The impact of 470-nm light on acute nocturnal melatonin suppression

by

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ABSTRACT

A model for human circadian phototransduction, published by Rea et al. (2005), incorporates the known neuroanatomy of the human circadian system. This mathematical model is a work in progress and is based on existing empirical studies. As more studies are performed, model calculations will be refined and adjusted to reflect the additional data. Currently the model has greater relative uncertainty for lower light levels, near the threshold response. The current study is an extension of the work begun by Figueiro et al. (2009), which investigated the impact of two corneal irradiances (11 and 74 μW/cm²) of 470-nm light. The overall purpose of this thesis study was to develop a complete data set for one peak wavelength with respect to irradiance and exposure duration. The experiment described in this thesis was conducted at the Lighting Research Center in Troy, NY over the course of five weeks. The study investigated the impact of four corneal irradiances (0.7, 2, 6, and 20 μW/cm²) of 470-nm light and one dark control condition on acute nocturnal melatonin suppression over the course of 90-minutes. Each week participants were exposed to one of the conditions for 90-minutes. The resulting data provide a more detailed understanding of melatonin suppression in response to 470-nm light. The experiment also provided data with which to check model predictions. In response to 0.7 and 2 μW/cm² mean relative melatonin concentrations were lower than in the dark condition, over the course of 90 minutes. The melatonin concentrations from the two lowest irradiance conditions resulted in higher concentrations after 90 minutes of exposure, compared to mean relative melatonin concentrations just prior to light exposure. Melatonin concentrations resulting from the higher irradiance conditions (6, 11, 20, and 74 μW/cm²) were lower after 90 minutes of exposure, compared to mean relative melatonin concentrations just prior to light exposure. The threshold for acute nocturnal melatonin suppression was revealed to be between 0.7 and 2 μW/cm² for a continuous 90-minute exposure. Significant melatonin suppression was found for: 11 μW/cm² for 20 and 60-minute exposure durations, for 20 μW/cm² for 30, 60, and 90-minute exposure durations and 74 μW/cm² for 60, 75, and 90-minute exposure durations. Calculated suppression for 60-minute exposures and 2005 model estimated suppression were compared through a correlation, which resulted in a slope of 0.81 and an R^2 value of 0.78. Since the completion of the experiment and the initial comparison, several

adjustments were made to the circadian phototransduction model with regard to lens transmission, threshold, and the spectral opponent response. A second correlation was completed between calculated suppression and new 2011 model estimated suppression resulting in a slope of 0.96 and an R^2 value of 0.75. The second correlation demonstrated an improvement in model estimates for 470-nm light.

1. Background

As the earth transformed over time into the planet we are familiar with today, the light dark (LD) cycle of day and night has remained (with seasonal variations) constant despite continental and atmospheric changes (Luboshitzky et al. 1998). Most organisms have developed circadian rhythms as a result of this stimulus (Reppert et al. 2002). The term circadian comes from the Latin: *circa* "around", and *dies* "day", meaning "approximately one day". Circadian rhythms are endogenous self-sustaining biological oscillations, with a cycle of approximately 24h. Biological rhythms, which follow a circadian cycle, are controlled by an internal master clock located in the brain's hypothalamus.

1.1 Circadian Timing System

The circadian timing system (CTS) is the specific neural system responsible for circadian rhythm generation and regulation. In humans, these rhythms are evident in physiology and behavior (Rusak et al. 1989) with an average period of 24.2h (Czeisler et al. 1999). External stimuli, of which the light/dark (LD) cycle is the strongest, help to synchronize the master clock with the solar day (Refinetti 2006). Other stimuli include scheduled sleep, activity, temperature and meals (Duffy et al. 2005). The CTS includes: the master clock, afferent pathways and efferent pathways.

1.2 Master Clock

The master clock is located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus in the brain. The SCN is the termination site of photic entrainment pathways. The SCN is comprised of approximately 20,000 neurons, where each neuron contains an oscillatory mechanism (Reppert et al. 2002), which means that these neurons exhibit a circadian rhythm *in vivo* as well as *in vitro*. Figure 1 shows a rat neuron from the SCN expressing regular oscillations *in vitro* for several weeks (Reppert et al. 2000).

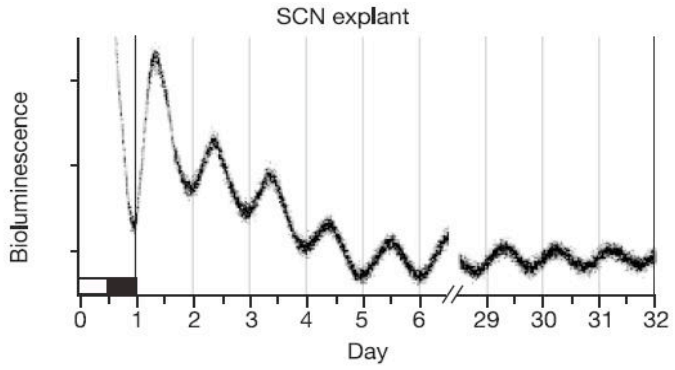


Figure 1: SCN neurons *in vitro* from a rat expressing a gene reporter that exhibits bioluminescence rhythms in culture. The black and white bars on the x-axis indicate the light/dark cycle at the time of tissue collection (Reppert et al. 2002).

In animals, lesions to the SCN lead to the dissipation of circadian rhythms, with the exception of expectant behavior before feeding. This demonstrates that the master clock is situated in the SCN and that these neural cells are crucial for the existence of circadian rhythms (Schwartz et al. 1986, Moore et al. 1997). SCN transplants to the brain can restore circadian rhythms in circadian arrhythmic animals. The term 'tau' is used to describe the length of a cycle in a free running animal while in constant dark. Ralph et al. (1990) transplanted neural SCN cells from hamsters with short circadian rhythms (20h tau) to arrhythmic hamsters with lesions to the SCN. The host animals with the transplanted neurons exhibited the short circadian rhythms of the donor animal, this provided evidence for SCN as the location of the master clock. Figure 2 shows the actogram (wheel running data) from an arrhythmic hamster prior to SCN ablation and after the SCN transplant, where a circadian rhythm was restored with a tau of 19.5h. There are also circadian oscillators scattered throughout the body, outside the SCN, where they are considered slave clocks. *In vivo* these secondary oscillators can only sustain regular oscillations for a few days without input from the master clock (Zylka et al. 1998, Reppert et al. 2002).

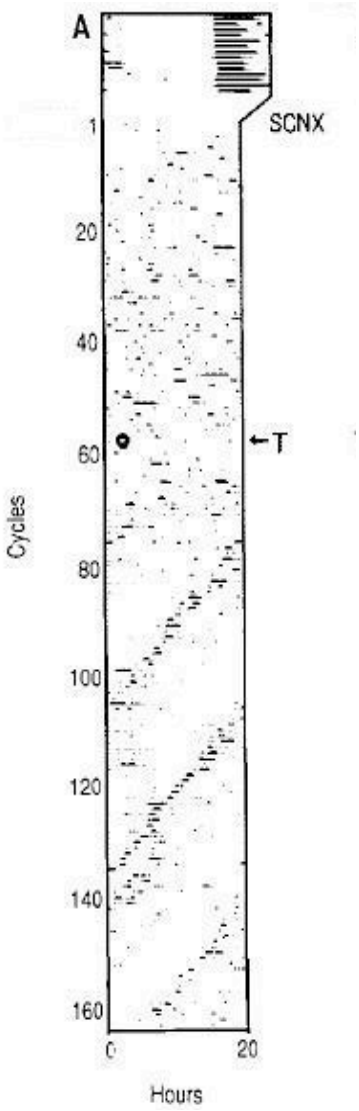


Figure 2: Actogram from a host, which initially exhibited a 24.05h tau. SCNX marks the ablation of the SCN. T marks the transplant of the SCN neural tissue to the now arrhythmic host from the mutant hamster with a 20h tau. A restored circadian rhythm is visible after cycle 80 (Ralph et al. 1990).

1.3 Afferent pathways

Synchronization of a circadian rhythm to the rhythm of external environment (ex. LD) is called 'entrainment'. There are two main pathways for entrainment: photic and non-photic.

1.3.1 Photic entrainment

Light is translated by photoreceptors into neural signals by rods, cones, and intrinsically photosensitive retinal ganglion cells (ipRGCs) in the retina. The light stimulus for the visual system passes from the retina, through the optic nerve, to the lateral geniculate nucleus (LGN) in the thalamus and then to the visual cortex in the posterior brain. The pretectal area (PT) receives information to control pupillary reflex. Eye movement is processed through the superior colliculus (SC). The SCN receives information directly from the retina via the retinohypothalamic tract (RHT). The SCN additionally receives information indirectly via the geniculohypothalamic tract (GHT) from the intergeniculate leaflet (IGL) in the thalamus (Figure 3) (Edelstein et al. 1999, Lowrey et al. 2000, Refinetti 2006).

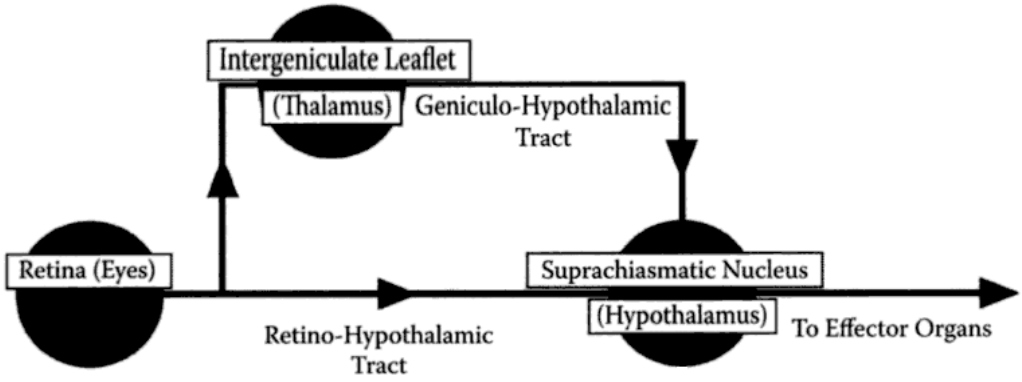


Figure 3: Afferent pathways (Refinetti 2006).

1.3.1.1 Retina & photoreceptors

The human eye has three main layers: (1) external layer - sclera and cornea; (2) intermediate layer – iris, ciliary body and choroid; and (3) the internal layer - the retina. Figure 4 shows a cross section of an eye with an exploded view of the retina.

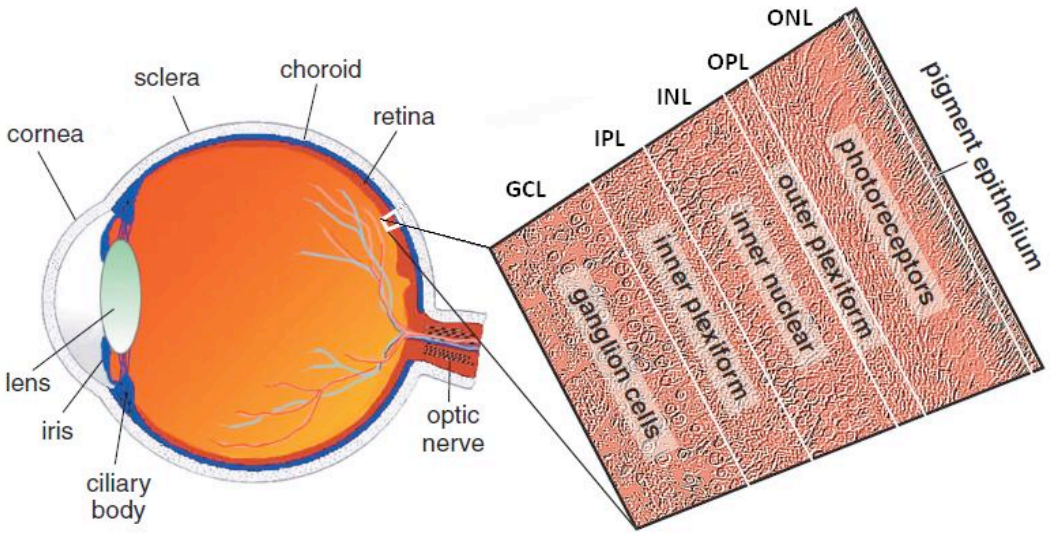


Figure 4: Cross section of the eye; layers in the retina are enlarged (Kolb 2003).

The retina itself has five main layers:

1) outer nuclear layer (ONL) - rods and cones
2) outer plexiform layer (OPL) – synapse layer between the rods, cones, and bipolar cells
3) inner nuclear layer (INL) – bipolar, horizontal, and amacrine cells
4) inner plexiform layer (IPL) – synapse layer between the bipolar, ganglion, and amacrine cells
5) ganglion cell layer (GCL) – ganglion cells

Light reaching the retina is mediated by the classic photoreceptors, which primarily provide signals to the visual system. There are three types of cones with different peak spectral sensitivities: short-wavelength cones (S-cones) have peak sensitivity at 420 nm, medium-wavelength cones (M-cones) have peak sensitivity at 534 nm, and long-wavelength cones (L-cones) have peak sensitivity at 594 nm (all prior to filtering by the crystalline lens). Rods have a peak spectral sensitivity at 498 nm (prior to filtering by the crystalline lens) (Bowmaker et al. 1980). The spectral sensitivities of the classic photoreceptors are shown in Figure 5.

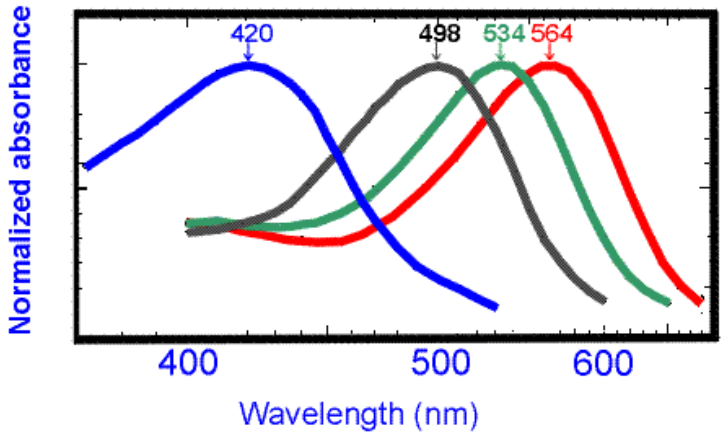


Figure 5: Spectral sensitivity of cones and rods, before filtering by the lens (Bowmaker et al. 1980).

Four types of horizontal cells laterally interconnect classic photoreceptors with bipolar cells. Horizontal cells form lateral inhibition that gives rise to the center-surround inhibition, which is apparent in retinal receptive fields. Lateral inhibition increases the contrast and sharpness in visual response. There are eleven types of bipolar cells, 22 types of amacrine cells, and 20 types of ganglion cells (Kolb 2003). Horizontal, amacrine and bipolar cells transfer information from rods and cones to retinal ganglion cells (Belenky et al. 2003). Parasol, midget, and bistratified ganglion cells are the three most common ganglion cells and project to the LGN. Retinal ganglion cells transmit image-forming and non-image forming information from the retina to the thalamus and hypothalamus. In addition to receiving light information from rods and cones, about 0.2% of the ganglion cells are photosensitive and project directly to the SCN in humans (Dacey et al. 2005). The ipRGCs express melanopsin, making them intrinsically photosensitive. IpRGCs play an important role in the non-visual impact of light (entrainment to L/D cycle) (Berson et al. 2002). Studies show that rods, cones, and ipRGCs participate in the retina's conversion of light into neural signals for the circadian system (Hattar et al. 2003). IpRGCs are less sensitive than rods and cones, and they depolarize in response to light (Berson 2003). The responses of ipRGCs are unique in that they are slow in responding to changes in light level (Hattar et al. 2002, Berson 2002, Wong et al. 2005). The peak sensitivity of ipRGCs has been shown to near 480

nm. Figure 6 illustrates the vertical pathways traveled by light signals, and shows that photoreception occurs in two layers in the retina.

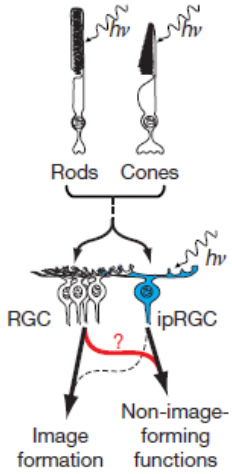


Figure 6: Diagram of rod-cone signaling through conventional RGCs or ipRGCs contributes to the image forming and non-image forming functions (Guler et al. 2008).

1.3.2 Nonphotic entrainment

The master clock, located in the SCN, sends neural signals throughout the nervous system to coordinate physiological and behavioral activities (Refinetti 2006). Non-photic stimuli are not primary zeitgebers, but they can still influence circadian timing. Zeitgebers that can shift circadian rhythms include: schedules, exogenous melatonin, and meal times (Refinetti 2006). Current research suggests that the IGL, a subdivision of the LGN, integrates photic and non-photic information to provide entraining information to the SCN. Under certain conditions pineal melatonin feedbacks into the SCN and can entrain the SCN (Edelstein et al. 1999).

1.4 Efferent pathways

There are four major outputs to the body from the SCN: subparaventricular zone of the hypothalamus (sPVZ) (which consists of the ventral (vSPZ) and dorsal (dSPZ) subparaventricular zones), dorsomedial nucleus of the hypothalamus (DMH),

paraventricular nucleus of the thalamus (PV), and arcuate hypothalamic nucleus (Arc). Four pathways send signals to reach target regions for the entrainment of secondary clocks (Figure 7). Signals from the sPVZ provide information for sleep and thermoregulation cycles. The DMH with input from the sPVZ regulates sleep cycles, activity, feeding, and corticosteroid secretion (Reppert et al. 2002, Saper et al. 2005).

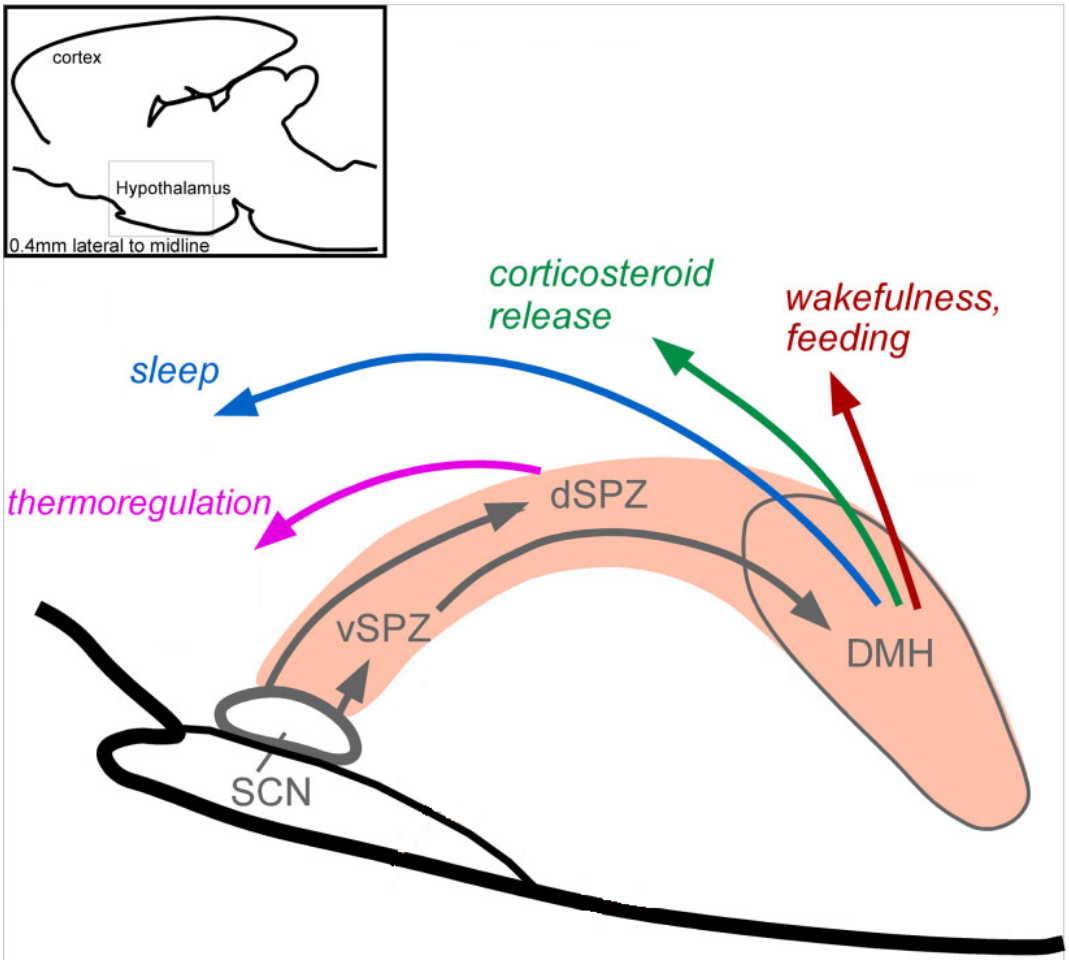


Figure 7: Projections from the master clock located in the SCN (Saper et al. 2005).

1.4.1 Melatonin

Additionally, the SCN projects neural signals to the pineal gland where the hormone melatonin is produced and released into the blood stream. Melatonin is normally produced at night when it is dark. It has been observed that melatonin affects

the timing of circadian rhythms and regulates other photoperiodic responses (Refinetti 2006). Melatonin concentration has been established as a reliable marker of the circadian clock (Lakin-Thomas et al. 1997, Lewy et al. 1999) and is found in easy to sample fluids: plasma and saliva. Average human plasma melatonin concentrations range between 50 and 70pg/ml, while melatonin found in saliva tends to have a concentration one third that of plasma (Refinetti 2006). The onset of melatonin secretion under dim light conditions is a marker used for assessing the timing of the circadian clock. Dim light melatonin onset (DLMO) is used for assessing phase delays or advances of circadian rhythms. For people with a regular sleep schedule, DLMO occurs about 2h prior to normal bedtime. Other phase markers include core body temperature (CBT) and the sleep/wake cycle. CBT tends to peak in the evening and has a minimum in the early morning hours. Figure 8 shows changes in melatonin concentration and CBT over the course of 24h. Cortisol levels, alpha amylase levels, brain activities, and subjective alertness are potentially influenced by the SCN. Cortisol levels tend to peak in the early morning and have a minimum 3-5h after sleep onset in the night. All of these outputs are easily masked by external factors, making melatonin one of the more reliable markers of the human circadian rhythm (Klerman et al. 2002). Light is most effective in reducing melatonin levels at night; this is called suppression (Macchi et al. 2004). Posture has been found to affect plasma and saliva melatonin concentrations. For instance, the impact of gravity on standing individuals causes a decrease in plasma concentration and a concentration increase for individuals lying down (Deacon et al. 1994). For average individuals night time and early morning light exposure suppresses melatonin production, while midday light exposure has no effect since melatonin concentrations are lowest during the day for individuals entrained to the solar day (Leproult et al. 2001).

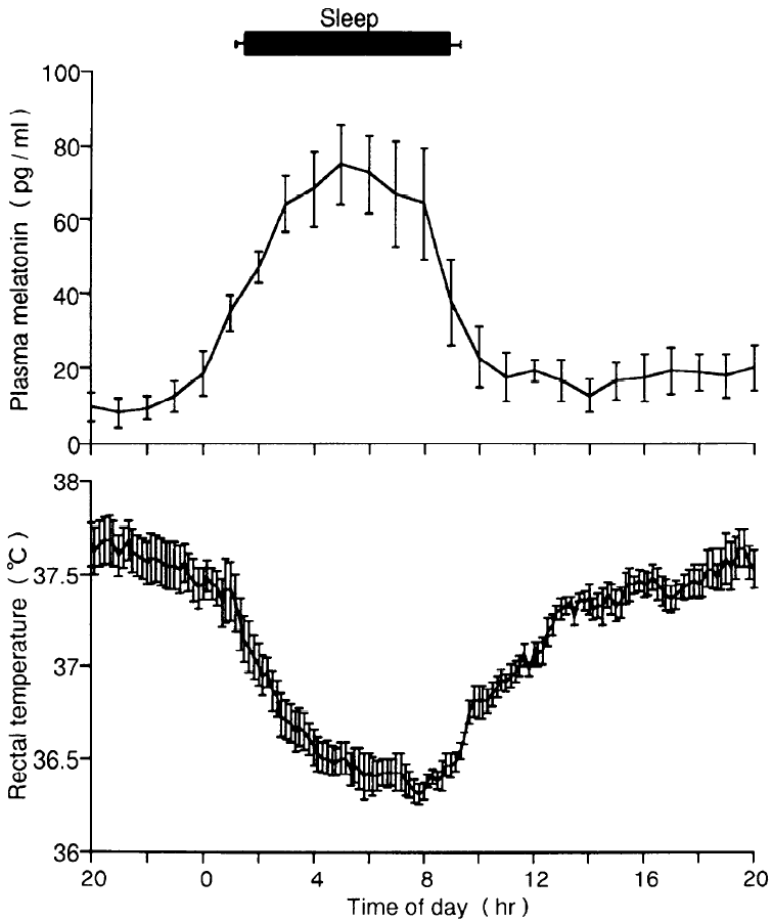


Figure 8: Melatonin and core body temperature in a human (Hashimoto et al. 1996).

1.4.2 Lighting characteristics which impact melatonin concentration

1.4.2.1 Quantity

There has been considerable research investigating the sensitivity of the circadian system to polychromatic 'white' light, and light's impact on melatonin production. In 1980, Lewy et al. (1980) sampled the melatonin suppression from exposure to multiple illuminances (lx): 500 lx (fluorescent lamp), 1500 lx (incandescent lamp), and 2500 lx (incandescent lamp) of polychromatic white light. Subjects were exposed to the light conditions from 2:00 am to 4:00 am while awake. It was demonstrated that high levels (at least 2500 lx at the cornea) of white light were needed to significantly suppress melatonin. These data indicated that 2h exposure to light suppressed melatonin

production in humans. Lewy et al. (1980) were the first to note that humans require higher light levels to suppress melatonin than laboratory animals. Figure 9 shows the mean melatonin concentrations from 500 and 2500 lx for six subjects. In this figure melatonin concentrations for the 2500 lx condition were significantly different from 500 lx, starting 30-minutes after light exposure. This sparked research into the correlation between light levels, melatonin suppression, and phase shifting in humans. It should be noted that this study made use of several different light sources. Incandescent and fluorescent lamps have different spectral power distributions (SPD).

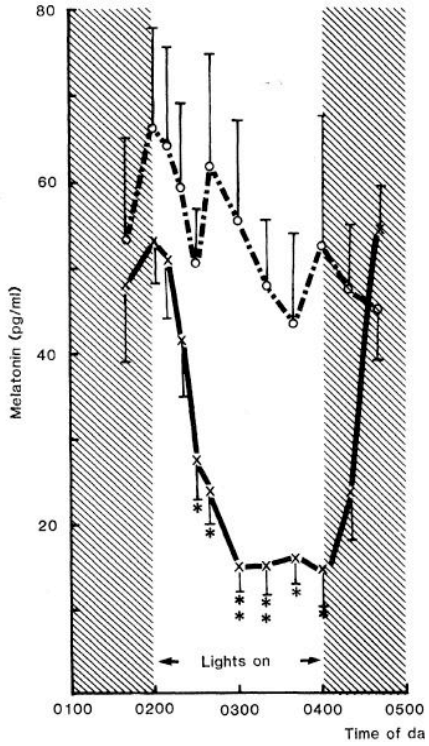


Figure 9: Change in melatonin concentration as a result of 500 (○) and 2500 (X) lx of polychromatic white light (Lewy et al. 1980).

McIntyre et al. (1989) conducted experiments using fluorescent lamps (5000K) to demonstrate that melatonin suppression is dependent on light level. Five illuminance levels were tested. 3000, 1000, 500, 350, and 200 lx resulted in 71%, 67%, 44%, 38%, and 16% suppression respectively after 60-minutes of exposure, light exposure began at midnight. Little was known about the interaction between light levels and exposure

duration. The results demonstrated that an increase in illuminance levels induced an increase in melatonin suppression. It is interesting to note that the lowest light level 200 lx, had reduced melatonin concentrations after 45-minutes of exposure (Figure 10A) and resulted in 16% melatonin suppression after 60-minutes of exposure. Figure 10B shows a dose response curve using suppression data from the 60-minute exposure.

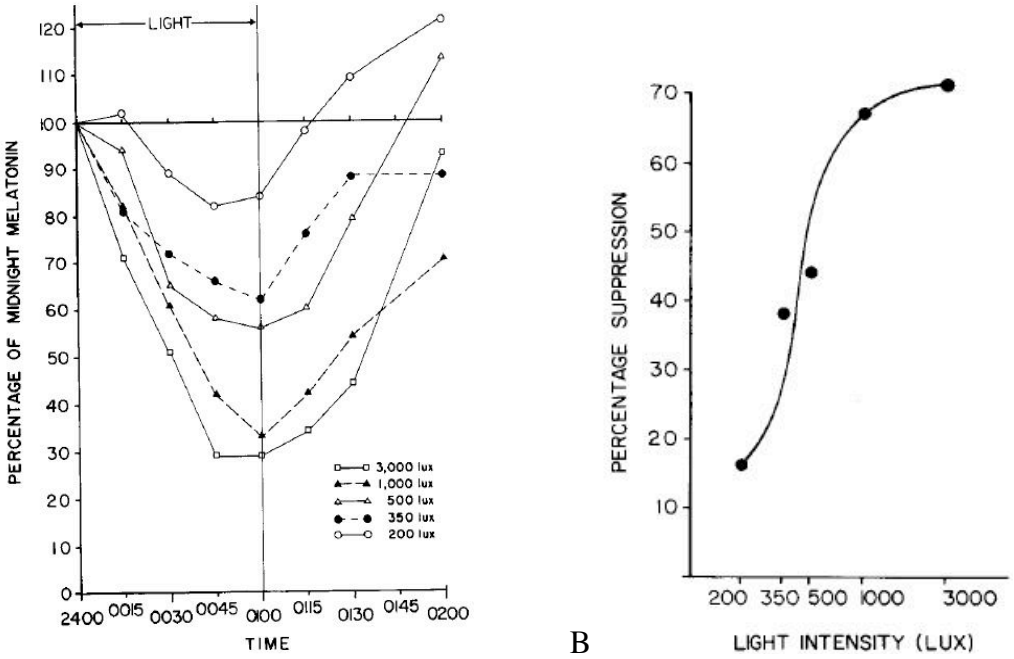


Figure 10: The left graph (A) shows the melatonin concentration response to five levels of 'white' light over two hours. The right graph (B) displays the curve for maximum percentage suppression of melatonin by light of different light levels after one hour of exposure (McIntyre et al. 1989).

Aoki et al. (1998) performed supplementary experiments to more closely describe the relationship between duration and light level. Five male subjects (mean age 33.6y) were exposed to 500, 1000, 2500, and 5000 lx of cool white fluorescent light for 2h while awake in the middle of the night (2:00am to 4:00am). Melatonin concentrations were sampled every 30-minutes. Adjusted suppression was calculated by subtracting melatonin suppression from light condition, from melatonin under the dim light condition (below 10 lx). The resulting suppression levels were used to derive a best-fit

curve to determine the minimum illuminance required to suppress melatonin for specific exposure durations (Figure 11).

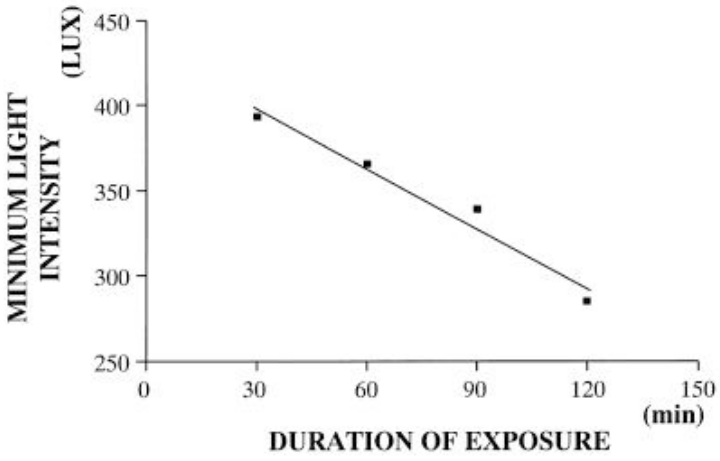


Figure 11: Relationship between light level and exposure duration, for an estimated threshold (significant melatonin suppression) (Aoki et al. 1998).

More recently, Zeitzer et al. (2000) used a single dose of light on healthy human subjects; the illuminances from 4000K fluorescent lamps ranged from 3 to 9100 lx. The 6.5h exposures were centered 3.5h prior to a subjects' measured minimum core body temperature (CBT_{min}). Based on the experiment results the authors suggested a threshold near 80 lx, stating that limited melatonin suppression was observed from lower light levels (Figure 12). The authors stated the half-maximal response for melatonin suppression to be between 50-130 lx and around 119 lx for phase shifting. The maximal response of melatonin suppression was predicted to be near 200 lx and for phase shifting it was near 550 lx. It should be noted that compared to the experiment conducted by McIntyre et al. (1989), the duration of this light pulse was 5.5h longer.

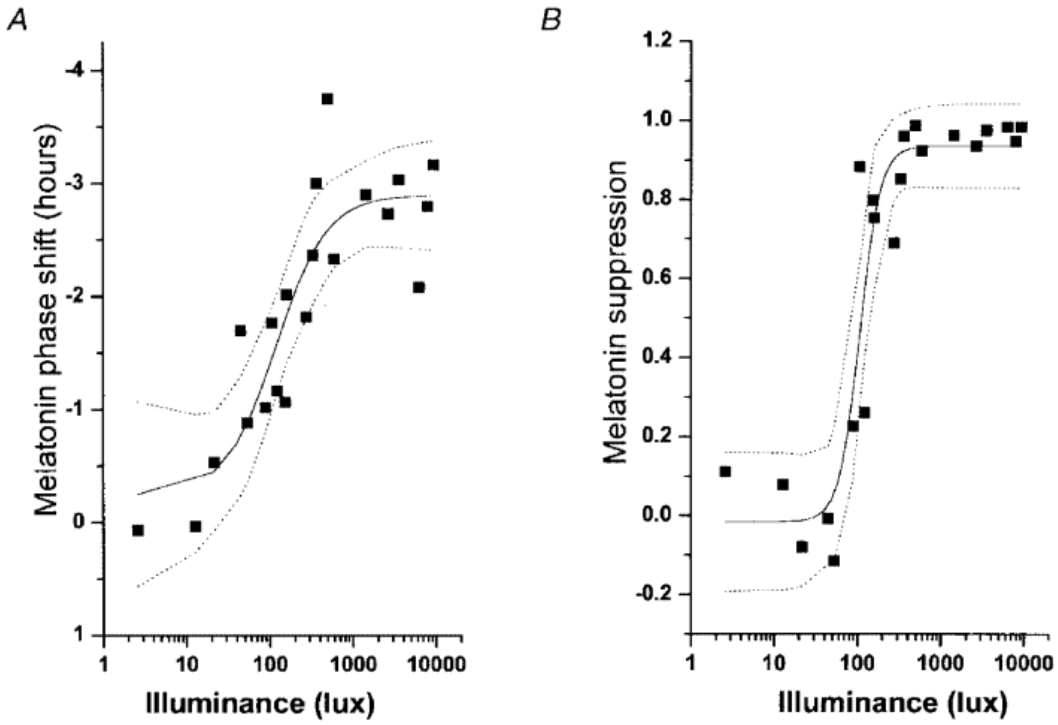


Figure 12: Illuminance response curve of the human circadian rhythm. The shift in the phase of the melatonin rhythm (A), as assessed on the day following exposure to a 6.5h experimental light stimulus. Acute suppression of plasma melatonin (B) during the light exposure. (Zeitzer et al. 2000).

1.4.2.2 Spectrum

Morita et al. (1996) showed that a 5h exposure (between 21:00 and 02:00) to 1000 lx at the cornea of a 6500K fluorescent lamp resulted in significantly more melatonin suppression than a 3000K fluorescent lamp. The lamp SPDs are shown in Figure 13. The 6500K lamp had more radiant energy in the short-wavelength region compared to the 3000K lamp. Urinary melatonin concentrations were reported in hour totals. A comparison of melatonin concentration at the beginning and at the end of exposure to the experimental conditions showed that exposure to the 6500K fluorescent lamp resulted in a 250pg/h decrease in melatonin concentration, while exposure to the 3000K fluorescent lamp resulted in an increase of about 350pg/h (Figure 14). Melatonin concentrations during the control condition increased (550pg/h). This indicates that both the 6500K and 3000K conditions suppressed melatonin and that the 6500K resulted in significantly greater suppression.

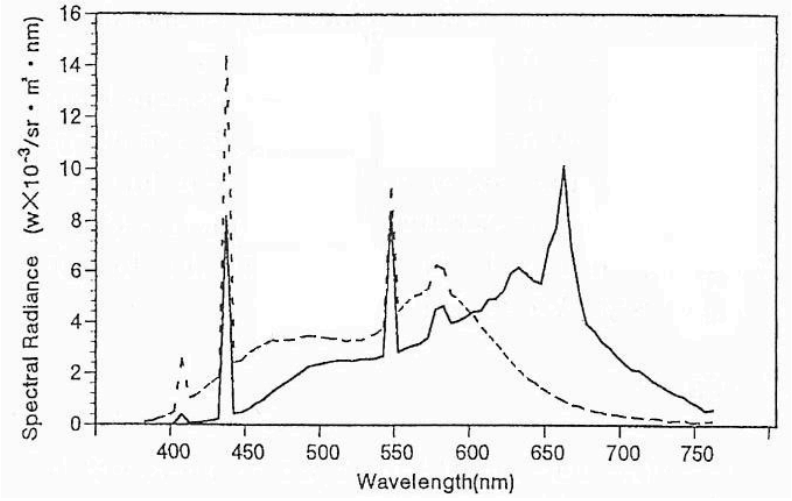


Figure 13: Spectral power distribution for the 1000 lx conditions generated using fluorescent lamps, 3000K fluorescent (—) and 6500K fluorescent (- - -) (Morita et al. 1996).

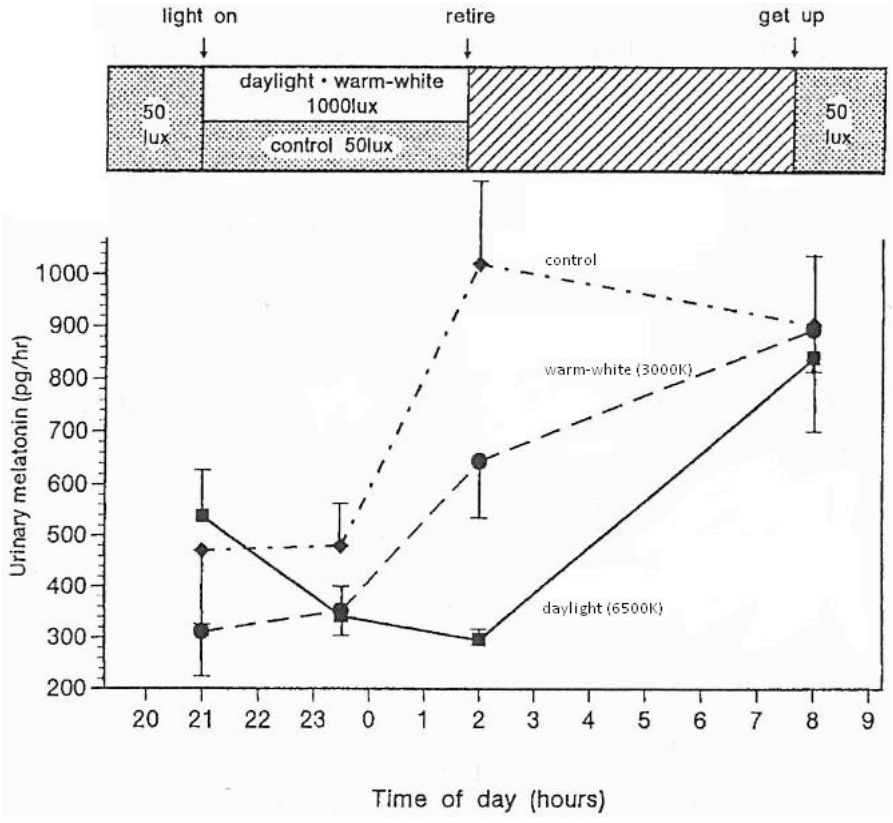


Figure 14: Urinary melatonin concentration as a result of three conditions: control (- · -), 6500K fluorescent lamp (—), and 3000K fluorescent lamp (– –) (Morita et al. 1996).

Under controlled laboratory conditions, Figueiro et al. (2006) exposed subjects with natural pupils to 30, 100, 300 and 1000 lx at the cornea with 4100K and 8000K from fluorescent lamps for 30 minutes. Acute melatonin suppression was found to be significantly greater after exposure to 8000K at 30, 100 and 300 lx, but not the 1000 lx conditions (Figure 15). This study demonstrates that light sources with more short-wavelength electromagnetic radiation are more effective in suppressing melatonin than sources with electromagnetic radiation predominantly in the longer wavelengths. Melatonin suppression increased with an increase in illuminance, with the exception of 1000 lx. Figure 15 shows suppression with respect to retinal illuminance (illuminance x pupil area). The experimenters and subjects found the highest light level (1000 lx) to be disturbingly bright; as a result subjects may have squinted during exposure to this condition, thus reducing actual retinal illuminance. Measured pupil area and retinal illuminance did not reflect the photophobic reaction of squinting. Reduction in retinal illuminance is most likely responsible for the reduction in melatonin suppression at 1000 lx.

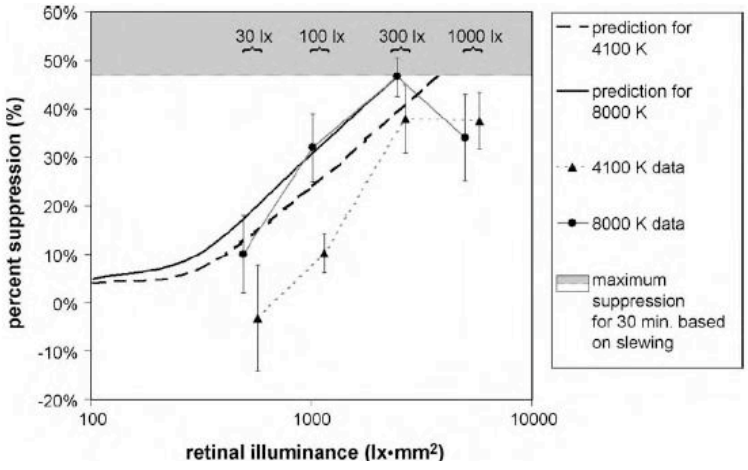


Figure 15: Melatonin suppression as a result of 4100K and 8000K light sources (Figueiro et al. 2006).

Recent studies have further explored light by assessing the ability of monochromatic light in stimulating the circadian system both in terms of melatonin

suppression and phase shifting, to characterize the spectral sensitivity of the circadian system. Recent research has found that the spectral sensitivity of the circadian system peaks at shorter wavelengths. However, the absolute threshold for the response by the circadian system at these wavelengths is not yet well delineated. Two studies in particular pointed to the participation of a novel photoreceptor and photopigment, ipRGCs and melanopsin, in the regulation of circadian rhythms. Both studies investigated the impact of long, middle, and short-wavelength light on acute melatonin suppression. In 2001, under controlled laboratory conditions, Brainard et al. (2001) showed that 460-nm light induced the greatest melatonin suppression compared to other wavelengths for the same irradiances. For 460-nm light, 3.1 $\mu W/cm^2$ and higher irradiances significantly suppressed melatonin compared to 0.01 $\mu W/cm^2$ after 90-minute exposure. Figure 16 shows melatonin suppression from 460-nm light stimulus for dilated pupils.

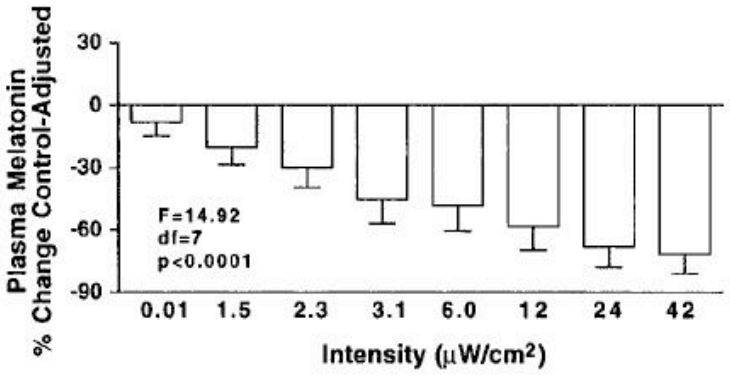


Figure 16: Percent change control–adjusted plasma melatonin suppression from 460-nm light (Brainard et al. 2001).

In the same year, Thapan et al. (2001) conducted a similar study using 30-minute exposures. Subjects participated in several three consecutive night protocols for each condition. On night three subjects were exposed to the light conditions for 30-minutes at a set circadian time (CT 16–18) (between 23:30 and 02:30h). The authors found melatonin to be maximally sensitive to short-wavelength radiation. Figure 17 presents irradiance response curves (IRCs) for six wavelength conditions. Additionally, the authors found the lowest irradiances necessary for significant suppression were 1.9, 2.0,

1.8, 3.0, 7.0, 7.2 $\mu W/cm^2$ for 424-, 456-, 472-, 496-, 520- and 548 nm. 456 nm required the lowest irradiance to induce 35 % melatonin suppression.

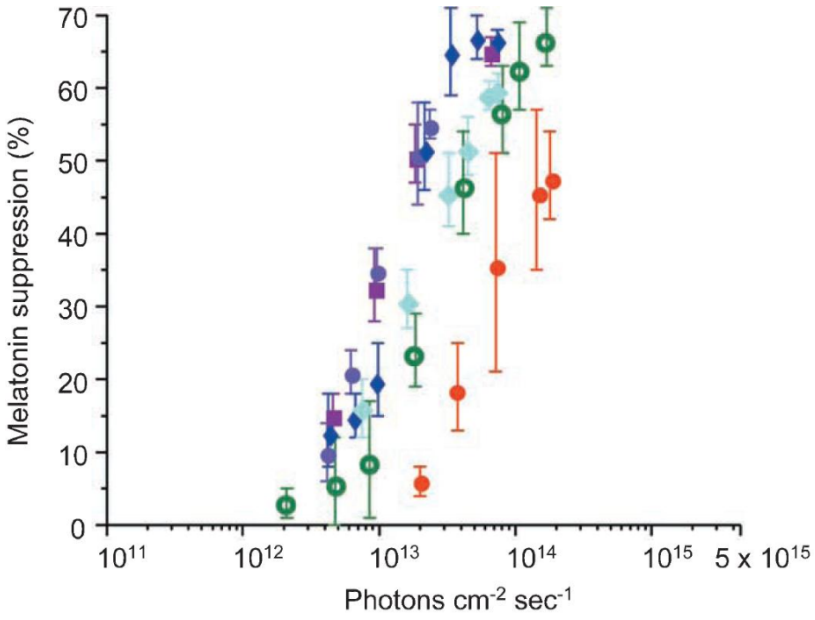


Figure 17: IRC from 420 nm (●), 456 nm (■), 472 nm (♦), 496 nm (♦), 520 nm (○), and 548 nm (●) (Thapan et al. 2001).

Brainard et al. (2001) and Thapan et al. (2001) separately published an action spectrum for melatonin suppression. They defined the nearly monochromatic light stimuli in terms of a response, acute nocturnal melatonin suppression. Both research groups tested several multiple wavelengths and light levels. A dose response curve (part of a sinusoidal curve) was created for each wavelength condition. For each wavelength dose response curve the half-maximum response was determined. Maximal percent melatonin suppression occurs around 70%. Both researchers used the half-maximal response called ED_{50} (35%) as the constant criterion. Relative efficiency in Figure 18 is 1/the constant criterion response. Figure 18 presents an overlay of the data results from Brainard et al. (2001) and Thapan et al. (2001), which clearly show peak sensitivity approximately between 440 and 470 nm.

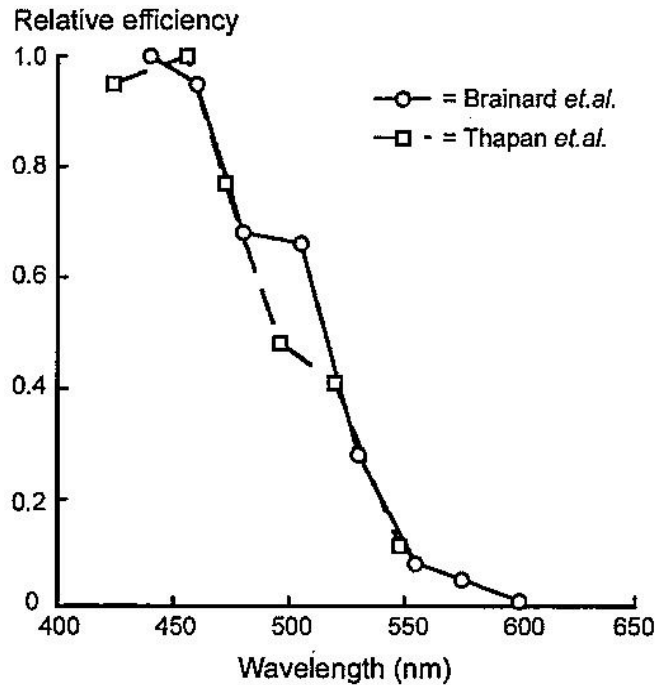
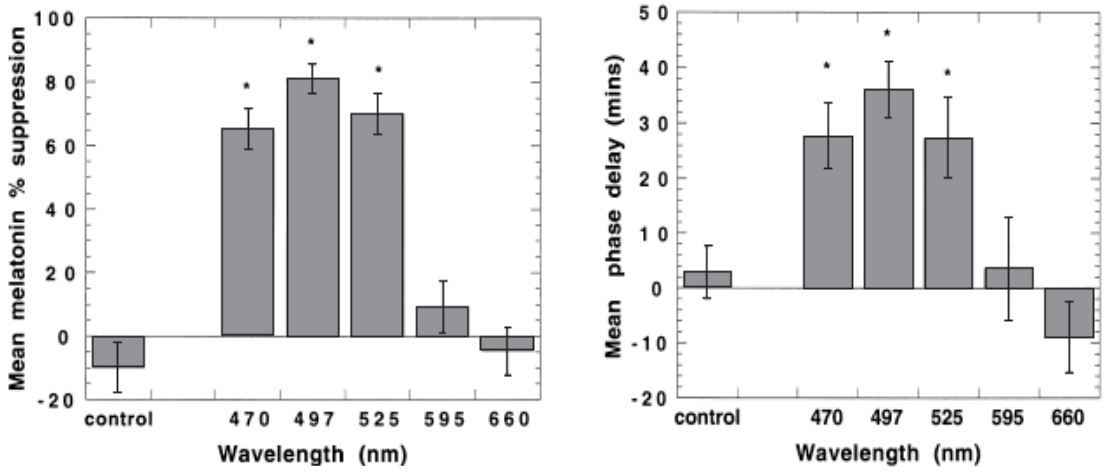


Figure 18: Measured relative efficiency of electromagnetic radiation at different wavelengths in stimulating the human circadian system, using melatonin suppression as a marker (after Brainard et al. 2001 and Thapan et al. 2001) (Boyce et al. 2003).

In the same year, Wright et al. (2001) examined the impact of five different wavelengths (470-, 497-, 525-, 595-, 600 nm) and one control condition on acute nocturnal melatonin suppression and on phase delay. During the first night (night 1), 15 subjects were exposed to the conditions for 2h starting at midnight. Melatonin concentration was sampled at the same times the following night (night 2). Each light condition exposed subjects to 130μW/cm^2 at the cornea, from narrowband light sources with a full width half maximum (FWHM) of 18-20 nm. The researchers did not disclose whether pupil size was controlled. Melatonin suppression was calculated against melatonin concentration just prior to light exposure [(Melatonin 24:00 - Melatonin 02:00/Melatonin 24:00) × 100]. The suppression and phase delay results demonstrated a greater sensitivity to shorter-wavelength light. The 470-, 497-, and 525-nm light conditions demonstrated statistical significance from the control in both melatonin suppression and phase delay (Figure 19). According to the results from Brainard et al. (2001), the human response to 24μW/cm^2 of 460-nm light exposure (90-minute

exposures) saturates for melatonin suppression. Thus 130μW/cm^2 must be at maximum saturation for melatonin suppression. This may also be the case for phase delay. Zeitzer et al. (2000) observed maximum saturation for melatonin suppression and phase delay at a similar illuminance for white polychromatic light (Figure 12). The high irradiance limited the ability to make greater distinctions between the three conditions that demonstrated significance. The data from Wright et al. (2001) indicate that melatonin suppression and phase delay have a similar maximum sensitivity as a result of exposure to narrowband light sources.



A **B**

Figure 19: Mean melatonin suppression and standard deviation for a 2h exposure (00:00 - 02:00) from five different wavelengths each delivering 130μW/cm^2 and the control condition (A). Mean phase delay (DLMO night 2 - DLMO night 1) for the same conditions (B). *Significantly different from the control condition (Wright et al. 2001).

In 2009, Figueiro et al. (2009) looked at much lower light levels using 74 and 11 μW/cm^2 to suppress melatonin. Subjects recruited for the study were between the age of 50 and 80y. Light emitting diodes (LEDs) with a peak wavelength of 470 nm were used in the experiment. After 1h of dark adaptation subjects were exposed to 470-nm light for 90-minutes starting at midnight. This study sampled plasma and saliva intermittently, which provided the investigators with 10 samples to track the change in melatonin concentrations during light exposure. Melatonin suppression was calculated against

melatonin concentration just prior to light exposure [(1 - Melatonin during light exposure/Melatonin 00:00) × 100]. Mean melatonin suppression after 90-minutes of continuous exposure to 470-nm light at 74 μW/cm^2 was 61% and 13% for 11 μW/cm^2 (Figure 20 & Figure 21).

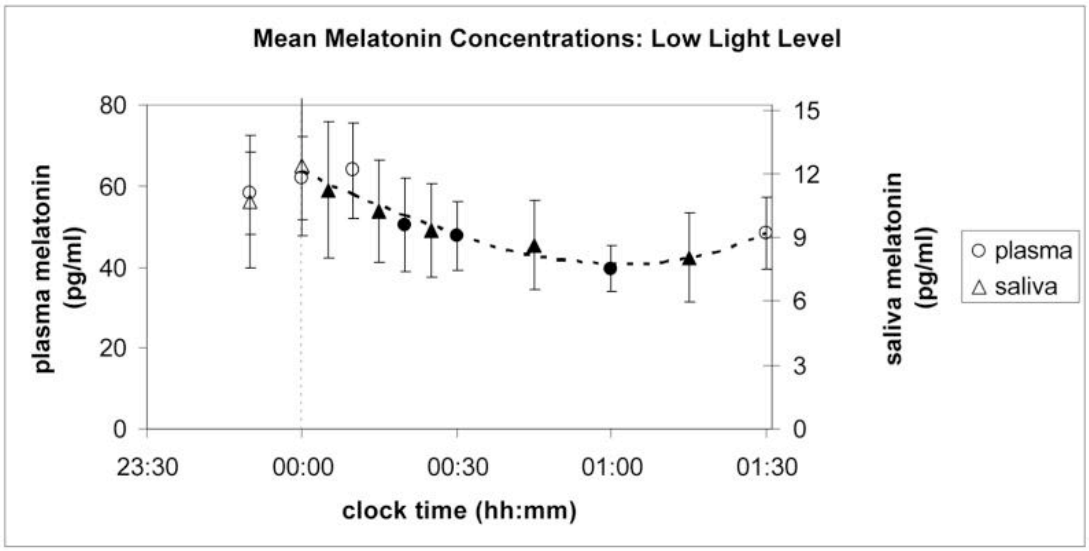


Figure 20: Mean melatonin concentrations (plasma and saliva) during exposure to 11 μW/cm^2 of 470-nm light (Figueiro et al. 2009).

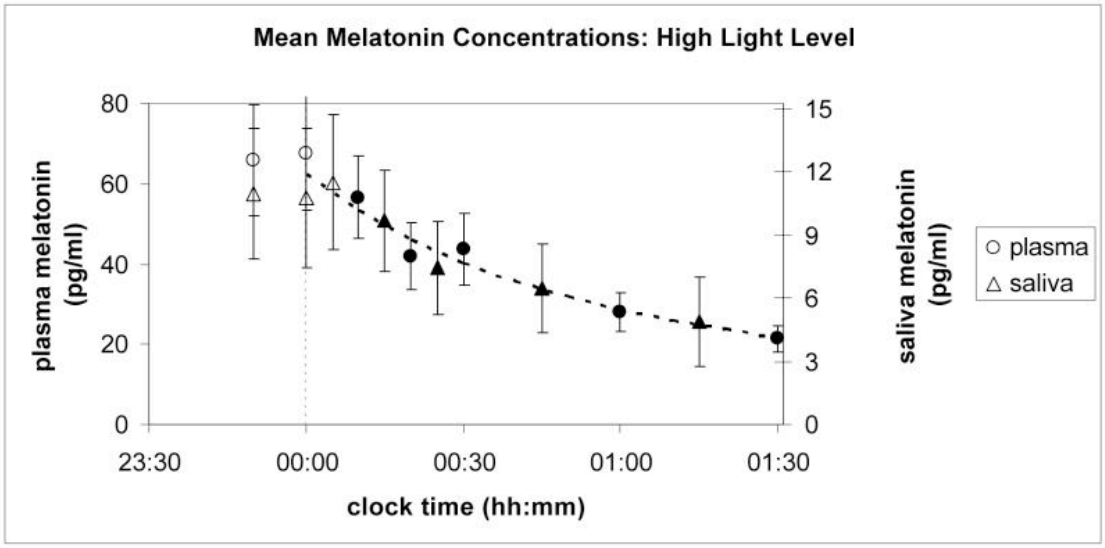


Figure 21: Mean melatonin concentrations (plasma and saliva) during exposure to 74 μW/cm^2 of 470-nm light (Figueiro et al. 2009).

1.4.2.3 Timing

Studies have shown that the circadian clock changes in sensitivity to light stimulus throughout a 24h cycle (Jewett et al. 1997). The timing of a light pulse may phase advance or delay a person's circadian rhythm. Generally, early morning light will advance the human circadian system, while evening light will cause a delay. A light dose 2-3h prior to CBT_{min} would delay the circadian system. Figure 22 shows a phase response curve (PRC) for humans. The PRC was generated from 21 subjects (mean age 27y) in a controlled laboratory setting with no external time cues. Subjects were exposed to a pattern of a 16h wake period (<150 lx) and an 8h sleep period in darkness for three 24h periods to establish a baseline. Light pulses of approximately 10,000 lx (5000-9000 lx during free-gaze periods) lasting 6.7h were delivered at varying circadian times. The resulting phase shift was assessed. The PRC illustrates the relationship between the timing of a light pulse and the resulting phase shift depending on the time separation from CBT_{min}. Phase advance values are positive and delay values are negative. They are plotted against the timing of the center of the light exposure relative to the midpoint of the lowest melatonin concentrations of a circadian rhythm prior to light stimulus (defined to be 22h). On average CBT_{min} is assumed to occur 2h after the 0h circadian phase point. The dashed line represents the predicted average drift of the master clock (Khalsa et al. 2003).

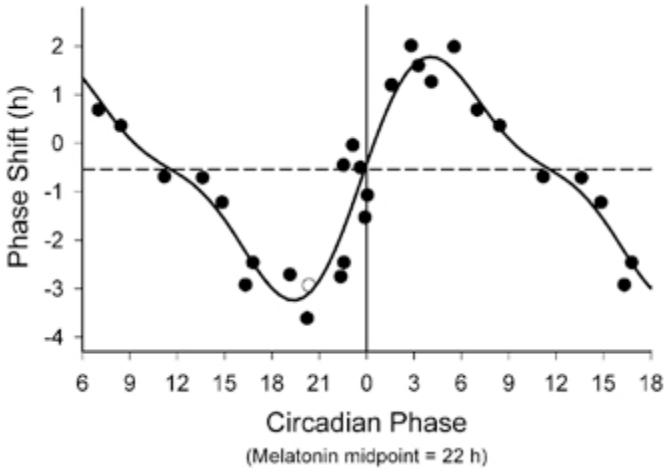


Figure 22: PRC for human (Khalsa et al. 2003).

1.4.2.4 Duration

There is a clear relationship between light level and exposure duration. The studies conducted by Zeitzer et al. (2000), Aoki et al. (1998), and McIntyre et al. (1989) all demonstrated that as illuminance decreased, a longer exposure time was required to obtain similar melatonin suppression. Figure 23 is based on two studies by McIntyre et al. (1989a, 1989b), both observed the impact of multiple illuminances on melatonin suppression over the course of a 60-minute exposure with intermediate sampling. Both studies employed the same procedures and setup, but tested different light levels.

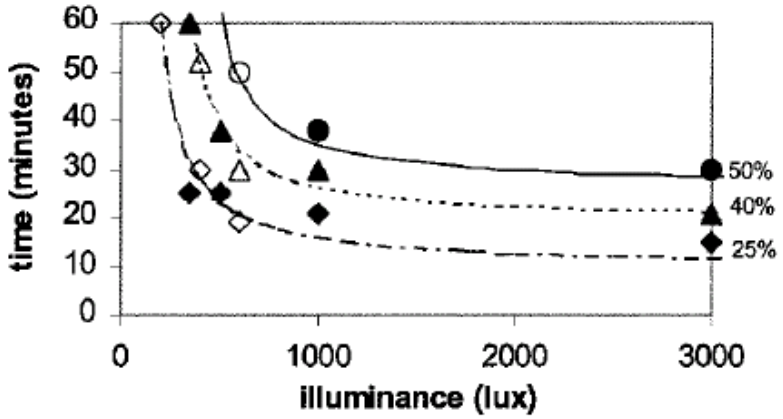


Figure 23: The duration of light exposure required to suppress a certain percent of melatonin as a function of illuminance. Solid figures show data from McIntyre et al. (1989a), while open figures show data from McIntyre et al. (1989b) (Rea et al. 2002).

Rea et al. (2002) combined and rearranged the data in terms of illuminance vs. exposure duration (time) using data generated by McIntyre et al. (1989a, 1989b). Data points with the same percent melatonin suppression were fit with a best-fit curve. The figure shows the relationship between duration and illuminance at the eye for polychromatic light, for three different levels of melatonin suppression: 25, 40, and 50%. This suppression data was fit with best-fit curves to estimate the interaction between other light levels and durations. Figure 23 tells us what combination of illuminance at the eye and duration exposure is needed to induce the desired melatonin suppression. For example, the transformed data demonstrate that 25% suppression could be reached after 20-minutes of exposure to ~500 lx and that a minimum illuminance of ~600 lx for 50-minutes is needed to achieve 50% suppression.

1.4.2.5 Light History

Sensitivity to light at night will change depending on the amount of light exposure during the day. Hebert et al. (2002) investigated the impact of light exposure history on nocturnal melatonin suppression by polychromatic light. A group of subjects was exposed to a bright week, where they were exposed to high light levels for a mean of 4.3h/day by going outside or using light boxes (5000-7000 lx fluorescent lamps)

indoor. The same group of subjects was exposed to a dim week; they were required to wear 2% transmittance goggles and were limited to a mean of 1.4h/day outside. To test nocturnal melatonin suppression after a week of controlled light exposure, subjects were exposed to a 500 lx (fluorescent lamps) 3h pulse given from 01:00 to 04:00. The 500 lx pulse resulted in a mean nocturnal melatonin suppression of 53% for the dim week, which was significantly greater than the 41% suppression after the bright week (Hebert et al. 2002). Though the effect of light history was significant, there were larger individual differences. A 2004 study controlled light exposure for 3 days in a laboratory setting with an inpatient protocol; subjects were exposed on average to 200 or 0.5 lx. Significantly greater melatonin suppression was found during exposure to a pulse after a photic history of 0.5 lx resulting in 85.7% suppression, compared with the 200 lx condition, which resulted in 71.2% suppression. This revealed that the human circadian system adapts to lower light levels (Smith et al. 2004).

1.4.2.6 Spatial Distribution

Research regarding the significance of light distribution on the retina compares nasal vs. temporal distribution or upper vs. lower visual field. One recent and conclusive study by Ruger et al. (2005) found a relationship between melatonin suppression and light exposure on the temporal region of the retina (Ruger et al. 2005). In this study subjects received either 100 lx on the nasal part of both retinas, 100 lx on the temporal part of both retinas, or less than 10 lx on the entire retina (control condition) from 00:00 to 04:00h. Though both conditions were significantly different from the control condition, exposure to the nasal portion of the retina resulted in a greater reduction of melatonin concentrations (Figure 24).

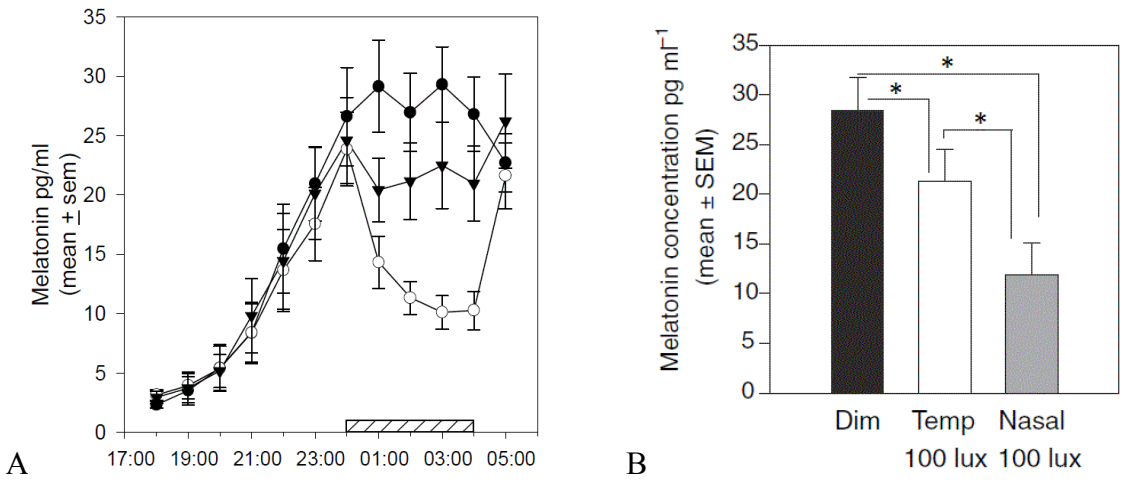


Figure 24: (A) Melatonin concentrations (mean ± sem) before, during and after light exposure; control (●), temporal (▼), and nasal (○) exposure. Light exposure is indicated by a hatched box. (B) Resulting melatonin concentrations (mean ± sem) after 4h exposure, *p < 0.01 (Ruger et al. 2005).

Lasko et al. (1999) used 500 lx from a fluorescent lamp to compare the sensitivity of the upper and lower retina. The study demonstrated that 500 lx in the lower retina significantly suppressed melatonin, as compared to 500 lx in the upper retina (Lasko et al. 1999). Glickman et al. (2003) also found a significant interaction between melatonin concentration and exposure of the lower retina using a 150W halogen lamp. Subjects with dilated pupils were exposed to 200 lx on the lower and upper retina on separate nights, the 1.5h exposures began at 02:22 after 2h of dark adaptation. An ophthalmological head holder was employed to ensure subjects' heads remained in a constant position. Only lower retinal exposure significantly lowered melatonin concentrations compared with melatonin concentrations prior to exposure (Figure 25). These studies point to higher melatonin suppression from illumination of the lower retina. Overall, these findings suggest the lower and nasal retina have a greater sensitivity or a larger density of photoreceptors, which are involved in circadian phototransduction (Glickman et al. 2003).

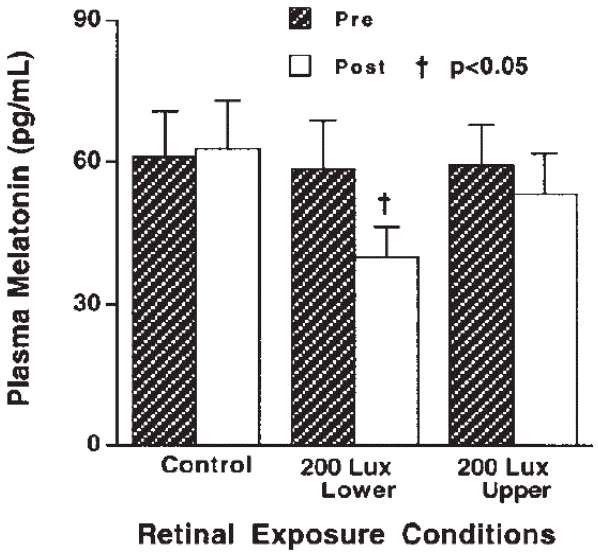


Figure 25: Melatonin concentrations (mean ± sem) before and after light exposure. Exposure of the lower retina resulted in a significant change in melatonin concentrations (Glickman et al. 2003).

1.5 Predicting melatonin suppression

Multiple attempts have been made to model human circadian spectral sensitivity incorporating the recent discovery of ipRGCs and the circadian response to narrowband light. The objective of mathematical models is to create reliable predictions for melatonin suppression and thus testable hypotheses. Some attempts specify a single opsin as responsible for the circadian response and present a single efficiency response function (Gall 2004). Multiple researchers have suggested that a single opsin cannot be responsible for the circadian response (Rea et al. 2002, Hattar et al. 2003, Figueiro et al. 2008). A single opsin is incapable of modeling the impact of all light sources. The model for human circadian phototransduction developed by Rea et al. (2005) considers the interaction of several opsins, from the two classic photoreceptors (rods and three cone types) and ipRGCs. The model was originally based on human nocturnal melatonin suppression data from several studies (McIntyre et al. 1989, Brainard et al. 2001, Thapan et al. 2001, Rea et al. 2001, Rea et al. 2002, Figueiro et al. 2004).

The model was revisited in 2010, when circadian phototransduction nomenclature was redefined: circadian light (CL), circadian light normalized to CIE standard illuminant A (CL_A), and circadian stimulus (CS) (Rea et al. 2010). As of 2010, CL

became the spectrally adjusted irradiance for the human circadian system. The CL value is normalized resulting in CL_A. Finally, the term CS is now used to describe the effect of a light stimulus on the circadian system as measured by acute melatonin suppression.

1.5.1 Spectral opponent reaction to polychromatic light

Spectral opponency plays an essential role in color vision (Kolb 2003) and is part of the circadian system (Rea et al. 2005, Figueiro et al. 2004, Figueiro et al. 2008). There are two opponent channels: red/green and blue/yellow. Evidence points to a non-additive spectral sensitivity function for melatonin suppression (Figueiro et al. 2004, Figueiro et al. 2005, Figueiro et al. 2008) and specifically to the blue/yellow channel as being responsible for the non-additive response of the circadian system to polychromatic light. Psychophysical and physiological studies show that blue/yellow opponency is created by S-cone signals in contrast to signals from L- and M-cones (Kolb 2003, Packer et al. 2010). S-cones differ from other cones by projecting deeper into the OPL (Webvision). Figure 26 illustrates the S-cone neural pathways.

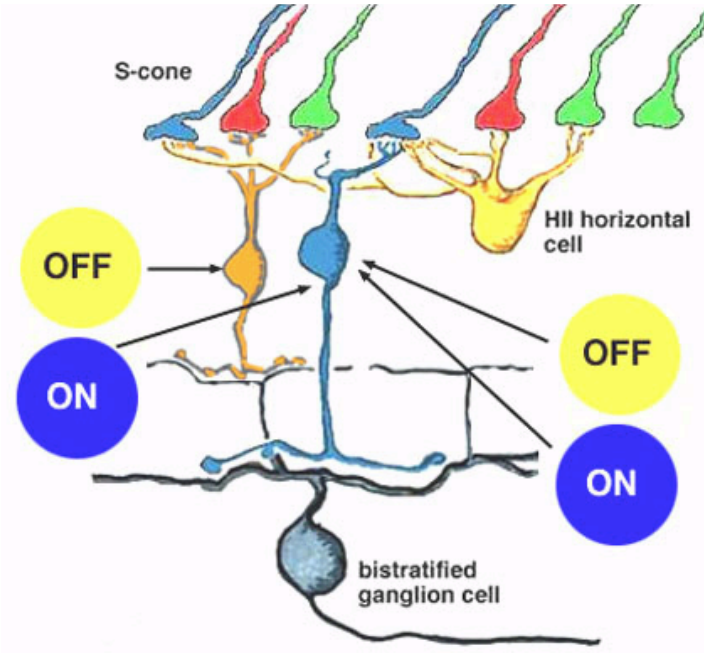


Figure 26: S-cone neural signal pathway (Webvision).

S-cones first encounter HII horizontal cells, which create communication pathways between cones in the surrounding field. Additionally, HII horizontal cells have been proven to be short-wavelength sensitive in primates (Dacey et al. 1996) by creating a feedback signal only to S-cones (Kolb 2003, Packer et al. 2010). Evidence suggests that the first stage of opponency begins with HII horizontal cell feedback to S-cones, where the horizontal cells collect information from a wide field of cones and provide bipolar cells with an opponent surround signal (Webvision). Additionally, horizontal treatment of information is processed by amacrine cells, which provide the pathways between ON and OFF bipolar cells and ganglion cells (Kolb 2003). S-cone signals are not processed in the same manner as L- and M-cone signals. There is a dedicated S-cone bipolar cell, which sends a signal to a specialized ganglion cell that can convey the depolarizing blue ON and the hyperpolarizing yellow OFF signal (Dacey et al. 1996, Kolb 2003). The S-cone bipolar cells carry the ON signals to the bistratified ganglion cells (Dacey et al. 2005). The ON reaction is determined by S-cones, while the OFF reaction is the sum of L- and M-cones responses (Kolb 2003). Short-wavelength radiation induces a depolarizing blue ON signal (Belenky et al. 2003).

Spectral opponency is active for polychromatic light when radiation is present in both the long and short-wavelengths. Therefore, the presence of certain long-wavelength radiation will diminish the impact short-wavelength radiation can have on the circadian system. The dashed line in Figure 27 shows the sensitivity curve to polychromatic light published Rea et al. (2010).

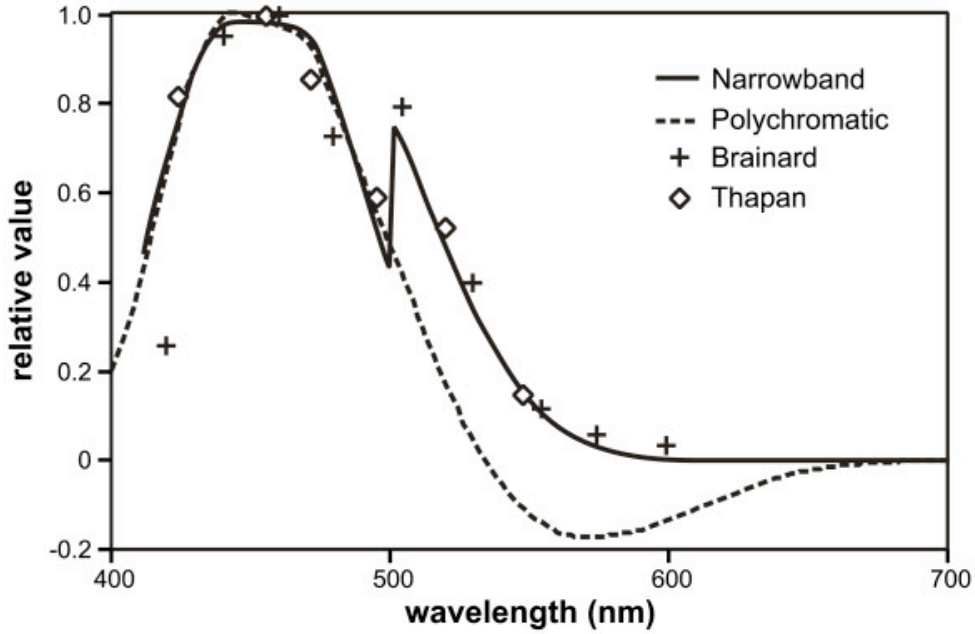


Figure 27: Spectral sensitivity to narrowband and polychromatic light is consistent with results from Brainard et al. (2001) and Thapan et al. (2001) (Rea et al. 2010).

The spectral sensitivity curve to narrowband light sources (solid line in Figure 27) exhibits a jump near 500 nm. This rift represents the disengagement of rod shunting for SPDs with dominant radiation above 500 nm. A depolarizing ON blue signal results in the ipRGCs receiving a signal curbed by rods. The rod shunting is conveyed by AII amacrine cells. When a hyperpolarizing OFF yellow signal is sent from the S-cone bipolar rods signal are not shunted (Rea et al. 2005).

Similar to S-cones, rods are diffusely spread in the retina. They also have a higher sensitivity to light than cones (Boyce 2003). Rod pathways involve several collector cell interactions, convergent and divergent synaptic cell contacts (Webvision). The intricate pathways are also another reason for low spatial resolution generated by these photoreceptors in comparison to one-to-one connections of the midget pathway. Multiple rods signal to rod bipolar cells in the OPL. Rod bipolar cells do not make direct synaptic contacts with ganglion cells (Rea et al. 2005); they are diverted through AII and the A18 amacrine cells. These amacrine cells make the synaptic connections to cone bipolar cells before sending the rod signal to ganglion cells (Webvision).

1.5.2 Circadian phototransduction model calculations (Rea et al. 2005)

The intent of the calculations is to model the vertical and horizontal signal interactions of retinal neural mechanisms and their impact on melatonin suppression. Currently, model predictions are for 60-minute exposure durations dependent on irradiance and light source SPD. Figure 28 shows a circuit diagram of the model, which is described by Equation 1. The model calculation for CL (spectrally weighted irradiance in W/m²), which is shown in Equation 1 with revised nomenclature, accounts for the participation of rods, cones and ipRGCs (Rea et al. 2010). The CL value is normalized to 1000 lx of CIE standard illuminant A (a blackbody radiator at 2856 K, similar to an incandescent lamp) (Rea et al. 2010), so that 1000 units CL_A may be numerically equal to 1000 lx (Equation 2). CS describes the effective light stimulus for the circadian system by estimating acute nocturnal melatonin suppression (Equation 3) (Rea et al. 2010). Whether spectral opponency is active (blue/yellow response) is defined by (part of Equation 1). The equation for CS is based on a three parameter logistic curve; it was made to fit the data on which the model was developed (Rea et al. 2005).

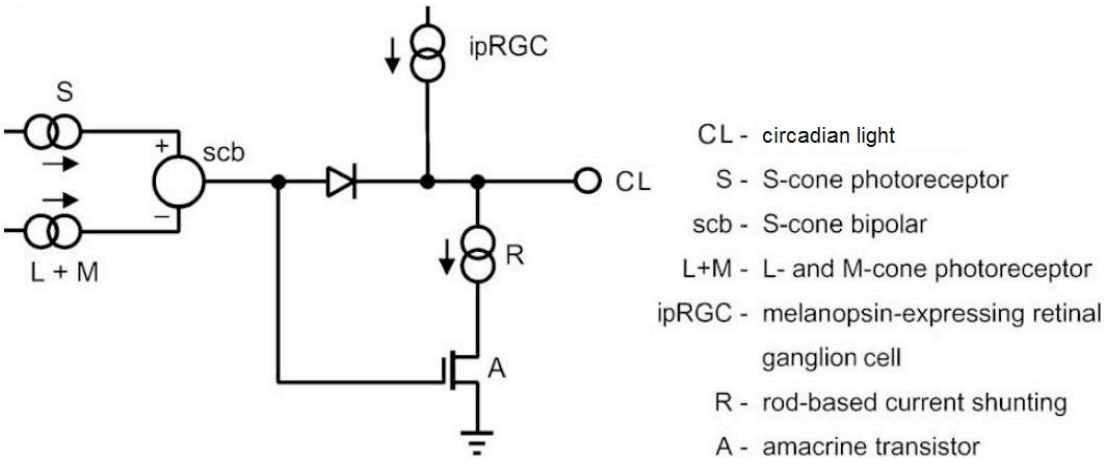


Figure 28: Revised circuit diagram of the circadian phototransduction model (Rea et al. 2005).

$$CL = \left[\left(a_1 \int P_\lambda M_\lambda d\lambda - b_1\right) + a_2\left(\int P_\lambda S_\lambda d\lambda - k\int P_\lambda V_{10\lambda} d\lambda\right) - b_2\right] - a_3\left(1 - e^{-\left(\frac{\int P_\lambda V'_\lambda d\lambda}{rodSat}\right)}\right)$$

for $\int P_\lambda S_\lambda d\lambda - k\int P_\lambda V_{10\lambda} d\lambda \geq 0$

$$CL = a_1 \int P_\lambda M_\lambda d\lambda - b_1$$

for $\int P_\lambda S_\lambda d\lambda - k\int P_\lambda V_{10\lambda} d\lambda < 0$

Equation 1: Equation defining CL (Rea et al. 2010).

$$CL_A = 5831CL$$

Equation 2: Equation defining CL_A normalized to CIE standard illuminant A (Rea et al. 2010).

$$CS = 0.75 - \frac{0.75}{1 + \left(\frac{CL_A}{215.75}\right)^{0.864}}$$

Equation 3: Equation used to determine CS based on the normalized CL_A value (Rea et al. 2010).

The variables in the equations are defined as follows: M_λ is the spectral efficiency function for melanopsin containing ipRGCs (peak λ 480 nm), $V10_\lambda$ is the large-field L- and M-cone spectral efficiency function, V'_λ represents the rod spectral efficiency function, S_λ is the S-cone spectral efficiency function, and P_λ is the spectral irradiance at the eye from a light source. The equations also contain a set of parameters: k = 0.31, a_1 = 0.285, a_2 = 0.2, and a_3 = 0.72. These parameters represent interactions between photoreceptors. There are three constants: b_1, b_2, and rodSat that set the threshold limits of photoreceptors in the mathematical model. The threshold limits for short-wavelength photoreception were set by b_1 at 0.01 for M_λ and b_2 at 0.001 for the blue/yellow response. The half-saturation constant for rod bleaching, rodSat, was set at 6.5 W/m^2 (Rea et al. 2005).

The calculation procedure can be broken down into six steps:

1. The responses of the four photoreceptors are calculated. The model weights the source SPD according to the M_λ, $V_{10\lambda}$, S_λ, and V'_λ (Figure 29).

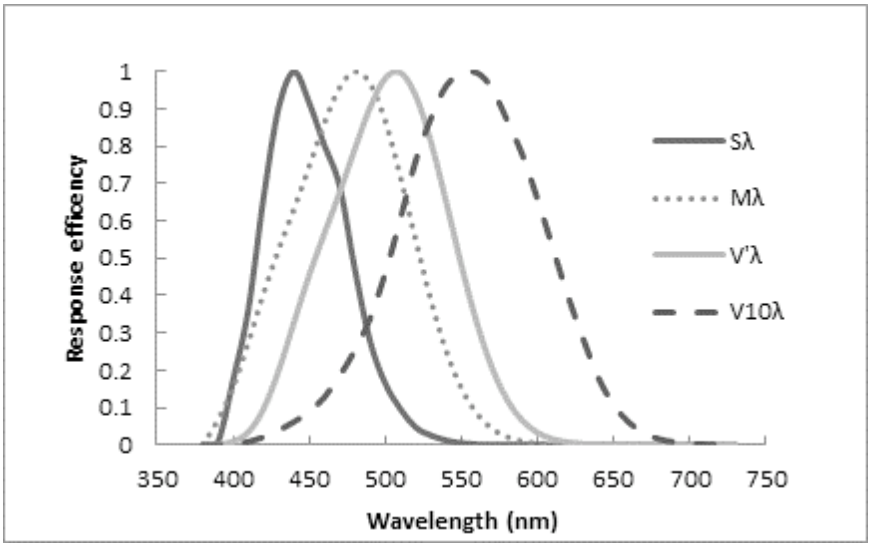


Figure 29: Spectral response functions used in the model (Rea et al. 2010).

2. It is determined if the blue/yellow spectral response function will provide input into the model. In other words, if opponency is active, the S-cone bipolar carries ON signals to a bistratified ganglion cell. These ganglion cells receive input mainly from S-cones (scb in Figure 28).
3. Rod shunting is determined (R in Figure 28), if spectral opponency is found to be active. Amacrine cells (A in Figure 28) liberate rod inhibition in response to an OFF signal from S-cone bipolar cells. When light levels increase, rods saturate and lose responsiveness.
4. The photoreceptor channels are combined into CL (Equation 1).
5. CL is multiplied by the constant 5831 to normalize it to Illuminant A (a blackbody radiator at 2856 K), resulting in CL_A (Equation 2).
6. Finally, CS is determined (Equation **3**). To find the estimated percent melatonin suppression, CS is multiplied by 100.

1.6 Further study of short-wavelength light

The literature review shows that exposure to narrowband radiation (peak wavelength between 440- and 470 nm) would result in a greater impact on nocturnal melatonin suppression than radiation peaking in any other portion of the visible spectrum. Further exploration is needed to understand how 470-nm light impacts the circadian system with respect to exposure duration and light level on melatonin suppression. Additionally, little work has been done to test for the threshold with respect to exposure duration and light level. Most experiments have been performed on subjects with dilated pupils. To ensure more applicable results, further study should be conducted employing the protocol and method developed by Figueiro et al. (2009), using the personal light treatment devices, which were developed under a grant from the National Institutes on Aging.

2. Methodology

2.1 Objectives

The aim of the present empirical study was to investigate the combined interactions of corneal irradiances and exposure duration on acute nocturnal melatonin suppression using a 470 nm narrowband light source. The secondary aim was to determine the threshold for acute melatonin suppression for 470-nm light with respect to duration and irradiance.

2.2 Light conditions

The circadian phototransduction model (introduced in the background) was used to predict melatonin suppression for 60-minute continuous exposure. Desired suppression percentages were selected to be near threshold, above threshold and near saturation. A back calculation was made using a program written in MatLab software (version 7.10.0.499, 64-bit) (CalculateIrradianceConditions.m). Several assumptions were made for the initial predictions with regard to pupil size. Included in the calculation a 5mm pupil diameter was assumed for 0.7 and 2 $\mu W/cm^2$, while a 2.3mm diameter was assumed for the two higher irradiance conditions. Light levels were selected based on the relative SPD of the light source and predicted suppression. Table 1 shows the predicted suppression for 1h, along with the target irradiance. All of the goggles used LEDs, which had a peak wavelength near 470 nm. The control condition required subjects to wear goggles, which were not energized.

Predicted melatonin suppression for 1h (%)	0 (Dark)	0.03	0.23	15	45
Target Irradiance ($\mu W/cm^2$)	0	0.7	2	6	20

Table 1: Predicted melatonin suppression for 1h and target light levels. The 470-nm light goggles were produced by Topbulb.com with LUXEON Rebel (LXML – PB01) light-emitting diodes (LEDs). As mentioned earlier, the goggles have already been tested at two light levels (11 and 74$\mu W/cm^2$) (Figueiro et al. 2009).

2.2.1 Goggles

Four LEDs with peak wavelength near 470 nm were mounted in the top inside rim of commercially available safety goggles (Figure 30). The heat from the LEDs was dissipated via a heat sync strip. The LEDs were placed in a translucent plastic tube to diffuse the light and to reduce glare from the point sources. The power source was located in a case extended by wire to allow for remote use. The power envelope and circuitry were placed in the small case (0.5 x 1.5 x 2.5inches). The irradiance could be adjusted with the LED driver (Maxim Evaluation Kit -1848 EVKIT). The goggles were each powered by two AA batteries for which a Texas Instruments' TPS77018DBVR 1.8V voltage regulator was selected. Batteries were checked prior to each experimental session and changed if the voltage was below 2.9V. The goggles were set to provide a consistent light level during the experiment.

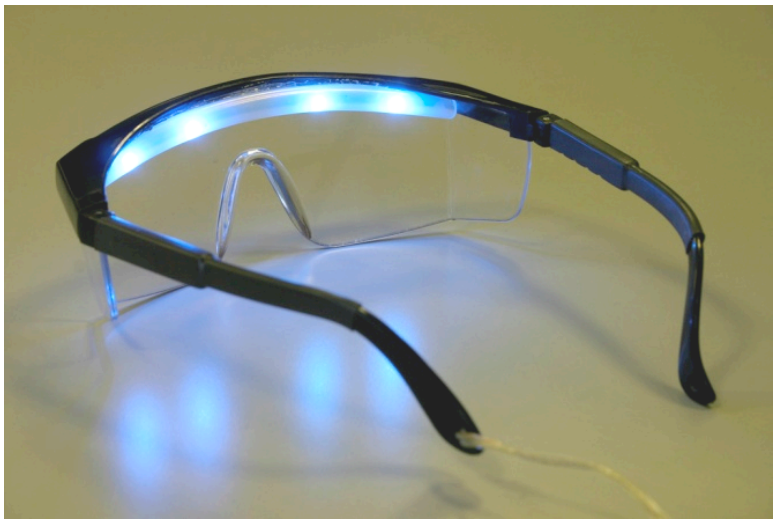


Figure 30: Prototype personal blue-light treatment goggles.

2.2.2 Calibration of measurement system

Prior to every experiment, goggle light output was checked using a double monochromator (Action Research double monochromator: 2300i) with a photomultiplier tube (PMT). The measurement system was calibrated prior to every use in a dark laboratory. The double monochromator limited stray light and has a high signal to noise

ratio, which was significant when measuring low light levels. A photomultiplier tube (PMT) enabled the measurement of low light levels thanks to low noise and high gain. Figure 31 shows the layout of the calibration system. A Lambertian diffuser at the end of the 5mm diameter optical fiber provided a cosine response for measuring irradiance from the standard lamp. A working standard lamp (28V 75W Q/CL halogen incandescent lamp no.12) was used to calibrate the double monochromator at a distance of one meter from the optic fiber diffuser. The working standard lamp was run on a constant current of 2.534A.

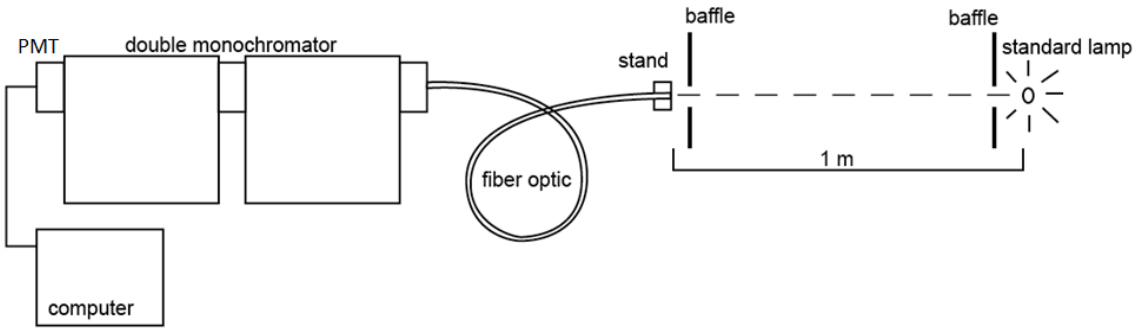


Figure 31: Layout of monochromator calibration.

The software SpectraSense (version 4.3.0) was used to control the monochromator and to record readings. The software set integration time, number of readings to average, and selected different wavelength ranges to scan. Integration time was set to 1000ms for high sensitivity with the scanning range set from 380 nm to 780 nm at a 1 nm step. Dark subtraction was selected to subtract the dark current from the PMT reading. To obtain a calibration factor, the reference standard lamp was first measured. Figure 32 shows the initial PMT signal reading of the standard lamp.

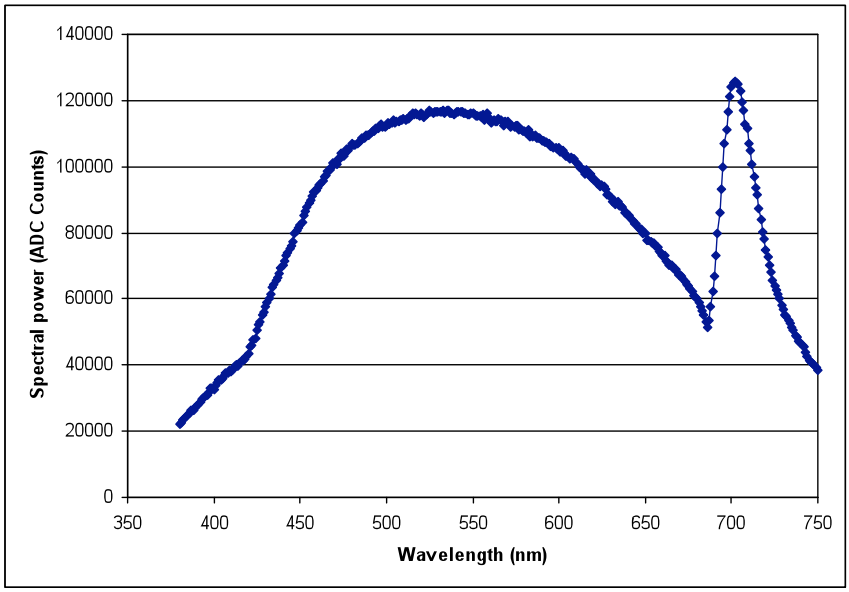


Figure 32: PMT signal from the standard lamp.

The initial reading was then divided by a reference file (Figure 33), which contained the standard lamp's known SPD. To create the reference file, the standard lamp was calibrated on the bar photometer using a National Institute of Standards and Technology (NIST) traceable spectral irradiance standard. This division resulted in a calibration factor (Figure 34).

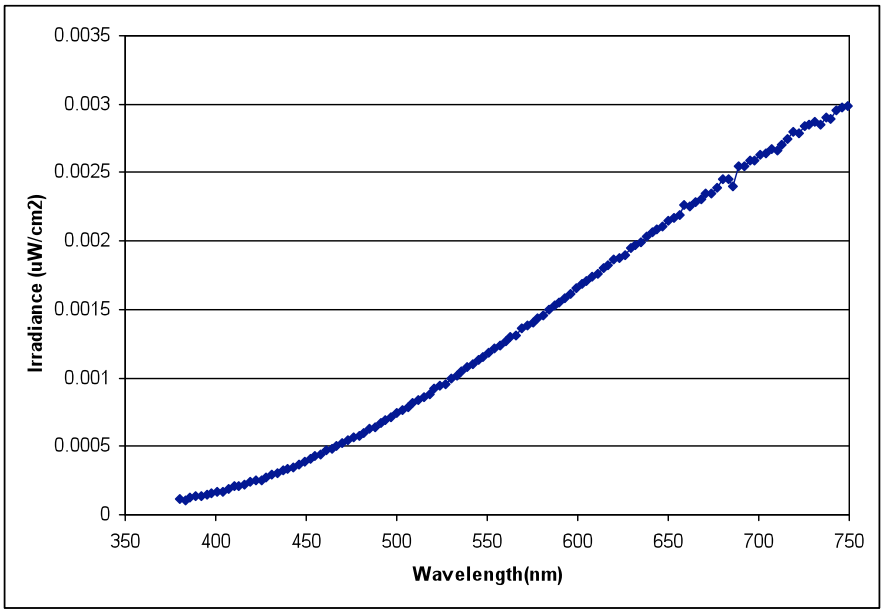


Figure 33: The known SPD of the calibration lamp.

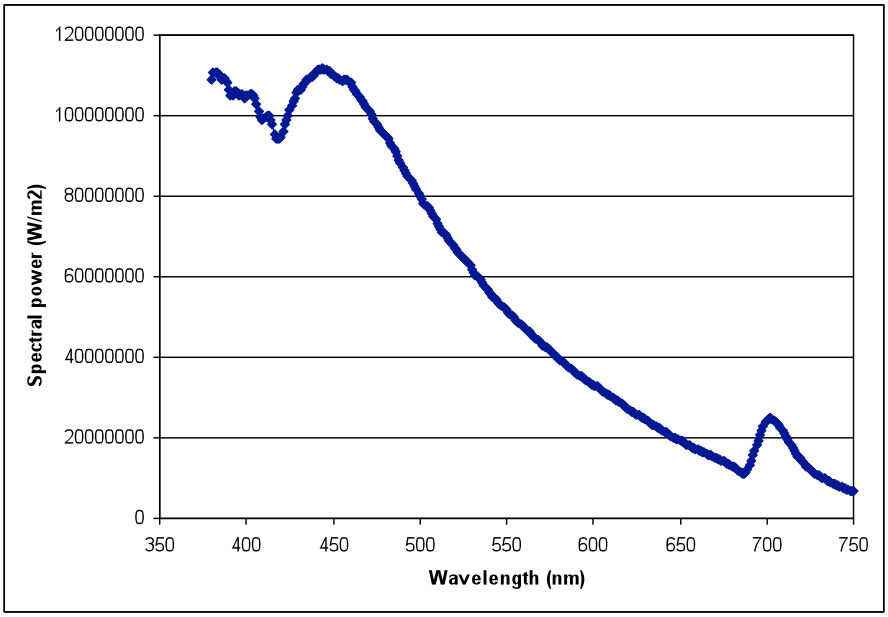


Figure 34: The calibration factor.

The newly obtained calibration factor (Figure 34) was then multiplied by the raw reading (Figure 32). The curve and calculated irradiance, from this multiplication, should be equal to the standard lamp's known SPD curve and irradiance (reference file).

If the calibration factor adjusted raw count of the standard lamp matched the reference file within 5 percent, the calibration factor was accepted. The standard lamp was measured again and the calibration was applied. The calibration system was then ready to be used to calibrate the goggles.

1.3.3 **Goggle calibration**

Once the monochromator calibration was set, the goggles were calibrated (Figure 35). Two alkaline type AA batteries (acceptable minimum measured voltage was 2.9v) were used in the goggles during calibration. The goggles were placed in a rigid stand (Figure 36) where the optical fiber end was positioned at the location of an average human pupil. Integration time was set to 500ms with the scanning range set to 430 to 540 nm at a 1 nm step. The resulting scan was then multiplied by a factor of 2 to match the 1000ms integration time of the calibration factor. Dark subtraction was selected during the scan to subtract the dark current from the PMT.

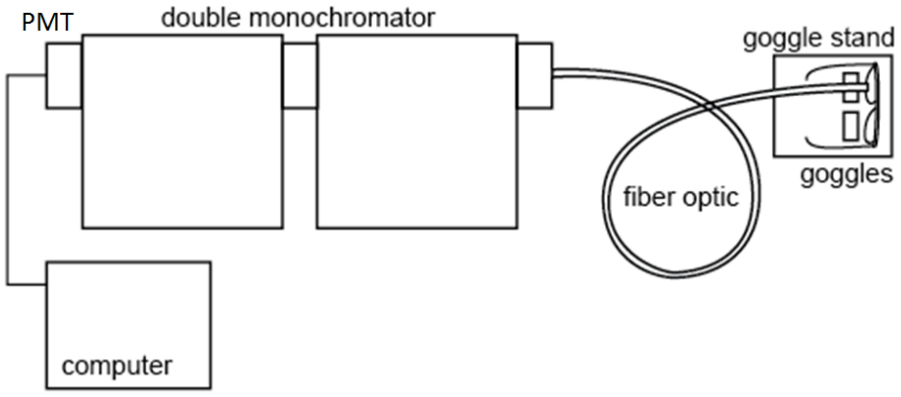


Figure 35: Goggle calibration setup.

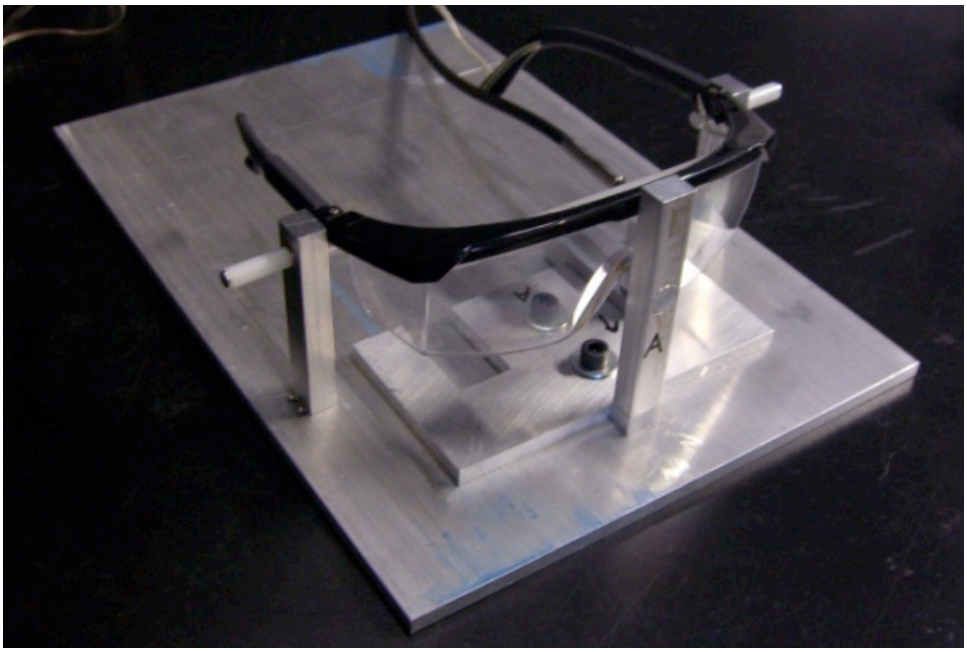


Figure 36: Goggles on calibration stand.

The electronic design of the goggles incorporated only one LED drive adjustment for both sides. As a result, during calibration, it was found that at lower irradiances the left and right sides of the goggles were not identical. To account for imbalances the left and the right pupil positions were measured separately. The goggle drive current was adjusted until the average irradiance (left and right sides) reached the desired irradiance. Goggles were adjusted until the resulting left and right average irradiances were within ±5% of the target values. Mean calibrated goggle measurements are shown in Table 2 with standard error of the mean (sem).

Irradiance (uW/cm^2)	Peak wavelength (nm)	FWHM (nm)
dark	NA	NA
0.7 ± 0.04	473 ± 0.4	18 ± 0.83
2 ± 0.08	467 ± 5.5	18 ± 0.29
6 ± 0.2	474 ± 0.3	20 ± 0.3
20 ± 2.0	466 ± 5.9	19 ± 1.1

Table 2: Results of the calibrated goggles worn by subjects during the experiment (mean ± sem).

2.3 Location

The experiment was conducted at the Lighting Research Center in Troy, NY. Figure 37 shows a sketch of the room where subjects remained during the experiment sessions. Each subject was assigned to a desk space. Desks were positioned 20 feet from the projection screen. Blackout shades were drawn to prevent exposure to stray light. Subjects were also allowed to leave if they needed to (e.g., for restrooms). Red light (peak wavelength 640 nm) traffic signals were placed on the floor behind where subjects were sitting, in the restroom and corridor. These traffic signals provided low-level indirect illumination in the space (less than 1 lx at the eye), which was sufficient for subjects and experimenters to safely navigate in the space. These red light traffic signals did not provide any short-wavelength light to stimulate the circadian system. During the experiment subjects watched a film projected on a screen in the dark. The measured illuminance at the subjects' cornea ranged from 0.14 to 0.2 lx of polychromatic light from the projected film. These low light levels were insufficient to stimulate the circadian system (Zeitzer et al. 2000) and were constant for all experimental conditions.

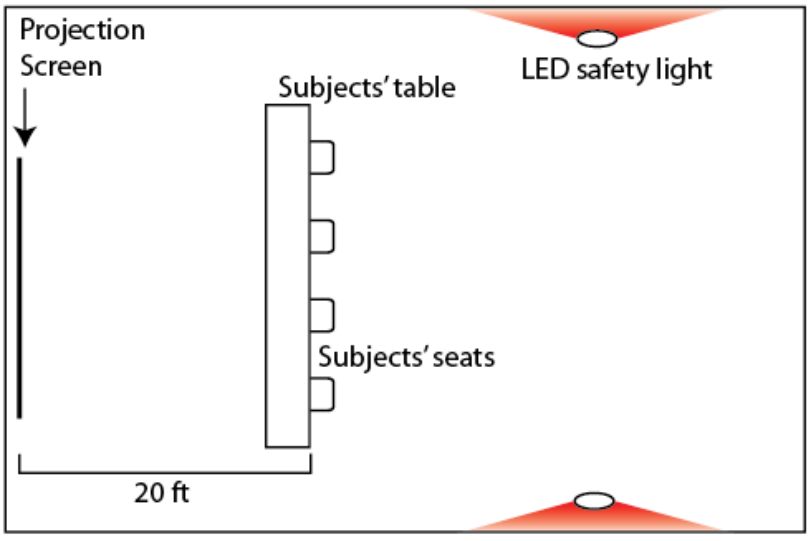


Figure 37: Experiment room layout.

2.4 Subjects

Eleven subjects were recruited into the study through e-mail notices, posters, and word-of-mouth. These subjects participated in the previous study, which explored suppression from 11 and 74 $\mu W/cm^2$ of 470-nm light. The experimental protocol was approved by Rensselaer's Institute Review Board (IRB) and all subjects were asked to sign a written informed consent form (Appendix 6.1). All subjects were between the ages of 51 and 62y (mean ± sem: 56.2 ± 3.9). 9 subjects completed all of the conditions. Eligibility for the study required subjects to be free of any major health problems such as cardiovascular disease, diabetes, or high blood pressure. They were excluded from the experiment if they were taking over-the-counter melatonin or prescription medication such as blood pressure medicine, antidepressants, sleep medicine, hormone replacement therapy or beta-blockers. Subjects were asked to self-report any eye diseases, such as cataract, glaucoma, and color blindness. Potential subjects who stated that they had any eye disease were not included in the study. Subjects were also selected for the experiment based on their responses to a Munich ChronoType Questionnaire (Appendix 6.2). The average ChronoType was moderate early (mean ± sem: 1.4 ± 0.4) (Table 3). Subjects kept a sleep/wake diary to ensure a regular sleep/wake schedule.

Number	ChronoType	Age	Gender
1	n/a	53	M
2	n/a	53	F
5	0	56	M
6	0	57	F
8	2	62	F
10	1	54	M
11	2	58	M
13	2	51	F
14	3	62	M

Table 3: Subject information.

2.5 Experimental Parameters

2.5.1 Independent variables

The independent variables for this experiment were corneal irradiances and exposure duration. Subjects were exposed to four irradiances (0.7, 2, 6, and 20 $\mu W/cm^2$) of 470-nm light and one control condition (dark). Exposure durations include: 0, 5, 10, 15, 20, 25, 30, 45, 60 and 90 minutes.

2.5.2 Dependent variables

The dependent variables for this experiment were melatonin levels in saliva and plasma. Saliva and blood samples were collected for melatonin assay; a total of 14 samples were taken from each subject during each session. Additionally, pupil diameters were measured while goggles were energized.

2.5.3 Extraneous variables

Extraneous variables included differences between subjects' light history. To account for this each subject was exposed to each condition and kept a regular schedule as requested by the experimenters. Actual corneal irradiances may have differed because of eye lashes blocking light and differences in facial profile. Again this was taken into account by exposing each subject to every condition. Data normalization (described in the results section) was used to correct for differences in measured melatonin concentration, which may have been caused by the use of separate melatonin radioimmunoassay test kits. Separate radioimmunoassay test kits were used since samples were sent out in batches.

2.6 Procedure

The current experiment followed the same protocol as the one conducted by Figueiro et al. (2009). Subjects took part in a five-night protocol. The sessions were always on Friday nights and scheduled at least one week apart. Data collection ran from September 2009 to March 2010. On the weeks of the experimental sessions, subjects were instructed to go to bed between 21:00 and 23:00. On the day of the experiment,

subjects were asked to arrive at the lab at 22:00. Subjects were also asked to refrain from caffeine and alcohol 12h prior to the start of the experiment.

A registered nurse inserted an indwelling catheter into an arm vein of the subjects. At 23:00 room lights were turned off, except for the red light (peak 640 nm) traffic signals placed on the floor behind the subjects. After lights were turned off, subjects viewed a film that was projected on a screen about 20 feet (ft) away from them. The movie kept subjects awake and looking up while wearing the LED goggles, which minimized likelihood of eye closures during the light exposure. Subjects were asked to remain sitting and were not allowed to drink or eat after 23:30.

	Dark	Dark	Goggles on	Goggles on	Goggles on	Goggles on	Goggles on
Blood	23:50	00:00	00:10	00:20	00:30	01:00	01:30
Saliva	23:50	00:00	00:05	00:15	00:25	00:45	01:15

Table 4: Collection times for the first subject.

As shown in Table 4, a total of seven blood and seven saliva samples were taken during each session. Three, 3ml blood samples were drawn at the prescribed times; samples were spun for 15-minutes at 1000 x g. Saliva samples were collected using the salivette system (ALPCO Diagnostics, Salem, NH, USA). Each subject moved a plain cotton cylinder around in the mouth until saturated, after which it was placed in a test tube and spun for 5-minutes at 1000 x g. The plasma and saliva samples were immediately frozen (–20°C) and later sent to a laboratory (Pharmasan Labs, Osceola, WI) for melatonin radioimmunoassay. Sampling was staggered by 2 minutes in both blood and saliva sample collection. Blood and saliva samples collected at 23:50 and 00:00 were taken while all subjects were in the dark. Immediately after the 00:00 data collection, subjects put on the 470-nm light goggles, which remained on for the remainder of the experiment. All subjects saw all lighting conditions in a counterbalanced manner as shown in Table 5. At the completion of pupil measurements, room lighting was turned on and the registered nursed removed the indwelling catheter from the subjects' arms and subjects were offered a ride home.

		Irradiance		Irradiance
subject #	Date	(μW/cm²)	Date	(μW/cm²)
11	9/18/09	6	9/25/09	20
1		20		6
2		6		20
14	10/2/09	6		20
13		20	10/9/09	6
9		6		20
5	10/16/09	6	10/23/09	20
6		20		6
10		6		20

		Irradiance		Irradiance		Irradiance
subject #	Date	(μW/cm²)	Date	(μW/cm²)	Date	(μW/cm²)
5	1/22/10	0.7	1/29/10	2	2/12/10	Dark
6		2		Dark		0.7
11		Dark		0.7		2
1	2/19/10	0.7	2/26/10	2	3/5/10	Dark
2		2		Dark		0.7
14		Dark		0.7		2
13		0.7		2		Dark
10	4/16/10	2	4/23/10	Dark	4/30/10	0.7
9		Dark		0.7		2

Table 5: Experiment dates and conditions.

2.7 Pupil photography

After 01:30, when a nurse had taken the last blood sample, subject pupils were photographed. One eye was photographed with a Sony video camera under infrared radiation and IMAQ Vision Builder software (version 6.1, National Instruments) with the goggles still energized. During photography, subjects held a small ruler just under an eye for reference (Figure 38). If subjects wore glasses during the experiment, their eye was photographed with both the goggles and glasses. It should be noted that subjects' pupils were not dilated at any point during the experiment. Since the right and left pupils are always the same, only one eye was photographed. Ten images of each pupil were taken.

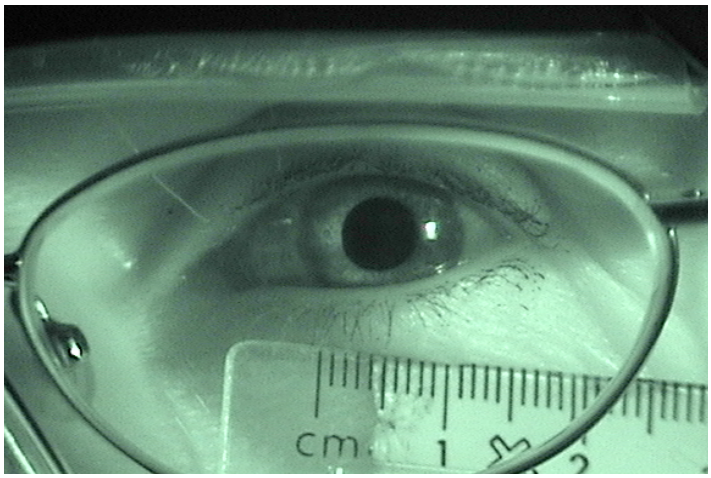


Figure 38: Sample of pupil photography.

3. Results

For the following analyses, data from the experiment conducted by Figueiro et al. (2009) were incorporated. The experiment conducted by Figueiro et al. (2009) used 11 and 74 μW/cm^2 of 470-nm light and the measured pupil diameters and melatonin concentrations of the nine subjects who completed all the experimental and control conditions. These measurements were included in the data analyses to provide a more detailed profile of acute nocturnal melatonin suppression. To better compare predicted melatonin suppression using the circadian phototransduction model predictions, additional irradiance conditions were critical for this comparison.

3.1 Pupil analysis

Pupil measurements are critical, since pupil size impacts the total amount of light entering the eye. The captured digital images of subject pupils were printed for each subject. Pupil size was determined by the ratio between the diameter of the pupil and the amount of millimeters on the ruler in the image using a caliper ruler. The mean of the three clearest images was used. The measured pupil diameters were proportionally smaller for higher light levels (Figure 39). The regression line in Figure 39, fit to the mean pupil diameters from the six 470-nm light conditions, describes a negative log relationship between corneal irradiances and pupil diameter ($R^2 = 0.98$) with a slope of -0.375. Six post hoc two-tailed t-tests were performed comparing pupil diameters from the 470-nm light levels to pupil diameters from the dark condition. The Sidak adjusted criterion probability of a Type 1 error is 0.008. There was no statistically significant difference between pupil diameters in the dark and 0.7 μW/cm^2 ($p = 0.48$) or 2 μW/cm^2 ($p = 0.03$) conditions. All other conditions compared to dark demonstrated a significantly reliable difference ($p < 0.008$).

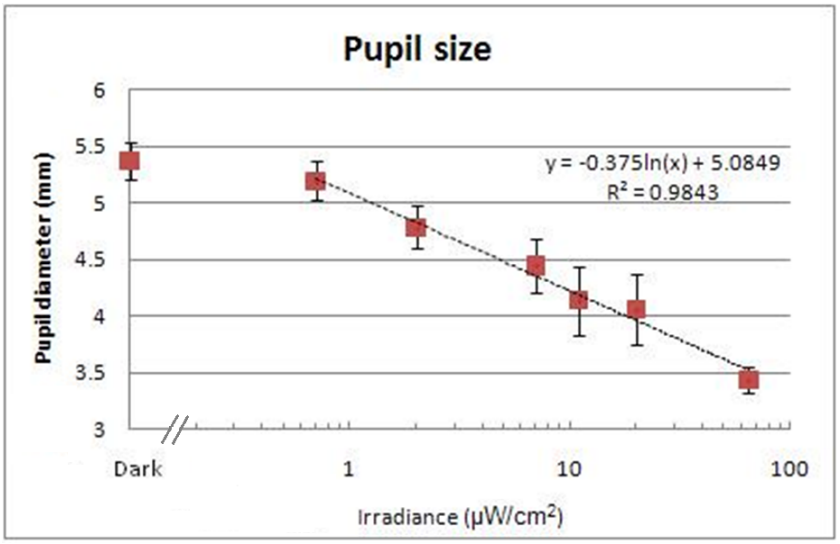


Figure 39: Relationship between pupil diameter (mean ± sem) in the control condition and the six 470-nm light conditions.

3.2 Melatonin radioimmunoassay

The plasma and saliva samples were immediately frozen (–20°C) and sent to a Pharmasan Labs in Osceola, WI for melatonin radioimmunoassay. The sensitivity of the saliva assay was 0.7 pg/ml and the intra- and inter-assay coefficients of variability (CVs) were 12.1% and 13.2% respectively. The sensitivity of the plasma assay was 3.5 pg/ml and the intra- and inter-assay CVs were 8.1% 14.8%, respectively.

3.3 Raw melatonin concentrations

For the following investigation, only the data from nine subjects who completed all the conditions were included for within-subject analyses. The melatonin samples were sent out for radioimmunoassay in three separate batches: 11 and 74 µW/cm^2; 6 and 20 µW/cm^2; dark, 0.7 and 2 µW/cm^2. The first batch was part of a separate experiment conducted by Figueiro et al. (2009). The present study was conducted in two sessions, in the fall of 2009 and the spring of 2010 (Table 5). To ensure that samples do not degrade prior to assay, melatonin samples were assayed within 30 days of collection (Pharmasan); thus the blood and saliva samples collected for this study were sent out for radio-immunoassay in two separate batches. The tested conditions were separated by

two months, this allowed for the immediate analysis of the melatonin concentrations. The mean resulting melatonin concentrations from blood and saliva samples are shown in Figure 40 and Figure 41. Illustrated are the large differences in the plasma melatonin concentrations between irradiance conditions sampled at midnight just prior to light exposure. This difference is especially prominent between the current experiment and the one conducted by Figueiro et al. (2009) (11 and 74 $\mu W/cm^2$), for plasma melatonin concentrations. The mean midnight plasma melatonin concentrations were 30-40pg/ml higher. Melatonin concentrations in saliva tend to contain around 30% of the concentrations found in plasma (Voultsios et al. 1997).

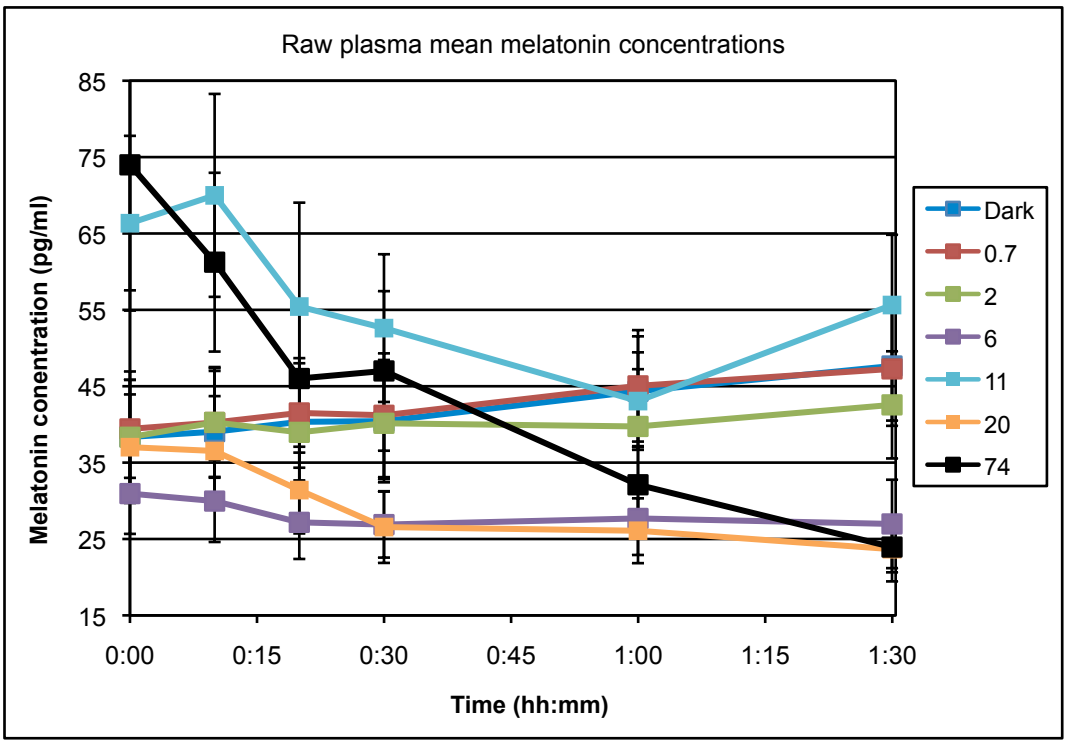


Figure 40: Raw plasma melatonin concentrations (mean ± sem) for 7 conditions.

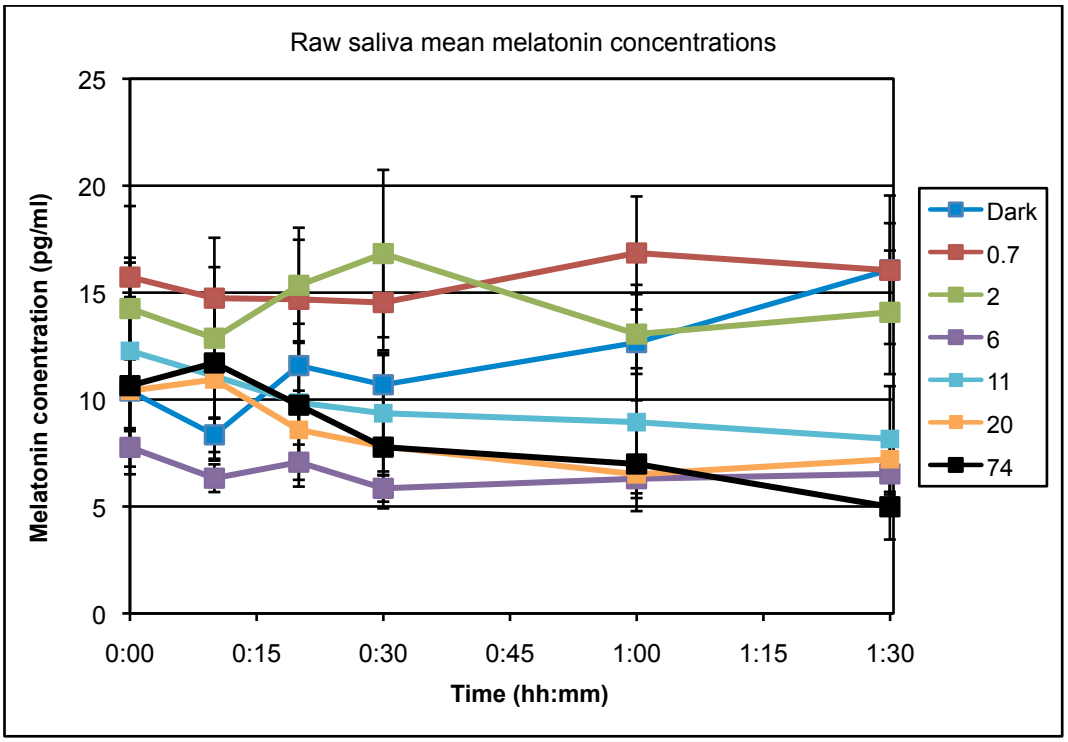


Figure 41: Raw saliva melatonin (mean ± sem) for 7 conditions.

3.4 Normalization of raw melatonin data

To better analyze the data, a four-step normalization procedure was applied. The first two steps corrected for differences between the three radioimmunoassay batches. First a grand mean was determined for all the data from each batch and for each subject a mean was determined for all the data from the same batch as the grand mean. Each subjects' data were multiplied by the ratio of the grand mean and the respective subjects' mean within the same batch. This procedure was completed separately for the three melatonin assay batches. The second step involved a similar procedure using melatonin concentrations from all three batches. Each subjects' data were multiplied by the ratio of the grand mean and the respective subjects' mean. Both plasma and saliva melatonin concentrations were normalized in the same manner. For the third step, saliva data were scaled by a ratio of the mean plasma and the mean saliva within a condition. Plasma melatonin levels are typically 3-4 times higher than saliva melatonin levels. Table 6 shows the saliva scaling factors used for each condition. Figure 42 shows the data after

the third step in the normalization process. These normalized observed mean plasma and scaled saliva values are shown in Appendix 6.3.

Condition (μW/cm²)	Dark	0.1	0.4	0.9	1.5	2.6	6.9
Saliva scaling factor	3.59	2.81	2.95	4.15	6.52	3.66	5.61

Table 6: Saliva melatonin concentration scaling factors were derived by dividing mean plasma melatonin concentrations by mean saliva melatonin concentrations for each condition.

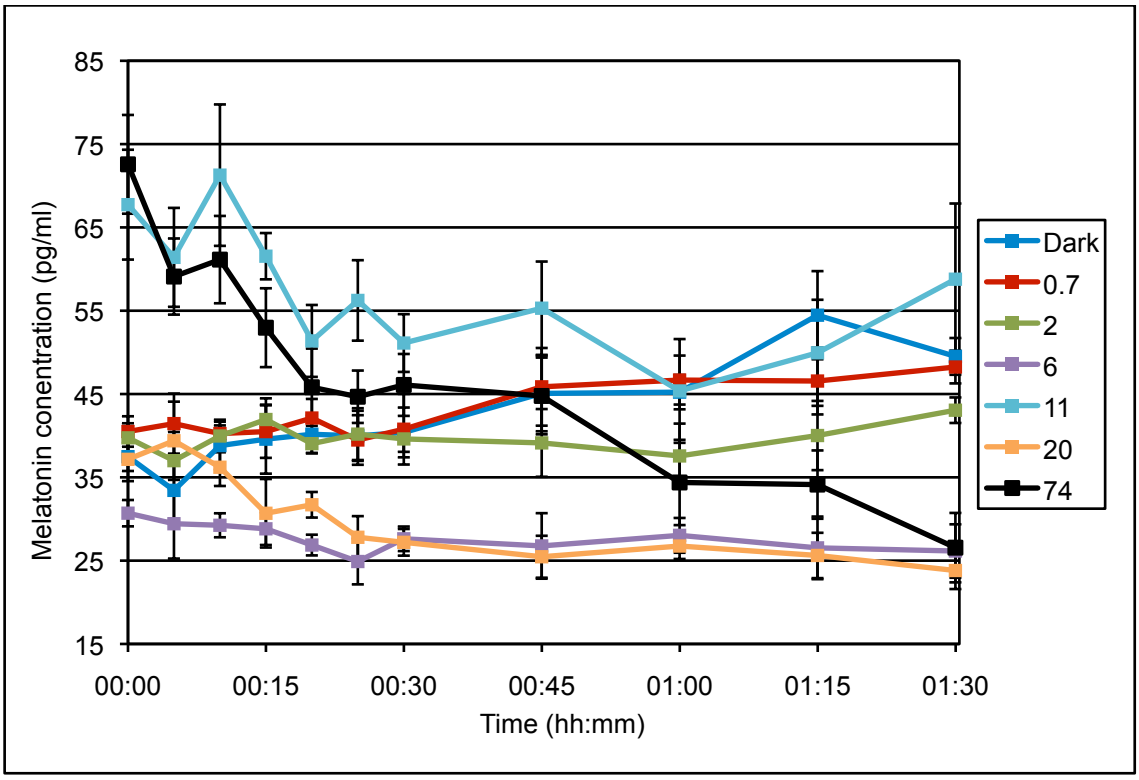


Figure 42: Normalized plasma and scaled saliva melatonin concentrations (mean ± sem).

Even after the third normalization step, the measured mean melatonin concentration values at midnight were noticeably different (Figure 42). Therefore, in the fourth step, the melatonin concentrations for each subject at each light level were scaled separately. For each subject and condition, a quadratic curve was fit to both plasma and saliva data from 00:00 until 01:30. Each subject had their own scaling values determined using the midnight crossing point from the quadratic curves for combined plasma and saliva data. These values were used in statistical analyses. Figure 43 shows mean relative

concentrations (Appendix 6.4) along with best-fit curves (Table 7) for all seven conditions. The multiple sampling times uniquely enabled experimenters to follow melatonin concentration changes in response to exposure duration and irradiance.

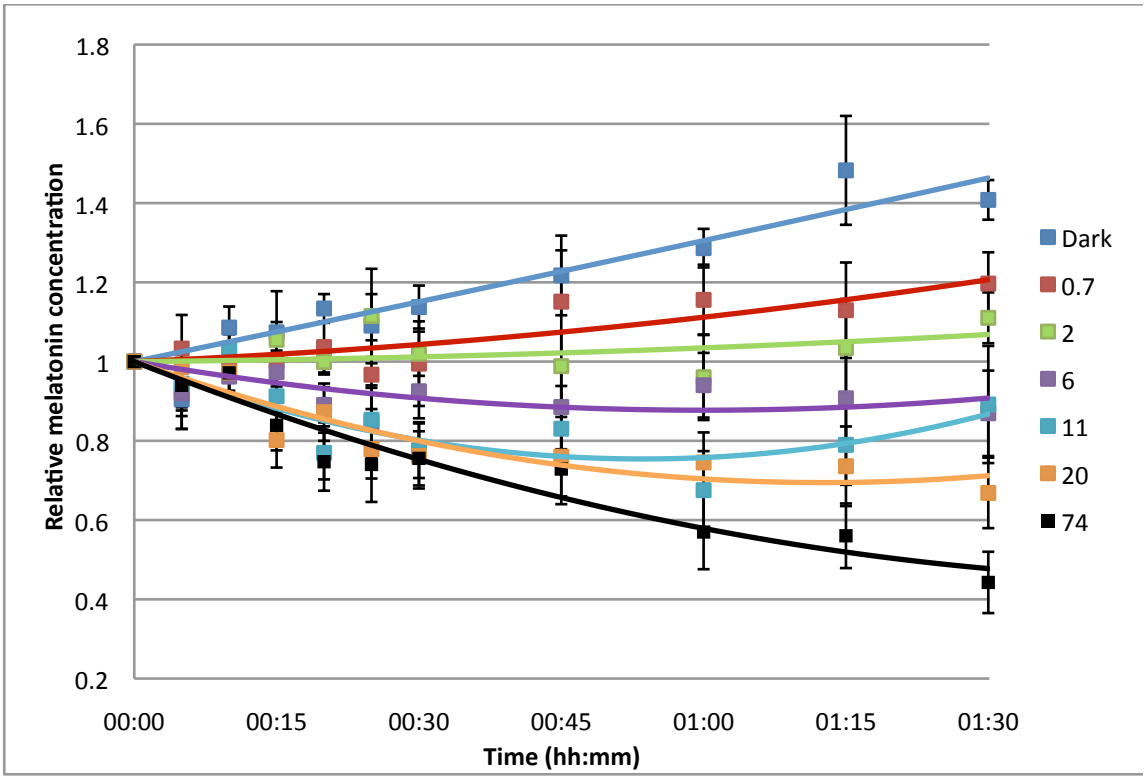


Figure 43: Normalized relative mean melatonin concentrations (mean ± sem) for 9 subjects (plasma and saliva samples) scaled to one at midnight.

Corneal irradiance (μW/cm²)	Quadratic equations
Dark	$y = 4.1078x^2 + 7.1554x + 1$
0.7	$y = 29.636x^2 + 1.4532x + 1$
2	$y = 12.619x^2 + 0.3047x + 1$
6	$y = 70.763x^2 - 5.8938x + 1$
11	$y = 177.45x^2 - 13.207x + 1$
20	$y = 119.98x^2 - 12.106x + 1$
74	$y = 83.734x^2 - 13.593x + 1$

Table 7: Best-fit quadratic equations for normalized relative melatonin concentrations.

3.5 Analyses of normalized plasma & saliva melatonin concentrations

A two-factor (Dark & 6 corneal irradiances x 11 sample times) analysis of variance (ANOVA) (SPSS version 13, Lead Tools, Apache Software Foundation) was used to determine the main effect of irradiance and exposure duration on normalized melatonin concentration (plasma and saliva) for the nine subjects who completed all conditions. The ANOVA showed a significant main effect of irradiance ($F_{6,42} = 17.401$, $p < 0.0001$) but not a significant main effect of exposure duration ($F_{10,70} = 1.556$, $p = 0.138$); there was a significant interaction between irradiance and exposure duration ($F_{60,420} = 3.756$, $p < 0.0001$). An ANOVA tests if the means of several groups are equal. No significant main effect for exposure duration was revealed because the duration means exhibited negligible differences (Appendix 6.4). Any potential significant effect was negated by high relative melatonin concentrations from dark and low irradiances in contrast to the relative low concentrations resulting from high irradiances during longer exposures. To further examine the significant results, 60 post hoc paired two-tailed t-tests were conducted comparing melatonin concentrations in the dark to collection times during 470-nm light exposure. All comparisons were conducted with a Sidak adjusted criterion probability of a Type 1 error. Table 8 illustrates significant interactions between exposure duration and corneal irradiance in the bottom right corner.

		Dark & condition ($\mu W/cm^2$)					
Sample type	Sample time (h:mm)	0.7	2	6	11	20	74
Saliva	0:05	0.3007	0.7681	0.9043	0.7322	0.3871	0.7224
Plasma	0:10	0.1753	0.3808	0.0759	0.5474	0.1274	0.1463
Saliva	0:15	0.5782	0.8702	0.4096	0.2406	0.0489	0.0900
Plasma	0:20	0.2053	0.0126	0.0006*	0.0002*	0.0052	0.0002*
Saliva	0:25	0.3248	0.8738	0.0635	0.0695	0.0138	0.0181
Plasma	0:30	0.2503	0.1494	0.0268	0.0009	0.0015	0.0005*
Saliva	0:45	0.7035	0.1268	0.0443	0.0227	0.0056	0.0040
Plasma	1:00	0.2160	0.0142	0.0022	0.0000*	0.0000*	0.0000*
Saliva	1:15	0.0794	0.0382	0.0062	0.0012	0.0006*	0.0001*
Plasma	1:30	0.0382	0.0021	0.0003*	0.0044	0.0000*	0.0000*

Table 8: Resulting p-values for two-tailed t-test of normalized melatonin concentrations. *Significantly different, with a Sidak adjustment for multiple comparisons.

3.6 Analyses of normalized plasma & saliva melatonin suppression

Percent melatonin suppression ($ms_n\%_e$) was calculated using normalized melatonin concentrations, where each subjects' data were scaled to one at midnight.

$ms_n\%_e = (1 - m_{n,i,e}/m_{n,d,e}) \times 100$

where: $m_{n,i,e}$ = a subject's (n) melatonin concentration (normalized pg/ml) after exposure to a given retinal irradiance (i) for a given duration after 00:00 (e)

$m_{n,d,e}$ = a subject's (n) melatonin concentration (normalized pg/ml) during the dark night (d) for a given duration after 00:00 (e)

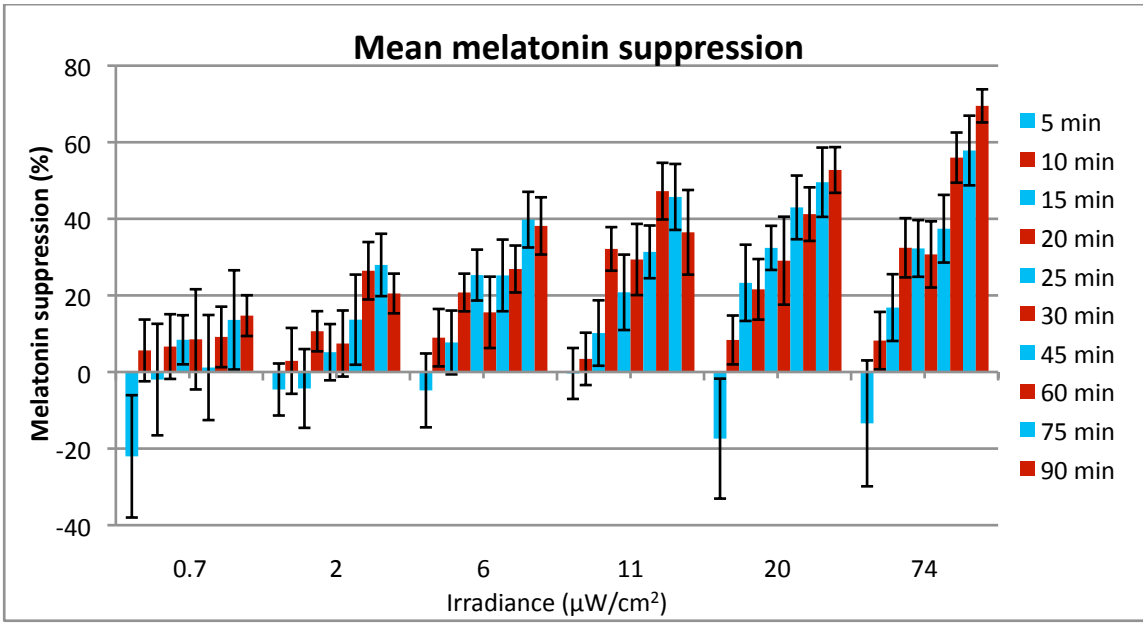


Figure 44: Melatonin suppression (mean ± sem) plasma samples are in red and saliva in blue.

The calculation resulted in ten exposure durations: 5, 10, 15, 20, 25, 30, 45, 60, 75 and 90 minutes. A two-factor ANOVA (6 corneal irradiances x 10 exposure durations after midnight) was performed using melatonin suppression data. The saliva data for subject 14 could not be used since subject 14 did not produce enough saliva in the dark condition to assay. The ANOVA revealed a significant main effect of corneal irradiance ($F_{5, 30} = 9.131$, $p < 0.0001$), a significant main effect of exposure duration ($F_{9, 54} = 5.731$, $p < 0.0001$), and a significant interaction between variables ($F_{45, 270} = 1.927$, $p < 0.001$).

Melatonin suppression was greater with the increase of exposure duration for the two highest corneal irradiances (20 and 74 μW/cm^2). The results indicate that the circadian system appears to habituate to the lower irradiances (2, 6, and 11 μW/cm^2) after an hour of exposure. Figure 44 shows melatonin suppression resulting from six corneal irradiances over ten exposure durations; the saliva samples are represented by the color blue, and red represents plasma samples (Appendix 6.5).

Table 9 shows the results of 60 post hoc one-sample t-tests, which were used to determine if the suppression levels were significantly different from zero. A Sidak adjusted criterion of a Type 1 error was used to correct for multiple comparisons. The bottom right corner of Table 10 illustrates the significant interactions between exposure durations over 20-minutes and corneal irradiances at 11μW/cm2 and above.

		Condition (μW/cm^2)					
Sample type	Exposure duration (min)	0.7	2	6	11	20	74
Saliva	5	0.21037	0.52271	0.63346	0.95525	0.30334	0.44157
Plasma	10	0.50433	0.74435	0.26594	0.62980	0.22635	0.30559
Saliva	15	0.89580	0.68834	0.38550	0.27269	0.05195	0.09503
Plasma	20	0.45470	0.07685	0.00292	0.00048*	0.02586	0.00302
Saliva	25	0.23115	0.50392	0.00666	0.07250	0.00079*	0.00332
Plasma	30	0.53289	0.41402	0.13324	0.01340	0.03526	0.00748
Saliva	45	0.93481	0.28355	0.03082	0.00262	0.00208	0.00387
Plasma	60	0.27964	0.00776	0.00232	0.00021*	0.00037*	0.00003*
Saliva	75	0.32880	0.01095	0.00093	0.00112	0.00093	0.00038*
Plasma	90	0.02457	0.00422	0.00093	0.01086	0.00002*	0.00000*

Table 9: Percent melatonin suppression: resulting p-values from one sample two-tailed t-tests. Test value = 0. *Significantly different from zero, with a Sidak adjusted criterion 0.0008.

3.7 Threshold analysis

In order to focus on the threshold values, measured melatonin concentration differences from the dark condition were calculated. Melatonin concentration data prior to normalization were used in the difference calculations. Figure 45 shows the plot of the mean differences for 9 subjects between the measured plasma melatonin concentrations

in the dark and those for 0.7 and 2 μW/cm^2 for the same exposure durations. These data were used since they were part of the same radioimmunoassay batch and subjects that participated at the same time period. Analysis of Figure 45 reveals that the melatonin concentrations for 2 μW/cm^2 are considerably lower than the melatonin concentrations in the dark condition for exposures of 1h and longer. Mean melatonin concentration differences from dark for 0.7 μW/cm^2 were never greater than 1.2 pg/ml. The negative difference between dark and 0.7 μW/cm^2 translates to higher melatonin concentrations for the lowest 470-nm light condition in comparison to the dark condition.

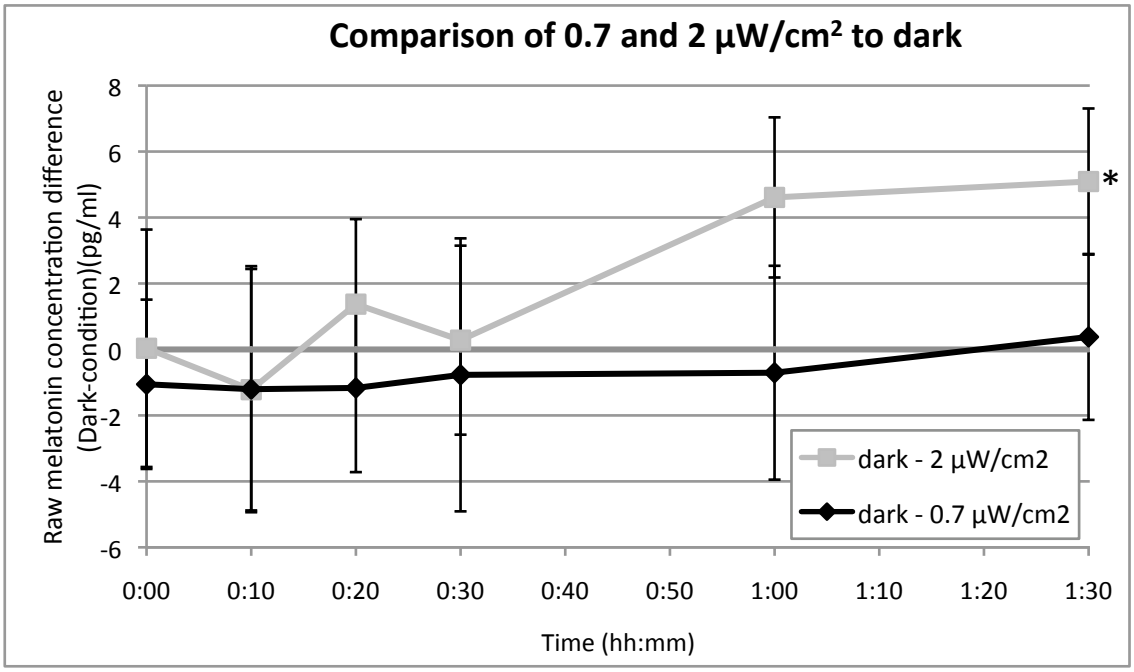


Figure 45: Melatonin concentration differences (mean ± sem). *Significantly different from zero.

The reliability of the differences was verified by comparing the two lowest irradiance conditions to the control condition. Table 10 presents the results of 10 post hoc one sample two-tailed t-tests, with the test value set at zero. According to the predictions generated using the circadian phototransduction model, the 0.7 and 2 μW/cm^2 conditions would induce minor melatonin suppression. The t-tests revealed a statistically significant difference from zero after a 1.5h exposure in response to 2μW/cm^2 ($p = 0.051$); therefore the threshold for acute nocturnal melatonin suppression

is below 2μW/cm^2 for 1.5h exposure. It can be speculated that for durations shorter than 1.5h the threshold is above 2μW/cm^2 and below 6μW/cm^2.

Dark- condition (μW/cm^2)	Sample time (h:mm)	Test Value = 0			
		t	df	Significance (2-tailed)	Mean Difference
0.7	0:10	-0.323	8	0.755	-1.2051
0.7	0:20	-0.457	8	0.660	-1.1668
0.7	0:30	-0.186	8	0.857	-0.7704
0.7	1:00	-0.218	8	0.833	-0.7054
0.7	1:30	0.151	8	0.884	0.3794
2	0:10	-0.333	8	0.747	-1.2203
2	0:20	0.533	8	0.609	1.3733
2	0:30	0.098	8	0.924	0.2814
2	1:00	1.899	8	0.094	4.6092
2	1:30	2.299	8	0.051*	5.0911

Table 10: Results for 10 one-sample two-tailed t-tests of melatonin concentration differences (before normalization). *Significantly different from zero.

4. Discussion

This thesis study is part of a larger framework of circadian research and its application in light therapy. The data presented here will be used to refine the human circadian phototransduction model, which will enable real-time light prescription. The model, published by Rea et al. (2005) is based on the relationship between radiation in the visible range and its effect on acute nocturnal melatonin suppression. The 2005 model was developed on the basis of several key studies (McIntyre et al. 1989, Rea et al. 2001, Rea et al. 2002, Brainard et al. 2001, Thapan et al. 2001, Figueiro et al. 2004). Since 2005, the model has been refined to reflect current knowledge of human circadian neuroanatomy by considering recently published data. For example, model predictions were previously tested against polychromatic light (Figueiro et al. 2006, Figueiro et al. 2011a, Figueiro et al. 2011b) and narrowband light (Figueiro et al. 2007, Figueiro et al. 2008, Figueiro et al. 2009). The results of these studies provided data to update and refine the circadian phototransduction model. This process is repeated, where the model is further refined by predicting acute melatonin suppression in new studies. The model will continue to evolve as new knowledge is discovered about the human circadian system and retinal neural pathways.

The present study provided data to update the model for near threshold light levels. In the present study, nocturnal melatonin suppression was determined from four 470 nm irradiances and ten continuous exposure durations up to 90 minutes. The final data results are a composite of melatonin suppression from 0.7, 2, 6, 20 $\mu W/cm^2$ and 11, 74 $\mu W/cm^2$ (Figueiro et al. 2009) from the same nine subjects exposed to narrowband 470-nm light. The results provided details about the relationship between irradiance and exposure duration on acute nocturnal melatonin suppression.

4.1 Comparison to the circadian phototransduction model

4.1.1 2005 model estimates

The purpose of comparing observed suppression results to model estimates is to verify the model and to determine if adjustments are necessary. Pupil size is a critical component, since it determines how much of a light stimulus may reach the retina. More

accurate model estimates are possible thanks to the availability of pupil measurements. A program written in MatLab (version 7.10.0.499, 64-bit) (Appendix 6.6) was used to estimate melatonin suppression (Appendix 6.7) and the calculation procedure was described in the Background section. Model estimates used the experiment goggle SPD (peak wavelength 470 nm).

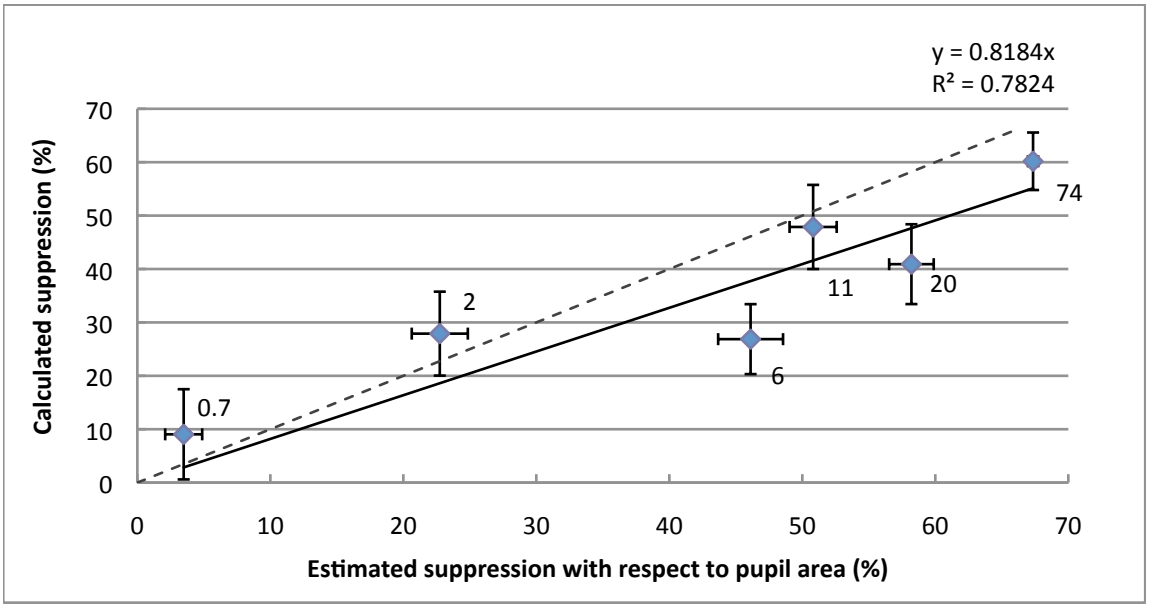


Figure 46: Correlation of calculated percent melatonin suppression and 2005 model suppression estimates for 60-minutes of continuous exposure (mean ± sem). An ideal correlation with a slope of 1 is represented by the dashed line.

Figure 46 shows a positive correlation between calculated melatonin suppression and model estimates incorporating observed pupil size for 60-minute exposures. In the correlation graph the best-fit line was forced through zero. The correlation resulted in a reliable R^2 value of 0.78 ($p < 0.05$). For 9 subjects the degree of freedom (df = N-2) is 7, which required a minimum R^2 value of 0.66 for the correlation to be reliable for a probability level of 0.05 (McGuigan 1993). 0.82 is the slope of the best-fit line confined to zero (solid line). The figure also shows a comparative dashed line with a slope of 1, representing an ideal correlation. The slope of the best-fit line demonstrates the model's drift from calculated melatonin suppression. To double-check the reliability of the correlations, a Bland-Altman analysis was conducted (Appendix 6.7.1). The purpose of

the analysis was to determine the range of differences and to look for systematic differences between the 2005 model estimated and calculated suppression. For this case there appears to be no systematic error, as data points are randomly dispersed in the Bland-Altman plot.

To look more closely at the general trends in the data, a second set of correlations was performed to confirm the slope and the R^2 value. This additional correlation used curve derived melatonin suppression (Appendix 6.9) obtained from quadratic regression curves (Table 7). This new correlation for 60-minute exposures is shown in Appendix 6.9.1. As expected, the correlation using curve derived data points demonstrated a higher R^2 value. The best-fit line (forced through zero) resulted in a slope of 0.8, which is very close to the 0.81 slope for the correlation using calculated mean suppression. This shows that despite large variations between subjects, the means are consistent with the general trend in the data and not influenced strongly by outliers.

This thesis study is noteworthy since it sampled melatonin concentrations multiple times during light exposure. It provided data to help understand to what extent melatonin suppression is affected by exposure duration and irradiance. Supplementary correlations were made for 30- and 90-minute exposures. This also allows for the comparison to other published research, which usually tests 30- or 90-minute exposure durations (Thapan et al. 2001, Brainard et al. 2001). These additional correlations were made for model estimated suppression versus calculated suppression (Appendix 6.7.2) and curve derived suppression (Appendix 6.9.1). The slope of the best-fit trend line was found to increase in relation to an increase in exposure duration for both calculated and curve derived suppression. The slope range for 30-minute exposures was 0.48-0.52, 0.81-0.80 for 60-minute exposures, and 0.92-0.91 for 90-minute exposures (calculated slope, curve derived slope respectively). The curve derived suppression correlations for 30- and 60-minute exposures demonstrated higher R^2 values, since the data points were obtained from regression curves, which produced more regularly spaced data points.

4.1.2 Revised 2011 model estimates

The model was created with several assumptions. First, it is based on nocturnal melatonin suppression from data taken near peak melatonin concentrations where the impact of a light pulse would be the greatest. Regarding the equations themselves, the threshold constants b_1, b_2, and rodSat were estimated from the literature and are subject to change with the addition of data from new studies. The parameters k, a_1, a_2, and a_3 were all determined empirically for a best-fit of the combined data from Brainard et al. (2001) and Thapan et al. (2001) (Rea et al. 2011).

Since the completion of the experiment and initial comparisons, several adjustments have been made to the circadian phototransduction model with regard to lens transmission, threshold, and the blue/yellow response function (Rea et al. 2011).

In the original 2005 model, the ipRGC melanopsin spectral efficiency function had not been adjusted for lens transmission. V_λ and V'_λ were already adjusted for lens transmission. Mc_λ is the ipRGC melanopsin spectral efficiency function (M_λ) adjusted by the human crystalline lens transmission. The crystalline lens transmissions for a standard observer were determined by Wyszecki and Stiles in 1982 (Rea et al. 2011).

Model equations included threshold constants for the ipRGC melanopsin (b_1) and blue/yellow response (b_2) efficiency functions (Rea et al. 2005, Rea et al. 2010). Changes have been made to address the models' limiting treatment of near threshold light levels. Recent data have shown that these two constants elevated the threshold limit too high. For the current 2011 formulation of the model, these limits have been removed (Rea et al. 2011).

The blue/yellow response has been modified to exclude spectral attenuation by the macular pigment. The macula is a 6mm diameter ovular layer of yellow pigment centered over the fovea (2mm diameter). Transmittance of the macular pigment begins to decrease for wavelengths below 585 nm (Rea et al. 2011). The V_λ and S_λ functions were based on 2° field stimulus centered on the fovea. This thesis study provided a wide field stimulus. To adjust for the field degree difference, V_λ and S_λ were divided by the macular pigment transmittance (mp_λ). The revised MatLab program (Appendix 6.10) was developed to estimate acute melatonin suppression for 60-minute exposure durations (Appendix 6.11).

Equation 4 and Equation 5 show the current version of the model. The internal equation, which determines if spectral opponency is active, remains mostly unchanged with the exception of the integration of macular pigment transmittance. This part of the equation specifies whether the opponency is active and contributing to the system. A diode operator specifies addition, unless $\left(\int \frac{S_\lambda}{mp_\lambda} P_\lambda d\lambda - k \int \frac{V_\lambda}{mp_\lambda} P_\lambda d\lambda\right) \leq 0$. In such a case, the whole term to the right of the diode operator is rejected, since the blue/yellow spectral opponent response is not contributing. The constant used to normalize CL to CIE standard illuminant A was adjusted to 5692. The calculation procedure remains unchanged. The empirically determined parameters k, a_1, a_2, and a_3 remain unchanged. The inclusion of low irradiance data created by the present study altered the constants of the three parameter sigmoid curve for calculating CS, affecting estimates primarily for lower irradiances.

$$CL_A = 5692\left[a_1 \int Mc_\lambda P_\lambda \, d\lambda \triangleleft \left(a_2\left(\int \frac{S_\lambda}{mp_\lambda} P_\lambda d\lambda - k \int \frac{V_\lambda}{mp_\lambda} P_\lambda d\lambda\right) - a_3\left(1 - e^{\frac{\int V'_\lambda P_\lambda d\lambda}{RodSat}}\right)\right)\right]$$

Equation 4: Revised equation for CL_A (Rea et al. 2011).

$$CS = 0.7 - \frac{0.7}{1 + \left(\frac{CL_A}{450}\right)^{1.21}}$$

Equation 5: Revised equation for CS (Rea et al. 2011).

Estimates were performed to incorporate adjustments to the model; they are compared to calculated melatonin suppression in a correlation graph. The estimated percent melatonin suppression used in the correlation graph was obtained by multiplying the calculated CS value by 100. Figure 47 shows a positive correlation between calculated suppression and 2011 model calculated suppression for 60-minute exposures. In the correlation graph the best-fit line was forced through zero. The figure also shows a comparative dashed line with a slope of 1, representing an ideal correlation. For 9 subjects, the degree of freedom (df = N-2) is 7, which required a minimum R^2 value of

0.66 for the correlation to be reliable for a probability level of 0.05 (McGuigan 1993). The correlation resulted in a reliable R^2 value of 0.76 ($p < 0.05$). The new model estimates saw a reduction in the R^2 value (from 0.78 to 0.76). However, there is an improvement regarding the slope, which increased from 0.82 to 0.97. A Bland-Altman analysis was completed to recheck the reliability of the correlation (Appendix 6.11.1). The limits of agreement improved from the original 2005 model estimates, indicating that 2011 model estimates have reduced variability. For this comparison there also appears to be no systematic error, since data points are randomly dispersed.

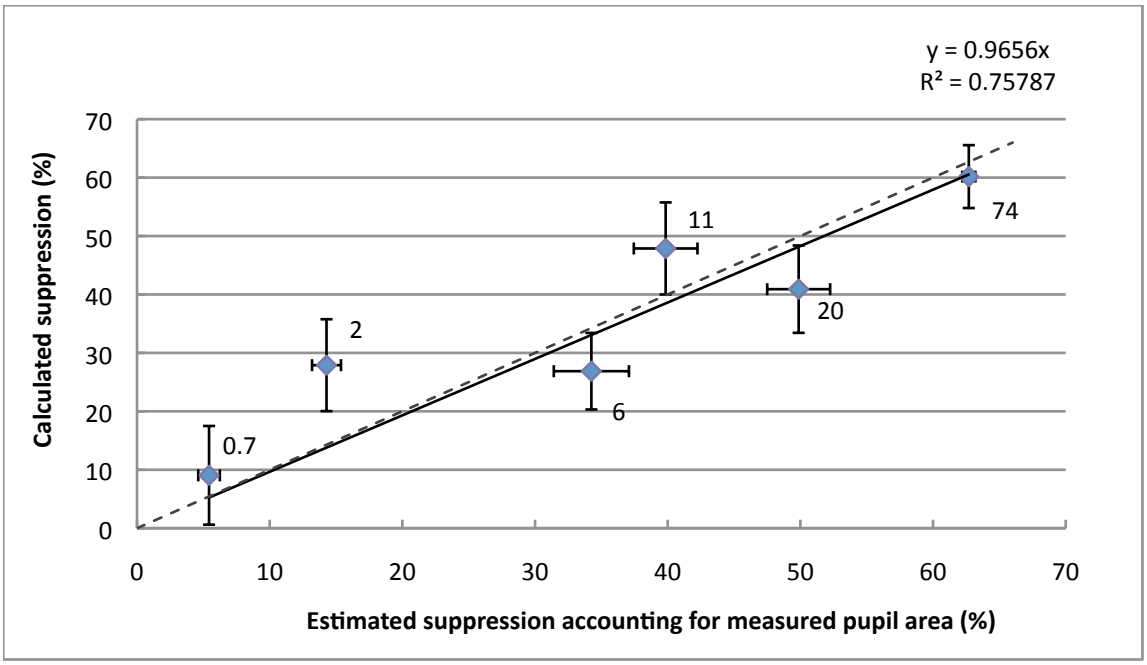


Figure 47: Correlation of calculated percent melatonin suppression and new 2011 model suppression estimates for 60-minutes of continuous exposure (mean ± sem). An ideal correlation with a slope of 1 is represented by the dashed line.

Curve derived suppression data (described earlier) was compared against the 2011 model estimates (Appendix 6.11.3). The correlation for 60-minute exposure resulted in a slope of 0.95, which is extremely close to the slope of 0.96 for the correlation using calculated mean suppression. As expected, the correlation using curve derived data points resulted in a higher R^2 value.

Additional correlations were made for 30- and 90-minute exposures (Appendix 6.11.2) to the 2011 model estimates for 60-minute exposures. The slopes of the best-fit trend lines increased in relation to increased exposure duration. Similar correlations were performed using the curve derived suppression (Appendix 6.11.3), which resulted in nearly identical slopes and demonstrated the same trends. The slope range for 30-minute exposures was 0.57-0.62, 0.96-0.95 for 60-minute exposures, and 1.1-1.0 for 90-minute exposures (calculated slope, curve derived slope respectively). The curve derived suppression correlations for 30-minute exposures demonstrated higher R^2 values. This was not the case for 90-minutes since there was a much greater separation between curve derived and model estimated suppression for the two lowest irradiance conditions.

4.2 Comparison to recently published study

A recently published paper by West et al. (2010), investigated the impact of similar corneal irradiances on melatonin concentrations. Under controlled laboratory conditions eight subjects (mean age 23.9 y) were exposed to eight levels (0.1, 0.5, 2, 10, 20, 75, 300, and 600 $\mu W/cm^2$ at the cornea) of narrowband short-wavelength light (peak wavelength 469 nm) from LEDs. Subjects sat approximately 35 cm from the light source. Subjects with natural pupils were exposed to the 469-nm light conditions for 90-minutes between 2:00 and 3:30 am. Interestingly, the mean pupil diameters in response to the numerous irradiance conditions (Figure 50) were quite similar for both subject groups despite a mean age difference of 30 y.

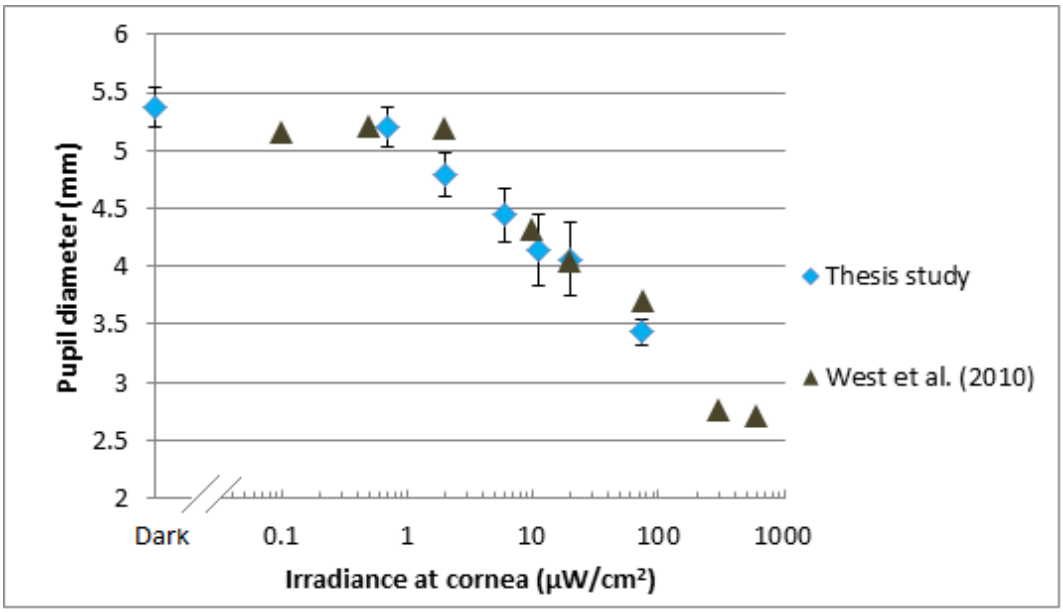


Figure 48: Mean pupil diameters published by West et al. (2010) and the thesis study (mean ± sem).

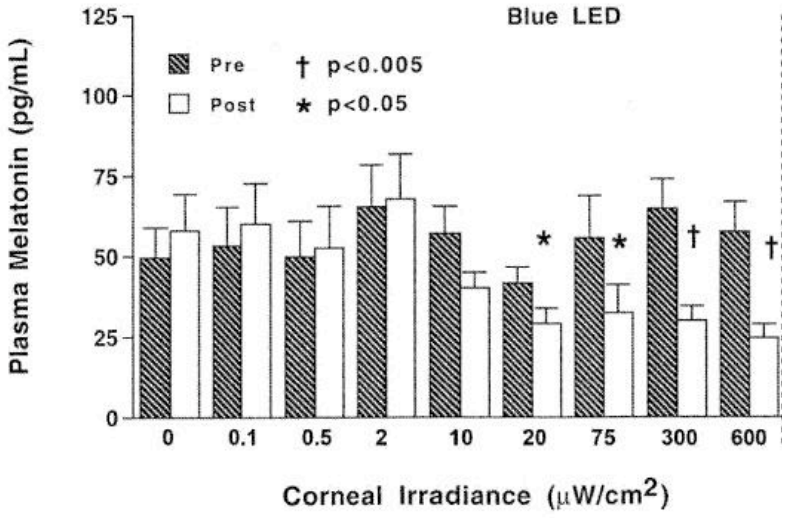


Figure 49: Mean plasma melatonin concentrations in the dark and after 1.5h of continuous exposure to 469-nm light (West et al. 2010).

Mean melatonin concentrations as published by West et al. (2010) are shown in Figure 49. In order to compare the results from West et al. (2010) to the new 2011 model using the same correlation method, melatonin suppression should be calculated employing the same procedure, which was used to determine melatonin suppression for

the nine subjects in the present 470-nm light study. Table 11 has the estimated and relative melatonin concentrations determined from Figure 49. These estimates are made for a coarse comparison, to employ the suppression calculation method used in the thesis study.

Irradiance (μW/cm²)	0 (Dark)	0.1	0.5	2	10	20	75	300	600
Melatonin (pg/ml) at 2:00	50	54	51	65	56	44	56	66	57
Melatonin (pg/ml) at 3:30	58	60	53	68	42	30	34	31	26
Relative melatonin at 3:30	1.16	1.11	1.04	1.05	0.75	0.68	0.61	0.47	0.46

Table 11: Estimated melatonin concentrations, from the study performed by West et al. (2010).

First, the melatonin concentrations in Table 11 were scaled, where the samples taken at 02:00 (in the dark) became 1. Percent melatonin suppression was calculated by subtracting a scaled sample during light exposure (at 03:30) divided by a sample concentration taken during the dark condition (at 03:30) subtracted from 1, then multiplied by 100 (Appendix 6.12). 2011 model prediction calculations were performed using the pupil measurements and irradiance values published by the authors (Appendix 6.13). Figure 50 shows a positive correlation between measured percent melatonin suppression and the 2011 model calculated suppression (▲). For 8 subjects the degree of freedom (df = N-2) is 6, which required a minimum r^2 value of 0.66 for the correlation to be reliably different for a probability level of 0.05 for a two-tailed test of reliability (McGuigan 1993). The correlation resulted in a reliable r^2 value of 0.96 ($p < 0.01$). The figure also shows a comparative dashed line with a slope of 1, representing an ideal correlation.

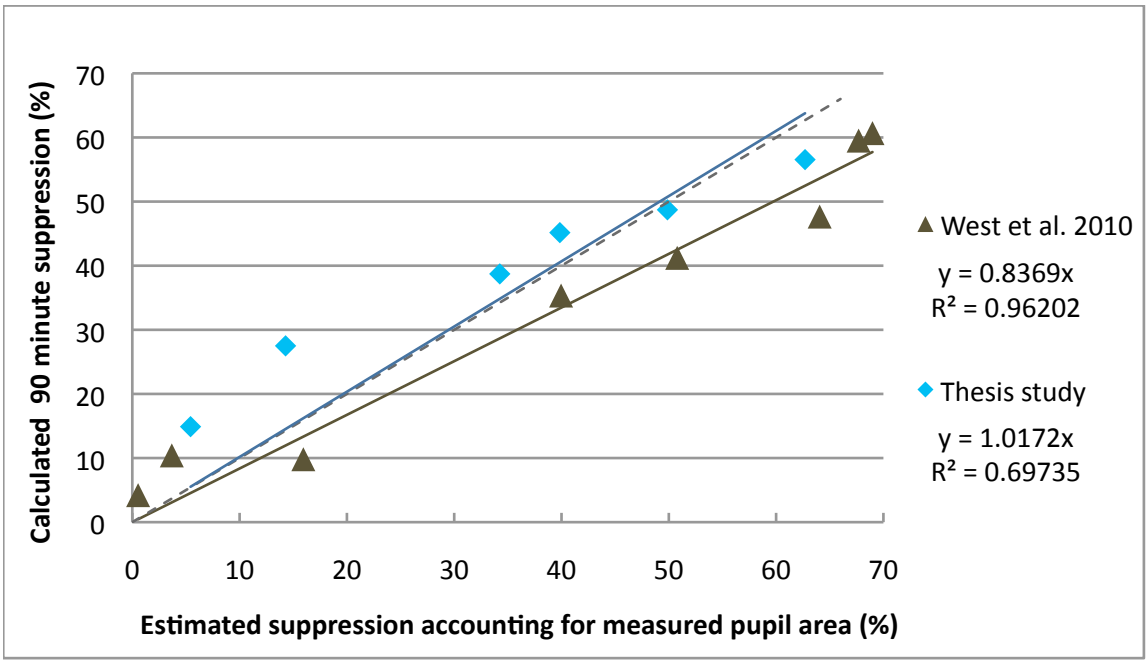


Figure 50: Correlation of calculated melatonin suppression for 90-minutes from West et al. (2010) with the 2011 adjusted model estimates and the thesis study suppression results for 90-minutes. An ideal correlation with a slope of 1 is represented by the dashed line.

In should be noted that the model was designed to make suppression estimates for 60-minute exposure durations. West et al. (2010) only sampled melatonin twice, directly prior to light exposure and after 90-minutes of exposure. Figure 50 shows the correlation between 2011 model estimates and measured melatonin suppression for 90-minute exposures for both West et al. (2010) (▲) and the thesis (♦) result. Compared to the present results the slope for West et al. (2010) is shallower. It is important to note that the melatonin concentrations for West et al. (2010) were estimated from a graph, so measured mean suppression could be different. The discrepancy in slopes can be explained by the low dark night melatonin concentrations for West et al. (2010). When their relative melatonin value (1.16 pg/ml) is replaced by the thesis study dark night value (1.41 pg/ml) after 90-minutes, the slope increases to 0.99. Figure 51 shows how closely 2011 model estimated and calculated suppression align for higher irradiances for both studies, with the dark value replacement. Since suppression calculations are dependent on the dark night, results are all relative to this measure. This comparison of

two independent studies illustrates the significance of a control condition and how it can distort results.

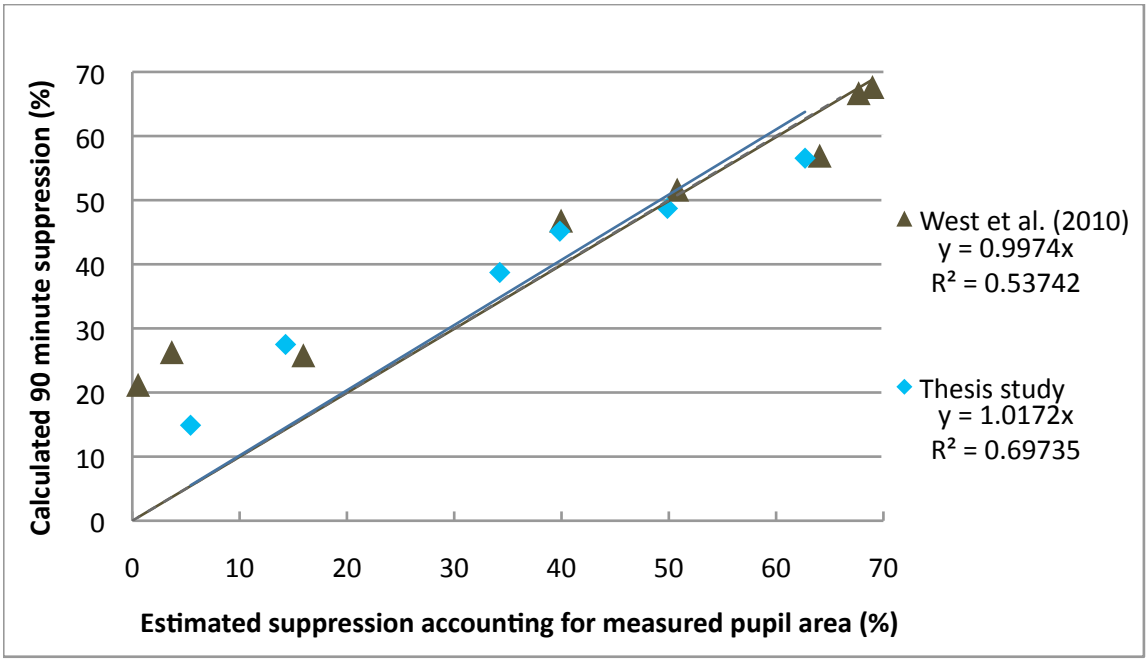


Figure 51: Correlation of calculated melatonin suppression for 90-minutes from West et al. (2010) (using the thesis study dark value) with the 2011 adjusted model estimates and the thesis study suppression results for 90-minutes. An ideal correlation with a slope of 1 is represented by the dashed line.

4.3 Threshold

In psychophysics and neuroscience there are multiple methods for defining a threshold. Absolute threshold is the lowest level at which a stimulus can be detected. An alternative definition of absolute threshold is the lowest light level at which a stimulus can be detected 50% of the time. Whereas a differential threshold is the level at which an increase in a detectable stimulus is perceived. For the purpose of this study the threshold has been defined as the light level and exposure duration, which yields statistically significant different raw melatonin concentration from the control condition (dark). Based on these criteria, 2 $\mu W/cm^2$ is the threshold light level and a 90-minute exposure is the duration threshold for the mentioned light level. Several factors may

have diminished the robustness of the impact on melatonin suppression. Actual corneal irradiances may have been lower because of extraneous variables; eye lashes blocking light, variation in goggle peak wavelength and brow size differences. Additionally, there were large variations in melatonin concentrations from person to person. Plasma melatonin concentration ranged between 7-85 pg/ml for one subject and between 27-200 pg/ml for another subject during a single session. Another limitation in determining the threshold is the rate at which melatonin concentrations rise during the dark night. The comparison of two independent studies in Discussion section 4.2 demonstrated that the rise of melatonin concentrations during the dark night greatly affects melatonin suppression calculations. This may also be the case for the threshold difference calculations, which used raw melatonin concentrations. Therefore, the light level and exposure duration that were determined to be the threshold are dependent on the change in melatonin concentrations sampled during the dark night.

4.4 Future research & implications for light therapy

Future research can explore the threshold relationship between melatonin suppression and phase shifting in response to a short duration light pulse. The study conducted by Zeitzer et al. (2000) observed the impact of polychromatic light on melatonin suppression and phase shifting. In this study light stimuli lasting 6.5 h were used. Such prolonged exposure durations incorporate the habituation of the circadian system to certain light levels (Gooley et al. 2010). The researchers demonstrated that phase delay and melatonin suppression have similar nonlinear responses to white light stimuli (Figure 12). Although the light level needed to reach saturation was found to be higher for phase delaying (550 lx) than for melatonin suppression (200 lx), perhaps this is also the case for narrowband light. Wright et al. (2001) published research demonstrating that phase delaying demonstrated the same sensitivity to short wavelength radiation as melatonin suppression (Figure 19). The authors used very high light levels (130 $\mu W/cm^2$) that have been shown to induce a maximal response in melatonin suppression. Potentially 130 $\mu W/cm^2$ induces a saturating response for phase shifting as well. Currently, the relationship between melatonin suppression and phase shifting is unclear and the threshold for phase shifting in response to narrowband light is still

unknown. The same spectral sensitivity of suppression and phase shifting suggests that the circadian phototransduction model may potentially be used to estimate phase shift. The maximal and minimal responses of phase shifting to three different pulse durations of light are recommended to test model estimates to better understand to what extent the circadian system is sensitive to the duration of a light pulse.

The ability to predict the impact a light stimulus will have on the circadian system is significant. It will enable clinicians to make precise light therapy prescriptions based on the desired melatonin suppression. Based on suppression estimates and further study, potential estimates could also be made for phase shifting. Once the relationship between melatonin suppression and phase shifting is more defined, model estimates can be applied to alleviate jetlag (Burgess et al. 2003), seasonal affective disorder (SAD), shift work disorder, and to help consolidate sleep for Alzheimer Disease (AD) patients (Riemersma-van der Lek et al. 2008, Figueiro et al. 2008).

Accepted traditional light treatments have been delivered through light boxes, which use fluorescent lamps. These light boxes typically deliver 10,000 lx of polychromatic light to the eye at distance of 30-35 cm. This light level induces a near maximal response (65% suppression estimated by the 2011 model) with nearly identical model estimated suppression as 74 $\mu W/cm^2$ of 470-nm light (62% suppression estimated by the 2011 model). The high level of white light tends to induce photophobic reactions often causing users to squint or look away, thus limiting the amount of light entering the eye. Participants from the present study commented that the higher 470-nm light irradiance conditions induced a claustrophobic effect when room light was turned off, the contrast of the LEDs to a completely dark room made it difficult for subjects to see their surroundings. This effect was eliminated when ambient lighting was turned on. The most appropriate light level to prescribe would be 20 $\mu W/cm^2$ of 470-nm light, since this was the highest acceptable irradiance to the study participants and would require the shortest exposure duration to substantially impact melatonin suppression and potentially phase shifting. The implementation of narrowband short-wavelength light is advantageous in many aspects compared to polychromatic white light (Glickman et al. 2006). The spectral sensitivity of the circadian system peaks between 440- and 470 nm, making short-wavelength light far more efficacious at suppressing melatonin compared

to polychromatic light. The benefit of selecting short-wavelength light is that it is enables the use of LEDs (peak wavelength 470 nm), which consume less energy. The small size of LEDs allows them to be mounted in goggles, close to the cornea, ensuring consistent light delivery and allows users to engage in other tasks while receiving a short-wavelength light treatment. This approach makes blue light treatment a cost effective alternative to pharmaceutical solutions and white light treatments.

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6. Appendices

6.1 Subject consent form

RPI Light Study: Sleep therapy headset for improving sleep quality of older adults

Consent Form from the Participant

Rensselaer Polytechnic Institute (RPI) is conducting a study to test if a sleep therapy headset that delivers blue light to the eye can suppress melatonin. Melatonin is a hormone produced at night and under conditions of darkness and helps a person fall asleep. Melatonin production will stop if enough light is presented at the eye. The experiment will be conducted at the Lighting Research Center (LRC) Laboratory.

The experiment will take place from 10:00 pm to 2:30 am. You will be asked to wake up and go to bed at a specific time on the day prior to the experiment, and wake up at a specific time on the day of the experiment. You will be asked not to consume caffeine products (coffee, tea, soda, or chocolate) on the day of the experiment. Before you start the experiment, a registered nurse will ask you about your general health. If your health status may place you or any participant in the experiment "at risk," you will not be allowed to participate in the study.

You will be exposed to a blue light that will delivered by a pair of light goggles. Two light levels will be used. The light levels that you will be exposed to are not harmful to your eyes. A registered nurse will place catheter in your arm at the beginning of the night and remove it after the last blood drawn, and this catheter will be used to draw the blood. Saliva samples (1 ml) will also be collected using the salivette system, in which you will chew on a plain (not citric impregnated) cotton cylinder. If you do not tolerate the salivette or require more than one cylinder on a consistent basis to produce adequate saliva, then you may chew parafilm to stimulate saliva and deposit it directly into a tube. After catheter placement you will be seated in a room illuminated with red light. Lights will be turned off at 11:00 pm. Blood collection will start at 11:50 pm. Blood will be collected approximately at the following times: 11:50 pm, 12:00 am, 12:10 am, 12:20 am, 12:30 am, 1:00 am and 1:30 am. Saliva samples will be collected approximately at the following times: 11:50 pm, 12:00am, 12:05am, 12:15am, 12:25am, 12:45am and 1:15am. Lights goggles will be turned on at 12:00 am. You will be asked to wear the goggles during the entire experiment. You will be allowed to read or watch a movie while being exposed to the light. A total of seven sets of blood samples (12 ml each set) will be collected by the registered nurse during the experiment. At the end of the night, you will have given 84 ml (about 0.15 pint), which is much less than what you give when donating blood (1 pint or 470-ml).

Throughout this process, participation is voluntary. You will get paid $80 per night. You are free to decide whether you are willing to participate or not, and may withdraw at any

time from the experiment, simply by notifying the experimenter. If you withdraw, your blood samples will be destroyed and your payment will be pro-rated. A registered nurse will be in charge of collecting the blood samples, and will be present during the entire experiment as well. Universal precaution procedures such as the use of gloves and disposal needles will be used. However, there are some minor risks involved such as bruise or infection. You will be instructed by the nurse to check for possible infection. An angio-catheter site infection may occur and you should follow up with your physician if size of redness around the original site increases or if pain around the site increases or the site becomes hot. If pain occurs in the area adjacent to the site where the angio-catheter was placed, you should seek medical care. In the event that you are harmed by participating in this study and this harm cannot be attributed to the fault and negligence of the investigator, compensation and/or medical treatment is not available from Rensselaer Polytechnic Institute. However, compensation and/or medical cost might be recovered by legal action.

All data collected during the experiment will be treated as confidential. The blood and saliva samples will be coded and the data will be kept in an archive at the Lighting Research Center, in Troy, NY. The data will not be disclosed to anyone outside the project team in such a way that you can be identified.

I ----------, have been asked to participate in a research study of the effectiveness of blue light goggles on the amount of melatonin production. I have read this consent form, understand it and got satisfactory answers to all my questions and agree to participate.

---------- ----------

Name Signature

Date

For further information, contact:
Dr. Mariana Figueiro
21 Union Street, Troy, NY 12180, 687-7100
518- 687-7142

Chair, Institutional Review Board, Rensselaer
Polytechnic Institute, CII 7015, 110 8th Street, Troy, NY 12180
Phone: 518- 276-4873

6.2 Munich Chronotype Questionnaire (MCTQ)

Munich ChronoType Questionnaire

Please enter your age, gender, etc., This information is important for our evaluations
Age: _____ female male __ Height: ___Weight: _____
On work days …
I have to get up at… __o'clock
I need… ___min to wake up
I regularly wake up… before the alarm/ with the alarm
From… ___o'clock I am fully awake
At around… ___o'clock, I have an energy dip
On nights before workdays, I go to bed at… __o'clock
…and it then takes me… _____min to fall asleep
If I get the chance I like to take a siesta/nap…
Correct I then sleep for _____
Not Correct I would feel terrible afterwards
On free days… (please only judge normal free days, I.e. without parties etc.)
My dream would be to sleep until… _____ o'clock
I normally wake up at… _____ o'clock
If I wake up at around the workday alarm time, I try to get back to sleep…
Correct
Not Correct
If I get back to sleep, I sleep for another… _____ min
I need… _____ min to wake up
From… ___ o'clock I am fully awake
At around… __o'clock I have an energy dip
On nights before free days, I go to bed at… _____ o'clock
…and it then takes me… _____ min to fall asleep
If I get the chance I like to take a siesta/nap…
Correct I then sleep for ____ hrs
Not Correct I would feel terrible afterwards
Once I am in bed, I would like to read for… __ min
…but generally fall asleep after no more than… ___ min
I prefer to sleep in a completely dark room
Correct - yes
Not Correct
I wake up more easily when morning light shines in my room
Correct - yes
Not Correct
How long per day do you spend on average outside (really outside) exposed to sunlight
On work days… ___ hrs _____ min
On free days… ___ hrs ___ min

Self-Assessment

After you have answered the preceding questions, you should have a feeling to which chronotype (time of day type) you belong to. If, for example, you like (and manage) to sleep quite a bit longer on free days than on workdays, or you cannot get out of bed on Monday mornings, even without a Sunday-night party, then you are more of a late type. However, if you regularly wake up and feel perky once you jump out of bed, and if you would rather go to bed early than to and evening concert then you are an early type. In the following questions, you should categorize yourself and your family members. Please select answer from the drop boxes.

Description of categories:

Extreme early type = 0
Moderate early type = 1
Slight early type = 2
Normal type = 3
Slight late type = 4
Moderate late type = 5
Extreme late type = 6

I am…

As a child, I was…

As a teenager, I was…

I case you are older than 65: In the middle of my life, I was…

My parents are/were…

Mother…

Father…

My siblings are/were…

My partner (girl/boyfriend, spouse, significant other) is/was…

6.3 Observed mean normalized melatonin concentrations

	Corneal irradiance (μW/cm²)						
Sample time (h:mm)	Dark	0.7	2	6	11	20	74
0:00	37.48	40.50	39.74	30.70	67.73	37.16	72.57
0:05	33.43	41.45	37.00	29.43	61.41	39.39	59.10
0:10	38.81	40.25	39.96	29.25	71.27	36.21	61.14
0:15	39.58	40.49	41.94	28.82	61.54	30.69	52.96
0:20	40.17	42.12	39.04	26.87	51.37	31.71	45.81
0:25	39.98	39.48	40.19	24.90	56.24	27.81	44.67
0:30	40.39	40.78	39.61	27.62	51.12	27.20	46.09
0:45	45.07	45.86	39.13	26.77	55.30	25.47	44.77
1:00	45.22	46.68	37.57	28.04	45.37	26.75	34.38
1:15	54.46	46.56	40.02	26.53	49.95	25.62	34.13
1:30	49.52	48.24	43.06	26.16	58.78	23.82	26.56

6.4 Mean relative melatonin concentrations (scaled to one)

	Corneal irradiance (μW/cm²)							
Sample time (h:mm)	Dark	0.7	2	6	11	20	74	Means
0:00	1	1	1	1	1	1	1	1.00
0:05	0.90	1.03	0.93	0.92	0.94	0.99	0.94	0.95
0:10	1.09	0.99	1.02	0.96	1.03	0.97	0.97	1.01
0:15	1.07	1.00	1.06	0.97	0.91	0.80	0.84	0.95
0:20	1.13	1.04	1.00	0.89	0.77	0.87	0.75	0.92
0:25	1.09	0.97	1.11	0.85	0.85	0.78	0.74	0.91
0:30	1.14	0.99	1.02	0.93	0.78	0.76	0.76	0.91
0:45	1.22	1.15	0.99	0.89	0.83	0.76	0.73	0.94
1:00	1.29	1.16	0.96	0.94	0.68	0.75	0.57	0.90
1:15	1.48	1.13	1.03	0.91	0.79	0.74	0.56	0.95
1:30	1.41	1.20	1.11	0.87	0.89	0.67	0.44	0.94
Means	1.17	1.06	1.02	0.92	0.86	0.83	0.75	

6.5 Mean calculated melatonin suppression (%)

	Corneal irradiance (μW/cm²)					
Duration exposure (min)	0.7	2	6	11	20	74
5	-23.21	-9.97	-8.44	-1.39	-16.71	-14.17
10	10.42	9.91	14.77	5.03	10.82	11.76
15	-0.64	-3.00	7.88	11.72	24.92	15.46
20	6.21	11.05	22.21	33.15	19.54	35.92
25	8.16	4.82	24.11	19.87	32.98	29.59
30	8.82	8.11	15.71	31.50	28.00	33.53
45	2.15	13.79	25.44	32.56	43.09	35.76
60	9.05	27.90	26.87	47.87	40.91	60.18
75	14.89	27.50	38.72	45.16	48.71	56.55
90	13.41	20.09	39.14	35.95	54.11	72.41
Means	4.93	11.02	20.64	26.14	28.64	33.70

6.6 MatLab program for 2005 model calculations

```
function [CS] = MSw(spd,pupil,w) %SPDs are relative SPDs with max = 1

% For a given relative SPD and target irradiance in (uW/cm^2) this
program calculates
% the meletonin suppression and CL for natural pupils based on Berman
model

wave = spd(:,1);
spdvalue = spd(:,2);

Vlamda = load('Vlamda.txt');

%next create a Vlamda Curve that has data points matching the input SPD
VlamdaI= interp1(Vlamda (:,1),Vlamda (:,2),wave,'linear',0.0);


%Convert relative SPD into absolute irradiance SPD

n=trapz(wave,spdvalue);     %find the relative power under the curve

a= w/n;                          %find the scaling factor to scale the
relative spd
```

```
                            %into   an   absolute   spd   based   on   an
irradiance value

spda=[spd(:,1),spd(:,2)*a]; %use scaling factor to convert relative spd
                            %to an absolute spd

%SPDirradiance=trapz(spda(:,1),spda(:,2))  %  resulting  irradiance  in
W/m^2



%Convert irradiance spd into illuminance spd (this is for calculating
pupil size and checking purposes)

        %spdb=[spda(:,1),spda(:,2).*VlamdaI];

        %spdc=[spdb(:,1),spdb(:,2)*683];

        %SPDilluminance=trapz(spdc(:,1),spdc(:,2))      %      resulting
illuminance in lux

        %pupil   =   PupilSize_Berman(SPDilluminance,spda)   %calculate
natural pupil diameter

[CL,CLa,CS]=Cstimulus07Nov08(spda,pupil,0,5);  %(spd  (in  W/m^2),pupil
diameter,draw time,exposure time)

CLa;
CS;
 end



function                            [CL,CLa,CS]                           =
Cstimulus07Nov08(spd,pupil,drawTime,lightDuration)
% Calculates the circadian light, stimulus, and melatonin suppression
for the given spd
% Pupil diameter (mm) is used to determine the total stimulus on the
retina (circadian flux)
% lightDuration is in minutes
% Corrections are made for the time of night when the sample is taken
(0:00
% to 6:00 am)-
% and the duration of exposure (minutes).
% spd must be in units of W/m^2/nm (spectral irradiance)
% CL is scaled to be equal to lux for illuminanct A (2856 K)
% spd is two column matrix with wavelength (nm) in the first column
% and spectral irradiance (W/(m^2 nm) in the second column

[row,columns] = size(spd);
if columns > 2
    error('Not column oriented data. Try transposing spd');
end
```

```
CL = CScalc28Mar07(spd); % Circadian Light (formerly circadian
stimulus)
%pupilD = 8; % pupil diameter in mm
%drawTime = 0; % 12:00 am blood draw time
%lightDuration = 30; % 30-minute light exposure

drawTimeAdj = 0.0017*drawTime^3 - 0.013*drawTime^2 + 0.059*drawTime -
0.11; % percentage points of suppression adjustment
pupilFactor = (pupil^2)/(2.3^2); % Ratio of pupil areas using 2.3 mm
diameter as baseline
exposureTimeFactor = lightDuration/60; % Suppression curvefit based on
60-minute exposure; suppression directly proportional to expousure time

% Equation used prior to June 26, 2009
%CS = CL*pupilFactor*exposureTimeFactor;% Circadian Stimulus including
pupil area and exposure duration
% Pupil area affects the physical stimulus before it is transduced so
it should modulate the spd intensity, not the nonlinear CL response
% Therefore, first adjust the physical amount of light, then calculate
circadian effectiveness
CLa                         =                         CScalc28Mar07([spd(:,1)
spd(:,2)*pupilFactor*exposureTimeFactor]);%      Circadian      Stimulus
including pupil area and exposure duration
%Suppression = ((0-0.75)./(1+(CS/(0.0435279*5830.6)).^0.85391))+0.75; %
Melatonin suppression
CS = 0.75-(0.75/(1+(CLa/215.75)^0.864)); % changed 16Feb2010 as per
Circadian Light Paper
 %CS = CS + drawTimeAdj; %*****Removed for Daylighting for schools
calcs.

end

function CL = CScalc28Mar07(spd,varargin)
% Calculates the circadian stimulus for the given spd
% spd is assumed to be in units of W/m^2
% CS is scaled to be equal to lux for illuminanct A (2856 K)
% spd is two column matrix with wavelength (nm) in the first column
% and spectral irradiance (W/(m^2 nm) in the second column
% OR spd is a column vector and start, end and increment wavelength
values
% are specified as additional arguements (e.g. f(spd,400,700,10))

if length(varargin)==0
    [rows columns] = size(spd);
    if columns > 2
        error('Not column oriented data. Try transposing spd');
    end
    wavelength_spd = spd(:,1);
    spd = spd(:,2);
else
    startw = varargin{1}
    endw = varargin{2}
    incrementw = varargin{3}
    wavelength_spd = (startw:incrementw:endw)';
    [rows columns] = size(spd);
    if columns > 1
```

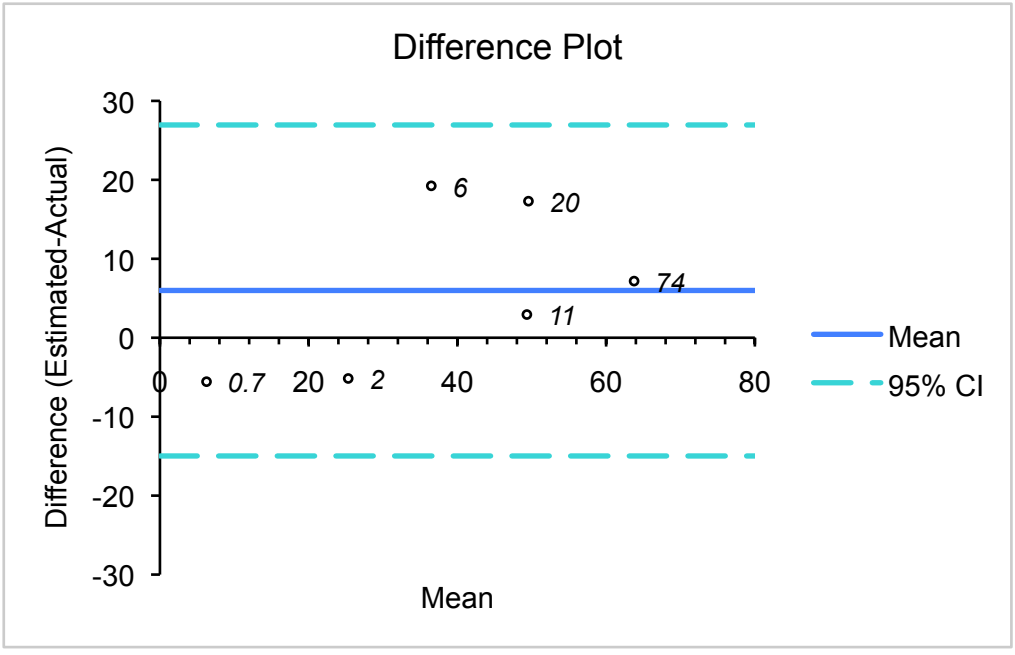
```
        error('Detected multiple columns of data. Try transposing
spd');
    end
end

Vlamda = load('Vlamda.txt');
Vlambda = interp1(Vlamda(:,1),Vlamda(:,2),wavelength_spd,'linear',0.0);

Vprime = load('Vprime.txt');
Vprime = interp1(Vprime(:,1),Vprime(:,2),wavelength_spd,'linear',0.0);

V10lamda = load('CIEY10_2nm.txt');
V10 = interp1(V10lamda(:,1),V10lamda(:,2),wavelength_spd,'linear',0.0);

wavesc = 390:10:730;
Scone = [0.0001 .00224 .00467 .00851 .01164 .01287 .01179 .01032 .00892
.00605 .00357 .00212 .00123 .00061 .00033 ...
        .00016 .00007 .00003 .00002 .00001 .00001 .00001 0 0 0 0 0 0 0
0 0 0 0 0 0];
Scone = interp1(wavesc,Scone,wavelength_spd,'linear',0.0);
Scone = Scone/max(Scone);

Melanopsin = load('Melanopsin.txt');
M                                                                    =
interp1(Melanopsin(:,1),Melanopsin(:,2),wavelength_spd,'linear',0.0);

rodSat = 35000; % Scotopic Trolands
retinalE = [1 3 10 30 100 300 1000 3000 10000 30000 100000];
pupilDiam = [7.1 7 6.9 6.8 6.7 6.5 6.3 5.65 5 3.65 2.3];
diam = interp1(retinalE,pupilDiam,rodSat,'linear');
rodSat = rodSat/(diam^2/4*pi)*pi/1700;

a1 = 0.285;                          %0.285
b1 = 0.01;                           %0.01
a2 = 0.2;                            %0.2
b2 = 0.001;                          %0.001
k = 0.31;                            %0.31
a3 = 0.72;                           %0.72

P = spd;
if  (trapz(wavelength_spd,Scone.*spd)-k*trapz(wavelength_spd,V10.*spd))
>= 0
    CS1 = a1*trapz(wavelength_spd,M.*spd)-b1;
    if CS1 < 0
        CS1 = 0; % remove negative values that are below threshold set
by constant b1.
    end
    CS2             =             a2*(trapz(wavelength_spd,Scone.*spd)-
k*trapz(wavelength_spd,V10.*spd))-b2;
    if CS2 < 0
        CS2 = 0; % This is the important diode operator, the (b-y) term
cannot be less than zero
    end
    Rod = a3*(1-exp(-trapz(wavelength_spd,Vprime.*spd)/rodSat));
    CS = (CS1 + CS2 - Rod);
```

```
    if CS < 0
        CS = 0; % Rod inhibition cannot make the CS less than zero
    end
    %disp('(B-Y) > 0')
else
    CS = (a1*trapz(wavelength_spd,M.*P)-b1);
    if CS < 0
        CS = 0; % Negative values mean stimulus is below threshold set
by constant b1
    end
    %disp('(B-Y) < 0')
end
CL = CS*5830.6; % sets equal to photopic value for 1000 lux of 2856 K
end
```

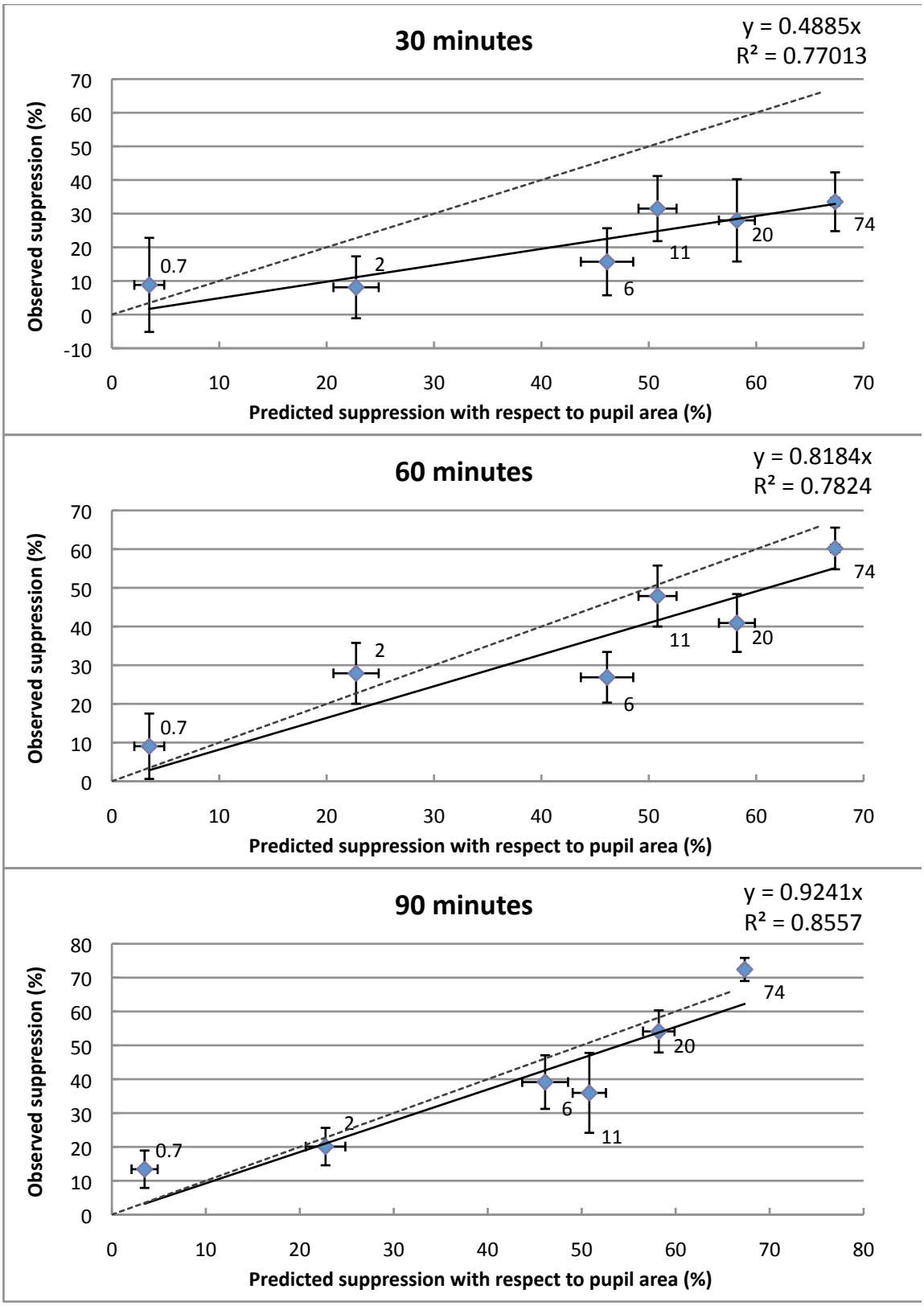
6.7 2005 model estimated melatonin suppression (%) with respect to pupil area

	Corneal irradiance (μW/cm²)					
Duration exposure (min)	0.7	2	6	11	20	74
60	3.48	22.74	46.11	50.81	58.20	67.35

6.7.1 Bland-Altman analysis of 2005 model estimated and calculated suppression



6.7.2 Correlation graphs of calculated mean melatonin suppression and 2005 model estimates for 60-minute exposure durations



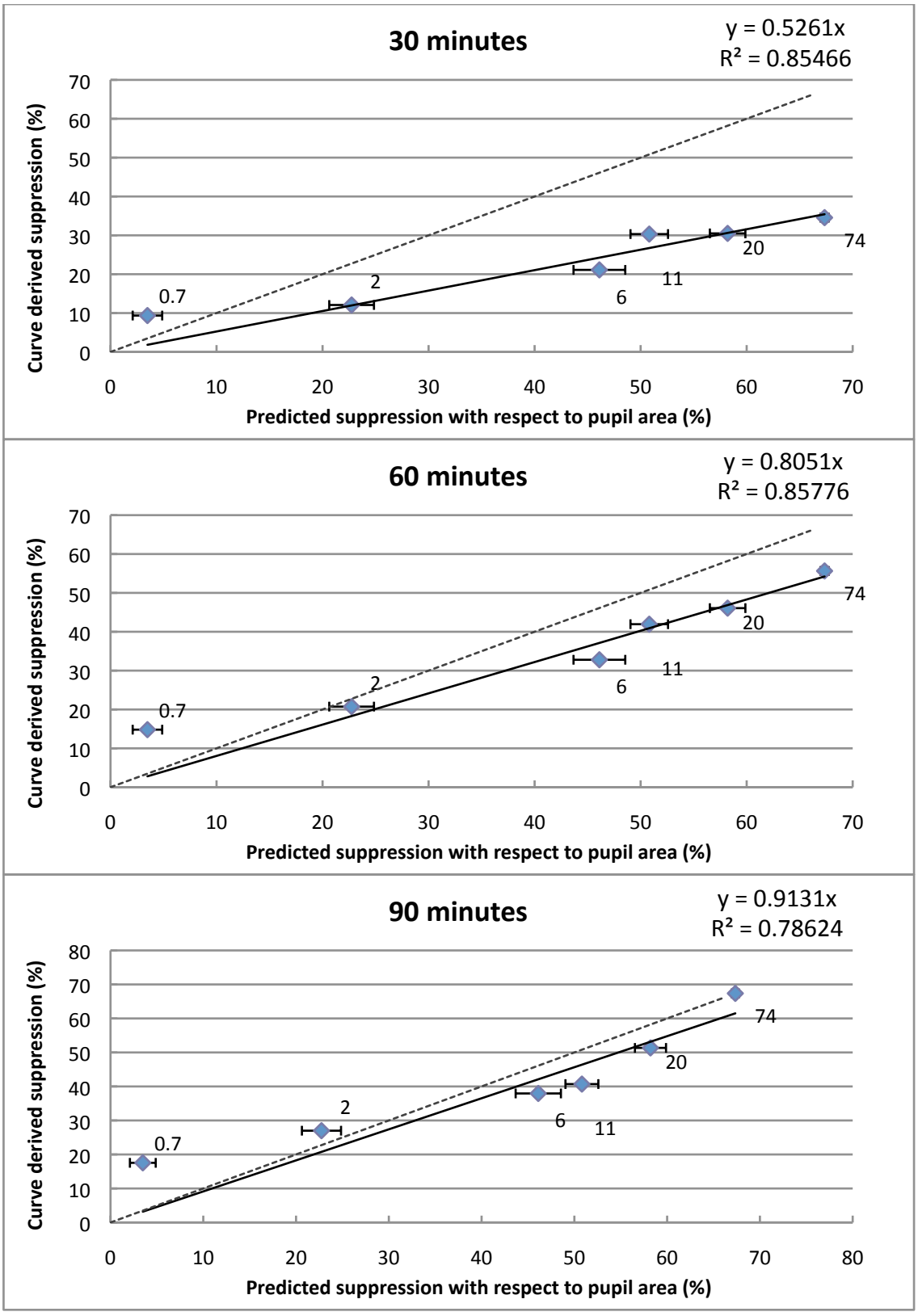
6.8 Relative melatonin concentrations derived from quadratic curves

	Corneal irradiance (µW/cm²)						
Sample time (h:mm)	Dark	0.7	2	6	11	20	72
0:30	1.15	1.04	1.01	0.91	0.80	0.80	0.75
1:00	1.31	1.11	1.03	0.88	0.76	0.70	0.58
1:30	1.46	1.21	1.07	0.91	0.87	0.71	0.48

6.9 Melatonin suppression (%) derived from quadratic curves

	Corneal irradiance (µW/cm²)					
Duration exposure (min)	0.7	2	6	11	20	72
30	9.36	12.08	21.11	30.32	30.50	34.56
60	14.81	20.74	32.79	41.94	46.07	55.64
90	17.54	26.99	37.94	40.70	51.34	67.37

6.9.1 Correlation graphs of curve derived melatonin suppression and 2005 model estimates for 60-minute exposure durations



6.10 MatLab program for 2011 model calculations

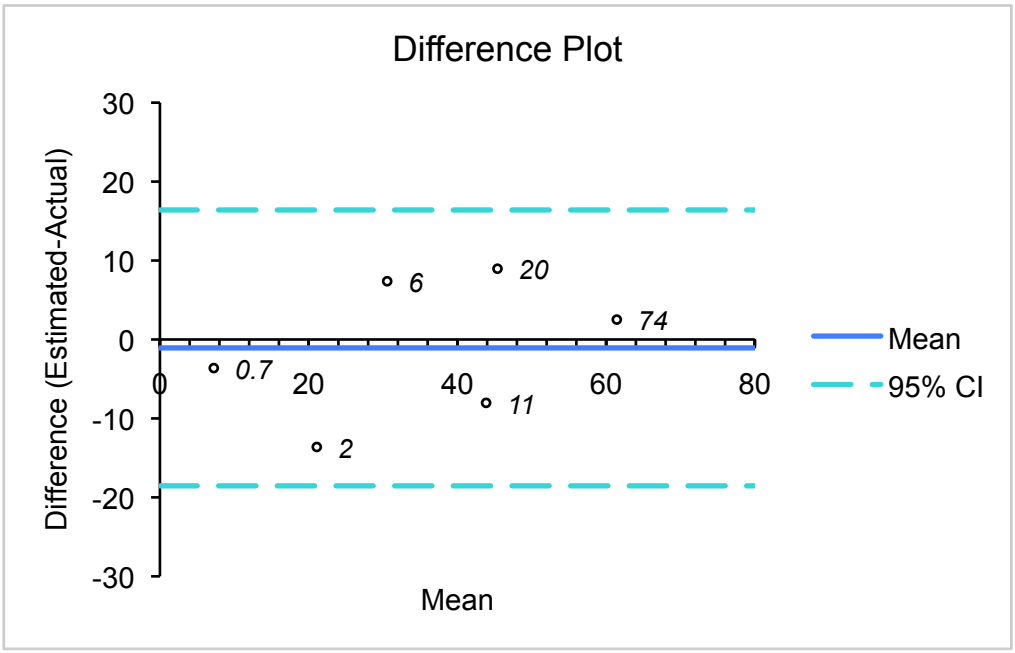
```
function [CS] = MSw(spd,pupilD,w) %SPDs are relative SPDs with max = 1

% For a given relative SPD and target irradiance in (uW/cm^2) this
program calculates
% the meletonin suppression and CL for natural pupils based on Berman
model

wave = spd(:,1);
spdvalue = spd(:,2);

Vlamda = load('Vlamda.txt');

%next create a Vlamda Curve that has data points matching the input SPD
VlamdaI= interp1(Vlamda (:,1),Vlamda (:,2),wave,'linear',0.0);


%Convert relative SPD into absolute irradiance SPD

n=trapz(wave,spdvalue);     %find the relative power under the curve

a= w/n;                     %find the scaling factor to scale the
relative spd
                            %into an absolute spd based on an
irradiance value

spda=[spd(:,1),spd(:,2)*a]; %use scaling factor to convert relative spd
                            %to an absolute spd

%SPDirradiance=trapz(spda(:,1),spda(:,2)) % resulting irradiance in
W/m^2



%Convert irradiance spd into illuminance spd (this is for calculating
pupil size and checking purposes)

        %spdb=[spda(:,1),spda(:,2).*VlamdaI];

        %spdc=[spdb(:,1),spdb(:,2)*683];

        %SPDilluminance=trapz(spdc(:,1),spdc(:,2)) % resulting
illuminance in lux

        %pupil = PupilSize_Berman(SPDilluminance,spda) %calculate
natural pupil diameter

[CLA,CSstd,CS] = CS_Berlin(spda,pupilD); %(spd (in W/m^2),pupil
diameter,draw time,exposure time)
 CLA;
 CSstd;
CS;
 end

 function [CLA,CSstd,CS] = CS_Berlin(spd,pupilD)
```

```
% Calculates the circadian light (CLA), Circadian stimulus (CSstd) for
% standard observer (2.3 mm diamter pupil, 60-minute exposure)
% and melatonin suppression for the given spd and pupil size (CS)
% Pupil diameter (mm) is used to determine the total stimulus on the
retina (circadian flux)
% lightDuration is in minutes

% spd must be in units of W/m^2/nm (spectral irradiance)
% CLA is scaled to be equal to lux for illuminanct A (2856 K)
% spd is two column matrix with wavelength (nm) in the first column
% and spectral irradiance (W/(m^2 nm) in the second column

[row,columns] = size(spd);
if columns > 2
    error('Not column oriented data. Try transposing spd');
end

CLA = CLA_Berlin(spd); % Circadian Light
CSstd = 0.7-(0.7/(1+(CLA/450)^1.21)); % Standard observer pupil
diameter of 2.3 mm; New threshold determination May 11, 2011

pupilFactor = (pupilD^2)/(2.3^2); % Ratio of pupil areas using 2.3 mm
diameter as baseline

% Pupil area affects the physical stimulus before it is transduced so
it should modulate the spd intensity, not the nonlinear CL response
% Therefore, first adjust the physical amount of light, then calculate
circadian effectiveness
CLApupil = CLA_Berlin([spd(:,1) spd(:,2)*pupilFactor]);% Circadian
Stimulus including pupil area and exposure duration
CS = 0.7-(0.7/(1+(CLApupil/450)^1.21)); % New threshold determination
May 11, 2011
 end
```

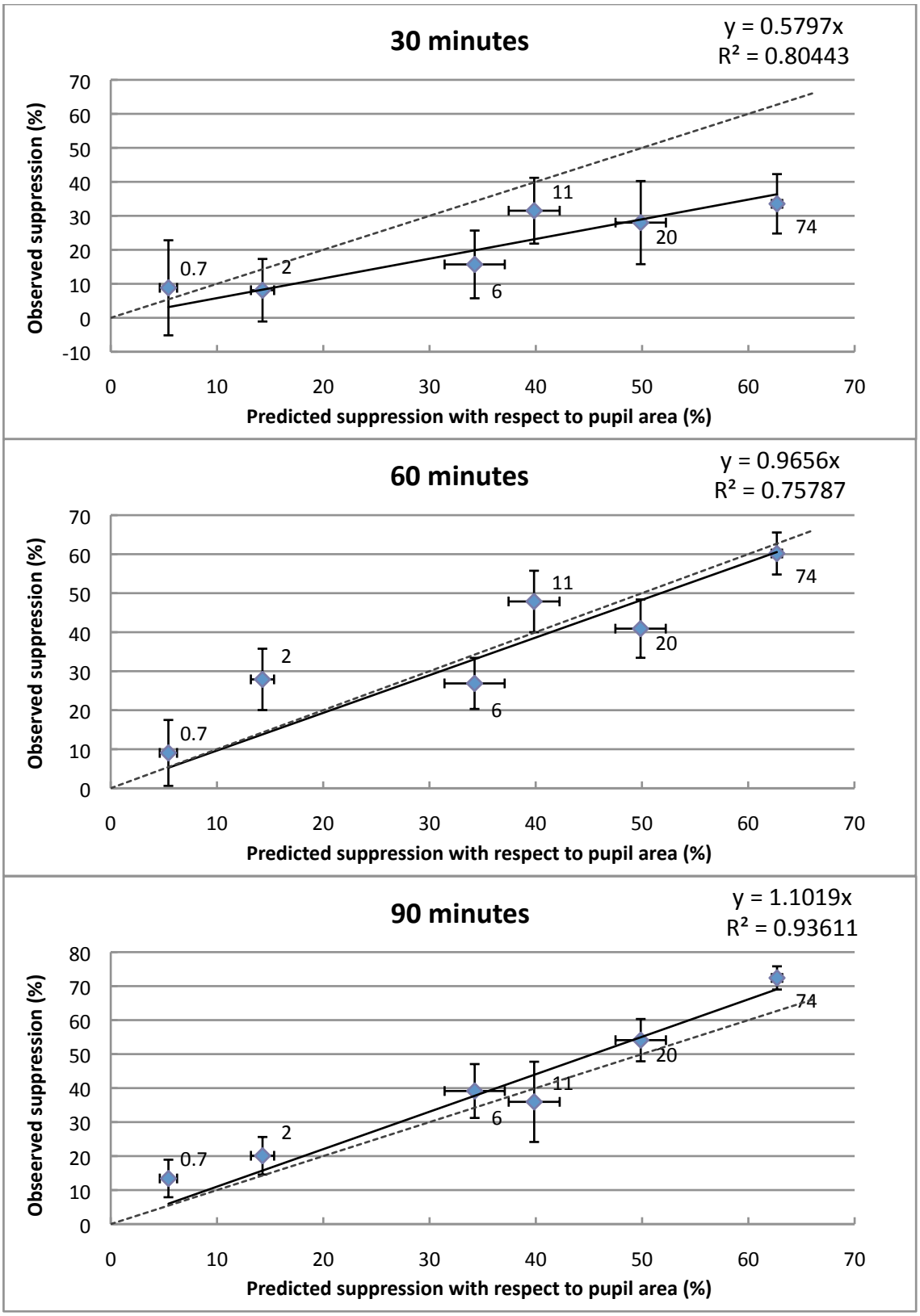
6.11 2011 model estimated melatonin suppression (%) with respect to pupil area

	Corneal irradiance (μW/cm²)					
Duration exposure (min)	0.7	2	6	11	20	74
60	5.43	14.28	34.25	39.85	49.87	62.70

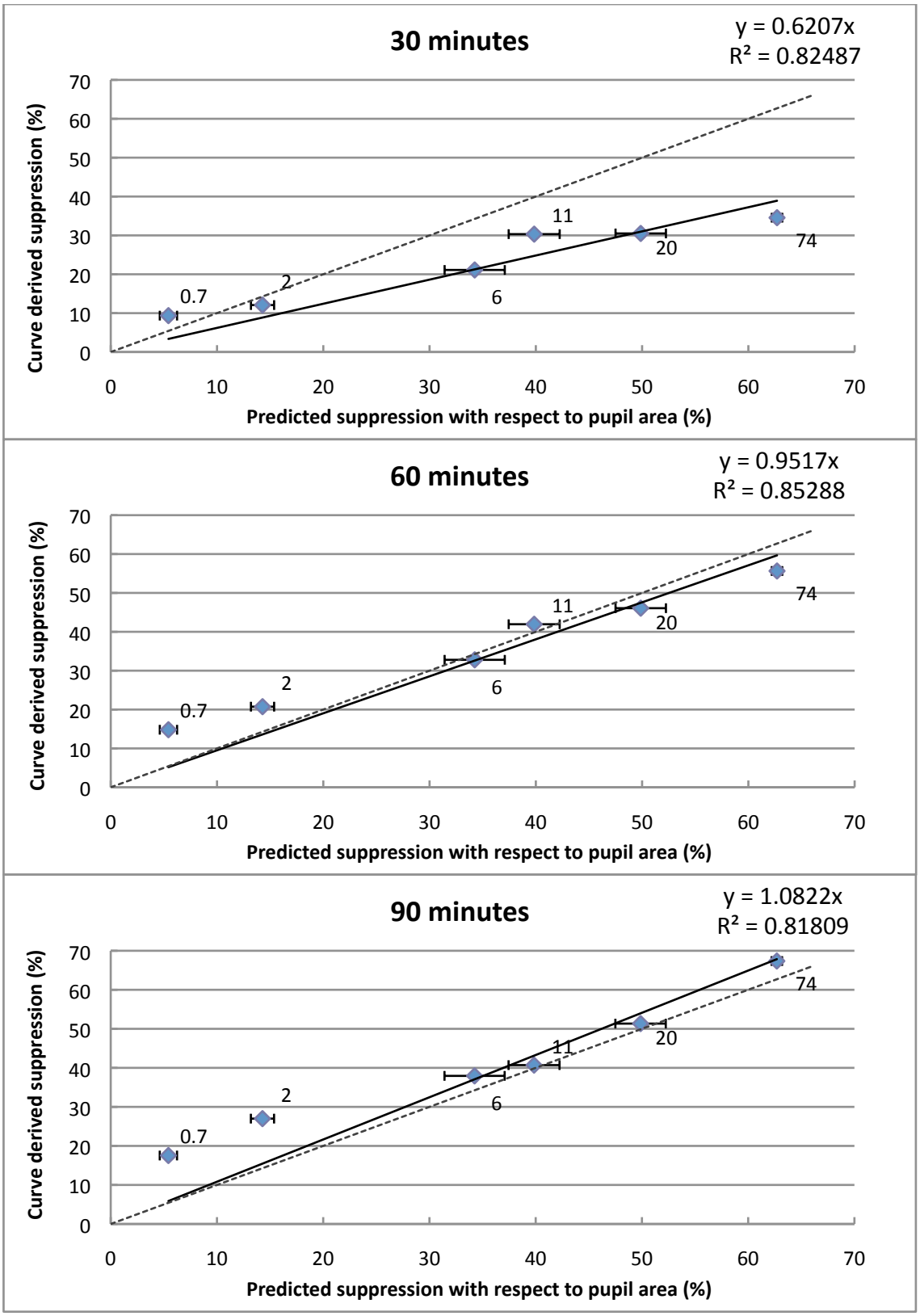
6.11.1 Bland-Altman analysis of 2011 model estimated and calculated suppression



6.11.2 Correlation graphs of calculated mean melatonin suppression and 2011 model estimates for 60-minute exposure durations



6.11.3 Correlation graphs of curve derived melatonin suppression and 2011 model estimates for 60-minute exposure durations



6.12 Melatonin suppression (%) for West et al. (2010)

	Corneal irradiance (μW/cm²)							
Duration exposure (min)	0.1	0.5	2	10	20	75	300	600
90	4.21	10.41	9.81	35.34	41.22	47.66	59.51	60.68

6.13 2011 model estimated melatonin suppression (%) with respect to pupil area for West et al. (2010)

	Corneal irradiance (μW/cm²)							
Duration exposure (min)	0.1	0.5	2	10	20	75	300	600
60	0.54	3.68	15.94	39.96	50.78	64.06	67.69	68.98