Circadian, Neuroendocrine and Neurobehavioral Effects of Polychromatic Light in Humans

by

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A thesis submitted in accordance with the requirements of the University of Surrey for the degree of Doctor of Philosophy

Thomas Jefferson University
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Abstract

In the last eighteen years there has been the identification of a novel photopigment, melanopsin, and its subsequent localization to human intrinsically photosensitive retinal ganglion cells (ipRGCs). Since melanopsin’s peak sensitivity is in the short wavelength portion of the visible spectrum (from 447 nm to 484 nm), there has been a steady increase in studies investigating the physiological effects of blue light. This thesis examines polychromatic light mixtures of blue light for circadian, neuroendocrine and neurobehavioral effects in humans.

White blue-enriched fluorescent lamps were tested at equal photon densities for increased efficacy for melatonin suppression, increased alertness, and circadian phase shifting. Results demonstrated that compared to white fluorescent light, blue-enriched fluorescent light was significantly stronger for suppressing melatonin and resulted in significantly reduced subjective sleepiness. Blue-enriched light, however, was not significantly stronger in eliciting circadian phase-delay or increasing objective measures of alertness.

Next, blue-appearing narrowband solid-state light was examined for its ability to acutely suppress nocturnal melatonin as well as enhance cognitive performance and alertness in healthy men and women when compared to dim white lighting. The results demonstrated that narrowband blue solid-state light was significantly stronger for melatonin suppression compared to dim white light. Subjective and objective assessments of alertness, however, were not significantly increased by blue-enriched light exposure.

The final study tested the hypothesis that certain combinations of light wavelengths are additive or opponent to the photoreceptor system that mediates the melatonin suppression. The results demonstrated that the melatonin suppression responses to dual narrow bandwidth light combinations were not significantly different from single wavelength exposures.

Taken together, the results suggest that melanopsin sensitivity is not the sole consideration for predicting the efficacy of white polychromatic lighting. The different effects of blue light on alertness, circadian phase-shifting and melatonin suppression imply a either a context dependent sensitivity and/or differential involvement of the classical photoreceptors in these light responses.
Acknowledgements

The support and encouragement of my family and friends goes without saying. I wish to dedicate this thesis to my family, especially my wife, Renee and daughters Megan and Nicole, who listened to my complaints and gave willingly of their time that I might further my education. Nicole and Megan inspired me with their dedication to their own studies.

I wish to thank the many colleagues and friends who have been so encouraging through the process, but chief among them are my supervisors Professors George Brainard and Debra Skene for their steadfast support, encouragement, and advice throughout. Sincere thanks to Dr. Steven Lockley and colleagues from Brigham and Women’s Hospital for training myself and the Thomas Jefferson University Light Research Program staff on the proper execution of the multiple day studies. I would also like to thank Dr. Namni Goel for her help and statistical expertise as well as Dr. Robert Levin, Dr. Joel Pokorny and Dr. Rigel Woida for their light source guidance.

The Thomas Jefferson University Light Research Program staff were invaluable to carrying out the experiments described in this thesis. A heartfelt thanks goes out to Benjamin Warfield; a true Renaissance man. His talents are on display throughout this thesis. So many colleagues were involved and I truly appreciate all of their efforts but specifically I would like to recognize the efforts of Melissa Ayers, Dr. Michael Jablonski and Kate Cecil.

I would like to express my sincere thanks to the funding agencies involved in sponsoring the research including the National Space Biomedical Research Institute, Philips Lighting B.V., Apollo Lighting, and OSRAM Sylvania. Finally, without the invaluable financial support of my employer, Thomas Jefferson University, none of this would have been possible.

My interest in science was sparked by quite a remarkable woman, Frances Hanifin. My Aunt Frances was one of only a handful of female engineers who worked on the NASA Apollo Lunar Module. She supported me through my early college years and was always fascinated by the work I was doing. Finally, to my late father, John, and mother, Mary, whom I lost during the early years of this journey, I know you would have been proud.
Statement of originality

This thesis and the work to which it refers are the results of my own efforts. Any ideas, data, images or text resulting from the work of other (whether published or unpublished) are fully identified as such within the work and attributed to their originator in the text, bibliography or in footnotes. This thesis has not been submitted in whole or in part for any other academic degree or professional qualification.

I agree that the University has the right to submit my work to the plagiarism detection service TurnitinUK for originality checks. Whether or not drafts have been so-assessed, the University reserves the right to require an electronic version of the final document (as submitted) for assessment as above.
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Abbreviations

AANAT  arylalkylamine-N-acetyltransferase
ANOVA  analysis of variance
AUC   area under the curve
BDI   Beck’s depression inventory
BWH   Brigham and Womens Hospital
CBC   complete blood count
CCFL  cold cathode fluorescent lamp
CCT   correlated color temperature
CP    constant posture
CR    constant routine
DD    constant darkness
DLMO  dim light melatonin onset
DMH   dorsomedial hypothalamic nucleus
dmSCN dorsomedian suprachiasmatic nuclei
DS    digit span
dSPZ  dorsal subparaventricular zone
DSST  digit symbol substitution task
ECG   electrocardiogram
ED    effective dose
EDTA  ethylenediaminetetraacetic acid
EEG   electroencephalogram
fMRI  functional magnetic resonance imaging
GHT   geniculo-hypothalamic tract
h     hour
HIOMT hydroxyindole-O-methyltransferase
IGL   intergeniculate leaflets
ipRGCs intrinsically photosensitive retinal ganglion cells
IRB   institutional review board
IV    intravenous
K     degrees Kelvin
KDT   Karolinska drowsiness test
KSS   Karolinska sleepiness scale
λ_max peak wavelength
LC    locus coeruleus
LD    light dark cycle
LE    light exposure
LED   light emitting diode
LL    constant light
MMPI  Minnesota multiphasic personality inventory
nm    nanometer
NTB   neurobehavioral testing battery
PACAP pituitary adenylate cyclase activating peptide
PDA   personal digital assistant
POMS  profile of mood states
POT   primary optic tract
PRC   phase response curve
PT    pars tuberalis
PTA   pretectal areas
<table>
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<td>PVT</td>
<td>psychomotor vigilance task</td>
</tr>
<tr>
<td>rd/rd</td>
<td>rodless</td>
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<tr>
<td>rd/rd cl</td>
<td>rodless and coneless</td>
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<tr>
<td>RHT</td>
<td>retinohypothalamic tract</td>
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<tr>
<td>RPE</td>
<td>retinal pigment epithelium</td>
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<tr>
<td>RT</td>
<td>reaction time</td>
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<tr>
<td>SAFTEE</td>
<td>systematic assessment for treatment emergent effects</td>
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<td>seasonal affective depression</td>
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<td>suprachiasmatic nuclei</td>
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<td>SD</td>
<td>standard deviation</td>
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<td>SEM</td>
<td>standard error of the mean</td>
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<td>Thomas Jefferson University</td>
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<tr>
<td>USUHS</td>
<td>Uniformed Services University of Health Sciences</td>
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<tr>
<td>VA</td>
<td>vertebrate ancient</td>
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<td>VLPO</td>
<td>ventrolateral preoptic nuclei</td>
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<td>vlSCN</td>
<td>ventrolateral suprachiasmatic nuclei</td>
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CHAPTER 1 INTRODUCTION

1.1 Light and the circadian system

Light is a key component to life on earth. The sun’s irradiance provides the biosphere with warmth, energy for photosynthesis, and provides signals that all living organisms use to link themselves to the natural day/night rhythm generated by the rotation of the planet (Devlin and Kay, 2001). Light supports vision and allows organisms to navigate their surroundings but it also operates at a level below consciousness controlling the daily hormonal rhythms that control homeostasis.

Rhythms range in length from microseconds such as heart rate (Bonnemeier et al., 2003) to yearly or circannual such as the fattening and hibernation of the ground squirrel (Pengelley, 1974). There are internal biological cycles such as menstrual as well as other cycles controlled by the moon or tides. Light can play a role in these other non-circadian rhythms but this review will not delve into the specifics of this.

Throughout nature, a range of physiological responses oscillate with the 24 hour rhythm of the day or a circadian rhythm. The circadian timing system controls daily rhythms such as sleep and wakefulness, body temperature, hormonal secretion, and other physiological parameters. These inherent rhythms persist even when the organism’s environment remains in a constant state. This indicates that these rhythms are under the control of endogenous oscillators or the circadian timing system. The circadian timing system allows the organism to anticipate and prepare for the profound changes in its natural environment at dawn and dusk. Although light is the primary stimulus for regulating the circadian system (Czeisler, 1995), other external stimuli such as the timing of sound, temperature, and social cues may also influence physiological functions (Aschoff, 1981; Wetterberg, 1993).

A rhythm can be represented as a repeating waveform on a continuous line running from the past on the left to the future on the right. A rhythm that continues unabated in constant conditions is described as freerunning. For example, if you were able to live in a time-free, light and temperature constant condition environment you would have a rhythm of sleeping and waking that would continue or freerun (Aschoff, 1981). The length of time in between
consecutive awakenings would be the period length of the freerun or a measure of your personal internal clock represented by the Greek letter tau (Binkley, 1990).

Figure 1. A representation of the waveform of a circadian rhythm. (Modified from Binkley, 1990)

When the timing of a freerunning rhythm is synchronized by a repeating external time signal, the rhythm is entrained (Aschoff, 1981). This signal that gives time cues is known by the German definition of zeitgeber or “time giver”. Light is the primary zeitgeber for most organisms on Earth (Czeisler, 1995). A particular time point on a rhythm can be used to mark circadian phase and the length between identical timepoints on a rhythm is known as period tau. The time difference between two phases is called the phase angle. When a rhythm is altered such that its peaks occur later in time then the phase of the rhythm is said to be delayed; when a rhythm is altered so that it peaks occur earlier in time then the rhythm is advanced (Binkley, 1990).

Circadian clocks all have the ability to respond to light at dusk and dawn. The appearance of light at dawn or diminishment of light at dusk causes changes to the phase of the clock to keep it synchronized. Light detected in the early morning advances the circadian oscillator
while light of an equal amount in the evening causes a delay. This response to light
information given at different times of the day results in a phase response curve (PRC). The
existence of a light PRC is evident in the vast majority of terrestrial plants, insects and
animals studied but varies greatly in shape between organisms (Johnson, 1990). The PRC
often is skewed to morning or evening with the appearance of dead zones or insensitivity to
light during the course of the day. However, a carefully controlled laboratory study dispelled
the notion of a dead zone and showed no evidence of clock insensitivity (Khalsa et al., 2003).
More recently, light PRCs have been constructed for a short, 1 h pulse of bright white light
(St. Hilaire et al., 2012) as well as for blue light (Revell et al., 2012).

1.1.1 Input pathways

1.1.1.1 Intrinsically photosensitive retinal ganglion cells

Intrinsically photosensitive retinal ganglion cells (ipRGCs) are the initial interactive point for
nonvisual responses between the nervous system and the light environment. Studies have
confirmed that the suprachiasmatic nuclei (SCN) receive environmental photic input from a
specialized subset of ipRGCs (Provencio et al., 2000; Gooley et al., 2001; Berson et al., 2002;
Hannibal et al., 2002; Hattar et al., 2002). Using immunohistochemistry and double labeling
studies, murine ipRGC anatomy has been described by multiple laboratories (Berson et al.,
2010; Ecker et al., 2010) and five types of ipRGCs, which have differing locations, dendritic
processes and cell bodies, have been identified.

In rodents, monkeys, and humans, melanopsin has been found in a specific subtype of
ipRGCs which have an expansive dendritic network and project to a number of nonvisual
nuclei, including the suprachiasmatic nuclei, intergeniculate leaflets, olivary pretectal nuclei,
preoptic nuclei and subparaventricular zones (Provencio et al., 2000; Gooley et al., 2001;
2003; Berson et al., 2002; Hannibal et al., 2002; Hattar et al., 2002; 2006; Provencio et al.,
2002). IpRGC's respond to light on their own when separated physically or blocked by drugs
from receiving input from other neurons (Berson et al., 2002; Hattar et al., 2002; Lucas et al.,
2003; Dacey et al., 2005). These unique cells comprise 1-3% of all retinal ganglion cells, and
their light responses appear to parallel those for circadian entrainment and melatonin suppression (Berson et al., 2002; Hattar et al., 2003).

In humans, these ipRGCs are found within the inner retina and have an extensive dendritic tree that forms a photoreceptive network (Rollag et al., 2003; Hannibal et al., 2004; Dacey et al., 2005). IpRGCs comprise approximately 0.2% to 0.8% of all ganglion cells present in the human retina (1-3% in rodents), and their dispersion of dendritic processes seems to encompass the entire retinal area as observed in earlier rodent studies (Hannibal et al., 2004; Dacey et al., 2005).

![Diagram of the neuroanatomy involved in sensory and nonvisual regulation.](Graphic design by Benjamin Warfield)

**Figure 2.** The diagram above provides a simplified layout of the neuroanatomy that is responsible for mediating both the sensory capacity of the visual system and the nonvisual regulation of circadian and neuroendocrine functions. Abbreviations: POT - primary optic tract; RHT - retinohypothalamic tract; ipRGC - intrinsically photosensitive retinal ganglion cell; IGL - intergeniculate leaflets; VLPO - ventrolateral preoptic nuclei; SCN - suprachiasmatic nuclei; PTA - pretectal areas; vSPZ - ventral subparaventricular zones.

**1.1.1.2 Retinohypothalamic tract**

Projections from the ipRGCs form the origin of the retinohypothalamic tract (RHT). The mammalian RHT is the primary neural projection to the circadian oscillator entraining circadian rhythms to the light-dark cycles of the environment (Moore, 1983). This pathway acts to convey information about external light conditions from the retina to several areas of
the hypothalamus, including the SCN, a primary site of the biological clock or central timing system in the brain. The RHT terminates primarily in the ventrolateral aspect of the SCN (Moore and Lenn, 1972; Moore et al., 1995). Even though it is known that the RHT projects to other areas of the hypothalamus (Pickard and Silverman, 1981) and that the SCN receives inputs from other visual areas of the brain, the retinal projection to the SCN via the RHT seems to be the primary pathway for photoperiod entrainment in mammals. This neural pathway is also directly involved in the suppression of melatonin production in the pineal gland in response to light (Klein and Moore, 1979). The retinal projection to the SCN has been studied for its role in synchronizing the endogenous oscillator which mediates circadian physiology.

Although the RHT has its densest projection in or around the SCN, this pathway has diffuse projections to other areas including the preoptic nuclei, anterior and lateral hypothalamic areas, retrochiasmatic area, dorsal hypothalamic nuclei, the intergeniculate leaflets, and the midbrain periaqueductal grey (Klein et al., 1991; Card, 1994; Hattar et al., 2006). Some of the functional roles of the other nonvisual projections from the retina have been elucidated (Gooley et al., 2003; Hattar et al., 2006). The ventrolateral preoptic nucleus (VLPO) is known to be integral in sleep/arousal state (Lu et al., 2002). Projections to the intergeniculate leaflet (IGL) are involved in regulation of circadian phase shifting and other integration of photoperiodic information (Morin, 1994). Projections to the ventral subparaventricular zone (vSPZ) are thought to be involved in the circadian and photic modulation of sleep and locomotor activity (Moore and Danchenko, 2002). The pretectal area (PTA) receives projections from the RHT and which contributes to the control of the pupillary light reflex in the absence of classical photoreceptors (Lucas, et al., 1999; 2003). Hattar and colleagues (2006) report a substantial projection to the dorsolateral area of the lateral habenula but this functional role is not well understood. In summary, these projections are thought to form an irradiance detection system providing photic information to several brain regions controlling numerous functions separate from areas of the brain that are involved in forming vision (Gooley et al., 2003).
1.1.1.3 Intergeniculate leaflet and geniculo-hypothalamic tract

The SCN receive a projection, the geniculo-hypothalamic tract (GHT), from neurons in the intergeniculate leaflet (IGL) and portions of the ventral lateral geniculate nucleus (vLGN) of the thalamus. When examined, the responses of putative GHT neurons to diffuse illumination using extracellular electrophysiological recordings the great majority of IGL neurons showed sustained ON responses to diffuse retinal illumination. These results indicate that GHT neurons may provide information about ambient light intensity to the suprachiasmatic nuclei (Morin, 1994). Although it is clear that the IGL is not necessary for photic entrainment, some changes on the ability of light signals effect phase and period control of the clock have been observed (Moore and Card, 1994). In addition to it being a secondary source of photic signaling to the circadian clock, the IGL plays a major role in the nonphotic regulation of the circadian system (Rosenwasser, 2003).

1.1.1.4 Other afferent inputs to the suprachiasmatic nuclei

A third major afferent projection to the SCN comes from the median raphe nucleus of the midbrain. These projections to the SCN are thought to have two functions. The first is the modulation of photic effects on the circadian pacemaker during the subjective night and the second being the mediation of nonphotic, behavioral state–related effects on the pacemaker during subjective day (Rosenwasser and Turek, 2005). For instance, photic entrainment is inhibited in the presence of high levels of arousal or locomotor activity, apparently through arousal related release of endogenous serotonin (Morin, 1999). In addition, it has been reported, in the rat, much like the hamster, over 40 brain regions have cells that project to the SCN (Krout et al., 2002) whose functions remain largely unknown.

1.1.2 Suprachiasmatic nuclei

The SCN are situated in the anterior part of the hypothalamus immediately dorsal and superior to the optic chiasm and bilateral to the third ventricle of the brain. They serve as the chief pacemaker of the circadian system controlling circadian rhythms in mammals (Klein et al., 1991). If this area is lesioned, the length and timing of sleep episodes become erratic.
while the total amount is maintained (Mistlberger et al., 1983). Other studies using metabolic mapping, tissue transplantation, and molecular rhythm analysis have shown the SCN to be able to maintain sustained rhythm as well as maintaining rhythms in peripheral tissues (Klein et al., 1991; Stokkan et al., 2001; Rossenwasser, 2003).

In mice, each of the two “pear-shaped” suprachiasmatic nuclei contains about 10,000 neurons (Abrahamson and Moore, 2001). The SCN can be subdivided into two parts: a ventrolateral (vlSCN, or core) and a dorsomedian part (dmSCN, or shell). Although early studies suggested that all SCN neurons were cell autonomous clocks (Welsh et al., 1995), recent studies demonstrated that they are not identical in function (Honma et al., 2012).

Figure 3. The flowchart above provides a simplified representation of the major inputs and outputs of the SCN. (Graphic design by Benjamin Warfield)

1.1.3 Output pathways

There are three major output pathways leading from the SCN. The largest portion of the efferent pathway travels through the regions just above the SCN (the ventral
subparaventricular zone (vSPZ)) and along the paraventricular hypothalamic nucleus (the
dorsal subparaventricular zone (dSPZ)) with a small portion ending in the dorsomedial
nucleus of the hypothalamus. Another pathway runs along the third ventricle, into the medial
preoptic area and then up into the paraventricular nucleus of the thalamus. The third pathway
runs along the base of the third ventricle, to the retrochiasmatic area and the capsule of the
ventromedial nucleus of the hypothalamus (Saper et al., 2005). These pathways are thought
to be necessary for body temperature regulation, locomotor activity, sleep-wake cyclicity and
feeding behavior.

A relatively small number of SCN axons also directly innervate areas also involved in
melatonin production. This pathway carries nonvisual information about light from the SCN
to the pineal gland via a multisynaptic pathway with connections being made sequentially in
the paraventricular hypothalamus, the upper thoracic intermediolateral cell column, and the
superior cervical ganglion. By way of this neuroanatomy, cycles of light and darkness which
are perceived through the eyes entrain SCN neural activity which, in turn, entrains the
rhythmic synthesis and production of melatonin from the pineal gland (Klein and Moore,
1979).

1.2 Circadian photoreception

Living organisms vary in their capacity to use visible and near-visible electromagnetic energy
for survival, however, a fundamental principle shared by all species is their ability to respond
to light stimuli: all photobiological responses are mediated by organic molecules that absorb
light quanta and then undergo physical-chemical changes. These light-induced changes
subsequently evoke broader physiological responses within the organism. This process is
termed phototransduction, and the specific organic molecules that absorb light energy to
initiate photobiological responses are called chromophores or photopigments. As a rule,
these photoactive molecules do not absorb energy equally across the electromagnetic
spectrum. Photopigment molecules or molecular complexes have their own characteristic
wavelength absorption spectrum that depends on their atomic structure (Grossweiner, 1989;
sensitivity, or its absorbance spectrum, is unique to that molecule.
1.2.1 Classical photoreceptors and opsins

Photoreceptors reside in a thin sheet of neural tissue located in the lining of the back of the eye called the retina. This layer extends forward toward the optical components of the eye which include the cornea, iris and lens. The retina was thought to be composed of five classes of neurons; photoreceptors, horizontal cells, bipolar cells, amacrine cells and ganglion cells (Rodieck, 1998). More recently, a sixth set of cells, the ipRGCs, have been identified and described (Hattar et al., 2002; Provencio et al., 2002). These cells are a small percentage of all ganglion cells, and their light responses will be discussed in detail later in this chapter. Each of the cell types, for the most part, occupies a layer in the retina as shown in Figure 4.

The retina contains over 100 million photoreceptors which serve, as their name suggests, to capture and to send information about the outside visual world to the brain. In vertebrates there are two general forms of visual photoreceptors called rods and cones. Rods are always cylindrical while cones, when found in the peripheral part of the retina are conical, hence their name.

The photoreceptors lie in the outer portion of the retina in the very back of the eye which results in light having to pass through the entirety of the retina in order to reach the outer segments of the photoreceptors. This may be due to the origins of the photoreceptors from neural tissue but it does have the advantage that they lay near the retinal pigment epithelium and choriocapillaris on either side of them. Thus, the photoreceptors, which are very metabolically active, have a rich supply of oxygen and nutrients. It is in these outer segments where the visual pigment molecules or opsins are located (Rodieck, 1998).

The membranous photoreceptor protein or opsin contains a pigment molecule called retinal. In rod cells, these together are called rhodopsin. In cone cells there are different types of opsins that combine with retinal to form pigments called photopsins. In humans, three different classes of photopsins (see Figure 5 in the cones react to different ranges of light frequency, long wavelength (red), middle wavelength (green) or short wavelength (blue). This number of photopsins and their sensitivity vary among species yet all serve to allow the visual system to distinguish color.
Figure 4. The basic cell types of the vertebrate retina. Light passes through the transparent ganglion layer to reach the rods and cones. The rods (R) and cones (C) convey visual information to the ganglion cells (G) through the bipolar cells (B). Horizontal cells (H) allow lateral connections between rods and cones. Amacrine cells (A) allow lateral connections between bipolar and ganglion cells. The optic nerve is formed from the axons of all the ganglion cells. A subset of ganglion cells (ipRGCs) also detects light directly and contain the mammalian photopigment melanopsin. (Modified from Foster, 2005)

Other alternate photoreceptors that were investigated to have a role in regulating the circadian system included both opsin-based molecules such as vertebrate ancient (VA) opsins (Philp et al., 2000) RPE-retinal G protein-coupled receptor (Jiang et al., 1993), peropsin (Sun et al., 1997), encephalopsin (Blackshaw and Snyder, 1999) as well as non-opsin molecules like biliverdin (Oren, 1996) and cryptochrome (Miyamoto and Sancar, 1998). An excellent
overview of nonclassical opsin photopigments found in the mammalian eye has been written by Kumbalasiri and Provencio (2005).

Figure 5. The approximate spectral sensitivity functions of the rods (rhodopsin) and three cones (short wavelength –blue; middle wavelength –green; and long wavelength –red) in humans. Peak sensitivities are 507 nm for rhodopsin, 440 nm for short wavelength cone, 540 nm for the middle wavelength cone and 565 nm for the long wavelength cone (Stockman and Sharpe, 1999).

1.2.2 Melanopsin

Melanopsin, a molecule found originally in frog-skin melanophore cells which sense and respond to light, was an early leading contender for the mammalian circadian photopigment,
since it is expressed in anatomical areas where nonvisual photoreceptors are likely to be present (Rollag, 1996; Provencio et al., 1998). Melanopsin shares predicted structural similarities with all known opsins, including seven transmembrane domains with a lysine residue in the 7th transmembrane domain needed for linkage of the retinoid chromophore.

Provencio and colleagues (2000) cloned both human and mouse melanopsins and showed predicted amino acid sequences of the transmembrane and loop domains to be 86% identical between mouse and human homologs and 57% identical to the frog homolog. Notably, human and all vertebrate melanopsins have a great sequence homology to invertebrate opsins.

This structural similarity also translates into functional harmony in that melanopsin is likely to operate with a biochemical independence allowing it to be located separate from chromophore regenerating tissues, such as the retinal pigment epithelium (RPE). In the human retina, it has been shown that melanopsin is co-stored with pituitary adenylate cyclase activating peptide (PACAP) which is a marker for the RHT (Hannibal et al., 2004). This identification and localization of melanopsin has ultimately led to a major change in our understanding of circadian photoreception.

Melanopsin is not required for normal circadian photoentrainment, and that other mechanisms for light input to the clock also play a role as concluded Panda and colleagues (2002) in the following study. Melanopsin-null (Opn4\(^{-/-}\)) mice were generated and exposed to constant darkness (DD), a 12-hour white light (800 lux)/12-hour dark (LD) cycle, and a normal LD cycle with 1 hour of 300 lux of white light during the dark phase. There were no significant differences between wild-type and Opn4\(^{-/-}\) mice regarding functioning of the SCN or the locomotor activity output of the clock in any of these conditions. However, when a 15-min pulse of monochromatic blue light (\(\lambda_{\text{max}} = 480\) nm, 10-nm half-peak bandwidth) of varying irradiance was administered 3 h after the onset of activity under constant darkness (a period when normal mice exhibit robust phase delays in response to light), it was found that there was a significant attenuation of phase delays compared to wild-type mice. Also, when placed in constant light (LL) cycle, Opn4\(^{-/-}\) mice exhibited a shorter period length than wild-type, though both were lengthened the LL cycle.
In contrast to rods and cones, melanopsin is thought to act as both a photopigment and a photoisomerase (Provencio et al., 2000). Following activation of melanopsin by light, a different portion of the light spectrum regenerates the chromophore and restores melanopsin photosensitivity (Melyan et al., 2005; Panda et al., 2005). Although it is well-established that melanopsin photopigment is most sensitive to short-wavelength light, recent studies suggest that melanopsin photoisomerase activity may increase this sensitivity by prior exposure to long-wavelength light (Koyanagi, et al., 2005; Mure et al., 2007; 2009). This potentiation by long wavelength (620 nm) light prior exposure to blue (480 nm) light as measured by ipRGC cell firing rates in mouse retina was not exhibited in a study by Mawad and Van Gelder (2008) and the physiological significance of melanopsin bistability remains in question.

1.2.3 Classical photoreceptor-rod/cone interaction

Abundant evidence shows that the melanopsin containing ipRGCs provide primary input for circadian and neuroendocrine regulation. It has also been shown, however, that rod and cone photoreceptors still play a role in this physiology (see Figure 5). Melanopsin- and cone-knockout mice show that the classical rod and cone photoreceptors can compensate for the loss of melanopsin and, at least partially mediate, light-induced circadian, neuroendocrine and neurobehavioral responses (Panda et al., 2002; Lucas et al., 2003; Dkhissi-Benyahya et al., 2007). By contrast, when both melanopsin is knocked out and the classical visual photoreceptors are compromised, animals lose all visual and nonvisual photoreceptive functions of the eye (Hattar et al., 2003; Panda et al., 2003). Further, cellular recording studies from nonhuman primate retinas have demonstrated that rod and cone cells can directly activate ipRGCs (Dacey et al., 2005). Data from human studies also suggest that the visual rods and cones may be involved in nonvisual light responses (Hebert et al., 2002; Figueiro et al., 2004; Jasser et al., 2006; Revell and Skene, 2007). Similar to classical retinal ganglion cells ipRGCs receive ON and OFF light signals from rods and cones (Belenky et al., 2003; Perez-Leon et al., 2006). Using mice where cone or rod photoreceptors were destroyed, rods were found to operate in two distinct retinal circuits to provide circadian photoentrainment over an extensive range of light intensities (Altimus et al., 2010). Using mice where human red cone opsin was expressed in the mice green cones, it was shown that rods drive circadian responses at very low irradiances and some circadian responses can be rod driven at even higher light intensities (Lall et al., 2010). In humans, interestingly, cones
are suggested to contribute substantially to circadian photoentrainment at low intensities and at the initiation of light exposure as described in a series of experiments where spectral content and irradiance were manipulated (Gooley et al., 2010). Different models of interaction between the melanopsin ganglion cells and classical rod and cone visual photoreceptors have been proposed, including the ideas that these photoreceptors are additive, stimulatory, opponent, or dynamic over time and intensity (Lucas et al., 2003; Lockley et al., 2003; Figueiro et al., 2004; Gall and Bieske, 2004; Rea et al., 2005; Dacey et al., 2005; Dkhissi-Benyahya et al., 2007; Revell et al., 2007; Zaidi et al., 2007; Pechacek et al., 2008; Mure et al., 2009; D.I.N, 2009; Gooley et al., 2010). Gooley and colleagues (2010) best summarize the current thinking that, much like other sensory systems such as touch and vision, multiple receptor subtypes respond differentially to the strength, frequency, and timing of stimuli to ensure appropriate physiologic responses.

1.3 Circadian physiology

Circadian physiology is essential in the daily functioning of animals, including humans. Biological activities such as body temperature, brain wave activity, hormone production, cell regeneration show regular patterns. The circadian timing system is integral to timing these biological events to the external light environment (Dunlap et al., 2004).

1.3.1 Primary oscillator – Suprachiasmatic nuclei

The SCN of the hypothalamus are the primary pacemakers for the human and mammalian circadian systems. The SCN distributes information about light, darkness, and biological time to other major central nervous system control centers including the limbic system, diencephalon, midbrain and spinal cord. The paired, bilateral SCN are heavily interconnected. Afferent projections from a multitude of nuclei reach the SCN, while efferent projections from the SCN influence endocrine responses, autonomic activity, and behavioral regulation (Klein et al., 1991; Dunlap et al., 2004).

As described earlier (section 1.1.2) cycles of light and darkness stimulate the retina, signals are sent from the retinohypothalamic tract (RHT) to multiple nuclei indicated in Figure 3.
While the SCN receives approximately 60% of the RHT afferents, other nuclei receive RHT afferents for other regulatory purposes.

1.3.2 Peripheral oscillators

Many tissues and organs in the body are able to generate circadian rhythms independently from the SCN (Yamazaki et al., 2000; Yoo et al., 2004). Liver cells, for example, appear to not need innervation from the SCN to maintain their rhythmicity and recent work has shown the inherent rhythms are sufficient to maintain functional significance (Atwood et al., 2011; Mohawk et al., 2012). There is evidence that the rhythms many of the peripheral oscillators are regulated both to each other as well as the external environment (Pezuk et al., 2010).

Humoral and neural signals are generated by the SCN which regulate the phases of peripheral oscillators (Kalsbeek et al., 2006; Prasai et al., 2011). Humoral signals in the form of melatonin or cortisol secretion as well as temperature changes have the capability of reaching every cell in the body. The autonomic nervous system has influence over rhythmicity in several organs including the liver, adrenals, pancreas, the gastrointestinal tract and adipose tissue (Buijs et al., 1999; 2001; Cailotto et al., 2009; Kreier et al., 2002; Malloy et al., 2012). This network of oscillators is quite complex and remains an area of intense research.

1.4 Pineal gland

The pineal gland is a small (in humans about the size of a pea) secretory organ located at base of the brain at the border between the mesencephalon and the diencephalon resembling a pine cone and closely associated with the 3rd ventricle. There are large variations in size and position even within species and this can vary according to time, age and physiological status (Arendt, 1995). It consists of two types of cells, the pinealocytes, which produce indoleamines and neuroglial cells (Vollrath, 1981).

During the development of some fish, reptiles and amphibians the pineal forms in two areas, an extracranial parietal organ and an intracranial pineal organ. The parietal organ in some of these lower vertebrates lies directly below the dorsal surface of the head and is often referred
to as the “third eye.” Thus, it can be directly photosensitive in some amphibians and fish.

The pineal maintains a dual role in avian species as well as reptiles as it maintains a self-generated rhythm that serves as a biological clock and is a secretory gland under the control of light input via the retina (review Arendt, 1995). The mammalian pineal gland acts as a secretory organ only, with the role of a self-sustaining rhythm generator being served by the SCN.

1.4.1 Anatomy

The structure of the pineal gland has been classified according to its form and relationship to the 3rd ventricle (Vollrath, 1981). Three types have been identified: (A) where the majority of pineal tissue lies near the 3rd ventricle; (AB) where the gland is elongated; and (ABC) where a substantial portion of the pineal lies superficially near the cerebellum.

The most important innervation of the mammalian pineal arises from post-ganglionic sympathetic fibers emanating from the superior cervical ganglion and is essential for the rhythmic production of melatonin, the primary hormone secreted from the pineal.

1.4.2 Indoleamine synthesis

There have been fifteen or more known 5-methoxyindoles described in the pineal gland. Their physiological significance is minor compared to melatonin. (Smith, 1983) Tryptophan, in addition to forming serotonin, undergoes decarboxylation to tryptamine and O-methylation by hydroxyindole-O-methyltransferase (HIOMT) to 5-methoxytryptamine. 5-methoxytryptamine may have some physiological role as it is photoperiod controlled with a day-night rhythm with high levels in the pineal during the day (Klein and Moore, 1979; Galzin et al., 1988). Some of the effects are similar to melatonin which may be due to N-acetylation and subsequent conversion to melatonin. 5-methoxytryptamine is metabolized by monoamine oxidase to 5-methoxindoleacetic acid and 5-methoxytryptophol. These indolic acids have similar concentrations and rhythms as 5-methoxytryptamine and may play a role in the retina (Skene, 1992).
1.5 Primary neuroendocrine hormone affected by light - melatonin

Melatonin, or N-acetyl-5-methoxytryptamine, is an indoleamine hormone synthesized and released by the pineal gland during the hours of darkness in all species studied to date. Melatonin was first isolated by dermatologist Aaron Lerner who was interested in skin pigmentation issues and the ability of pineal extracts to lighten frog skin (Lerner et al., 1958; Brainard, 1978). Numerous physiological functions are associated with melatonin, chief among them circadian rhythm regulation and seasonal reproduction (Arendt, 1995). Other physiological roles of melatonin include protection of tissues from oxidation (Reiter et al., 1994) and oncostatic effects (Blask et al., 2002). It is used as a therapeutic agent for jet lag following rapid time-zone changes, shiftwork sleep disturbances (Skene et al., 1996; Arendt et al., 1997) as well as metastatic cancer (Lissoni et al., 1995). Marketed as a dietary supplement, it is widely available in a number of countries including the USA (Bonn, 1996).

1.5.1 Melatonin synthesis and metabolism

The initial step in the production of melatonin is the uptake of the amino acids tryptophan or 5-hydroxytryptophan from the general circulation into the pineal gland. From there tryptophan is converted to 5-hydroxytryptophan by tryptophan-5-hydroxylase and/or 5-hydroxytryptophan is then decarboxylated to serotonin. Serotonin is then converted to melatonin by sequential actions of the enzymes arylalkylamine-N-acetyltransferase (AANAT) which provides the N-acetylation and HIOMT which provides the O-methylation (Ganguly et al., 2002). There is much species differentiation in the transcriptional and post-transcriptional mechanisms of regulation of AANAT and this enzymatic reaction is considered to be the rate limiting step in the synthesis of melatonin (Klein et al., 1997).

The liver breaks down melatonin into 6-sulfatoxymelatonin using 6-hydroxlation by the P450 enzyme CYP1A2 which is then excreted in the urine (Arendt et al., 1985; Skene et al., 2001; Ma et al., 2005). The half-life of melatonin in plasma is about 10 minutes due to this rapid hydroxylation in the liver (Iguchi et al., 1982). Melatonin is not stored anywhere systemically in the body and as a result melatonin levels in the blood directly mimic the rates of production and metabolism. This very precise and changeable rhythmic production and degradation of melatonin proves to be a very efficient way for transmitting the message of
darkness throughout the body. The target tissues mount their own responses to this chemical messenger of darkness.

1.5.2 Melatonin rhythm

As the signal for darkness to the organism, melatonin rhythm in all species studied is invariant in that it rises with periods of darkness and falls to low levels during periods of light. In this way, it acts as the hormonal message of darkness (Arendt, 1995). This holds true despite the species differences in the mechanisms regulating the production of melatonin. The duration of melatonin secretion at night varies in proportion to the length of dark phase and in this way propagates the signal of season to the organism. The timing of the melatonin rhythm is considered to be the best physiological marker of circadian phase and its normal rise in concentration during an evening dim light exposure, named dim light melatonin onset (DLMO), is used both clinically to characterize circadian rhythm disorders and experimentally as you will read in Chapters 3 and 4 (Lewy et al., 1999).

1.5.3 Melatonin receptors

The identification and localization of melatonin receptors with use of the radioligand 2-[\(^{125}\text{I}\)]iodomelatonin became widely used for identification of sites with high affinity for melatonin (Weaver, 1999). Two types of 7-transmembrane G-protein coupled receptors have been identified and cloned in mammals and have labeled MT\(_1\) and MT\(_2\) formerly designated with other nomenclature such as Mel\(_{1a}\) and Mel\(_{1b}\) (Reppert et al., 1995). These two types of receptors have distinct molecular structures and pharmacological characteristics with differential affinity for certain ligands (Dubocovich, 2007). High affinity receptors are highly variable among species yet not surprisingly they are present in the SCN of most mammalian species studied (Weaver, 1999). Sites with high density of melatonin receptors in mammals include various brain regions including most importantly, the SCN, the pars tuberalis (PT) of the pituitary gland as well as the retina, and several regions of the cardiovascular system. Melatonin receptors located in the SCN are thought to mediate circadian response to melatonin while those located in the PT are believed to contribute to reproductive responses (Weaver, 1999). Other regions containing melatonin receptors have been identified with their
putative functions, including biological roles and clinical relevance, currently being elucidated (Chan and Wong, 2013).

1.5.4 Light effects on melatonin

A well-defined neural pathway carrying photic information extends from the SCN to the pineal gland via a multisynaptic pathway with connections being made sequentially in the paraventricular hypothalamus, the upper thoracic intermediolateral cell column, and the superior cervical ganglion (Moore, 1983). By way of this neuroanatomy, cycles of light and dark which are perceived through the eyes entrain SCN neural activity which, in turn, entrains the rhythmic synthesis and secretion of melatonin from the pineal gland. In virtually all species including humans, high levels of melatonin are secreted during the night and low levels are secreted during the day (Arendt, 1995).

1.5.4.1 Acute suppression

In addition to entraining melatonin synthesis by the pineal gland, light can acutely suppress melatonin synthesis. Specifically, exposure of the eyes to light during the night can cause a rapid decrease in the high activity of the pineal enzyme AANAT and subsequent synthesis of melatonin. This reduction in AANAT activity has been shown to be paralleled by reduction in protein amount which, in turn, is mediated by the rapid, reversible process of proteasomal proteolysis (Gastel et al., 1998). The acute light-induced suppression of nocturnal melatonin synthesis was first observed in rats (Klein and Weller, 1972) and has been used in numerous animal studies to help determine the neural and biochemical mechanisms of melatonin regulation.

1.5.4.2 Intensity and duration of light

There is a well-known dose-response relationship between the intensity of light and the resultant magnitude of melatonin suppression. (Lynch et al., 1981; Brainard et al., 1983; 1988; Bojkowski et al., 1987; McIntyre et al., 1989; Aoki et al., 1998; Zeitzer et al., 2000).
Zeitzer and colleagues (2000) performed a study examining the sensitivity of the circadian pacemaker to a single nighttime exposure (6.5 h) of dim broadband fluorescent lighting (~100 lux) using a 9 day phase shift protocol. Exposure to a single 6.5 h episode of ~100 lux of light generated half of the response observed for a stimulus that is nearly 100-fold brighter (~9000 lux). Lighting was provided by overhead cool white fluorescent designed to provide uniform illuminance to the whole experimental room. In a recent retrospective comparison study using similar lighting conditions of cool white fluorescent at either a typical room light (<200 lux) versus dim light (<3 lux) given over long durations (8 h or 16 h) as part lengthier circadian study, melatonin was shown to be suppressed by more than 50% in the majority of subjects (Gooley et al., 2011).

1.5.4.3 Timing of light

As mentioned earlier in section 1.1, light is the most important zeitgeber for entraining circadian oscillators. The effect of light on melatonin production can be compared to the effect of light on other circadian oscillators which respond differently to light presented at different phases of its cycle. These phase responses can be plotted as a curve of phase shift of a circadian rhythm, such as melatonin production, as a function of the circadian phase that a stimulus, or zeitgeber, is given. Khalsa and colleagues (2003) elegantly presented the PRC for single lengthy (6.7 h) bright light exposures in a carefully controlled laboratory setting using fluorescent light. Recently, a comparison using a similar fluorescent light source but of a shorter duration (1 h) showed a PRC whose magnitude was 40% of that for the 6.7 h PRC of Khalsa and colleagues (St. Hilaire et al., 2012). When comparing a monochromatic blue (480 nm) light PRC, this was shown to be 75% as strong of that for the bright polychromatic light PRC of Khalsa and colleagues (Ruger et al., 2013). Another blue light PRC with the source being from a small, portable, commercially available device has been published (Revell et al., 2012).

1.5.4.4 Previous light history

In an initial important study by Lynch and colleagues (1981) that showed that with adaptation, rats could respond to a dim stimulus as either light or dark in terms of circadian
regulation depending on what light intensity was available for the rest of the day. In this study, urinary melatonin rhythms were studied in rats housed individually in metabolism cages. For 17 days one group was exposed to alternating 12-hour periods of dim light and total darkness (dim:dark) while a second group was exposed alternately to dim light and bright light (dim:bright). Both groups were then exposed to constant dim light for 15 days, returned to their original lighting conditions and 18 days later, half of each group was sacrificed at the midpoint of the dim light phase, and the other half 12 h later. Both groups excreted melatonin when exposed to daily cycles in light intensity; the dim:bright rats excreted 69%; of the total daily melatonin output during the dim light phase while the dim:dark group of rats excreted 70% during the dark phase.

Additionally, studies in animal models show high sensitivity to phase shifting by light after exposure to extended periods of darkness (Shimomura and Menaker, 1994; Refinetti, 2003). Two human studies showed that a higher intensity light history over a given period of time dampens the magnitude of subsequent melatonin suppression by acute light exposure. In one study, one week of controlled dim light (less than 200 lux) resulted in 53% subsequent suppression of melatonin by 500 lux white light compared with only 41% after a week of bright light (5000-7000 lux) exposure (Hebert et al., 2002). In another study, a light history of 0.5 lux for over 63 h prior to a 6.5 hour 200 lux white light exposure resulted in a mean melatonin suppression of 85.7%, compared with 71.2% after a constant light history of 200 lux over an equal period (Smith et al., 2004).

Aggelopolous and Meissl (2000) showed that light acting via rod and cone pathways could have state-dependent, opposing actions on the electrical activity of SCN neurons and thus potentially influence the response of these neurons to light stimuli. Using an endpoint of electrical activity as measured by extracellular electrodes, the investigators found that the sensitivity of SCN neurons to light became proportionally higher, with a lower threshold for excitation, with increased time of dark-adaptation to scotopic conditions. They also concluded from the significantly divergent sensitivities of SCN neurons to 505 nm light under dark- and light-adapted pre-conditions that there are separate cone and rod inputs to the SCN, and that most SCN neurons receive both rod and cone input. Their extensive findings suggest a complex, dynamic interrelationship between the traditional visual photoreceptors and SCN cell sensitivity to light stimuli, relative to various adaptive lighting conditions (Aggelopolous and Meissl, 2000).
Jasser et al. (2006) demonstrated that exposure to white light at levels as low as 18 lux for 2 h at night can significantly decrease the magnitude of subsequent melatonin suppression by 460 nm monochromatic light up to 46% in healthy male and female volunteers. Thus, pre-exposure to photopic light levels appear to diminish phototransduction as measured by plasma melatonin suppression. This finding supports the observations by Aggelopolous and Meissl (2000) in rats.

1.5.4.5 Wavelength

Several years after Lewy and colleagues discovered that light at 2500 lux can suppress melatonin in humans (Lewy et al., 1980), Brainard worked with Lewy and others to more precisely determine the dosages of light needed to suppress melatonin in normal volunteers (Brainard et al., 1988). In that study, six healthy males were exposed to carefully controlled intensities of monochromatic light at 509 nm for one hour during the night. The data demonstrated that light at 509 nm affects melatonin in a fluence-response (dose-response) fashion (i.e., the brighter the photic stimulus the greater the suppression of melatonin).

Studies using monochromatic light exposures in humans, monkeys and rodents have shown that nonvisual responses are maximally sensitive to blue light (between 459-483 nm) (Brainard et al., 2001a; Brainard et al., 2001b; Thapan et al., 2001; Foster, 2005; Gamlin et al., 2007). This sensitivity is different than that of the combined photopic response of classical photoreceptors for vision which peak in the green part of the spectrum ($\lambda_{\text{max}}$ 555 nm).

1.6 Other neuroendocrine and neurobehavioral responses to light

Earlier sections have addressed how light affects the neuroendocrine and circadian systems primarily through melatonin suppression. Another neuroendocrine hormone with a clear circadian rhythm is cortisol (Buijs et al., 1999; Kudielka and Kirschbaum, 2003). Light exposure during the morning has been shown to accentuate the normal daily morning rise in
cortisol levels (Scheer and Buijs, 1999; Leproult et al., 2001). Two other physiological responses to bright light include heart rate and core body temperature. When bright white light is presented during the night, heart rate increases (Scheer et al., 1999) and core body temperature elevates (Badia et al., 1991; Ruger et al., 2006).

Neurobehavioral responses such as alertness also have a circadian rhythm (Dijk et al., 1992), but the exact mechanism behind the acute alerting effects of light is not known. Measures for alertness include neurobehavioral performance testing, subjective sleepiness scales, and electroencephalogram (EEG) via polysomnography. Exposure to a sufficient light stimulus often results in increasing performance on neurobehavioral testing, lower subjective sleepiness ratings, and increased EEG frequencies in the alpha range, all pointing to increased alertness (French et al., 1990; Badia et al., 1991; Cajochen et al., 1999; Wright et al., 2002; Phipps-Nelson et al., 2003; Cajochen et al., 2005; Lockley et al., 2006).

Melatonin was thought to mediate part of the alerting effects but Ruger and colleagues (2005) showed that there is only a weak relationship between suppression of melatonin and reduction of sleepiness. Other laboratories (Krauchi et al., 1997; Krauchi and Wirz-Justice, 2001) have demonstrated a functional link between the degree of heat loss (distal vasodilation) and subjective sleepiness and the link between core body temperature and alertness continues to be elucidated (Wright et al., 2002).

Alerting effects of light may use the afferent pathways leading from the SCN to other areas of the brain. Indirect projections to the locus coeruleus (LC), an area of the brain associated with arousal and sleep-wake regulation, are relayed via the dorsomedial hypothalamic nucleus (DMH) have been shown in rodents (Aston-Jones et al., 2001). By lesioning these connections, the circadian variation in the LC was eliminated (Usher et al., 1999; Aston-Jones et al., 2001). In humans, the LC is influenced by bright light exposure during the night in blood flow studies performed by Perrin and colleagues (Perrin et al., 2004). The DMH also projects indirectly and directly to the ventrolateral preoptic nucleus (VLPO) (Chou et al., 2002) which is also a target region for ipRGCs identified by Gooley and colleagues (Gooley et al., 2003). Taken together, these indirect projections from the SCN, as well as direct projections from the ipRGCs, are the more likely mechanism by which bright light exposure transduces alerting affects.
1.7 Action spectra for circadian, neuroendocrine and neurobehavioral responses

In the field of photobiology, an action spectrum is one of the principal tools for identifying the chromophore or photopigment that initiates a light-induced response. The concept of an action spectrum was first introduced in the 19th century when biologists observed that plant growth depended on the spectrum of light to which plants were exposed. Strictly defined, an action spectrum is the relative response of an organism to different wavelengths of visible and near-visible electromagnetic radiation. Over the years, photobiologists have evolved a set of refined approaches for determining action spectra that are applicable to all light responsive organisms (Grossweiner, 1989; Coohill, 1991; 1999; Horspool and Song, 1994).

Throughout the photobiological literature, there are two basic types of action spectra: polychromatic and analytic (Coohill, 1999). In general, when investigators begin exploring light sensitive biological reactions, they typically begin by determining polychromatic action spectra. Such action spectra are developed using broader bandwidth light stimuli that either have half-peak bandwidths greater than 15 - 20 nm or by emphasizing particular wavelengths against backgrounds of "white" artificial or natural light. These types of action spectra are useful for: 1) identifying interactions of biological responses to multiple wavelengths; 2) elucidating how organisms respond to light in more natural settings; and 3) guiding the development of the more sophisticated analytic action spectra. By themselves, however, polychromatic action spectra have limited utility for identifying the specific chromophores or photopigments that initiate photobiological responses (Coohill, 1999).

Beginning in the latter half of the 20th century, several advances led to the development of the first mammalian neuroendocrine and circadian action spectra. A series of discoveries determined that light detected by the eyes was the primary stimulus that regulated pineal gland morphology and melatonin biosynthesis (Quay, 1961; 1963; Wurtman et al., 1964). Soon after that, it was shown that light detected by the eyes regulated robust pineal-reproductive photoperiodic responses in hamsters (Hoffman and Reiter, 1965). Following those advances, a discrete, light sensitive neural pathway extending from the retina into the SCN for circadian regulation was described (Moore and Lenn, 1972; Hendrickson et al., 1972). These discoveries were built on a growing understanding that environmental light played a fundamental role in regulating homeostasis, and quickly fostered further advances
that elucidated many important aspects of how light regulates neuroendocrine and circadian physiology (for reviews, see Brainard, 1978; Oksche, 1991). Cumulatively, those early scientific breakthroughs enabled investigators to begin using the tools of photobiology to explore neuroendocrine and circadian physiology.

1.7.1 Polychromatic

As in other fields of photobiology, the earliest action spectra studies on neuroendocrine and circadian responses to light utilized polychromatic stimuli. Table 1 summarizes the methods and results of both early and more recent polychromatic action spectra. Across this set of publications, both humans and rodents were studied in terms of pineal melatonin synthesis, circadian phase shifting or photoperiodic responses.

The polychromatic action spectra in Table 1 are reasonably consistent in suggesting that the general spectral region between 450 nm and 550 nm provides the strongest stimulation of circadian and neuroendocrine responses in mammals. These action spectra, however, are limited in determining refined peak sensitivities for given responses because the test light stimuli have broader bandwidths.

Table 1. Selected polychromatic action spectra for mammalian neuroendocrine and circadian responses

<table>
<thead>
<tr>
<th>Species</th>
<th>Biological response</th>
<th>Stimuli tested</th>
<th>Peak sensitivity *</th>
<th>First author</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Pineal gland</td>
<td>Single irradiances of 5 restricted bandwidths (1/2 peaks 19-80 nm)</td>
<td>508-553</td>
<td>Cardinali</td>
<td>1972</td>
</tr>
<tr>
<td>Deer mouse</td>
<td>Photoperiodic reproductive development (juvenile)</td>
<td>Single irradiances of 3 restricted bandwidths (1/2 peaks 45-50 nm)</td>
<td>No peak detected</td>
<td>Friend</td>
<td>1973</td>
</tr>
<tr>
<td>Rat</td>
<td>Core temperature rhythm phase shift</td>
<td>Single irradiances of 5 restricted bandwidths (1/2 peaks 19-80 nm)</td>
<td>508-553</td>
<td>McGuire</td>
<td>1973</td>
</tr>
<tr>
<td>Hamster</td>
<td>Pineal gland melatonin suppression</td>
<td>Two to 4 irradiances of 5 restricted bandwidths (1/2 peaks 15-78 nm)</td>
<td>435-500</td>
<td>Brainard</td>
<td>1984</td>
</tr>
<tr>
<td>Hamster</td>
<td>Photoperiodic reproductive response (adult)</td>
<td>Single irradiances of 5 restricted bandwidths (1/2 peaks 15-90 nm)</td>
<td>350-555</td>
<td>Brainard</td>
<td>1986</td>
</tr>
<tr>
<td>Human</td>
<td>Salivary melatonin suppression and phase shift (delay)</td>
<td>Single irradiances of 5 restricted bandwidths (1/2 peaks 18-35 nm)</td>
<td>455-539</td>
<td>Wright</td>
<td>2001</td>
</tr>
</tbody>
</table>

* Half peak domain (s) of the stimulus (i) producing the strongest responses in nm.
HIOMT = hydroxyindole-O-methyltransferase
1.7.2 Analytic

The first analytic action spectrum developed in the circadian field was the groundbreaking work of Takahashi and colleagues (1984) who tested the effects of monochromatic light on wheel-running behavior of hamsters. Following their lead, other circadian and neuroendocrine researchers began employing monochromatic wavelengths and increasingly sophisticated photobiological techniques for determining analytic action spectra. The earliest analytic action spectra are listed in Table 2. In 1998, the seminal discovery of melanopsin in frog skin, and in the mammalian retina, created a major breakthrough in our understanding of human circadian phototransduction (Provencio et al., 1998; 2000). Analytic action spectra published during and after discovery of melanopsin are shown in Table 3.

Analytical action spectra are determined comparing the effects of two or more monochromatic light stimuli with half-peak bandwidths of 15 - 20 nm or less. It is optimum to develop analytic action spectra by establishing a set of dose-response curves (fluence-response curves) at different wavelengths for a specific biological response. An action spectrum is then formed by plotting the reciprocal of incident photons required to produce the biological response versus wavelength (Grossweiner, 1989; Coohill, 1991; 1999; Horspool and Song, 1994). This approach can become very labor intensive as more and more wavelengths are compared. An acceptable, although potentially less definitive alternate technique is to compare the effects of different wavelengths at an equal photon density. Two principal advantages of analytic action spectra over polychromatic action spectra is that they bring greater precision to defining the wavelength sensitivity of a given biological response and they permit a better opportunity to identify the photopigment molecules that mediate phototransduction.

The analytic action spectrum for circadian regulation described by Takahashi and colleagues (1984) utilized the classic photobiological approach of establishing fluence-response functions for a set of monochromatic wavelengths and then forming an action spectrum from the half-saturation constants of those functions. The resulting action spectrum had a \( \lambda_{\text{max}} \) around 500 nm and seemed similar in shape to the absorption spectrum for rhodopsin. Although Takahashi and colleagues found these data supported the hypothesis that a rhodopsin-based photopigment and rod cells in the retina mediate circadian entrainment in
hamsters, they were careful to point out that the participation of a cone mechanism could not be ruled out.

Table 2. Early (pre-1998) analytic action spectra for mammalian neuroendocrine and circadian responses

<table>
<thead>
<tr>
<th>Species</th>
<th>Biological response</th>
<th>Stimuli tested</th>
<th>Peak sensitivity*</th>
<th>First author</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamster Mesocricetus auratus</td>
<td>Locomotor rhythm phase shift</td>
<td>3 full and 4 partial fluence response curves 7 monochromatic bands (1/2 peaks 9-16 nm)</td>
<td>469-522</td>
<td>Takahashi</td>
<td>1984</td>
</tr>
<tr>
<td>Hamster Mesocricetus auratus</td>
<td>Pineal gland melatonin suppression</td>
<td>2 full fluence response curves 2 monochromatic bands (1/2 peaks 10 nm)</td>
<td>495-505</td>
<td>Podolin</td>
<td>1987</td>
</tr>
<tr>
<td>Rat Rattus norvegicus</td>
<td>Pineal gland AANAT suppression</td>
<td>2 partial fluence response curves 2 monochromatic bands (1/2 peaks 10 nm)</td>
<td>455-465</td>
<td>Bronstein</td>
<td>1987</td>
</tr>
<tr>
<td>White-footed Mouse Peromyscus leucopus</td>
<td>Pineal gland melatonin suppression</td>
<td>Single irradiances of 6 monochromatic bands (1/2 peaks 9-10 nm)</td>
<td>355-565</td>
<td>Benshoff</td>
<td>1987</td>
</tr>
<tr>
<td>Human Homo sapiens</td>
<td>Plasma melatonin suppression</td>
<td>1 fluence response curve and 5 single irradiances of 6 monochromatic bands (1/2 peaks 9-16 nm)</td>
<td>504-514</td>
<td>Brainard</td>
<td>1988</td>
</tr>
<tr>
<td>Mouse Mus musculus</td>
<td>Locomotor rhythm phase shift</td>
<td>6 full fluence response curves (+/+ and rd/rd) of 6 monochromatic bands (1/2 peaks 6-12 nm)</td>
<td>475-521</td>
<td>Provencio</td>
<td>1995</td>
</tr>
<tr>
<td>Mouse Mus musculus</td>
<td>Locomotor rhythm phase shift</td>
<td>7 full fluence response curves (rd/rd) of 7 monochromatic bands (1/2 peaks 6-12 nm)</td>
<td>454-521</td>
<td>Yoshimura</td>
<td>1996</td>
</tr>
</tbody>
</table>

* Half peak domain (s) of the stimulus (i) producing the strongest responses in nm.

AANAT = serotonin-N-acetyltransferase

† Estimated (Est) $\lambda_{max}$ from fitting data to spectral sensitivity curves or to visual photopigment nomograms.

Wild type and retinally degenerate strains are indicated by (+/+ and rd/rd), respectively.

The studies in Table 3 indicate shorter wavelength peak sensitivities in the blue portion of the visible spectrum. This was first demonstrated prior to the discovery of melanopsin and ipRGCs when Yoshimura and Ebihara (1996) showed that circadian phase shifting of rd/rd mice had a peak response at 480 nm. Given the differences in laboratories, animal models,
physiological endpoints and specific investigative techniques, this consistent identification of peak responses across the 459 nm to 484 nm range in the blue spectrum is remarkable.

Table 3. Recent (post-1998) analytic action spectra for circadian, ipRGC and ocular responses

<table>
<thead>
<tr>
<th>Species</th>
<th>Biological Stimuli</th>
<th>Peak Response</th>
<th>First Stimuli Tested</th>
<th>Peak Sensitivity</th>
<th>First Author Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Plasma melatonin suppression</td>
<td>8 fluence response curves (1/2 peaks 10-15 nm)</td>
<td>Est $\lambda_{\text{max}} = 464$ nm, (446-477 nm)</td>
<td>Brainard 2001b</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>Plasma melatonin suppression</td>
<td>6 fluence response curves (1/2 peaks 5-13 nm)</td>
<td>Est $\lambda_{\text{max}} = 459$ nm, (457-462 nm)</td>
<td>Thapan 2001</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>Pupillary light reflexes</td>
<td>6 fluence response curves (1/2 peaks ≤ 10 nm)</td>
<td>(+/+)$\lambda_{\text{max}} = 498$ nm or 508 nm</td>
<td>Lucas 2001</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>Cone cell ERG-wave</td>
<td>7 fluence response curves (1/2 peaks ≤ 10 nm)</td>
<td>Est $\lambda_{\text{max}} = 483$ nm</td>
<td>Hankins 2002</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>ipRGC cellular depolarization</td>
<td>6/10 fluence response curves (1/2 peaks 10 nm)</td>
<td>Est $\lambda_{\text{max}} = 484$ nm</td>
<td>Berson 2002</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>Circadian phase shift</td>
<td>7 fluence response curves (1/2 peaks 10 nm) (rd/rd cl) $\lambda_{\text{max}} = 481$ nm</td>
<td>Hattar 2003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monkey</td>
<td>ipRGC cellular depolarization</td>
<td>10 fluence response curves (1/2 peaks 15-20 nm)</td>
<td>Est $\lambda_{\text{max}} = 482$ nm</td>
<td>Dacey 2005</td>
<td></td>
</tr>
<tr>
<td>Monkey/Human</td>
<td>Pupillary light reflexes</td>
<td>9 fluence response curves (1/2 peaks 8-10 nm)</td>
<td>Est $\lambda_{\text{max}} = 482$ nm</td>
<td>Gamlin 2007</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>Pupillary light reflexes</td>
<td>8 fluence response curves (1/2 peaks 10 nm)</td>
<td>Est $\lambda_{\text{max}} = 480$ nm</td>
<td>Zaidi 2007</td>
<td></td>
</tr>
</tbody>
</table>

Wild type and retinally degenerate strains are indicated by (+/+) and (rd/rd cl), respectively. Intrinsically photosensitive retinal ganglion cells (ipRGC).

The only study in Table 3 that does not identify a $\lambda_{\text{max}}$ in the blue part of the spectrum is the action spectrum that identifies a peak at 498 nm to 508 nm in pupillary responses of wild type mice (Lucas et al., 2001). Earlier analytic action spectra with wild type mice and hamsters also showed peaks in the 500 nm to 511 nm range for phase shifting locomotor activity (Takahashi et al., 1984; Provencio and Foster, 1995; Yoshimura and Ebihara, 1996). It may be that the intact rodent retina combines input from ipRGCs and classical visual photoreceptors for circadian phase shifting and pupillary responses. In contrast, when mice do not have functioning visual photoreceptors (such as in the rd/rd and rd/rd cl models), their
retinas appear to shift to the shorter wavelength sensitivity for circadian and pupillary responses (Yoshimura and Ebihara, 1996, Lucas et al., 2001; Hattar et al., 2003).

Other lines of evidence described earlier in section 1.2.3 support the concept that ipRGCs are not the exclusive photoreceptors involved in circadian regulation. Despite rapid experimental progress on ipRGC physiology, it is currently unknown precisely how these newly discovered photoreceptors work with the classical visual photoreceptors in transducing light in the dynamic, complex polychromatic light environments where humans carry out their daily activities.

1.8 Light source comparison studies

On the heels of defining the spectral sensitivity of the melanopsin photopigment in the ipRGCs, multiple laboratories embarked on wavelength comparison studies on melatonin suppression, circadian phase changes and alertness using narrowband (15 nm or greater halfpeak bandwidth) or monochromatic (less than 15 nm halfpeak bandwidth) light sources.

1.8.1 Narrowband or monochromatic source comparisons

Exposure to 6.5 h of monochromatic light at 460 nm induced a two-fold greater circadian phase delay than 6.5 h of 555 nm monochromatic light of equal photon density. Also, 460 nm light caused twice the amount of melatonin suppression as 555 nm light, dependent on the duration of exposure (Lockley et al., 2003). Therefore, it was concluded that the efficacy of light in phase shifting human circadian rhythms is wavelength dependent, with a blue-shift in sensitivity.

A four hour pulse of very low levels of short-wavelength (combined 436 nm and 456 nm) presented immediately after habitual wake time was shown to produce a phase advance similar in size to very bright polychromatic light despite the large (185-fold) difference in light photons (Warman et al., 2003).
Revell and colleagues (2005) tested a phase advance protocol and compared 440 nm, 470 nm and 600 nm monochromatic light. Although the results were not entirely clear, 470 nm light was more effective than 600 nm light and perhaps, though not statistically significant, more effective than 440 nm light in phase advancing the offset of the human melatonin rhythm.

Wright and colleagues (2004) performed a study which falls into both monochromatic and polychromatic source comparisons since the wavelengths used (470, 495, 525, 595 and 660 nm) were between 10-18 nm. The lights used were portable light emitting diode (LED) devices mounted on spectacle frames. A 2 h light pulse was administered starting at 06:00 on two consecutive mornings. DLMO, using saliva, showed that shorter wavelengths of 470, 495 and 525 nm had greatest DLMO advances ranging from approximately 40-65 min while the longer wavelengths (595 nm and 660 nm) produced no significant phase advance.

Like melatonin suppression and phase changes, monochromatic blue light has been shown to increase subjective and objective measures of alertness, improve performance measures and affect waking EEG over monochromatic green light (Cajochen et al., 2005; Lockley et al., 2006). Specifically, 460 nm monochromatic light was either compared with 555 nm monochromatic light over a 6.5 h exposure (Lockley et al., 2006) or with 550 nm monochromatic light over a 2 h exposure (Cajochen et al., 2005).

Finally, a study from our laboratory using the identical light source studied in Chapter 4 results demonstrated that increasing irradiances of narrowband blue light can elicit increasing plasma melatonin suppression in healthy subjects (p<0.0001) (West et al., 2011). The data were fit to a sigmoidal fluence-response curve ($R^2=0.99$; $ED_{50}= 14.19 \ \mu W/cm^2$). A comparison of with 40 $\mu W/cm^2$ from 4,000 K broadband white fluorescent light, still currently used in most general lighting fixtures, suggests that narrow bandwidth blue LED light may be stronger than 4,000 K white fluorescent light for suppressing melatonin (West et al., 2011).

1.8.2 Broadband fluorescent light source comparisons

Fluorescent lighting still remains the standard in many indoor environments, including the workplace. Broadband, white fluorescent light sources have been studied for comparison
studies on alertness, neurobehavioral testing, melatonin suppression, and circadian phase changes. Early studies have shown that bright white polychromatic light exposure has been shown to acutely increase alertness and reduce fatigue during light exposure (French et al., 1990; Dawson and Campbell, 1991; Badia et al., 1991). Bright, overhead, white polychromatic light (Philips FB40/CW/3 cool white fluorescent) at 3,000 lux, when compared to 100 lux dim white light, increased measures of subjects’ performance on seven of ten neurobehavioral tests during a 16 hour overnight light exposure in 9 male subjects (French et al., 1990). A similar study, simulating night shift work, compared a four hour period of 6,000 lux white light to dim white light at less than 200 lux throughout an 8 hour shift in 13 male and female subjects. The group that received the four hours of bright light on the first night of the study exhibited a smaller decline in alertness during the second half of the night shift than the group that received dim light the entire time (Dawson and Campbell, 1991). Another, larger, study compared 43 male subjects in a between subjects design in four different lighting conditions. An increase in brain wave frequencies associated with alertness and improvement in performance on the neurobehavioral test battery were found for the bright light exposures (Badia et al., 1991). In all of these studies the light source was not highly characterized if described at all.

Many investigators continued to perform acute dose-dependent alerting effects to broadband fluorescent light sources presented to subjects at night. Measures taken include subjective sleepiness, psychomotor vigilance reaction times, reduced lapses, reduction of attentional failures indicated by electrooculogram-derived slow rolling eye movements, and suppression theta-alpha activity in waking EEG (Burgess et al., 2001; Campbell and Dawson, 1990; Badia et al., 1991; Daurat et al., 1993; Myers and Badia, 1993; Campbell et al., 1995; Wright et al., 1997; Cajochen et al., 2000; Lavoie et al., 2003). One study compared daytime broadband fluorescent light exposure presented to subjects for 5 h and reported enhanced alertness and performance as well as reduced incidents of slow eye movements (Phipps-Nelson et al., 2003).

Broadband fluorescent light sources are described in terms of correlated color temperature or CCT. CCT is a specification of the color appearance of a lamp in reference to a heated referenced black surface. It is measured in degrees Kelvin (K), and only gives information about the color of the light, not the spectral power distribution. Most of the studies described above do not report CCT but only describe the kinds of lamps used. A cool white fluorescent
lamp, for example, would have a color temperature of approximately 4,100 K, while a warm white fluorescent lamp would have a CCT of approximately 3,000 K (DiLaura et al., 2011). The CCT of fluorescent lamps not only influences visual discrimination, but it can alter neuroendocrine and neurobehavioral effects. CCT is commonly used in architectural lighting, but is just now being used in photobiological research to classify light sources. Table 4 presents a partial listing of light sources in terms of approximate CCT (DiLaura et al., 2011).

Multiple studies carried out at the same time in Japan, manipulating numerous parameters, have documented neuroendocrine and neurobehavioral effects using different color temperature lamps. Specifically, higher color temperature lamps evoked stronger melatonin suppression than lower correlated color temperature bulbs (Morita and Tokura, 1998; Sato et al., 2005). Further, core body temperature, blood pressure, and EEG frequency increased under higher color temperature when compared to lower color temperatures (Morita and Tokura, 1998; Yasukouchi et al., 2000; Noguchi and Sakaguchi, 1999; Yasukouchi and Ishibashi, 2005). Reductions in deep, slow-wave sleep have also been observed in the first half of the sleep period when subjects are exposed to high

<table>
<thead>
<tr>
<th>Light Source</th>
<th>CCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>North, Light Blue Sky</td>
<td>8,000 K</td>
</tr>
<tr>
<td>Daylight Fluorescent</td>
<td>6,500 K</td>
</tr>
<tr>
<td>Clear Mercury Lamp</td>
<td>6,000 K</td>
</tr>
<tr>
<td>Sunlight at Noon</td>
<td>5,500 K</td>
</tr>
<tr>
<td>Cool White Fluorescent</td>
<td>4,100 K</td>
</tr>
<tr>
<td>Warm White Fluorescent</td>
<td>3,000 K</td>
</tr>
<tr>
<td>High Pressure Sodium</td>
<td>2,200 K</td>
</tr>
<tr>
<td>Incandescent</td>
<td>2,800 K</td>
</tr>
<tr>
<td>Sunset</td>
<td>2,500 K</td>
</tr>
<tr>
<td>Candlelight</td>
<td>1,900 K</td>
</tr>
</tbody>
</table>
color temperature lamps and when these lights were given (Kozaki et al., 2005). Generally, these results are complementary to our understanding of the analytic action spectra in that higher color temperature (richer in blue wavelengths) fluorescent bulbs induce stronger neurobehavioral and neuroendocrine effects than lower color temperature lamps. These studies are summarized in Table 5.

More refined studies comparing polychromatic light with more short-wavelength energy (8,000 K CCT) to typical fluorescent lighting (4,100 K CCT) for melatonin suppression revealed differences but only at one of several low light levels that were compared (Figuero et al., 2006). Smith and Eastman (2009) compared 4,100 K and 17,000 K CCT lamps at equal photon densities for its phase-delaying effect.

Table 5. CCT effects on neurobehavioral, neuroendocrine, and circadian responses.

<table>
<thead>
<tr>
<th>Parameters Measured</th>
<th>CCT Comparisons</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Time</td>
<td>7500 K, 5000 K, 3000 K</td>
<td>Deguchi and Sato, 1992</td>
</tr>
<tr>
<td>Heart Rate Variability</td>
<td>6700 K, 5000 K, 3000 K</td>
<td>Mukae and Sato, 1992</td>
</tr>
<tr>
<td>Core Temperature, Melatonin</td>
<td>6500 K, 3000 K</td>
<td>Morita and Tokura, 1998</td>
</tr>
<tr>
<td>Heart Rate Variability, EEG, Subjective Drowsiness</td>
<td>5000 K, 3000 K</td>
<td>Noguchi and Sakaguchi, 1999</td>
</tr>
<tr>
<td>Skin Temperature, Rectal Temperature, Expired Gas</td>
<td>7500 K, 5000 K, 3000 K</td>
<td>Yasukouchi et al., 2000</td>
</tr>
<tr>
<td>Polysomnography, EEG, EOG, EMG</td>
<td>6700 K, 5000 K, 3000 K</td>
<td>Kozaki et al., 2005</td>
</tr>
<tr>
<td>Rectal Temperature, Salivary Melatonin</td>
<td>6480 K, 3150 K</td>
<td>Sato et al., 2005</td>
</tr>
<tr>
<td>Arousal Level, Heart Rate Variability, Blood Pressure, Body Temperature Reg., Sleep Architecture</td>
<td>7500 K, 6700 K, 5000 K, 3000 K</td>
<td>Yasukouchi and Ishibashi, 2005</td>
</tr>
<tr>
<td>Heart Rate Variability</td>
<td>6700 K, 5000 K, 3000 K</td>
<td>Ishibashi et al., 2007</td>
</tr>
</tbody>
</table>

Bedtime, 2 h bright light exposures were scheduled according to each subject’s baseline melatonin offset phase or circadian time versus typical studies that employ clock time. Light
exposure and bedtime moved 2 h later sequentially each day with no greater phase delays seen in the 17,000 K CCT condition. Smith and colleagues (2009) compared 4,100 K and 17,000 K CCT lamps at relatively equal irradiances (commonly used light therapeutic lux levels) for its phase-advancing potential. Early morning bright light exposures were scheduled according to each subject’s baseline melatonin onset phase or circadian time versus typical studies that employ clock time. Light exposure and wake-up moved one hour earlier sequentially each day with no greater phase advances seen in 17,000 K CCT condition observed (Smith et al., 2009).

In a combined field and laboratory study, the laboratory portion examined five polychromatic fluorescent light conditions for effects on the evening rise of melatonin, subjective measures of sleepiness as well as EEG measured sleep parameters. The field observations allowed a comparison of the laboratory-controlled polychromatic exposures and suggests that typical illumination used in the evening has the potential to disrupt sleep (Santhi et al, 2011).

1.8.3 Mixed light source comparisons

Several other studies have compared the efficacy of light emitted from LEDs versus fluorescent lamps for neuroendocrine, circadian and neurobehavioral regulation. An early study with rats found no significant differences in pineal melatonin suppression between broad spectrum white LEDs and cool white fluorescent lamps across five different illuminances (Heeke et al., 1999). A human study compared 2,000 lux light emitted by broad spectrum white LEDs, blue/green LEDs, and white fluorescent lamps (Wright et al., 2001). The results showed no significant differences between light from white LEDs and white fluorescent lamps for acute melatonin suppression and phase delay of the melatonin rhythm, but broad spectrum blue/green light appeared to be stronger than both white light sources. In a follow up to that finding, it was shown that 65 μW/cm² of narrow bandwidth LED light with peaks at 470, 495, and 525 nm could phase advance human circadian rhythms, but longer wavelength peaks of LED light at 595 and 660 nm had no effect at this same intensity (Wright et al., 2004). A different study with 4 healthy human subjects showed that narrow bandwidth blue LED exposure was a stronger than clear mercury vapor lamps for melatonin suppression (Figueiro et al., 2004). Finally, an experiment testing four illuminances of narrow bandwidth blue LED light peaking at 470 nm showed that increased illuminances
evoked increased acute alertness in volunteers in both objective and subjective assessments (Figueiro et al., 2007). Paul and colleagues (2007) compared several commonly marketed phototherapeutic devices that emitted enhanced light in the blue-green portion of the spectrum. All devices produced significant phase-delays with no one device producing a more significantly phase response.

1.8.4 Light source comparisons of typically used polychromatic light

A recent study compared typical domestic and workplace lamps over a short term exposure as one might experience in an extended bathroom visit prior to bed for effects on melatonin and subjective measures of alertness. Data revealed that the evening rise in salivary melatonin was reduced by lamps containing an abundance of blue wavelengths and subjectively measure alertness was increased (Wahnshaffe et al., 2013).

In the past few years there has been an emphasis on examining light sources more typically encountered in the home or office for their neuroendocrine, circadian and neurobehavioral regulatory effects. As LED technology is likely to become a greater presence in our homes, work, and general environment, many of the studies used LEDs as the light sources. In a fascinating study, given the huge use of electronic devices in the evening, the spectral composition commonly used in computer screens which employ a LED backlight was compared to computer screens back illuminated with a cold cathode fluorescent lamp (CCFL). The photon flux of blue light of the LED backlit computer screen was three times higher than that of the CCFL computer screen. A 5 hour evening exposure resulted in a significant suppression in the evening rise of salivary melatonin levels as well as EEG measures of objective sleepiness (Cajochen et al., 2011).

1.9 Aims and Hypotheses

Since the discovery of ipRGCs and the photopigment, melanopsin, with its wavelength sensitivity in the blue portion of the visible spectrum, there has been a steady increase in studying blue light for its circadian, neuroendocrine and neurobehavioral effects. The aim of this set of studies is to study the effects of blue light, either in combination with other light
wavelengths, or refined to specific blue wavelengths, in experiments to test the immediate, alerting or longer term, circadian effects.

The initial study (Chapter 3) will test the hypothesis that white polychromatic fluorescent lamps will have increased efficacy for melatonin suppression, alertness, and circadian phase shifting when they are enriched in the blue portion of the spectrum. This will be done by comparing the magnitude of melatonin suppression, changes in alertness, and phase resetting of circadian rhythms in healthy male and female subjects following exposure to equal photon densities of blue-enriched fluorescent lights compared to standard white fluorescent lights.

The overall aim of the second project (Chapter 4) is to test the ability of blue-appearing narrowband solid-state light for its ability to acutely suppress nocturnal melatonin as well as enhance cognitive performance and alertness in healthy men and women. The specific hypotheses tested were that compared to dim light exposure, blue narrowband light would: 1) suppress nocturnal melatonin, 2) decrease reaction time and lower number of lapses in a 10-min auditory psychomotor vigilance task, 3) increase number of correct responses in two working memory tasks (Digit symbol substitution task (DSST), digit span forward and reverse entry (DS)), 4) lower subjective levels of sleepiness as measured by the Karolinska sleepiness scale (KSS), and 5) show differences in EEG in delta-theta frequency band (0.5 – 5.5 Hz) and high alpha frequency band (9.5 – 10.5 Hz) during the Karolinska drowsiness test (KDT).

The third study (Chapter 5) will test the hypothesis that certain combinations of light wavelengths are additive or opponent to the circadian receptor system that mediates the suppression of nighttime synthesis of the pineal hormone, melatonin, in healthy human subjects. Specific wavelength combinations will be given under highly controlled experimental conditions to healthy men and women and plasma melatonin concentration will be determined.
CHAPTER 2 GENERAL METHODS

2.1 General human use/ethical approval

All human use protocols used in this thesis were submitted and approved under my collaborative supervisor and the study’s Principle Investigator, Dr. George Brainard, and followed the Institutional Review Board (IRB) guidelines in place at Thomas Jefferson University (TJU). The IRBs are established and empowered under the authority of the President of TJU and the University’s Federal Wide Assurance with the U.S. Department of Health and Human Services. TJU requires that all research involving human subjects, or material or personal information from living humans, be reviewed and approved by one of the TJU’s IRBs prior to initiation of any research activities. This includes recruitment and screening activities.

Each protocol in this thesis was done at TJU and all human subjects research was designed and carried out in a manner that protected the rights, welfare and privacy of the subjects. Each protocol was approved after modifications to secure approval, and underwent annual reviews to insure compliance to all TJU IRB guidelines.

2.1.1 Consent process

Subjects were recruited by a member of the laboratory on an individual basis after some general invitation to join the study (i.e. poster, classroom announcement, personal referral, media advertisement, etc.). Each potential subject was interviewed by phone and asked questions concerning their current and past health status, sleep/wake habits, travel history and job description. If their answers did not exclude them immediately from further consideration then a brief description of the protocol was given. Initial questions and concerns were addressed and a copy of the TJU approved IRB stamped consent form was then forwarded to the potential subject. A follow up email or phone call was initiated after a few days to schedule a laboratory visit. Potential subjects then received a lengthy and detailed explanation of the special procedures involved in the study. They were given a tour of the laboratory, the area in which they were to be studied and shown the lights, needles, meters
and the like, before making a commitment to participate. Subjects were informed that a camera would be present. Every attempt was made to acquaint each prospective subject with all of the procedures involved in this study in order to minimize the possible effect of uncertainty about the experimental procedures on the results. They were informed that they were free to discontinue participation in the experiment at any time, should they wish to do so. Written informed consent was then obtained from subjects before the pre-study screening began.

**2.1.2 Screening process – acute study**

In order to be included in the protocol, volunteers underwent ophthalmological and psychological assessments, and a toxicological screen for drugs of abuse. Evaluation of ophthalmologic condition included visual acuity test, measurement of intraocular pressure, slit lamp exam, external exam, fundus exam, and ophthalmological and self-reported medical history of the subject. Color vision and color discrimination of all subjects was tested by the Ishihara and Farnsworth Munsell D-100 tests. Female subjects were interviewed with the study psychologist to screen for premenstrual dysphoric disorder before being admitted into the study. Subjects with no history of drug or alcohol dependency were instructed to avoid prescription or non-prescription drugs, over-the-counter drugs, recreational/street drugs, and other foreign substances before entering and during the running of the studies. The Jefferson Hospital Clinical Testing Service verified compliance with urine toxicology screens for common drugs of abuse.

Sleep:wake cycle screening consisted of a minimum of ten days and a maximum of three weeks of self-reported sleep:wake times on paper sleep logs and calls into a voicemail box just before going to bed and just after waking. Subjects also wore an actigraph (BASIC Motionlogger, Ambulatory Monitoring, Inc., Ardsley, NY) to verify routine bedtime and wake time of eight hours allowed sleep time. The voice mailbox recorded the time and date of each call and these messages were compared to the subjects' sleep/wake logs and actigraphic data at least one day before they began any light exposure protocol.
2.1.3 Screening process – multiday studies

In order to be included in the multiday protocols, volunteers underwent the same sleep:wake verification, ophthalmological, psychological, and toxicological screens for drugs of abuse described above as well as a full history and physical given by the study physician. Furthermore, subjects underwent toxicological screening of blood and urine for caffeine, pregnancy in females, basic metabolic panel, alcohol, thyroid function, and a complete blood count (CBC). Physical screening by the study physician included an eye exam, electrocardiogram (ECG), general health exam, and verification of lack of medications, as well as acute, chronic, or debilitating conditions. Additional psychological screening was performed for the subjects in the multiday study consisting of the Minnesota Multiphasic Personality Inventory (MMPI) II, Beck’s Depression Inventory (BDI) II, and an interview with Brenda Byrne, Ph.D., the study clinical psychologist (Butcher et al., 1989; Beck et al., 1996).

2.1.4 Inclusion criteria – acute study

Subjects must have passed a urine screen for common drugs of abuse and reported no history of drug or alcohol dependency. Subjects must have had a consistent and stable sleep:wake cycle verified by call-ins and actigraphy. Subjects must have had a sleeping pattern with routine bedtime starting at midnight or earlier, have had no plans to travel outside the time zone during the length of the active protocol, or engaged in evening or night-time shift work. This was initially discussed and emphasized during the phone screen. Sleep-wake stability and timing of sleep were critical requirements in our recruitment since our melatonin suppression protocols are based on fixed clock times (section 5.3.3).

Subjects’ age was limited to 18-30 years old to avoid a confound of reduced short wavelength transmission to the retina due to aging of the lens of the eye after age 30 years (Brainard et al., 1997). Normal color vision was a requirement. Ishihara and Farnsworth Munsell D-100 tests were administered and scored to quantify subject's color discrimination or to discern if a color anomaly is present. Female subjects were interviewed with the study psychologist and had to show no evidence of premenstrual dysphoric disorder. If a female subject was using birth control medication, she must have been using it consistently for at least three months.
prior to the beginning of the study and continued use during the study. Evaluation of ophthalmologic condition included passing a visual acuity test, normal intraocular pressure, normal slit lamp exam, normal external exam, normal fundus exam, and reviewing ophthalmological and medical histories of volunteer and volunteer’s family.

2.1.5 Inclusion criteria – multiday studies

Inclusion criteria for the multiday studies included all of the criteria from the acute studies described above (section 2.1.4). Furthermore, subjects had to have no caffeine or alcohol in their blood and urine, no pregnancy hormones detected in females, basic metabolic panel results were to be at normal levels, thyroid function results to be normal, and CBC results to be in normal ranges. Normality was established on the basis of clinical history, ECG, clinical biochemical screening tests of blood and urine, eye and physical examination. Physical screenings by the study physician were normal, general health exam results were normal, medications, as well as acute, chronic, or debilitating conditions were absent. Psychological screening scores from the MMPI and the BDI were within normal ranges (Butcher et al., 1989; Beck et al., 1996), and the subject passed an interview with the study psychologist.

2.1.6 Exclusion criteria – acute study

If there was a lack of sleep:wake stability on visual inspection of the actigraphy data or if there was more than one significant deviation from standard bed times, the subject was excluded from starting the protocol at that time. If the subject’s routine bedtime shifted significantly past midnight, or they made plans to travel beyond three time zones during the length of the active protocol, or they began evening or night-time shift work the subject was excluded from starting the protocol at that time. Any positive results of the urine toxicology screen for drugs of abuse resulted in the subject being excluded from the study. If the subject reported a history of abusing alcohol or drugs they were also excluded from the study.

Normal color vision was a requirement. If Ishihara and Farnsworth Munsell D-100 tests discerned that a color anomaly was present, the subject was excluded. Female subjects who showed evidence of premenstrual dysphoric disorder were excluded. If a female subject was
using birth control medication for less than three months prior to the beginning of the study or discontinued use during the study, she was excluded. If the ophthalmologic evaluation included failures of a visual acuity test, abnormal intraocular pressure, abnormal slit lamp exam, abnormal external exam, abnormal fundus exam, or an abnormal ophthalmological and medical history of the volunteer or volunteer’s family, the subject was excluded.

2.1.7 Exclusion criteria – multi day studies

Exclusion criteria for the multiday studies were inclusive of all of the criteria from the acute studies described above (section 2.1.6) with additional exclusions listed below. Furthermore, any subject with symptoms of acute or active illness upon entering the protocol was excluded from study. Individuals who were unaware of specific psychiatric diagnoses but who had a history of having been treated with antidepressant, neuroleptic medications or major tranquilizers were excluded from studies. Given the wide range of illnesses that are encountered in medical practice, it would not have been possible to provide a comprehensive list of each and every disease that could have served as grounds for exclusion for the subject. The following was a list, however, of current illnesses that would have certainly been grounds for exclusion:

- **Prescription medications:** At the discretion of the study physician;
- **Sleep disorders:** narcolepsy, sleep apnea, periodic limb movement, nocturnal paroxysmal dystonia, REM-sleep behavior disorder and nocturnal enuresis;
- **Diseases of the cardiovascular system:** hypertension, heart failure, cardiomyopathy, ischemic heart disease, valvular heart disease, history of heart transplantation, cardiac tumors and pericardial disease;
- **Disorders of the respiratory system:** asthma, cystic fibrosis, chronic bronchitis, emphysema and airway obstruction, interstitial lung diseases, pulmonary hypertension, lung neoplasm;
- **Disorders of the kidney and urinary tract:** acute or chronic renal failure, history of renal transplantation, tubulointerstitial diseases of the kidney, urinary tract obstruction, and tumors of the urinary tract;
- **Infectious diseases:** infective endocarditis, HIV infection, sexually transmitted
diseases, osteomyelitis, brucellosis, tuberculosis, leptospirosis, Lyme disease, mononucleosis, parasitic infections;

- Disorders of the gastrointestinal system: esophagitis, peptic ulcer and gastritis, disorders of absorption, inflammatory bowel disease, diseases of the small and large intestine, acute appendicitis, hepatitis, liver cirrhosis, history of liver transplantation, diseases of the gallbladder and bile ducts, and pancreatic disease;

- Disorders of the immune system, connective tissue and joints: AIDS, systemic lupus erythematosus, rheumatoid arthritis, scleroderma, ankylosing spondylitis, vasculitis and sarcoidosis, fibromyalgia;

- Disorders of the hematopoietic system: anemia, leukemia, myeloproliferative diseases, and history of bone marrow transplantation;

- Neoplastic diseases: lymphoma, carcinoma, melanoma, or any other neoplastic diseases;

- Endocrine and metabolic diseases: thyroid disease, Addison's Disease, Cushing's Syndrome, aldosteronism, hypoaldosteronism, pheochromocytoma, diabetes, disorders of sexual differentiation, disorders of neuroendocrine regulation, diseases of the anterior pituitary and hypothalamus, hemochromatosis porphyria, Wilson's Disease, glycogen storage diseases, diseases of the parathyroid gland, metabolic bone disease, disorders of phosphorus or magnesium metabolism and Paget's Disease;

- Neurologic disorders: epilepsy and disorders of consciousness, dementia, amnesic disorders, demyelinating diseases, Parkinson's Disease, muscular dystrophy, myasthenia gravis, periodic paralysis, dermatomyositis, polymyositis, infections of the nervous system, stroke, history of transient ischemic attacks, hydrocephalus, tumors of the pituitary gland, pinealoma, intervertebral disc disease, ataxia, Gilles de la Tourette Syndrome, Huntington's Disease, tardive dyskinesia, history of recurrent migraine or cluster headaches, and neuromuscular disease.

- Mental health: Each subject completed a MMPI II, Symptom Checklist-90, BDI and State Anxiety Scale (Butcher et al., 1989; Beck et al., 1996) and received a comprehensive psychological examination by the study psychologist. Individuals with evidence of psychopathology on the standardized questionnaires or in a structured clinical interview were excluded from study.
2.2 Radiometry and photometry

The spectral power distribution measurements were taken using a Model FSHH 325-1075P FieldSpec handheld spectroradiometer (Analytical Spectral Devices, Inc., Boulder, CO). Irradiance measures were taken using an IL-1400BL radiometer/photometer (International Light Technologies, Inc., Peabody, MA). This irradiance meter has a silicon diode detector head (Model #SEL033) wide angle input optic (W#6874) and a filter (F#14299) to provide a flat response and was annually given a full scanned calibration between 250 and 1100 nm. The meter was received in tolerance (standard readings within 5% of previous year) when calibrated during the course of running this protocol. All calibrations were traceable to the U.S. National Institute of Standards and Technology.

Illuminance measures were taken using an IL-1400BL radiometer/photometer (International Light Technologies, Inc.). This meter has a silicon diode detector head (Model #SEL033) with a wide angle input optic (W#11437) and a filter (Y#27475) to provide a photopic illuminance response and was annually given a full scanned calibration between 400 and 700 nm. This meter was also received in tolerance when calibrated during the course of running this protocol. All calibrations were traceable to the U.S. National Institute of Standards and Technology.

2.3 Blood samples

In multiday studies, blood samples (2-3 ml) were collected every 20-60 minutes through an indwelling intravenous catheter located in a forearm vein into 3 ml polystyrene tubes which contained K2 ethylenediaminetetraacetic acid (EDTA) (BD Diagnostics, Franklin Lakes, NJ). In the acute study, blood samples (10-12 ml) were collected before and after the 90 minute light exposure via antecubital venipuncture into 7 ml glass tubes which contained K2 EDTA (BD Diagnostics, Franklin Lakes, NJ). Plasma was separated by refrigerated centrifugation (2,000 RPM for 15 minutes), aliquoted into polypropylene cryogenic vials (Thermo Fisher Scientific, Rochester, NY) and stored at -20˚C until assay. Plasma samples were shipped overnight on dry ice to the laboratories running the radioimmunoassays.
2.4 Saliva samples

In multiday studies, saliva samples (1-2 ml) were collected at the same time as the blood draws to serve as backup for the measurement of plasma melatonin should indwelling intravenous lines fail to flow. The saliva samples were collected with the Salivette sampling device (Sarstedt, Inc., Hayward, CA) which consisted of a small cotton swab and two plastic tubes. Any drinks or food with the exception of water were removed from the subject for 30 minutes prior to sampling. The swab was left in the mouth for 2-5 minutes while the subjects gently chewed on it to stimulate saliva flow. After collection the Salivette was centrifuged at 3000 RPMs for 2 minutes to yield clear and watery samples. Aliquots of at least 1.2 ml were placed into cryogenic vials described above and stored at -20˚ C.

2.5 Urine samples

Urine was collected during each void for the entire duration of the multiday studies subject for possible later analysis of the major metabolite of melatonin, 6-sulphatoxymelatonin. Total urine volume was recorded for each sample and a 10 ml aliquot stored at -20˚ C in sterile polypropylene tubes (Thermo Fisher Scientific, Rochester, NY).

2.6 Melatonin radioimmunoassays

Melatonin concentrations were assayed in one of the multiday studies (section 3.2.6) and the acute study (section 5.3.4) by radioimmunoassay using antiserum described by Rollag and Niswender (1976). I was trained in this assay system by Dr. Rollag but did not run the assays for this thesis.

Melatonin concentrations were assayed in other multiday study (section 4.3.6) by radioimmunoassay using the Kennaway G280 antimelatonin antibody in an assay system modified by Vaughan (1993). I was never trained in this assay system and did not run the assays for this thesis.
2.6.1 Rollag radioimmunoassay

The Rollag radioimmunoassay was performed in the Department of Anatomy at the Uniformed Services University of Health Sciences located in Bethesda, Maryland. Radiolabeled ligand was prepared by adding 100 µl of pyridine containing 60 nmole 5-methoxytryptamine to 250 µCi (0.1 nmole) Bolton-Hunter Reagent (New England Nuclear Corp., Boston, MA) in approximately 100 µl benzene. The reaction was allowed to proceed for 20 minutes before adding 500 µl of water. After waiting five minutes to ensure that the unreacted Bolton Hunter reagent was destroyed, the radioactivity was diluted to approximately 200 cpm/µl with assay buffer (Rollag and Niswender, 1976). Duplicate aliquots of 200 µl of each unknown plasma sample and quality control sample were extracted into 2 ml of chloroform. The chloroform was removed in a centrifuge (SpeedVac, Savant Instruments, Holbrook, NY) and the residue resuspended in 200 µl of assay buffer (phosphate buffered saline, pH 7.4, containing 0.1% gelatin with 100 mg thimerosal/liter as a preservative). The extracts were washed twice with 3 ml of petroleum ether, and then evaporated to dryness in a centrifuge before being resuspended in 100 µl of deionized water. Approximately 10,000 cpm of radiolabeled ligand in 50 µl Phosphate Buffered Saline-Gel and 50 µl of a 1:64,000 dilution of antiserum (R1055, 9/16/74; 1:300,000 final dilution) was added to each unknown and a triplicate 2-fold geometric series of standards ranging in concentration from 250 to 0.5 pg melatonin per 100 µl assay buffer. The final assay volume of buffer in each tube was 200 µl. At the end of the 48 hour incubation period, three ml of 95% ethanol (4°C) was added to each assay tube and the bound radioactivity precipitated by centrifugation at 2000 x g for 30 minutes. The supernatant was decanted and radioactivity in the precipitate was quantified. The quantity of melatonin immunoreactivity in the samples was calculated with the use of a computer program to give a four parameter logistical fit of the data (M.L. Jaffe and Associates, Silver Spring, MD; see Davis et al., 1980). All solutions were maintained at 4°C throughout the radioimmunoassay procedure. Assay results were not corrected for recovery (which has proven to be >95% in independent trials). The minimum detection limit of the assay is 0.5-2.0 pg/ml melatonin.
2.6.2 Modified Kennaway radioimmunoassay

The modified Kennaway radioimmunoassay was performed in the Division of Endocrinology, Diabetes and Hypertension at the Brigham and Women’s Hospital in Boston, Massachusetts. The melatonin radioimmunoassay kit made by American Laboratory Products Company (Windham, NH) measures melatonin by a double-antibody radioimmunoassay based on the Kennaway G280 antimelatonin antibody. Reversed-phase column extracted samples and controls and reconstituted calibrated dilutions are incubated with the anti-melatonin antibody and $^{125}$I-melatonin. $^{125}$I-melatonin competes with melatonin present in samples, calibrators and controls. After 20 hours of incubation, solid-phase second antibody is added to the mixture in order to precipitate the antibody-bound fraction. After aspiration of the unbound fraction, the antibody bound fraction of $^{125}$-I-melatonin is counted. Assay results were not corrected for recovery (which has proven to be >95% in independent trials). The minimum detection limit of the assay is < 0.2 pg/ml melatonin.

2.7 Behavioral measures

The behavioral measures included in the multiday studies (sections 3.2.7 and 4.3.7) included KSS (Akerstedt & Gillberg, 1990), 5 and 10 minute Psychomotor Vigilance Tasks (PVT) (Dinges and Kribbs, 1991; Dinges and Powell, 1985), Neurobehavioral Test Batteries (Profile of Mood States, DSST, DS Forward entry tasks, DS Reverse entry tasks) (Kane and Reeves, 1997; McNair et al., 1992), and a side effects form (Terman and Terman, 1999; Goel and Etwaroo, 2006). No behavioral measures were used during the acute study.

During the week prior to admission, subjects came into the laboratory and completed each of the individual tests at least four times to ensure familiarity prior to the start of the study. Additional tests occurred on baseline days in each of the multiday studies to give subjects additional practice. Neurobehavioral testing was done on a laptop using software from Pulsar Informatics, Inc. of Philadelphia, Pennsylvania. Files were uploaded to Pulsar after each study run, and the extracted data was then emailed to our laboratory from Pulsar. The tests in this battery were the DSST, DS Forward task, DS Reverse task, and Profile of Mood States (POMS).
2.7.1 Karolinska sleepiness scale

The KSS is a subjective measure of a person’s perceived sleepiness based on a 9-number scale where 1 = very alert, 3 = alert, 5 = neither alert nor sleepy, 7 = sleepy (but not fighting sleep), and 9 = very sleepy (fighting sleep) ((Akerstedt & Gillberg, 1990). Subjects completed the KSS by pressing the appropriate number on a computer keyboard during the beginning of all testing batteries throughout the multiday studies.

2.7.2 Psychomotor vigilance tasks

The 5- and 10- minute PVTs are sustained-attention reaction time tasks that were developed by Dr. Dave Dinges (Dinges and Kribbs, 1991; Dinges and Powell, 1985). Subjects were given these tests visually or by audio throughout the multiday studies and were instructed to monitor a red rectangular box on a computer screen or listen for a beep and press a response button as soon as a yellow stimulus counter appears inside the box or a beep is sounded. The button stops the counter, which is displayed in milliseconds for a 1 second period. Subjects were instructed to press the button as soon as each stimulus occurs, but not to press the button too soon, which will register as a false start. The stimuli were presented at random intervals shorter in the 5-minute PVT and at much longer intervals during the 10-minute PVT. Mean reaction times and lapses (reaction times greater than 500 ms) were counted per testing session as a measure of performance impairment indicative of reduced behavioral alertness.

2.7.3 Digit symbol substitution task

The primary test of working memory was the DSST (Kane and Reeves, 1997). The DSST is a subject-paced task that involves the matching of digits (0–9) to symbols. The number of correct responses in 1.5 min was counted as a measure working memory performance.
2.7.4 Digit span forward and digit span reverse

Secondary measures of working memory included the DS Forward and Reverse tasks (Kane and Reeves, 1997). In the DS test, subjects were presented with a series of digits and must immediately type them back in forward or reverse order. If they do this successfully, they are given a longer list (e.g., '9, 2, 4, 0'). The length of the longest list a person can remember is that person's digit span.

2.7.5 Profile of moods states

The POMS is a psychological rating scale that surveys transient mood states of subjects. Its six-factor scales of mood assessment can be used to track changes in mood states within a given subject over time, offering objective intra-subject measure of mental state tolerance and response to experimental exposures (McNair et al., 1992). Each item inventory in the POMS is scored with a maximum of 4 points from 0-4. The 6-subscale factor analysis includes 9 such tension-anxiety items (scored 0-36), 15 depression-dejection items (scored 0-60), 12 anger-hostility items (scored 0-48), 8 vigor-activity items (scored 0-32), 7 fatigue-inertia items (scored 0-28), and 7 confusion-bewilderment items (scored 0-28). Each administration of the scale can be scored to reflect a subject’s “total mood disturbance” at a given point in time by adding the 5 subscale scores other than vigor-activity and subtracting the subscale score for vigor-activity from the other 5 totaled.

2.7.6 Side effects

All subjects were given, before and after all light exposures, a Systematic Assessment for Treatment Emergent Effects (SAFTEE) questionnaire. This questionnaire was designed to be used to assess effects of light in patients diagnosed with Seasonal Affective Depression (SAD) (Terman and Terman, 1999) and has been used in successfully in non-depressed subjects exposed to light (Goel and Etwaroo, 2006). A computerized version was installed on the subject testing laptops and 52 questions were asked, organized by organ system and body areas. Subjects were asked to evaluate how they felt according to the following scale: 1) - not at all; 2) - a little bit; 3) – moderate; 4) - quite a bit; and 5) – extreme.
CHAPTER 3 POLYCHROMATIC BLUE-ENRICHED FLUORESCENT LIGHT FOR MELATONIN SUPPRESSION, ALERTNESS, AND CIRCADIAN PHASE Resetting

3.1 Introduction

The human circadian pacemaker is exquisitely responsive to ocular light exposure (Czeisler et al., 1999; Zeitzer et al., 2000) (section 1.1). Photic information about light is carried along a well studied neural pathway that extends from the SCN to the pineal gland via a multisynaptic pathway with connections being made sequentially in the paraventricular hypothalamus, the upper thoracic intermediolateral cell column, and the superior cervical ganglion (Moore, 1983). By way of this neuroanatomy, cycles of light and dark which are perceived through the eyes entrain SCN neural activity which, in turn, entrains the rhythmic synthesis and secretion of melatonin from the pineal gland. In virtually all species including humans, high levels of melatonin are synthesized during the night with low to undetectable amounts produced during the day (Arendt, 1995).

The elucidation of light-induced melatonin suppression in humans led to the demonstration that bright white light could be used to phase-shift human circadian rhythms (Czeisler et al., 1986; Lewy et al., 1987). Measurement of melatonin responses to light has been used extensively as a tool to examine the ocular, neural, and biochemical physiology of melatonin regulation and circadian rhythms (Klein et al., 1991; Brainard et al., 1997). Similarly, studies of the melatonin responses to light have been used to develop light as a treatment for circadian disruption, jet lag, shiftwork, sleep disturbances, and mood disorders (Lam, 1998; CIE, 2006; IESNA, 2008).

Before 1990, most investigators thought that the photoreceptors for vision were responsible for circadian regulation and melatonin control. Strong evidence from animal and human studies, however, indicated that a novel photoreceptor is primarily responsible for transduction of photic stimuli in circadian regulation (Foster et al., 1991; Czeisler et al., 1995; Ruberg et al., 1996; Freedman et al., 1999; Lucas et al., 1999; Brainard et al., 2001a; Klerman et al., 2002) (section 1.2.3). The action spectrum for melatonin suppression in normally sighted humans appears to be independent of each of the individual visual
photoreceptors (rods and cones) that support day and night vision (Brainard et al., 2001b; 2008b; Thapan et al., 2001). Other action spectra developed in rat, mice, monkey, and human found a common 446-484 nm region of peak sensitivity for circadian, neuroendocrine, and neurobehavioral responses (Brainard and Hanifin 2005, for review; Gamlin et al., 2007, Zaidi et al., 2007) (section 1.6.2). Each of these action spectra suggested that a novel photopigment mediates circadian phototransduction.

Working from the spectral sensitivity of melanopsin containing ipRGCs, multiple laboratories have performed wavelength comparison studies on melatonin suppression, circadian phase change and acute alertness using monochromatic (less than 15 nm halfpeak bandwidth) or narrowband (15 nm or greater halfpeak bandwidth) light sources (review, Brainard and Hanifin, 2005). Monochromatic blue light has been shown to cause greater phase delay over monochromatic green light (Lockley et al., 2003). The phase delay shift of the 460 nm monochromatic light presented in this study was similar to what was observed in a study using much more in intense (approximately 10,000 lux) polychromatic white light (Khalsa et al., 2003). Another study showed that very low levels of short-wavelength light (combined 436 nm and 456 nm) presented immediately after habitual wake time produced a phase-advance similar in size to very bright polychromatic light despite the large (185-fold) difference in light photons (Warman et al., 2003). A comparison of 440, 470 and 600 nm monochromatic light presented with intermittent exposure revealed that the 470 nm light was more effective in phase advancing than 600 nm and 440 nm light although the phase shifts were not conclusive (Revell et al., 2005).

Studies carried out at the same time in Japan, manipulating numerous parameters, have documented neuroendocrine and neurobehavioral effects using different color temperature lamps (Jablonski et al., 2008 for review). Specifically, higher correlated color temperature (CCT) (richer in blue wavelengths) lamps evoked stronger melatonin suppression than lower CCT bulbs (Morita and Tokura, 1998; Sato et al., 2005). Generally, these results are complementary to our understanding of the analytic action spectra in that higher color temperature fluorescent bulbs induce stronger neurobehavioral and neuroendocrine effects than lower color temperature lamps.

More recent studies comparing polychromatic fluorescent light with more short-wavelength energy (8,000 K CCT) to typical fluorescent lighting (4,100 K CCT) for melatonin
suppression revealed differences but only at one of several low light levels that were studied (Figuero et al., 2006). Smith and Eastman (2009) compared 4,100 K and 17,000 K CCT lamps at equal photon densities for circadian phase-delay with no greater phase-delays seen in the 17,000 K CCT condition. Smith and colleagues (2009) compared 4,100 K and 17,000 K CCT lamps at relatively equal irradiances (commonly used light therapeutic lux levels) for phase-advancing potential with no greater phase-advances observed in the 17,000 K CCT condition (Smith et al., 2009). These studies used very high, potentially saturating, levels of light to mimic a real world therapeutic light situation.

3.2 Aims and Hypothesis

In the following study, we tested the hypothesis that blue-enriched polychromatic fluorescent light at lowered light levels (e.g. room light levels) is more effective than standard white polychromatic fluorescent light (4,000 K) light for eliciting a circadian phase-delay, acutely suppressing melatonin, and acutely reducing sleepiness and increasing alertness.

3.3 Methods

3.3.1 Test facility

The study was conducted at TJU in a facility built specifically for inpatient studies with experimental light exposures. As illustrated in Figure 6, this facility has two identical interior rooms, with a hallway connecting them with a shared bathroom, an experimental control room, and a kitchen for preparing subject meals. A white noise machine (Cambridge Sound Management, Cambridge, MA) has been placed in the drop ceilings of the subject rooms and runs constantly. Heating, ventilating and air conditioning systems located above each of the doors to the subject rooms were set to 75º F (24º C) at all times. The subjects did not have access to the kitchen nor the control room, where there is a wet laboratory bench for processing samples and the lighting controls.
Each subject room contained a bed which can be raised to a 45° angle, a dresser, and a desk with an ophthalmological head holder 11.8” in front of a 4’ x 4’ lighting panel. A television and DVD player were also included in each subject room and allowed to be used during the periods of normal room lighting. Each room also had a video camera connected to a monitor in the control room, as well as several intercom buttons to signal the staff in the control room when the subject needed something or had completed testing.

Subjects were not permitted to know the date nor time throughout the study. The subject rooms have neither windows nor clocks, and subjects were forbidden to have time pieces, cell phones, or any other electronics displaying date and/or time. Portable music players were permitted, but any clock functions were disabled by laboratory staff upon admission. Laboratory staff members were instructed not to use any day, time, or time of day specific language when interacting with subjects. The windows in the bathroom that the subjects were allowed to use at specific points in the study as well the windows in the hallway were covered in blackout shades. The staff members were also not permitted to wear watches or carry cell phones into subject rooms. Scrubs and laboratory coats were provided for the staff to wear to further eliminate weather or other cues from the outside world.

Figure 6. Floor plan of the laboratory study facility. Subject areas were equipped with a remotely controlled zone lighting system, audio/video monitors and a dedicated heating, ventilating and air conditioning system.
3.3.2 Ambient light stimuli and measurement

Ambient light in the facility was produced by ceiling-mounted standard cool-white (4,100 K CCT) 40 watt fluorescent lamps (F40T12/841ALTO, Philips Lighting, B.V., Eindhoven, Netherlands) placed above matte white acrylic 0.080 inch diffuser panels and controlled by a full range dimming system (Model #HLT-2000/3 Dimmer Module with HILUME Electronic dimming ballast OPSCU Series, DP-1 dimmer panel, VA-3-4A Control Panel, Lutron Corp., Coopersburg, PA).

The light intensities in the study rooms were < 80 lux in any direction of gaze on study day 1 as well as during the first eight hours of study day 2. For the rest of the waking hours of the study until the day of release, subjects were kept in constant dim light with illuminances < 5 lux in any direction of gaze. Sleep opportunities totaling 8 h were in complete darkness in the study rooms. Daily illuminance/irradiance measures were taken using meters described earlier in section 2.2 that were centered in the room pointed in the horizontal direction of gaze at approximately 4’ from the floor. Four readings were taken facing each of the four walls in the room and averaged.

3.3.3 Experimental light stimuli and measurement

Polychromatic light exposures occurred on the night of study day 4 and subjects underwent a 6.5 hour light exposure that began 9.25 h before their habitual wake time. Light exposure was timed to start 9.25 h before habitual waketime which was assumed to correspond to approximately 6.75 h before core body temperature minimum, a phase where previous studies of polychromatic white light produced phase delays (Zeitzer et al., 2000; Lockley et al., 2003). Two 4’ x 4’ light exposure systems pictured in Figure 8 held both sets of 54 W fluorescent lamps in each. A diffuser cover on the front of the panel allowed for a uniform light exposure for the subject. The panel was attached to an analog dimmer switch to adjust intensity. The lamps, as well as the lighting panel unit, diffuser cover, and dimmers were constructed and donated by Philips Lighting, B.V. (Eindhoven, Netherlands). An additional neutral density filter panel (0.3 ND 50% transmission) that covered the entire light panel surface was used to further adjust the intensity of the 17,000 K light source (Rosco Laboratories, Stamford, CT). Experimental illuminance/irradiance measures were taken
using an IL-1400BL radiometer/photometer (International Light Technologies, Inc., Peabody, MA) previously described which was held in place by a clear, plastic holder attached in an ophthalmologic head holder 11.8 inches from the panel at approximate eye level. The spectral power distribution measurements determinations shown in Figure 7 were taken using a Model FSHH 325-1075P FieldSpec handheld spectroradiometer (Analytical Spectral Devices, Inc., Boulder, CO).

Figure 7. Spectral power distribution for 17,000 K (upper panel) and 4,000 K (lower panel) light sources. Profiles are relatively similar, with the primary difference between 400 and 500 nm. Compared to the 4,000 K light source, the 17,000 K light source emitted substantially more power in the short wavelength part of the spectrum.
The target intensity of the 17,000 K lamp was chosen from previous melatonin suppression testing, which resulted in a fluence-response curve (Brainard et al., 2007). The ED90, or 90% of the saturating dose of light for melatonin suppression for the 17,000 K lamp, was chosen for this study. It was assumed based on laboratory pilot data that this would give a partial (approximately 50%) suppression of melatonin for the 4,000 K exposure condition yielding a clear difference in melatonin suppression between the two light conditions. This resulted in an equal photon density of $1 \times 10^{14}$ photons/cm²/s for both exposure conditions. The equivalent irradiance/illuminance of light for the 4,000 K and 17,000 K lamps were 40.7 μW/cm² (134.3 lux) and 50.0 μW/cm² (128.9 lux), respectively.

### 3.3.4 Subject screening

Over 400 subjects contacted the study recruiter in response to local newspaper or internet advertisements via phone (63%) or e-mail (37%). Approximately 50% of the respondents who were given additional study requirements and details over e-mail or phone either declined to proceed further or never contacted the laboratory again. Initial general questioning on age, health, work schedule and medications eliminated approximately 30% of the contacts. Of the 123 individuals who participated in a full phone screen, 87 were found to have exclusion criteria or declined to further participate in the screening process. Thirty-five individuals met with the study recruiter to tour the laboratory facility. At this point, 33 subjects were instructed to schedule appointments with the study physician and study psychologist, have a urine drug screen for drugs of abuse performed, have a blood drawn for a basic metabolism panel and keep a strict sleep/wake cycle with daily phone calls to the laboratory voicemail upon sleeping and waking. For at least 10 days prior to entry into the seven day protocol subjects maintained a self-selected 8-hour sleep/wake schedule with calls to a time- and date-stamped voicemail at bedtime and wake. Subjects also wore an actigraph (BASIC Motionlogger, Ambulatory Monitoring, Inc., Ardsley, NY) to verify bed and wake times during this time period. The inclusion criteria for medication were no recreational, over-the-counter, or prescription medications inclusive of caffeine and alcohol. Toxicological screening of blood and urine for caffeine, pregnancy in females, basic metabolic panel, alcohol, drugs of abuse, thyroid function, and a CBC was conducted during screening and on the day of entry in the facility.
Figure 8. The two 4’ x 4’ light exposure systems used are shown above (17,000 K top; 4,000 K bottom). Please note that during actual light exposure the subjects head would be in an ophthalmologic head holder 11.8” from the panel.
Three subjects were eliminated due to physical or psychological exclusion criteria. Nine subjects failed to keep regular sleep/wake times, tested positive for drugs of abuse, or declined to participate further. After undergoing psychological, toxicological, physical, ophthalmological examinations including color vision testing, and sleep/wake cycle screenings the remaining 21 subjects met study requirements and chose to be entered into the seven day inpatient study protocol. On the day of the study, I reviewed in great detail all that was expected of the research subject and consented the subject. As proposed, 20 healthy men and women completed the full study. One subject asked to be released from the protocol on the 5th day of the study. A total of 11 males and 9 females ranging in age from 19 to 26 years old, with a mean age (± SEM) of 22.1 ± 1.8 years were studied in the Light Research Laboratory of TJU.

3.3.5 Study protocol

This study was approved by the IRB of TJU and all subjects gave written informed consent prior to entering the protocol. Figure 9 provides a diagrammatic representation of the 7-day study protocol. The phase shifting protocol used in this study was adapted from the 9-day protocol used at the Brigham and Women’s Hospital (BWH) (Zeitzer et al., 2000). Subjects were kept in a time-free environment (no windows, timepieces, TV, or internet access with continual supervision by trained staff) and remained in their assigned room for the duration of the study, except for scheduled showers or when they were allowed bathroom breaks in the suite bathroom. If a second subject was in the facility, there was no interaction between the subjects at any point during the study. The schedule consisted of a 2-day baseline (8-hour:16-hour sleep-wake cycle based on each subject’s self-selected sleep-wake times), an initial 26 hour constant routine, a 16 hour light-exposure day, and a second 30 hour constant routine, each preceded and followed by an 8-hour sleep opportunity. During the constant-routine period, subjects remained awake in dim light in a semirecumbent position while under direct supervision with isocaloric meals (basal energy expenditure x 1.3) being served every two hours. Basal energy expenditure calculations were done by dieticians from the Nutrition and Dietetics department at TJU Hospital and were corrected for age and gender.
Figure 9. Overview of 7-day phase-shifting protocol for a subject with a midnight to eight AM sleep:wake schedule. This protocol was adapted from the 9-day phase-shifting protocol frequently used by Brigham and Women’s Hospital (Zeitzer et al., 2000).

Subjects were randomly assigned in groups of 4 throughout the length of the protocol to polychromatic light exposure from either the 4,000 K or the 17,000 K fluorescent lamps (N=10, 5 females, both conditions). Subjects were seated for 140 minutes prior to, and for the full duration of the 6.5 h light exposure. Subjects wore blindfolds for approximately 30 minutes prior to light exposure while the lights stabilized and intensities were verified. Subjects were seated 11.8 inches in front of the light panel with their chin placed in an ophthalmologic head holder and monitored continuously. Subjects were asked to maintain a fixed gaze toward the panel for 90 minutes before resting their head and neck out of the headholder for 10 minutes while remaining seated. This sequence was repeated throughout the entire 6.5 hour exposure.

3.3.6 Measurement of melatonin

Blood samples (3 ml) were collected through an indwelling intravenous catheter located in a forearm vein into 3 ml polystyrene tubes which contained 5.4 mg of K2 EDTA (BD Diagnostics, Franklin Lakes, NJ). Plasma was separated by refrigerated centrifugation (2,000
RPM for 15 minutes), aliquoted into polypropylene cryogenic vials (Thermo Fisher Scientific, Rochester, NY) and stored at -20˚ C until assay (section 2.3). Plasma melatonin samples were collected every 60 minutes during the constant-routine periods on Days 3/5 and every 20 minutes during light exposure (Figure 9). Plasma melatonin concentrations were assayed by radioimmunoassay using antiserum described by Rollag and Niswender (1976). A full description of the method can be found in section 2.6.1.

Salivary samples were collected as backup to the primary plasma measure and were used when there were problems with patency of the intravenous catheter. Salivary samples (1.2-2 ml) were collected with the "Salivette" sampling device (Sarstedt, Inc., Hayward, CA). The specific protocol for this is described in section 2.4. Salivary melatonin samples were collected every 60 minutes during the constant-routine periods on Days 3/5 as well as during light exposure. Salivary melatonin concentrations were assayed by radioimmunoassay (Vaughan, 1993). A full description of the method can be found in section 2.6.2.

3.3.7 Neurobehavioral testing

Neurobehavioral testing was organized and initiated by collaborators at Brigham and Womens Hospital (BWH) in Boston. I was trained on this laboratory technique but did not do the preparation of the instruments for the protocol.

3.3.7.1 Visual psychomotor vigilance task

During every hour of wakefulness, subjects completed a 5-min visual psychomotor vigilance tasks (PVT) (Dinges and Powell, 1985; Dinges and Kribbs, 1991). The visual PVT was done on a personal digital assistant (PDA) (Tungsten E2, Palm, Inc, Sunnyvale, CA). The screen showed successive black bull's eye targets on white backgrounds; the subject was asked to press the button at the bottom of the right hand side of the PDA as quickly as possible once the target appeared (section 2.7.2). The subject's reaction time in milliseconds appeared overlaid on the target briefly before the screen went blank white until the next target appeared. This modified visual PVT lasted for five minutes, at the end of which the mean reaction time for that trial was displayed for the subject. The main measures for each trial for
each subject were mean reaction time in milliseconds and number of lapses greater than 500 milliseconds (i.e. number of times the subject took 500 milliseconds or longer to react to the target appearing) (Dinges and Powell, 1985; Lim and Dinges, 2008; Lamond et al., 2008).

### 3.3.7.2 Auditory psychomotor vigilance task

During every hour of wakefulness, subjects completed a 10-min auditory psychomotor vigilance task (PVT) (Dinges and Powell, 1985; Dinges and Kribbs, 1991). The auditory PVT was completed on a PVT-192 by Ambulatory Monitoring, Inc. (Ardsley, NY). Subjects were instructed to complete the KSS on the PDA first, then the auditory PVT using the PVT-192, and finally the visual PVT on the PDA. Subjects completed the auditory PVT by wearing a set of headphones attached to the PVT-192 and pressing a button on the right side of the device as quickly as possible when a tone was heard (section 2.7.2). The auditory PVT trial was 10 minutes in length. The LED panel at the top of the device that can be used for a visual PVT was covered in several layers of tape so that any visual changes were not visible to the subject. The main measures for each trial for each subject were mean reaction time in milliseconds and number of lapses greater than 400 milliseconds (Dinges and Kribbs, 1991).

### 3.3.7.3 Karolinska sleepiness scale

The first screen on the PDA prior to the visual PVT asked the subject to rate their subjective sleepiness at that point using the KSS (Akerstedt and Gillberg, 1990). The KSS is a subjective measure of a person’s perceived sleepiness based on a 9-number scale where 1 = very alert, 3 = alert, 5 = neither alert nor sleepy, 7 = sleepy (but not fighting sleep), and 9 = very sleepy (fighting sleep).

### 3.3.8 Data reduction and statistics

Neurobehavioral testing results were analyzed by collaborators at BWH. I was trained on this laboratory technique but did not do analysis.
3.3.8.1 Melatonin data reduction and statistics

The plotting of the melatonin rhythm of each subject for CR1 and CR2 was determined through the use of a three-harmonic fit program created by collaborators at BWH. Phase-shift was determined from differences in clock time between 25% dim light onset of melatonin secretion during the first constant routine (CR1) as compared to the second constant routine (CR2) (Duffy et al., 1996). Unpaired, two tailed Student’s t-tests were used to compare differences in phase shifts as well as differences in variance by exposure to 17,000 K lamps compared to 4,000 K lamps.

Melatonin suppression (mean ± SD) was calculated from the difference in area under the curve (AUC) between melatonin profiles during light exposure and the corresponding clock times during the previous melatonin cycle on CR1 (Lockley et al., 2003). Unpaired, two tailed t-tests were used to compare differences in melatonin suppression by exposure to 17,000 K lamps compared to 4,000 K lamps.

3.3.8.2 PVT data reduction and statistics

The data from the visual PVT and the auditory PVT-192 devices were extracted by our collaborators at BWH using the PVTCOMM for Windows Version 2.10.1.1 software from Ambulatory Monitoring, Inc (Ardsley, NY). The extracted data was then read using the REACT Version 1.105 software from Ambulatory Monitoring, Inc. The data for each trial were copied from REACT and transferred into an Excel spreadsheet for each subject. Date and time stamps were converted to h since CR1 began, light exposure began, and CR2 began, respectively. Mixed analysis of variance (ANOVA) comparing light sources and time was completed using SAS 9.0 (SAS, Cary, NC). Graphs were made using SigmaPlot 10.0 (Systat, San Jose, CA) after binning the data by hour since the respective block of the study began and taking the average value of each measure per hour.
3.3.8.3 KSS data reduction and statistics

KSS data were also binned by the hour with Mixed ANOVA analysis comparing light sources and time completed using SAS 9.0 (SAS, Cary, NC). Graphs were made using SigmaPlot 10.0 (Systat, San Jose, CA) after taking the average value of each measure per hour.

3.3.8.4 Sample size determination

To determine sample size a priori, data from a previous study of the phase-shifting effects of 460 nm and 555 nm monochromatic light on melatonin rhythms were used (Lockley et al., 2003) to perform a power analysis. The power analysis was performed by our colleagues at BWH. Using these data, a 1 h phase shifting difference was assumed, with a standard deviation of 0.62 h, $\alpha$ value of 0.05, and $\beta$ value of 0.9. With these assumptions, 10 subjects per group were calculated to be needed to detect the 1 h phase difference (Cohen, 1988).

3.4 Results

3.4.1 Ambient light stimuli measurement

The measured illuminances (± SEM) for the baseline days were 52.7 ± 1.6 lux and for the CR conditions illuminances were 1.6 ± 0.1 lux.

3.4.2 Experimental light stimuli measurement

The measured irradiances (± SEM) over the 6.5 h exposure were 40.9 ± 0.1 $\mu$W/cm$^2$ for the 4,000 K exposure and 50.0 ± 0.1 $\mu$W/cm$^2$ for the 17,000 K exposure.
3.4.3 Measurement of melatonin

Polychromatic fluorescent light exposure caused a phase-delay of the DLMO in all but of the one of the subjects. Exposure to 6.5 hours of 17,000 K light caused a greater phase-delay shift (mean (±SD) 2.1 ± 0.6 h, n=10) than did exposure to 4,000 K light (mean (± SD) 1.7 ± 1.3 h, n=10). This phase-delay shift, however, was not statistically significant (p=0.22) (Figure 10). Further comparison of the data revealed that 17,000 K light provided a more consistent phase-delay than 4,000 K light when comparing standard deviations (p<0.05).

As shown in Figure 11, all subjects exposed to 17,000 K polychromatic fluorescent light at 1 x 10^{14} photons/cm²/s had at least 29% suppression of the melatonin as calculated by AUC during the 6.5 h light exposure (range 29-91%) compared to the corresponding clock times during the previous melatonin cycle on CR1. Suppression was more variable among subjects exposed to the 4,000 K polychromatic fluorescent light at 1 x 10^{14} photons/cm²/s (range 0-82%), including 2 individuals with 1% and 0% melatonin suppression. On average, exposure to 6.5 hours of 17,000 K light caused a significantly greater suppression of melatonin (mean (±SD) 70.9 ± 19.6%; n=10) compared with 4,000 K light (mean (±SD) 42.8 ± 29.1%; n=10) (p<0.05).

The inter-assay coefficient of variation from the 15 plasma assays run for this experiment using the assay from Rollag and Niswender (1976) was 15.9% for 50 pg/ml and 14.9% for 125 pg/ml control samples (n=4 replicates). Coefficient of variation calculated from a control sample of 100 pg/ml assayed had 12.3% for intra-assay coefficient of variation (n=4 replicates). The minimum detection limit of the assay is 0.5-2.0 pg/ml melatonin. The inter-assay coefficient of variation from the 7 salivary assays run for this experiment using the assay from Vaughan (1993) was 11.6% for 2.1 pg/ml and 8.4% for 18.5 pg/ml control samples (n=20 replicates). Coefficient of variation calculated from control samples of 1.83 and 18.99 pg/ml assayed had 2.7% and 6.9% intra-assay coefficient of variation respectively (n=2 replicates). The minimum detection limit of the assay is 0.5-2.0 pg/ml melatonin.
Figure 10. Phase-delay shift (±SD) of the plasma (filled circles) or salivary (empty circles) melatonin rhythm as assessed by DLMO following exposure to 6.5 h of white 4,000 K fluorescent light and blue-enriched 17,000 K fluorescent light. There was not a significant difference between DLMO’s (p=0.22). There was, however, a significantly more consistent delay in DLMO under the 17,000 K fluorescent light compared to 4,000 K fluorescent light.
3.4.4 Neurobehavioral testing

3.4.4.1 Visual psychomotor vigilance task

As shown in Table 6, the mean reaction time (RT) for the visual PVT for each hour since light exposure began were compared between the two light groups using ANOVA and were not significantly different. There was a significant difference over time for the light exposure period (p<0.02), but no interaction between light source and time. Figure 12 shows the mean

Figure 11. Melatonin suppression (±SD) as calculated by AUC with plasma (filled circles) or salivary (empty circles) levels following exposure to 6.5 h of white 4,000 K fluorescent light or blue-enriched 17,000 K fluorescent light compared to corresponding clock times during the previous melatonin cycle on CR1. A significantly greater suppression of plasma melatonin occurred under 17,000 K fluorescent light compared to 4,000 K fluorescent light (p<0.05).
RT averaged per hour. Note that mean RT remained low before and throughout light exposure for both light groups. There was no significant difference over time or between light sources for the 3 hours post light exposure as shown in Table 7.

Table 6. Mixed repeated ANOVA on mean RT for visual PVT during light exposure.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F value</th>
<th>Pr &gt; F</th>
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<td>1.36</td>
<td>0.260</td>
</tr>
<tr>
<td>time</td>
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<td>83</td>
<td>2.88</td>
<td>0.014</td>
</tr>
<tr>
<td>light source*time</td>
<td>6</td>
<td>83</td>
<td>0.75</td>
<td>0.612</td>
</tr>
</tbody>
</table>

Table 7. Mixed repeated ANOVA on mean RT for visual PVT 3 hours post light exposure.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>light source</td>
<td>1</td>
<td>16</td>
<td>2.64</td>
<td>0.124</td>
</tr>
<tr>
<td>time</td>
<td>2</td>
<td>30</td>
<td>1.88</td>
<td>0.169</td>
</tr>
<tr>
<td>light source*time</td>
<td>2</td>
<td>30</td>
<td>0.90</td>
<td>0.418</td>
</tr>
</tbody>
</table>
Figure 12. Average mean RT (±SE) of the 17,000 K fluorescent light group (empty circles) or the 4,000 K fluorescent light group (filled circles) per hour for visual PVT before, during, and after light exposure. The period of light exposure is indicated by the box.

As shown in Tables 8 and 9, the lapses greater than 500 milliseconds for the visual PVT for each hour since light exposure began were compared between the two light groups using ANOVA and were not significantly different for the light exposure period (p=0.70) or for 3 hours post light exposure (p=0.35). There was a significant difference over time for the light exposure period (p<0.02) and for 3 hours post light exposure (p<0.03), but no interaction between light sources and time. As seen in Figure 13, the number of lapses for both groups increase linearly before, during, and for 3 hours post light exposure.
Table 8. Mixed repeated ANOVA of lapses for visual PVT during light exposure.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>light source</td>
<td>1</td>
<td>16</td>
<td>0.15</td>
<td>0.704</td>
</tr>
<tr>
<td>time</td>
<td>6</td>
<td>83</td>
<td>2.90</td>
<td>0.013</td>
</tr>
<tr>
<td>light source*time</td>
<td>6</td>
<td>83</td>
<td>0.55</td>
<td>0.767</td>
</tr>
</tbody>
</table>

Table 9. Mixed repeated ANOVA of lapses for visual PVT for 3 hours post light exposure.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>light source</td>
<td>1</td>
<td>16</td>
<td>0.90</td>
<td>0.356</td>
</tr>
<tr>
<td>time</td>
<td>2</td>
<td>30</td>
<td>4.43</td>
<td>0.021</td>
</tr>
<tr>
<td>light source*time</td>
<td>2</td>
<td>30</td>
<td>1.29</td>
<td>0.290</td>
</tr>
</tbody>
</table>
Figure 13. Average visual PVT lapses (±SE) of the 17,000 K fluorescent light group (empty circles) or the 4,000 K fluorescent light group (filled circles) per hour during the exposure day. The period of light exposure is indicated by the box.

3.4.4.2 Auditory psychomotor vigilance task

Three subjects were eliminated from the auditory PVT analysis for the 4,000 K light group as their data files were corrupted due to equipment malfunction. The mean RT for each hour during light exposure were compared between the two groups using ANOVA and were not significantly different for the light exposure period for light source, time, nor an interaction between light source and time, as shown in Table 10. There was a significant difference (P<0.03) between light sources, however, for the 3 hours post light exposure, but no interaction between light sources and time, also shown below in Table 11. Figure 14 shows that the mean RTs were consistently low for both light source groups before and during light exposure, as with the visual PVT.
Table 10. Mixed repeated ANOVA of mean RT for auditory PVT during light exposure.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>light source</td>
<td>1</td>
<td>13</td>
<td>0.03</td>
<td>0.861</td>
</tr>
<tr>
<td>time</td>
<td>6</td>
<td>68</td>
<td>0.95</td>
<td>0.467</td>
</tr>
<tr>
<td>light source*time</td>
<td>6</td>
<td>68</td>
<td>0.56</td>
<td>0.759</td>
</tr>
</tbody>
</table>

Table 11. Mixed repeated ANOVA of mean RT for auditory PVT 3 hours post light exposure.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F value</th>
<th>Pr &gt; F</th>
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<td>1</td>
<td>13</td>
<td>6.60</td>
<td>0.023</td>
</tr>
<tr>
<td>time</td>
<td>2</td>
<td>19</td>
<td>1.32</td>
<td>0.290</td>
</tr>
<tr>
<td>light source*time</td>
<td>2</td>
<td>19</td>
<td>0.65</td>
<td>0.531</td>
</tr>
</tbody>
</table>
Figure 14. Average mean RT (±SE) of the 17,000 K fluorescent light group (empty circles) or the 4,000 K fluorescent light group (filled circles) per hour for auditory PVT during light exposure study day, light exposure indicated by box.

The lapses greater than 400 milliseconds for each hour since light exposure began were compared between the two groups using ANOVA and were not significantly different for the light exposure period for light source, time, nor an interaction between light source and time, as shown below in Table 12. Figure 15 shows the number of lapses was consistently low for both groups before and during light exposure. Also seen in Figure 15 and Table 13 below, the number of lapses for both groups increased linearly for 3 hours post light exposure with a significant difference between light sources (p<0.003).
Table 12. Mixed repeated ANOVA for lapses of auditory PVT during light exposure.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>light source</td>
<td>1</td>
<td>13</td>
<td>0.03</td>
<td>0.861</td>
</tr>
<tr>
<td>time</td>
<td>6</td>
<td>68</td>
<td>0.95</td>
<td>0.467</td>
</tr>
<tr>
<td>light source*time</td>
<td>6</td>
<td>68</td>
<td>0.56</td>
<td>0.759</td>
</tr>
</tbody>
</table>

Table 13. Mixed repeated ANOVA for lapses for auditory PVT 3 hours post light exposure.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>light source</td>
<td>1</td>
<td>13</td>
<td>14.1</td>
<td>0.002</td>
</tr>
<tr>
<td>time</td>
<td>2</td>
<td>19</td>
<td>0.79</td>
<td>0.469</td>
</tr>
<tr>
<td>light source*time</td>
<td>2</td>
<td>19</td>
<td>0.52</td>
<td>0.601</td>
</tr>
</tbody>
</table>
Figure 15. Average auditory PVT lapses (±SE) of the 17,000 K fluorescent light group (empty circles) or the 4,000 K fluorescent light group (filled circles) per hour before, during, and after light exposure. The period of light exposure is indicated by box.

### 3.4.4.3 Karolinska sleepiness scale

The results of a mixed ANOVA for the KSS values are shown in Tables 14 and 15. As shown in Figure 16, subjective, self-rated sleepiness as measured by the KSS revealed that scores were lower during light exposure for the 17,000 K group versus the 4,000 K group (both p<0.04). Subjects reported getting sleepier over time (p<0.05) with the 4,000 K light group consistently reporting being sleepier at all time points (p<0.05) compared to the 17,000 K light group. KSS scores were lower during light exposure and for 3 hours post light exposure.
for the 17,000 K exposure group versus the 4,000 K exposure group (both p<0.05) indicating lower subjective sleepiness levels in the 17,000 K exposure group.

Table 14. Mixed repeated ANOVA for KSS during light exposure; Num DF = numerator degrees of freedom (number of groups in effect - 1); Den DF = denominator degrees of freedom (number of data points for each effect - 2).

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>light source</td>
<td>1</td>
<td>16</td>
<td>8.49</td>
<td>0.010</td>
</tr>
<tr>
<td>time</td>
<td>7</td>
<td>101</td>
<td>5.68</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>light source*time</td>
<td>7</td>
<td>101</td>
<td>1.55</td>
<td>0.159</td>
</tr>
</tbody>
</table>

Post-hoc analysis showed that this effect of lighting condition and time continued through for the 3 hours post light exposure (p<0.05).

Table 15. Mixed repeated ANOVA for KSS 3 hours post light exposure.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>light source</td>
<td>1</td>
<td>16</td>
<td>5.65</td>
<td>0.030</td>
</tr>
<tr>
<td>time</td>
<td>3</td>
<td>33</td>
<td>3.45</td>
<td>0.028</td>
</tr>
<tr>
<td>light source*time</td>
<td>3</td>
<td>33</td>
<td>0.88</td>
<td>0.459</td>
</tr>
</tbody>
</table>
3.5 Discussion

One limitation of the study design was fact that two active conditions were compared without a control condition. A variety of logistics made the inclusion of a control group not possible. If we did involve a control, no light exposure group, results would likely have shown a group small phase-delay due to their tau or natural freerunning circadian period known to be 24.2 on average in humans (Czeisler et al., 1999). Some laboratories have accounted for this in reporting their data (St. Hilaire et al., 2012), however, we chose not to adjust the calculated phase-delays.
3.5.1 Melatonin suppression and phase shifting

Our results demonstrate that both 4,000 K and blue-enriched 17,000 K fluorescent light at an equal photon density suppress plasma melatonin in healthy young subjects (42.8% and 70.8%, respectively) with 17,000 K resulting in a significantly stronger hormonal suppression. By contrast, the phase-delay data do not support the hypothesis that blue-enriched 17,000 K fluorescent light is a more potent circadian stimulus than the white 4,000 K fluorescent light. The 17,000 K fluorescent light, however, provided a significantly more consistent phase-delay than 4,000 K fluorescent light when comparing variances suggesting a greater effect on the circadian system. A post study power analysis revealed that the phase-delay study at these light intensities would need 82 subjects (41 in each lighting condition) to capture a significant difference between the two lights. Thus, the a priori power analysis was not sufficient to properly design the present study. At the time this study was done the best available data set on wavelength comparisons for phase-shifting was a study comparing two monochromatic wavelengths at an equal photon density (Lockley et al., 2003). Perhaps comparisons of monochromatic lights are not appropriate for power analyses for studies employing polychromatic light sources. Alternatively, the light intensities selection may not have been optimal. The light intensities chosen for our study intensity were based on two dose-response curves for melatonin suppression run in our laboratory which informed our team as to which photon density of 4,000 K or 17,000 K light would show the most difference (Brainard et al., 2006; 2008; Hanifin et al., 2009). It was decided that we would use the ED$_{90}$ for the 17,000 K dose response curve with its matching 4,000 K dose (approximately ED$_{50}$).

A study comparing these two polychromatic light sources at high light irradiances (1,640 μW/cm$^2$, 17,000 K; 1,741 μW/cm$^2$, 4,000 K) typically used for light therapy found no difference between the two light sources for phase advancing salivary melatonin rhythms (Smith, et al., 2009). A follow up study from the same laboratory examined phase-delaying rhythms using similar light sources in a within subjects counterbalanced design and found no difference in the capacity of 17,000 K blue-enriched lamps for phase-delaying salivary melatonin rhythms (Smith and Eastman, 2009). This study also employed high irradiances of light (1,640 μW/cm$^2$, 17,000 K; 1,475 μW/cm$^2$, 4,000 K) that are commonly used for
circadian resetting. While these data are interesting, they are not surprising given the very high light levels tested.

Monochromatic and narrowband blue light has been used in several laboratories for phase shifting the circadian clock (Lockley et al., 2003; Revell et al., 2005; Wright and Lack, 2001; Wright et al., 2004). An example is that of Warman et al. (2003) in which dim blue light (28 μW/cm²) produced similar phase shifts as bright polychromatic white light over 150 times the irradiance (4,300 μW/cm²). Another laboratory was able to produce similar circadian effects using only a dim irradiance (65 μW/cm²) of narrowband blue LED light combined with advancing the wake time in delayed sleep disorder patients (Lack et al., 2007). Another study comparing fluorescent lamps with increased short wavelength energy (8,000 K) versus typical fluorescent lamps (4,100 K) and compared at much lower illuminances, more like evening room light levels (100 lux), showed that 8,000 K blue enriched induced a significantly greater melatonin suppression versus 4,100 K light. (Figuiero et al., 2006). This significant difference in melatonin suppression was lost at the next two higher illuminances of 300 and 1,000 lux.

At low irradiances blue enriched lamps would be expected to produce a greater phase shift than typical fluorescent lighting. This difference will likely be lost as irradiance levels increase. Comparisons of blue enriched versus typical fluorescent lamps at higher irradiances typically used for circadian resetting little difference has been seen (Smith and Eastman, 2009; Smith et al., 2009). Our comparison was done at much lower irradiances, but further work is needed to determine whether polychromatic light shifted toward the shorter wavelengths of the spectrum is more effective for phase shifting circadian rhythms than standard fluorescent lighting at typical room lighting levels. Indeed several other studies have made comparisons of the light sources used in this study for other parameters such as daytime alertness, performance, mood, as well effects on SAD (Geerdrick and Schlangen, 2006; Mills et al., 2007, Viola et al., 2008, Meesters et al., 2011).

3.5.2 Neurobehavioral parameters

In the study reported here, objective and subjective measures of alertness yielded conflicting results. Subjective sleepiness measures with the KSS supports the hypothesis that 17,000 K
light exposure significantly reduced sleepiness compared to 4,000 K light exposure. Similarly, post hoc analysis of subjective sleepiness indicated that 17,000 K light exposure appeared to significantly reduce sleepiness for 3 hours after light exposure compared to 4,000 K light exposure. By contrast, the four sets of objective PVT data during light exposure do not support the hypothesis that 17,000 K light is more effective at acutely enhancing psychovigilance reaction time than 4,000 K light during light exposure. Post hoc analysis of the PVT data, however, indicated that 17,000 K light exposure appears to enhance psychovigilance reaction time for the 3 hours after light exposure compared to 4,000 K light exposure. It should be noted that statistical power was lost for some of the comparisons due to equipment malfunction or technician error causing loss of data.

Many, but not all, studies have shown that light increases alertness and improves performance in neurobehavioral tests. Early studies used bright versus dim white polychromatic fluorescent light for evoking behavioral effects. Bright white polychromatic fluorescent light at 3,000 lux, when compared to 100 lux dim white light, increased measures of subjects’ performance on seven of ten neurobehavioral tests during a 16 hour light exposure (French et al., 1990). A similar study, simulating night shift work, compared a four hour period of 6,000 lux white light to dim white light at less than 200 lux throughout an 8 hour shift (Dawson and Campbell, 1991). The four hours of bright light was given during the first night of three consecutive nights of simulated work. The group that received the four hours of bright light on the first night of the study exhibited a significantly smaller decline in alertness during the second half of the night shift than the group that received dim light the entire time. Another study compared four different lighting conditions: alternating 90 minute exposures to 5,000 lux bright white light and 50 lux dim white light during nighttime hours, alternating 90 minute exposures to bright and dim light during daytime hours, continuous bright light, or continuous dim light (Badia et al., 1991). An EEG analysis and a neurobehavioral test battery were used to assess alertness and performance throughout the experiment. An increase in brain wave frequencies associated with alertness (beta band, approximately 19 Hz) and improvement in performance on the neurobehavioral test battery were found for the bright light exposures.

Other investigators performed a dose response curve for a 6.5 h light exposure and subjective and objective measures of alertness, with a 50 hour CR before and 30 hour CR after the light exposure day (Cajochen et al., 2000). Light exposures were randomly assigned single
illuminances from 3 to 9100 lux of white light. The saturation response for KSS was approximately 200 lux, and the saturation response for quantitative EEG was approximately 300 lux. This coherence between KSS and quantitative EEG in saturating light level responses shows a similar response to light between subjective and objective alertness. According to these results, normal room light levels can elicit subjective and objective alerting responses.

Selected monochromatic wavelengths have also been tested for their behavioral effects. One such study compared equal photon doses to either 460 nm or 550 nm monochromatic light, and no light (Cajochen et al., 2005). A decrease in subjective sleepiness was found during 460 nm light exposure, but not with 550 nm light exposure or no light exposure. In a similar study (Lockley et al., 2006), exposure to equal photon doses of blue (460 nm) monochromatic light (5.0 lux), compared to 10.0 μW/cm² green (555 nm) monochromatic light (68.1 lux) for 6.5 hours, showed decreased PVT mean RT, fewer PVT lapses, increases in brain wave frequencies associated with alertness and decreases in brain waves associated with drowsiness.

Like the current study, some experiments have failed to demonstrate the effect of light improving alertness or performance measures. For example, in the study by French and colleagues described above, three tests were unaffected by bright light: column addition and subtraction, Wilkinson reaction time, and tower puzzle (French et al., 1990). Another study compared cognitive performance on selected tests from computerized test batteries to a night (2100 h to 0800 h) of either bright (5,000 lux) or dim (50 lux) common white fluorescent light in a counter-balanced design using young, male subjects. The majority of the 39 performance variables tested in these two test batteries showed a trend toward bright light exposure improving performance with only a few reaching statistically significant improvement (Brainard et al., 1996). In another study, 13.5 hour light exposures of white fluorescent light at 300, 1,500, or 3,000 lux were compared for performance on a neurobehavioral test battery (Dollins et al., 1993). Lighting condition had no effect on performance on a battery of neurobehavioral tests. Performance on the test battery declined on successive testing days, regardless of light condition.

Despite the inconsistencies discussed above, the bulk of the published literature supports the idea that light exposure can cause significant improvement in alertness and behavioral
performance measures. Although the responses appear to depend on circadian timing, light intensity, wavelength and duration, the field does not have a complete knowledge of all relevant factors behind such effects. A number of models have been developed to try to predict the relative efficacy of a particular light stimulus in terms of its relative circadian, neuroendocrine, and neurobehavioral efficacy (Gall and Bieske, 2004; Rea et al., 2005; D.I.N., 2009; Pechacek et al., 2008). Caution must be exercised, however, in relying on these and other preliminary models of circadian phototransduction. Reasons for this include the fact that the models may only be based on one behavioral or biological measure, action spectra of the responses measured may be incomplete, and light source differences on which the model is based may be quite different than the application being tested. These models will be discussed further in the General Discussion chapter of the thesis (Chapter 6). Empirical verification of specific light stimuli on a defined biological parameter will remain the most accurate way to determine the potency of light sources.
CHAPTER 4 EFFECTS OF SHORT WAVELENGTH SOLID-STATE LIGHTING ON MELATONIN SUPPRESSION AND ALERTNESS

4.1 Introduction

Daily rhythms of sleep, alertness, and physiology are controlled by the SCN in the anterior hypothalamus of the brain. A small subset of ipRGCs project directly to the SCN as well as other regulatory nuclei and synchronize the circadian timing system, ensuring that daily changes in behavior are timed with their photic environment. IpRGCs contain the photopigment melanopsin which has a maximal response between 459-484 nm (Brainard et al., 2001b; Thapan et al., 2001; Lucas et al., 2001; Hankins and Lucas, 2002; Berson et al., 2002; Hattar et al., 2003; Dacey et al., 2005; Zaidi et al., 2007; Gamlin et al., 2007). As discussed earlier in section 1.7, action spectra studies and experiments on selected wavelength comparisons have indicated that photic melatonin suppression and alerting responses are strongest in the blue-appearing portion of the spectrum.

Initial studies using broad spectrum white light reported alerting effects when used at night (French et al., 1990; Badia et al., 1991; Myers and Badia, 1993; Wright et al., 1997; Cajochen et al., 2000). Neurobehavioral and physiological parameters affected by nocturnal bright light exposure include subjective sleepiness, EEG frequencies in the beta, theta and alpha ranges, psychomotor vigilance performance, and a variety of cognitive tests. In addition, these effects were observed with exposure to bright light given during the day (Phipps-Nelson et al., 2003; Ruger et al., 2006).

As discussed earlier in chapter 3, not all studies that assessed alertness by bright broad spectrum white light exposure demonstrated enhancements in all measures of alertness (French et al., 1990; Brainard et al., 1996; Dollins et al., 1993). The fact that Dollins and colleagues (1993) found no bright light effects on alertness yet achieved suppression of melatonin levels suggested that the two responses have different methods of action. Indeed, the alerting affects observed during the day by Ruger et al. (2006) and Phipps-Nelson and colleagues (2003) further support this theory as melatonin levels during the day are...
negligible. Conversely, Cajochen and colleagues (2000) positively correlated the degree of melatonin suppression by bright light with a positive alerting response.

Whether there exist differing levels of sensitivity to light responses for melatonin suppression and alertness remains an area of ongoing investigation. It has been shown light in the blue-appearing portion of the spectrum (446-477 nm) is highly effective at suppressing melatonin levels (Brainard et al., 2001b; Thapan et al., 2001). In addition, neurobehavioral responses to narrow bandwidth light exposure as quantified by such objective measures as functional magnetic resonance imaging (fMRI), waking EEG and subjective alertness measures have demonstrated light in the blue-appearing portion of the spectrum to have the most enhanced response (Cajochen et al., 2005; Lockley et al., 2006; Revell et al., 2006; Vandewalle et al., 2007a; 2007b; Phipps-Nelson et al., 2009; Rahman et al., 2014).

Experimental light sources studied have different spectral power distributions which can be described as monochromatic (<15 nm half-peak bandwidth), narrowband (15-30 nm half-peak bandwidth) or broadband (>30 nm half-peak bandwidth). Light sources used for testing the effects of light emitted in the blue-appearing region of the visible spectrum (446-477 nm) on melatonin suppression and alertness have been varied including fluorescent (Smith and Eastman, 2009; Smith et al., 2009), compact fluorescent (Chellappa et al., 2011), monochromatic light from arc lamps (Lockley et al., 2003; 2006; Brainard et al., 2001b), as well as solid-state (Wright et al., 2001; 2004; West et al., 2011).

The development of solid-state lighting technology is advancing rapidly and will likely be the future of installed lighting in the home, workplace and the general built environment due to increased energy efficiency and durability (Schubert and Kim, 2005). Currently under development are adjustable lighting systems with narrow and/or broad bandwidth LEDs that can be controlled for changes in intensity, spectrum and duration. These lighting systems are predicted to be better able to regulate circadian, neuroendocrine, and neurobehavioral physiology, although this remains to be proven. NASA, the funding agency for this study, is looking to develop an in-flight lighting countermeasure for enhancing alertness in astronauts as well as NASA ground crew utilizing LED technology.
4.2 Aims and Hypotheses

The overall goal of this study was to test the ability of blue-appearing narrowband solid-state light for its ability to acutely suppress nocturnal melatonin as well as enhance cognitive performance and alertness in healthy men and women. The specific hypotheses tested were that compared to dim light exposure, blue narrowband light would: 1) suppress nocturnal melatonin, 2) decrease reaction time and lower number of lapses in a 10-min auditory PVT, 3) increase number of correct responses in two working memory tasks (DSST, DS forward and reverse entry), 4) lower subjective levels of sleepiness as measured by the KSS, and 5) show differences in EEG in delta-theta frequency band (0.5 – 5.5 Hz) and high alpha frequency band (9.5 – 10.5 Hz) during the KDT.

4.3 Methods

4.3.1 Test facility

The study was performed at TJU in the same facility previously described in section 3.3.1.

4.3.2 Ambient light stimuli and measurement

Ambient light in the facility was produced by the same lighting system outlined in section 3.3.2.

After 4 hours in the laboratory, overhead fluorescent room lighting was dimmed to less than 4 lux in the direction of gaze and remained at that level for the duration of the study, except for the 4-hour sleep opportunity on night 1 which occurred in complete darkness. Daily illuminance/irradiance measures were taken in the same previously described method (see section 3.3.2).
4.3.3 Experimental light stimuli and measurement

The blue LED system consisted of a 122 cm² blue LED array, $\lambda_{\text{max}} = 469$ nm, 26 nm half-peak bandwidth, with a lens diffuser that resulted in a relatively even distribution of narrow bandwidth blue light (Figure 17). The light source used in the study was identical in size as in the previous chapter and was designed to provide a full field retinal exposure. The light intensity used in this study was based on a previous melatonin fluence-response curve performed with this lighting system (West et al., 2011). Subjects either viewed the blue solid-state light exposure system at 30 cm for 4 hours beginning at their habitual sleep time to achieve a full visual field corneal exposure of 75 µW/cm² or they remained in dim white ambient room light (< 4 lux). Based on study by West and colleagues (2011), the light source was expected to produce a significant suppression of melatonin. Figure 18 shows the spectral power distribution of this polychromatic blue-appearing LED light source. The measured photon density was calculated to be $1.9 \times 10^{14}$ photons/cm²/sec.

Subjects were randomly assigned to exposures of narrow bandwidth blue LED light or dim white light (N=12 blue LED, N=10 dim white light). For the full duration of the light exposure subjects were seated 30 cm in front of the light panel with their chin placed in an ophthalmologic head holder and monitored continuously. In this position, subjects experienced an exposure that encompassed their full retinal field. Subjects were asked to maintain a fixed gaze toward the panel for 125 minutes before resting their head and neck out of the head holder for 10 minutes while remaining seated with the light remaining on. The subject then went back into the head holder for the remainder of the 4 hour light exposure (105 minutes).

Experimental irradiance measures were taken using an IL-1400BL radiometer/photometer (International Light Technologies, Inc., Peabody, MA) previously described in section 2.2 which was held in place by a clear, plastic holder attached in an ophthalmologic head holder 30 cm from the panel at approximate eye level. The spectral power distribution measurement shown in Figure 18 was taken using a Model FSHH 325-1075P FieldSpec handheld spectroradiometer (Analytical Spectral Devices, Inc., Boulder, CO).
Figure 17. The 122 cm$^2$ blue LED array light exposure system that was used. Note that during the actual light exposure for the study reported here the subject’s head would be in an ophthalmologic head holder 30 cm from the panel.
Figure 18. Spectral power distribution of the polychromatic blue-appearing LED light source. This narrow bandwidth blue LED array emitted light in three color bandwidths (CIE, 1987).

4.3.4 Subject screening

Over 300 subjects were initially contacted by the study recruiter in response to local newspaper or internet advertisements via e-mail. Approximately 30% of the respondents who were given additional study requirements and details over e-mail either declined to proceed further or never contacted the laboratory again. Initial general questioning on age, health, work schedule and medications eliminated approximately 60% of the remaining contacts. Thirty individuals then met with the study recruiter to tour the laboratory facility.

At this point, 28 subjects were instructed to schedule appointments with the study physician and study psychologist, have a urine drug screen for drugs of abuse, have blood drawn for a basic metabolism panel and keep a strict sleep/wake cycle with daily phone calls to the laboratory voicemail upon sleeping and waking. Subjects chose the timing of their own 8 hour sleep schedule. Three subjects were eliminated due to physical or psychological exclusionary criteria. Nine subjects failed to keep regular sleep/wake times, tested positive for drugs of abuse, or declined to participate further.
After undergoing psychological, toxicological, physical, and sleep/wake cycle screenings the remaining 24 subjects met study requirements and chose to be entered into the three day inpatient study protocol. Twenty-two healthy men and women completed the full study. One subject asked to be released from the protocol on the first day of the study due to extreme fatigue and the other subject failed to keep their sleep/wake cycle stable for the last few nights prior to entry. A total of 10 males and 12 females ranging in age from 21 to 30 years old, with a mean age (± SEM) of 25.1 ± 0.6 years were studied in the Light Research Laboratory of TJU.

### 4.3.5 Study protocol

The study was approved by the IRB of TJU and all subjects gave written informed consent prior to entering the protocol. For at least 10 days prior to admission to the laboratory, subjects were asked to maintain a regular 8:16 h sleep:wake schedule and dark-light exposure, complete a daily sleep-wake log, and phone into a voice mailbox to record the time of awakening and time to bed each day. Subjects were asked to wear a wrist-borne, non-invasive activity and light monitor for at least two weeks prior to admission to the laboratory study to ensure compliance with the screening criteria for maintaining a consistent sleep-wake schedule (Motion Logger Sleep Watch-L, AMI, Ardsley, NY). The subjects’ pre-study sleep-wake schedule was determined from the actigraph data, the sleep logs and voice mail logs for the 14 days prior to admission and were used to schedule the timing of sleep during baseline days in the laboratory and, subsequently, all the events in the study including the timing of light exposure. Subjects who had a general lack of sleep-wake stability or more than one deviation of more than 30 minutes from their regular bedtime or wake-time were excluded from the study or asked to extend the pre-study sleep-wake maintenance duration. Subjects were restricted to 4 hours of sleep in the second half of their usual 8 h sleep period on the night prior to admission in the study facility. This was done because sleep and circadian problems have been documented in space flight missions as short as 10 days (Dijk et al., 2001). The inclusionary criteria for medication were no recreational, over-the-counter, nor prescription medications inclusive of caffeine and alcohol. Toxicological screening of blood and urine for caffeine, pregnancy in females, basic metabolic panel, alcohol, drugs of abuse, thyroid function, and a CBC was conducted during screening and on the day of entry into the facility.
Figure 19 provides a diagrammatic representation of the 3-day study protocol. Subjects were limited to 4 hours of time in bed at home for the night prior to admission as well as Night 1 in the laboratory. After entering the laboratory, subjects lived in a relatively isolated environment in a windowless facility but maintained either direct or intercom contact with the study staff. The first four hours was an adaptation period where subjects entered the laboratory and got acquainted with the test facility. Neurobehavioral and performance testing was begun 2 hours after admission and continued during all wake periods while in the laboratory. After 4 hours in the laboratory, overhead room lighting was dimmed to less than 4 lux and remained at that level for the duration of the study, except for the 4-hour sleep opportunity on Night 1 when all lights were off. PSG leads were put on and constant posture (CP) was started approximately 12 hours after admission to the laboratory. CP occurred for 4 hours on Night 1 until a four-hour sleep opportunity, which coincided with the latter half of the subject’s 8-hour sleep schedule. Dim room light levels, PSG recording, and performance and neurobehavioral testing were continued on Day 2. For the first four hours of the subject’s usual 8 hour sleep schedule, the subject was randomized to receive a blue light exposure or a control dim white light exposure. The subject was in CP during this period and the four-hour period following the experimental light exposure period.

4.3.6 Measurement of melatonin

Blood samples (3 ml) were collected through an indwelling intravenous (IV) catheter located in a forearm vein into 3 ml polystyrene tubes which contained 5.4 mg of K2 EDTA (BD Diagnostics, Franklin Lakes, NJ). Plasma was separated by refrigerated centrifugation (2,000 RPM for 15 minutes), aliquoted into polypropylene cryogenic vials (Thermo Fisher Scientific, Rochester, NY) and stored at -20° C until assay. Plasma melatonin samples were
Figure 19. Overview of 3-day blue light alerting protocol for a subject with a midnight to 08:00 sleep:wake schedule. Abbreviations are CP = constant posture, PSG = polysomnography, IV = intravenous blood sampling, q20, q30, q60 = taken at 20 min, 30 min, 60 min, NTB = neurobehavioral testing battery, PVT = psychomotor vigilance task, KSS = Karolinska sleepiness scale, KDT = Karolinska drowsiness test

drawn every 30 minutes before and after the 4 hour blue LED exposure or dim light condition and drawn every 20 minutes during the 4 hour blue LED exposure or dim light condition (see Figure 19). Plasma melatonin concentrations were assayed by radioimmunoassay (Vaughan, 1993). A full description of the method can be found in section 2.6.2.

Salivary samples were collected as backup to the primary plasma measure and were used when there were problems with patency of the IV line. Salivary samples (1.2-2 ml) were collected with the "Salivette" sampling device (Sarstedt, Inc., Hayward, CA). The specific protocol for this is described earlier in section 2.4. Salivary melatonin samples were collected every 30 minutes during the 4 hour blue LED exposure or dim light condition.
Salivary melatonin concentrations were assayed by radioimmunoassay (Vaughan, 1993). A full description of the method can be found in section 2.6.2.

4.3.7 Neurobehavioral testing

Neurobehavioral testing was done on a laptop using software from our collaborators at the University of Pennsylvania. I was trained in this laboratory technique but testing was done by TJU research technicians. Subjects completed cognitive performance and working memory tests throughout the inpatient study. These tests included DSST), and DS forward and reverse entry tasks. During the week prior to admission, subjects came into the laboratory and completed each of the individual tests at least four times to ensure familiarity with the tests prior to the start of the study.

4.3.7.1 Psychomotor vigilance task

Subjects completed a 10-min auditory PVT at regular intervals during wakefulness (Dinges and Powell, 1985). These intervals were more frequent prior to, during, and post the four hours of blue light exposure or a control dim light exposure. Subjects were instructed to listen via headphones placed over their entire ear and press a response button as soon as a beeping sound was heard. The button stops the counter, which is displayed in milliseconds for a 1 second period. Subjects were instructed to press the button as soon as each stimulus sounded, but to not press the button too soon, which would register as a false start. The auditory stimulus was presented at random intervals during the 10-minute PVT. Mean reaction times and lapses (reaction times greater than 400 ms) were counted per testing session as a measure of performance impairment indicative of reduced behavioral alertness.

4.3.7.2 Digit symbol substitution task digit span forward and reverse entry tasks

The primary test of working memory was the DSST (Kane and Reeves, 1997). The DSST is a subject-paced task that involves the matching of digits (0–9) to symbols. The number of correct responses in 1.5 min was counted as a measure of working memory performance.
Secondary measures of working memory included the D Span Forward and Reverse tasks (Kane and Reeves, 1997). In the DS test, subjects were presented with a series of digits and must immediately type them back in forward or reverse order. If they do this successfully, they are given a longer list (e.g., '9, 2, 4, 0'). The length of the longest list a person can remember is that person's digit span.

4.3.8 Karolinska sleepiness scale

The KSS is a subjective measure of a person’s perceived sleepiness based on a 9-number scale where 1 = very alert, 3 = alert, 5 = neither alert nor sleepy, 7 = sleepy (but not fighting sleep), and 9 = very sleepy (fighting sleep) (Akerstedt & Gillberg, 1990). Subjects completed the KSS by marking next to the appropriate number on a printed KSS scale every 30 minutes during waking hours.

4.3.9 Karolinska drowsiness test

An objective assessment of sleepiness was attained with the KDT which is a non-invasive test based upon EEG in specific spectral power bands monitored over an extended period of hours or days (Aeschbach et al., 1999; Cajochen et al., 2000; Lockley et al., 2006). Subjects were asked to perform the KDT periodically during waking hours. During the KDT, subjects were instructed to relax and fixate on a 5-cm black dot 1-m away for 3 minutes with their eyes open. These tests were performed every 60 minutes during what would have been the subject’s 4-hour sleep period, and every two hours during all other waking hours. The increased frequency of testing during light exposures was to potentially capture alerting or non-alerting effects of the light changes.

4.3.10 Profile of mood states

The POMS is a psychological rating scale that surveys transient mood states of subjects. Its six-factor scales of mood assessment can be used to track changes in mood states within a given subject over time, offering an objective intra-subject measure of mental state tolerance.
and response to experimental exposures. These scales are intended not for global or clinical diagnosis of pathological states, but rather to reflect snapshot assessments of complex mood factors in quantifiable terms within a given subject over time (McNair et al., 1992).

Each item inventory in the POMS is scored with a maximum of 4 points from 0-4, with 0 reflecting endorsement of the given mood state as “very slightly or not at all”, 1 reflecting “a little” endorsement of a state, 2 reflecting “moderately” endorsing a given mood state, 3 reflecting “quite a bit,” and 4 reflecting “extremely” experiencing the mood state item surveyed. The 6-subscale factor analysis include 9 tension-anxiety items (scored 0-36), 15 depression-dejection items (scored 0-60), 12 anger-hostility items (scored 0-48), 8 vigor-activity items (scored 0-32), 7 fatigue-inertia items (scored 0-28), and 7 confusion-bewilderment items (scored 0-28). Finally, each administration of the scale can be scored to reflect a subject’s “total mood disturbance” at a given point in time by adding the 5 subscale scores other than vigor-activity and subtracting the subscale score for vigor-activity from the other 5 totaled.

For the purposes of this study, the intended purpose of POMS administration was one of monitoring subjects’ mood states as a secondary measure of tolerance and mood responses to experimental protocol, rather than as a primary outcome measure. Thus, subjects having already been screened by the study protocol for the absence of clinical-level mood conditions were used as their own controls for POMS variability comparisons throughout the study.

4.3.11 Side effects

All subjects were given before and after the experimental period, a SAFTEE questionnaire. This questionnaire was designed to be used to assess effects of light in SAD patients (Terman and Terman, 1999) although it has been used successfully in non-depressed subjects exposed to light of shorter durations (Goel and Etwaroo, unpublished). Subjects were asked to evaluate how they felt according to the following scale: 1) - not at all; 2) - a little bit; 3) - moderate; 4) - quite a bit; and 5) - extreme. Fifty-two different questions were asked organized by organ system and different areas of the body. Although probing for side effects has potential importance, this was not a primary dependent variable of the study.
4.3.12 Data reduction and statistics

Data for the various tests used in this experiment was separated into three groups prior to analyses; Day 2 pre-light exposure period, light exposure period, and post-light exposure period. There was variability in the number and frequency of tests run throughout this experiment and, therefore each of the tests had a distinct breakdown.

The neurobehavioral data analysis was completed by co-investigator Namni Goel, Ph.D. from the University of Pennsylvania using within subjects repeated measures ANOVAs. The tests analyzed by Dr. Goel were the DSST, DS Forward task, DS Reverse task, and POMS. I am familiar with the analyses used but did not do the analysis for this part of the study.

4.3.12.1 Melatonin data reduction and statistics

Light-induced melatonin suppression (mean ± SEM) was measured after 120 and 240 minutes of light exposure. Unpaired, two tailed Student’s t-tests were used to compare differences between initial melatonin values at the beginning of light exposure between the two groups as well as 120 and 240 minute post-exposure values. The change in melatonin for each condition was expressed as a percent change from the pre-exposure melatonin level, with a positive percent change indicating an increase and a negative percent change demonstrating a decrease in melatonin levels. Percent change values were then analyzed by unpaired, two tailed Student’s t-tests (Gaddy et al., 1993; Ruberg et al., 1996; Brainard et al., 2001a; 2001b).

For each subject’s partial melatonin profile, dim light melatonin onset (DLMO) was calculated by calculating where DLMO reaches a 25% threshold rise in plasma melatonin from peak to trough in the pre-light exposure condition (Benloucif et al., 2007).
4.3.12.2 PVT data reduction and statistics

The data from the auditory PVT were downloaded by research technicians at TJU throughout the study using the PVTCOMM for Windows Version 2.10.1.1 software from Ambulatory Monitoring, Inc. The extracted data were then read using the REACT Version 1.105 software, also from Ambulatory Monitoring, Inc. (Ardsley, NY). The data for each trial were copied from REACT and transferred into an Excel spreadsheet for each subject by research technicians at TJU. Mixed-model ANOVAs (time: within-subjects factor; experimental group: between-group factor) were run by co-investigator Namni Goel, Ph.D. on PVT lapses (> 400 ms reaction times) for pre-light exposure period, light exposure period, and post-light exposure periods. Greenhouse-Geisser corrections were applied to all within-subjects effects.

4.3.12.3 Digit symbol substitution task, digit span forward and reverse entry tasks data reduction and statistics

Mixed-model ANOVAs (time: within-subjects factor; experimental group: between-group factor) were run by co-investigator Namni Goel, Ph.D. on mean number of correct responses for pre-light exposure period (pre-LE), light exposure (LE) period, and post-light exposure periods (post-LE). Greenhouse-Geisser corrections were applied to all within-subjects effects. Graphs were made using Excel.

4.3.12.4 KSS data reduction and statistics

Mixed-model ANOVAs (time: within-subjects factor; experimental group: between-group factor) were run by co-investigator Namni Goel, Ph.D. on KSS scores for pre-LE, LE, and post-LE. Greenhouse-Geisser corrections were applied to all within-subjects effects.

4.3.12.5 KDT data reduction and statistics

The KDT data analysis was performed by co-investigator John Balaicuis, M.D. from TJU. The waking EEG signals derived from Cz/Ax during the KDT were visually inspected, and 2-
second epochs containing muscle artifact, eye blinks, and eye movements were discarded from further analysis. Artifact-free 2-second epochs were subjected to off-line spectral analysis using a fast-Fourier transformation and a 10% cosine window. Data were further reduced by discarding spectra above 20 Hz. I was trained in this technique but did not perform the tests or analysis. Frequencies were compared in the delta-theta band (0.5 – 5.5 Hz) and high alpha band (9.5 – 10.5 Hz).

4.3.12.6 Profile of mood states data reduction and statistics

Mixed-model ANOVAs (time: within-subjects factor; experimental group: between-group factor) were run by co-investigator Namni Goel, Ph.D. for all 6-subscales as well as the total mood disturbance scores for Day 2, during light exposure and post-light exposure periods. Greenhouse-Geisser corrections were applied to all within-subjects effects.

4.4 Results

4.4.1 Ambient light stimuli measurement

The measured illuminance (± SEM) for the baseline day was 69.7 ± 1.3 lux and for the dim light condition illuminance was 2.5 ± 0.1 lux. The measured irradiance (± SEM) for the baseline day was 19.6 ± 0.5 μW/cm² and for the dim light condition irradiance was 0.8 ± 0.02 μW/cm².

4.4.2 Experimental light stimuli measurement

The measured irradiance at subject’s corneas (± SEM) over the 4 h exposure was 75.2 ± 0.1 μW/cm² for the blue LED exposure and 0.7 ± 0.1 μW/cm² for the dim light control exposure. Illuminance measures were not recorded during the 4 h blue LED exposure. Data from a previous study run in this laboratory with illuminance measures taken identically averaged (± SEM) 79.5 ± 1.7 lux for the blue LED exposure (West et al., 2011). The measured
illuminance for the dim light control exposure (± SEM) was 2.1 ± 0.1 lux for the dim light control exposure.

4.4.3 Measurement of melatonin

One female subject was eliminated from the analysis due to an error in giving the appropriate intensity of the narrowband blue LED light. Two of the subjects yielded very high melatonin levels that were outliers from the mean (greater than two standard deviations). Three subjects had only salivary melatonin data due to intravenous line failure and staffing issues. In total, sixteen (eight in each group) subjects were included for plasma melatonin data analysis. Please see the subject information table in the Appendix.

Typical melatonin profiles observed in this study from dim white ambient light and blue LED light exposures are shown in Figure 20. The group data are shown in Figures 21 and 22. Unpaired, equal variances, two-tailed Student’s t-tests indicated no significant variations across mean melatonin pre-light exposure values (t=0.69, p=0.50) between the dim light and blue LED exposure groups. Exposure to narrow bandwidth blue LED light at 75 µW/cm² caused a significant suppression of melatonin when measured after 2 and 4 hours while dim white ambient light allowed the normal rise in melatonin to progress. Unpaired, equal variances, two-tailed t-tests indicated significant suppression of melatonin with blue LED exposure after 2 hours (t=6.17, p<0.0001) and 4 hours (t=5.02, p<0.0005) when compared to the dim light condition. In addition, percent melatonin changes were significantly different after exposure to narrow bandwidth blue LED light at 75 µW/cm² after 2 hours (t=6.45, p<0.0001) and 4 hours (t=3.62, p<0.005) when compared to the dim condition.

DLMOs were calculated for the subjects included in the melatonin analyses by 25% threshold rise in plasma melatonin from peak to trough and are given in Table 16 below. No significant differences were found between exposure groups (t=2.36, p=0.92)
Figure 20. Plasma melatonin profiles from two different individuals: a) subject (NA03) exposed to dim white ambient fluorescent light (4,000 K) at 0.7 μW/cm²; b) subject (NA16) exposed to narrow bandwidth blue LED light at 75 μW/cm².

Figure 21. Plasma melatonin values (mean ± SEM) after exposure to a blue LED panel light exposure 2 hours (a) and 4 hours (b) at 75 μW/cm² or dim white ambient fluorescent light (4,000 K) in the control condition (0.7 μW/cm²). Unpaired, equal variances, two-tailed t-tests indicated significant suppression of melatonin with blue LED exposure after 2 hours (t=6.17, p<0.0001) and 4 hours (t=5.02, p<0.0005) when compared to the dim light condition.
Figure 22. Group mean (± SEM) plasma percent melatonin change scores after 120 minutes or 240 minutes of exposure to blue LED light exposure at 75 µW/cm² or from dim white ambient fluorescent light (4,000 K) at < 4 lux. Significant differences were found after 120 minutes (t=6.45, p<0.0001) and 240 minutes (t=3.62, p<0.005).

Table 16. DLMOs calculated in decimal hours by condition.

<table>
<thead>
<tr>
<th></th>
<th>BLUE LED</th>
<th>DIM WHITE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subject</strong></td>
<td><strong>DLMO 25%</strong></td>
<td><strong>Subject</strong></td>
</tr>
<tr>
<td>NA01</td>
<td>21.52</td>
<td>NA04</td>
</tr>
<tr>
<td>NA02</td>
<td>23.38</td>
<td>NA05</td>
</tr>
<tr>
<td>NA07</td>
<td>21.95</td>
<td>NA06</td>
</tr>
<tr>
<td>NA08</td>
<td>22.53</td>
<td>NA09</td>
</tr>
<tr>
<td>NA10</td>
<td>22.00</td>
<td>NA14</td>
</tr>
<tr>
<td>NA16</td>
<td>21.35</td>
<td>NA15</td>
</tr>
<tr>
<td>NA18</td>
<td>23.20</td>
<td>NA19</td>
</tr>
<tr>
<td>NA22</td>
<td>21.53</td>
<td>NA20</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>22.18</strong></td>
<td><strong>Mean</strong></td>
</tr>
<tr>
<td><strong>SE</strong></td>
<td><strong>0.28</strong></td>
<td><strong>SE</strong></td>
</tr>
</tbody>
</table>
4.4.4 Auditory psychomotor vigilance task

One female subject was eliminated from the auditory PVT analysis for the dim light group as her performance data were non-compliant as determined by co-investigator Namni Goel, Ph.D. The mean number of lapses for each of the three time periods (Day 2 Pre- LE, LE, and post-LE) were compared separately between the two groups using mixed-model ANOVAs (time: within-subjects factor; experimental group: between-group factor). Greenhouse-Geisser corrections were applied to all within-subjects effects. The results are presented in Table 17.

Lapses were not significantly different in any of the time periods for light source, nor were there significant interactions between light source and time, as shown in Table 17 and Figure 23. There was a significant difference (p<0.05) over time during the LE period and post-LE period as shown in Table 17 and Figure 23. Mean lapses significantly increase over time in the LE and post-LE time periods indicating a deterioration of objectively measured performance.

Table 17. Mixed model ANOVA on lapses for auditory PVT Day 2 pre-LE, during LE and post-LE.

<table>
<thead>
<tr>
<th>Pre LE</th>
<th>Effect</th>
<th>DF</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>light source</td>
<td>1</td>
<td>0.08</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>2.9</td>
<td>0.63</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>light source*time</td>
<td>2.9</td>
<td>0.63</td>
<td>0.59</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LE</th>
<th>Effect</th>
<th>DF</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>light source</td>
<td>1</td>
<td>0.17</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>2.3</td>
<td>4.99</td>
<td>0.01</td>
</tr>
<tr>
<td>Pre LE</td>
<td>Effect</td>
<td>DF</td>
<td>F value</td>
<td>P value</td>
</tr>
<tr>
<td>-----------------</td>
<td>------------------</td>
<td>-----</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>light source*time</td>
<td>2.3</td>
<td>0.16</td>
<td>0.87</td>
</tr>
<tr>
<td>Post LE</td>
<td>Effect</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>light source</td>
<td>1</td>
<td>0.07</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>2.7</td>
<td>4.07</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>light source*time</td>
<td>2.7</td>
<td>0.70</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Figure 23. Average PVT lapses (±SE) of the blue LED light group (empty circles, dashed line) or the dim white light group (filled circles, solid line) before, during, and after light exposure. The period of light exposure is indicated by box.
4.4.5 Digit Symbol Substitution Task (DSST), Digit Span (DS) Forward and Reverse Entry Tasks

For both the DSST and DS the mean number correct for each of the three time periods (Day 2 pre-LE, LE, and post-LE) were compared separately between the two groups using mixed-model ANOVAs (time: within-subjects factor; experimental group: between-group factor). Greenhouse-Geisser corrections were applied to all within-subjects effects. The following tables show these results. N=21 for this set of tests.

The mean number correct for DSST were not significantly different in any of the time periods for light source, nor were there significant interactions between light source and time, as shown in Table 18 and Figure 24. There were non-significant trends observed for the interactions between light source and time prior to (p=0.06) and through the light exposure periods (p=0.09) with the blue LED outperforming the dim light group prior to and through the LE time periods.

Table 18. Mixed model ANOVA on total correct for DSST Day 2 pre-LE, during LE and post-LE.

<table>
<thead>
<tr>
<th></th>
<th>Effect</th>
<th>DF</th>
<th>F value</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td><strong>Pre LE</strong></td>
<td>light source</td>
<td>1</td>
<td>0.44</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>1</td>
<td>0.17</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>light source*time</td>
<td>1</td>
<td>4.00</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>LE</strong></td>
<td>light source</td>
<td>1</td>
<td>1.36</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>1.9</td>
<td>0.25</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>light source*time</td>
<td>1.9</td>
<td>2.61</td>
<td>0.09</td>
</tr>
<tr>
<td>Post LE</td>
<td>Effect</td>
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<td>F value</td>
<td>P value</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------</td>
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<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>light source</td>
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<td>0.80</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>1.9</td>
<td>1.68</td>
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<td>light source*time</td>
<td>1.9</td>
<td>0.40</td>
<td>0.67</td>
</tr>
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</table>

Figure 24. Mean number of correct answers on the DSST (±SE) of the blue LED light group (empty circles, dashed line) or the dim white light group (filled circles, solid line) before, during, and after light exposure. The period of light exposure is indicated by box.

The mean number correct for DS forward and reverse task were not significantly different in any of the time periods for light source, nor were there significant interactions between light source and time, as shown in Table 19 and Figure 25. The data from the post-LE period, however, did reveal a significant effect of time (p<0.05) indicating a worsening of performance over time.
Table 19. Mixed model ANOVA on total correct for DS Day 2 pre-LE, during LE and post-LE.

<table>
<thead>
<tr>
<th>Pre LE</th>
<th>Effect</th>
<th>DF</th>
<th>F value</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>light source</td>
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<td>0.87</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>time</td>
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<table>
<thead>
<tr>
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<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>light source</td>
<td>1</td>
<td>0.14</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>2.0</td>
<td>1.01</td>
<td>0.37</td>
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<tr>
<td></td>
<td>light source*time</td>
<td>2.0</td>
<td>0.16</td>
<td>0.85</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Post LE</th>
<th>Effect</th>
<th>DF</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>light source</td>
<td>1</td>
<td>0.42</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>1.9</td>
<td>5.48</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>light source*time</td>
<td>1.9</td>
<td>0.70</td>
<td>0.51</td>
</tr>
</tbody>
</table>
4.4.6 Karolinska sleepiness scale

Subjective sleepiness as measured by the Karolinska sleepiness scale for each of the three time periods (Day 2 pre-LE, LE, and post-LE) were compared separately between the two groups using mixed-model ANOVAs (time: within-subjects factor; experimental group: between-group factor). Greenhouse-Geisser corrections were applied to all within-subjects effects. These analyses are shown in the following Table 20 and Figure 26. N=17 for this set of scores.

The Day 2 Blue LED exposure group had significantly higher average scores than the dim light exposure group on Day 2 before LE (p<0.05) and showed a non-significant trend in increasing sleepiness over time across the day (p=0.08). During the LE period, there were no group differences, though there was a significant time difference with scores increasing over time (p<0.005). During the post-LE period there were no group differences, nor time of day differences. There were no significant interactions between light source and time of day in any of the exposure periods. These data are shown below in Table 20 and Figure 26.
Table 20. Mixed model ANOVA on KSS scores Day 2 pre-LE, during LE and post-LE.

<table>
<thead>
<tr>
<th>Pre LE</th>
<th>Effect</th>
<th>DF</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>light source</td>
<td>1</td>
<td>6.14</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>5.5</td>
<td>1.97</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>light source*time</td>
<td>5.5</td>
<td>0.94</td>
<td>0.47</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LE</th>
<th>Effect</th>
<th>DF</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>light source</td>
<td>1</td>
<td>1.64</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>2.8</td>
<td>7.31</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>light source*time</td>
<td>2.8</td>
<td>0.43</td>
<td>0.71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Post LE</th>
<th>Effect</th>
<th>DF</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>light source</td>
<td>1</td>
<td>0.56</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>1</td>
<td>2.50</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>light source*time</td>
<td>1</td>
<td>0.28</td>
<td>0.63</td>
</tr>
</tbody>
</table>
Figure 26. KSS scores (±SE) before, during and after light exposure blue LED light group (empty circles, dashed line) or the dim white light group (filled circles, solid line). The period of light exposure is indicated by box.

4.4.6 Karolinska drowsiness test

Of the 21 subjects recorded, only KDTs from 8 subjects (4 each in dim and Blue LED exposure conditions) were analyzed due to an unexpected reduction in funding for the project. Given the dramatically reduced data set, no significant differences were expected for KDT. Indeed, there were no significant differences between nights or light exposure groups in the delta-theta (0.5 – 5.5 Hz) or high alpha (9.5 – 10.5 Hz) frequencies recorded during KDTs on Night 1 and 2. The results from the limited data set are summarized below in Tables 21 and 22.
Table 21. Repeated mixed model ANOVA on delta-theta band during KDTs on Nights 1 and 2

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>F value</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>light source</td>
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<td>0.11</td>
<td>0.75</td>
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<td>night</td>
<td>1</td>
<td>0.84</td>
<td>0.39</td>
</tr>
<tr>
<td>light source*night</td>
<td>1</td>
<td>1.45</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Table 22. Repeated mixed model ANOVA on high alpha band during KDTs on Nights 1 and 2

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>light source</td>
<td>1</td>
<td>0.90</td>
<td>0.38</td>
</tr>
<tr>
<td>night</td>
<td>1</td>
<td>0.00</td>
<td>0.99</td>
</tr>
<tr>
<td>light source*night</td>
<td>1</td>
<td>1.01</td>
<td>0.92</td>
</tr>
</tbody>
</table>

4.4.7 Profile of mood states

Mixed model ANOVAs were run for all 6 subscales for POMS as well as the total mood disturbance scores for Day 2 during LE in both the dim and blue LED groups. Shown below are results of 2 of the 6 subscales that were near significant or significant. The remaining 4 subscales for POMS were not statistically significant: anger/hostility (light source p=0.15, time p=0.63, light source*time p=0.93); vigor/activity (light source p=0.61, time p=0.16, light source*time p=0.88); fatigue/inertia (light source p=0.60, time p=0.89, light source*time p=0.46) and confusion/bewilderment (light source p=0.17, time p=0.77, light
source*time p=0.14). Total mood scores were also not significant (light source p=0.29, time p=0.67, light source*time p=0.46). N=17 for this set of scales.

The blue LED LE group had a non-significant trend for higher tension/anxiety (Table 23) scores during LE (p=0.06) compared to the dim light group. As shown in Table 24, when inclusive of the post-LE period with the LE period, the blue LED LE group had significantly higher depression/dejection ratings (p=0.05) compared to the dim light group.

Table 23. Mixed model ANOVA on tension/anxiety subscale for POMS during LE.

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>light source</td>
<td>1</td>
<td>4.21</td>
<td>0.06</td>
</tr>
<tr>
<td>time</td>
<td>1.54</td>
<td>1.33</td>
<td>0.28</td>
</tr>
<tr>
<td>light source*time</td>
<td>1.54</td>
<td>0.28</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Table 24. Mixed model ANOVA on depression/dejection subscale for POMS during LE and post LE combined.

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>light source</td>
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<td>4.87</td>
<td>0.05</td>
</tr>
<tr>
<td>time</td>
<td>1.23</td>
<td>1.54</td>
<td>0.24</td>
</tr>
<tr>
<td>light source*time</td>
<td>1.23</td>
<td>2.47</td>
<td>0.13</td>
</tr>
</tbody>
</table>
4.4.8 Side effects

One subject failed to complete a side effect form in the baseline portion of the dim test condition due to a technician oversight. Very few side effects scaled moderate or greater were reported in any light exposure condition (less than 8% of all responses). Table 25 lists number of responses scaled moderate to extreme reported by the two lighting conditions. No analysis was necessary to compare across or within light conditions due to the overall lack of side effects observed. Tiredness and fatigue were reported in 21 of 22 subjects studied. Trouble thinking was the next most common complaint occurring in approximately half the individuals studied. Other, less reported, side effects such as eye issues, restlessness, muscular pain, anxiousness, and irritability comprised the remainder of side effects reported.

Table 25. Number of responses scaled moderate to extreme reported on the SAFTEE questionnaire at baseline and in the different light exposure groups.

<table>
<thead>
<tr>
<th>Class</th>
<th>Symptom</th>
<th>Baseline</th>
<th>Dim White</th>
<th>Blue LED</th>
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</thead>
<tbody>
<tr>
<td>Head</td>
<td>Headaches</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Dizziness/faintness</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Eyes</td>
<td>Eye irritation</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Eye swelling</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Blurred vision</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Double vision</td>
<td>1</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Poor vision</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Light bothersome</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Mouth</td>
<td>Dry mouth</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Excess salivation</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Stomach</td>
<td>Discomfort</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>Pain</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Numbness</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Restlessness</td>
<td>3</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Rigidity</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Skin</td>
<td>Itching</td>
<td>-</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Depression</td>
<td>Fatigue</td>
<td>14</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Condition</td>
<td>Score</td>
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</tr>
<tr>
<td>--------------------</td>
<td>-------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excessive energy</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jittery</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excited</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thought problems</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irritability</td>
<td>2</td>
<td></td>
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</tr>
</tbody>
</table>

4.5 Discussion

The overall goal of this study was to test the hypothesis that blue-appearing narrowband solid-state light would acutely suppress nocturnal melatonin as well as enhance cognitive performance and alertness in healthy men and women. The results clearly demonstrate that narrowband blue LED light has a strong effect on melatonin suppression when compared to dim white light. The data demonstrate that both the dim light control group and blue LED exposure groups had similar evening rises in melatonin levels with no differences in DLMOs between the two groups. The blue LED exposure significantly suppressed plasma melatonin after 2 h and continued through 4 h while the dim control light exposure permitted a continued rise in plasma melatonin. Monochromatic blue light, narrowband blue light as well as polychromatic light enhanced with blue light can suppress melatonin production (Brainard et al., 2001a;b; Thapan et al., 2001; Wright et al., 2001; 2004; Lockley et al., 2003; 2006; Smith and Eastman, 2009; Smith et al., 2009; Revell et al., 2010; Chellappa et al., 2011). The current results are consistent with the published data as well as a previous dose-response study performed on this identical lighting system in our laboratory (West et al., 2011). As reported in the present study, a corneal exposure of 75 µW/cm² of the blue LED light significantly suppressed melatonin as was the case in the dose-response study (West et al., 2011).

Data on subjective alertness as measured by KSS and objective assessments of alertness as measured by auditory PVT did not support the hypothesis that blue LED exposure would shorten reaction time and lower number of lapses in a 10-min auditory PVT or lower
subjective levels of sleepiness. Exposure to narrowband blue LED light for 4 h did not significantly improve auditory PVT performance during both the day and night as compared to dim white light exposure. Both groups, however, showed a significant time effect during the LE and post LE periods with increasing number of lapses and higher KSS scores (p<0.05). No significant differences were found between the blue LED light group and the dim light control group during the light exposure period or the post-light exposure interval for either KSS or PVT measures. There were also no significant time x group interactions. With very limited data, EEG correlates of alertness as measured by KDT also did not reveal any differences in a small group comparison between the narrowband blue LED light group when compared to the dim white light group.

Data on cognitive throughput as measured by DSST and DS did not support the hypothesis that blue LED exposure would increase the number of correct responses in those working memory tasks. DSST and DS data showed no significant differences between the light groups before or during LE but post-LE there was an effect of time for DS but not for DSST.

The observed significant time effect on PVT number of lapses, higher KSS scores and lower correct responses on DS could be representative of a circadian effect and homeostatic sleep/wake buildup as described by Borbely (Borbely et al., 1984). The homeostatic sleep/wake-dependent process (Process S) would interact with the intrinsic circadian signal for sleep timing (Process C) such that there likely would be a high need for sleep at this point. Elements of Process S that would have contributed to this need for sleep includes the fact that subjects were restricted to 4 hours of sleep in the second half of their usual 8 h sleep period on the night prior to testing and the subjects would have been awake for 16 to 24 h during the LE and post LE periods. Relevant to Process C, the LE and post LE periods are inclusive of circadian times where the subjects normally would be sleeping. Perhaps the effects of sleep restriction were too great to overcome by a lighting intervention.

Numerous studies examining the effects of blue light sources, both monochromatic and polychromatic, on both melatonin suppression and alerting effects, have yielded similar results for melatonin suppression and mixed results for alertness. Exposure to compact fluorescent light of 6500 K led to greater melatonin suppression and faster reaction times on PVT yet yielded negative results on a Paced Visual Serial Addition Task—a task relying on executive function similar to the DS and DSST used in our study (Chellappa et al., 2011).
Similarly, in a study using a monochromatic source peaking at 479 nm, significant melatonin suppression was observed yet this wavelength failed to yield any significant alerting enhancement (Revell et al., 2010). Conversely, in a study using a blue LED source (460 nm) at a much dimmer intensity (2 µW/cm²) than used in our study showed no significant melatonin suppression yet had a significant effect on alertness as measured by PVT (Phipps-Nelson et al., 2009).

With regard to mood states, both groups showed increases in fatigue scores across time. The blue LED group experienced some tension and anxiety during the light exposure period. Depression/dejection ratings were higher both during the light exposure and the post exposure period in the blue LED group. This result at first may be puzzling as blue LEDs and blue-enriched fluorescent lighting has been used for therapy in Seasonal Affective Depressed (SAD) patients but the intensity of light presented here (70 lux) was at least a factor of 2 lower than the lowest intensity used in the SAD trials (Glickman et al., 2006; Anderson et al., 2009; Strong et al., 2009). Perhaps the introduction of the blue light stimulus versus remaining in a more comfortable dim lighting environment could be a reason why this was not observed in the dim condition.

Notably, tiredness and fatigue were the most common side effects reported which is attributable to the partial sleep deprivation conditions that the subjects in both groups were asked to maintain. Interestingly, common side effects such as headache and nausea which are often noted as the two major side effects to bright light therapy (Lam and Levitt, 1999) were not widely reported in the current study perhaps due to the fact that all light intensities used in the study were well below standard bright light therapy levels (2,500 – 10,000 lux of white light).

Limitations of the study include the small sample size and a non-crossover study design. There is strong evidence that inter-individual differences in neurobehavioral deficits during sleep deprivation exist and can contribute to variation in sustained attention performance tests such as the PVT (Van Dongen et al., 2004). In addition, the intensity of testing was reported to be too much by many of the subjects and perhaps fatigued our subjects. Indeed, the POMS fatigue scores increased across time in both groups. Future investigations into the effects of light wavelength on alertness would benefit from larger sample sizes, a crossover study design, and fewer tests with better pacing between the tests.
As mentioned earlier (section 3.4), in examining the published data on the effects of light on alertness, some, but not all, studies show enhancements of alertness. Multiple studies have shown that light exposure can positively influence alertness. The timing of the light exposure during the day or night did not affect these responses differently. For nighttime light exposure effects on alertness, it has been hypothesized that the mechanism of action is related to the suppression of melatonin (Cajochen et al., 2003; 2005; Perrin et al., 2004; Lockley et al., 2006; Chellappa et al., 2011) while others have shown that there is no relationship between melatonin suppression and alertness (Ruger et al., 2006; Revell et al., 2010). Daytime improvements in alertness to bright white light exposure cannot be explained in a similar manner as daytime melatonin levels are undetectable. Further, another study suggested an alternate pathway, with different neural processing as well as spectral sensitivity for the two light responses (Revell et al., 2006). It was observed in this study that exposure to short wavelength light of 420 nm had a greater effect on subjectively measured alertness and mood than the 470 nm light exposure. Indeed, acute effects of light on circadian phase and alertness may not be mediated by melatonin suppression. The data reported here are consistent with the hypothesis that the alerting effects of light are independent of the melatonin suppression effects of light. Indeed, alerting effects of light may be mediated by independent neural pathways with differing spectral sensitivities.

As outlined in the Introduction (section 1.1.3), one of the SCN’s major output pathways leads to the vSPZ which has been shown to contribute to the alerting effects of light presented at night (Saper et al., 2005) in rats. Also in rodents, this area, as well as the VLPO, has been shown to receive direct input from the ipRGCs (Hattar et al., 2006). As mentioned earlier (section 1.1.1.2), the VLPO is known to be integral in the sleep/arousal state (Lu et al., 2002). The anatomical circuitry is thus in place, at least in rodents, to serve as a separate pathway with likely independent wavelength sensitivities and regulation for alerting effects of light.

Studies on healthy human subjects who have had their brains imaged during the daytime have shown that exposure to blue light (470 nm or 473 nm) when compared to green light (550 nm or 527 nm) has a greater activation of certain neural structures such as the hypothalamus, thalamus, amygdala, brainstem and hippocampus (Vandewalle et al., 2007a; 2007b). These fMRI studies suggest the existence of previously unidentified retinal pathways in humans that may be involved in the behavioral or alerting effects of light independent of the pineal gland.
These additional networks remain under investigation. Further research is needed to clarify which of the biological and behavioral responses to light share common underlying neural physiology.
CHAPTER 5 ADDITIVE AND OPPONENT SPECTRAL EFFECTS ON MELATONIN REGULATION IN HEALTHY HUMANS

5.1 Introduction

As previously mentioned in parts of the thesis (sections 1.7, 3.1, 4.1), numerous studies have confirmed that shorter wavelength monochromatic light in the blue portion of the spectrum is more potent than equal photon densities of longer wavelength light for evoking circadian phase shifts, suppressing melatonin, enhancing subjective and objective correlates of alertness, increasing heart rate, and increasing body temperature (Brainard et al., 2001b; Thapan et al., 2001; Lockley et al., 2003; Warman et al., 2003; Cajochen et al., 2005; Revell et al., 2005; Lockley et al., 2006; Revell et al., 2006).

Also, as reviewed earlier (section 1.2.3), despite abundant evidence that melanopsin-containing ipRGCs provide the primary input for circadian and neurobehavioral phototransduction, it is clear from studies on rodents, monkeys, and humans that the visual rod and cone photoreceptors also play a role in this physiology (Berson et al., 2002; Hattar et al., 2003; Lockley et al., 2003; Lucas et al., 2003; Figueiro et al., 2004; Smith et al., 2004; Dacey et al., 2005; Jasser et al., 2006; Gooley et al., 2010; Lucas et al., 2014).

In addition, as mentioned earlier (section 1.2.2), melanopsin does not behave like a typical vertebrate opsin. The response to light is delayed compared to rods and cones, though once engaged, the response to light is strong and ongoing (Berson et al., 2002; Wong et al., 2005). The structure of melanopsin more closely resembles an invertebrate opsin, both in form and function. Invertebrate opsins have the capacity to regenerate the chromophore that is initially photoisomerized from the 11-cis to all-trans configuration back to 11-cis by absorbing a second, different wavelength of light (Hillman et al., 1983), known as bistability. Observations in mouse SCN single cell recordings (Mure et al., 2007) as well as the pupil light response in humans (Mure et al., 2009) have shown that pre-exposure to 620 nm light increases the response to doses of monochromatic blue light at 480 nm administered afterwards. This increased response to blue light was explained by the authors as being due to
the increased pool of melanopsin in the 11-cis state. With regard to polychromatic light sources, one would expect an increase in melatonin suppression due to the increase availability of melanopsin in the 11-cis state. Not all studies, however, have shown bistability to be present (Mawad and Van Gelder, 2008; Papamichael et al., 2012) and its biological significance has yet to be determined.

Typical daily lighting conditions in homes and workplaces are comprised of broadband polychromatic light. In such lighting conditions, melanopsin would be stimulated by long and short wavelength light simultaneously. Previous melanopsin bistability studies mentioned above employed consecutive exposures of short and long wavelength monochromatic light (Mure et al., 2007; 2009). Revell and colleagues (2010), when examining the predicative qualities of their melanopsin photosensitivity function for nocturnal melatonin suppression, employed simultaneous exposures of narrowband wavelengths (437, 479 and 532 nm) but found no significant additive or opponent interactions of these combinations of monochromatic wavelengths. When polychromatic sources (4,000 K and 17,000 K) were compared, however, melatonin suppression response was significantly lower than predicted by the melanopsin photosensitivity function suggesting an involvement of the cone photopigments (Revell et al., 2010).

Different models of interaction between the melanopsin ganglion cells and classical rod and cone visual photoreceptors have been proposed, including the ideas that these photoreceptors are additive (Revell and Skene, 2007; Dkhissi-Benyahya et al., 2007; Mure et al., 2009; Revell et al., 2010) , opponent (Figueiro et al., 2004; Rea et al., 2005; Dacey et al., 2005), complementary (Lucas et al., 2003; Lockley et al., 2003; Zaidi et al., 2007) or dynamic over time and intensity (Gooley et al., 2010). These interactions span a variety of species and physiological measures. Due to the current uncertainty about these interactions, it is imperative to continue to empirically measure circadian, neuroendocrine and neurobehavioral efficacies of various combinations of light wavelengths presented at the same time.

Specifically, in order to assess the type of short wavelength cone (S-cone) retinal opponency physiology that may be responsible for contribution to acute plasma melatonin suppression, combinations of narrow bandwidth light with nominal peaks at 460 nm combined with 570 nm versus narrow bandwidth light with nominal peaks at 500 nm combined with 570 nm
were used in the experiment. In the opponency model proposed by Figueiro et al. (2005), 460/570 nm would show a reduced suppression of melatonin, while condition 500/570 nm would show an increased suppression of melatonin compared to 460 nm alone. The opposite responses would be observed, however, if the conclusions of Dacey and colleagues (2005) opponency model held true.

5.2 Aims and Hypothesis

The overall goal of this study was to test whether specific combinations of wavelengths indicate an opponent cone contribution to the suppression of nighttime synthesis of the pineal hormone, melatonin, in healthy human subjects. Specifically, we tested whether the acute suppression of melatonin by short-wavelength light at 460 nm and 500 nm could be reduced by simultaneous exposure of long-wavelength light at 570 nm.

5.3 Methods

5.3.1 Experimental light stimuli and measurement

A Kilo Arc Illumination system using a 1000 W Xenon compact arc lamp source with a grating monochromator from Optical Building Blocks (OBB) Corporation (Birmingham, NJ) was used. The system was fitted with an optical mask system capable of providing two variable narrow spectral bands simultaneously. The development of a dual wavelength mask was based on our prior work with Peter Smith, Ph.D. and Rigel Woida at the Lunar and Planetary Lab (LPL) at the University of Arizona. A diffraction grating first spreads out the spectrum prior to contacting the mask, which blocks the undesired wavelengths. Using MatLab computer software from MathWorks (Natick, MA), a mask was designed for each of the two dual wavelength conditions and transferred onto acetate. The acetate mask was attached at the exit port of the optical array which was coupled to an eight inch diameter Ganzfeld integrating sphere engineered by Labsphere (North Sutton, NH) to give a full visual field patternless exposure (see Figure 27 for diagram). I trained in using this optical system and mask design but the construction of additional masks was done by Michael Jablonski, a
research technician in our laboratory. Michael also staffed the majority of nighttime exposure conditions along with several technicians from the Light Research Program at TJU. I have visited the LPL and interacted with both Dr. Smith and Rigel Woida where this unique optical system was constructed.

The six light exposure conditions were: 1) no light exposure, 2) narrow bandwidth light with a peak of 460 nm at 12.1 µW/cm² (Stimulus A), 3) narrow bandwidth light with a peak of 500 nm at 8.9 µW/cm² (Stimulus B), 4) narrow bandwidth light with a peak of 570 nm at 18.5 µW/cm² (Stimulus C), 5) Stimulus A & C combined at 31.0 µW/cm², and 6) Stimulus B & C combined at 26.7 µW/cm². A more detailed explanation can be found in section 5.3.3 below and is diagramed in Figure 27 below.

![Diagram of the light exposure system used in the study. A 1000 W Xenon compact arc lamp source generated the light that then passed through a grating monochromator. An acetate mask was attached at the exit port of the optical array which was coupled to an eight inch diameter Ganzfeld integrating sphere.](image)

Irradiance measures were taken using an IL-1400BL radiometer/photometer (International Light Technologies, Inc., Peabody, MA) previously described in section 2.2 which was held in place at the front of the Ganzfeld dome by a clear, plastic holder. The spectral power
distribution measurements were taken using a Model FSHH 325-1075P FieldSpec handheld spectroradiometer (Analytical Spectral Devices, Inc., Boulder, CO) (section 2.2).

Relative spectral power distribution measurements were made on the three single peak spectra and on the two double peak spectra. Figure 28 illustrates one measurement of a double peak spectrum with peaks at 460 nm and 570 nm. Absolute radiometric measurements were made on each of the five test spectra in terms of irradiance on the plane of the pupil at the eye exposure position in front of the Ganzfeld integrating sphere. These absolute values together with the relative spectral distributions were used to develop the absolute spectral irradiance distributions. Figure 29 shows the five spectra used in these experiments. Because the double peak spectra were generated independent of the single peak spectra generation, there are slight displacements of the respective peaks. The three individual peaks are identified as A, B, and C while the double peak spectra are identified as A + C and B + C. Table 26 shows the spectral and irradiance characteristics of the light sources used in the study.

These measures were taken by Michael Jablonski who worked with our Co-investigator Robert Levin, Ph.D. on Figure 29. Further, Robert Levin, Ph.D. first proposed to fund our laboratory to study spectral opponency in melatonin regulation, provided funding through Osram-Sylvania and teamed with Joel Pokorny, Ph.D. to define experimental lighting conditions.
Figure 28. The figure shows the wavelength peaks at 460 nm and 570 nm with the appropriate intensity contribution from each peak. Irradiance was determined based on the melanopsin sensitivity curve.

The irradiance of the 460 nm light source (12.1 μW/cm²) was chosen based on our laboratory’s action spectrum for melatonin suppression (Brainard et al., 2001b). Joel Pokorny, Ph.D. (Co-Investigator) calculated equal melanopsin absorptions for our experimental conditions based on the 483 nm nomogram for melanopsin absorption of visible light (Gamlin et al., 2007; Dacey et al., 2005). He also provided the estimated contributions of each light stimulus for activating cone photoreceptors. The results of his analysis were the basis for the irradiance settings for the various light exposure conditions. This information then was applied to the optical mask making system.
Figure 29. Absolute spectral irradiance distribution on the plane at the eye position in front of the Ganzfeld sphere. This figure was developed by Robert Levin, Ph.D (Co-Investigator).

Table 26. Spectral and irradiance characteristics at the plane of the subjects’ eyes in front of the Ganzfeld integrating sphere.

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>A + C</th>
<th>B + C</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nominal Peak</td>
<td>460</td>
<td>500</td>
<td>570</td>
<td>460 + 570</td>
<td>500 + 570</td>
<td>nm</td>
</tr>
<tr>
<td>Actual Peak</td>
<td>465</td>
<td>497</td>
<td>568</td>
<td>463 + 572</td>
<td>500 + 566</td>
<td>nm</td>
</tr>
<tr>
<td>FWHM</td>
<td>26.5</td>
<td>26</td>
<td>27.5</td>
<td>28.5 + 34.5</td>
<td>26.5 + 26.5</td>
<td>nm</td>
</tr>
<tr>
<td>Irradiance</td>
<td>12.1</td>
<td>8.9</td>
<td>18.5</td>
<td>31.0</td>
<td>26.7</td>
<td>µW/cm²</td>
</tr>
<tr>
<td>Photon Irradiance</td>
<td>2.82E13</td>
<td>2.23E13</td>
<td>5.29E13</td>
<td>8.23E13</td>
<td>7.32E13</td>
<td>photons/s/cm²</td>
</tr>
</tbody>
</table>

FWHM = Full Width at Half Maximum
5.3.2 Subject screening

Healthy volunteers of either sex between the ages of 18 and 30 were included. A cohort of 8 subjects was selected from a pool of subjects who had recently completed other similar nighttime studies in the laboratory for this project. Initial screening questions on age, health, work schedule and medications were on file and were updated to exclude any individuals who had changes to these conditions. No factor of race or ethnic background was used for acceptance or rejection of a volunteer. Ocular health of subjects was determined by eye examination from our consulting physician, Edward Gerner, M.D. Color vision of all subjects was tested by the Ishihara test for color-blindness and Farnsworth Munsell D-100 tests. A total of 4 males and 4 females ranging in age from 21 to 28 years old, with a mean age (± SEM) of 24.0 ± 1.0 years were studied in the Light Research Laboratory of TJU.

5.3.3 Study protocol

The study was approved by the Institutional Review Board of TJU and all subjects gave written informed consent prior to entering the protocol. The study protocol is shown in Figure 30.

Each subject was studied on six different nights in a randomized order, with at least one week between study nights. The six exposure conditions were described in the previous section. Each experiment began at approximately 23:45 when subjects had their pupils dilated with one drop in each eye of 0.5% HCl Cyclopentolate. The subjects were then blindfolded. Just before 02:00 h, a blood sample was taken by venipuncture in the arm. The subjects’ heads were then placed in a head holder in front of the experimental light and blindfolds were removed. The subjects were exposed to the light from 02:00 until 03:30 h. During this 90 minute exposure, the subjects sat quietly, keeping their eyes open and looking at a fixed target dot in the center of this light field. At 03:30 h, a second blood sample was taken and the subjects were permitted to leave the laboratory. Each subject was randomly exposed to the different combinations of light and one night they remained blindfolded throughout the night with no exposure to light.
5.3.4 Measurement of melatonin

Blood samples for this acute study were collected as described earlier (section 2.3). Plasma melatonin concentrations were assayed by radioimmunoassay using antiserum described by Rollag and Niswender (1976). Mark Rollag, Ph.D. assayed the samples in his laboratory at the Uniformed Services University of Health Sciences (USUHS). Previously, Dr. Rollag trained me to perform this assay in his laboratory at USUHS. A full description of the method can be found in section 2.6.1.

5.3.5 Melatonin data analysis and statistics

Two-tailed paired Student’s t tests were used to determine statistical significance of the change in raw melatonin levels from 02:00 to 03:30 h. Next, change in melatonin levels and percent change were calculated for each subject using the data for subject plasma melatonin levels at 02:00 and 03:30 h for each study night. The mean and the standard error of the mean were then calculated for each light exposure condition. Percent change in melatonin data values were then normalized to percent control-adjusted change scores. This was done by subtracting percent change scores for each subject’s control condition (no light exposure) from the percent melatonin change value for each condition for that same subject. This adjusts the plasma melatonin values to account for the normal rise in melatonin levels during this time of night (Gaddy et al., 1993; Brainard et al., 1997). Sets of pre-exposure melatonin values, percent change and percent control-adjusted melatonin change scores were analyzed with one-way repeated measures ANOVA. Significant differences between light exposure groups were assessed with post-hoc Fisher PLSD test with alpha set at 0.05. The statistics were run in StatView (SAS Institute Inc, Cary, NC).
Figure 30. The study protocol for each experimental light exposure and the dark, control night. The light stimulus nights corresponded to either the single narrow bandwidth exposures or the combined two-peak exposures, as listed above.

A power analysis for a future study based on collected data was performed using G*Power (freeware): http://www.gpower.hhu.de. Training on this statistical technique was provided by research assistant Melissa Ayers.
5.4 Results

A comparison of the pre-light exposure values across all light conditions showed no statistically significant variation in plasma melatonin based on exposure night indicating that baseline levels of melatonin were consistent for all nights of the study (Figure 31).

Figure 31. Pre-treatment plasma melatonin values (mean ± SEM, n=8) for each light exposure group. There was no significant difference between the pre-treatment melatonin values (p = 0.85).

When comparing pre-treatment melatonin values to post-treatment melatonin values, there was a significant rise in plasma melatonin in the control night (p < 0.05, no light exposure) as shown in Figure 32. As expected based on the human action spectrum for melatonin regulation in humans (Brainard et al., 2001b), there was a significant suppression of plasma melatonin in the 460 nm condition (p < 0.01) and there was slight suppression in the 500 nm and 570 nm conditions, although not statistically significant. In the comparisons between pre-treatment and post-treatment values in the 460 nm + 570 nm condition and the 500 nm + 570 nm condition, there was significant suppression of plasma melatonin (p < 0.05).
Figure 32. Plasma melatonin values (mean ± SEM, n=8) before and after light exposure.

Based on the percent change scores of plasma melatonin (Figure 33), there was significant melatonin suppression of the 460 nm light condition compared to the no light control (p < 0.001). There was no significant melatonin suppression by 500 nm, 570 nm, or 460 + 570 nm light conditions compared to the no light control. There was, however, a significant melatonin suppression in the 500 + 570 nm light condition compared to the no light control (p < 0.05).

Figure 33. Plasma melatonin percent change scores (mean ± SEM, n=8). Compared to the control night, both the 460 nm and 500 + 570 nm light groups exhibited significantly suppressed melatonin (p<0.001 and p<0.05, respectively).
When considering the control adjusted plasma melatonin percent change scores (Figure 34), there was no significant differences between the light exposure conditions (p=0.08).

![Figure 34. Plasma melatonin percent control-adjusted change scores (mean – SEM, n=8). Differences between the light exposure groups were not significant.](image)

Table 27 lists the mean ± SEM for Figures 31, 32, 33 and 34.

A sample size, post-hoc power analysis on the five means from the control-adjusted change score data from Table 27 when powered at 0.95 with an alpha of 0.05 determined that a sample size of 10 would be sufficient to detect significant differences between the light exposure conditions.
Table 27. Mean (± SEM) plasma melatonin values (pg/mL), percent change scores and control adjusted percent change scores in the different light exposure groups.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>460 nm</th>
<th>500 nm</th>
<th>570 nm</th>
<th>460+570 nm</th>
<th>500+570 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Exposure Mean</td>
<td>102.5</td>
<td>92.5</td>
<td>103.5</td>
<td>102.8</td>
<td>90.1</td>
<td>90</td>
</tr>
<tr>
<td>Pre-Exposure SEM</td>
<td>12.9</td>
<td>18</td>
<td>17.9</td>
<td>19.7</td>
<td>25.1</td>
<td>13.9</td>
</tr>
<tr>
<td>Post-Exposure Mean</td>
<td>123.5</td>
<td>48.8</td>
<td>90.4</td>
<td>93</td>
<td>55.8</td>
<td>67.9</td>
</tr>
<tr>
<td>Post-Exposure SEM</td>
<td>18.6</td>
<td>10.9</td>
<td>14</td>
<td>11.9</td>
<td>15.8</td>
<td>13.9</td>
</tr>
<tr>
<td>% Change Mean</td>
<td>18.7</td>
<td>-46.4</td>
<td>-5.6</td>
<td>6.7</td>
<td>-11.6</td>
<td>-20.5</td>
</tr>
<tr>
<td>% Change SEM</td>
<td>5.2</td>
<td>7.6</td>
<td>10.9</td>
<td>18.8</td>
<td>18</td>
<td>13.3</td>
</tr>
<tr>
<td>% Control Adjusted Mean</td>
<td>-65.2</td>
<td>-24.4</td>
<td>-12.1</td>
<td>-30.3</td>
<td>-39.2</td>
<td></td>
</tr>
<tr>
<td>% Control Adjusted SEM</td>
<td>10.6</td>
<td>15.2</td>
<td>18.8</td>
<td>17.1</td>
<td>12.4</td>
<td></td>
</tr>
</tbody>
</table>

5.5 Discussion

A novel human light exposure system was developed that permits exposure of the eyes to either single wavelengths of narrow bandwidth light or simultaneous exposure to two narrow bandwidth wavelengths. Based on the raw pre-treatment plasma melatonin values obtained from the 8 subjects on each of their 6 nighttime exposures, the data indicate that baseline nocturnal melatonin levels were consistent across the different nights of the study (p=0.85). This indicates that the subjects had normal, elevated levels of nocturnal melatonin during their participation in the study. Furthermore, in accordance with previous data, the narrow bandwidth light wavelength conditions of equal photon density at 460 nm (12.1 µW/cm²), 500 nm (8.9 µW/cm²), and 570 nm (18.5 µW/cm²), produced anticipated results, showing strong melatonin suppression in the short wavelength portion of the spectrum (460 nm) and moderate suppression in the longer wavelength ranges (500 and 570 nm). These results
indicate that the study is coherent with our prior studies as well as data published by other laboratories (Brainard et al., 2001b; Thapan et al., 2001).

For the conditions with combined narrow bandwidth stimuli, plasma melatonin percent indicated that there was strong melatonin suppression with the 460 nm light condition that was significantly different from control values. By contrast, the combined 460 nm + 570 nm stimulus did not significantly suppress melatonin compared to control values. This result is suggestive that the addition of the 570 nm wavelength blunted the strong response to 460 nm. Unfortunately, this finding is uncertain since post hoc analysis does not demonstrate a statistical difference between the 460 nm condition alone versus the 460 nm + 570 nm condition. Further, when examining the percent control-adjusted change score data, the ANOVA approaches, but does not reach statistical significance (p = 0.08). Hence, at this level of analysis, none of the groups are significantly different from one another. Importantly, in more than 20 years of peer-reviewed publications, our research group has used the control adjusted plasma melatonin percent change scores as our most definitive assessment of the efficacy of light stimuli for melatonin suppression in human subjects (Gaddy et al., 1993; Brainard et al., 2013).

A previous study using simultaneous exposures of narrowband wavelengths (437, 479 and 532 nm) found no significant additive or opponent interactions of these combinations of wavelengths (Revell et al., 2010). Similarly, another study using simultaneous administration of narrowband sources in the blue (479 nm) and red (627 nm) wavelength ranges showed no evidence of opponent or additive responses (Papamichael et al., 2012). The longest wavelengths used (532 nm and 627 nm, respectively) in these studies, however, perhaps were far enough removed from the wavelengths used previously by Figueiro and colleagues (2004) (546 nm and 578 nm) the spectral peaks of a mercury vapor lamp, as well as the long wavelength narrowband used in the study presented here (570 nm) to not have an opponent effect.

The results reported here do not provide evidence that is consistent with a theory of spectral opponency for light regulation of the normal human neuroendocrine system. While there is a trend approaching statistical significance between the light exposure conditions, these findings cannot be interpreted as a significant difference. This data is consistent with an
earlier publication that compared melatonin suppression with either a broadband LED source or high pressure mercury vapor lamp with 3 distinct narrowband emissions (Figueiro et al., 2004). In that study, the irradiance at the eye was significantly higher for the mercury vapor lamp yet the melatonin suppression was only half that of the LED source. This reduction in the effectiveness of short wavelength light from the 436 nm peak for the melatonin production at night was interpreted as evidence that the circadian system does not work additively and indeed suggested an opponent mechanism to the spectral sensitivity to light for melatonin suppression (Figueiro et al., 2004). Specifically, the same group concluded that a S-on, blue minus yellow (b-y) mechanism provided input to the ipRGCs (Rea et al., 2005). By contrast, Dacey and colleagues identified a S-off, blue minus yellow (b-y) response in the LGN in the primate using electrophysiology (Dacey et al., 2005). While the data reported here are more suggestive of the model proposed by Rea and colleagues (2005), they are not conclusive. The challenge of using a melatonin suppression paradigm so far removed from the retinal circuitry and involving many intervening processes may prove to be too much. Indeed, electrophysiological recording such as used by Dacey and colleagues (2005) yields a higher degree of certainty.

Two follow up approaches are worthy of consideration. One approach would be simply to repeat the study with a larger set of subjects to the exact protocol described above. Indeed, the power analysis performed from the control-adjusted change score data indicated that a sample size of 10 would be sufficient to see significant differences. Alternatively, a parsimonious experimental design could be conducted that employs only three study conditions: 1) a control, dark exposure night; 2) exposure to narrow bandwidth light peaking at 460 nm; and 3) simultaneous exposure to two narrow bandwidths of light with one peak at 460 nm and one at 570 nm. While the former design could potentially yield evidence as to which type of color opponency is operating, the latter design could substantially lend further evidence of the existence of opponency in light-induced melatonin suppression. In either case, and based on the data presented here, certain assumptions that polychromatic light sources will suppress melatonin modeled on the two action spectra published on monochromatic exposures (Thapan et al., 2001; Brainard et al., 2001b) alone are likely prone to error. This conclusion is in harmony with a study based on predicting the effects of broadband light sources on light-induced melatonin suppression (Revell et al., 2010).
The overarching aim of this set of studies was to examine the biological and behavioral effects of short wavelength light in the blue-appearing portion of the visible spectrum either in combination with other light wavelengths or refined to specific blue wavelengths. These studies assessed immediate alerting responses, acute melatonin suppression, or longer term circadian effects in healthy human subjects. Three experiments outlined in Chapters 3, 4 and 5 were performed with different light stimuli.

6.1 Polychromatic blue-enriched light

This first study (Chapter 3) tested the hypothesis that white polychromatic fluorescent lamps have increased efficacy for melatonin suppression, alertness, and circadian phase-shifting when they are enriched in the blue-appearing portion of the visible spectrum. This was done by comparing the magnitude of melatonin suppression, changes in alertness, and phase resetting of circadian melatonin rhythms in healthy male and female subjects following exposure to equal photon densities of blue-enriched fluorescent lights (17,000 K) compared to standard cool white fluorescent light (4,000 K).

6.1.1 Circadian and neuroendocrine effects

Blue-enriched polychromatic fluorescent light presented at relatively low room lighting levels was more effective than standard white polychromatic fluorescent light for acutely suppressing melatonin. This comparison proves that blue-enriched polychromatic fluorescent light is more efficient at this low light level than typical fluorescent lighting. There was a non-significant result for circadian phase-delay in melatonin timing. In previous studies that have compared blue-enriched versus typical fluorescent lamps at higher irradiances, both light sources caused circadian phase-shifts with little difference in the degree of effect (Smith and Eastman, 2009; Smith et al., 2009).
By contrast, the data reported here are not in line with a number of studies showing that monochromatic or narrowband light in the blue region of the spectrum is more efficient than other wavelengths for phase-shifting the circadian system (Wright & Lack, 2001; Warman et al., 2003; Lockley et al., 2003; Wright et al., 2004; Revell et al., 2005).

Our results reveal that both polychromatic 4,000 K and blue-enriched 17,000 K fluorescent light at equal photon density of $1 \times 10^{14}$ photons/cm$^2$/s suppress plasma melatonin in healthy young subjects (42.8% and 70.8%, respectively), with 17,000 K exposure condition resulting in a significantly stronger hormonal suppression. The equivalent irradiance/illuminance of light for the 4,000 K and 17,000 K lamps were 40.7 $\mu$W/cm$^2$ (134.3 lux) and 50.0 $\mu$W/cm$^2$ (128.9 lux), respectively.

When examining the magnitude of phase-delay shifts of the 4,000 K and blue-enriched 17,000 K fluorescent light at equal photon density of $1 \times 10^{14}$ photons/cm$^2$/s, the 17,000 K condition caused a mean phase-delay shift of 2.1 h while the 4,000 K condition caused a mean phase-delay shift of 1.7 h. Statistical testing indicated no significant difference for this number of subjects likely due to the variability of the 4,000 K condition. The spread of the data points was nearly twice as much for the 4,000 K condition when compared to the 17,000 K condition. Nonetheless, our study found that blue-enriched polychromatic light did not produce larger phase-delays than bright white polychromatic light of equal photon density of $1 \times 10^{14}$ photons/cm$^2$/s.

Previous studies support these findings. In a within-subjects crossover design, 13 healthy young subjects were given 4,100 K and blue-enriched 17,000 K polychromatic fluorescent light at an equal photon density of $4.2 \times 10^{15}$ photons/cm$^2$/s. In this study, an inpatient circadian phase assessment on salivary melatonin levels was performed after 4 consecutive, 2 h exposures beginning on Day 1, 3 h after their predetermined DLMO with each light exposure being delayed by 2 h. Phase-delay shifts after 17,000 K condition (-4.45 h) were not significantly different than the 4,100 K condition (-4.48 h) (Smith and Eastman, 2009). In another study on elderly subjects, using the same fluorescent lamps as above in a similar phase-delaying protocol (2 h experimental light exposures on 4 consecutive days) at an equal photon density of $1 \times 10^{15}$ photons/cm$^2$/s, a phase-delay of -1.93 h was observed in the
17,000 K condition and -1.67 h in the 4,100 K condition with no significant difference detected (Munch et al., 2011).

Eighteen young subjects (18-30 years) were studied in a similar protocol to construct a phase response curve (PRC) to monochromatic blue (480 nm) light (Ruger et al., 2013). Results were compared with a prior white light PRC (Khalsa et al., 2003) and indicated that the monochromatic blue light stimulus achieved approximately 75% of the resetting response of the much brighter (3,000 µW/cm²) white light stimulus with the maximum phase-delay of -2.6 h. The authors went on to suggest that monochromatic blue light was more efficient at phase resetting as the blue light used comprised approximately 4% of the energy of the white light stimulus yet achieved similar results. Examining our data for the 17,000 K exposure we found the average phase-delay of -2.1 h which approached 80% of maximum potential phase resetting. In our study, we used only 1.7% (50 µW/cm²) of the energy of the white light stimulus, so the 17,000 K blue-enriched stimulus appears to be similarly efficient at producing photic resetting as observed in previous studies.

When comparing our melatonin suppression data to a study using 17,000 K polychromatic fluorescent light mounted in an experimental light box, our melatonin suppression data values are approximately twice as much as reported for their blue-enhanced condition (Santhi et al., 2012). Santhi and colleagues reported half-hourly averaged area under the curve (AUC) plasma melatonin concentrations multiplied by duration of exposure (pg/ml*min) represented graphically in the 1,200-1,400 range. Calculation of our averaged 17,000 K AUC values of plasma melatonin captured in the first 4 hours of light exposure multiplied by the total exposure yielded a value of 2,844 pg/ml*min. One possible reason for this difference is the timing of light exposure. Our exposure was 9.25 h before habitual wake time, well into the evening rise of endogenous melatonin while their evening timing occurred 4.25 before the subject’s habitual sleep time, an approximate 3 h difference. Consequently, their evening melatonin values were suppressed from what would naturally be an already low daytime value while our initial starting values were after the normal evening rise in melatonin and were much higher.

When assessing the melatonin suppression data of unpublished dose-response curves run at similar intensities on the same 4,000 K and 17,000 K fluorescent lights in our laboratory
(Brainard et al., 2007), it was not possible to perform a reasonable comparison since the exposure time of a 1.5 h light exposure was given from 0200 h to 0330 h in the unpublished fluence-response studies, while a 6.5 h exposure was given 9.25 h prior to habitual waketime in the phase-shift study (Chapter 3). Essentially, the phase-shift study examines the rising part of the melatonin curve versus the fluence-response study where the timing of light exposure was close to the peak of the melatonin rhythm. In addition, the prior photic histories were different; dim polychromatic fluorescent light at <4 lux in the phase-shift study for 4.75 h versus a 2 h blindfolded period in the fluence-response study. In the dose-response study, 4,000 K light at 39.5 μW/cm² caused a percent suppression of melatonin of 29.7% compared to the phase-shifting study, where the 40.9 μW/cm² exposure elicited melatonin suppression of 42.8%. Similar to the phase-shift study where a significantly higher melatonin suppression of 70.9% was found with the 17,000 K, 50 μW/cm² light exposure, the exact same intensity 17,000 K light in the dose-response study elicited 54.9% melatonin suppression. These differences in melatonin suppression emphasize the importance of the circadian time of light exposure and prior photic history in determining the neuroendocrine response to light as noted in previous studies (Hebert et al., 2002; Khalsa et al., 2003; Smith et al., 2004).

6.1.2 Neurobehavioral effects

Measures of both objective and subjective alertness gave varying results (Chapter 3). KSS data supported the hypothesis that 17,000 K light exposure significantly reduces sleepiness compared to 4,000 K light exposure. Post hoc analysis of subjective sleepiness revealed that 17,000 K light exposure significantly reduced sleepiness for 3 hours after light exposure compared to 4,000 K light exposure. PVT data during the light exposure, however, do not support the hypothesis that 17,000 K light is more effective at acutely enhancing reaction time than 4,000 K light. Post hoc analysis of the PVT data indicated that 17,000 K light exposure appears to statistically enhance psychovigilance reaction time significantly for the 3 hours after light exposure compared to 4,000 K light exposure.

Our results for alerting effects found with the 17,000 K light are in harmony with some of the results from Chellappa and colleagues (2011) who found alerting responses to polychromatic light exposure in the evening with 6,500 K light versus exposure to 2,500 K or 3,500 K light.
Subjective sleepiness was reduced and cognitive performance enhanced after 90 minutes of exposure continuing 3 h into post-light exposure. As with their results, our data showed post light exposure effects for 3 h in auditory PVT lapses and reaction time as well as subjective sleepiness as measured by KSS.

In a crossover field trial in an office setting that used the same polychromatic fluorescent sources employed in our study, significant reductions in sleepiness measured during the day were detected in the 17,000 K light group versus the 4,000 K light group (Viola et al., 2008). The results were consistent across 4 weeks, yet the time of day that they were acquired was in the morning, only a few hours after awakening, which differed greatly from when our data was collected.

Smith and colleagues (2008) used the Stanford Sleepiness Scale during 2 h morning exposures of the same polychromatic fluorescent sources in a phase advancing protocol and showed no significant difference in subjective alertness between the two light stimuli (Smith et al., 2008). This is in contrast to our KSS findings but the timing of testing (upon awakening) was in stark contrast to ours (beginning after several hours of being awake).

### 6.2 Narrowband blue light

The overall goal of the second project (Chapter 4) was to study the efficacy of blue-appearing narrowband solid-state light versus dim white light exposure for acutely suppressing melatonin as well as enhancing alertness and cognitive performance in healthy men and women. Multiple types of data were collected including PSG, KSS, KDT, 10 minute auditory PVT, and a modified NTB. Results from subjects exposed to the blue solid-state light source were compared to subjects who remained in a standard dim white fluorescent lighting condition.

With the current exploding development of solid state technology for lighting, many recent studies have employed narrowband blue LEDs as a readily available experimental blue light source. The light emitted from many commercially available blue LEDs have peaks that approximately match the spectral sensitivity of melanopsin (Lucas et al., 2014).
6.2.1 Neuroendocrine effects

Narrowband blue LED light at 75 μW/cm² exhibited a strong suppressive effect on melatonin levels when compared to dim white light at 0.7 μW/cm². Plasma melatonin was suppressed after 2 h and continued for 4 h while the dim white light exposure permitted a rise in endogenous plasma melatonin. Blue light, both monochromatic and narrowband, as well as polychromatic light sources with enhanced output in the blue-appearing part of the spectrum can suppress melatonin production (Brainard et al., 2001a;b; Thapan et al., 2001; Wright et al., 2001; 2004; Lockley et al., 2003; 2006; Smith and Eastman, 2009; Smith et al., 2009; Revell et al., 2010; Chellappa et al., 2011). Our results are consistent with these published data as well as a dose-response study performed earlier in our laboratory (West et al., 2011). A corneal exposure of 75 μW/cm² of the blue LED light used in the current study, elicited similar significant melatonin suppression as in the earlier study (West et al., 2011).

Taking LED technology into everyday application by testing a low intensity blue LED light (λmax=465 nm) indirectly illuminating the interior automobile space, Lerchl and colleagues (2009) found no effect on salivary melatonin levels. This finding was compared incorrectly with our action spectrum of melatonin suppression data (Brainard et al., 2001b) with a claim that the intensities used were comparable. Indeed this is not the case for three reasons. The intensities used (0.22 and 1.25 lx) in the Lerchl study were comparable to the only the 2 lowest monochromatic exposures used in the 460 nm dose-response curve (Brainard et al., 2001b). Pupils were freely reactive as no mydriatic agent was employed so indeed corresponding retinal illuminances were quite different. Lastly, the indirect exposure of the eyes also led to much lower retinal illuminances when one considers the exposures used in our study were full field given in an integrating sphere.

Similar findings were reported by Phipps-Nelson and colleagues (2009) using low intensity LED sources at 460 nm (25 nm half-peak bandwidth) with a reported irradiance of 2.05-2.07 μW/cm² in an interior automobile space. Salivary melatonin was found not to be significantly suppressed by these irradiances. The authors’ conclusions resonate with those above regarding the use of mydriatic agents, full field versus partial field exposure and fixed versus variable gaze behavior (Phipps-Nelson et al., 2009).
6.2.2 Neurobehavioral effects

Alertness as measured objectively by auditory PVT revealed no statistically significant results in reaction time or lapses during light exposure between the blue LED light subjects compared to subjects exposed to dim white light conditions. Cognitive performance testing using DSST and DS tests showed that the blue LED light subjects outperformed the dim white light subjects prior to and throughout light exposure. With regard to subjective sleepiness as measured by KSS, the blue LED group was sleepier prior to light exposure, remained sleepier through light exposure when compared to the dim white light subjects.

The study of narrowband blue LED sources with regard to alertness reaffirms that the link between exposure to short wavelength light and reduction in subjective sleepiness is tenuous. The assumption that because short wavelength light is more effective than other wavelengths at suppressing melatonin than it is equally effective at causing a reduction in subjective sleepiness and alertness may be flawed.

The mechanism by which sleepiness is reduced by light exposure has been suggested to be through melatonin suppression (Badia et al., 1991; Cajochen et al., 1999; 2000). More recent studies have suggested that the mechanism may be independent of melatonin suppression and may involve the VLPO which innervates key brain areas that play a role in wakefulness (Lu et al., 2002; Saper et al., 2005). The discovery of neuroanatomical pathways existing from ipRGCs to the VLPO confirm this is feasible (Hattar et al., 2006).

Indeed, light presented during the daytime, when melatonin levels are generally undetectable, enhanced alertness (Phipps-Nelson et al., 2003; Ruger et al., 2006; Vanderwalle et al., 2007a;b). Similarly, in a fMRI study, a brief bright light pulse prevented sleepiness while subjects were in continuous darkness (Vandewalle et al, 2006). Light exposures given during this study induced increased activity in the cortical brain areas involved in whatever task the subject were performing at the time of fMRI.

Cognitive performance results in the current study revealed that the blue LED subjects outperformed dim white light subjects prior to and throughout light exposure. Differential
effects of light on subjective sleepiness, behavioral and physiological measures of alertness and sleepiness have also been observed in individuals in other studies (Galliaud et al., 2008; Leproult et al., 2003). As was the case with earlier studies performed with bright white fluorescent light, not all studies show alerting effects of narrowband blue light.

Cajochen and colleagues (2011) tested computer screens that were backlit by LEDs versus being backlit by cold cathode fluorescent lamps on alertness and cognitive performance. LED backlit computer screens emitted 3.32 times more light in the blue range between 440 and 470 nm. In contrast to our findings, alertness levels were sensitive to this portion of the spectrum with the LED-backlit computer screens significantly improving sustained attention reaction times as measured in the GO/NOGO task (Cajochen et al., 2011). Notably, this result was achieved using a relatively dim, narrowband LED light source in contrast to previous studies which employed higher intensity monochromatic light sources. There are several differences between the studies that could explain our differential findings. We performed our testing in sleep-deprived individuals kept awake during the middle of their normal sleep episode while in the Cajochen study subjects were regularly rested individuals performing testing in the evening prior to their normal bedtime. Also, a 30 minute adaptation period of dim, red light was included prior to testing (Cajochen et al., 2011). Prior photic history, particularly, the use of red light prior to short wavelength exposure possibly impacted the results attained (Hebert et al., 2002; Smith et al., 2004; Mure et al., 2009; Chang et al., 2013; Chellappa et al., 2014).

Studies testing the neurobehavioral effects of short wavelength light presented as part of either broadband polychromatic fluorescent light or narrowband blue LED light exposure often give varying, conflicting results. A well-rested experimental group tested in the early portion of their day may not show differences in contrast to a sleep deprived group who are tested in the middle of the habitual sleep time. Clearly, the timing of testing and the test subject’s time awake inherently play major roles in the resulting data. In fact, it is plausible that many studies designed to detect an alerting effect of short wavelength light do not see a significant effect. This could be due to the effect being a subtle –hard to measure- outcome in which a large sample size would be needed to see a difference. In fact, this is a limitation of this study as the logistics demanded a small sample size and a non-crossover study design. Inter-individual differences in neurobehavioral deficits during sleep deprivation do exist and
can contribute to variation in sustained attention performance tests such as the PVT (Van Dongen et al., 2004). Due to the bias of some journals only publishing positive results, studies that fail to detect effects of light on alertness may not reach the scientific community.

6.3 Combinations of narrow bandwidth light

The third study (Chapter 5) tested whether certain combinations of wavelengths are additive or opponent to the circadian photoreceptor system that mediates the suppression of nighttime synthesis of the pineal hormone, melatonin, in human subjects. Specific narrowband wavelength combinations were given under highly controlled experimental conditions to healthy men and women and melatonin content of plasma samples was quantified.

6.3.1 Neuroendocrine effects

Narrow bandwidth light wavelength conditions of 460 nm, 500 nm, and 570 nm showed strong melatonin suppression in the short wavelength portion of the spectrum and moderate suppression in the longer wavelength range. These results are in parallel with melatonin action spectra data (Brainard et al., 2001b; Thapan et al., 2001). For the conditions with combined narrow bandwidth stimuli, there was strong melatonin suppression with the 460 nm light condition that is significantly different from control values. By contrast, the 460 nm + 570 nm stimulus did not significantly suppress melatonin compared to control values. This result suggests that the addition of the narrow bandwidth light wavelength at 570 nm wavelength blunted the strong response of the narrow bandwidth light wavelength at 460 nm and is suggestive of opponency. However, the results are in agreement with previous studies where simultaneous exposure of short and long narrowband wavelengths found no significant interaction in the form of additivity or opponency (Revell et al., 2010; Papamichael et al., 2012).

Spectral opponency in the visual system occurs in the layers of bipolar, amacrine and horizontal cells (Rodieck, 1999). Since rod and cone cells do not directly connect to the ipRGCs then the theoretical likelihood of detecting opponency is high. Exquisite murine work has revealed that opponent processes involving ipRGCs are at play but they do not use
chromatic processes evident in primates as described by Dacey (Weng et al., 2013; Dacey et al., 2005). If opponent physiology is found to exist in the ipRGC circuitry then broad spectrum polychromatic light environments that are enriched in the blue portion of the spectrum would not have greater neuroendocrine efficacy than standard fluorescent light (4,000 K). Unpublished fluence-response curves for melatonin suppression with 17,000 K and 4,000 K broad spectrum fluorescent light, however, show stronger melatonin suppression for 17,000 K blue enriched light exposures versus 4,000 K light exposure (Brainard et al., 2007; 2009). Revell and Skene (2007) also found that polychromatic light was more effective at suppressing melatonin than monochromatic blue light when matched for melanopsin stimulation, implying that the melatonin suppression response is not solely driven by melanopsin. Hence, the full spectrum of a light source’s distribution must be considered when speculating on its biological efficacy.

Clearly the ipRGCs are key to phototransduction to the circadian system as determined by suppression of melatonin synthesis. How the classic photoreceptors participate in this phototransduction process remains an area of intensive investigation. Recent finding of different types of ipRGCs with distinct retinal connections (Schmidt and Kofuji, 2010) further complicate the picture as these differing classes are likely to combine rod and cone inputs differentially leading to changing spectral sensitivities for the different evoked light responses.

### 6.4 Measurement models of circadian photoreception

Several laboratories have developed light measurement models based on data obtained in studies that were either run in their laboratory or based on an analysis of available data or some combination of both. These models have sought to develop functions to quantify the biological and behavioral effects of light mediated by melanopsin within the ipRGCs in terms a single measurement unit. It is challenging, however, to specify the contribution of classic photoreceptors and melanopsin containing ipRGCs across intensities in these models.

An initial attempt to design a measurement model for circadian photoreception was based strictly on the first two human action spectra for melatonin suppression (Brainard et al.,
2001b; Thapan et al., 2001). The circadian action function c(\(\lambda\)), claimed to give a quick estimation effect of different light sources on the circadian system (Gall and Bieske, 2004). Published in an international standards proceeding (CIE, 2004), this function garnered much attention in the scientific community.

Rea and colleagues have continued to attempt to model the spectral sensitivity of the human circadian system based on the two published action spectra on human melatonin suppression along with one small (N=4) study run in their facility by combining the spectral efficiency of rods, cones and melanopsin and combining it with the known neurophysiology of the retina for supporting vision (Rea et al., 2005; 2012).

Revell and Skene (2007) compared polychromatic light to monochromatic light when matched for equal “melanopsin stimulating” photons for melatonin suppression. Their comparison assumed a human melanopsin sensitivity curve with a lambda max of 480 nm. Their data revealed that melanopsin cannot be the only photopigment to contribute to melatonin suppression in humans with cones being implicated as contributing to this physiology at the wavelengths and intensities used in this study (Revell and Skene, 2007).

Furthering their work on the predictive ability of a melanopsin photosensitivity function with a lambda max of 480 nm, Revell and colleagues compared multiple monochromatic and polychromatic light sources for acute neurobehavioral and neuroendocrine effects (Revell et al., 2010). The results confirmed their previous findings that this melanopsin photosensitivity function at 480 nm cannot predict nocturnal neuroendocrine and neurobehavioral responses to polychromatic light. Further, the potential influence of short and middle wavelength cones on this physiology could not be determined at the intensities studied.

Gooley and colleagues (2010), however, were able to determine the relative contributions of cones to phototransduction for melatonin suppression and circadian phase-shifting of two selected monochromatic exposures of 460 and 555 nm. Cones were shown to contribute to melatonin suppression at onset lasting with a calculated half-life of 37.85 min and for circadian phase-shifting at low irradiance (< 13 log photons cm\(^{-2}\) s\(^{-1}\)) levels. As the light exposure continues or increases in intensity this contribution was no longer sustained (Gooley et al., 2010).
Turning to rodent studies to further identify the relative contributions of rods and cones to phototransduction for non-visual responses, several laboratories have made tremendous contributions (Panda et al., 2002; Lucas et al., 2003; Hattar et al., 2003; Panda et al., 2003; Dkhissi-Benyahya et al., 2007; Altimus et al., 2010; Lall et al., 2010; Brown et al., 2013). By genetically lesioning rod and cone or melanopsin input to circadian photoreception, these animal studies offer more power than human studies to delineate the relative contributions of the three photoreceptor types.

Using mice that lack functional rods or where rods are the lone functional photoreceptor, rods were found to operate in two distinct retinal circuits to provide circadian photoentrainment over an extensive range of light intensities (Altimus et al., 2010). One circuit for the rods was found to operate through cones at high light intensities (500 lux) but not at low light intensities (1 lux of less) where they signal through a rod-bipolar cell circuit.

In order not to disrupt the integrity of a normally functioning retina, as may be the case in retinally degenerate or knockout mice, Lall and colleagues (2010) carried out a series of experiments on mice in which their normal M-cone opsin was transgenically changed to a human red cone opsin. This shift to a longer wavelength cone spectral response also enabled a delineation of rod, cone and melanopsin contributions. They observed distinct phases of rod (approximately $10^7$ photons/cm$^2$/sec), cone (approximately $10^8$ photons/cm$^2$/sec) and melanopsin (approximately $10^{12}$ photons/cm$^2$/sec) input to non-visual photoreception with stimulus irradiance at 500 nm (Lall et al., 2010).

Using electrophysiology and pupillometry on rodless and coneless mice, a $V^Z\lambda$ melanopic sensitivity function was first described and tested by al Enezi and colleagues (al Enezi et al., 2011; Brown et al., 2013). The $V^Z\lambda$ melanopic sensitivity function is based on a Vitamin A opsin nomogram at 480 nm corrected for prereceptoral filtering by the lens and ocular media. Their data demonstrated that under diverse lighting conditions their $V^Z\lambda$ function accounted for responses in mice under diverse lighting conditions. Further, they tested light-driven firing activity of the LGN in mice with rods and cones and found $V^Z\lambda$ provided the best available predictor.
The many, assorted studies on physiological responses in both animals and humans have led to varied, sometime opposing conclusions (e.g. Gooley et al., 2010; Altimus et al., 2010). One should remain cognizant of the fundamental structural and physiological differences between nocturnal rodent and diurnal human retinas, with nocturnal animals having rod dominated retinas and diurnal species having cone-rich retinas. The number and structure of cones between rodents and humans are also quite different (Mustafi et al., 2009).

Abundant evidence shows that the melanopsin ipRGCs are anatomically and functionally interconnected with the rods and cones that support vision. Light-induced physiological responses reflect input from all of the retinal photoreceptor classes, with the relative importance of each being highly labile within and between response types. This complex interconnection is represented nicely in Figure 35 below from Lucas and colleagues (Lucas et al., 2014).

Figure 35. A) Schematic of the relevant retinal circuitry in humans. On cone bipolar cells (on CBCs) amacrine cells (AII) and rod bipolar cells (RBC); B) photoreceptive mechanisms R (rod opsin) M (melanopsin) SC (S-cone opsin) MC (m-cone opsin) LC (l-cone opsin) with plots of log sensitivity against wavelength to generate a distinct measure of illuminance (Lucas et al., 2014). Figure reprinted with permission from Elsevier.

The lack of a consistent and adequate method of quantifying light between different laboratories publishing data on photic regulation of biological and behavioral responses can make it challenging to replicate experimental conditions or to compare across studies. The leading researchers in the field have met and developed a consensus paper about measuring
light for circadian, neuroendocrine and neurobehavioral regulation based on current and accepted sensitivity functions for rods, cones and melanopsin integrating the spectral power distribution of the source (Lucas et al., 2014). To this end, source data from Chapter 3 is reported in the Appendix using the Toolbox described by Lucas and colleagues (Lucas et al., 2014). While quantification of the biological potency of light for non-visual photoreception remains elusive, collecting source descriptions on one platform will help for comparing and developing testable hypotheses that predict spectral characteristics for targeted physiological responses to polychromatic light.

6.5 Conclusion and future directions for lighting

Within the last decade and half, a new photosensory system in the eye has been discovered; a truly remarkable development in human physiology. A small set of retinal ganglion cells, previously thought to function as an output neuron, have been shown to be directly photosensitive and act quite differently from the classic photoreceptors of the visual system. Since the discovery of melanopsin (Provencio et al., 1996) and then the localization of that photopigment to ipRGCs (Berson et al., 2002; Hattar et al., 2002) there has been a dramatic increase in studies utilizing light as an independent variable for testing hypotheses. With the peak wavelength sensitivity of melanopsin in the blue portion of the visible spectrum, there has been a focus on studying blue light for physiological effects in animals and humans. The aim of the studies in this thesis was to test polychromatic light, inclusive of specific blue wavelengths thought to stimulate melanopsin maximally, in experiments on circadian, neuroendocrine and neurobehavioral effects in humans.

White polychromatic fluorescent lamps enriched in the blue portion of the spectrum were tested for increased efficacy for melatonin suppression, alertness, and circadian phase. Blue enriched fluorescent light resulted in a significantly stronger hormonal suppression and enhanced subjectively measured alertness. The phase-delay data did not support the hypothesis that blue-enriched fluorescent light is a more potent stimulus for the circadian system nor did objective alertness responses increase under blue-enriched fluorescent lights compared to standard white fluorescent lights.
Next we examined the efficacy of blue-enriched narrowband solid-state light for acutely enhancing alertness and cognitive performance in healthy men and women. The results demonstrated that narrowband blue light has a strong effect on melatonin suppression when compared to dim white light while objective and subjective measures of alertness, however, did not show any significant enhancement.

Lastly focusing strictly on a single neuroendocrine parameter, we tested combinations of narrow-bandwidth light wavelengths on melatonin suppression in healthy human subjects. Results showed strong melatonin suppression in the short wavelength portion of the spectrum, moderate suppression in the longer wavelength range of the spectrum with a nonsignificant attenuation of the strong short wavelength melatonin suppression with the addition of long wavelength light. Demonstration that the melatonin suppression response to this bichromatic light combination was lower than expected suggests that melanopsin sensitivity is not the sole consideration when trying to predict the efficacy of broadband lighting. In general, the different effects of short wavelength enriched polychromatic light on alertness, circadian phase-shift and melatonin suppression imply a differential involvement of the classical photoreceptors.

The results also show, as several parameters approached statistical significance, that there is need for focused, larger sample studies to tease out the answers to specific questions related to clarifying circadian, neuroendocrine and neurobehavioral effects in humans. Smaller numbers of research subjects have been adequate over the years for melatonin suppression studies due to the large amplitude of the physiological response. The lower amplitude response inherent in circadian phase-shift and neurobehavioral testing coupled with significant inter-individual variability to performance testing, however, require larger, targeted studies, preferably in a cross-over design.

This tradeoff between tightly controlled, laboratory based studies with small numbers of participants (typically N=20 or lower) and larger, field based, limited control studies (N=50 or more) is not a new dilemma but often emerges when bringing a basic research finding into application. The dilemma faced by the scientific photobiological community is that LED technology is evolving at an exponential rate, lighting companies are scrambling for
diminishing profits, and claims of healthy lighting for improving human wellbeing are not entirely based on sufficiently powered studies or peer-reviewed data.

Not surprising to any research scientist who studies human physiology, the more one looks at a sensory system, the more complex the system becomes. In a little over a decade since first being discovered, ipRGC’s contribution to phototransduction for circadian, neuroendocrine and neurobehavioral effects continues to be elucidated. At least five distinct types of ipRGCs have been identified with each having distinct central projection patterns. Indeed biological and behavioral effects are likely governed one or more ipRGCs in combination with classic photoreceptors. The likelihood that the many downstream physiological responses share the same sensitivity to light irradiance, the same spectral sensitivity, and the same adaptation rate is remote. The most prudent course that photobiological researchers can take is to carefully measure the incident light spectrum being studied and report this in a systematic way. To this end, the consensus methodology described by Lucas and colleagues (2014) with use of a common nomenclature based on current and accepted sensitivity functions will open the door for researchers to continue to unravel this complex photosensory system.


References


hypothalamic centers that are involved in the regulation of food intake. *J Comp Neurol* 431:405-423.


Weng S, Estevez ME and Berson DM (2013) Mouse ganglion-cell photoreceptors are driven by the most sensitive rod pathway and by both types of cones. PLoS ONE 8:e66480.


### Appendix I

**Chapter 3 Subject Information**

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Individual melatonin profiles are presented on the following pages. The top graph shows the two constant routine days overlayed. CR1 shown with filled circles and CR2 shown with empty circles. The bottom graph illustrates melatonin values following exposure to 6.5 h of white 4,000 K fluorescent light or blue-enriched 17,000 K fluorescent light (empty circles) compared to corresponding clock times during the previous melatonin cycle on CR1 (filled circles). The type of fluorescent light source is indicated in the upper right corner.
PS21 saliva

Constant Routines

Phase shift
DLMO_{25} = -1.83 h

Light exposure day & corresponding clock time for CR1

Suppression 42.48 %
Constant Routines

Phase shift
DLMO$_{25}$ = -1.88 h

Light exposure day & corresponding clock time for CR1

Suppression 29.03 %
Constant Routines

Phase shift
$\text{DLMO}_{25} = -1.88 \, \text{h}$

Light exposure day & corresponding clock time for CR1

Suppression $77.71\%$
Constant Routines

Phase shift
$DLMO_{25} = -1.70 \, \text{h}$

Light exposure day & corresponding clock time for CR1

Suppression $80.83\%$
Constant Routines

Phase shift
$DLMO_{25} = -2.67\ \text{h}$

Light exposure day &
corresponding clock time for CR1

Suppression
90.70 %
Constant Routines

Phase shift
$DLMO_{25} = -2.10 \, h$

Light exposure day & corresponding clock time for CR1

Suppression
$74.81\%$
Constant Routines

Phase shift
DLMO_{25} = -2.99 h

Light exposure day & corresponding clock time for CR1

Suppression 74.38 %
Constant Routines

Phase shift
\( DLMO_{25} = -2.46 \, h \)

Light exposure day & corresponding clock time for CR1

Suppression 80.04 %
PS04 Saliva

Constant Routines

Phase shift
\( \text{DLMO}_{25} = -2.60 \) h

Light exposure day & corresponding clock time for CR1

Suppression 85.55 %
Constant Routines

Phase shift
$DLMO_{25} = -0.84 \text{ h}$

Light exposure day & corresponding clock time for CR1

Suppression 80.7 %
Constant Routines

Phase shift
DLMO$_{25}$ = + 0.43 h

Light exposure day & corresponding clock time for CR1

Suppression
37.77 %
Constant Routines

Phase shift
DLMO_{25} = - 3.83 h

Light exposure day & corresponding clock time for CR1

Suppression 81.19 %
PS17 Saliva

Constant Routines

Phase shift
DLMO_{25} = -3.14 h

Light exposure day & corresponding clock time for CR1

Suppression 49.12 %
Constant Routines

Phase shift
$DLMO_{25} = -1.30 \text{ h}$

Light exposure day & corresponding clock time for CR1

Suppression
$-14.62\% (0\%)$
Constant Routines

Phase shift
DLMO<sub>25</sub> = - 0.23 h

Light exposure day & corresponding clock time for CR1

Suppression 18.11 %
Constant Routines

Phase shift
$DLMO_{25} = -1.30\ h$

Light exposure day & corresponding clock time for CR1

Suppression 57.94 %
Constant Routines

Phase shift
$DLMO_{25} = -2.05\ h$

Suppression
43.43 %

Light exposure day & corresponding clock time for CR1
Constant Routines

Phase shift
\[ DLMO_{25} = -2.89 \text{ h} \]

Light exposure day & corresponding clock time for CR1

Suppression 58.49 %
Constant Routines

Phase shift

$DLMO_{25} = -0.80 \text{ h}$

Light exposure day & corresponding clock time for CR1

Suppression

1.2 %
PS01

**Constant Routines**

Phase shift
\[ \text{DLMO}_{25} = -2.08 \text{ h} \]

**Light exposure day & corresponding clock time for CR1**

Suppression
80.4 %
## Appendix II

### Chapter 4 Subject Information

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<td>Dim White</td>
<td>I.V. line failure</td>
</tr>
<tr>
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<td>22</td>
<td>Blue LED</td>
<td>Light intensity issue</td>
</tr>
<tr>
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<td>Outlier</td>
</tr>
<tr>
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</tr>
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<td>NA15</td>
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<td>NA16</td>
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<td>30</td>
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</tr>
<tr>
<td>NA17</td>
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<td></td>
<td></td>
<td>Too fatigued to continue</td>
</tr>
<tr>
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<td>Blue LED</td>
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<td>NA19</td>
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<td>Dim White</td>
<td></td>
</tr>
<tr>
<td>NA20</td>
<td>Female</td>
<td>25</td>
<td>Dim White</td>
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</tr>
<tr>
<td>NA21</td>
<td>Disempaneled</td>
<td></td>
<td></td>
<td>Actigraphy non-compliance</td>
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<td></td>
</tr>
<tr>
<td>NA23</td>
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<td>Blue LED</td>
<td>No blood collected – staffing issue</td>
</tr>
<tr>
<td>NA24</td>
<td>Female</td>
<td>23</td>
<td>Blue LED</td>
<td>No blood collected – staffing issue</td>
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## Appendix III

### Chapter 5 Subject Information

<table>
<thead>
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<th>Subject Code</th>
<th>Sex</th>
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</tr>
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<tbody>
<tr>
<td>OS01</td>
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<td>OS02</td>
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<tr>
<td>OS08</td>
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Appendix IV

Sample Toolbox Source Comparison

<table>
<thead>
<tr>
<th>Radiometric and Photometric Values (380 - 780 nm inclusive)</th>
<th>Retinal Photopigment Weighted Illuminances (α-opic lux)</th>
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</thead>
<tbody>
<tr>
<td>Photon Flux (photons/cm²/s)</td>
<td>S Cone</td>
</tr>
<tr>
<td>Irradiance (μW/cm²)</td>
<td></td>
</tr>
<tr>
<td>Photopic Illuminance (lux)</td>
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</table>

<table>
<thead>
<tr>
<th>Light Source</th>
<th>Photon Flux</th>
<th>Irradiance</th>
<th>Photopic Illuminance</th>
<th>S Cone</th>
<th>Melanopsin ipRGC</th>
<th>Rod</th>
<th>M Cone</th>
<th>L Cone</th>
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<tr>
<td>4,000 K Light</td>
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<td>109</td>
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<td>17,000 K Light</td>
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<td>133</td>
<td>122</td>
<td>107</td>
<td>96</td>
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</table>

Below are toolbox calculations for the two sources used in Chapter 3.

Calculated irradiances, photopic illuminances v(λ), and human photopigment illuminances relative to the two polychromatic light source used in Chapter 3 (Lucas et al., 2014).