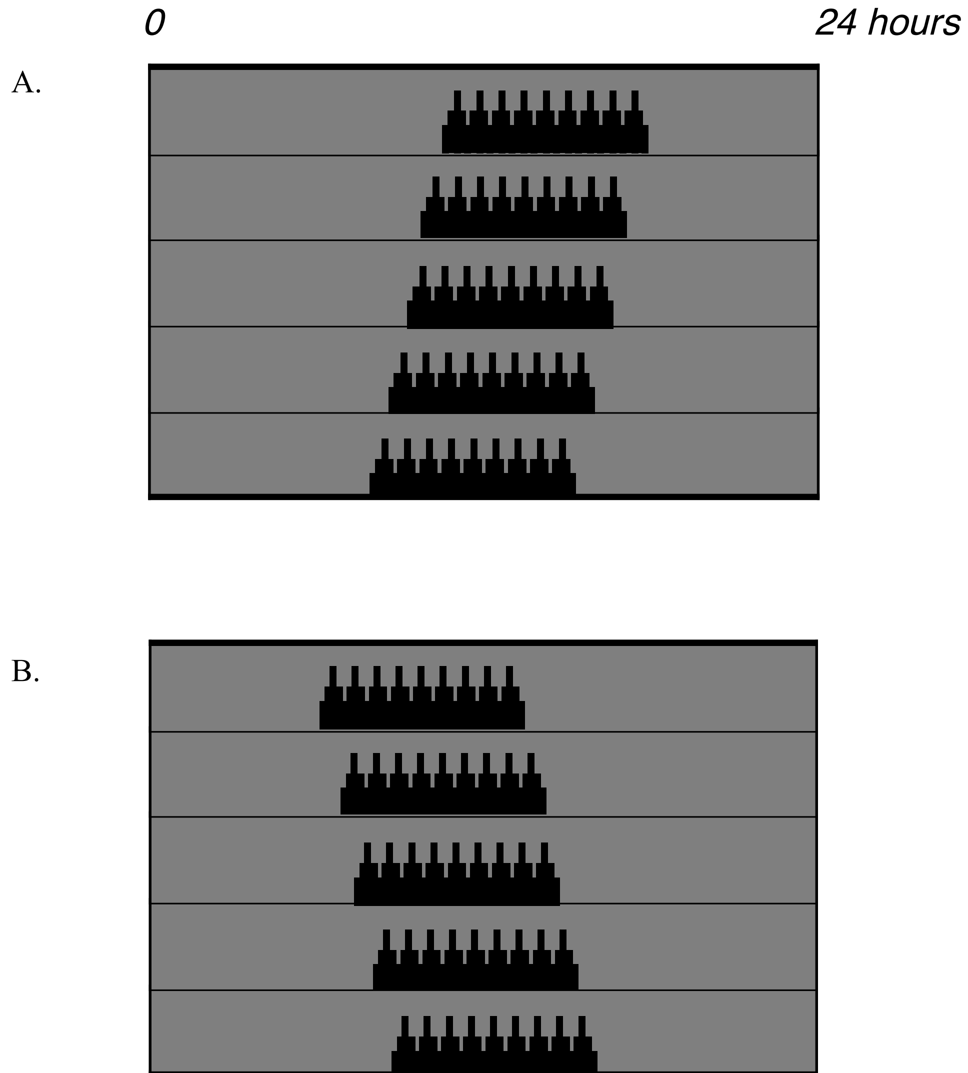

Figure 1.1. Free running period (τ) in A) nocturnal and B) diurnal animals. Each line represents a 24 hour day, with 5 days illustrated for each example. Grey bars indicate the animal is in constant darkness. Black patterns represent activity.

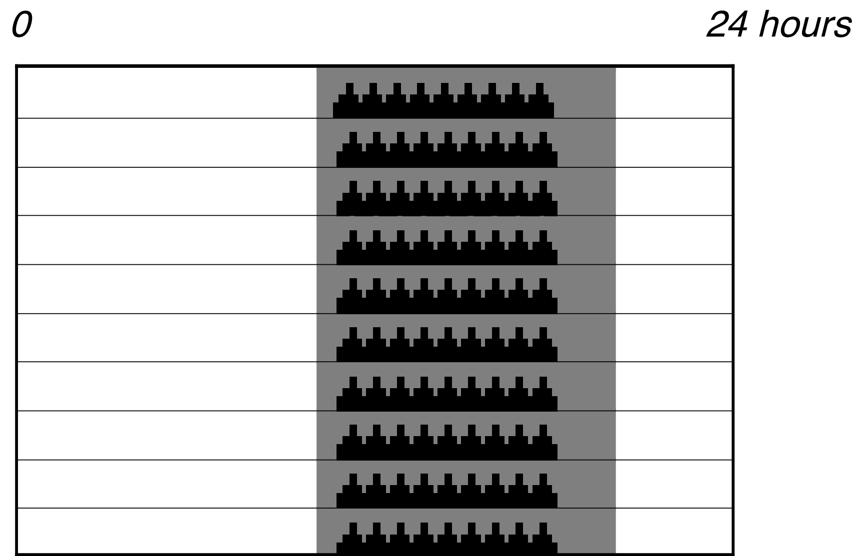

Figure 1.2. Entrainment of a nocturnal rodent to LD14:10. Each line represents a 24 hour day. White bars represent periods of light (photophase), and gray bars represent periods of darkness (scotophase). Black patterns illustrate activity.

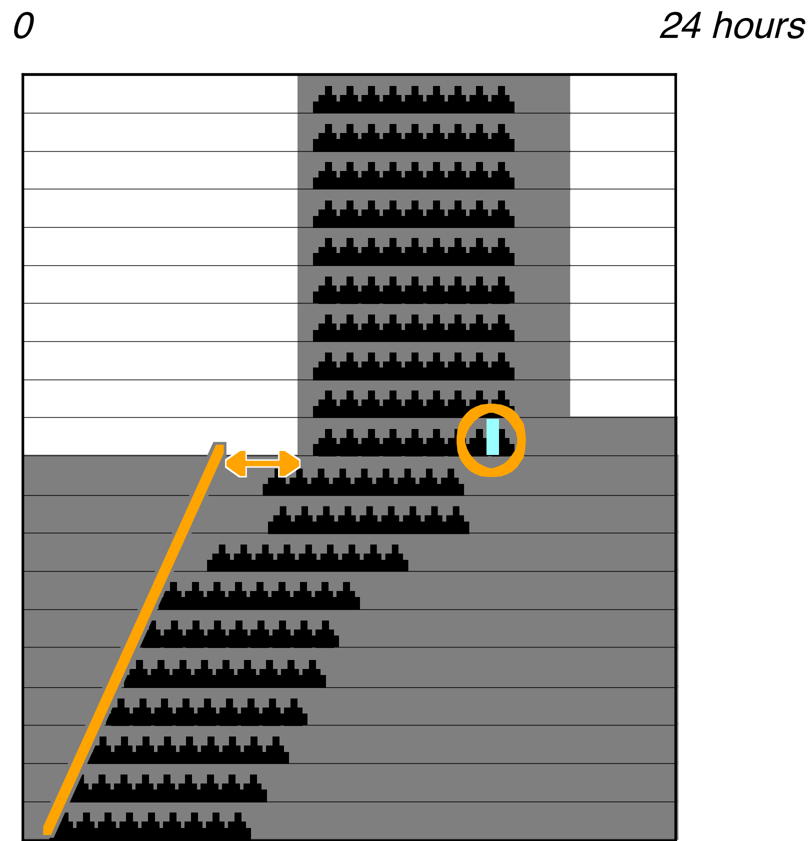

Figure 1.3. Light-induced phase advance of activity rhythms in a nocturnal rodent. Each line represents a 24 hour day. White bars indicate periods of light, and grey bars indicate periods of darkness. Black patterns represent patterns of activity. The circled square represents the light pulse, and the arrow spans the amount of time in which the activity rhythm has shifted. Calculations of phase shift magnitude typically include subtracting the activity onset on the day after the light pulse from the onset in the day(s) of and/or before the pulse. When measuring phase advances, in particular, the first 3 days following a pulse are usually eliminated in these calculations due to transient effects. Therefore, a regression line for the following several days is created to estimate the new phase on the day after the pulse, as indicated by the orange line.

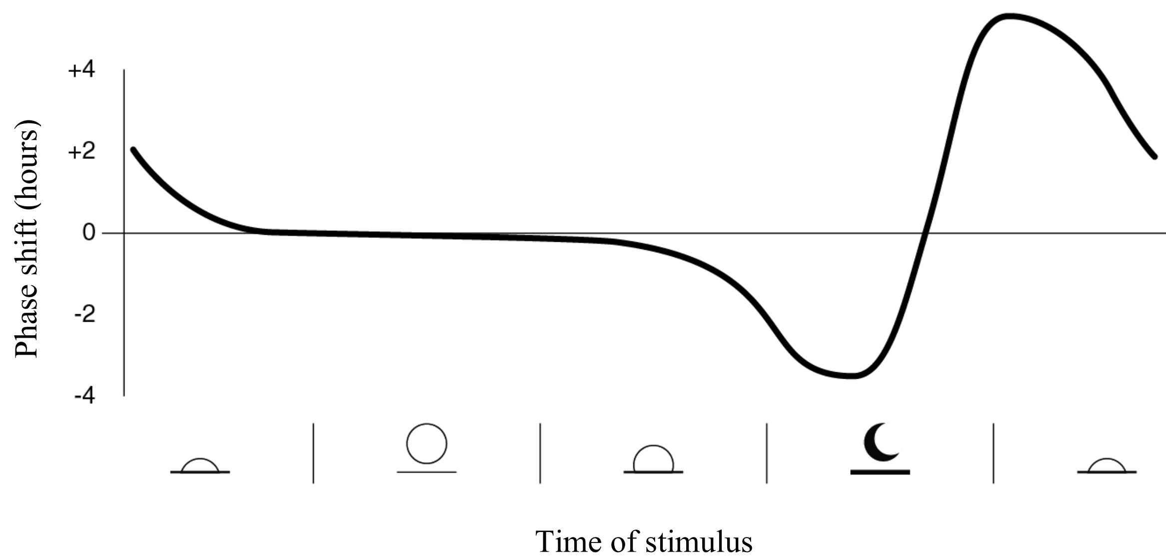

Figure 1.4. Phase response curve. This illustration describes the relationship between phase shift magnitude and circadian phase. Light does not cause a phase shift during the subjective day, which is often referred to as the "dead zone." However, significant shifts are obtained with light during the subjective night, with systematic changes in magnitude and direction. Specifically, light administered earlier in the night produces a phase delay to a later time (indicated by a negative phase shift) whereas light later in the night causes a phase advance (indicated by a positive phase shift).

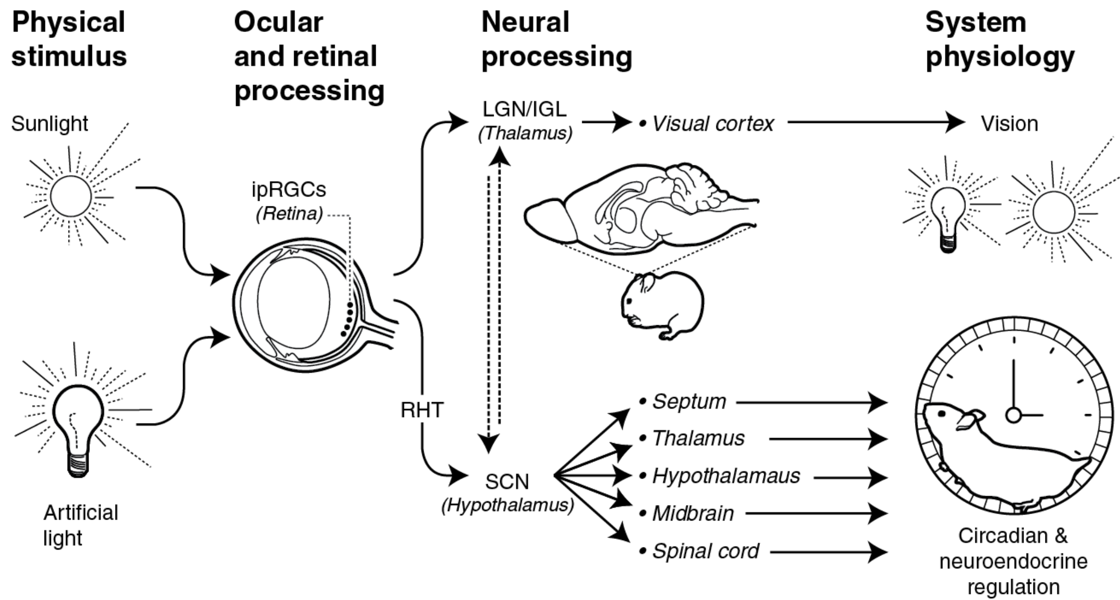

Figure 1.5. Diagram of the neural pathway by which light regulates circadian rhythms in mammals. Light enters the eye and is transduced by intrinsically photosensitive ganglion cells in the retina (ipRGCs). Photic information is conveyed to the suprachiasmatic nucleus (SCN) in the hypothalamus via the retinohypothalmic tract (RHT). The SCN then projects to other regions in the brain to regulate circadian and neuroendocrine functions.

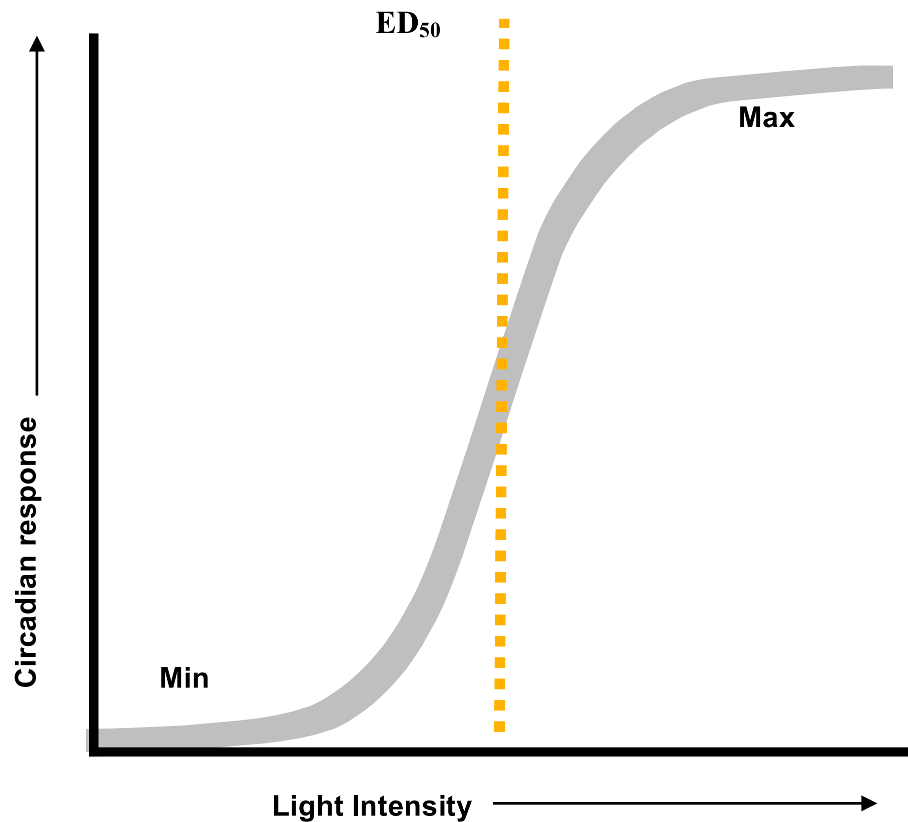

Figure 1.6 Dose response function. This figure illustrates the relationship between circadian response and light intensity. It follows a sigmoid function, with a particular threshold sensitivity, as a certain amount of light is necessary to obtain a measurable outcome. There is also a saturation point (Max) wherein an increase in intensity will not cause a greater response. Finally, the ED_{50} is denoted via the dotted orange line and represents the intensity required to elicit a response halfway between the Min and Max, enabling a point of comparison between fluence response curves. As an example, a lower ED_{50} would indicate a relative increased sensitivity.

CHAPTER 2. INCREASED PHOTIC SENSITIVITY FOR CIRCADIAN PHASE RESETTING UNDER SHORTER DAYS

INTRODUCTION

Hamsters entrained to short photoperiods show an approximately two-fold greater maximal phase shift to a bright white light pulse as compared to animals with long photoperiodic histories (Goldman and Elliott, 1988). More recently, our lab found a similar difference in phase shifts elicited by bright light pulses at two different regions of the PRC (Evans et al., 2004). Although a difference in phase shift magnitude has been observed with bright white light, there have been no studies testing for differences in *sensitivity* based on response to sub-saturating light pulses. The two sets of experiments described in this chapter aim to test the hypothesis that photoperiod alters photic sensitivity for circadian phase resetting of activity rhythms.

In the first experiment, complete fluence-response curves were constructed for short wavelength light-induced phase advances in Syrian hamsters with short versus long photoperiodic histories. Here we tested for photoperiod differences in sensitivity to light for phase resetting, as defined by the ED_{50}. The results from this initial study, however, may be limited to phase advances resulting from light administered late in the subjective night. In order to determine if photoperiod dependent changes in sensitivity to light vary by circadian phase, we also test phase resetting in response to subsaturating light in the phase delay portion of the PRC. In addition, this study of phase shifting in response to light earlier in the subjective night serves to inform our

later described molecular work, for which time course information for photic induction of proposed markers had already been quantified at a similar phase.

Possible Outcomes

Phase Advances:

We anticipated a characteristic sigmoid fluence-response function for each condition in the phase advance experiment. Examining full dose-response curves relative to one another allows for comparisons of threshold sensitivity, half saturation and maximal response magnitude as well as the rate of increase (i.e. the slope of the steep portion of the curve), each representing theoretically separable dimensions of a given response. Enhanced maximal phase shifts for short versus long photoperiod cohorts were expected based on prior work (Goldman and Elliott, 1988; Evans et al., 2004); however, to our knowledge, other parameters had not been previously studied as a function of photoperiod.

Possible response dynamics for long and short photoperiod histories are diagrammed in Figure 2.1. For example, it was possible that short photoperiods would result in not just an increased maximal phase shift, but also higher sensitivity (i.e. lower half-saturation constant/ED_{50}, Figure 2.1.B). Alternatively, the slope as well as the sensitivity may be shared for both long and short photoperiod conditions, even though the maximal phase shift is greater in the short photoperiod cohort (Figure 2.1.A). Finally, other scenarios could exist wherein the short and long photoperiod conditions have different slopes and/or unexpected maximal responses (Figure 2.1.D).

Even more unlikely, but still theoretically possible, is that animals with long photoperiod histories show an increased sensitivity, with an enhanced maximal response in the short photoperiod condition (Figure 2.1.C).

Phase Delays:

If the dynamics for phase advances do not generalize to a delay condition, this would suggest photoperiod effects on a given parameter may be limited to a particular circadian phase. Prior work has shown that sensitivity, as based on the ED_{50}, does not vary by phase (Nelson and Takahashi, 1991); however, the ED_{50} has not been compared across phases in animals with different photoperiod histories. In contrast, confirming similar photoperiod influences for phase advances and delays would suggest an effect on resetting of rhythms that is independent of circadian phase. In addition, the phase delay protocol, with pulses administered two hours after release into darkness for both photoperiod conditions, allows us to determine whether dark adaptation plays a role in sensitivity differences for phase advances. Finally, testing photoperiod influence on response to light at this time, earlier in the subjective night, serves to inform our design and interpretation of results for the photoperiod studies to follow.

METHODS

Male Syrian hamsters (Harlan Sprague Dawley, Inc., Indianapolis, IN), 3-4 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 opaque white cages (27 cm X 20 cm X 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 ½ peak bandwidth;7.9 x 10^{-6} $\mu W/cm^2$). 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 ½ 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 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 $\mu W/cm^2$ and converted to photon density ($photons/cm^2/sec$) 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 2.2). This method includes a period of re-entrainment between each test pulse as well as an extended period of time under constant conditions immediately preceding, and in the days following, administration of light. The administration of test pulses is timed according to the environmental light-dark cycle. This paradigm was chosen instead of an Aschoff Type I (wherein multiple light pulses are administered under constant conditions without an intervening period of re-entrainment and are timed relative to each individual animal's activity rhythm) to ensure consistent prior entrainment status as well as to standardize the 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 re-entrained to their original photoperiod for an additional 10 days. Cages were changed on day 1 of re-entrainment 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, but animals are 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 x 36 cm x 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, 68.03 $\mu W/cm^2$. 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 the aforementioned parametric model.

Photoperiodic Modulation of Phase Delays:

In order to test the generality of phase-advance results to a phase-delay condition, a separate sample of animals was entrained to LP (n = 11) or SP (n = 11) in

two separate large ventilated, light-tight, matte white interior chambers. Light pulses were administered 2 hours 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 one-hour phase advance under SP and LP, as determined by the results of the phase advance study (i.e., 0.14 uW/cm^2 or 3.38016 x 10^{11} $photons/cm^2$ sec and 3.5 uW/cm^2 or 8.4504 x 10^{12} $photons/cm^2/sec$, respectively; see Figure 2.3) 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 two 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 ANOVA.

Statistical Analyses:

SPSS was used to perform ANOVA and post-hoc tests (Version 13.0.0). GraphPad Prism software (Version 5; San Diego, CA) was utilized 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.

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 two progressively increasing irradiances of 0.003 and 0.03 $\mu W/cm^2$ (Figure 2.2). All irradiances $\geq$ 0.25 $\mu W/cm^2$ elicited phase advances that were significantly greater than those under 0.03 $\mu W/cm^2$ ($p<0.01$), and 68 $\mu W/cm^2$ induced larger phase advances than all other irradiances ($p<0.0001$). SP animals showed relatively larger light-induced phase-shifts as compared to LP animals at all irradiances ≥ 0.03 $\mu W/cm^2$, although comparisons at individual irradiances showed a statistical difference in phase advances only at 0.25, 1.31 and 68 $\mu W/cm^2$ ($p<0.05$). The phase advance data were well fit by four-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 2.3).

LP vs SP for Phase Delays:

Phase delays 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. Phase shifting also 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 ($p<0.001$). In addition, LP response to the lower irradiance condition was not different from 0, whereas all other conditions produced significant phase delays ($p<0.05$). The two irradiances that were calculated to elicit comparable 1 h advances in LP and SP (3.5 $\mu W/cm^2$ and 0.14 $\mu W/cm^2$, respectively) produced phase delays that did not differ significantly between conditions. Indeed, the values were very closely matched (mean shift ± SE for LP-3.5 $\mu W/cm^2$ = -0.28 ± 0.11 and SP-0.14 $\mu W/cm^2$ = -0.36 ± 0.11; see Figure 2.4).

DISCUSSION

This is the first study to demonstrate that the measured sensitivity to light is increased 1.4 log units under a short photoperiod. In addition, the sensitivity to light for shifting of activity rhythms appears to be maintained regardless of circadian phase.

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 phase advancing light pulses under short and long photoperiods were selected based on roughly

comparable proportions of the subjective night, they cannot be considered to represent precisely identical phases. However, Nelson and Takahashi (1991) found no evidence for variation in ED_{50} with circadian phase. Furthermore, the lower threshold for phase shifting (delays) 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., PRCs). 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; Evans et al., 2004). 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 non-mammalian 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 known 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.

In conclusion, photic history potently alters subsequent circadian response to light. This is the first account of photoperiod influence on photic sensitivity, refining our understanding of how seasonal changes in photoperiod modulate circadian response. Specifically, prior exposure to short photoperiods substantially increases sensitivity to light for circadian phase resetting.

Acknowledgements: Portions of this chapter appear in Photic sensitivity for circadian response to light varies with photoperiod in Journal of Biological Rhythms 2012. Glickman, Gena; Webb, Ian; Elliott, Jeff; Baltazar, Ricardo; Rheale, Meghan; Lehman, Michael; Gorman, Michael.

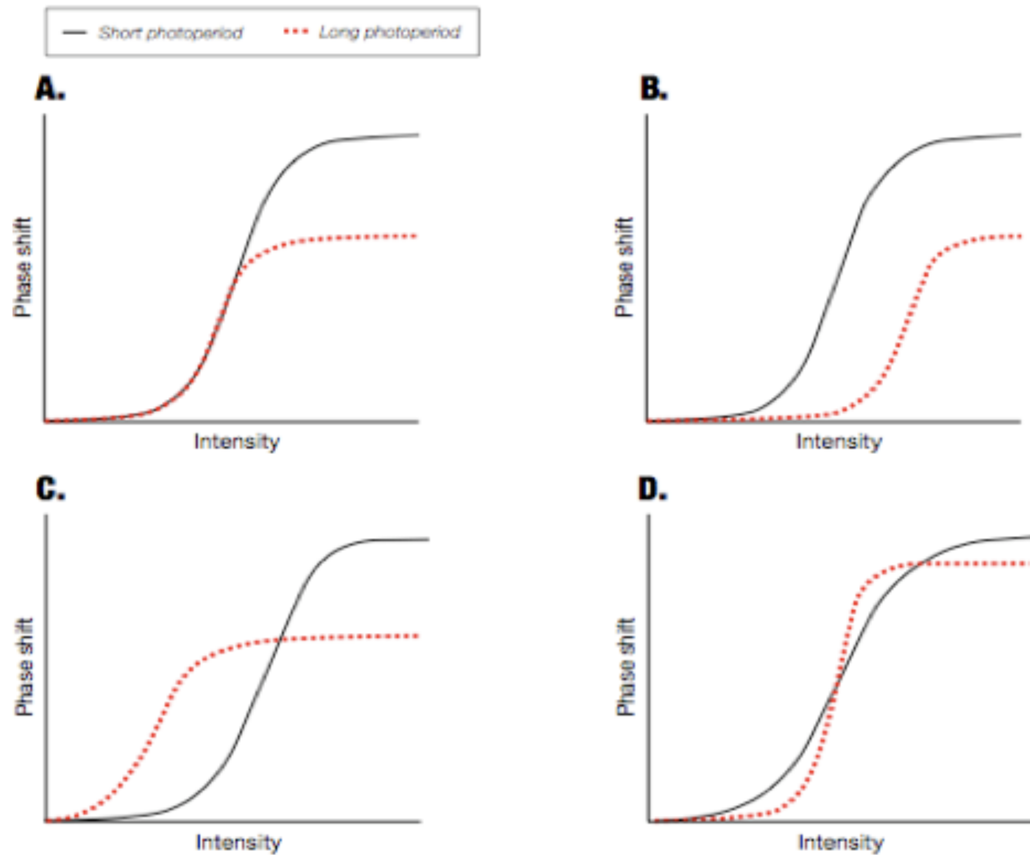

Figure 2.1. Graphical representations of the possible outcomes for the phase advance experiment. Each figure shows the different relationships between fluence-response curves for phase shifting, illustrating how various parameters of interest (e.g. ED50, maximum dose, etc.) represent separable dimensions of a response**.** Black curves represent short photoperiod (SP), and red curves represent long photoperiod (LP) conditions.

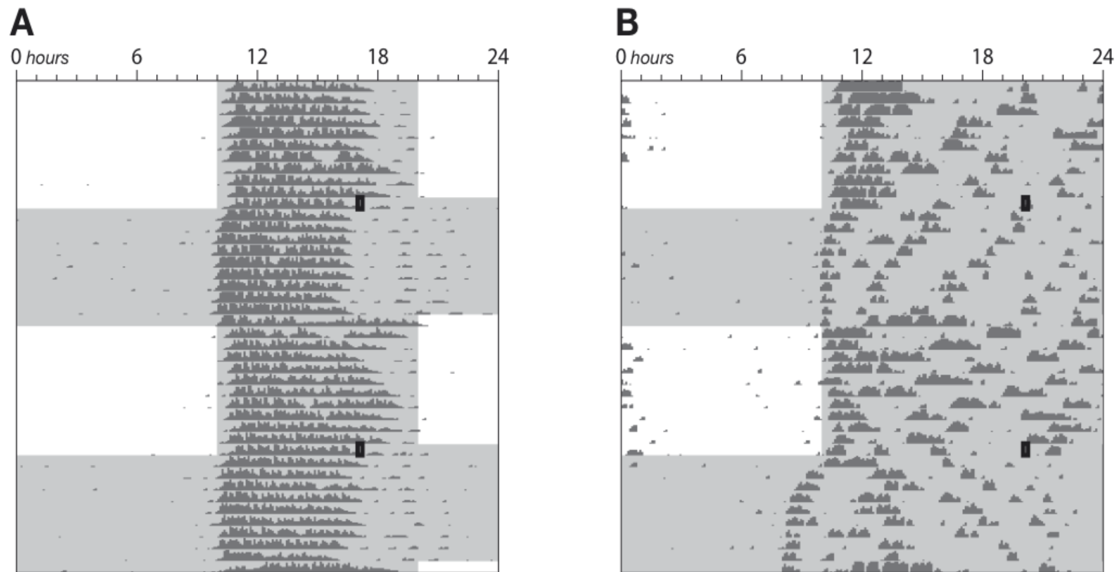

Figure 2.2. Representative actograms for phase advance study. Activity rhythms for animals under A) long photoperiod (LP) and B) short photoperiod (SP) at progressively increasing irradiances of 0.003 and 0.03 µW/cm2. Each line represents a 24-h day. Light gray areas represent dim light, white areas signify bright fluorescent white light, and the 2 black blocks each represent a 15-min short wavelength light pulse. Dark gray patterns show wheel-running activity.

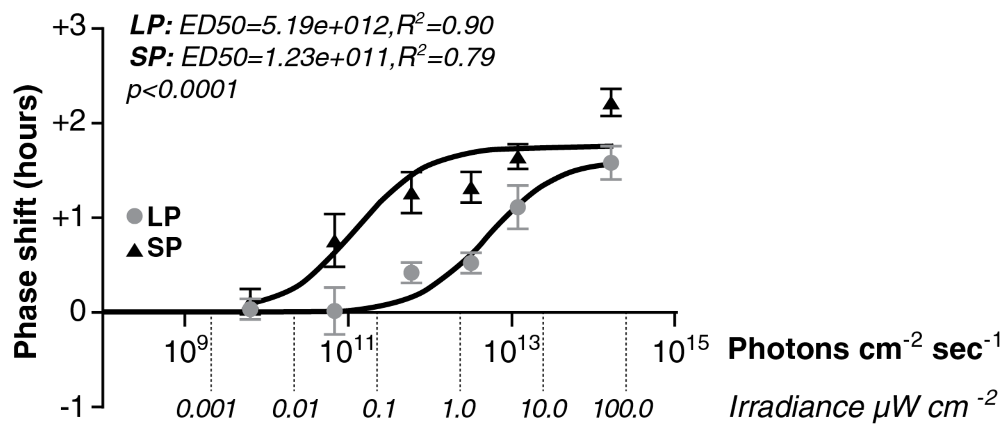

Figure 2.3. Fluence-response curves for phase advances. Phase shifts (in hours) for animals previously entrained to long photoperiod (LP; gray circles) or short photoperiod (SP; black triangles). SP animals are 40-fold more sensitive to light than LP, as indicated by the lower ED_{50} ($p < 0.0001$).

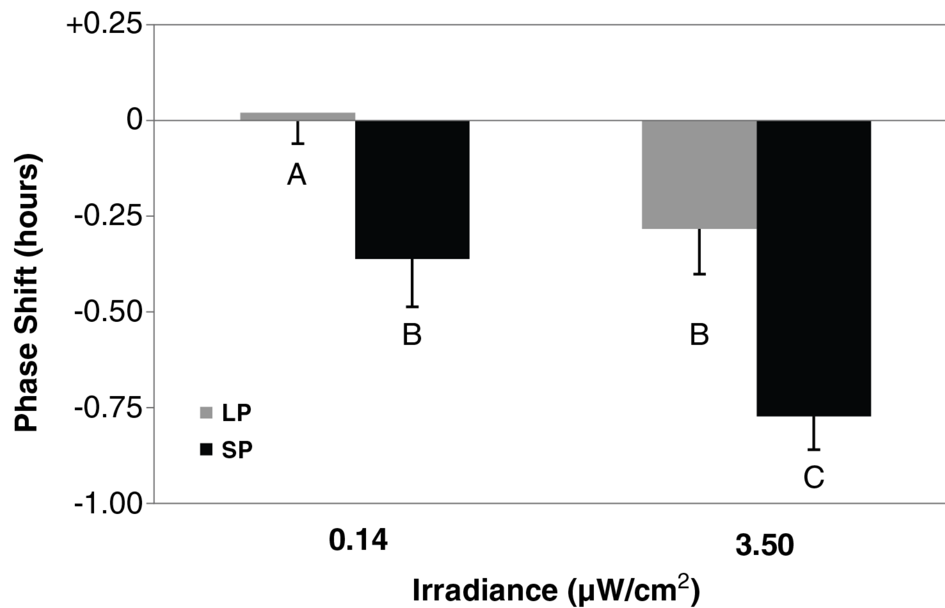

Figure 2.4. Photoperiod Effects on Phase Delays. Mean ± SE phase delay data for long photoperiod (LP; gray) versus short photoperiod (SP; black) (n = 11 per condition). Phase-shift scores were expressed relative to each animal's phase shift on the dark control night. Different letters illustrate statistically significant mean differences ($p < 0.05$).

CHAPTER 3. LIGHT SAMPLING: IMPACT OF PHOTOPERIOD HISTORY, IRRADIANCE AND CIRCADIAN PHASE

INTRODUCTION

Studies of circadian response to light often measure the effects of photic exposure under highly artificial conditions. For example, laboratory studies typically maintain completely dark nights except for the duration of the experimental light pulse; light intensity of the photophase and pulsing stimuli are held constant; narrowband or monochromatic light may be employed to control for the effects of different wavelengths; and the precise timing of light exposure can be selected to maximize circadian response. The amount of light received by the eyes is sometimes further standardized via transfer to a separate pulsing chamber, restraint for the duration of a test pulse and/or pharmacological pupil dilation (e.g. Takahashi et al., 1984; Nelson and Takahashi, 1991a, 1991b, 1999; Brainard et al., 2001; Glickman et al., 2003). Though these methods serve to optimize the amount of light hitting the retina, they may elicit stress and/or provide cues that serve to alter the circadian system independent of the light itself. Indeed, light is the primary environmental signal for entrainment, but the mammalian clock may also be altered by non-photic cues (e.g. handling) and those cues may further interact with light stimuli (Mrosovsky and Salmon, 1987, 1990; Mrosovsky, 1991; Joy and Turek, 1992). In addition, such highly controlled exposures represent an environment quite different from the varied patterns of illumination encountered under more naturalistic conditions, making

extrapolation of study results to real world applications difficult.

Recent work has shown that at least one of these methods, pharmacological pupil dilation, may be an unnecessary control for most studies of circadian response to light. Early work in humans found an influence of pupillary response on light-induced melatonin suppression (Gaddy et al., 1993), which is consistent with later evidence of a common photoreceptor system mediating light for both functions (Brainard et al., 2001; Thapan et al., 2001; Lucas et al., 2002). More recently, however, phase shifting in Syrian hamsters was shown to reach saturation at intensities lower than the threshold required for pupillary constriction, indicating minimal overlap (if any) in the dynamic range of response (Hut et al., 2008). Since melatonin suppression achieves saturation at relatively lower irradiances than phase shifting (Nelson and Takahashi, 1991a), it is also unlikely to be affected by pupillary response in this species. A number of studies (including our own) have employed a more ecologically relevant paradigm for examination of photobiological function, where the eyes are freely constricting and animals may continue to roam in their home cage during administration of a light pulse. It remains unknown how the freedom to move within the cage environment influences photic exposure. Certainly, previous work has shown that the position of a light source relative to the retina can have a significant impact on the magnitude of melatonin suppression and phase shifting responses in humans (Lasko et al., 1999; Visser et al., 1999; Glickman et al., 2003; Rueger et al., 2005).

The overall amount of light received by the eyes will depend on light sampling behavior, in general. The term “light sampling” is used here merely to indicate

changes in position of the animal relative to the light source, without any inferences made regarding the basis for this behavior. Light sampling patterns may be due to relative levels of comfort, engagement in particular activities (e.g. grooming, feeding, etc.) or any number of additional reasons. There is a large literature describing light aversion behaviors, wherein both rats and mice tend to move away from bright light towards relatively dimmer areas when possible (Crozier and Pincus, 1927; Keller, 1941; Welker, 1959; Crawley and Goodwin, 1980; Misslin et al., 1989; Mrosovsky and Hampton, 1997); however, studies documenting this behavior in a hamster model were not found.

To the contrary, though precise quantification is not reported, Nelson and Takahashi note that Syrian hamsters keep their eyes opened and directed up at the light source when experimental control of eyelids and gaze behavior are not implemented (1991a). These animals were, however, removed from their home cage and restrained for the duration of the pulse within a separate cylindrical chamber. In these studies, a dose response curve for phase shifting by 5 minute pulses of 503 nm light was constructed for animals that were previously entrained to LD14:10 (Nelson and Takahashi, 1991a). Sensitivity of the response as defined by the ED_{50} was found to be $3.1x10^{11}$ photons/cm^2/sec, which is considerably more sensitive than photic phase advances for our studies under identical photoperiod conditions in this species ($5.19x10^{12}$ photons/cm^2/sec, see Chapter 2). It is worth noting, however, that the light-induced phase shifts they obtained under longer days still show lower sensitivity as compared to our short photoperiod animals (ED_{50} =$1.23x10^{11}$ photons/cm^2/sec). The

characteristics of pulsing stimuli employed are not likely to explain the absolute sensitivity differences across studies and if anything, greater sensitivity would be predicted for our studies, which used longer duration 15-minute pulses of 480 nm light. Methodological differences in the pulsing environment, however, may be a factor. Having an estimate of the percent of light to which animals are exposed when unrestrained within a typical cage environment may be useful for making comparisons with study results using alternative methods, where exposures are optimized and expected to result in close to a full dose of light.

Although our studies obtained a reasonably homogenous exposure within the home cage via the geometry of setup in combination with the use of diffusers, better understanding light sampling behaviors will serve to clarify the relatively greater phase shifts under short photoperiods as well as the ecological relevance of those findings. Hamsters show a forty-fold increase in sensitivity to equal photon doses of light when under a short versus a long photoperiod (Glickman et al., 2012). This robust effect may be reduced or even completely eliminated under more naturalistic conditions if animals also receive less photic input in the winter due to differences in sampling behaviors. Such a scenario might suggest a biological need for enhanced photic sensitivity in order to maintain entrainment with less environmental light during winter months. Alternatively, if short day animals receive more light than those under longer days due to photoperiod differences in sampling behaviors, this could potentially contribute to our findings and/or serve to further potentiate the already heightened response found for phase resetting under short photoperiods. Since

irradiance and phase were also manipulated in our phase shifting studies, it is important to know if those factors influence light sampling behavior as well.

Limited previous work has formally probed light sampling behaviors of *Glaucomys volans* (flying squirrels) as well as Syrian hamsters within the context of circadian rhythms in an effort to obtain more ecologically valid understandings of photic entrainment (DeCoursey, 1986; Pratt and Goldman, 1986). Those studies focused on sampling of the photophase of a light-dark cycle rather than sampling of a shorter discrete light pulse administered during the nighttime scotophase, such as those employed experimentally to elicit phase shifts or melatonin suppression. This is the first known account of light sampling behavior during a discrete light pulse presented in the subjective night. We aimed to test the hypothesis that light sampling of a nighttime test pulse varies as a function of photoperiod, irradiance and/or circadian phase. Further understanding light sampling will provide practical insights into the ways in which volitional behaviors shape the ability of light to influence the circadian system.

Possible Outcomes:

Any differences in light sampling as a function of photoperiod, phase or irradiance would need to be considered within the context of previous reported results and future study design. Though our past study setup optimized photic exposure so that the amount of light entering the eye was relatively equivalent regardless of location within the cage, some variation was inevitable. In the event that sampling

varies systematically according to any of the variables being tested, studies to follow may need to account for such factors and potentially, incorporate appropriate modifications to planned methods. For example, if the actual amount of light received differs greatly according to photoperiod, future planned work of photoperiod influences on light response may benefit from restraining animals during test pulses to eliminate light sampling as a potential confound. If no differences in light sampling exist, the observed photic responses in our other experiments (with a more highly controlled exposure setup) are not likely due to the amount of light received and instead, confirm physiological differences in photic sensitivity. It would also suggest that restraint of animals during studies of circadian response to light is not necessary, at least when the variables of interest include photoperiod history, circadian phase and/or irradiance.

METHODS

Male Syrian hamsters were entrained to LD14:10 (LP) or LD10:14 (SP) for 6 weeks while individually housed in clear polypropylene cages (28 cm x 19 cm x 18 cm) (n=16 total, n=8 per photoperiod condition) located in ventilated, light-tight, matte white interior cabinets (43 cm x 36 cm x 46 cm). 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 cabinet (560 nm, 23 nm ½ peak bandwidth; 7.9×10^{-6} $\mu W/cm^2$).

Animals in each photoperiod group received 15 minute pulses of light on 4 different nights, each separated by one week. Immediately preceding exposure to test pulses, animals were transferred to a cage that was marked to delineate six separate sections of equal area, with each section corresponding to a specific irradiance level.

Two different 480 nm light sources were employed. Test pulses consisted of a 480 nm (23 nm ½ peak bandwidth) 8-LED or 24-LED lamp positioned atop the cage lid on the side furthest from the water bottle. The 8-LED lamp was further modified with neutral density filters. Spectral power distributions and half-peak bandwidth of the LED lamps were determined via an Ocean Optics spectral radiometer (model USB2000, Dunedin, FL). Irradiances were measured with an IL1700 radiometer (International Light, Inc., Newburyport, MA) with the sensor head positioned at the center of each of 6 sections in the cage. Measures were made in terms of $\mu W/cm^2$ and converted to photon density based on the energy per photon for 480 nm.

Large gradations in light levels across the six cage regions were obtained (see Figure 3.1). Light pulses were administered at two different irradiance conditions during prescribed zeitgeber times in the animal's scotophase (ZT19 and ZT22 for animals entrained to LP and SP, respectively; ZT14 for both LP and SP conditions, where ZT12= lights off). One irradiance condition approximated a range of intensities containing levels of threshold sensitivity for SP but not LP phase shifts (subsaturating, SUB). The other irradiance condition included a relatively brighter range of intensities potent enough to elicit significant phase shifts under both LP and SP and further, which reflected a saturating dose in half of the cage environment (saturating, SAT).

Animals were digitally recorded for the duration of each light pulse and returned to their individual home cage within assigned entrainment conditions until the next test. After collecting recordings for each animal in all four conditions, video footage was uploaded and formatted for scoring with J-Watcher software (Version 1.0, Blumstein et al., 2010). Each individual recording was scored according to the amount of time the animal's eyes were located within each of the six cage sections. A precise estimate of total light exposure for each animal was determined via integrating the duration of time spent within each section with respective photon doses. All scoring was performed by the same researcher, who was blind to the conditions being tested until the end of the study.

Mean exposure levels were calculated for each of the eight different conditions (LP-SUB and LP-SAT at ZT14 and ZT19; SP-SUB and SP-SAT at ZT14 and ZT22). Those levels were then expressed as a proportion of the total maximal dose possible, as determined by dividing the total light exposure for a given condition by the maximal possible light exposure for that particular irradiance (if the animal remained in the brightest section of the cage for the entire 15 minute pulsing duration). ANOVA was used to determine differences in mean proportion of total maximal photons between photoperiod groups as well as changes with irradiance and circadian phase (repeated measures). Statistical differences between groups were considered significant if $p<0.05$.

RESULTS

There is a trend for a greater proportion of maximal photon exposure under

long versus short photoperiods ($F_{1,12}$=1.986, p=0.059), but no significant phase differences ($F_{1,12}$=0.083, p>0.7), during a sub-saturating light pulse (see Figures 3.2 and 3.3). There are also no statistical differences in the proportion of the maximal photons received as a function of circadian phase ($F_{1,14}$=3.04, p>0.1) or photoperiod ($F_{1,14}$=0.658, p>0.4) for the SAT condition (Figures 3.4 and 3.5). Light sampling is, however, differentially affected by irradiance, with approximately 1/2 (48%) and 1/3 (32%) of the maximal available photons received when pulsed with sub-saturating and saturating light pulses, respectively ($F_{1,13}$=88.12, p<0.0001) (Figure 3.6).

DISCUSSION

This is the first study to characterize the amount of light to which animals are exposed when unrestrained within a typical cage environment and administered discreet pulses of light during the night. In general, only a fraction of the maximum possible exposure was received. Though there were no significant effects of photoperiod or circadian phase, a greater proportion of total light was received for the relatively low (SUB) versus high (SAT) irradiance condition.

Significantly more light is likely required to elicit a comparable response when an animal is free to roam in its home cage (and perhaps, in the wild) as compared to optimized laboratory conditions. Given only a fraction of the maximal possible dose was received for all conditions in this study, information obtained regarding key parameters of photobiological functions (e.g. threshold and sensitivity) in the lab may represent a gross underestimate. Instead, these values may be closer to approximately 2-3 times the reported dose for a given parameter under more naturalistic conditions.

However, assuming the proportion of light received in the more homogenous exposure set up of our phase shifting studies (in Chapter 2) was as low as 1/3, light sampling still cannot fully account for the differences in ED_{50} with studies in which animals were restrained for the duration of the pulse (Nelson and Takahashi, 1991a). Additional unknown factors must be contributing to the absolute differences in sensitivity across these dose response studies. Therefore, examining subsaturating responses within a new setup requires direct measure across a range of irradiances rather than extrapolating data from previous work.

Hamsters only received 1/3 of the possible photon dose from the bright irradiance (SAT) condition, which is consistent with reports of bright light aversion (BLA) in other rodent species (Crozier and Pincus, 1927; Keller, 1941; Welker, 1959; Crawley and Goodwin, 1980; Mrosovsky and Hampton, 1997). Though the phenomenon has been used in a variety of applications, including drug development and mouse models of migraine (Misslin et al., 1989; Russo et al., 2009), it has not been extremely well characterized. A more recent study described the temporal kinetics of BLA in mice with varying combinations of photoreceptors, demonstrating an aversion response within as little as 5 minutes of exposure in wild type animals and a slower onset of ~15-20 minutes in those containing only melanopsin ipRGCs (Semo et al., 2010). A 15-minute bright light pulse may have been enough to elicit an aversion response in hamsters as well. The fact that a relatively greater proportion of light was received for the SUB versus SAT condition raises the interesting possibility that the light aversion response may be dose-dependent. To our knowledge, the

response has only been studied within bright light environments, and it is not known if there is some critical threshold irradiance that elicits an aversion response. In view of the contribution of melanopsin ipRGCs to bright light aversion in mice (Semo et al., 2010), it would be useful to know if aversion behaviors are only triggered at irradiances brighter than those required to elicit saturating circadian responses and/or full pupillary constriction.

The SUB condition does not appear to be bright enough to elicit an aversion response. In this experiment, an attempt was made to simulate a typical laboratory setup, including a clear cage with bedding and access to food and water from the upper lid region. In addition, diffusers were not employed for the distinct purpose of creating further variability in illuminance levels within different sections of the cage environment. As illustrated in Figure 3.1, this results in significant shadow on one half of the cage (the left side of the cage in the photo). Given the consequent distribution of light, animals would need to spend the entire time in the upper right corner of the cage (just below the light source) in order to obtain an optimal exposure. It might be expected that animals would not spend a significant amount of time in this region due to light aversion. Not only is this the brightest area of the cage, but it also requires additional physical effort to maintain the eyes in this region because an animal must be in an upright position. Yet, the eyes of animals were in this area closest to the light source for the majority of the pulsing time in the SUB condition. This can be appreciated by noting the proportion of the total light that was calculated for SUB (48%) and recognizing that the next brightest section is less than half (2/5) as bright.

The proportion of total light in the SAT condition was significantly lower (32%), suggesting some threshold intensity may exist in which the animal begins to spend more time in relatively dimmer regions. Though animals still received more overall light in SAT versus SUB, the irradiance difference in proportions suggests the dynamic range of response for experiments where animals are free to roam in their cages may be artificially compressed. Yet, further comparisons between our phase advance data and that of Nelson and Takahashi (1991a) do not find this to be true, as the dynamic range of the response is quite similar for the two studies.

Sampling behaviors do not appear to be among the myriad effects of photoperiod on circadian response to light. Though not statistically significant, light sampling of sub-saturating irradiances alone would predict results in the opposite direction of the heightened sensitivity for phase shifts under short photoperiods. The trend for less light under short photoperiods (in the SUB condition) may reduce the impact of heightened sensitivity when exposures are not optimized, under more naturalistic conditions. It thus raises the possibility that enhanced photic sensitivity is necessary in order to maintain entrainment with less environmental light (due to sampling behavior) during winter months. On the other hand, there was no detectable effect of photoperiod for the SAT condition. Not only was the mean proportion of light received statistically equivalent for the LP and SP groups, but there were no photoperiod differences in the incidence of other activities that were noted during scoring (e.g. gaze behavior, feeding, grooming, and burrowing). This may be somewhat surprising considering the robust seasonal changes in physiology and

behavior that occur in this species (Goldman, 2001); however, activities were only examined over the 15 minutes for which the light pulse was administered.

Phase differences in light sampling do not lead to differential photic exposures. Specifically, at two different times in the subjective night, the same animals received roughly equivalent amounts of light. The timing of administration of light pulses for this study (LP: ZT14 and ZT19 SP: ZT14 and ZT22) was selected to match the protocols of our other photoperiod experiments for phase resetting. These times further represent circadian phases that have been well established to elicit bright light-induced phase shifts of different magnitude and direction (Goldman and Elliott, 1988; Moore, 1995; Glickman et al., 2012). Though light sampling of a nighttime pulse could not explain why a rhythm would shift to an earlier or later time, systematic changes in the amount of light received as a function of circadian phase could contribute to magnitude differences. Previous work has shown phase differences in light sampling of the photophase in flying squirrels housed in a dark simulated den environment, with greatest sampling at dusk (DeCoursey, 1986). Our study setup was more akin to those of studies that have constructed phase response curves and therefore, suggests light sampling does not contribute to the differential effects in the magnitude of phase shifts by light administered at different times in the subjective night.

Considering the impossibility of establishing phase equivalence for two different waveforms (as discussed in Chapter 1), lack of phase differences in light sampling is also relevant to our photoperiod comparison of phase resetting. For

example, since we cannot definitively know that ZT19 under LP and ZT22 under SP represent identical circadian phases, it is important to determine whether circadian phase alters the proportion of a light pulse that is received for these two conditions. In view of the comparable light sampling for conditions identical to those employed in our study of phase advances, phase differences in the amount of light received by the eye cannot account for the increased photic sensitivity for phase resetting under short photoperiods.

In conclusion, though the amount of light received is significantly reduced from the total possible when animals are free to move within their home cage, this reduction is remarkably consistent across different circadian phases and photoperiods. The disproportionately higher reduction for the SAT condition suggests a possible bright light aversion response in hamsters. Further studies are necessary to establish whether bright light aversion occurs in response to subsaturating irradiances of light. Ultimately, restraint of animals does not appear to be necessary but a homogenous exposure within the entire cage environment should be employed when characterizing dose response to light.

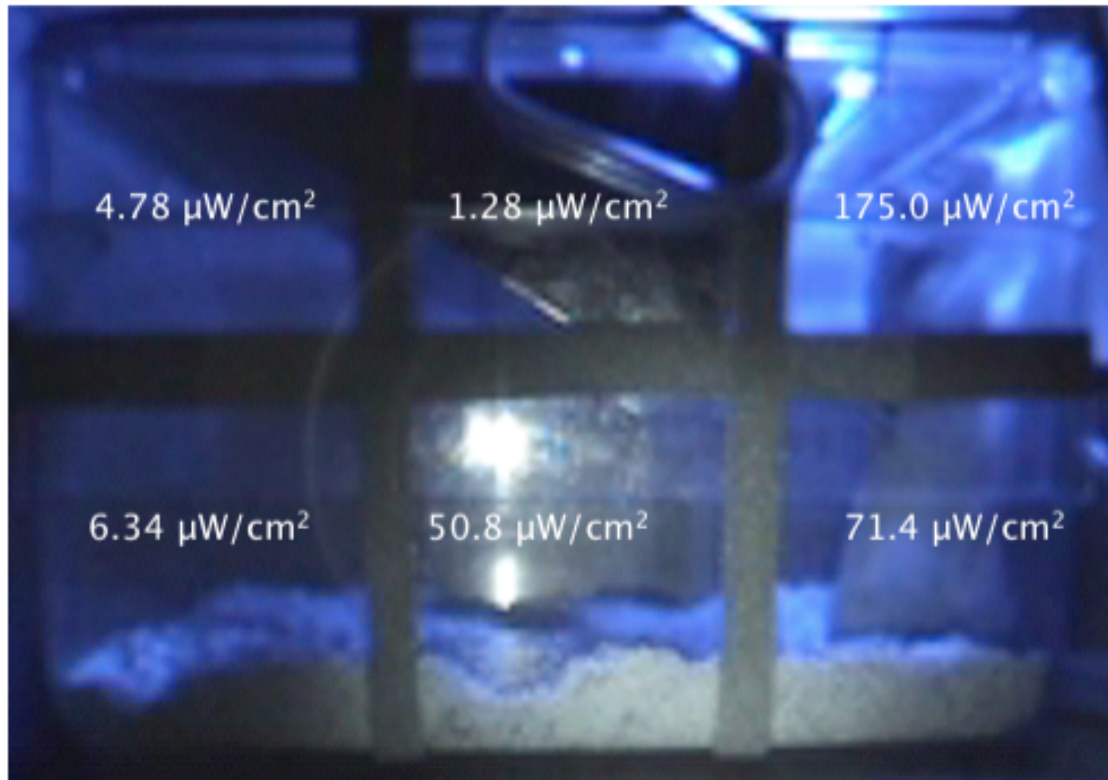

Figure 3.1. Experimental setup for light sampling study. Six equal area regions are demarcated on the cage exterior. The irradiance was measured in the center of each region, as indicated. Scoring of time spent in each region was based on continuous monitoring of the location of the eyes (e.g. upper right in this picture).

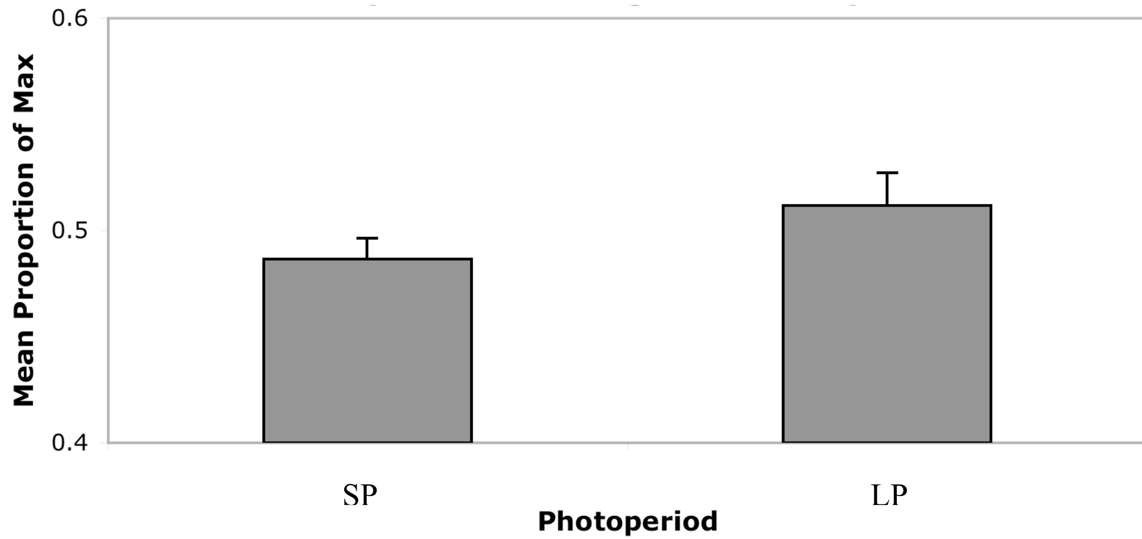

Figure 3.2. Proportion of a sub-saturating light pulse received as a function of photoperiod. Mean ± SE proportion of the maximum possible photon dose of subsaturating light for short photoperiod (SP) versus long photoperiod (LP) conditions (n = 8 per condition). Though not statistically significant based on our criteria, there is a trend for a greater proportion of maximal photon exposure under long versus short photoperiods ($p=0.059$).

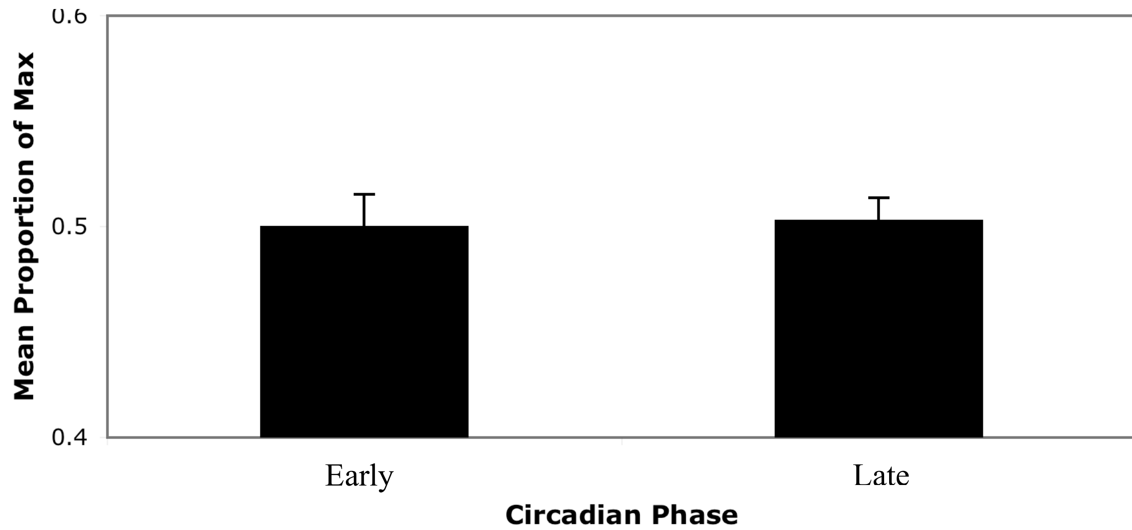

Figure 3.3. Proportion of a sub-saturating light pulse received as a function of circadian phase. Mean ± SE proportion of the maximum possible photon dose of subsaturating light within subjects early (ZT14) and late (ZT19 and ZT22 for LP and SP, respectively) in the subjective night. There were no significant phase differences in the proportion of total photons received during a sub-saturating light pulse ($p>0.7$).

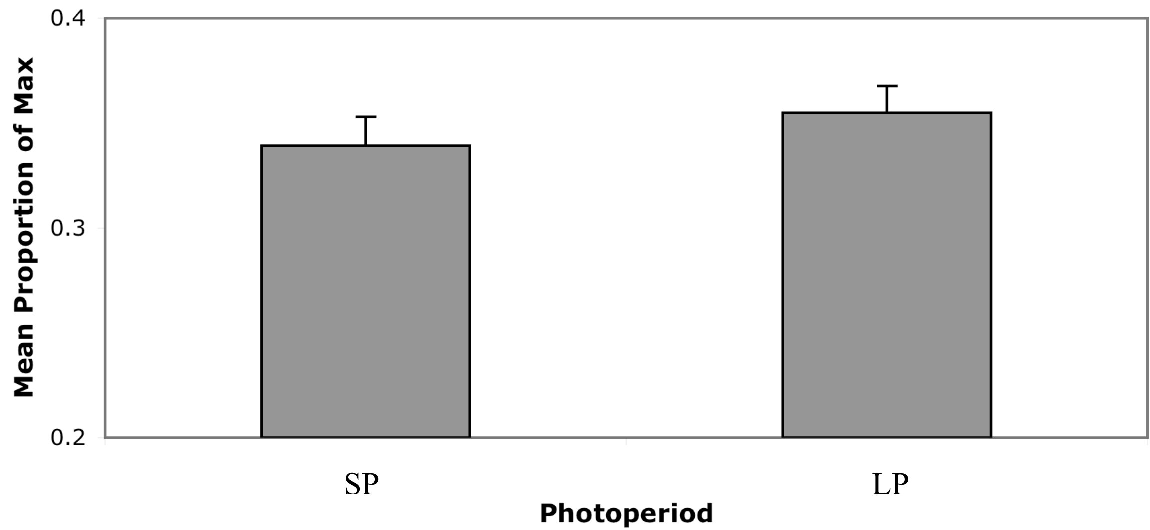

Figure 3.4. Proportion of saturating light pulse received as a function of photoperiod. Mean ± SE proportion of the maximum possible photon dose of saturating light for short photoperiod (SP) versus long photoperiod (LP) conditions. There were no significant photoperiod differences in the proportion of total photons received during a saturating light pulse ($p>0.4$)

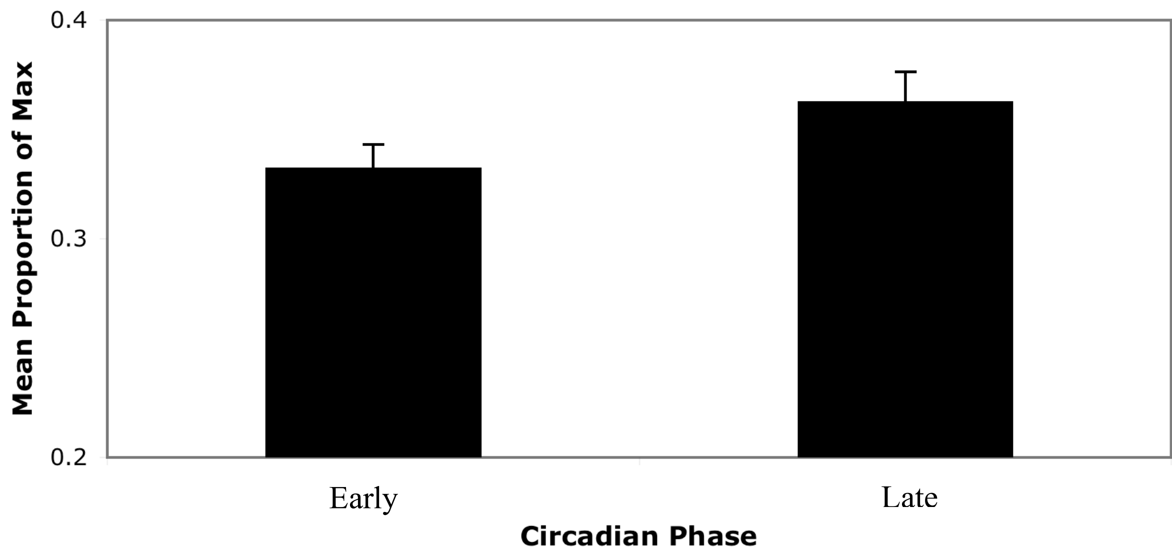

Figure 3.5. Proportion of saturating light pulse received as a function of circadian phase. Mean ± SE proportion of the maximum possible photon dose of saturating light within subjects early (ZT14) and late (ZT19 and ZT22 for LP and SP, respectively) in the subjective night. There were no significant phase differences in the proportion of total photons received during a saturating light pulse ($p>0.1$)

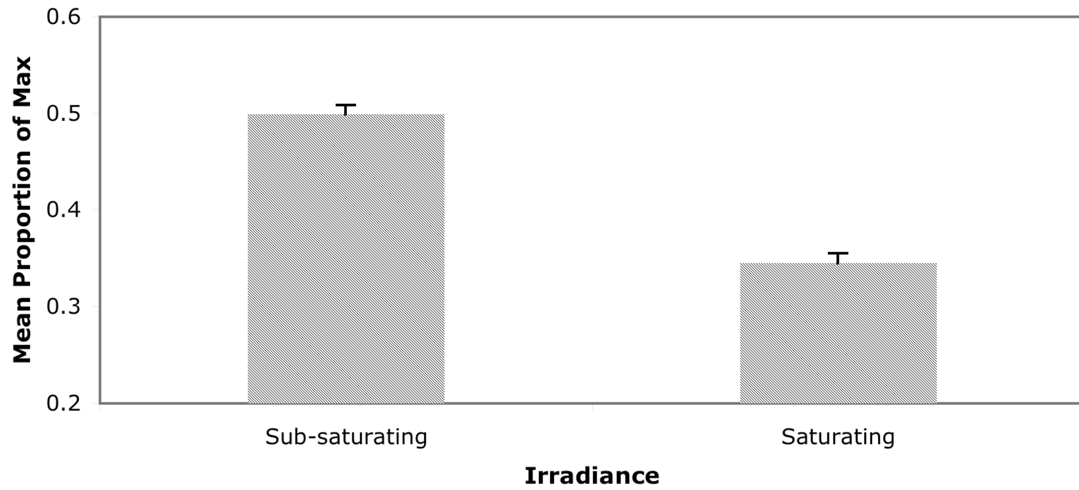

Figure 3.6. Proportion of total photons received as a function of irradiance. Mean ± SE proportion of the maximum possible photon dose of subsaturating and saturating light received based on light sampling behaviors. Only ~1/2 and 1/3 of the maximal available photons received when pulsed with sub-saturating and saturating light pulses, respectively ($p<0.0001$)

CHAPTER 4. PHOTOPERIOD MODULATES SENSITIVITY TO LIGHT IN THE SCN

INTRODUCTION

Previous experiments of photoperiod changes in the SCN have focused primarily on the *interval* of induction of molecular makers by bright light pulses, with a longer interval of photic induction of *Per1* and *cFos* under short versus long photoperiods in the SCN of both Syrian hamsters and rats (Sumová and Illnerová, 1996; Jacob et al., 1997; Tournier et al., 2003; Sumová et al., 2003). Photoperiod history effects on light-induced pERK expression have not been examined. Moreover, none of these molecular markers have been studied in terms of induction by sub-saturating light pulses in order to better understand the physiology underlying photoperiod changes in photic sensitivity, as reported in Chapter 2. We aim to test the hypothesis that photoperiod modulation of light sensitivity is occurring at the level of the SCN. Studying photic induction of pERK, PER1 and cFOS in the SCN will further serve to identify the mechanisms underlying photoperiod regulation of light sensitivity. Quantitative expression of these photoinducible molecules will be examined in response to light pulses administered after entrainment to short versus long photoperiods in Syrian hamsters. Results reveal the level of regulation by which photic history influences circadian response to light.

Possible Outcomes:

Testing photic induction of various proteins in the SCN in response to equal photon doses as well as light irradiances that elicit comparable phase shifts in SP and LP animals allows us to determine if photoperiod changes in photic sensitivity for phase resetting are reflected at the level of the SCN. Furthermore, comparing patterns of induction between the three different proteins- pERK, PER1 and cFOS- will help to identify where along the signal transduction cascade photoperiod modulation of light sensitivity might occur. For example, at the same light dose, pERK induction may not differ between photoperiods, but PER1 induction may be greater in SP vs LP. This result would imply that increased sensitivity occurs at some point between these two events along the signal transduction cascade. Alternatively, it could be that all three markers show photoperiod differences, in which case, modulation is likely occurring upstream of these events (e.g. retina or post-synaptic receptor level). Finally, none of these markers may vary by photoperiod history. Such findings would indicate that regulation of light responsiveness via photic history is occurring downstream of PER1. In sum, this project provides a first account of the neural mechanisms underlying photoperiod modulation of phase resetting by light in hamsters.

METHODS

In order 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 (in Chapter 2) 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 uW/cm^2 or 3.5 uW/cm^2, to match the irradiances of the behavioral phase-delay study (In Chapter 2). 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 uW/cm^2 (n=6 at 15 min; n=6 at 60 min post-pulse), 2) SP-0.14 uW/cm^2 (n=6 at 15 min; n=6 at 60 min post-pulse), 3) LP-3.5 uW/cm^2 cm^2 (n=6 at 15 min; n=6 at 60 min post-pulse), 4) SP-3.5 uW/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 (diagrammed in Figure 4.1). 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.

Light Sources:

The photoperiod entrainment conditions used broad spectrum white fluorescent bulbs (F4T5) (105 μW/cm^2) for the photophase and narrowband light-emitting diodes (LEDs) affixed to the back wall of each chamber (560 nm, 23 nm ½ peak bandwidth; 7.9 x 106 μW/cm^2) for the scotophase. This dim scotopic illumination was included to match the experimental design of our other studies. A 480 nm (23 nm ½ peak bandwidth) 8-LED lamp source with diffuser was positioned atop the center of each cage lid. Irradiance levels of lamps were manipulated with neutral density filters. Spectral power distributions and half-peak bandwidth were determined 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.

Tissue Collection:

At the assigned post-pulse time, animals were deeply anesthetized in complete darkness with sodium pentobarbital (i.p.) and transcardially perfused with 100 ml 0.1M phosphate buffered saline (PBS) followed by 100 ml 4% paraformaldehyde in 0.1M phosphate buffer (PB) with eyes covered, under dim red light. Brains were then removed, post-fixed overnight and cryoprotected in 30% sucrose with 0.01% sodium azide in 0.1M PB prior to sectioning with a microtome. Four parallel series of 35μm 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 0.1M 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 hr 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 cFOS 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 for pERK and cFOS) or a biotin-conjugated rabbit anti-goat IgG (1:500, Vector Laboratories for PER1) for 1 hr 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 3.2) and mouse SCN (Webb & 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. Pre-adsorption 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 the Lehman laboratory as well as several published reports from other laboratories using this antibody demonstrate that it detects rhythmic PER1 expression in the hamster SCN (Yan et al., 2005; Yan & Silver, 2008; Ian Webb, personal communication).

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 atlas (Morin and Wood, 2001) and imaged via a digital camera (Microfire, Optronics, CA, USA) 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 three molecules are analyzed and reported in detail. Cell counts are included for PER1 and cFOS. Using ImageJ software (National Institutes of Health, Bethesda, MD, USA), boundaries encapsulating the SCN were consistently applied to each image (See Figure 4.2), and the percent 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.

RESULTS

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). Light pulses that elicited phase shifts in the behavioral studies (LP at 3.5 $\mu W/cm^2$ 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≥0.398) but instead were closely matched in numerical terms (see Figure 4.2).

DISCUSSION

Light pulses of sufficient irradiance to generate robust phase delays and induction of pERK, PER1, and cFOS in the SCN under short photoperiods were ineffective at eliciting a similar response under longer days. Furthermore, SCN activation equivalent to that 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.

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 photoperiod 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; Morin et al., 2003; Dacey et al., 2005). In addition, glutamatergic input from the RHT is modulated by serotonin (5-HT) receptors (Sollars et al., 2006b). 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 photoperiod 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, the robust enhancement of sensitivity under shorter photoperiods is reflected very early in the signal transduction cascade, at the level of the SCN.

Acknowledgements: Portions of this chapter appear in Photic sensitivity for circadian response to light varies with photoperiod in Journal of Biological Rhythms 2012. Glickman, Gena; Webb, Ian; Elliott, Jeff; Baltazar, Ricardo; Rheale, Meghan; Lehman, Michael; Gorman, Michael.

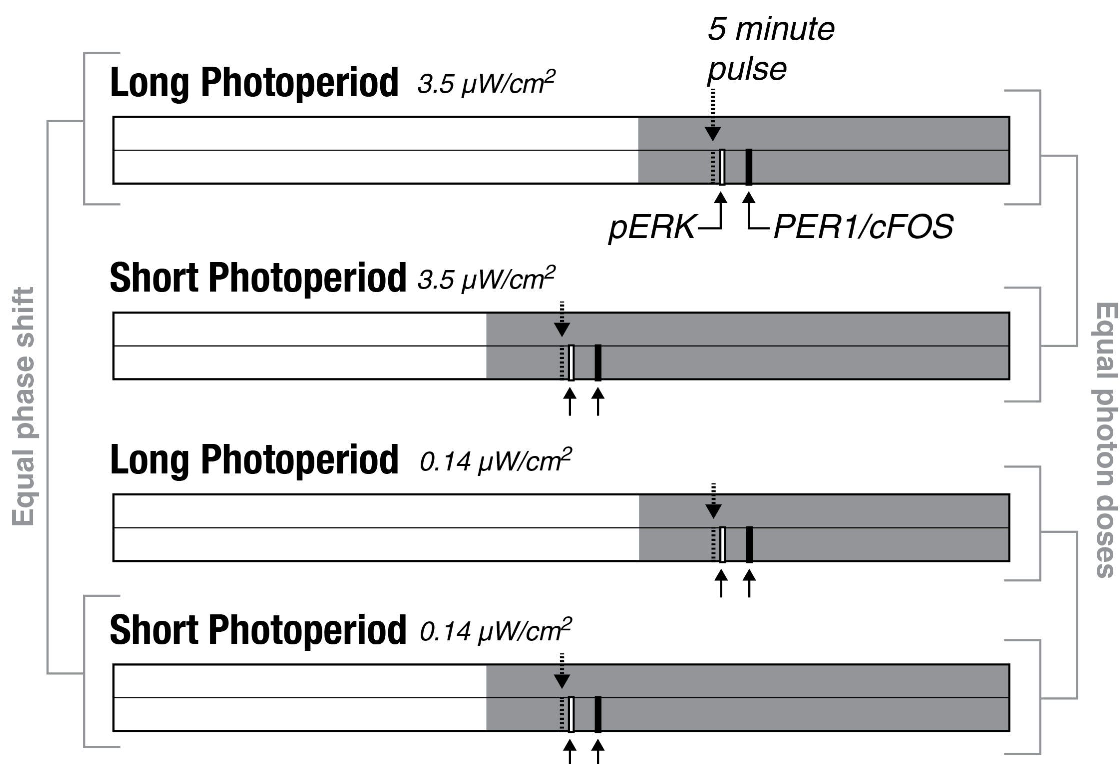

Figure 4.1. Schematic of the protocol for the immunocytochemistry experiment. The dotted lines represent the administration of a 5-min short-wavelength light pulse at ZT14, 2 h after lights-out. The white and black bars illustrate the timing of brain collections for study of pERK (15 min post-pulse) 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.

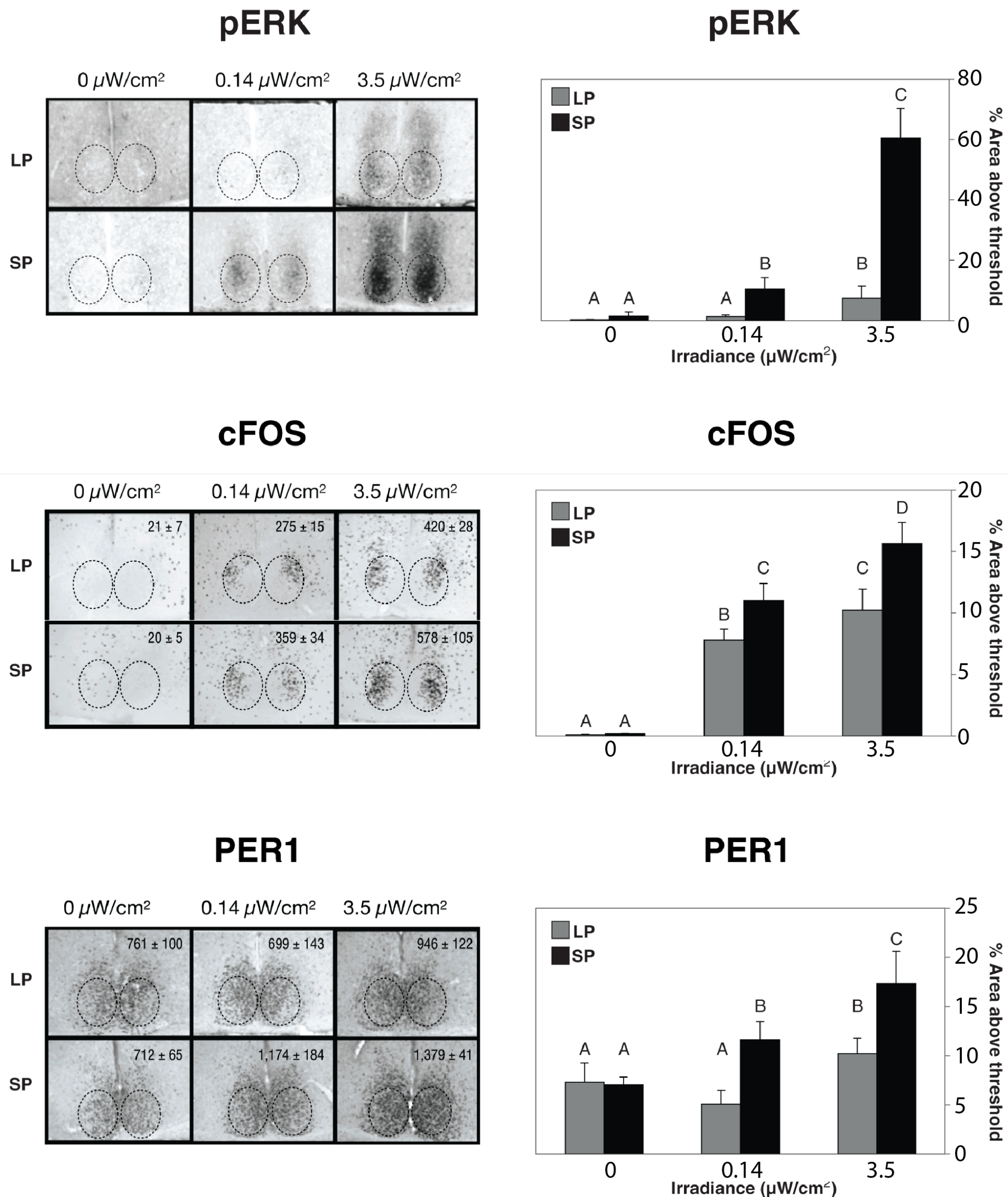

Figure 4.2. Representative histology for pERK, cFOS, and PER1 in the SCN of animals previously maintained under long photoperiod (LP) or short photoperiod (SP). Dotted circles illustrate the area that was used for analyses. Data in the upper right corner of each section for cFOS and PER1 provide cell 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 ($p < 0.05$).

CHAPTER 5. SENSITIVITY TO LIGHT FOR MELATONIN SUPPRESSION DOES NOT VARY BY PHOTOPERIOD IN SIBERIAN HAMSTERS

INTRODUCTION

Melatonin suppression by light shares similar properties to phase resetting and has therefore been a useful method for studying 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 certain amount of light is required to elicit a response (threshold); 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., 1983, 2001; Takahashi et al., 1984; Zetzer 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 shift of the melatonin fluence response curve to lower irradiances relative to that for phase shifts (Nelson and Takahashi, 1991; Lewy et al., 1980; Czeisler et al., 1982).

Not only is the timing of 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) signals seasonal timing of physiology and behavior. In hamsters, reproduction, body weight and pelage are all regulated by seasonal changes in photoperiod (Goldman, 2001). The waveform, or shape, of circadian rhythms change

as a function of photoperiod as well. In nocturnal and diurnal animals, the pineal gland synthesizes and secretes high levels of melatonin at night and low levels during the day (Reiter, 1991; Arendt, 1998). Consequently, the melatonin waveform reflects seasonal alterations in day length, with the interval of elevated nocturnal levels increasing in direct proportion to duration of darkness across mammalian species (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 (Elliot and Tamarkin, 1994) along with SCN neuronal activity (Meijer, 2010) indicates that these photoperiod influences are due to changes in pacemaker network organization. Light-induced phase shifts of rhythms also occur across a broader range of the circadian cycle and with increased amplitude in hamsters previously entrained to shorter photoperiods (Milette and Turek, 1986; Puchalski and Lynch, 1988; Goldman and Elliott, 1988). Even after release into constant conditions for many days, the period and waveform of the activity rhythm for a given photoperiod is maintained, further suggesting entrainment effects rather than a consequence of immediate light history (Pittendrigh and Daan, 1976).

It was recently shown that Syrian hamsters previously entrained to a short photoperiod demonstrate an impressive 40-fold increase in sensitivity (as defined by the ED_{50}) to light for phase resetting of activity rhythms (Glickman et al., 2012). Furthermore, those photoperiod differences are found at the level of the SCN and early in the signal transduction cascade as 25 times more light is 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 short day pacemaker is not universally more responsive to resetting cues since a non-photic stimulus did not alter phase shifting as a function of photoperiod (Evans et al., 2004). Thus, the enhanced short day sensitivity may be specific to the light input pathway.

The influence of photoperiod on light sensitivity for melatonin suppression has not previously been studied in an animal model wherein light history can be precisely controlled and monitored. Siberian hamsters are a particularly good model for studies of circadian function as they demonstrate distinct rhythms in locomotor activity and melatonin secretion, both of which also show robust and predictable changes in waveform as a function of photoperiod. In addition, Siberian hamsters are a highly photoperiodic species in their display of seasonal changes in reproductive function, body weight fluctuations, and pelage changes. Though circadian and photoperiod responses have been studied extensively in the Siberian hamster, there is a surprising lack of quantitative data describing its photic sensitivity.

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 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, photoperiod differences 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 that was established in our laboratory in 1994. 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 photoperiod of short day animals was achieved by extending the time of lights off by 4 hours. Food and water were available ad libitum. All procedures were approved by UCSD Institutional Animal Care and Use Committee.

Melatonin Suppression Study:

Hamsters (n= 106 total) were group housed in clear polypropylene cages (48 cm x 27 cm x 20 cm) and maintained in large ventilated, light-tight, matte white interior chambers on LD14:10 (LP) or LD10:14 (SP) for 6 weeks prior to melatonin suppression tests. On the night of the light pulse, a 480 nm (20 nm half-peak bandwidth) light source of a pre-quantified irradiance was administered in a separate matte white interior pulsing cabinet (43 cm x 36 cm x 46 cm), which was optimized for a homogenous, highly controlled exposure. Each animal was transferred to a smaller polypropylene cage (28 cm x 19 cm x 18 cm) immediately before placement

in the cabinet in order to be pulsed individually for 15 minutes with 480 nm light at ZT18 (LP) or ZT20 (SP) (with ZT12 representing the time of lights out) at one of the following prescribed intensities: 0, 0.003, 0.03, 0.3, 1.31, 4.8 or 68 uW/cm2 (n=6-8 per irradiance for each photoperiod condition). The light pulse was timed to occur approximately 60% of the way through the scotophase (60% for LP and 57% for SP) at a time known to produce near-peak phase advance shifts and when circulating melatonin is at its peak under each photoperiod (Jeff Elliott, personal communication; personal observations). Precisely one hour following the start time of each light pulse, animals were anesthetized with isofluorane and a single retinal-orbital bleed was performed, using heparinized caraway micro collecting tubes (Fisherbrand). Animals were then euthanized. Blood was transferred to tubes containing 40 uL of heparin, centrifuged, and plasma was stored at -70°C until assay.

Phase Shifting Study:

An Aschoff type II randomized within-subjects design was employed in a separate group of Siberian hamsters that were entrained to LP (n =10) or SP (n = 10) for 6 weeks. Animals were individually housed in clear polypropylene cages (28 cm x 19 cm x 18 cm) equipped with 12 cm diameter wheels from when they first entered the entrainment period. Cages resided in 2 separate large ventilated, light-tight, matte white interior chambers. To examine photosensitivity associated with phase delay shifts, light pulses were administered 2 h into the dark (ZT14) for both LP and SP, with each home cage being transferred to individual cabinets (as described in the

melatonin suppression section) for the duration of the pulse and returned to chambers afterwards. Each animal received 15 minutes of short wavelength light at 0.11 and 3.14 μW/cm2 as well as a sham (dark control) condition. For each light or sham pulse, animals were exposed to constant conditions beginning at the normal time of lights-off. Animals remained under constant conditions for 10 days following the test 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, using dim red illumination.

Each one-half wheel revolution generates 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 by eye fit. 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. 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 activity onset regression line, created from the three days 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. Photic phase-shift scores were expressed relative to each animal's phase shift for the sham control condition. All group values are expressed as means ±

SEM and analyzed with analysis of variance (ANOVA).

Light Sources:

Photophases were illuminated with broad-spectrum white fluorescent bulbs (F4T5) (100-150 $\mu W/cm^2$). To facilitate rapid and full entrainment to SP, 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; 7.9×10^{-6} $\mu W/cm^2$, ~0.01 lux). This dim scotopic illumination is comparable in irradiance to natural ambient light at night and represents a very small fraction (~1/380) of our lowest irradiance test pulse. In addition, dim light scotophases have been shown to reduce the incidence of short day non-responder Siberian hamsters (Gorman and Elliott, 2004). Experimental light pulses were administered via 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 $\mu W/cm^2$), 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 W/m^2 and converted to photon

density (photons/cm^2/sec) based on the energy per photon for 480 nm.

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 Kenaway anti-melatonin antibody and 125I-melatonin for 18-24 hours at 2-8° C. Second antibody was then added, and samples were incubated for 15 minutes at 2-8° 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., Hunstsville, AL). The gamma counter software utilizes the standard curve to compute 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.

Statistical Analyses:

Melatonin data were analyzed as concentrations (pg/mL) and percent control-

adjusted melatonin suppression (mean melatonin for control – mean melatonin at each irradiance/mean melatonin for control x 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 (Nelson and Takahashi, 1991; Owen and Arendt, 1992; Brainard et al., 1984, 2001). SPSS (version 13.0.0) was used to perform ANOVA and post hoc tests. 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.

RESULTS

Melatonin Suppression Study:

Two-way between subjects ANOVA demonstrates a significant effect of both irradiance ($F_{7,92}=7.492$, $p<0.001$) and photoperiod ($F_{1,92}=10.511$, $p<0.005$) on melatonin concentrations. Figure 5.1 shows the mean +/- SEM melatonin for the two photoperiod conditions (mean range, 4.89 to 277.65 pg/ml). Melatonin concentrations do not differ between the dark control and 0.003 $\mu W/cm^2$ exposure conditions ($p=1.0$) but levels in both groups are higher compared to irradiances >0.03 $\mu W/cm^2$ ($p \leq 0.05$). Mean melatonin at 0.03 $\mu W/cm^2$ is also greater than the remaining higher irradiance conditions ($p<0.005$). Finally, melatonin levels at irradiances ≥1.31 $\mu W/cm^2$ were not statistically different, and likely represent a saturating response in both LP and SP.

Melatonin is higher in SP versus LP animals at all but the highest irradiance condition ($p \leq 0.5$).

Suppression scores varied with irradiance ($F_{6,84}=4.964$, $p<0.001$) but not by photoperiod ($F_{1,84}=0.003$, $p= .954$). Post hoc tests show that all intensities above 0.03 $\mu W/cm^2$ suppress melatonin more than 0.003 $\mu W/cm^2$ ($p< 0.05$). In addition, irradiances $\geq$ 1.31 $\mu W/cm^2$ reduce circulating melatonin to a greater extent than the two lowest irradiances ($p<0.05$) (see Figure 5.3). 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 5.4). The formula for the curve is: Y= Minimum (0) response dose+(Maximal response dose-Minimum (0) response dose)/1+10^(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 SD= 0.97 and LD= 0.95). However, there are no photoperiod differences in ED_{50} ($p=0.74$) or maximum response ($p=0.99$).

Phase Shifting Study:

Repeated measures ANOVA showed that phase delays differ as a function of light intensity ($F_{1,18}= 25.5$, $p < 0.001$), with the brighter pulse eliciting a greater phase shift. Phase shifting also varies by photoperiod condition ($F_{1,18} = 6.91$, $p<0.02$), with greater delays in SP versus LP at both tested irradiances ($p<0.001$). In addition, LP

response to the lower irradiance condition was not different from 0, whereas all other conditions produce significant phase delays ($p<0.05$) (see Figure 5.6).

The mean phase shift induced by the sham dark control was not statistically different from 0 for either photoperiod condition (95% CI LP, $-0.280<\mu 1<0.248$ and SP, $-0.528<\mu 2<0.272$), confirming that the practice of administering the pulse (independent of the light) was minimally disruptive to the animals. Differences in the duration of wheel-running activity (α) between long and short day conditions verifies that animals were entrained to assigned photoperiods (mean ± SE α for LP: 9.03 ± 0.22 hours and SP: 13.07 ± 0.23 hours, see Figure 5.5).

DISCUSSION

There is virtually complete overlap of the melatonin suppression fluence response curves for LP and SP conditions. Thus, key parameters of the dose-dependent function do not vary by photoperiod history. In contrast, there is a robust photoperiod effect on resetting of activity rhythms, with a greater phase shift under shorter days at both tested irradiances. Furthermore, the same irradiance that was capable of eliciting a phase delay under a short photoperiod failed to produce a significant response under longer days. In combination, these results suggest that seasonal modulation of photic sensitivity differentially affects melatonin suppression and phase resetting of activity rhythms.

These are the first fluence response curves for photic response in Siberian hamsters, which demonstrate that melatonin suppression is well fit to the sigmoid function obtained in other species. Melatonin suppression dose response curves under LD14:10- but not shorter photoperiods- have been described in Syrian hamsters and show a comparable ED_{50} to the one calculated here for Siberian hamsters entrained to the same photoperiod (Brainard et al., 1984; Nelson and Takahashi, 1991a). Consistent with one previous report (Panke et al., 1980), initial examination of melatonin in Syrian hamsters revealed a relatively sharp nighttime peak in the hormone under both SP and LP, yielding them a poor model for suppression tests across conditions with different waveforms (personal observations). In contrast, Siberian hamsters have a broad rise in melatonin levels, with continued elevation during our selected times of light pulse and blood collection under both photoperiod conditions (Lerchl and Schlatt, 1993; Niklowitz et al., 1994; Lerchl, 1995; personal observations). Because quantitative data on circadian light sensitivity of Siberian hamsters were lacking, irradiances for these studies were selected on the basis of comparable experiments in Syrian hamster, where SP induces a 40 fold increase in sensitivity (Glickman et al., 2012). In Syrian hamsters previously entrained to a short photoperiod, an irradiance of 0.14 $\mu W/cm^2$ induces robust phase shifting, PER1, pERK and cFOS, but is without effect in long day animals. Furthermore, though the higher intensity condition (3.5 $\mu W/cm^2$) elicits photic responses under both photoperiod conditions, responses are significantly greater in SP versus LP across measures (Glickman et al., 2012).

In the present study, we do not see analogous results for melatonin suppression. Instead, response curves for the two photoperiod conditions are not significantly different with regard to variables of interest, such as threshold, sensitivity, or maximum response. The absolute change in melatonin may give the illusion of increased photic sensitivity under shorter days; however, the control-adjusted scores reveal that this apparent photoperiod effect is being completely driven by baseline differences (see Figures 1 and 2). The fluence response curves for suppression in LP and SP show mathematically similar ED_{50}s of 4.78 x 10^{10} and 4.98 x 10^{10} photons/cm^2/sec, respectively. The much higher baseline melatonin in the short versus long day control group confirms most but not all previous reports in hamsters (Hoffman et al., 1981, 1985; Illnerová et al., 1984; Skene et al., 1987). Threshold levels of light required to significantly reduce melatonin as well as maximum melatonin suppression are also statistically similar regardless of photoperiod history. Identical maximal response is perhaps not as surprising since there is an obvious ceiling effect that renders it a less robust comparison measure than the ED_{50}. Ultimately, no matter how the two conditions are compared, photoperiod history does not appear to modulate melatonin suppression by light in Siberian hamsters.

We do not know of any studies examining photoperiod effects on light-induced melatonin suppression in other mammalian models; however, studies in humans have found this acute response to be significantly influenced by various other dimensions of previous light history (Thompson et al., 1990; Owen and Arendt, 1992; Hebert et al., 2002). Specifically, prior exposure to higher daytime light intensities over a one-week

period decreases the melatonin suppressing effects of test light 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, increased melatonin suppression by light has been found in the winter of some individuals (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 is what accounts for the results.

In contrast to melatonin suppression, analysis of activity rhythms reveals that threshold response to light for phase resetting is altered by photoperiod history in Siberian hamsters. Phase shifts elicited by the lower irradiance condition appear to represent a sub-saturating response since the brighter pulse yields a relatively greater magnitude phase shift for both photoperiod conditions. These photoperiod differences in phase delays are consistent with the enhanced sensitivity found in Syrian hamsters maintained under shorter days (Glickman et al., 2012). Moreover, the lower threshold for phase delays (at very similar irradiances) in both Syrian and Siberian hamsters maintained under SP suggests a general enhancement of photic sensitivity for phase shifting that is common to both species. Previous reports demonstrate additional shared photoperiod effects on the activity rhythms of these two species of hamsters, including alpha expansion and a relatively greater PRC amplitude under short versus long days (Milette and Turek, 1986; Puchalski and Lynch, 1988; Goldman and Elliott, 1988).

In any study of photoperiod effects on light sensitivity, two potential confounds are the amount of time in darkness prior to a test pulse and the equivalence of circadian phase between experimental groups. With regard to possible adaptation effects, both melanopsin-containing ipRGCs and SCN neurons demonstrate hours-long dark-adaptation kinetics (Aggelopoulos and Meissl, 2000; Wong et al., 2005) and therefore, it is possible that animals were not completely dark-adapted prior to light pulsing. Because the timing of the light pulse for the melatonin study was designed to sample 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 photoperiod effect on light sensitivity. In contrast, 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 do not likely account for the increased phase shifting by light under shorter days.

Photoperiod continues to influence phase resetting after a number of days in constant darkness, suggesting the effects are not a consequence of immediate light history but rather due to changes in entrainment state (Goldman and Elliott, 1988). In view of this likelihood, it is important to note that matching the phase of two different waveforms is an impossible task. For example, as soon as two different photoperiods are aligned based on one phase indicator (e.g. activity onset), they become misaligned for another (e.g. offset). In both reported experiments, our selected pulse times do not necessarily represent precisely equivalent circadian phases for the SP and LP

conditions. Consequently, examination of response to bright light pulses alone would require testing across the subjective night for both photoperiods, since the magnitude and direction of photic responses vary systematically by phase. However, there is no evidence indicating the timing of a light pulse contributes significantly to parameters of threshold response or sensitivity. On the contrary, full dose response curves to light for phase shifting of activity rhythms do not reveal phase differences in sensitivity (i.e. ED_{50}) (Nelson and Takahashi, 1991a). 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 a dimly lit scotophase serves to enhance photoperiod entrainment but is not potent enough alone to elicit phase resetting (Evans et al., 2007). Comparable levels of dim light pulses have been relatively ineffective at suppressing melatonin in Syrian hamsters (Brainard et al., 1982, 1984; Nelson and Takahsahi, 1991). Similarly, 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 hamster. Our combined study findings are, however, consistent with reports of lower levels of light being required for melatonin suppression as compared to phase shifting (Nelson and Takahashi 1991). Indeed, the lower irradiance for 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 disassociation between the photoperiod effects on light response for phase resetting and melatonin suppression further refines our understanding of the mechanistic basis for this plasticity. It has been suggested that light induction of per1 and melatonin suppression follow two separate transduction cascades within the SCN, as NMDA receptor activation is required for the former but not the latter (Paul et al., 2003). Since we now know that photoperiod modulation of photic sensitivity is observed at the level of the SCN (very early in the signal transduction cascade) (Glickman et al., 2012) but not for melatonin suppression, we suggest that only one of these pathways may be photoperiod sensitive. Further studies are needed to confirm this hypothesis.

In sum, these studies suggest that photic responses of phase resetting and melatonin suppression are modulated differently by photoperiod history. After entrainment to a short day, phase shifting of activity rhythms is substantially increased in response to subsaturating levels of light. In contrast, melatonin suppression by light appears unaffected by photoperiod history, even though photoperiod alters the waveform of circadian fluctuations in the hormone. Manipulating waveform has been proposed as a potentially useful approach for facilitating circadian adjustment to challenging schedules (Harrison and Gorman, 2012). Better understanding the mechanistic basis for the profound impact of photoperiod on light response may ultimately lead to new strategies for optimizing therapeutic applications.

Acknowledgements:

Portions of this chapter appear within a manuscript in preparation, Sensitivity to light for melatonin suppression does not vary by photoperiod Glickman, Gena; Harrison, Elizabeth; Elliott, Jeffrey; Gorman, Michael.

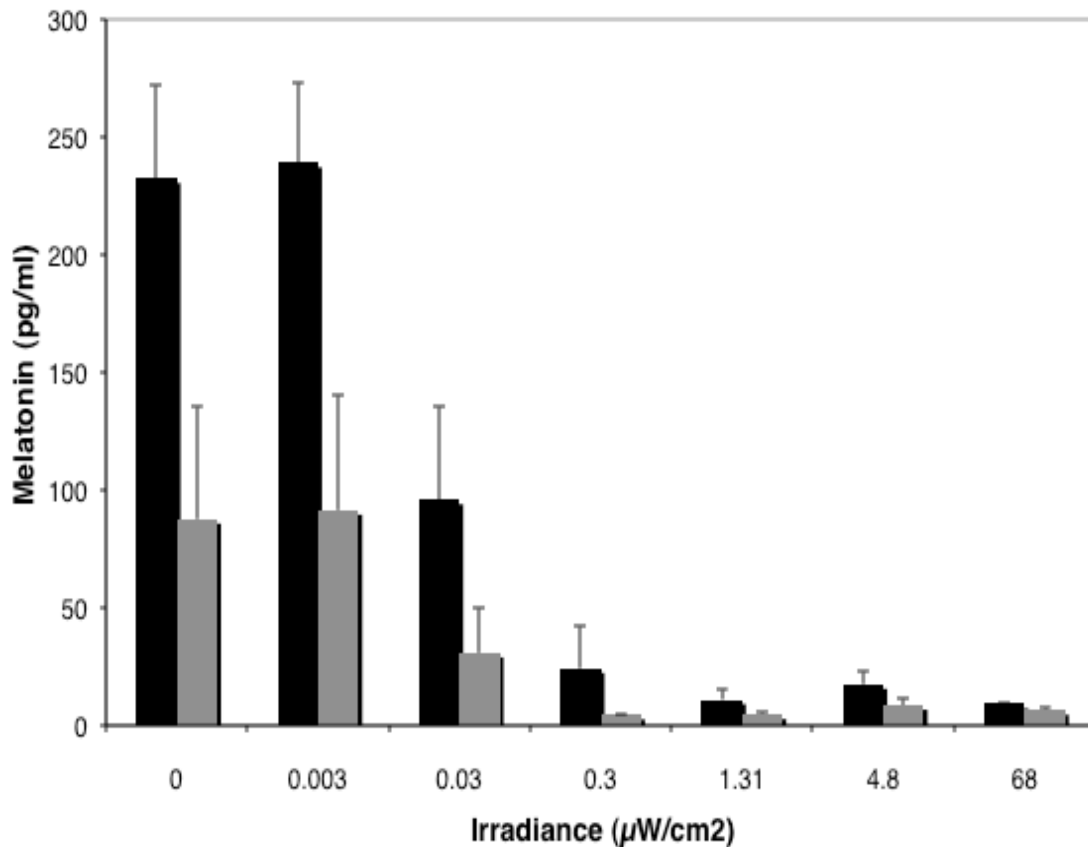

Figure 5.1. Short day versus long day: mean melatonin scores. Mean ± SE melatonin concentrations (pg/ml) for long photoperiod (LP; gray) versus short photoperiod (SP; black). Two-way between subjects ANOVA demonstrates a significant effect of both irradiance ($p<0.001$) and photoperiod ($p<0.005$) on melatonin concentrations.

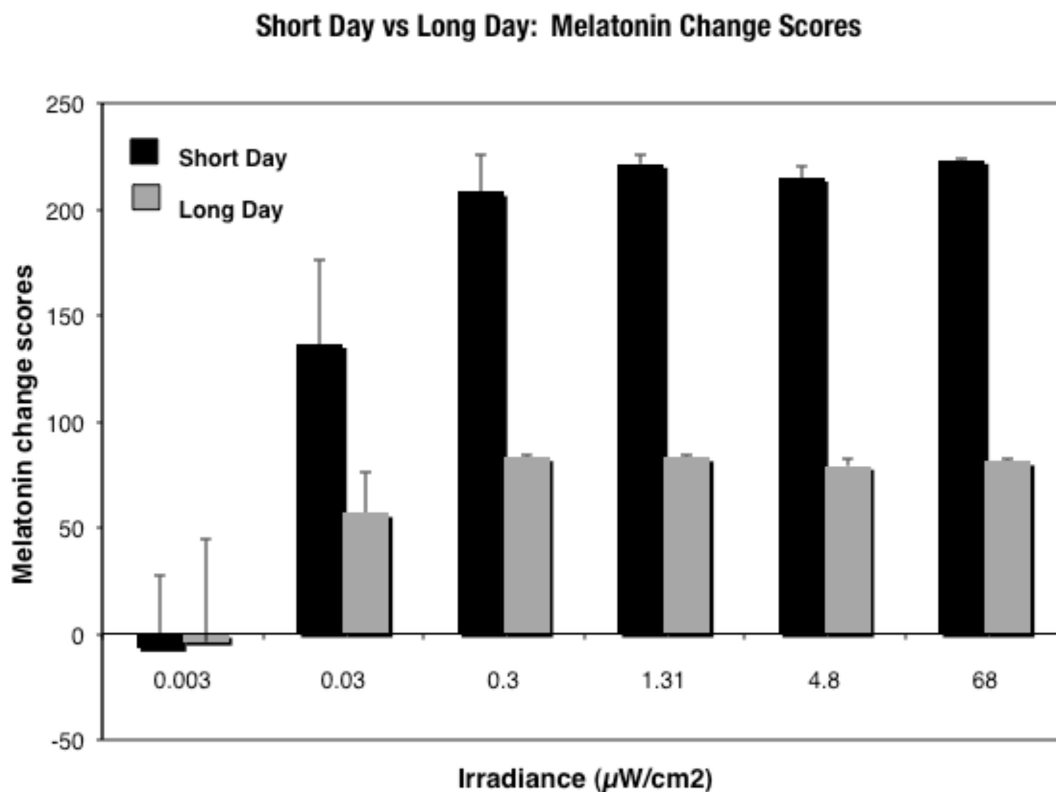

Figure 5.2. Short day versus long day: mean melatonin change scores. Mean ± SE phase melatonin change scores for long photoperiod (LP; gray) versus short photoperiod (SP; black). Melatonin change scores were calculated by subtracting the mean melatonin levels for each irradiance from the mean melatonin levels for the dark control photoperiod-matched condition. Statistically significant mean differences between photoperiod condition were found for every irradiance except 0.003 $\mu W/cm^2$ ($p < 0.05$).

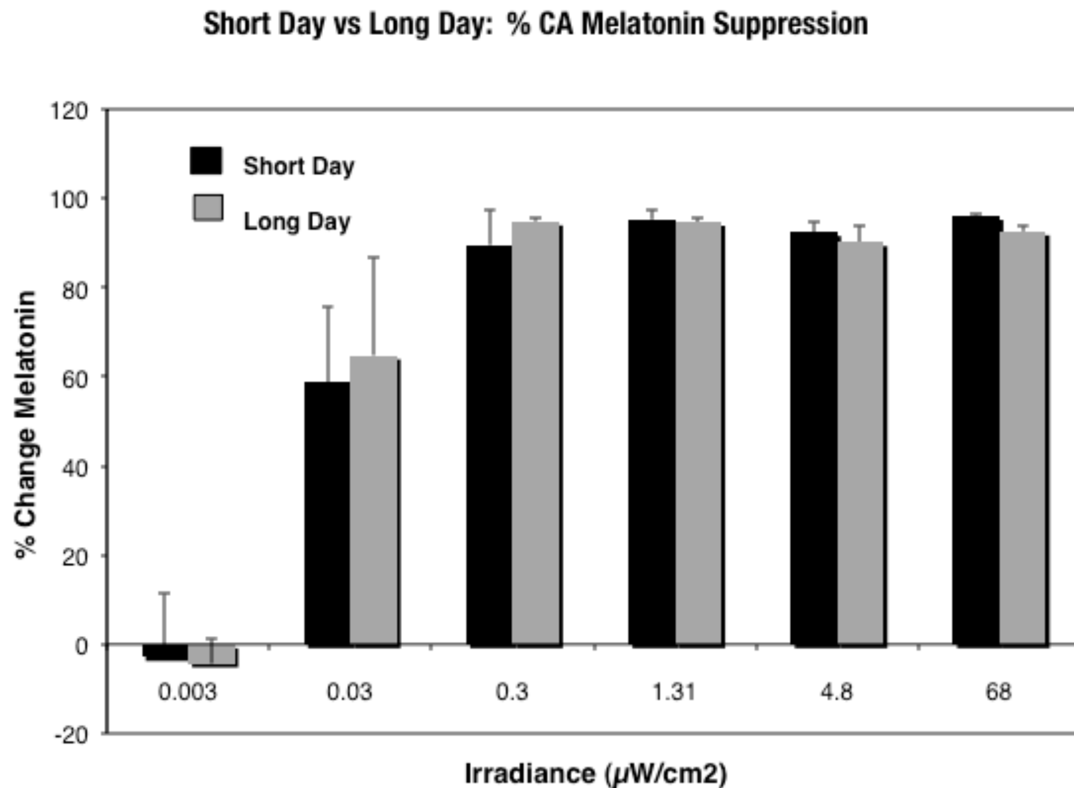

Figure 5.3. Short day versus long day: percent control-adjusted melatonin. Mean ± SE control-adjusted percent change scores for long photoperiod (LP; gray) versus short photoperiod (SP; black). In order to account for baseline differences, these means are expressed as the percent change in melatonin from the dark control for the photoperiod-matched condition. Suppression scores varied with irradiance ($p<0.001$) but not by photoperiod ($p= .954$).

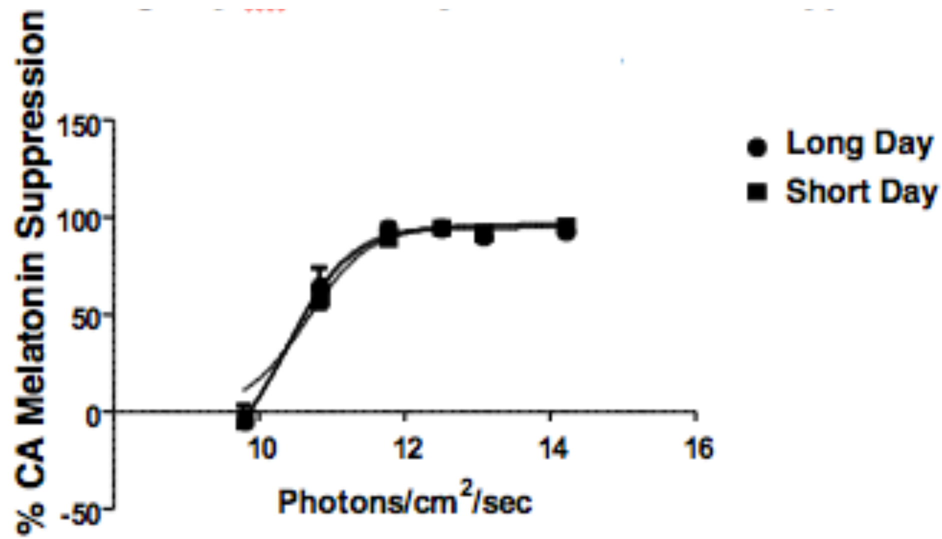

Figure 5.4. Fluence-response curves for melatonin suppression. Percent control-adjusted melatonin suppression for Siberian hamsters previously entrained to long photoperiod (LP; black circles) or short photoperiod (SP; black squares). Data for both conditions were well fit to this sigmoid function, as demonstrated by high coefficients of correlation (R^2 for SD= 0.97 and LD= 0.95). However, there are no photoperiod differences in ED_{50} (p=0.74) or maximum response (p=0.99).

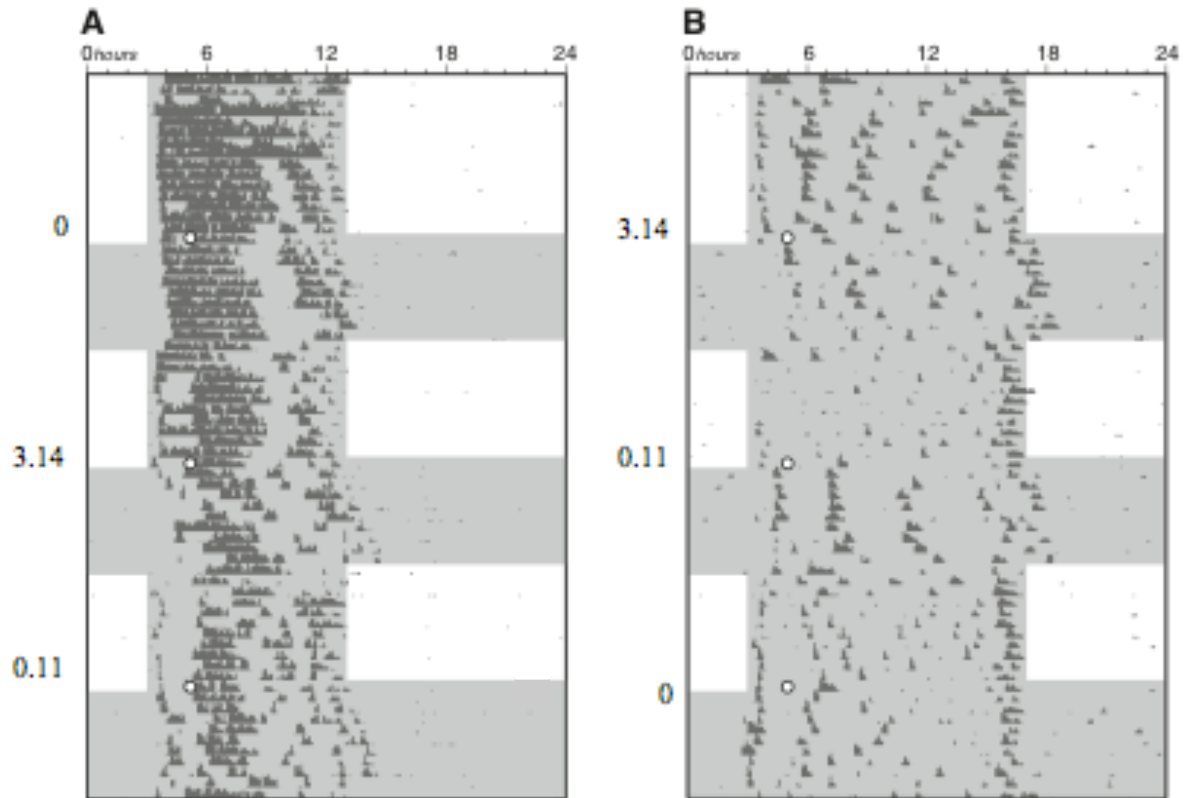

Figure 5.5. Representative actograms for phase shifting in Siberian hamsters. Activity rhythms for animals under A) long photoperiod (LP) and B) short photoperiod (SP) at three different irradiances- 0, 0.11 and 3.14 $\mu W/cm^2$. Each line represents a 24-h day. Light gray areas represent dim light, white areas signify bright fluorescent white light, and the 3 small white circles each represent a 15-min short wavelength light pulse. Dark gray patterns show wheel-running activity.

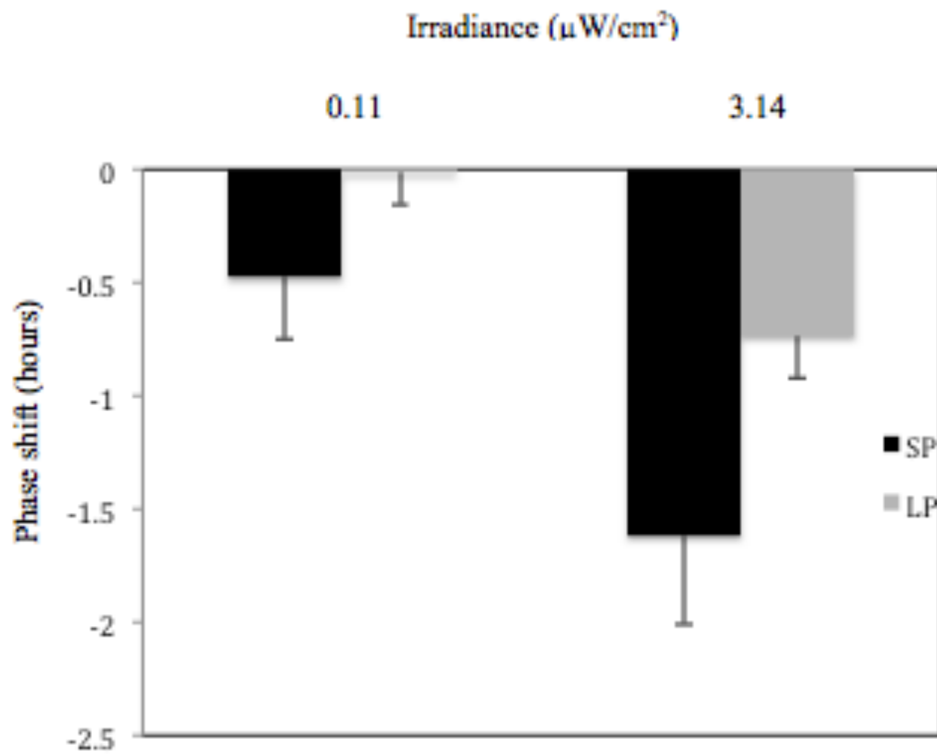

Figure 5.6. Photoperiod effects on phase delays in Siberian hamsters. Mean ± SE phase delay data for long photoperiod (LP; gray) versus short photoperiod (SP; black) (n = 10 per condition). Phase-shift scores are expressed relative to each animal's phase shift on the dark control night.

CHAPTER 6. CONCLUSIONS

In sum, the present studies demonstrate the following:

1) Prior entrainment to a short winter-like photoperiod results in a 40-fold increase in photic sensitivity for phase resetting. The characteristic dose-response to light is maintained for phase advances in animals previously housed under shorter days; however, the curve is shifted to significantly lower irradiances as compared with the long day function.

2) Increased sensitivity to light for phase shifting under short photoperiods does not appear to be limited to a particular circadian phase. Instead, phase delays were also significantly greater for short versus long days when animals were pulsed with the same dose of light earlier in the subjective night. In addition, the phase delay comparison controlled for the duration of time in darkness prior to administration of the light pulse and therefore, these findings further suggest that dark adaptation does not account for the photoperiod effect on photic sensitivity.

3) Light sampling behavior does not lead to disparities in photon dose as a function of prior photoperiod history or circadian phase of administration. Consequently, the heightened photic sensitivity observed in short day animals

likely represents an ecologically relevant change in the physiology underlying circadian response to light. However, a lower proportion of light is received at relatively higher irradiances, suggesting a possible bright light aversion in hamsters. Future work will need to more directly test for bright light aversion response in this species and if present, determine the irradiances at which it occurs. Of note, this possible light aversion was not implied in the data at irradiances that were separately shown to elicit threshold phase shifting responses.

4) Photoperiod modulation of photic sensitivity originates at or upstream of the SCN, as we demonstrate robust history-dependent differences in expression of light-induced pERK, PER1, and cFOS within the pacemaker. Specifically, photic induction of all of these proteins by the same light dose is greater in animals previously maintained under a short versus long photoperiod. Moreover, patterns of relative immunoreactivity for long and short days at select irradiances correspond to the behavioral phase-shift data under nearly identical conditions.

5) Photoperiod history does not affect melatonin suppression by light in Siberian hamsters. Overlapping fluence response curves for melatonin suppression in long and short day animals could be explained by potential species differences given all prior related work had been done in the Syrian hamster model.

However, follow up studies confirming enhanced phase shifting under short days in Siberian hamsters indicate otherwise. The apparent disassociation between the photoperiod effects on light response for phase resetting and melatonin suppression suggests a mechanism downstream of the common retinal input pathway.

Together, these studies not only characterize sensitivity under different photoperiods for the first time but also, begin to hone in on the mechanism responsible for this plasticity. Specifically, there is a consistent robust short day increase in sensitivity to light for phase resetting at different circadian phases and in different hamster species. The first key to identifying the physiology underlying the marked increase in light sensitivity under short days comes from our SCN studies. Photoperiod differences in photic induction of all three proteins, which reflect different points along the signal transduction cascade, indicate the responsible mechanism must lie upstream of or at the postsynaptic membrane of the SCN. However, this still leaves a large number of potential candidate mechanisms, from the behavior of the animal regulating the light available to be transduced by photoreceptors to the photoreceptors themselves to the post-synaptic receptor of the SCN.

Our light sampling study provides evidence that photoperiod effects on photic sensitivity are not due to some change in overt behavior but rather suggest that there must be some physiological mechanism underlying the phenomenon. Photoperiod

influences have to be occurring at least at the level of the retina since the amount of light received does not vary by either photoperiod or circadian phase. If the photoperiod differences in sensitivity were in fact due to relative photon dose to the eye, photoperiod differences for all light responses- phase shifting, induction of proteins and melatonin suppression- would be expected. Therefore, a lack of photoperiod difference in melatonin suppression is consistent with findings from our light sampling study.

The melatonin suppression results further suggest the mechanism must reside downstream of the common retinal input pathway. Consistent with this idea, the effects of photoperiod on phase resetting are maintained when prior duration of darkness is controlled (e.g. phase delay study) and therefore, not due to differences in adaptation at the photoreceptor level. In fact, the combined findings of photoperiod effects on photic induction of SCN proteins (especially pERK) but not melatonin suppression leave us with only a narrow set of candidate mechanisms since so much of the physiology for these responses in shared.

Light for photic resetting and melatonin suppression are both thought to be transduced by melanopsin ipRGCs (Gooley et al., 2001; Brainard et al., 2001; Thapan et al., 2001; Provencio et al., 2002; Hattar et al., 2002, 2003). Information about light for these responses is then conveyed from the ganglion cells to the SCN via the same monosynaptic projection, the RHT (Moore and Klein, 1974; Gooley et al., 2001). Glutamate mediates the effects of light on the circadian system through its role as transmitter at the synapse between the RHT and SCN (Liou et al. 1986; Meijer et al.

1988; Colwell and Menaker 1992; Ding et al. 1994; Shirakawa and Moore 1994; Ebling 1996; Mintz et al. 1999; Obrietan et al. 1998), acting on both N-methyl-d-aspartate (NMDA) and amino-methyl proprionic acid/kainate (AMPA/KA) iontotropic glutamate receptors (GluRs) (Colwell and Menaker 1992; Rea et al. 1993; Ebling 1996; Colwell 2001; Pennartz et al. 2001). One study in Syrian hamsters has found that NMDA receptor activation is necessary for photic induction of per1 but not melatonin suppression, suggesting these particular circadian responses to light may follow separate transduction cascades within the SCN (Paul et al., 2003). Since we now know that photoperiod modulation of photic sensitivity is observed for Per1 (and even earlier in the transduction cascade, for pERK) but not for melatonin suppression, it is possible that only one of these pathways is photoperiod sensitive.

Another potential mechanism by which photoperiod adjustments to light sensitivity may occur includes changes in postsynaptic receptor density or sensitivity. It is also possible that the photoperiod effects of light act via a mechanism that is specific to a particular population of neurons within the SCN. Certainly, a number of studies have identified distinct sub-regions within the ventrolateral (vl) and dorsomedial (dm) SCN that demonstrate a differential response to light (Leak and Moore, 2001; Schwartz, 2009). One study in rats has further suggested that the vlSCN and dmSCN neurons are responsible for circadian entrainment and acute suppression of melatonin, respectively (Schwartz et al., 2009). Therefore, photoperiod influences on photic sensitivity may specifically involve the vlSCN (and not the dlSCN) subpopulation of neurons.

Serotonin also modulates photic response via direct innervation of the SCN from the midbrain raphe nucleus (Moore et al., 1978; Meyer-Bernstein and Morin, 1996; Muscat et al., 2005; Sollars et al., 2006a, 2006b). In particular, when serotonergic neurons of the mid raphe nucleus are lesioned, photic sensitivity for phase resetting of activity rhythms is altered in hamsters (Muscat et al., 2005). Though this neurotransmitter may modulate RHT transmission through both pre- and post-synaptic mechanisms in the SCN (Morin and Blanchard, 1991; Glass et al., 1994; Pickard et al., 1996; Weber et al., 1998), activation of one particular receptor subtype on the presynaptic receptors of RHT terminals (5HT-1B) has been shown to dampen a variety of light responses, including the effects of light on phase resetting, induction of the immediate early gene cfos and melatonin suppression (Pickard et al., 1996; Rea and Pickard, 2000). Again, there are robust effects of photoperiod on light sensitivity for photic phase shifts and cfos induction in the SCN; however, melatonin suppression does not differ between long and short day animals. Therefore, a presynaptic 5HT-1B receptor mechanism is unlikely to account for our combined findings. Yet, it is still possible that serotonin plays a role in the physiology underlying photoperiod modulation of photic sensitivity via a different receptor subtype and/or along postsynaptic SCN processes.

Photoperiod changes in photic sensitivity may relate to Seasonal Affective Disorder (SAD), a form of depression that demonstrates a seasonal pattern. Symptoms of SAD typically present during Fall and Winter months and spontaneously remit in the Spring, including depressed mood, increased need for sleep, weight gain and

carbohydrate cravings. Within a short time span, bright white light was found to suppress melatonin in humans (Lewy et al., 1980) and effectively treat SAD (Lewy et al., 1981). It has therefore been suggested that the pathophysiology underlying the disorder may be associated with the effects of light on the circadian system (Sohn and Lam, 2005 for review; Glickman et al., 2006).

Interestingly, those with SAD show an increased duration of melatonin secretion in winter akin to the photoperiod changes in waveform found in hamster models (Wehr et al., 2001). Since healthy subjects do not show this same seasonal change in nocturnal melatonin secretion outside of laboratory conditions, it was suggested that patterns of light exposure and/or alterations in photic sensitivity may be different in those with SAD (Wehr et al., 2001). Separate studies that have assessed exposure to natural light across seasons find winter levels to be statistically similar in SAD patients and normal healthy subjects (Guillemette et al., 1998; Graw et al., 1999). ERG studies show sensitivity differences in the opposite direction of the photoperiod effects demonstrated here in hamsters, with significantly lower retinal sensitivity in patients with SAD as compared to healthy controls during winter months (Hebert et al., 2004). Since our melatonin findings suggest *photoperiod* modulation of light sensitivity is occurring downstream of the retina, ERG data in these patients may be due to a seasonal variable other than daylength. In addition, there may be species differences in the photoperiod effects on circadian response to light.

Future studies should determine whether short photoperiod histories could serve to enhance photic sensitivity for phase resetting in humans. Evidence suggesting

it might comes from studies examining the effects of sleep duration on phase resetting by bright light pulses, which demonstrate greater phase shifts within subjects after sleeping for 9 versus 6 hours. This enhanced response to light with longer periods of sleep was maintained in two different regions of the PRC (Burgess and Eastman, 2005, 2006). Since sleep largely determines the duration of darkness experienced by humans, these findings could represent a similar photoperiod effect to what we report here in hamsters.

In addition to the well-established efficacy in alleviating the symptoms associated with SAD, light therapy has been successfully employed for a number of other applications in humans, including treatment of circadian sleep disturbances. Normal patterns of sleep and circadian rhythms may become disrupted as a result of a misalignment between the circadian system and the sleep–wake cycle or via direct impairment of the circadian system. Recognized circadian sleep disorders include delayed sleep-phase syndrome (DSPS), advanced sleep-phase syndrome (ASPS), free-running type, and irregular sleep–wake type (ICSD-2, 2005). In general, these disorders represent impairments in the generation of circadian rhythms and/or circadian entrainment processes.

Though healthy individuals are able to maintain a 24-hour period that closely matches environmental cycles most of the time, circadian rhythms can occasionally become transiently misaligned when the system is particularly taxed. Intercontinental travel across multiple time zones and shift work schedules are two common examples of such situations. In both cases, sleep times begin to deviate from the usual 24-hour

pattern and become desynchronized relative to other circadian functions (Kronauer et al., 1982). Furthermore, both jet lag and shift work have been associated with a variety of negative health consequences that are thought to be at least partly due to desynchronization of the circadian system, including cardiovascular disease, gastrointestinal disorders and cognitive as well as psychological disturbances (Moore-Ede et al., 1983; Czeisler et al., 1990; Eastman, 1990; Eastman et al., 1995).

Thus far, light therapy has been the most effective countermeasure for these circadian disturbances (Eastman, 1990; Rosenthal et al., 1990; Eastman et al., 1995; Samel and Wegmann, 1997; Boulos et al., 2002; Lack et al., 2005). Manipulating waveform has been proposed as a potential strategy for further facilitating circadian adjustment in humans (Harrison and Gorman, 2012). The present work suggests photoperiod history may be a particularly effective tool for modulating the potent effects of light.

REFERENCES

Aggelopoulos N and Meissl H (2000) Responses of neurones of the rat suprachiasmatic nucleus to retinal illumination under photopic and scotopic conditions. J Physiol 523:211-222

Agostino P, Ferreyra G, Murad A, Watanabe Y, Golombek D (2004) Diurnal, circadian and photic regulation of calcium/calmodulin-dependent kinase II and neuronal nitric oxide synthase in the hamster suprachiasmatic nuclei. Neurochem Int 44:617-625

Albrecht U, Sun Z, Eichele G, Lee C (1997) A differential response of two putative mammalian circadian regulators, mper1 and mper2, to light. Cell 91:1055-1064

Albrecht U, Zheng B, Larkin D, Sun Z, Lee C (2001) MPer1 and mper2 are essential for normal resetting of the circadian clock. J Biol Rhythms 16:100-104

Arendt J (1995) Melatonin and the Mammalian Pineal Gland, Chapman & Hall, London

Bartness T, Powers J, Hastings M, Bittman E, Goldman B (1993) The timed infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception and the photoperiod control of seasonal responses? J Pineal Res 15:161-190

Berson D, Dunn F, Takao M (2002) Phototransduction by retinal ganglion cells that set the circadian clock. Science 295:1070-1073

Binkley S, Stephens J, Riebman J, Reilly K (1977) Regulation of pineal rhythms in chickens: photoperiod and dark-time sensitivity. Gen Comp Endorcinol 32:411-416

Blumstein D, Daniel J, Evans C (2010) JWatcher Software, http://www.jwatcher.ucla.edu/

Boulos Z, Macchi M, Sturchler M, Stewart K, Brainard G, Suhner A (2002) Light visor treatment for jet lag after westward travel across six time zones. Aviat Space Environ Med 73:953–963

Brainard G, Richardson B, Peterborg L, Reiter R (1982) The effect of different light intensities on pineal melatonin content. Brain Res 233:75-81

Brainard G, Richardson B, Hurlbut E, Steinlechner S, Matthews S, Reiter R (1984) The influence of various irradiances of artificial light, twilight and moonlight on suppression of pineal melatonin content in the Syrian hamster. J Pineal Res 1:105-119

Brainard G, Hanifin J, Greeson J, Byrne B, Glickman G, Gerner E, Rollag M (2001) Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. J Neurosci 21:6405-6412

Burgess H and Eastman C (2005) Short nights attenuate light induced circadian phase advances. J Clin Endocrinol Metab 90:4437-4440

Burgess H and Eastman C (2006) Short nights reduce light-induced circadian phase delays in humans. Sleep 29:25-30

Butcher G, Doner J, Dziema H, Collamore M, Burgoon P, Obrietan K (2002) The p42/44 mitogen-activated protein kinase pathway couples photic input to circadian clock entrainment. J Biol Chem 277:29519-29525

Butcher G, Lee B, Obrietan K (2003) Temporal regulation of light-induced extracellular signal-regulated kinase activation in the suprachiasmatic nucleus. J Neurophysiol 90:3854-3863

Cardinali D, Larin F, Wurtman R (1972) Control of the rat pineal glad by light spectra. Proc Nat Acad Sci, 69:2003-2005

Chiesa J, Angles-Pujolras M, Diez-Noguera A, Cambras T (2006) History-dependent changes in entrainment of the activity rhythm in the Syrian hamster (mesocricetus auratus). J Biol Rhythms 21:45-57

Colwell C (2001) NMDA-evoked calcium transients and currents in the suprachiasmatic nucleus: gating by the circadian system. Eur J Neurosci 13:1420-1428

. Colwell C and Menaker M (1992) NMDA as well as non-NMDA receptor antagonists can prevent the phase-shifting effects of light on the circadian system of the golden hamster. J Biol Rhythms 7:125-136

Comas M, Beersma D, Spoelstra K, Daan S (2006) Phase and period responses of the circadian system of mice (mus musculus) to light stimuli of different duration. J Biol Rhythms 21:362-372

Coogan A, Piggins H (2003) Circadian and photic regulation of phosphorylation of ERK1/2 and Elk-1 in the suprachiasmatic nuclei of the Syrian hamster. J Neurosci 23:3085-3093

Cooper H, Tessonneaud A, Caldani A, Locatelli A, Richard S, Viguier-Martinez M (1993) Morphology and distribution of retinal ganglion cells (RGC) projecting to the suprachiasmatic nucleus in the sheep. Am Soc Neurosci Abstr 19:1704

Crawley J, Goodwin F (1980) Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol Biochem Behav 13:167-170

Cronly-Dillon J and Sharma S (1968) Effect of season and sex on the photopic spectral sensitivity of the three-spined stickleback. J Exp Biol 49:679-687

Crozier W and Pincus G (1927) Phototropism in Young Rats. J Gen Physiol 10:407-417

Czeisler C, Moore-Ede M and Coleman R (1982) Rotating shift-work schedules that disrupt sleep are improved by applying circadian principles. Science 217:460-463

Czeisler C, Johnson M, Duffy J, Brown E, Ronda J, Kronauer R (1990) Exposure to bright light and darkness to treat physiologic maladaptation to night work. New Engl J Med 322:1253-1259

Dacey D, Liao H-W, Peterson B, Robinson F, Smith V, Pokorny J, Yau K-W, Gamlin P (2005) Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. Nature 433:749-754

DeCoursey P (1986) Light-sampling behavior in photoentrainment of a rodent circadian rhythm. J Comp Physiol 159:161-169

Ding J, Chen D, Weber E, Faiman L, Rea M, Gillette M (1994) Resetting the biological clock: mediation of nocturnal circadian shifts by glutamate and NO. Science 266:1713-1717

Dunlap J (1999) Molecular bases for circadian clocks. Cell 96:271-290

Eastman C (1990) Circadian rhythms and bright light: recommendations for shift work. Work Stress 4:245-260

Eastman C, Boulos Z, Terman M, Campbell S, Dijk D.-J, Lewy A (1995) Light treatment for sleep disorders: consensus report. VI. Shift work. J Biol Rhythms 10:157-164

Ebling F (1996) The role of glutamate in the photic regulation of the suprachiasmatic nucleus. Prog Neurobiol 50:109-132

Elliott J and Tamarkin L (1994) Complex circadian regulation of pineal melatonin and wheel-running in Syrian hamsters. J Comp Physiol 174:469-484

Evans J, Elliott J, Gorman M (2004) Photoperiod differentially modulates photic and nonphotic phase response curves of hamsters. Am J Physiol Regul Integr Comp Physiol. 286:R539-546

Evans J, Elliott J, Gorman M (2005) Circadian entrainment and phase resetting differ markedly under dimly illuminated versus completely dark nights. Behavioural Brain Res 162:116-126

Evans A, Elliott J, Gorman M (2007) Circadian effects of light no brighter than moonlight. J Biol Rhythms 22:356

Evans J, Elliott J, Gorman M (2009) Dim nighttime illumination accelerates adjustment to timezone travel in an animal model. Curr Biol 19:R156-157

Field M, Maywood E, O'Brien J, Weaver D, Reppert S, Hastings M (2000) Analysis of clock proteins in mouse SCN demonstrates phylogenetic divergence of circadian mechanisms. Neuron 25:437-447

Frank D, Evans J, Gorman M (2010) Time-dependent effects of dim light at night on re-entrainment and masking of hamster activity. J Biol Rhythms 25:103-112

Gaddy J, Rollag M, Brainard G (1993) Pupil size regulation of threshold of light-induced melatonin suppression. J Clin Endocrinol Metab 77:1398-1401

Gamlin P, McDougal D, Pokorny J, Smith V, Yau K-W, Dacey D (2007) Human and macaque pupil responses driven by melanopsin-containing retinal ganglion cells. Vision Res 47:946-954

Glass J, Selim M, Rea M (1994) Modulation of light- induced c-fos expression in the suprachiasmatic nuclei by 5-HT1A receptor agonists. Brain Res 638:235-242

Glickman G, Hanifin J, Rollag M, Wang J, Cooper H, Brainard G (2003) Inferior retinal light exposure is more effective than superior retinal exposure in suppressing melatonin in humans. J Biol Rhythms 18:71-79

Glickman G, Byrne B, Pineda C, Hauck W, Brainard G (2006) Light therapy for seasonal affective disorder with blue narrow-band light emitting diodes (LEDs). Biol Psychiatry 59:502-507

Glickman G, Webb I, Elliott JA, Rheale M, Baltazar R, Lehman M, Gorman M (2012) Photic sensitivity for circadian response to light varies with photoperiod. J Biol Rhythms 27:308-318

Goldman B and Elliott J (1988) Photoperiodism and seasonality in hamsters: role of the pineal gland. In: Processing of Environmental Information in Vertebrates, edited by Stetson M, Springer-Verlag New York, Inc. USA. p. 203-218

Goldman B (2001) Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement. J Biol Rhythms 16:283-301

Gooley J, Lu J, Chou T, Scammell T, Saper C (2001) Melanopsin in cells of origin of the retinohypothalamic tract. Nature Neurosci 4:1165

Gooley J, Lu J, Fischer D, Saper C (2003) A broad role for melanopsin in nonvisual photoreception. J Neurosci 23:7093-7106

Gorman M, Elliot J, Evans J (2003) Plasticity of hamster circadian entrainment depends on light intensity Chronobio Int 20:233-248

Gorman M and Elliot J (2004) Dim light illumination alters coupling of circadian pacemakers in Siberian Hamsters, Phodopus sungorus. J Comp Physiol 190:631-639

Gorman M, Kendall M, Elliot J (2005) Scotopic illumination enhances entrainment of circadian rhythms to lengthening light: dark cycles. J Biol Rhythms 20:38-48

Graw P, Recker S, Sand L, Krauchi K, Wirz-Justice A (1999) Winter and summer outdoor light exposure in women with and without seasonal affective disorder. J Affect Disord 56:163-169

Guido M, Goguen D, De Guido L, Robertson H, Rusak B (1999) Circadian and photic regulation of immediate-early gene expression in the hamster suprachiasmatic nucleus. Neuroscience 90:555-571

Guillemette J, Hebert M, Paquet J, Dumont M (1998) Natural bright light exposure in the summer and winter in subjects with and without complaints of seasonal mood variations. Biol Psychiatry 44:622-628

Güler A, Ecker J, Lall G, Haq S, Altimus C, Liao H-W, Barnard A, Cahill H, Badea T, Zhao H, Hankins M, Berson D, Lucas R, Yau K-W, Hattar S (2008)

Melanopsin cells are the principle conduits for rod-cone input to non-image-forming vision. Nature 453:102-105

Hankins M and Lucas R (2002) The primary visual pathway in humans is regulated according to long-term light exposure through the action of a nonclassical photopigment. Curr Biol 12:191-198

Hannibal J, Jamen F, Nielsen H, Journot L, Brabet P, Fahrenkrug J (2001) Dissociation between light induced phase shift of the circadian rhythm and clock gene expression in mice lacking the PACAP type 1 receptor (PAC1). J Neurosci 21:4883-4890

Hannibal J, HIndersson P, Knudsen S, Georg B, Fahrenkrug J (2002) The photopigment melanopsin is exclusively present in pituitary adenylate cyclase-activating polypeptide-containing retinal ganglion cells of the retinohypothalanic tract. J Neurosci 22:RC191-197

Harrison E and Gorman M (2012) Changing the waveform of circadian rhythms: considerations for shift-work. Front Neurol 3:72

Hattar S, Liao H-W, Takao M, Berson D, Yau K-W (2002) Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. Science 295:1065-1070

Hattar S, Lucas R, Mrosovsky N, Thompson S, Douglas R, Hankins M, Lem J, Biel M, Hofmann F, Foster R, Yau K-W (2003) Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. Nature 424:76-81

Hebert M, Martin S, Lee C, Eastman C (2002) The effects of prior light history on the suppression of melatonin by light in humans. J Pineal Res 33:198-203

Hebert M, Beattie A, Tam B, Yatham L, Lam R (2004) Electroretinography in patients with winter seasonal affective disorder. Psychiatry Res 127:37-34

Hoffman K, Illnerová H, Vanecek J (1981) Effect of photoperiod and of one minute light at night-time on the pineal rhythm on N-acetyltransferase activity in the Djungarian Hamster Phodopus sungorus. Biology of Reproduction 24:551-556

Hoffman K, Illnerová H, Vanecek J (1985) Comparison of pineal melatonin rhythms in young adult and old djungarian hamsters (phodopus sungorus) under long and short photoperiods. Neurosci Letters 56:39-43

Honrado G, Johnson R, Golombek D, Spiegelman B, Papaioannou V, Ralph M (1996) The circadian system of c-fos deficient mice. J Comp Physiol 178:563-570

Hut R, Oklejewicz M, Rieux C, Cooper H (2008) Photic sensitivity ranges of hamster pupillary and circadian phase responses do not overlap. J Biol Rhythms 23:37-48

Illnerová H, Hoffman K, Vanecek J (1984) Adjustment of pineal melatonin and n-acetyltransferase rhythms to change from long to short photoperiod in the Djungarian hamster Phodopus sungorus. Neuroendocrinology 38:226-231

Jacob N, Vuillez P, Pevet P (1997) Photoperiod does not act on the suprachiasmatic nucleus photosensitive phase through the endogenous melatonin in the Syrian hamster. Neurosci Lett 229:117–120

Jasser S, Hanifin J, Rollag M, Brainard G (2006) Dim light adaptation attenuates acute melatonin suppression in humans. J Biol Rhythms 21:394-404

Johnson R, Moore R, Morin L (1988) Loss of entrainment and anatomical plasticity after lesions of the hamster retinohypothalamic tract. Brain Res Bull 460:297-313

Joy J and Turek F (1992) Combined effects on the circadian clock of agents with different phase response curves: phase shifting effects of triazolam and light. J Biol Rhythms 7:51-63

Keller F (1941) Light aversion in the white rat. Psychological Record 4:235-250

Klein D and Weller (1972) Rapid light-induced decrease in pineal serotonin N-acetyltransferase. Science 177:523-533

Klein D, Moore R, Reppert S, editors (1991) Suprachiasmatic Nucleus: The Mind's Clock. Oxford: Oxford University Press.

Kornhauser J, Nelson D, Mayo K, Takahashi J (1990) Photic and circadian regulation of c-fos gene expression in the hamster suprachiasmatic nucleus. Neuron 5:127-134

Kronauer R, Czeisler C, Pilato S, Moore-Ed M, Weitzman E (1982) Mathematical model of the human circadian system with two interacting oscillators. Am J Physiol Regul Integr Comp Physiol 242:3-17

Krout K, Kawano J, Mettenleiter T, Loewy A (2002) CNS inputs to the suprachiasmatic nucleus of the rat. Neuroscience 110:73-92

Lack L, Wright H, Kemp K, Gibbon S (2005) The treatment of early morning awakening insomnia with 2 evenings of bright light. Sleep 28:616-623

Lam R and Levitan R (2000) Pathophysiology of seasonal affective disorder: a review. J Psychiatry Neurosci 25:469-480

Lasko T, Kripke D, Elliott J (1999) Melatonin suppression by illumination of upper and lower visual fields. J Biol Rhythms 14:122-125

Lavoie M, Lam R, Bouchard G., Sasseville A., Charron M, Gagne A, Tremblay P, Filteau M, Hebert M (2009) Evidence of a biological effect of light therapy on the retina of patients with seasonal affective disorder. Biol Psychiatry 66:253-258

Leak R and Moore R (2001) Topographic organization of suprachiasmatic nucleus projection neurons. J Comp Neurol 433:312–334

Lee C, Etchegaray J, Cagampang F, Loudon A, Reppert S (2001) Posttranslational Mechanisms Regulate the Mammalian Circadian Clock. Cell 107:855-867

Lee H, Nelms J, Nguyen M, Silver R, Lehman M (2003) The eye is necessary for a circadian rhythm in the suprachiasmatic nucleus. Nature Neurosci 6:111-112

Lerchl A and Schlatt S (1993) Influence of photoperiod on pineal melatonin synthesis, fur color, body weight, and reproductive function in the female Djungarian hamster, Phodopus sungorus. Neuroendocrinology 57:359–364

Lerchl A (1995) Sustained response of pineal melatonin synthesis to a single one-minute light pulse during night in Djungarian hamster (*Phodopus sungorus*). Neurosci Lett 198:65-67

Lewy A, Wehr T, Goodwin F, Newsome D, Markey S (1980) Light suppresses melatonin secretion in humans. Science 210, 1267-1269

Lewy A, Wehr T, Goodwin F, Newsome D, Rosenthal N (1981) Manic-depressive patients may be supersensitive to light Lancet 1:383-384

Liou S, Shibata S, Iwasaki K, Ueki S (1986) Optic nerve stimulation-induced increase of release of 3H-glutamate and 3H-apratate but not 3H-GABA from the suprachiasmatic nucleus in slices of rat hypothalamus. Brain Res Bulletin 16:527-531

Lucas R, Douglas R, Foster R (2001) Characterization of an ocular photopigment capable of driving pupillary constriction in mice. Nature Neurosci 4:621-626

Lucas R, Hattar S, Takao M, Berson D, Foster R, Yau K-W (2003) Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. Science 299:245-247

Mathes A, Engel L, Holthues H, Wolloscheck T, Spessert R (2007) Daily profile in melanopsin transcripts depends on seasonal lighting conditions in the rat retina. J Neuroendocrinol 19:952-957

Maywood E, Mrosovsky N, Field M, Hastings M (1999) Rapid down-regulation of mammalian Period genes during behavioral resetting of the circadian clock. PNAS 96:15211-15216

Meijer J, van der Zee E, Dietz M (1988) Glutamate phase shifts circadian activity rhythms in hamsters, Neurosci Lett 86:177-183

Messager S, Ross A, Barrett P, Morgan P (1999) Decoding photoperiodic time through Per1 and ICER gene amplitude. Proc Natl Acad Sci USA 96:9938-9943

Messager S, Garabette M, Hastings, M, Hazlerigg D (2001) Tissue-specific abolition of Per1 expression in the pars tuberalis by pinealectomy in the Syrian hamster. NeuroReport 12:579-582

Meyer-Bernstein E and Morin L (1996) Differential serotonergic innervation of the suprachiasmatic nucleus and the intergeniculate leaflet and its role in circadian rhythm modulation. J. Neurosci. 16: 2097-2111

Milette J and Turek F (1986) Circadian and photoperiod effects of brief light pulses in male Djungarian hamsters. Biol Reprod 35:327-335

Minneman K, Lynch H, Wurtman R (1974) Relationship between environmental light intensity and retina-mediated suppression of rat pineal serotonin-N-acetyltransferase. Life Sci 15:1791-1796

Mintz E, Marvel C, Gillepsie C, Price K, Albers E (1999) Activation of NMDA receptors in the suprachiasmatic nucleus produces light-like phase shifts of the circadian clock in vivo. J Neurosci 19:5124-5130

Misslin R, Belzung C, Vogel E (1989) Behavioural validation of a light/dark choice procedure for testing anti-anxiety agents. Behavioural Process 18:119–132

Moga M and Moore R (1997) Organization of neural inputs to the suprachiasmatic nucleus in the rat. J Comp Neurol 389:508–534

Moore R (1995) Organization of the mammalian circadian system. CIBA Found Symp 183: 88-106

Moore R and Klein D (1974) Visual pathways and the central neural control of a circadian rhythm in pineal melatonin N-acetyltransferase activity. Brain Res 71:17-33

Moore R and Lenn N (1972) A retinohypothalamic projection in the rat. J Comp Neurol 146:1–14

Moore R, Halaris A, Jones B (1978) Serotonin neurons of the midbrain raphe: ascending projections. J Comp Neurol 180:417–438

Moore R, Weis R, Moga M (2000) Efferent projections of the intergeniculate leaflet and the ventral lateral geniculate nucleus in the rat. J Comp Neurol 420:398-418

Moore-Ede M, Czeisler C, Richardson G (1983) Circadian timekeeping in health and disease. N Engl J Med 309:530

Morin L and Blanchard J (1999) Forebrain connections of the hamster intergeniculate leaflet: comparison with those of lateral geniculate nucleus and retina. Vis Neurosci 16:1037-1054

Morin L and Wood R (2001) Stereotaxic Atlas of the Golden Hamster Brain. San Diego, CA: Academic Press

Morin L, Blanchard J, Provencio I (2003) Retinal ganglion cell projections to the hamster suprachiasmatic nucleus, intergeniculate leaflet and visual midbrain: bifurcation and melanopsin immunoreactivity. J Comp Neurol 465:401-416

Moriya T, Horikawa K, Akiyama M, Shibata S (2000) Correlative association between N-Methyl-D-Aspartate receptor mediated expression of period genes in the suprachiasmatic nucleus and phase shifts in behavior with photic entrainment of clock in hamsters. Molecular Pharmacology 58:1554-1562

Mrosovsky N and Hampton R (1997) Spatial responses to light in mice with severe retinal degeneration. Neurosci Lett 222:204–206

Mrosovsky N and Salmon P (1987) A behavioural method for accelerating re-entrainment of rhythms to new light-dark cycles. Nature 330:372-373

Mrosovsky N and Salmon P (1990) Triazolam and phase-shifting acceleration re-evaluated. Chronobiol Int 7:35-41

Mrosovsky N, Foster R, Salmon P (1999) Thresholds for masking responses to light in three strains of retinally degenerate mice. Jo Comp Physiol 184:423-428

Muscat L, Tischler R, Morin L (2005) Functional analysis of the role of the median raphe as a regulator of hamster circadian sensitivity to light. Brain Res 1044:59-66

Nelson D and Takahashi J (1991a) Comparison of visual sensitivity for suppression of pineal melatonin and circadian phase-shifting in the golden hamster. Brain Research 554:272-277

Nelson D and Takahashi J (1991b) Sensitivity and integration in a visual pathway for circadian entrainment in the hamster (Mesocricetus auratus) J Physiol 439:115-145

Nelson D and Takahashi J (1999) Integration and saturation within the circadian photic entrainment pathway of hamsters. Am J Physiol 277:R1351-1361

Niklowitz P, Lerchl A, Nieschlag E (1994) Photoperiodic responses in Djungarian hamsters (Phodopus sungorus): Importance of light history for pineal and serum melatonin profiles. Biology of Reproduction 51:714-724

Nuesslein-Hildesheim B, O'Brien J, Ebling F, Maywood E, Hastings M (2000) The circadian cycle of mPER clock gene product in the suprachiasmatic nucleus of the Siberian hamster encodes both daily and seasonal time E J Neurosci 12:2856-2864

Obrietan K, Impey S, Storm D (1998) Light and circadian rhythmicity regulate MAP kinase activation in the suprachiasmatic nuclei. Nat Neurosci 1:693–700

Owen J and Arendt J (1992) Melatonin suppression in human subjects by bright and dim light in Antarctica: time and season-dependent effects. Neurosci Lett 137:181-184

Panda S, Sato T, Castrucci A, Rollag M, DeGrip W, Hogenesch J, Provencio I, Kay S (2002) Melanopsin (Opn4) requirement for normal light-induced circadian phase shifting. Science 298:2213-2216

Panke E, Reiter R, Rollag M, Panke T (1978) Pineal serotonin N-acetyltransferase activity and melatonin concentrations in prepubertal and adult Syrian hamsters exposed to short daily photoperiods. Endocr Res Commun 5:311-324

Panke E, Rollag M, Reiter R (1980) Effects of photoperiod on hamster pineal melatonin concentrations. Comp Biochem and Physiol A: Physiol 4:691-693

Paul K, Fukuhara C, Tosini G, Albers H (2003) Transduction of light in the suprachiasmatic nucleus: evidence for two different neurochemical cascades regulating the levels of Per1 mRNA and pineal melatonin. Neuroscience 119:137-144

Pennartz C, Hamstra R, Geurtsen A (2001) Enhanced NMDA receptor activity in retinal inputs to the rat suprachiasmatic nucleus during the subjective night. J Physiol 532:181–194

Pickard G, Weber T, Scott P, Riberdy A, Rea M (1996) 5HT1B receptor agonists inhibit light-induced phase shifts of behavioral circadian rhythms and expression of the immediate-early gene c-fos in the suprachiasmatic nucleus. J Neurosci 16:8208-8220

Pittendrigh C and Daan S (1976) A functional analysis of circadian pacemakers in nocturnal rodents IV. Entrainment:Pacemaker as clock. J Comp Physiol 106:291-351

Pratt B and Goldman B (1986) Activity rhythms and photoperiodism of Syrian hamsters in a simulated burrow system. J Physiology and Behavior 36:83-89

Provencio I, Rodriguez I, Jiang G, Hayes W, Moreira E, Rollag M (2000) A novel human opsin in the inner retina. J Neurosci 20:600-605

Provencio I, Rollag M, Castrucci A (2002) Photoreceptive net in the mammalian retina. Nature 415:493

Puchalski W and Lynch G (1988) Daily melatonin injections affect the expression of circadian rhythmicity in Djungarian hamsters kept under a long-day photoperiod. Neuroendocrinology 48:280-286

Rea M and Pickard G (2000) A 5HT1B receptor agonist inhibits light-induced suppression of pineal melatonin. Brain Res 858: 424-428

Rea M, Buckley B, Lutton L (1993) Local administration of EAA antagonists blocks light-induced phase shifts and c-fos expression in hamster SCN. J Physiol 265:R1191–R1198

Refinetti R (2003) Effects of prolonged exposure to darkness on circadian photic responsiveness in the mouse. Chronobiol Int 20:417-440

Reme C, Wirz-Justice A, Rhyner A, Hofmann S (1986) Circadian rhythms in

light response of retinal disk-shedding and autophagy. Brain Res 369:356-360

Reppert S and Weaver D (2001) Molecular analysis of mammalian circadian rhythms. Ann Rev Physiol 63:647-676

Rollag, M, Panke E, Reiter R (1980) Pineal melatonin content in male hamster throughout the seasonal reproductive cycle. Proc. Soc. Exp. Biol. Med. 165:330-334

Rosenthal N, Joseph-Vanderpool J, Levendosky A, Johnston S, Allen R, Kelly K (1990) Phase-shifting effects of bright morning light as treatment for delayed sleep phase syndrome. Sleep 13:354-361

Ruger M, Gordin M, Beersma D, deVries B, Daan S (2005) Nasal versus temporal illumination of the human retina: effects on core body temperature, melatonin and circadian phase. J Biol Rhythms 20:60-70

Russo A, Kuburas A, Kaiser E, Raddant A, Recober A (2009) A Potential Preclinical Migraine Model: CGRP-Sensitized Mice. Mol Cell Pharmacol 1:264–270

Samel A and Wegmann H (1997) Bright light: a countermeasure for jet lag? Chronobiol Int 14:173-183

Schwartz W (2009) Circadian rhythms: A tale of two nuclei. Curr Biol 19:R460-462

Schwartz M, Wotus C, Liu T, Friesen W, Borjigin J, Oda G, de la Iglesia H (2009) Disassociation of circadian and light inhibition of melatonin release through forced desynchronization in the rat. Proc Natl Acad Sci USA 106:17540-17545

Sekaran S., Foster R, Lucas R, Hankins M (2003) Calcium imaging reveals a network of intrinsically light-sensitive inner-retinal neurons. Curr Biol 13:1290-1298

Semo M, Clas C, Ahmado A, Sugano E, Allen AE, Lawrence JM, Tomita H, Coffey P, Vugler A (2010) Dissecting a role for melanopsin in behavioural light aversion reveals a response independent of conventional photoreception. PLoS One 29:5

Shigeyoshi Y, Taguchi K, Yamamoto S, Takekida S, Yan L, Tei H, Moriya T, Shibata S, Loros J, Dunlap J, Okamura H (1997) Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the mPer1 transcript. Cell 91:1043-1053

Shimomura K and Menaker M (1994) Light-Induced Phase Shifts in tau Mutant Hamsters. J Biol Rhythms 9:97-110

Shirakawa T and Moore R (1994) Glutamate shifts the phase of the circadian neuronal firing rhythm in the rat suprachiasmatic nucleus in vitro. Neurosci Lett 178:47-50

Skene D, Pevet P, Vivien-Roels B, Masson-Pevet M, Arendt J (1987) Effect of different photoperiods on concentration of 5-methoxtryptophol and melatonin in the pineal gland of the Syrian hamster. J Endocrinol 114:301-309

Sohn C and Lam R (2005) Update on the biology of seasonal affective disorder. CNS Spectr 10:635-646

Sollars P, Simpson A, Ogilvie M, Pickard G (2006a) Light-induced Fos expression is attenuated in the suprachiasmatic nucleus of serotonin 1B receptor knockout mice. Neurosci Lett 401:209-213

Sollars P, Ogilvie M, Simpson A, Pickard G (2006b) Photic entrainment is altered in the 5-HT1B receptor knockout mouse. J Biol Rhythms 21:21-32

Sumová A, Trávnícková Z, Illnerová H (1995) Memory on long but not on short days is stored in the rat suprachiasmatic nucleus. Neurosci Lett 200:191-194

Sumová A, Trávnicková Z, Peters R, Schwarts W, Illnerová H (1995) The rat suprachiasmatic nucleus is a clock for all seasons. PNAS 92:7754-7758

Sumová A and Illnerová H (1996) Endogenous melatonin signal. does not mediate the effect of photoperiod on the rat suprachiasmatic nucleus. Brain Res 725:281-283

Sumová A, Jác M, Sládek M, Sauman I, Illnerová H (2003) Clock gene daily profiles and their phase relationship in the rat suprachiasmatic nucleus are affected by photoperiod. J Biological Rhythms 18:134-144

Takahashi J, DeCoursey P, Bauman L, Menaker M (1984) Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. Nature 308:166-168

Takahiko H and Yasuo T (1988) Seasonal variation of spectral sensitivity in cray fish retinula cells. Comp Biochem Physiol 81A:529-533

Takumi T, Matsubara C, Shigeyoshi Y (1998) A new mammalian period gene predominantly expressed in the suprachiasmatic nucleus. Genes to Cells 3:167-176

Tei H, Okamura H, Shigeyoshi Y, Fukuhara C, Ozawa R, Hirose M, Sakaki Y (1997) Circadian oscillation of a mammalian homologue of the Drosophila period gene. Nature 389:512-516

Thapan K, Arendt J, Skene D (2001) An action spectrum for melatonin suppression: evidence for a novel non-rod, non-cone photoreceptor system in humans. J Physiol 535:261-267

Thompson C, Stinson D, Smith A (1990) Seasonal affective disorder and season dependent abnormalities of melatonin suppression by light. Lancet 336:703-706

Tosini G and Menaker M (1996) Circadian rhythms in cultured mammalian retina. Science 272:419-421

Tournier B, Menet J, Dardente H, Poirel V, Malan A, Masson-Pevet M, Pevet P, Vuillez P (2003) Photoperiod differentially regulates clock genes' expression in the suprachiasmatic nucleus of Syrian hamster. Neurosci 118:317-322

Trávnicková Z, Sumová A, Peters R, Schwartz W, Illnerová H (1996) Photoperiod-dependent correlation between light-induced SCN c-Fos expression and resetting of circadian phase. Am J Physiol 271:R825-31

vanderLeest H, Houben T, Michel S, Deboer T, Albus H, Vansteensel M, Block G, Meiier J (2007) Seasonal encoding by the circadian pacemaker of the SCN Current Biol 17:468-473

vanderLeest H, Rohling J, Michel S, Meijer J (2009) Phase shifting capacity of the circadian pacemaker determined by the SCN neuronal network organization. PLoS ONE 4:e4976

Visser E, Beersma D, Daan S (1999) Melatonin suppression by light in humans is maximal when the nasal part of the retina is illuminated. J Biol Rhythms 14:116-121

vonGall C, Noton E, Lee C, and Weaver D (2003) Light does not degrade the constitutively expressed BMAL1 protein in the suprachiasmatic nucleus. Eur J Neurosci 18:125-133

Watanabe T, Naito E, Nakao N, Tei H, Yoshimura T, Ebihara S (2007) Bimodal clock gene expression in mouse suprachiasmatic nucleus and peripheral tissues under a 7-hour light and 5-hour dark condition. J Biol Rhythms 22:58-68

Watson R, Wiegand S, Clough R, Hoffman G (1986) Use of cryoprotectant to maintain long-term peptide immunoreactivity and tissue morphology. Peptides 7:155-159

Weber E, Gannon R, Rea M (1998) Local administration of serotonin agonists blocks light-induced phase advances of the circadian activity rhythm in the hamster. J Biol Rhythms 13:209-218

Wehr T, Moul D, Barbato G, Giesen H, Seidel J, Barker C, Bender C (1993) Conservation of photoperiod responsive mechanisms in humans. Am J Physiol 265:R846-857

Wehr R, Duncan W, Sher L, Aeschbach, Schwatrz P, Turner E, Postolache T, Rosenthal N (2001) A circadian signal of change of season in patients with Seasonal Affective Disorder. Arch Gen Psychiatry 58:1108-1114

Welker W (1959) Escape, exploratory, and food-seeking responses of rats in a novel situation. J Comp Physiol Psychol 52:106-111

Wong K, Dunn F, Berson D (2005) Photoreceptor adaptation in intrinsically photosensitive retinal ganglion cells. Neuron 48:1001-1010

Yan L and Silver R (2008) Day-length encoding through tonic photic effects in the retinorecipient SCN region. E J Neurosci 28:2108-2115

Yan L, Foley N, Bobula J, Kreigsfeld L, Silver R (2005) Two antiphase oscillators occur in each suprachiasmatic nucleus of behaviorally split hamsters. J Neurosci 25:9017-9026

Yan L, Silver R, Gorman M (2010) Reorganization of suprachiasmatic nucleus networks under 24-h LDLD conditions. J Biol Rhythms 25:19-27

Zawilska J, Lorenc A, Berezinska M, Vivien-Roels B, Pevet P, Skene D (2007) Photoperiod-dependent changes in melatonin synthesis in the turkey pineal gland and retina. Poult Sci 86:1397-1405

Zeitzer J, Dijk D-J, Kronauer R, Brown E, Czeisler C (2000) Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. J Physiol 526:695-702